

1998

## Exclusion mapping of polycystic kidney disease: A third locus

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**EXCLUSION MAPPING OF POLYCYSTIC KIDNEY DISEASE:**

**A THIRD LOCUS**

**BY**

Eoin Thompson

A thesis submitted in partial fulfilment of the requirements for the award  
of Masters of Science (Human Biology)

at the Faculty of Communications, Health and Science,

Edith Cowan University.

Date of submission:

## **ACKNOWLEDGEMENTS**

Firstly, I would like to thank the Australian Kidney Foundation for providing the funding for this project.

I would also like to thank my supervisor, Dr. Luba Kalaydjieva, for her expert advice and assistance in completing this project. Also for having patience with me. It could certainly not have been completed without her.

I would also like to thank Dr. Nadja Bogdanova of the University of Muenster, Germany, who gave me enormous help completing the genotyping and collecting of the DNA samples for this project.

I would especially like to thank Associate Professor Joachim Hallmayer for his excellent advice and assistance in completing the statistical calculations for this project. Also for helping me to understand their significance.

Thanks also to David Chandler for his valued advice, technical assistance and continued help throughout this project. Thank you to Dr. Dora Angelicheva and Danielle Dye for their valued help in a time of need.

Thank you to everyone at the Centre for Human Genetics, Edith Cowan University, who helped me through this project and encouraged me every step of the way. Good luck to you all.

Finally I want to thank my family and friends for their moral support throughout this project. Especially my parents, because without their support I would not have completed this project.

## **ABSTRACT**

The aim of this research was to perform exclusion on a rare form of hereditary autosomal dominant polycystic kidney disease (ADPKD). To-date, two genes for ADPKD have been identified: PKD1 which has been localized to the short arm of chromosome 16 and PKD2 which has been localized to the long arm of chromosome 4. However, a small number of families have been reported that have not shown linkage to either of these two loci, thus suggesting the existence of at least one additional locus (PKD3). Two families that are affected with ADPKD and do not show linkage to PKD1 or PKD2 have participated in this study. Both families are of Bulgarian origin and between them contain 9 affected individuals. Even though these families are small, they are large enough to exclude a large proportion of the genome as the location of the PKD3 gene. The availability of these families and the discovery of other non PKD1/PKD2 families has provided a rare opportunity to perform exclusion mapping of the PKD3 gene which will eventually lead to mapping and cloning of this gene.

A systematic search of the human genome was performed using polymorphic markers (from the Research Genetics screening set version 4a) which span the human genome at an average distance of 16cM. The results were then analysed statistically using the computer programs EXCLUDE, LINKAGE and GENEHUNTER. From this study the highest Lod score obtained was a multipoint Lod score of 2.95 on 22q11.21 at marker D22S446. Two other regions also produced relatively high Lod scores, the first being on 4q22.1 at marker D4S1647

which produced a Lod score of 1.42 and the second region being on 11p15.2 at marker D11S902 which produced a Lod score of 1.41.

These results provide a strong indication as to the location of the PKD3 gene however, before linkage can be claimed with any degree of certainty the sample size must be increased with the addition of other non PKD1/PKD2 families. This increases the possibility of obtaining a Lod score of 3.00 or greater and thus proving linkage to the PKD3 gene.

## DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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## TABLE OF ABBREVIATIONS

ADPKD	Autosomal Dominant Polycystic Kidney Disease
ESRF	End Stage Renal Failure
PKD	Polycystic Kidney Disease
ICA	Intracranial Aneurysms
HVR	Hypervariable Region
cpk	congenital polycystic kidney
pcy	polycystic kidneys
jck	juvenile cystic kidneys
kat	kidney anemia testis
PKDr	Polycystic Kidney Disease rat
PCR	Polymerase Chain Reaction
PAGE	Polyacrylamide Gel Electrophoresis
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide tri-phosphate
dATP	Deoxyadenosine tri-phosphate
dCTP	Deoxycytidine tri-phosphate
dGTP	Deoxyguanosine tri-phosphate
dTTP	Deoxythymidine tri-phosphate
mM	Milimolar ( $10^{-3}$ )
ml	Mililitre ( $10^{-3}$ )
ul	Microlitre
uM	Micromolar
TEMED	N,N,N',N'-tetramethylethylene-diamine
mm	Milimetre

M	Molar
bp	Base Pair
TBE	Tris-borate electrophoresis buffer
ABI	Applied Biosystems Inc.
APM	Affected pedigree member
IBS	Identity by state
IBD	Identity by descent
$\theta$	Theta

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## **INTRODUCTION AND LITERATURE REVIEW**

### **1.1 Introduction**

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic diseases, affecting approximately 1 in 1000 individuals in Caucasian populations (Dalgaard, 1957). It accounts for approximately 8-10% of all patients requiring haemodialysis (Gabow *et al.*, 1992).

The main clinical expression of the disease is multiple fluid-filled cysts present in the kidneys and in some cases, also in the liver, spleen, ovaries and pancreas. Other symptoms of polycystic kidney disease include hypertension, cardiac valve defects, herniae and intracranial aneurysms. The phenotypic expression of polycystic kidney disease varies quite considerably: some individuals remain mildly affected into their 60's and 70's, while others suffer from end stage renal failure (ESRF) in early childhood. According to a study by Parfrey *et al.* (1990) 25% of patients reach end stage renal failure (ESRF) by the age of 47, 50% by the age of 59 and 75% of patients by the age of 70 (Parfrey *et al.*, 1990). However, not all patients reach ESRF and even though cysts may be present, deterioration of kidney function may not be obvious (Churchill *et al.*, 1984). This wide variation in clinical severity is due mainly to the genetic heterogeneity of ADPKD.

So far, two different genes have been found that are responsible for autosomal dominant polycystic kidney disease. The first gene, PKD1 has been mapped to the short arm of chromosome 16 (Reeders, *et al.*, 1985) and further localised to 16p13.3 (Somlo *et al.*, 1992). The second of these loci, PKD2, has been mapped to the long arm of chromosome 4 at position 4q21-23 (Kimberling, *et al.*, 1993, Peters *et al.*, 1993). In 1994, the PKD1 gene was cloned and the entire nucleotide sequence identified (The European Polycystic Kidney Disease Consortium, 1994). The product of the PKD1 gene, Polycystin, is involved in cell-cell and/or cell-cellular matrix interactions. The product of the PKD2 gene (Polycystin 2) has recently been determined (Mochizuki *et al.*, 1996) and found to be very similar to Polycystin. The role of Polycystin 2 is that it associates with Polycystin as part of a common signal transduction pathway (Qian *et al.*, 1997). The knowledge of the structure of both genes and their products is invaluable in understanding the pathogenesis of polycystic kidney disease.

To date, research carried out on polycystic kidney disease has focused on PKD1 and PKD2. However, studies in different countries have revealed the existence of a small number of families affected with polycystic kidney disease that do not show linkage to either the PKD1 or the PKD2 locus (de Almeida *et al.*, 1995, Bogdanova *et al.*, 1995, Daoust *et al.*, 1995). This indicates further locus heterogeneity, and the existence of at least one additional gene responsible for polycystic

kidney disease, (provisionally designated PKD3). In this study, two families which do not show linkage to PKD1 and PKD2 will be analysed. Computer simulations using the package SLINK (Ott, 1989, and Weeks et al., 1990) have shown that the maximum likely Lod score that can be obtained using the first family is 1.43 and the maximum Lod score that can be reached with the second family is 1.08. Combined, these scores gives a total Lod score of 2.51 which indicates that these families are not powerful enough to perform linkage, however they allow the exclusion of a large proportion of the genome as the location of the PKD3 gene. Pooling the results of this study with those obtained by other groups may provide enough linkage power to map PKD3, thus paving the way for refined mapping and the eventual cloning of the PKD3 gene.

The identification of the gene will shed more light on the pathogenesis of the disease and provide a better understanding of its molecular mechanisms. ADPKD is a complex disorder whose phenotype may possibly result from defects in a number of different signalling and/or growth regulation pathways. Understanding PKD3 may result in the identification of a new pathway in the regulation of cell growth and differentiation.

## **1.2 LITERATURE REVIEW**

### **1.2.1 Clinical Features**

Polycystic kidney disease is characterised by progressive swelling of multiple tubular segments and, as the name suggests, the development of multiple fluid filled cysts in the kidneys. These cysts vary greatly in size and some may be exceedingly large. They seem to be scattered equally in the cortex and medulla of the kidney and generally there is no evidence of any intact parenchyma (Wardener, 1985). In more severe cases, extrarenal cysts may be evident in organs such as the liver, spleen and pancreas (Chapman *et al.*, 1991).

Hypertension is a common manifestation of ADPKD and is an important factor in the overall patient mortality (Chapman *et al.*, 1991). Studies on the incidence of hypertension in ADPKD patients have shown an elevated number of hypertensive patients in this population compared to the non-ADPKD population. Higgins (1952) reported a 73% incidence of hypertension among 94 patients and, in another study, Hansson *et al.*, (1974) reported a 75% incidence among ADPKD individuals. Recently, it has been shown that the incidence of hypertension in ADPKD patients increases with age. In a study of 17 families, 25% of patients aged under 40 years had hypertension, this increased to 55% in patients between 40 and 59 years of age, and to 75% in patients over the age of 60 (Parfrey *et al.*, 1995). A classical study carried out by Dalgaard (1957) reported that 64% of ADPKD patients

died of cardiovascular causes and, of these, 52% had increased left ventricular mass, a known consequence of prolonged hypertension and a risk factor for patient death.

Liver cysts are frequently found in ADPKD patients, with more women being affected than men (Levey *et al.*, 1983). The prevalence of liver cysts increases with age, with one study reporting that 18% of ADPKD patients under the age of 30 years had liver cysts while 70% of ADPKD patients over the age of 60 displayed liver cysts (Levey *et al.*, 1983).

Another important clinical characteristic of ADPKD is the development of intracranial aneurysms (ICA). There is some variation in the reported frequency of ICAs within the ADPKD population. Levey *et al.*, (1983) reported 10-30% and a subsequent study by a Japanese group found a 41% prevalence of ICAs among 17 ADPKD patients (Wakabayashi *et al.*, 1983). More recently however, Kaehny *et al.*, (1991) reported a 6-10% prevalence of ICAs among 431 ADPKD patients. Other characteristics of ADPKD include diverticulosis of the colon, herniae and cardiac valve defects.

Polycystic kidney disease gives rise to considerable morbidity and mortality in affected individuals and is an important cause of end-stage renal failure (ESRF) in both adults and children worldwide (Avner, 1994). Death can be caused by end stage renal failure (ESRF) due to compression of the renal parenchyma by the enlarging cysts, or from

hypertensive cardiac failure (Uldall, 1988). The age of onset of symptoms of PKD is generally in the 3<sup>rd</sup> or 4<sup>th</sup> decade and the severity varies from patient to patient and more importantly between families with different forms of the disease. ESRF usually develops midlife ie. about 55 years of age.

### **1.2.2 Clinical Heterogeneity**

Studies have shown that there is a great degree of phenotypic variation between PKD1 and non PKD1 individuals. In a study of phenotype-genotype heterogeneity in autosomal dominant polycystic kidney disease, Ravine et al. (1992) reported that non-PKD1 families displayed a milder phenotype than families linked to PKD1. Non-PKD1 patients lived longer with a median survival age of 71.5 yrs. (compared to 56.0 yrs. in PKD1), had a lower risk of progression to renal failure, were less likely to develop hypertension and were diagnosed at an older age (median 69.1 yrs. vs. 44.8 yrs.). Non-PKD1 patients also showed fewer cysts than PKD1 patients. In a large European study conducted by Peters et al. (1993), it was shown that affected individuals from families that were not linked to PKD1 were diagnosed at an older age and had fewer cysts at the time of diagnosis. These patients were less likely to have hypertension, lived longer and had a slower progression towards kidney failure than patients with PKD1. These findings also suggested that non-PKD1 has a less severe phenotype than PKD1 thus supporting

the findings of Ravine *et al.* (1992). Further evidence to support the claim that PKD1 is a more severe type of ADPKD than non-PKD1 was presented by Jeffery *et al.* (1993) who reported that a family of Sicilian origin that did not link to PKD1 had a milder phenotypic expression than families that linked to PKD1. In this family, a 75 year old woman had no clinical symptoms and was normotensive; the only member of the family to have elevated blood pressure was a male in his seventies.

The clinical severity of PKD in families not linked to either PKD1 or PKD2 varies between the different reports. Daoust *et al.*, (1995) described a family not linked to PKD1 or PKD2 that showed progression to renal failure similar to that found among patients with PKD1. Another paper by de Almeida *et al.*, (1995) reported a Portuguese family not linked to PKD1 or PKD2 that showed a slower progression to renal failure than that of PKD1. The clinical severity of PKD3 also varies between individuals within families, with some showing a faster rate of progression to renal failure than others (Daoust *et al.*, 1995). More recently Turco *et al.*, (1996) reported a small family of Italian origin that did not show linkage to PKD1 or PKD2 and presented with a mild phenotype.

### **1.2.3 Locus Heterogeneity**

#### **1.2.3.1 Linkage To Chromosome 16 : The PKD1 Gene**

Mutations in PKD1 are by far the most common cause of ADPKD. It has been estimated that in the European population, PKD1 accounts for about 86% of the families with ADPKD (Peters *et al.*, 1992). In 1985, the PKD1 gene was localised to the tip of the short arm of chromosome 16 (Reeders *et al.*, 1985). In a study of nine families with a mean age at onset of kidney failure of 48yrs, a highly polymorphic probe from the 3'HVR, 8kb beyond the 3'  $\alpha$ -globin region was used in the search for the ADPKD gene. All of these families showed linkage to this locus with a maximum Lod score of 25.85 at  $\theta = 0.05$ , confidence interval 99%. A subsequent study in Wales involving 24 families diagnosed with ADPKD, the 3'HVR and alpha-globin probes produced a maximum Lod score of 24.18 ( $\theta = 0.03$ ) (Lazarou *et al.*, 1987). Further linkage of PKD1 to this region was proven when the 24-1 marker which flanks the PKD1 gene on the opposite side to the  $\alpha$ -globin gene was discovered (Breuning *et al.*, 1987). In 1988, Reeders *et al.* refined the localisation of PKD1. Using 19 affected families, PKD1 was found to lie proximal to the alpha-globin gene cluster and somatic cell hybrid mapping placed it within the region 16p13.11-16pter. Further studies using additional polymorphic markers allowed the



refined mapping of PKD1 to 16p13.3 (Somlo *et al.*, 1992, Germino *et al.*, 1992).

In 1994, the complete structure of the PKD1 gene and its protein was identified by the European Polycystic Kidney Disease Consortium. The PKD1 transcript has a length of 14.5 kb and encodes a 4304 amino acid protein that contains 14 units of a novel 80 amino acid domain. It was proposed by the Consortium that Polycystin is a multifunctional protein that is involved in protein - protein interactions in the extracellular compartment. The presence of numerous binding sites on Polycystin suggests that it binds to components of the extracellular matrix thus mediating cell - cell or cell - matrix interactions.

#### 1.2.3.2 Linkage To Chromosome 4: The PKD2 Gene

Initially, in 1987, Reeders *et al.* reported among 28 unrelated families of northern European decent no evidence to suggest locus heterogeneity within the ADPKD population. In 1988 however, Romeo *et al.* reported a single Italian family with ADPKD that failed to show linkage to the PKD1 gene. Using the 3'HVR and 090a probes, Lod scores of -2.76 and -2.36 respectively were obtained. Thus linkage to the PKD1 gene was effectively ruled out. This indicated the existence of a second gene responsible for ADPKD. More evidence of genetic heterogeneity was revealed by the study of three Icelandic families and a large three generation family in Northern Ireland with ADPKD which

did not show linkage to chromosome 16p13.3 (Fossdal *et al.*, 1993, Wright, *et al.*, 1993).

Peters *et al.* (1993) localized the second gene for ADPKD to chromosome 4. Using the polymorphic DNA markers D4S423 and D4S231, positive Lod scores were detected in two-point linkage analysis. Multipoint linkage analysis was then carried out, producing an even higher Lod score and placing the gene centromeric to D4S231. It has since been shown by refined mapping that this gene, now known as PKD2, lies in the region 4q.21-23 (Kimberling *et al.*, 1993, Peters *et al.*, 1993).

In 1996 Mochizuki *et al.* determined the product of the PKD2 gene. Using the group of clones cTM-4, the PKD2 protein was discovered. It is a 968 amino acid sequence that contains six transmembrane domains with intracellular amino and carboxyl termini. There is 50% homology between the translation product of PKD2 and 450 amino acids of polycystin. Qian *et al.*, (1997) described a previously unrecognized coiled-coil domain within the C terminus of Polycystin and demonstrated that it binds specifically to the C terminus of the PKD2 gene product. This suggested that PKD1 and PKD2 interact and may be partners in a common signal transduction pathway involved in tubular morphogenesis.

### 1.2.3.3 The PKD3 Gene

Recently it has been shown that there are definitely more than two loci responsible for ADPKD. In 1995 a study of 23 affected French-Canadian families reported one that failed to show linkage to either PKD1 or PKD2. This led the researchers to acknowledge the existence of a third PKD locus (Daoust, *et al.*, 1995).

This finding was supported by de Almeida *et al.* (1995) who reported a large Portuguese family that did not show linkage to PKD1 or PKD2. Bogdanova *et al.* (1995) reported that of 22 Bulgarian families with ADPKD, 67% were linked to PKD1 and 33% unlinked to PKD1. Of the six families unlinked to PKD1, five showed linkage to the PKD2 locus and 1 family failed to show linkage to PKD1 or PKD2.

More recently, a two generation family of Italian origin has been reported to be unlinked to either the PKD1 or the PKD2 loci (Turco *et al.*, 1996).

### 1.2.4 Animal Models

Animal models are often very useful in understanding the pathogenesis and morphology of human genetic diseases. Mouse models in particular have been helpful in understanding ADPKD. The first mouse model to be identified was the cpk (congenital polycystic kidney) mutation of autosomal recessive polycystic kidney disease

(ARPKD). The disorder in these mice is fatal within 25 days (Fry *et al.*, 1985). The gene has been located to the proximal region of mouse chromosome 12 (Davisson *et al.*, 1991). A different mutation, pcy, (polycystic kidneys) was then discovered in a KK mouse. This gene was also inherited recessively and the phenotype and course of the disease is similar to that of adult PKD (Takahashi *et al.*, 1985). In 1991 Takahashi *et al.*, localised this gene to mouse chromosome 9 using linkage studies.

A new recessive mutation in the mouse which also causes polycystic kidney disease was discovered in 1993 by Atala *et al.*, and is not allelic to any of the previously described mutations. This mutation known as jck (juvenile cystic kidneys) was localized to mouse chromosome 11 (Jakoubova *et al.*, 1995). Further studies on mouse models of polycystic kidney disease by Olsson *et al.*, (1996) revealed the mouse homologue of the PKD1 gene on mouse chromosome 17. This gene however is a single copy gene unlike the PKD1 gene which is triplicated and clustered on 16p13.1. The mouse PKD1 homologue was sequenced in 1997 by Lohning *et al.*, and the predicted protein showed a 79% similarity to human PKD1. Recent studies have revealed another mouse mutation 'kat' (kidney anemia and testis) that causes polycystic kidney disease. This mutation mapped to the mouse chromosome 8 which is homologous to human chromosome 19p (Janaswami *et al.*, 1997).

Recently a PKD causing mutation has been discovered in the rat. This mutation (PKDr1) has been mapped to rat chromosome 5 in a region homologous to human chromosome 8 (Bihoreau *et al.*, 1997).

#### **1.2.5 Conclusion of the Literature Review**

From the published evidence on both human disease and animal models, it can be concluded that there are definitely more than two loci responsible for ADPKD. To date all research conducted has been focused on the PKD1 and PKD2 gene and the identification of their respective proteins. No research has been conducted into the third locus however, publications in the last few years have revealed the existence of families affected with ADPKD but do not show linkage to the PKD1 or PKD2 gene. As additional non PKD1/PKD2 families are being discovered, the need to carry out further research in this area becomes more and more urgent.

#### **1.3 OBJECTIVES**

The major objective of this study is to exclude a large part of the genome and to demonstrate chromosomal regions which are likely to contain the PKD3 gene. The study is divided into the following stages: Stage one is an initial genome screening of the first family where a large proportion of the human genome will be excluded leaving a number of candidate regions. Stage two of the study involves the saturation of

these candidate regions with extra markers in an attempt to exclude most of them and thus limit the search further. A second PKD3 family of Bulgarian origin will also be introduced at this stage. Stage three of this study is the setting up of international collaboration in an attempt to map the PKD3 gene.

## **2. MATERIALS AND METHODS**

### **2.1 FAMILIES**

#### **2.1.1 Pedigree Structure**

Both families participating in this study are of Bulgarian origin. DNA extraction has been done at the laboratory of Molecular Pathology, Medical School in Sofia, Bulgaria. The analysis of polymorphic markers linked to PKD1 and PKD2 has been conducted at the laboratory of Molecular Pathology and subsequently at the Institute of Human Genetics at the Medical Faculty of Muenster University, Germany.

Pedigree one consists of 11 individuals of whom 5 are affected. The second Bulgarian family consists of 7 individuals with 4 individuals being affected. In both these families linkage to the PKD1 and PKD2 genes has been excluded with deeply negative Lod scores. Family 1 produced Lod scores of -7.757 for markers linked to the PKD1 gene and Lod scores of -2.697 for markers linked to the PKD2 gene. Family 2 also showed non linkage to these two genes with Lod scores of -1.999 to markers linked to the PKD1 gene and Lod scores of -2.389 to the PKD2 gene.

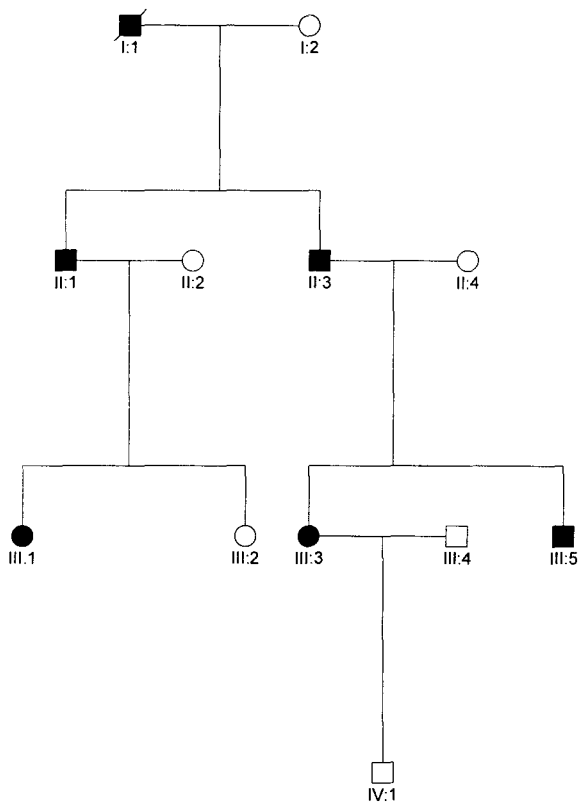


Figure 2.1: Diagram of Bulgarian pedigree 1



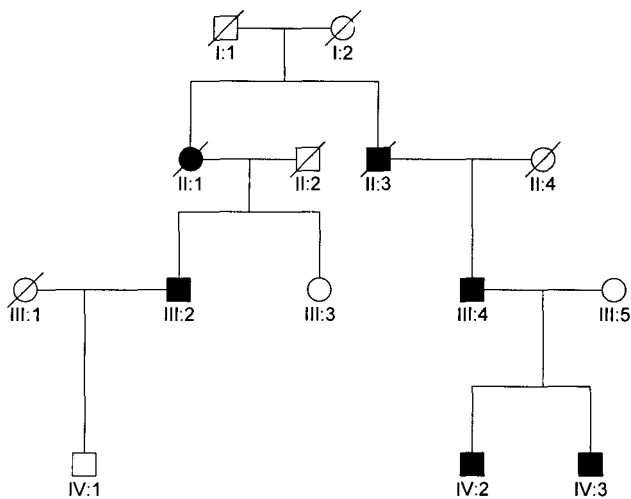


Figure 2.2: Diagram of Bulgarian pedigree 2

### **2.1.2 Clinical Phenotype**

The clinical course of polycystic kidney disease in these two families resembles that of PKD1, with a more rapid deterioration of kidney function observed in family 2. Two individuals in family 1 (III1 and III3) were in their early to mid 50's at the onset of chronic renal failure, both of these individuals had numerous kidney cysts present. Three other individuals (III11, III13 and III15) had normal creatinine levels in their late 20's and early 30's however did have numerous cysts present in their kidneys. All 5 affected individuals did not show liver cysts.

Family 2 showed an earlier onset of PKD than that displayed by family 1. Individual III2, developed chronic renal failure in the early 50's with rapid (6 years) progression to end stage renal failure (compared to the average) of 10 yrs. While individual III4 developed chronic renal failure at the even earlier age of 46 and the time taken to reach end stage renal failure in this individual was even shorter (5 yrs).

### **2.1.3 Linkage Simulations**

Computer simulations were carried out using SLINK (Ott, 1989, Weeks *et al.*, 1990) to determine the power of exclusion of family 1 and the power of linkage of both families combined, thus providing an indication of what proportion of the genome could be excluded.

Conducting 1000 multipoint simulations on family 1 under the assumption of non-linkage, the maximum Lod score obtained for this

family was 1.44. These Lod scores were obtained for theta values 0.00 and 0.05. Simulations were also performed on this family under the assumption of an unlinked marker. Using the same number of simulations as before (1000) it was shown that the maximum was again 1.44 while the minimum and average Lod scores were negative infinity.

The simulations also gave an indication of the probability of obtaining a Lod score greater than 1.00 with family 1 under the assumption of both linkage and non-linkage to a marker. The probability of obtaining a Lod score greater than 1.00 under the assumption of linkage was 62.8% while the probability of obtaining a Lod score greater than 1.00 under the assumption of non-linkage was 3.4%. This indicates that, for a linked marker, a Lod score of 1.00 or greater will be obtained in 62.8% of cases, whereas, the percentage of false positive results using this cut-off point is expected to be quite low (3.4%).

To determine the power to detect linkage using both Bulgarian families, 1000 simulations were performed under the assumption of linkage and the maximum Lod score was obtained. At theta 0.05 and 0.00 the maximum Lod score attainable for family 1 was 1.44 while the maximum for family 2 was 1.08. This proves that the gene cannot be linked however, with the two families combined, a reasonable indication can be obtained as to where the gene is located.

## **2.2 STRATEGY**

This study involves two non-PKD1/PKD2 families and is based on the working assumption that in both families the disease is caused by mutations in the same gene.

This study can be divided into three stages:

Stage 1 is the initial screening stage and involves the first Bulgarian family only. The DNA for this family was analysed using 234 polymorphic markers from the Research Genetics screening set version 4a, spanning the entire human genome and spaced on average about 16cM apart. The genotyping results were analysed using the program MLINK of the LINKAGE package (Lathrop *et al.*, 1984). This package performs two-point analysis producing two-point Lod scores. These two-point Lod scores were represented graphically in exclusion maps using the EXCLUDE program (available from Dr J.H. Edwards). These graphs show areas that can be excluded, represented as flat regions on the chromosome graph. Multipoint linkage analysis was carried out using the Genehunter program (Kruglyak *et al.*, 1996). Any region that produced a Lod score of 1 or greater was considered a candidate region requiring further investigation.

The second stage of the study involved a detailed investigation of the candidate regions from stage one. The investigation was carried out, by saturating the candidate regions with additional markers and including the second family in the analyses. The aim of this stage was to

exclude these regions or to reconfirm them as possible candidates. As in stage one of the study, linkage analysis was performed on the genotyping results obtained. Stage two also focussed on genomic regions that could not be excluded during the initial screening because of large distances between markers. These large distances were investigated by adding extra markers and analysing them on the first Bulgarian family.

The third stage of the study was to set up an international network of collaboration between research groups working on non-PKD1/PKD2 families.

### **2.3 LABORATORY PROCEDURES**

The polymorphic markers used to screen the human genome in our study belong to the category of microsatellites. These microsatellites are short tandem repeats (less than 500bp) with the repeats varying in length from 1 to 5 nucleotides (Weber and May, 1989). Several types of microsatellites have been described, such as poly (G) and poly (A), however the most common is purine/pyrimidine repeats poly (CA/GT) which are thought to constitute 57% of the total purine/pyrimidine repeats. The polymorphic nature of these microsatellites is due to the number of tandem repeats presented in a block and it is widely noted that the greater the number of repeats at a locus the more informative the marker (Weber, 1990). The mutation rate of microsatellites is between  $5 \times 10^{-3}$  and  $10^{-4}$  (Hearne *et al.*, 1992). Microsatellite markers are ideal for

linkage studies not only because of their polymorphic nature but also because of the possibility to conduct the analyses in PCR format.

The laboratory procedures used in this study were as follows:

- Polymerase Chain Reaction (PCR)
- Size separation of the PCR products by polyacrylamide gel electrophoresis (PAGE)
- Detection of the PCR fragments and assignment of polymorphic alleles

### **2.3.1 Polymerase Chain Reaction (PCR)**

The polymerase chain reaction is a technique for the *in vitro* amplification of specific DNA sequences. The underlying principle of PCR is the simultaneous extension of complementary strands of DNA, flanked by two primers, each complementary to part of one of the two strands of the DNA fragment being amplified. The extension of these primers is performed by DNA polymerase. The primers are arranged so that each primer extension directs the synthesis of DNA towards the other, resulting in a synthesised fragment of DNA flanked by the two primers. Initially, synthesis will go beyond the sequence complementary to the primer on the other strand. However, with each cycle, the amount of DNA flanked by each primer increases exponentially while the amount of longer sequences will accumulate linearly. Thus the predominant segment of DNA will be that which is flanked by the primers. DNA

amplification will continue until one of the reaction products is exhausted.

PCR is performed in cycles consisting of the following steps: denaturation, annealing, synthesis. Denaturation is usually performed at high temperatures (usually 94°C). These high temperatures cause the double stranded DNA to dissociate into two single strands. The annealing temperature of the PCR cycle varies depending on the primers being used and is usually around 55°C. This promotes annealing of the unique primer sequence to its complementary site on the DNA. The extension step (usually performed at approximately 72°C) allows the enzyme to replicate the template DNA. The number of cycles required for optimum amplification varies, but generally 25-35 cycles are sufficient.

The reagents required for performing PCR are: deoxynucleotides (dNTPs), to provide the nucleosides for the synthesis of DNA; DNA polymerase; primers; genomic DNA as template and buffer. The dNTPs and primers are present in excess so as not to limit the rate of the reaction. The reaction buffer that was used in our experiments varied, depending on where it was ordered. Two commercial sources were used: Molecular Sigma Biology and Perkin Elmer. The buffer ordered from Molecular Sigma Biology contained the following components:

67mM Tris-HCl (pH 8.8 at 25°C)

16.6mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

0.45% TritonX-100

0.2mg/ml gelatin

The buffer ordered from Perkin Elmer contained:

500mM KCl

100mM Tris-HCl (pH 8.3)

0.1% (w/v) gelatin

The DNA polymerase most commonly used in PCR is *Taq* polymerase, extracted from the bacterium *Thermophilus aquaticus*. This enzyme is most popular due to its high heat stability. The *Taq* DNA polymerase was supplied by (Molecular Sigma Biology or Perkin Elmer) at a concentration of 5.5 units per ul.

The dNTPs (Molecular Sigma Biology.) used were contained in separate tubes of dATP, dCTP, dGTP and dTTP each at a concentration of 100mM.

The Magnesium Chloride (Molecular Sigma Biology and Perkin Elmer) was packaged at a concentration of 25mM.

The volumes and final concentrations of the PCR components, for a 5ul reaction were as follows:



Table 2.1: Protocol for a 5ul PCR reaction.

Component	Stock Concentration	Volume
H <sub>2</sub> O	-	1.64ul
5.1 <sup>20</sup> Buffer	5mM MgCl <sub>2</sub> , 100uM dNTPs	1.00ul
Primer (Forward)	20uM	0.125ul
(Reverse)	20uM	0.125ul
<i>Taq</i>	5.5 units/ul	0.05ul
<sup>32</sup> P(3000Ci/mmol)	3.3uM	0.06ul
DNA	5ng/ul	2.00ul

Pipetting was performed using a Tri Continent Scientific Automated Pipette. The automatic pipette uses capillary tubes (Australian Biosearch). The PCR was performed on a Bresatec minicycler.

### **2.3.2 Methods of labelling the PCR Product**

The two techniques for detection of DNA used in this study were:

1. Incorporation of radioactively labelled nucleotides into PCR products which allows visualisation by autoradiography.
2. Incorporation of fluorescently labelled primers into PCR products with subsequent automated laser detection.

### 2.3.2.1 Incorporation of $\alpha$ - $^{32}\text{P}$ Deoxycytosine 5'-Triphosphate into PCR

#### Products

The majority of markers in this study were analysed using the radioactive labelling technique. This technique involves the incorporation of  $\alpha$ - $^{32}\text{P}$  Deoxycytosine 5'-Triphosphate ( $^{32}\text{P}$ ) (3000 Ci/mmol) (Bresatec) into the PCR product. The  $\alpha$  phosphate of the dCTP is labelled with radioactive phosphorus hence the name  $\alpha$ - $^{32}\text{P}$ . During PCR, the radioactively labelled dCTP molecules are incorporated into the DNA as well as the unlabelled dCTP so in a sense they are competing with each other. Incorporation of the labelled dCTP is enhanced by the low concentrations of the dNTPs.

### 2.3.2.2 Incorporation of Fluorescently Labelled Primers into PCR

#### Products

Fluorescent labelling was the other method used to detect the PCR products. This method involves the use of fluorescently labelled primers in the PCR followed by laser detection of the product.

A primer is labelled fluorescently using a phosphoramidite derivative with a six carbon chain and amine group. This amine group acts as a linker and can be incorporated at the 5' terminal position of the oligonucleotide. The amine group can then react with various activated fluorophores and chromophores. The primers used were commercially

labelled (Applied Biosystems Inc.) with three fluorescent dyes: FAM, TET and HEX.

The commercially labelled primers were incorporated into the PCR products essentially as described in section 3.1 with some modifications in the protocols as described below. fluorescently labelled primers and multiplex amplification of the microsatellites was used to analyse chromosomes 15 and 16. Multiplexing increases the output of results by reducing the number of individual PCR reactions required. In this study, up to 6 microsatellites were amplified in one reaction. The multiplex PCR requires a higher concentration of magnesium chloride than individual amplification of microsatellites, in order to cater for the increased number of primers in the reaction mix. An increased level of magnesium chloride in the PCR however, may cause mispriming, thus producing high background interference or artifact bands. To counteract this effect, Amplitaq GOLD<sup>TM</sup> (Perkin Elmer) *Taq* polymerase was used. This specific enzyme requires a longer start time at 94°C which serves to reduce the non-specific background bands caused by mispriming, by preventing annealing of primers until the enzyme has become active. Amplitaq GOLD<sup>TM</sup> also continues to activate during the PCR thus maintaining the level of enzyme activity throughout the PCR reaction. This ensures the highest yield possible by preventing enzyme degradation becoming a limiting factor during the reaction.

The multiplex PCR buffer (appendix A) comprised of Amplitaq 10X buffer (Perkin Elmer), 25mM magnesium chloride (Perkin Elmer), 5mM dNTPs, Amplitaq GOLD™ Taq DNA polymerase, primers used for each individual reaction and dH<sub>2</sub>O. This was made up to a volume of 4.2ul and 0.8ul of 50ug/ml target DNA was added to make a final volume of 5ul. The multiplex reaction protocols are listed in appendix A.

### **2.3.3 Cycling Conditions**

#### **2.3.3.1 Radioactive PCR Cycling Conditions**

In most of our experiments, a touchdown thermocycling program was used according to the following conditions: on completion of an initial denaturation for 5min at 94°C, 15 cycles were carried out with the annealing temperature starting at 63°C for 1 min and decreasing by 0.5°C every cycle. Thus at the end of 15 cycles, the annealing temperature reached 55°C. A further 15 cycles were performed with the annealing temperature kept at 55°C. The first 15 cycles were: 94°C for 20sec, 63°C for 1 min (-0.5°C/cycle), 72°C for 30sec. The final 15 cycles were: 94°C for 20sec, 55°C for 20sec and 72°C for 30 sec, with a final extension performed at 72°C for 5min. The reason a touchdown program was chosen as the standard protocol was that the higher annealing temperatures in the first half of the program promote more specific binding of the primer to the DNA template, while the relatively lower

annealing temperature in the second half of the program ensures a high product yield.

These conditions are not rigid and where amplification of DNA was unsuccessful, they had to be adjusted in order to maximise amplification at that particular marker. In the event of a lack of signal, the annealing temperature was reduced to facilitate annealing. Conversely, if an abundance of background bands were present as a result of a high amount of non-specific binding, the annealing temperature was increased.

As mentioned before, the initial start time or denaturing time needs to be longer when using Amplitaq GOLD<sup>1M</sup> for multiplex PCR. The PCR program for the multiplexing of microsatellites, comprising of a total of 30 cycles, was as follows: an initial denaturing time of 15min at 94°C, and for the first 10 cycles 94°C for 15sec, 55°C for 15sec and 72°C for 30sec. The next 20 cycles comprise of 89°C for 15sec, 55°C for 15sec, 72°C for 30sec, followed by a final extension time of 10min at 72°C.

#### **2.3.4 Size Separation of PCR Products by Polyacrylamide Gel**

##### **Electrophoresis (PAGE)**

Polyacrylamide gel electrophoresis (PAGE) offers high resolution of small fragments of DNA (<500bp) and allows the

separation of DNA fragments that have a length difference as small as 2bp.

Acrylamide is a monomer and in the presence of free radicals provided by ammonium persulfate and TEMED (N,N,N',N'-tetramethylethylene-diamine), it polymerizes into long chains. These long chains are further covalently linked when the cross linking agent N,N'-methylenebisacrylamide is included, thus forming a gel.

PAGE works on the theory that negatively charged DNA will migrate from a negatively charged terminal to a positively charged terminal. To achieve this, a current must be passed through the gel thus causing the DNA to migrate down the gel by passing through the pores of the polymer. Length separation of the DNA bands is achieved because of the fact that smaller bands will pass through the pores more quickly than larger bands in a given time. As a result of this, smaller bands will move further down the gel than the larger bands.

### **2.3.5 Preparation and Pouring a Polyacrylamide Gel for Radioactive PCR Products**

The gel is poured between two glass plates of different size. The larger plate is 37 x 44cm while the smaller plate is 37 x 41cm. Diversey Liquid Pyronex was used to clean both plates thoroughly in order to remove any grease and dust which may cause bubbles when pouring.

Once rinsed with water and drip dried, both plates were sprayed and washed lightly with 70% ethanol to clean any soap that may not have been removed during rinsing. The inside of the front plate (the smaller plate) was treated with Sigmacote (Molecular Sigma Biology) which serves to cover this surface with silicon. This is necessary to prevent the gel from sticking to the smaller plate.

The plates were placed together with the silanized surface on the inside and separated with 0.35mm spacers placed at the long edge of the plates. The bottom third of the plates was taped with masking tape and the top two thirds of the plates were held firmly together with "bulldog" clamps. In order to facilitate pouring, a wedge was placed between the two plates creating a small gap.

Fifty five ml of 6% acrylamide 7M urea (Molecular Sigma Biology) were mixed in a 100ml beaker with 170ul of 25% ammonium persulfate (Molecular Sigma Biology) and 170ul of TEMED (Molecular Sigma Biology) and drawn up into an 80ml syringe (The protocol for preparing 6% acrylamide can be seen in appendix A). In some instances, where the size of the product was larger than 300bp, a 4% acrylamide gel was used. Conversely where product size was less than 100bp, an 8% acrylamide gel was used (The protocol for both 8% acrylamide and 4% acrylamide can also be seen in appendix A). The mix was immediately injected between the plates at the point where the wedge separates the plates, by applying constant pressure to the syringe and gradually filling

the space between the plates. After pouring the gel, the wedge was removed and a well former inserted at the top between the two plates in order to form a well approximately 5 mm deep. The plates were clamped at the top and the gel allowed to set for 30-45 minutes. The gel can be stored at 4°C up to 72 hours.

### **2.3.6 Electrophoresis**

#### **2.3.6.1 Radioactive Electrophoresis**

##### **2.3.6.1.1 Loading and Running a Polyacrylamide Gel for Radioactively Labelled PCR Products**

The electrophoretic apparatus used was a Hoeffer Pokerface Instrument. The gel was placed against a backing plate containing a top tank. Two long clamps were used to hold the backing plate and the gel together. Foam stoppers coated with petroleum jelly were placed at the top edges of the plates in order to prevent leakage. This assembly was placed in a stand containing a bottom tank. Both top and bottom tanks were filled with 1 X TBE and the well former removed (The protocol for 1 X TBE can be seen in appendix A). The well was thoroughly flushed to remove any urea that will have gathered during storage. A sharks tooth comb was inserted into the well, forming individual wells. The Pokerface apparatus was connected to a Biorad 3000Xi power supply and the gel was pre-warmed for 30 min. at 1400V.



The PCR products were injected from the capillary into 0.65ml microcentrifuge tubes (Australian Biosearch) containing 8ul of formamide loading buffer (1ul of 10M NaOH, 5ul 5% Bromophenol Blue/5% Xylene cyanol). Before loading onto the pre-warmed gel, the PCR products were denatured at 94°C for 5 minutes on a heating block and snap-chilled on ice. 2ul of each product was injected into a well and the gel was run at 1400V for the required time. The running time depends on the length of the PCR products being separated.

#### 2.3.6.1.2 Fixing a Polyacrylamide Gel

After completing the electrophoretic run, the equipment was disassembled by unhooking the electrodes and emptying the buffer from the top tank. The long clamps were removed and the backing plate separated from the glass plates. With the plates lying flat, they were separated with a wedge inserted between them at one of the top corners. The front plate and spacers were removed and the gel was fixed in 10% acetic acid, 10% methanol. The gel was allowed to air dry for 15-20min after which it was lifted with blotting paper and covered with clear plastic gladwrap. The gel was placed on a Savant SGD4050 slab gel dryer (Selby Bio Labs) and left to dry for 30 minutes at 80°C.

### 2.3.6.2 Fluorescent Electrophoresis

#### 2.3.6.2.1 Preparation and Pouring a Polyacrylamide Gel for

#### Fluorescently Labelled PCR Products

A detailed account of preparing and pouring a polyacrylamide gel is given in the 373 DNA Sequencing System User's Manual distributed by Applied Biosystems Inc.

In brief the glass plates were washed carefully with Alconox and allowed to drip dry. The plates were placed face to face and separated with 0.4mm spacers. Both sides of the plates were taped as was the bottom and the corners to avoid leakage.

80ml of 6% acrylamide-urea (6% acrylamide, 8.3M urea [Molecular Sigma Biology] in 1 X TBE) were poured into a 150ml beaker, filtered and degassed for 5 min. 400ul of ammonium persulfate and 45ul of TEMED were added to the acrylamide. Using a 60ml syringe the acrylamide solution was injected between the plates taking care to avoid bubbles forming. The well former was immediately inserted between the plates in order to form a well and the gel was left to set for 2 hours.

#### 2.3.6.2.2 The ABI 373 DNA Sequencer

The ABI 373 DNA sequencer is an automated gel-scanning instrument which consists of an electrophoresis module and a Macintosh

computer containing the appropriate software for data collection and analysis.

Three different fluorescent dyes are used to distinguish different products. When the dye-labelled DNA fragments electrophorese through the acrylamide gel they pass through an argon ion laser beam. This beam excites the fluorescence of the dyes which is detected by a photo multiplier tube and converts it to an electrical signal. These signals are transferred to the computer and stored for analysis.

### 3 6 2 3 Loading and Running the Polyacrylamide Gel

Before the gel was run, the tape, clamps and comb were removed and the plates cleaned to remove any dried acrylamide. To avoid background fluorescence due to particles on the plates, the gel was pre-scanned with the laser beam. The top and bottom chambers were filled with 1 X TBE buffer. Using a transfer pipette, the well was flushed and a sharkstooth comb was inserted into the gel to form individual wells.

PCR products were prepared for the electrophoresis as follows: 0.5ul of the internal lane size standard (ROX 1000 or Tamra, Applied Biosystems Inc.) and 1 to 3ul of PCR product were added to 2ul of deionized formamide and 0.5ul of blue dextran (50mm EDTA, 50mg/ml blue dextran). These samples were denatured at 95°C for 5min, snap chilled on ice and one sample loaded into each lane. The gel was then

run for the required length of time at 800V, 40mA and 30W with a laser setting of 40mW and a photo multiplier tube voltage of 720-760V.

### **2.3.7 Detection and Assignment of Polymorphic Alleles**

#### **2.3.7.1 Autoradiography**

The dry gel was placed in an X-ray cassette (Amersham Hypercassette<sup>TM</sup>), facing a sheet of Agfa X-ray film and exposed overnight. The film was developed using a Curix automatic processor (Agfa). In the event of a faint signal, the gel was either exposed for a longer time or exposed with the aid of an intensifying screen. In this case the film was sandwiched between the gel and the screen and exposed overnight at -80°C. The white intensifying screen reflects the radiation particles back through the X-ray film thus increasing the intensity of the bands on the X-ray film.

#### **2.3.7.2 Allele analysis**

The alleles were assigned in a “top-down” fashion with the largest or uppermost PCR fragment being assigned allele number 1. The genotyping data was then entered into the appropriate pedigree using the CYRILLIC programme (Chapman, 1993).

### 2.3.7.3 Allele Assignment of Fluorescent PCR Products

The GENESCAN 672 software package automatically recognised the internal size standards labelled with the ROX or Tamra dye. All other DNA fragments labelled with the three other dyes are sized relative to the lane standard. The PCR fragments are represented as peaks. The peak size was determined by highlighting them and recording the size displayed by the computer. Allele assignment was performed by labelling the peak with the largest size as number 1.

The genotyping information was entered on to pedigrees using CYRILLIC (Chapman, 1993).

## **2.4 Linkage Analysis**

Linkage analysis aims to extract all available inheritance information from pedigrees and to test for coinheritance of polymorphic alleles with a particular phenotypic trait. The aim is to discover how often two loci are separated by meiotic recombination. The further apart two loci are on a chromosome, the greater the chance of a recombination occurring between these loci thus separating them. The distance between two loci can be represented by a recombination fraction. This recombination fraction is denoted by  $\theta = k/n$  where 'k' is the total number of observed recombinations and 'n' is the total number of possible recombination events, with  $\theta$  yielding a maximum value of 0.5.

A 'θ' value of 0.01 (1% recombination) is defined as 1 centimorgan (cM). To define the relationship between recombination fraction and genetic distance, mapping functions are necessary. The simplest function is Haldane's function, which assumes that crossovers occur at random and is denoted by:

$$w = -1/2 \ln(1-2\theta)$$

where w is the map distance and ln stands for the natural logarithm.

Of course it is now known that the assumption that crossovers occur at random is not true and that interference (the presence of one chisma can inhibit the formation of another) occurs. To combat this phenomenon several mapping functions have been devised. However, the most widely used mapping function is Kosambi's function:

$$w = 1/4 \ln [(1+2\theta)/(1-2\theta)]$$

Where w is the map distance and ln is the natural logarithm.

Two methods are commonly used in linkage analysis: (1) parametric methods and (2) nonparametric methods (Kruglyak *et al.*, 1996). Parametric analysis, also called co-segregation analysis, allows for specification of parameters such as mode of inheritance, phase, mutation rate, allele frequencies, degrees of phenocopy and penetrance. The greater the degree of specification, the greater the power of analysis and the more accurate the results. This gives parametric analysis a distinct advantage over non-parametric methods. However if disease are

inaccurately specified then the results can be misleading. This type of analysis utilizes the Lod score method. Using this method, two-point and multipoint Lod scores can be calculated and many computer programs are now available that can quickly and accurately carry out the computation of these Lod scores (Lathrop *et al.*, 1984). Non-parametric linkage analysis also focusses on affected individuals within a pedigree, however as the name suggests it does not attempt to model parameters such phenocopy, incomplete penetrance or locus heterogeneity. Non-parametric linkage analysis is performed using two methods. The first method breaks the pedigree in nuclear families and applies sib pair analysis. The second approach is the affected pedigree member method (APM) which focuses on whether affected relatives have the same allele at a locus (identity by state, [IBS]) regardless of whether the allele is inherited from the same ancestor (identity by descent, [IBD]) (Kruglyak *et al.*, 1996).

#### **2.4.1 The Lod Score Method**

The Lod score is the logarithm of the odds that two genetic loci are linked and is based on the likelihood ratio test. The likelihood ratio can be called odds for linkage as it indicates, for a given value of  $\theta < 1/2$ , how much higher the likelihood of the data is under linkage than under absence of linkage. This is denoted by:

$$L(\theta) = L(\theta)/L(1/2).$$

However it is more convenient to work with the base 10 logarithm or the Lod score. This is denoted by :

$$Z(\theta) = \log_{10}[L(\theta)/L(1/2)]$$

Lod scores are rarely calculated manually because of factors such as missing information, incomplete penetrance, unequal male and female recombination fractions. Instead, programs are used to carry out this process. A Lod score of -2.00 or less for a particular marker is generally considered conclusive evidence of non-linkage. On the other hand a Lod score of 3.00 or more strongly suggests linkage in 999 cases out of 1000. Definite conclusions cannot be drawn if, for a particular marker, a Lod score between -2.00 and 3.00 is obtained. In this case further investigation is necessary.

In this study, MLINK of the LINKAGE program (Lathrop *et al.*, 1984) was used to calculate two-point lod scores. Firstly a pedigree file was set up in which the pedigree is described. This pedigree file contains the following information:

Column 1: Pedigree identifier

Column 2: Individual's ID number

Column 3: The individual's father

Column 4: The individual's mother

Column 5: Sex of the individual



#### Column 6: Genetic data

It must be noted that if the individual is the founder then '0' should be placed in columns 3 and 4. The sex of the individual in column 5 is shown as '1' denoting male and '2' denoting female. Column 6 and every column following contains the genetic data which comprises of the disease and marker phenotypes.

This file was then processed using the MAKEPED program so that the file is readable by the LINKAGE programs. A parameter file was then set up in which the model parameters for each locus are defined. These parameters include number of loci, whether autosomal or X-linked markers are being used, recombinations values which allows the user to set recombination fractions at which Lod scores can be calculated. This is done by using the PREPLINK program. The MLINK program in which each locus and the recombination fractions ( $\theta$  values) are specified is run. This program produces Lod scores at each locus for the range of  $\theta$  values specified. For a more detailed account of the LINKAGE package refer to: Terwilliger and Ott, "Handbook of Human Genetic Linkage", 1994.

#### **2.4.2 Multipoint Lod Scores and Genehunter**

Multipoint linkage analysis is the simultaneous analysis of several loci. In this study, multipoint analysis was carried out using

Genehunter (Kruglyak *et al.*, 1996). Genehunter is a computer package that performs multipoint linkage analysis by calculating Lod scores for individual chromosomes rather than just several loci for a particular region on a chromosome.

Genehunter performs several steps to calculate multipoint lod scores. Firstly all inheritance information is extracted from the pedigree by using a multipoint algorithm that determines the probability distribution over possible inheritance patterns at each point in the genome. Secondly, Genehunter performs a rapid multipoint linkage algorithm for Lod score calculations as well as a new approach to pedigree analysis called nonparametric linkage (NPL) analysis. This provides a unified multipoint framework for both parametric and nonparametric linkage analysis. This framework also allows reconstruction of haplotypes (Kruglyak *et al.*, 1996). Genehunter is particularly relevant to this study because the methods used are computational for pedigrees of moderate size and not for large multigenerational pedigrees (Kruglyak *et al.*, 1996).

## **2.5 Exclusion Mapping**

Linkage analysis provides the localisation of a disease gene relative to marker loci. The focus of such analysis is obtaining a positive Lod score for linkage to a particular locus ( $Z(\theta) > 3.00$ ). By contrast,

exclusion mapping focuses on the probability of the disease gene not being at a particular position. The test statistic being applied is :  $Z_{\max} = \log_{10} L(\theta)/L(\theta = 1/2)$  (Terwilliger & Ott, 1994).

In this study, exclusion was obtained using the results from both two-point and multipoint mapping. The two-point Lod scores will also be represented graphically using the computer package EXCLUDE (Edwards, 1987). The number of recombinations and meiotic events are calculated from the two-point Lod scores using the program ZEQ. This information, along with map distance and the marker name is set up in an execution file. EXCLUDE then runs this program and provides a graphical account of the exclusion regions on any given chromosome (Edwards, 1987).

### **3. RESULTS**

#### **3.1 Chromosome 1**

The analysis of chromosome 1 included 20 markers over a distance of 315 cM, at an average distance of 15 cM. The two point Lod scores obtained with the individual markers are shown in table 3.1.

As shown in the table, the highest two-point Lod score obtained for chromosome 1 was with marker D1S186 at 1p34.1 with a value of 0.727 at  $\theta = 0.00$ . Two other markers, D1S214 (telomeric to D1S186 on 1p34.1) and D1S188 (on 1p21.3), also produced positive lod scores of 0.204 and 0.236 respectively. All other markers gave deeply negative two-point Lod scores at  $\theta = 0.00$  and Lod score values ranging between -2.28 and -0.38 at recombination distances 0.1.

The two-point Lod scores shown in table 3.1 were used to construct the exclusion map in figure 3.1. As demonstrated by the map, most of chromosome 1 is excluded apart from the two small areas of non-exclusion defined by D1S214 and D1S186 and representing the moderately positive Lod scores obtained with these markers.

Multipoint linkage analysis was performed using the Genehunter program. The results in figure 3.2 provide strong support for the exclusion of chromosome 1 as the possible location of the PKD3 gene in our family. This analysis allowed the exclusion of the interval containing marker D1S214 which, in the two-point analysis, produced an inconclusive Lod score of 0.204. The multipoint Lod score obtained for this interval was -

1.7. The only persisting region of non-exclusion was at 1p34.1, around marker D1S186, where the low positive Lod score of 0.727 was confirmed in the multipoint analysis.

To examine this region further, we have analysed the inheritance of haplotypes on chromosome 1 by the members of the family. Haplotype analysis identified two recombinations centromeric to D1S186, in affected individuals II2 and III3 and another recombination in unaffected individual III2 which cannot be placed on either side of D1S186. None of the individuals shows a double recombination. Thus a very small interval on 1p cannot be excluded as the possible location of PKD3, however the probability that it does contain the gene is low.

Table 3.1: Two-point Lod scores for all markers used to analyse chromosome 1.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D1S243	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
15	D1S214	0.204	0.172	0.141	0.112	0.085	0.061	0.04	0.023	0.01	0.003	0
14	D1S228	-99.999	-2.163	-1.331	-0.887	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
24	D1S552	-99.999	-1.177	-0.658	-0.393	-0.233	-0.132	-0.068	-0.03	-0.01	-0.002	0
13	D1S233	-99.999	0.008	-0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
8	D1S186	0.727	0.65	0.573	0.495	0.417	0.34	0.265	0.193	0.124	0.06	0
14	MYCL1	-99.999	-0.885	-0.377	-0.124	-0.021	0.102	0.141	0.146	0.123	0.074	0
7	D1S162	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
16	D1S550	-99.999	-1.575	-1.024	-0.721	-0.521	-0.375	-0.265	-0.17	-0.106	-0.048	0
13	D1S207	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.069	-0.025	-0.006	0
14	D1S188	0.236	0.2	0.165	0.132	0.1	0.072	0.048	0.027	0.012	0.003	0
6	D1S534	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
9	D1S305	-99.999	-3.442	-2.285	-1.63	-1.183	-0.851	-0.595	-0.392	-0.229	-0.1	0
25	D1S318	-99.999	-1.245	-0.725	-0.455	-0.289	-0.179	-0.105	-0.055	-0.023	-0.006	0
9	ATA4E02	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
12	D1S518	-99.999	-1.184	-0.672	-0.411	-0.254	-0.153	-0.087	-0.044	-0.018	-0.004	0
22	D1S249	-99.999	-1.163	-0.632	-0.354	-0.183	-0.074	-0.005	0.032	0.044	0.033	0
17	D1S549	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
9	D1S103	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
31	D1S102	-99.999	-2.721	-1.841	-1.338	-0.989	0.727	-0.519	-0.351	-0.211	-0.096	0

CHROMOSOME 1 EXCLUSION MAP

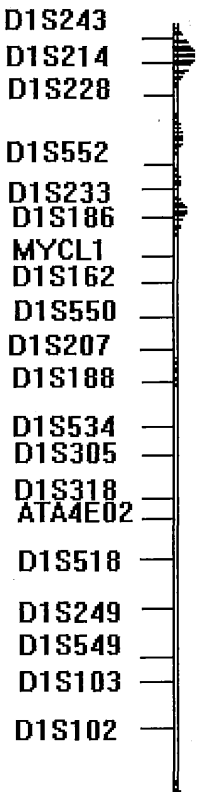


Figure 3.1: Exclusion map of chromosome 1.

MULTIPOINT LOD SCORES FOR CHROMOSOME 1

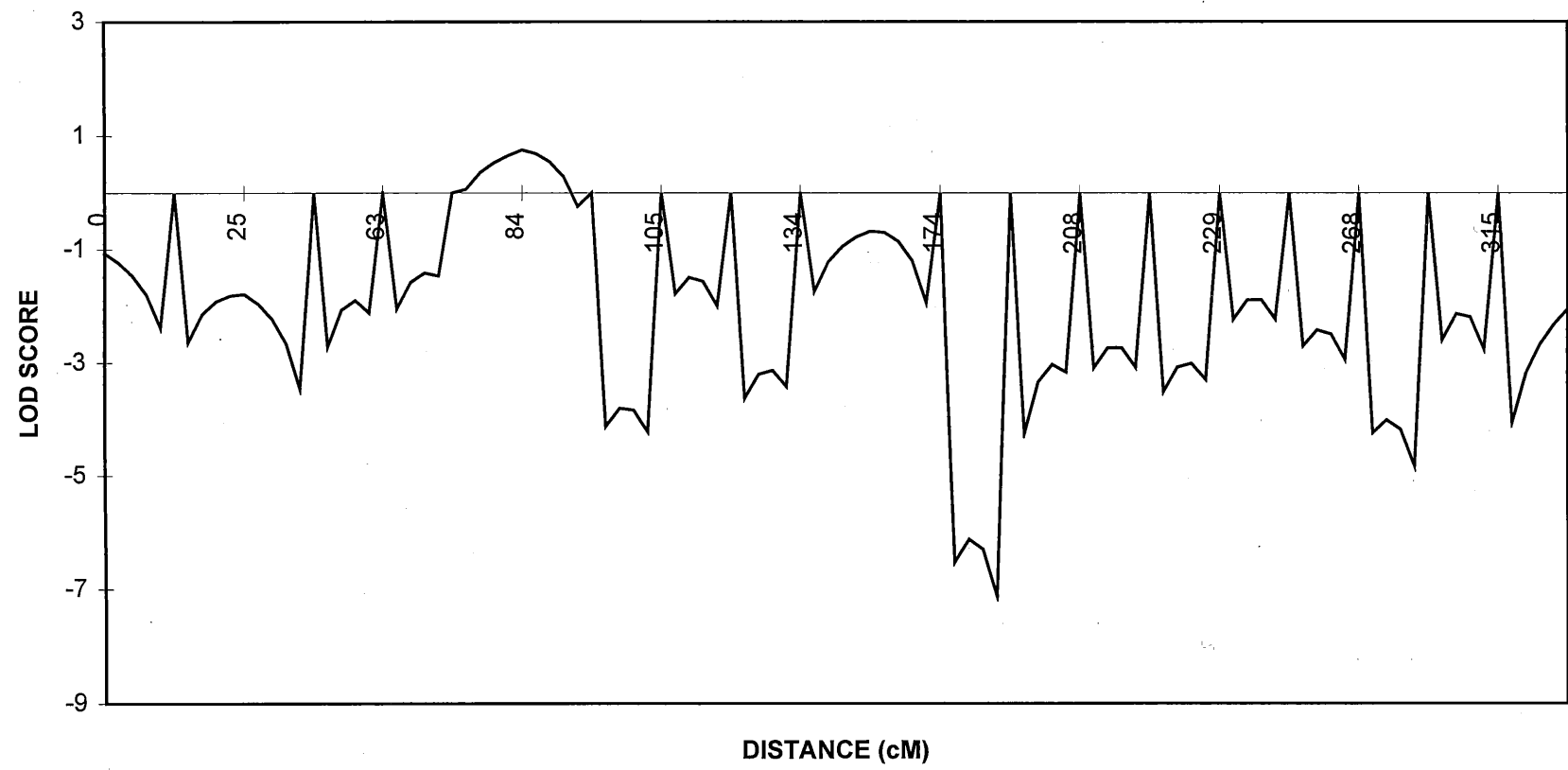


Figure 3.2: Graph of multipoint Lod scores for chromosome 1.



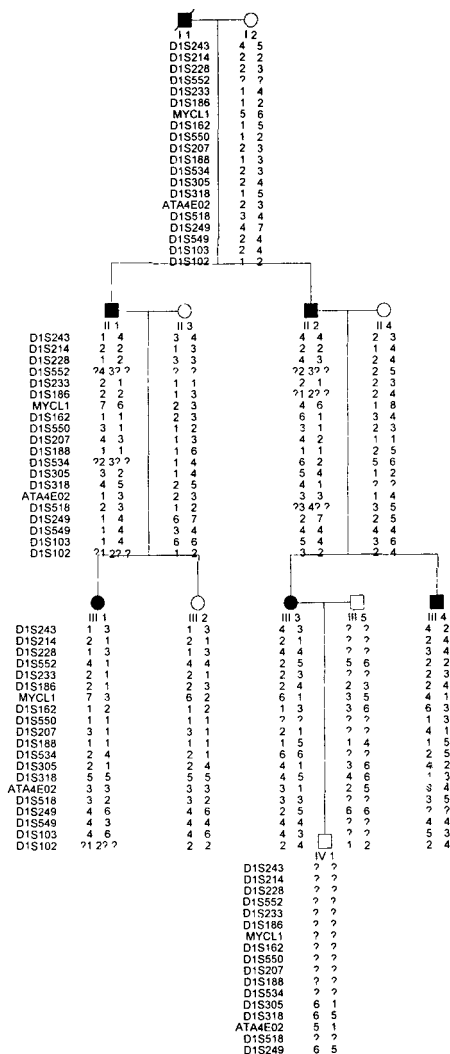


Figure 3.3: Haplotype of family 1 for chromosome 1.

### **3.2 Chromosome 2**

Chromosome 2 was analysed using 22 markers spaced approximately 14cM apart and covering a total distance of 323cM. The two-point Lod scores are shown in table 3.2.

Three markers spanning a region of approximately 29cM on 2p produced slightly positive two-point Lod scores. The interval was defined by markers D2S405 at 2p22.1 and D2S406 at 2p15, with D2S177(2p16.3) bracketed in the middle. The maximum Lod score obtained in this interval was 0.204 at  $\theta = 0.00$  for marker D2S406. D2S405 and D2S177 produced two-point Lod scores of 0.125. Strongly negative results were obtained at D2S131, at 17 cM telomeric to D2S405 on 2p24.3, with a Lod score value of -1.84 at  $\theta = 0.1$  and -1.34 at  $\theta = 0.15$ . Proximally, the region is flanked by D2S441, at 10 cM centromeric to D2S406, where the two-point Lod score at  $\theta = 0.1$  was -0.77. Thus the weakly positive region is within the D2S405-D2S406 interval, with negative Lod scores on either side. Two other markers, namely D2S417(2p11.1) and D2S110(2q21.2) also produced marginally positive results at  $\theta = 0.00$ , with two-point Lod scores of 0.177 and 0.204 respectively. D2S126 gave an inconclusive result of 0.099. Most other markers on chromosome 2 produced Lod scores of - infinity at  $\theta = 0.00$ . However, definite exclusion at  $\theta = 0.05$  could be achieved at only two markers, whereas for most of the remaining intervals the results at this

and at higher  $\theta$  values were inconclusive, ranging from -0.145 to -0.88 at recombination distance 0.1.

The exclusion map of chromosome 2 is shown in figure 3.4. As shown on the map, a minor peak of non-exclusion is located in the area around D2S177, resulting from the consistently positive, although low two-point Lod scores discussed above.

The large peak evident between D2S125 and D2S102 is due to the large 43 cM distance between these markers. The two-point Lod scores at each of the markers are - infinity at recombination fraction zero, however they are only slightly negative, -0.56 to -0.62 at  $\theta = 0.05$ , hence the interval cannot be excluded.

Multipoint analysis (Fig. 3.5) produced convincingly negative Lod scores for most of chromosome 2. The region between D2S405 and D2S406 also showed negative multipoint lod scores of -1.5 to -1.3. Although these scores do not reach the level necessary for definite exclusion, they classify the region among the highly unlikely locations of PKD3.

Negative results were also obtained for the interval defined by markers D2S125 at 2q37.3 and D2S102 at 2qter. The multipoint results for this interval were in the range of -0.3 to -1.1 thus placing the region 2q37.3 - 2qter in the group of nonexcluded regions which however are unlikely locations of PKD3.

Table 3.2: Table of two-point Lod scores for markers on chromosome 2.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D2S207	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.006	0
13	D2S423	-99.999	-0.36	-0.145	-0.053	-0.011	0.007	0.011	0.009	0.005	0.001	0
10	D2S131	-99.999	-2.721	-1.841	-1.338	-0.989	0.727	-0.519	-0.351	-0.211	-0.096	0
17	D2S405	0.125	0.104	0.084	0.066	0.049	0.035	0.023	0.013	0.006	0.001	0
13	D2S177	0.125	0.104	0.084	0.066	0.049	0.035	0.023	0.013	0.006	0.001	0
16	D2S406	0.204	0.172	0.141	0.112	0.085	0.061	0.04	0.023	0.01	0.003	0
10	D2S441	-99.999	-1.294	-0.766	-0.489	-0.315	-0.198	-0.118	-0.063	-0.027	-0.007	0
17	D2S428	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.006	0
5	D2S417	0.177	0.148	0.121	0.096	0.072	0.051	0.034	0.019	0.009	0.002	0
11	D2S436	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
14	D2S110	0.204	0.172	0.141	0.112	0.085	0.061	0.04	0.023	0.01	0.003	0
14	D2S117	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
6	D2S222	-99.999	-2.163	-1.331	-0.887	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
11	D2S142	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
11	D2S111	-99.999	-0.362	-0.148	-0.058	-0.015	0.003	0.008	0.007	0.004	0.001	0
36	D2S72	-99.999	-0.573	-0.322	-0.197	-0.121	-0.073	-0.042	-0.022	-0.009	-0.002	0
5	D2S155	-3.699	-0.721	-0.443	-0.292	-0.194	-0.125	-0.076	-0.041	-0.018	-0.004	0
12	D2S434	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
7	D2S116	0.099	0.121	0.121	0.108	0.088	0.065	0.042	0.023	0.01	0.002	0
18	D2S427	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
24	D2S125	-99.999	-0.617	-0.36	-0.227	-0.145	-0.09	-0.053	-0.028	-0.012	-0.003	0
43	D2S102	-99.999	-0.559	-0.321	-0.205	-0.137	-0.093	-0.063	-0.041	-0.024	-0.01	0

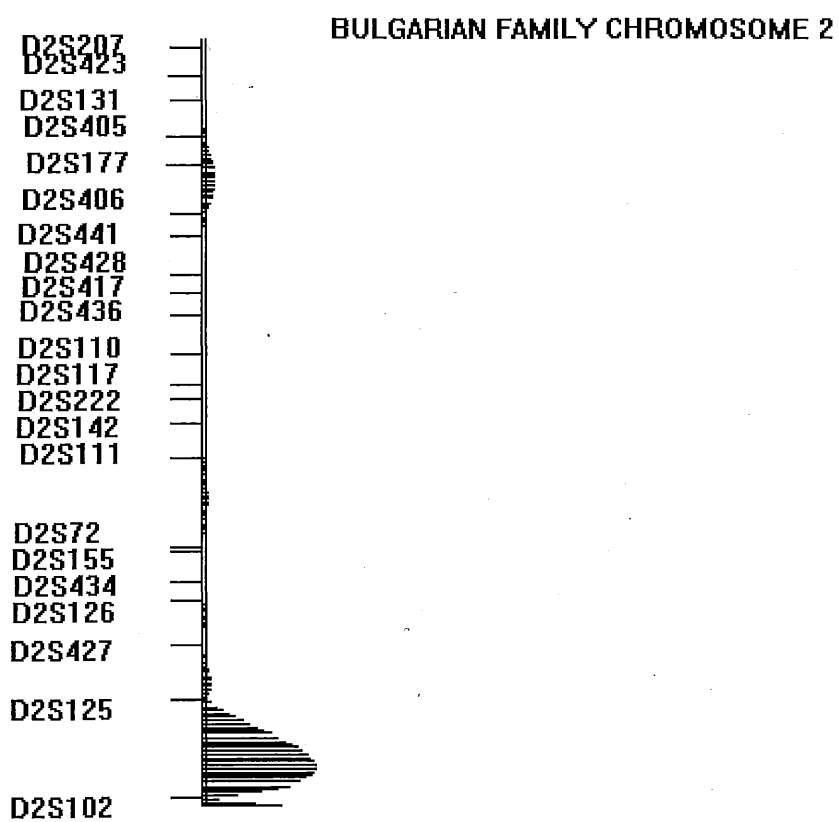


Figure 3.4: Exclusion map of chromosome 2.

MULTIPOINT LOD SCORES FOR CHROMOSOME 2

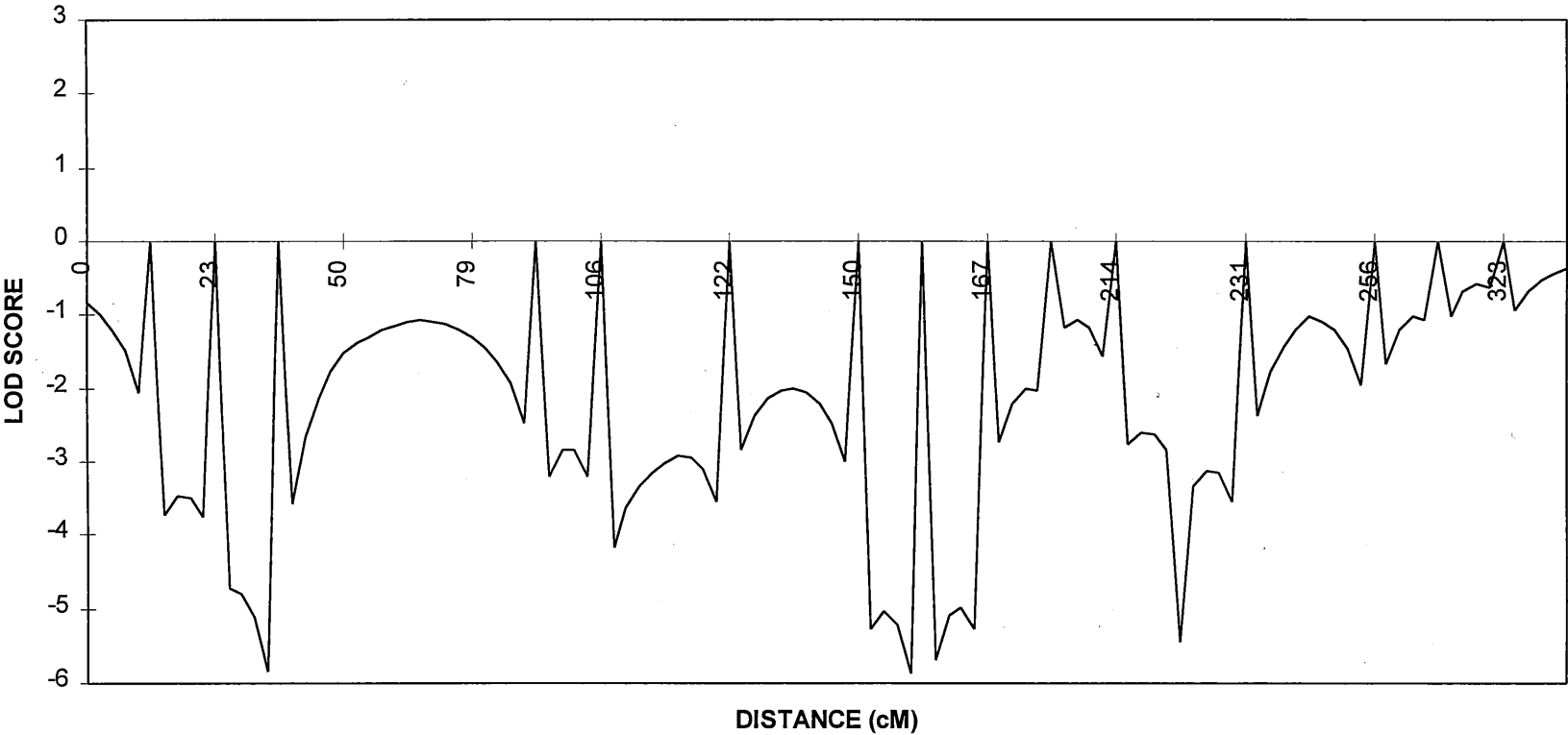


Figure 3.5: Graph of multipoint Lod scores for chromosome 2.



### **3.3 Chromosome 3**

A total of 21 markers covering a distance of 278cM and spaced approximately 13cM apart were used to analyse chromosome 3. The results of the two-point Lod scores are shown in Table 3.3.

The highest Lod score obtained was 0.683 at  $\theta = 0.00$  for marker D3S1269 at 3q21.2, followed by 0.174 at  $\theta = 0$  for D3S1282 at 3q26.1. Deeply negative Lod scores were obtained at  $\theta = 0.00$  for the remaining 19 markers. However, two-point Lod scores were slightly positive for a significant number of the chromosome 3 markers at  $\theta > 0.05$ , resulting in lack of exclusion of 3p26.3 - 3q21.1, evidenced by the map in figure 3.7.

Multipoint linkage analysis produced negative scores for the interval flanked by D3S1266 (3p24.3) and D3S1284 (3p12.2), i.e. the largest peak on the exclusion map in figure 3.7. The multipoint Lod scores ranged between -0.79 and -1.13 for the whole interval, thus placing it marginally in category 2, i.e. non-excluded but highly unlikely to contain the gene. Multipoint analysis also excluded the interval around D3S1269, the marker which yielded the highest two-point Lod score on chromosome 3. At D3S1269, multipoint linkage analysis produced a Lod score of -1.33 and the flanking intervals was definitely excluded with Lod score values around -2.00.



Table 3.3: Two-point Lod scores for all markers used to analyse chromosome 3.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D3S1307	-99.999	-0.885	-0.377	-0.124	-0.021	0.102	0.141	0.146	0.123	0.074	0
16	D3S1304	-99.999	-1.497	-0.913	-0.588	-0.375	-0.227	-0.123	-0.053	-0.011	-0.006	0
19	D3S1293	-99.999	0.024	0.221	0.291	0.308	0.294	0.26	0.212	0.152	0.081	0
7	D3S1266	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.209	0.151	0.081	0
11	D3S1768	-99.999	-0.359	-0.145	-0.053	-0.011	0.007	0.011	0.009	0.005	0.001	0
18	D3S1766	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.209	0.151	0.081	0
14	D3S1285	-99.999	0.024	0.221	0.291	0.308	0.294	0.26	0.212	0.152	0.081	0
13	D3S1284	-99.999	-0.617	-0.36	-0.227	-0.145	-0.09	-0.053	-0.028	-0.012	-0.003	0
12	D3S1752	-3.87	-0.477	-0.252	-0.148	-0.092	-0.058	-0.036	-0.021	-0.009	-0.003	0
24	D3S1769	-2.802	0.088	0.275	0.335	0.342	0.319	0.276	0.221	0.155	0.081	0
6	D3S1269	0.683	0.61	0.536	0.462	0.388	0.315	0.245	0.177	0.114	0.055	0
12	D3S1238	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
7	D3S1764	-3.699	-0.721	-0.443	-0.292	-0.194	-0.125	-0.076	-0.041	-0.018	-0.004	0
11	D3S1744	-4.324	-0.537	-0.292	-0.172	-0.103	-0.059	-0.033	-0.016	-0.007	-0.002	0
9	D3S1763	-99.999	-2.163	-1.331	-0.887	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
7	D3S1282	0.174	0.146	0.119	0.094	0.071	0.05	0.033	0.019	0.008	0.002	0
9	D3S1754	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
10	D3S1262	-99.999	-2.163	-1.331	-0.887	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
8	GATA6G12	-99.999	-0.654	-0.39	-0.251	-0.163	-0.103	-0.062	-0.033	-0.014	-0.003	0
4	D3S1314	-99.999	-2.163	-1.331	-0.887	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
12	D3S1311	-99.999	-2.534	-1.687	-1.215	-0.896	-0.66	-0.475	-0.325	-0.2	-0.093	0

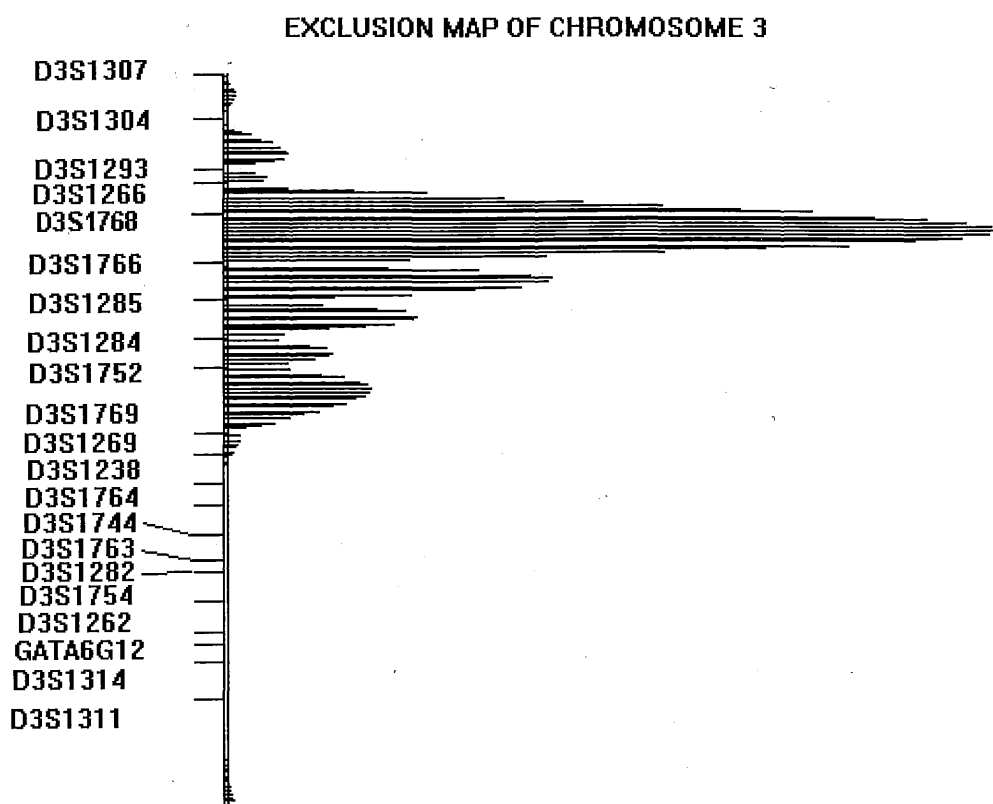


Figure 3.7: Exclusion map of chromosome 3.

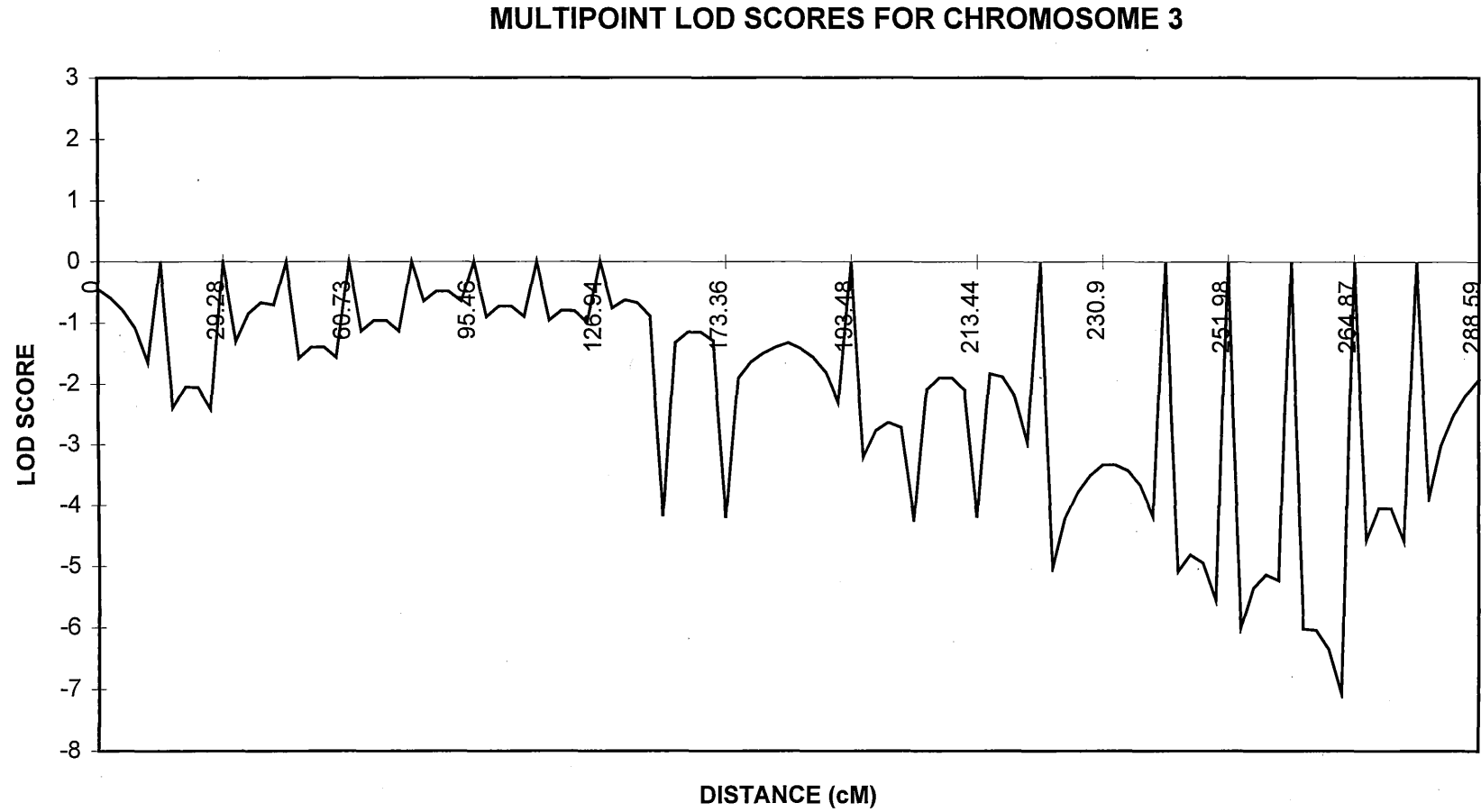


Figure 3.8: Graph of multipoint Lod scores for chromosome 3.

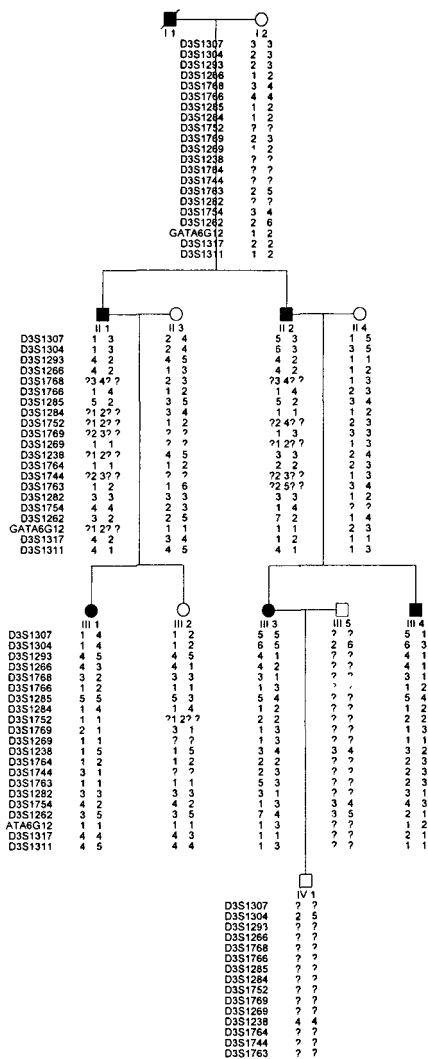


Figure 3.9: Haplotype of pedigree 1 for chromosome 3.

### **3.4 Chromosome 4**

#### **3.4.1 Stage 1**

Stage 1 of the study on chromosome 4 involved a total of 16 markers at an average distance of 18cM apart. These 16 markers covered a total of 291cM. The two-point Lod scores for chromosome 4 are shown in table 3.4.

As can be seen from the table, all markers except two, D4S394 and D4S403 which are discussed below, gave deeply negative Lod scores at  $\theta = 0$ . At higher recombination fractions, a number of chromosome 4 markers produced marginally positive two-point Lod score values around 0.3.

The map for chromosome 4 (figure 3.10) reveals two peaks, one large close to the end of the short arm (see below) and three sequential smaller peaks further to the centromeric region resulting from the combination of inconclusive Lod scores at recombination fraction 0.05 and above plus large distances between some of the markers.

Multipoint linkage analysis provided Lod scores of -1.00 or lower for most of the chromosome. Slightly positive values persisted for the regions around marker D4S175 at 4p15.1 and marker ATA2E03 at 4q21.1.

As mentioned above, two chromosome 4 markers (D4S394 at 4p16.1 and D4S403 at 4p15.33) produced positive Lod scores above 1.00. The distance between these two markers is 11cM. This positive region is presented in the large peak of non-exclusion in the map on

figure 3.10. These findings were confirmed by multipoint linkage analysis where Lod scores of 1.18 to 1.19 were obtained for the interval. These results justified a more detailed analysis of chromosome 4, specifically of the D4S394-D4S403 interval in stage 2 of the study.

Table 3.4: Table of two-point Lod scores on chromosome 4 for family 1 for stage 1 of the study.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D4S412	-99.99	0.008	0.208	0.28	0.299	0.288	0.256	0.209	0.151	0.081	0
12	D4S394	1.426	1.302	1.175	1.044	0.91	0.771	0.628	0.481	0.328	0.168	0
11	D4S403	1.408	1.287	1.162	1.034	0.901	0.765	0.624	0.479	0.327	0.168	0
17	D4S404	-99.999	-2.041	-1.456	-1.113	-0.865	-0.668	-0.501	-0.356	-0.225	-0.107	0
18	D4S174	-99.999	0.033	0.229	0.298	0.313	0.298	0.263	0.214	0.153	0.081	0
5	D4S1627	-99.999	0.024	0.221	0.291	0.308	0.294	0.26	0.212	0.152	0.081	0
21	D4S392	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.209	0.151	0.081	0
15	ATA2A03	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.001	0
37	D4S175	-99.999	-1.721	-1.142	-0.815	-0.592	-0.426	-0.23	-0.196	-0.115	-0.05	0
11	D4S1625	-99.999	-0.885	-0.377	-0.124	0.021	0.102	0.141	0.146	0.123	0.074	0
16	D4S1090	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
13	D4S243	-99.999	0.024	0.221	0.291	0.308	0.294	0.261	0.212	0.152	0.081	0
12	D4S415	-99.999	0.024	0.221	0.291	0.308	0.294	0.261	0.212	0.152	0.081	0
10	D4S408	-99.999	-0.663	-0.397	-0.257	-0.168	-0.107	-0.065	-0.035	-0.016	-0.004	0
16	D4S1652	-99.999	-0.798	-0.503	-0.337	-0.226	-0.147	-0.09	-0.049	-0.021	-0.005	0

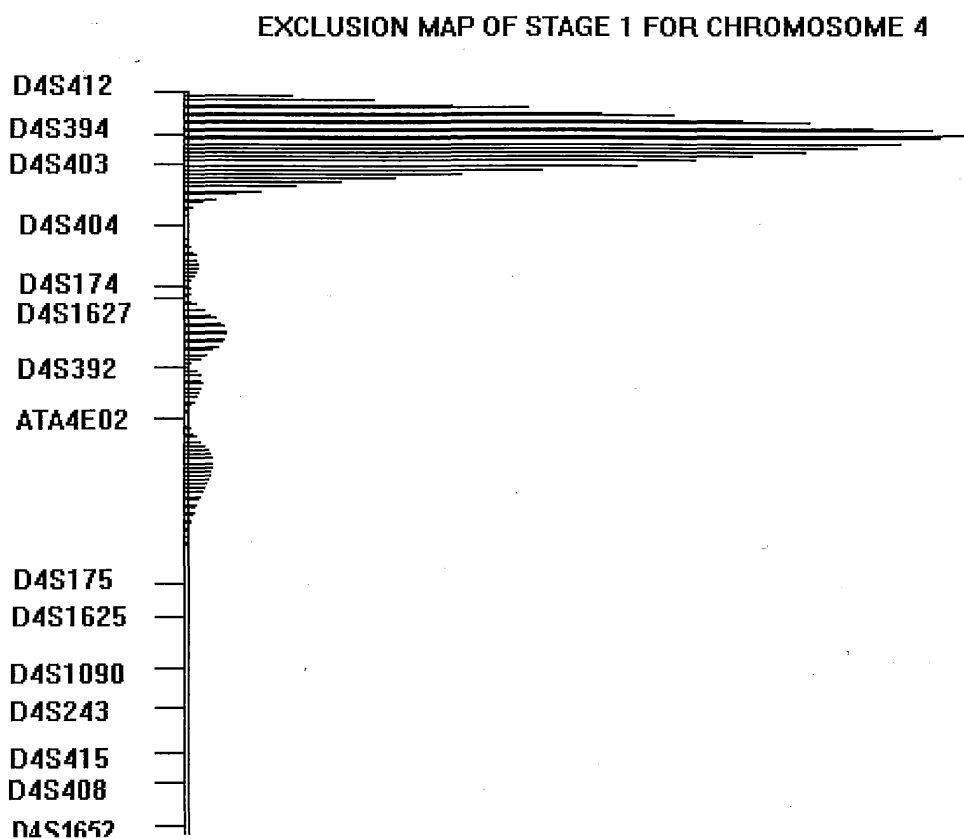


Figure 3.10: Exclusion map of chromosome 4 for stage 1 of the study.



### MULTIPOINT LOD SCORES FOR CHROMOSOME 4 FOR STAGE 1

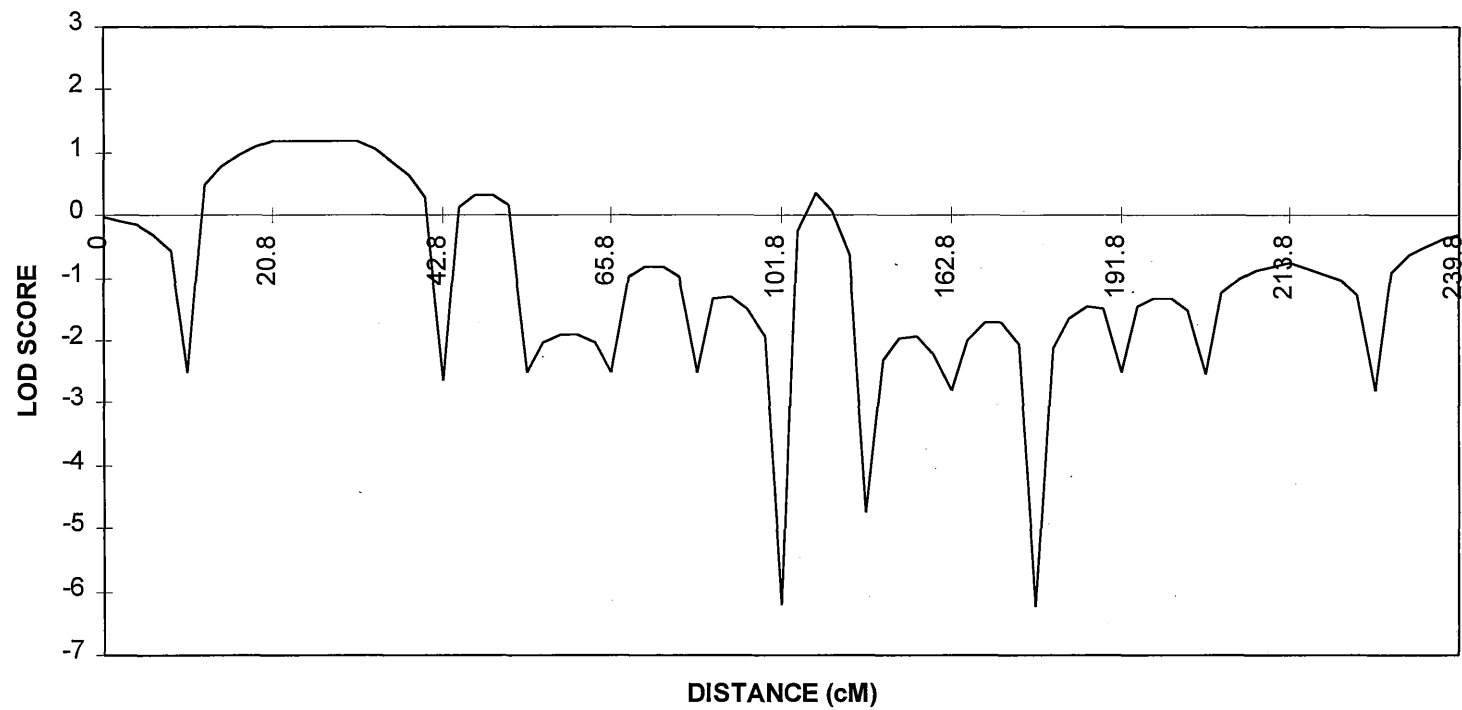


Figure 3.11: Graph of multipoint Lod scores on chromosome 4 for family 1.

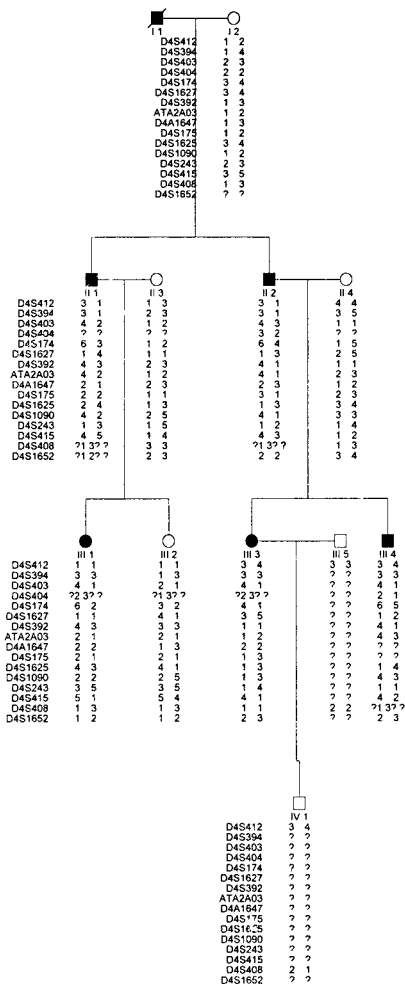


Figure 3.12: Haplotype of family 1 for markers used in stage 1 of the study to analyse chromosome 4.

### 3.4.2 Stage 2

Stage 2 of the study on chromosome 4 involved the investigation of the candidate region from stage 1. The investigation was conducted by adding extra markers to this region and by including the second Bulgarian pedigree in the study. A total of 4 markers were added to the region between D4S394 and D4S403, namely D4S3007 at 4p16.1, 2.2 cM telomeric of D4S394; D4S2983 and D4S2944 located inside the interval and D4S3048, 3.3 cM centromeric to D4S403 at position 4p15.31 (see Table 3.5 for distances). One marker (D4S1647) was also added to the large gap of 50cM between ATA2A03 and D4S175 further down the chromosome.

The two-point Lod scores obtained from the investigation of family 1 with the newly added markers were positive. Two of the 4 markers added produced Lod scores greater than 1.00 for family 1. Marker D4S3007 produced a lower positive Lod score of 0.199 at  $\theta = 0$ . The highest Lod scores of 1.4 were still obtained at D4S394 and D4S403. One of the additional markers, D4S3048, which flanked this region centromerically, produced a negative Lod score. Thus the interval D4S394-D4S403 remained positive in family 1 with the additional markers studied in stage 2.

Haplotype analysis in family 1 (figure 3.15) revealed a recombination between D4S403 and D4S3048 in individual III3 (family 1), thus placing D4S3048 outside the candidate region. No other recombinations were revealed by haplotype analysis.

Multipoint analysis on family 1 showed that positive Lod scores were obtained for the region between markers D4S3007 (4p16.1) and D4S403 (4p15.31). These Lod scores ranged from 1.07 to 1.50 with the maximum Lod score of 1.50 being obtained for several markers in this region (namely D4S394, D4S2983, D4S2944 and D4S403).

In addition to saturating the candidate region with additional polymorphic markers, stage 2 of the study involved the analysis of the second non-PKD1/non-PKD2 Bulgarian family.

The only positive two-point Lod score obtained for family 2 was 0.01 for marker D4S3007 at  $\theta = 0.35$ . All other markers used to investigate family 2 produced negative Lod scores ranging from -0.15 to -0.619.

The investigation of the chromosome 4 candidate region in the second family demonstrated that affected individuals 1 and 2 have inherited different haplotypes for the markers in the region.

The multipoint Lod scores for this family were deeply negative thus excluding the whole interval. The Lod scores for the region around D4S394 ranged from -0.6 to -1.63 thus making it unlikely that the PKD3 gene would be located in this region.

The combined two-point Lod scores for the markers used to analyse both families, namely D4S3007, D4S2983, D4S2944 and D4S3048, ranged from 0.20 to 0.79. Markers D4S394 and D4S403 reduced as a result of the addition of the second family however the Lod scores for these markers were still above 1.00 (1.309 and 1.201

respectively). In the case of D4S2944 the Lod score was negative infinity at  $\theta = 0$ .

As a result of the addition of the second family the combined multipoint Lod scores for the interval D4S2983 and D4S403 were negative, with values ranging from -0.1 to -2.8 thus excluding a significant part of the candidate region on chromosome 4p. A positive multipoint Lod score was retained for a small part of this interval, around markers D4S3007 and D4S394, with the highest Lod score of 0.83 at D4S3007 (4p16.1).

As mentioned before, marker D4S1647 at 4q22.1 was added in stage 2 to the interval between ATA2E03 at 4p15.1 and D4S175 at 4q21.1. This marker gave a two-point Lod score of 1.07 for family 1 and a two point Lod score of 0.01 for family 2. The combined Lod score for D4S1647 was 1.08. The markers flanking D4S1647 both produced negative two-point Lod scores of negative infinity at  $\theta = 0$ . The positive Lod score of 1.08 for marker D4S1647 is visualised by the small peak of non-exclusion on the map in figure 3.13. The exclusion map shows this as a small peak, since negative Lod scores were obtained for the flanking markers at recombination fractions  $> 0$ , namely ATA2E03, -0.75 at  $\theta = 0.05$  and D4S175, -1.142 at  $\theta = 0.1$  and -0.59 at  $\theta = 0.2$ .

Looking at the multipoint Lod scores for each family separately, it can be seen that family 1 produced a positive Lod score of 1.46 at D4S1647 while the flanking markers produced negative Lod scores. Family 2 also produced a positive Lod score of 0.02 which is far less than

the maximum Lod score of 1.2 that can be attained with this family. The flanking markers also produced positive multipoint Lod scores, however these scores (0.02 for ATA2E03 and 0.004 for D4S175) are only marginally positive.

Multipoint linkage analysis on both families combined showed that D4S1647 retained a positive Lod score and even increased slightly from the two-point result of 1.08. The combined multipoint result for both families at marker D4S1647 is 1.42, however the Lod scores for the markers flanking D4S1647 (namely ATA2E03 and D4S175) were negative infinity. Inside this region the results were positive ranging from 0.08 to 1.42 as mentioned at D4S1647.

The results obtained during stage 2 indicate that a strong candidate region on chromosome 4 has been excluded as a result of the addition of new markers in the region and the addition of the second family. At the same time, a candidate region of approximately 16cM around marker D4S1647 has been defined, with a maximum Lod score of 1.42 at D4S1647.

Table 3.5: Table of two-point Lod scores on chromosome 4 for family 1 for stage 2 of the study.

TWO POINT LOD SCORES FOR MARKERS ON CHROMOSOME 4 FOR PEDIGREE 1												
DISTANCE	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D4S3007	0.199	0.151	0.118	0.094	0.071	0.05	0.033	0.019	0.008	0.002	0
2.2	D4S394	1.426	1.302	1.175	1.044	0.91	0.771	0.628	0.481	0.328	0.168	0
1.6	D4S2913	1.134	0.942	0.836	0.732	0.599	0.465	0.333	0.209	0.01	0.027	0
8.2	D4S2944	1.418	1.364	1.192	1.058	0.921	0.779	0.634	0.484	0.329	0.169	0
0.6	D4S403	1.408	1.287	1.162	1.034	0.901	0.765	0.624	0.479	0.327	0.168	0
3.3	D4S3048	-99.999	-0.103	-0.017	0.075	0.114	0.126	0.12	0.101	0.074	0.04	0

Table 3.6: Table of two-point lod scores on chromosome 4 for family 2 for stage 2 of the study.

TWO POINT LOD SCORES FOR MARKERS ON CHROMOSOME 4 FOR PEDIGREE 2												
DISTANCE	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	<b>D4S3007</b>	0.003	0.003	0.004	0.004	0.005	0.007	0.009	0.01	0.008	0.005	0
2.2	<b>D4S394</b>	-0.117	-0.101	-0.08	-0.065	-0.038	-0.014	-0.008	-0.005	-0.003	-0.001	0
1.6	<b>D4S2983</b>	-0.546	-0.379	-0.313	-0.189	-0.115	-0.068	-0.038	-0.019	0.008	0.062	0
8.2	<b>D4S2944</b>	-0.619	-0.558	-0.385	-0.25	-0.171	-0.121	-0.087	-0.062	-0.041	-0.02	0
0.6	<b>D4S403</b>	-0.117	-0.101	-0.08	-0.065	-0.038	-0.014	-0.008	-0.005	-0.003	-0.001	0
3.3	<b>D4S3048</b>	-99.999	-0.193	-0.152	-0.041	0.013	0.036	0.04	0.033	0.022	0.009	0



Table 3.7: Two-point Lod scores for chromosome 4 for family 1 and family 2 combined in stage 2 of the study. Additional markers added to the region are shown in bold.

TWO-POINT LOD SCORES FOR CHROMOSOME 4 FOR STAGE 2												
		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D4S412	-99.99	0.008	0.208	0.28	0.299	0.288	0.256	0.209	0.151	0.081	0
8.6	D4S3007	0.202	0.1543	0.1235	0.0977	0.0763	0.0581	0.0422	0.0285	0.0167	0.0071	0
2.2	D4S394	1.309	1.201	1.095	0.979	0.872	0.757	0.62	0.476	0.325	0.167	0
1.6	D4S2983	0.5877	0.5634	0.5495	0.5432	0.4841	0.3969	0.295	0.1896	0.0942	0.0254	0
8.2	D4S2944	0.799	0.8052	0.807	0.8082	0.75	0.6586	0.5469	0.4222	0.2889	0.1483	0
0.6	D4S403	1.291	1.186	1.082	0.969	0.863	0.751	0.616	0.474	0.324	0.167	0
3.3	D4S3048	-99.999	-0.2963	-0.1696	0.0334	0.1276	0.162	0.1599	0.1351	0.0963	0.0498	0
8.3	D4S404	-99.999	-2.041	-1.456	-1.113	-0.865	-0.668	-0.501	-0.356	-0.225	-0.107	0
18	D4S174	-99.999	0.033	0.229	0.298	0.313	0.298	0.263	0.214	0.153	0.081	0
5	D4S1627	-99.999	0.024	0.221	0.291	0.308	0.294	0.26	0.212	0.152	0.081	0
21	D4S392	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.209	0.151	0.081	0
15	ATA2A03	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.001	0
13	D4S1647	1.08	0.985	0.887	0.787	0.685	0.58	0.472	0.361	0.246	0.126	0
37	D4S175	-99.999	-1.721	-1.142	-0.815	-0.592	-0.426	-0.23	-0.196	-0.115	-0.05	0
11	D4S1625	-99.999	-0.885	-0.377	-0.124	0.021	0.102	0.141	0.146	0.123	0.074	0
16	D4S1090	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
13	D4S243	-99.999	0.024	0.221	0.291	0.308	0.294	0.261	0.212	0.152	0.081	0
12	D4S415	-99.999	0.024	0.221	0.291	0.308	0.294	0.261	0.212	0.152	0.081	0
10	D4S408	-99.999	-0.663	-0.397	-0.257	-0.168	-0.107	-0.065	-0.035	-0.016	-0.004	0
16	D4S1652	-99.999	-0.798	-0.503	-0.337	-0.226	-0.147	-0.09	-0.049	-0.021	-0.005	0

# CHROMOSOME 4 - SECOND ROUND EXCLUSION MAP

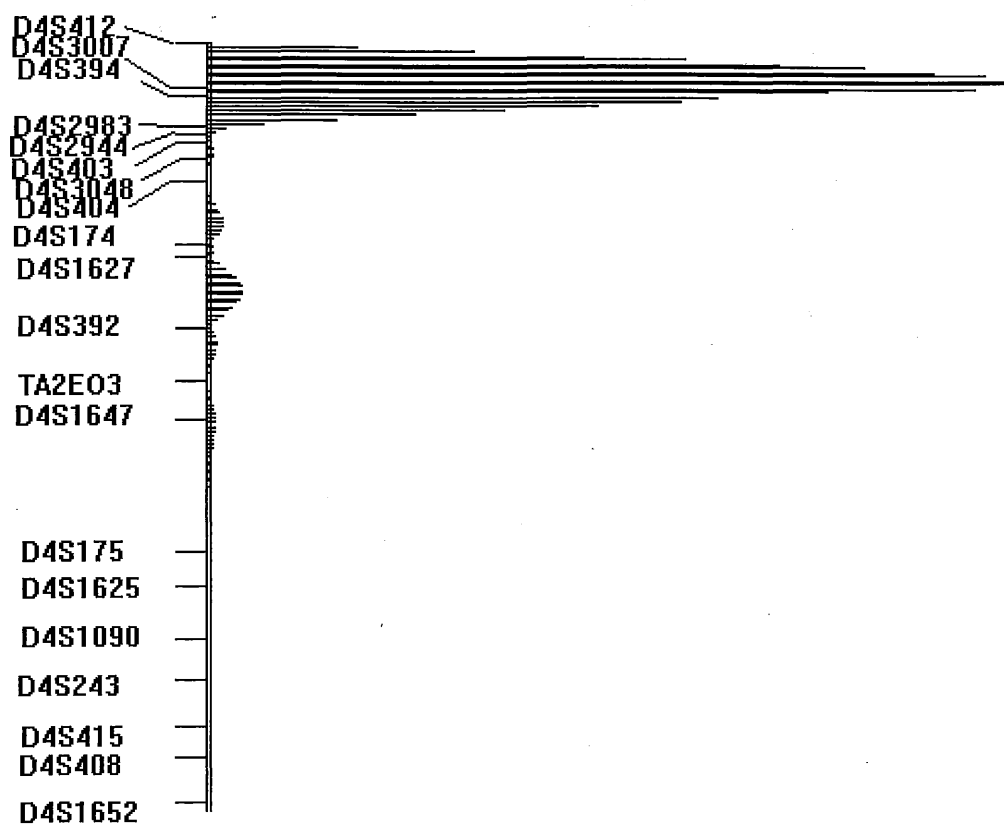


Figure 3.13: Exclusion map of chromosome 4 for the second stage of exclusion.

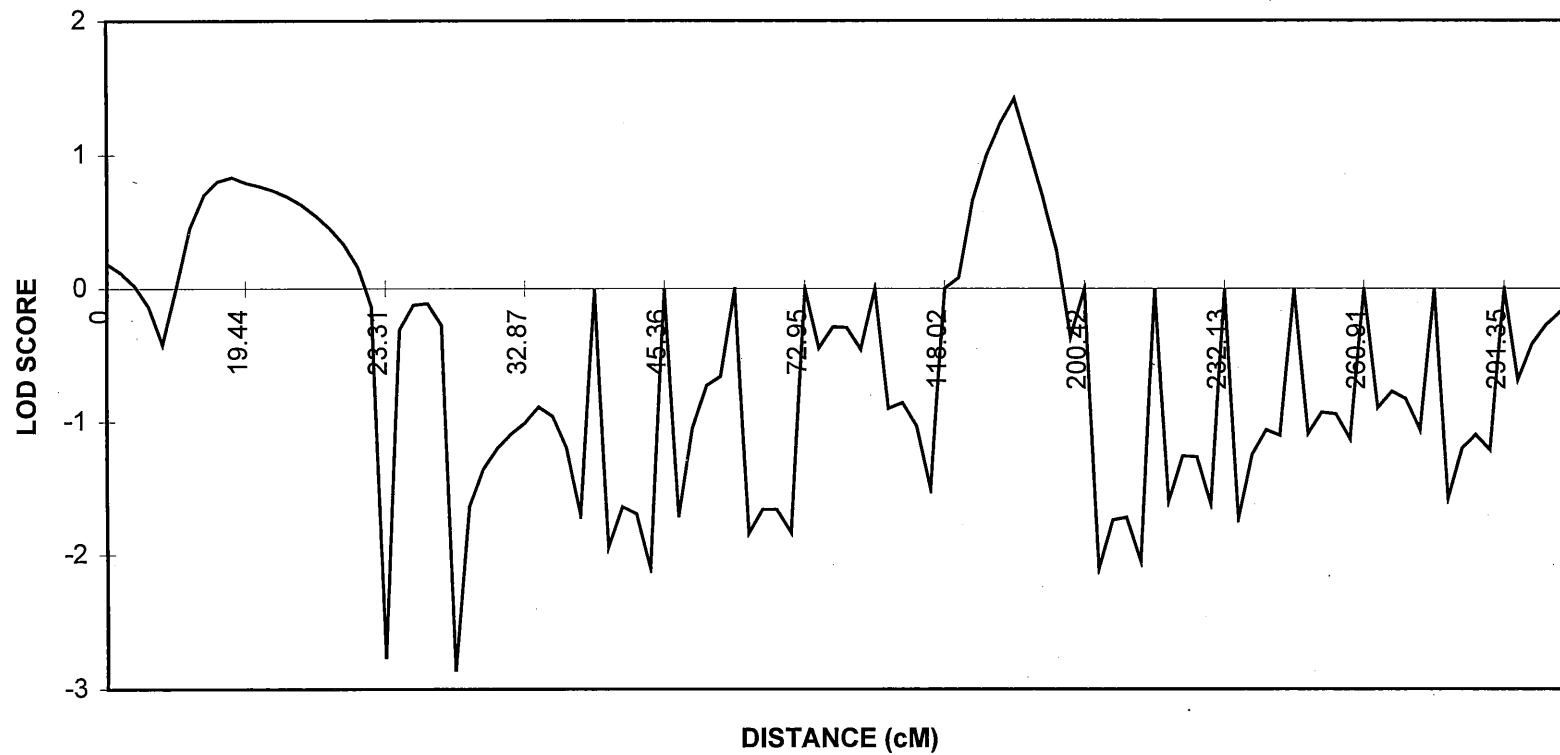
**COMBINED MULTIPOINT LOD SCORES FOR CHROMOSOME 4**

Figure 3.14: Graph of multipoint Lod scores for family 1 and family 2 combined for chromosome 4.

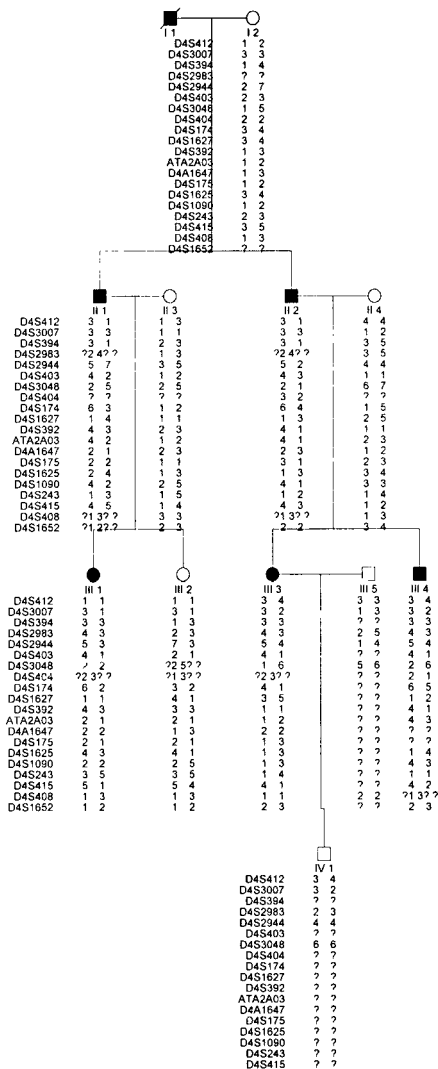


Figure 3.15: Haplotype of pedigree 1 for all markers used to analyse chromosome 4 in stage 2 of the study.

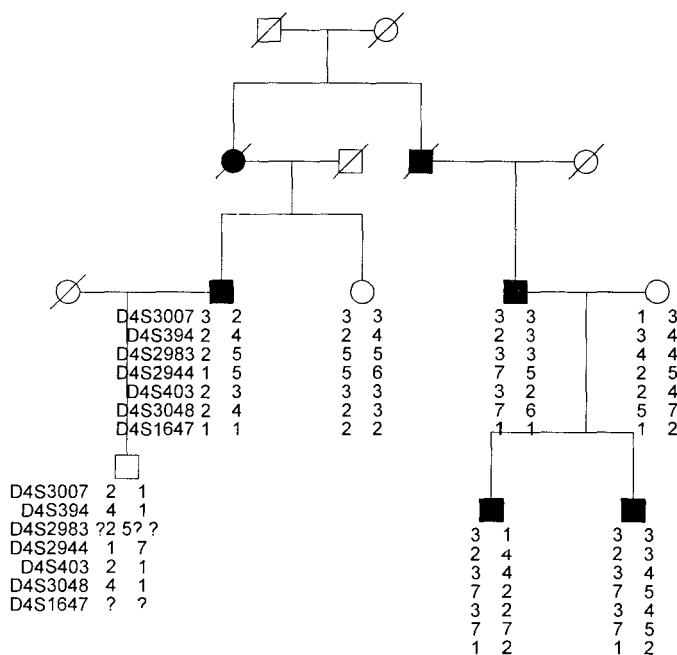


Figure 3.16: Haplotype of pedigree 2 showing markers run on chromosome 4.

### **3.5 Chromosome 5**

Nineteen markers spaced at approximately 12cM intervals were used to analyse chromosome 5. At recombination fraction 0, all markers without exception gave deeply negative Lod scores (table 3.8). The highest two-point Lod score observed was 0.308 at  $\theta = 0.2$  for marker D5S428 at 5q14.1. The exclusion map in figure 3.17 confirms that most of the chromosome is excluded. The peak near D5S392 is a result of the slightly positive Lod scores of approximately 0.02 at  $\theta$  values greater than 0.25 for this marker.

The peak between GATA11A11 and GABRA1 is due to the marginal Lod scores of 0.016-0.021 at  $\theta > 0.2$  and to the large distance of 47cM between the two markers. Haplotype examination for GATA11A11 - GABRA1 (Figure 3.19) revealed that the affected cousins in generation III have inherited different haplotypes from their affected parents who are sibs. Moreover, an identical haplotype was shared by III1 and III2, one of whom is affected and the other unaffected.

Multipoint linkage analysis provided evidence of definite exclusion of a very large proportion of chromosome 5 (Fig. 3.18). The two regions mentioned above, i.e around D5S392 and the interval flanked by GATA11A11 and GABRA1, produced Lod scores of -0.98 and -1.64 respectively. These findings classify both intervals among the genomic regions which have not been definitely excluded but are highly unlikely to contain the gene.

Table 3.8: Two-point Lod scores for markers used to analyse chromosome 5.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D5S392	-99 999	-0 306	-0.1	-0 017	0.016	0 027	0 025	0 017	0 009	0.002	0
10	D5S406	-3 398	-0 463	-0 229	-0 119	-0 06	-0 028	-0 011	-0 003	-0.001	0	0
10	D5S807	-3 097	-0 163	0.067	0 168	0.214	0 227	0 216	0 187	0 141	0.078	0
12	D5S416	-99 999	-0 885	-0.377	-0.124	0.021	0 102	0 141	0 146	0.123	0.074	0
25	D5S426	-99 999	-1 442	-0.887	-0 585	-0.388	-0 25	-0 151	-0 082	-0.035	-0.009	0
13	D5S407	-99 999	-3 442	-2 285	-1 63	-1.183	-0 851	-0.595	-0 392	-0.229	-0.1	0
20	D5S424	-99 999	-2 549	-1 7	-1 226	-0.905	-0 666	-0 48	-0 328	-0.201	-0 093	0
12	D5S428	-99 999	0 024	0 221	0.291	0 308	0 294	0 26	0 212	0 152	0 081	0
6	D5S815	-99 999	-2.549	-1.7	-1 226	-0 905	-0 666	-0 48	-0.328	-0 201	-0.093	0
7	D5S409	-99 999	-0 573	-0 322	-0 197	-0 121	-0.073	-0 042	-0.022	-0.009	-0.002	0
6	ATA40107	-99 999	-2 534	-1 687	-1 215	-0 996	-0 66	-0 475	-0 325	-0.2	-0.093	0
7	D5S421	-99 999	-2 573	-1.721	-1 242	-0 917	-0 675	-0 486	-0.331	-0.203	-0.093	0
11	D5S818	-99 999	-2 575	-1 723	-1 244	-0 919	-0 677	-0.486	-0 332	-0 203	-0.094	0
9	D5S816	-99 999	-1 573	-1 021	-0 719	-0.519	-0 374	-0.264	-0 177	-0 106	-0 048	0
9	D5S210	-99 999	-2 549	-1 7	-1.226	-0 905	-0 666	-0 48	-0 328	-0.201	-0.093	0
14	D5S820	-99 999	-1 442	-0 887	-0 585	-0.388	-0 25	-0.151	-0 082	-0 035	-0 009	0
10	D5S415	-99 999	-0 885	-0.377	-0.124	0.021	0 102	0.141	0.146	0 123	0 074	0
5	GATA11A11	-99 999	-0 306	-0 1	-0 017	0.016	0 027	0.025	0 017	0.009	0.002	0
47	GABRA1	-99 999	-0 885	-0 377	-0 124	0.021	0.102	0 141	0 146	0 123	0.074	0

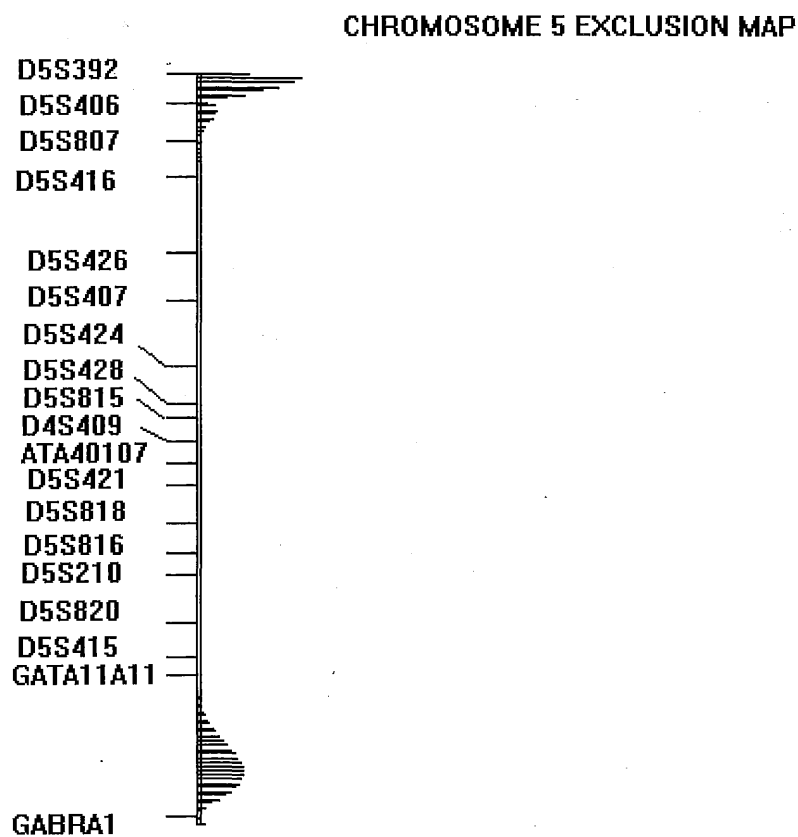


Figure 3.17: Exclusion map of chromosome 5.



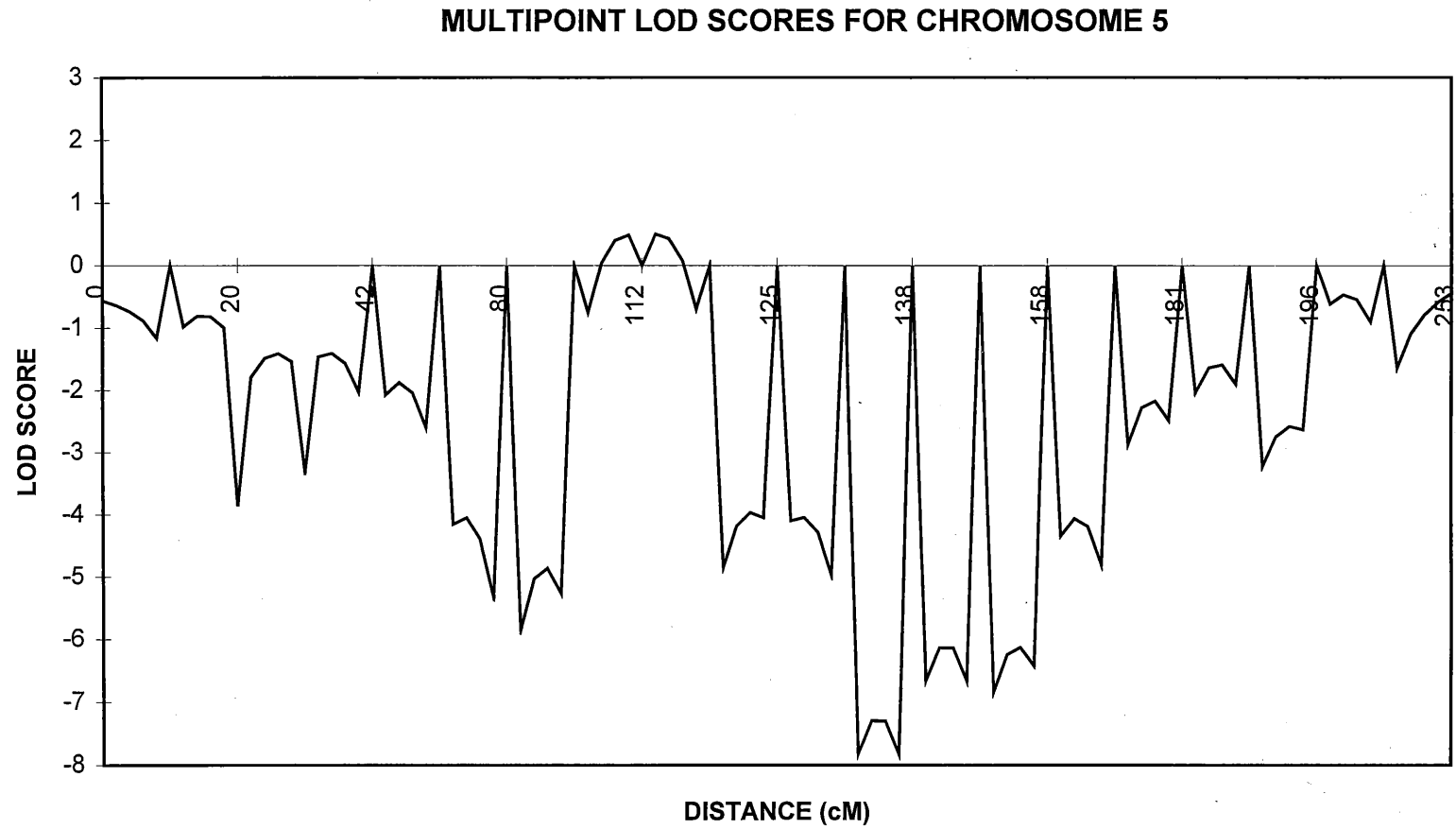
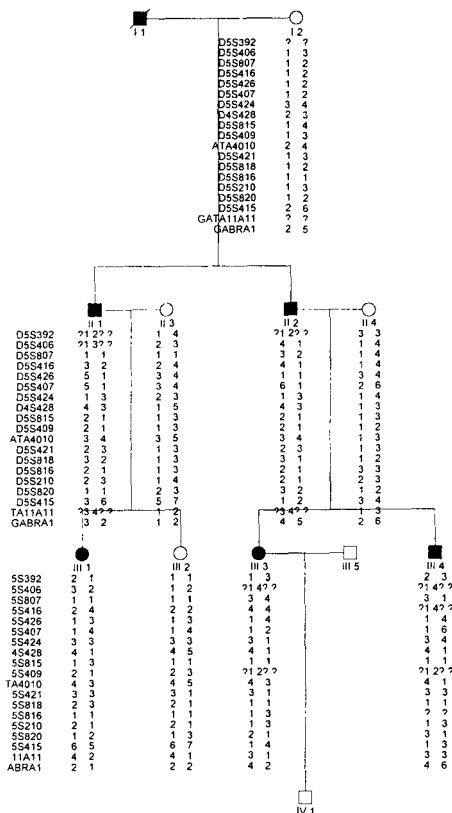


Figure 3.18: Graph of the multipoint Lod scores for chromosome 5.



### **3.6 Chromosome 6**

#### **3.6.1 Stage 1**

A total of 15 markers at an average distance of approximately 16cM were used to analyse chromosome 6. The list of markers and two-point Lod scores is shown in Table 3.9.

The highest Lod score for this chromosome was 1.4 at marker D6S271 on 6p21.1. This marker was flanked by D6S285, telomeric, at a distance of 39cM which gave a Lod score of  $-\infty$  at  $\theta = 0$  and -0.36 at  $\theta = 0.05$  and D6S257 at 6p11.1, 19cM centromeric, with a Lod score of 0.174 at recombination distance zero. A Lod score around 0 (0.068) at  $\theta = 0.00$  was obtained for D6S264 on 6q27. For the remaining markers on this chromosome, deeply negative two-point Lod scores were obtained at  $\theta = 0$  and, for most markers, also at higher theta values.

The map in figure 3.20 shows two peaks of non-exclusion on chromosome 6. The larger peak is at marker D6S271 and corresponds to the highest Lod score of 1.4 observed on this chromosome. The smaller peak visible between D6S477 and D6S259 is due to the large distance of 30cM between these markers and to the slightly positive two-point Lod scores at  $\theta$  values  $> 0.15$  (for D6S477) and  $> 0.05$  (for D6S259).

Multipoint linkage analysis definitely excluded the short arm of chromosome 6. The 6pter region gave multipoint scores ranging from -0.58 to -2.50. The positive results in the interval D6S271 - D6S257 (6p21.1 - 6p11.1) were confirmed, with the Lod scores values peaking at

1.19 for D6S271. These findings placed the short arm of chromosome 6 among the candidate regions which require further investigation.

Table 3.9: Two-point Lod scores for chromosome 6 for family 1 in stage 1 of the study.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D6S477	-99.999	-0.27	-0.047	0.05	0.095	0.112	0.11	0.096	0.072	0.04	0
30	D6S259	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
5	D6S285	-99.999	-0.36	-0.145	-0.054	-0.011	0.007	0.011	0.009	0.005	0.001	0
39	D6S271	1.408	1.287	1.162	1.034	0.901	0.765	0.624	0.479	0.327	0.168	0
19	D6S257	0.174	0.146	0.119	0.094	0.071	0.05	0.033	0.019	0.008	0.002	0
9	D6S254	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
16	D6S252	-99.999	-1.184	-0.672	-0.412	-0.254	-0.153	-0.087	-0.004	-0.018	-0.004	0
16	D6S474	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
13	D6S262	-99.999	-3.442	-2.285	-1.631	-1.183	-0.852	-0.595	-0.392	-0.229	-0.1	0
16	ATA1F08	-99.999	-3.721	-2.54	-1.861	-1.861	-1.028	-0.741	-0.506	-0.308	-0.142	0
22	D6S255	-99.999	-3.828	-2.655	-1.979	-1.979	-1.143	-0.848	-0.597	-0.377	-0.18	0
7	D6S305	-3.875	-0.897	-0.619	-0.465	-0.465	-0.283	-0.22	-0.163	-0.11	-0.056	0
11	D6S264	0.068	0.065	0.06	0.051	0.051	0.031	0.021	0.012	0.006	0.001	0
8	D6S503	-3.875	-0.897	-0.619	-0.465	-0.465	-0.283	0.22	-0.163	-0.11	-0.056	0

# MULTIPOINT LOD SCORES FOR CHROMOSOME 6 FOR STAGE 1

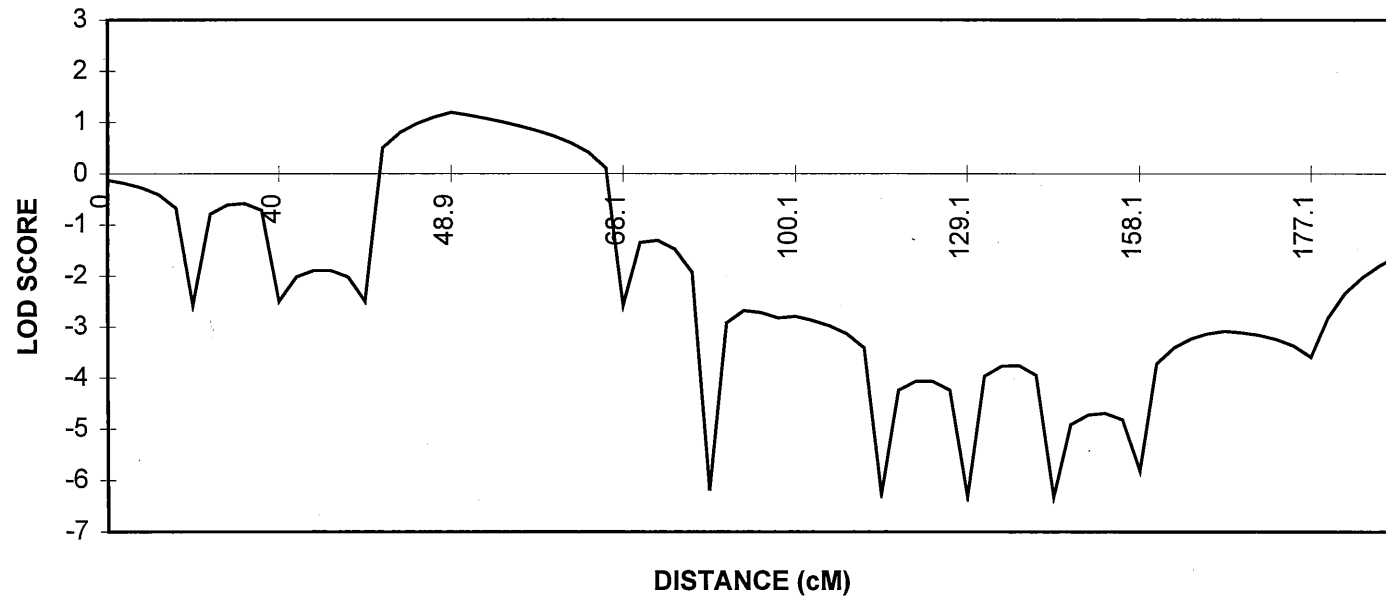


Figure 3.21: Graph of multipoint Lod scores on chromosome 6 for family 1.

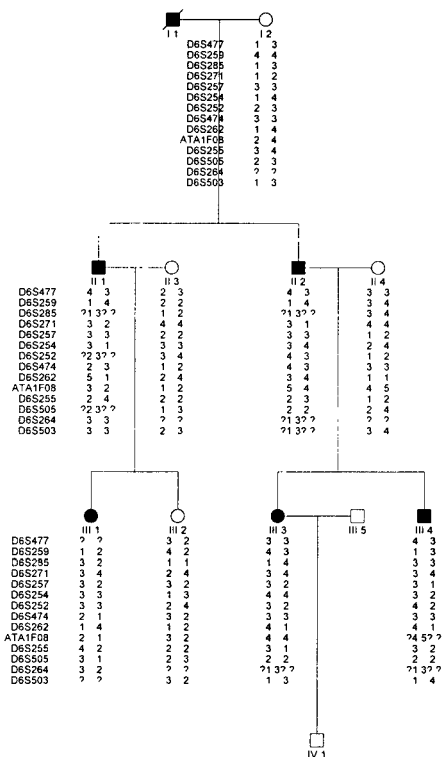


Figure 3.22: Haplotype of family 1 for markers used to analyse chromosome 6 in stage 1 of the study.

### 3.6.2 Stage 2

In the second stage, 4 markers were added to the region between D6S271 and D6S257, namely D6S1650, D6S1669, D6S466 and D6S1661, at an average distance of 2.7cM. Both family 1 and family 2 were genotyped for these markers. The combined two-point Lod scores are shown in table 3.10.

The results of the two-point Lod scores for the additional markers for family 1 ranged from -0.27 to 0.89 for  $\theta = 0$ . The maximum Lod score for this family remained at D6S2271 and one of the new markers, D6S1669, produced a Lod score of 0.89 at  $\theta = 0$ . Conversely D6S1661 produced a negative Lod score of -0.27.

The analysis of marker haplotypes for this region in family 1, revealed a crossover between D6S271 and D1650 in individual III3 which was not detectable in the first stage of the screening due to the fact that both affected individuals in generation II are uninformative for the proximal flanking marker D6S257 (Fig. 3.25).

Multipoint analysis of this region for family 1 showed that the majority of this region was excluded for the first family. The maximum Lod score gained was 1.49 at marker D6S271, thus confirming the positive Lod score achieved at this marker in stage 1. However D6S257, which also gave a positive Lod score in stage 1, became negative. All new markers added to this region produced negative results ranging from -1.59 to -3.05 thus excluding most of this region for family 1.



The addition of the second family did not increase the Lod scores for D6S271. In this family, the values for the new markers in the telomeric part of the interval were moderately positive (around 0.50), whereas the most centromeric new marker, D6S1661 produced slightly negative values ranging from -0.037 to -0.313 for  $\theta = 0.00$  to 0.1. Analysis of haplotypes for family 2 show a crossover between markers D6S466 and D6S1661 in individual 3. Individual 1 who is affected, does not display the same haplotype as the other affected individuals thus suggesting that the gene is not in this region for family 2.

Multipoint analysis on the second family shows that the Lod scores for this region range from -1.84 to -4.30. The negative Lod scores allow exclusion of this region for family 2.

The lack of linkage of family 2 to the candidate region between 6p21.1 and 6p11.1, together with the recombination event detected in the distal part of this region in family 1, both suggested a false positive finding in the initial stage of the study. Multipoint linkage analysis for both families combined produced negative results for the entire interval spanned by the newly added markers, in the range of -3.5 to -5.9 thus definitely excluding most of the region. The positive Lod score of 1.19 at marker D6S271 from the first stage declined to 0.5 in the second stage due to the addition of the second family and the negative Lod scores obtained for this family at D6S271. The Lod scores between this marker and its flanking markers, namely D6S285 and D6S1650, remained positive, ranging from 0.3 to 0.9.

Table 3.10: Table of Lod scores for markers on chromosome 6 for family 1 in stage 2.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D6S477	-99.999	-0.27	-0.047	0.05	0.095	0.112	0.11	0.096	0.072	0.04	0
30	D6S259	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
5	D6S285	-99.999	-0.36	-0.145	-0.054	-0.011	0.007	0.011	0.009	0.005	0.001	0
39	D6S271	1.408	1.287	1.162	1.034	0.901	0.765	0.624	0.479	0.327	0.168	0
2.3	D6S1650	0.202	0.183	0.154	0.122	0.093	0.067	0.044	0.025	0.011	0.003	0
1.3	D6S1669	1.08	0.932	0.862	0.732	0.559	0.465	0.333	0.209	0.101	0.027	0
4.8	D6S466	0.538	0.501	0.438	0.396	0.322	0.284	0.198	0.098	0.042	0.098	0
1.8	D6S1661	-0.01	-0.019	-0.026	0.067	0.109	0.122	0.117	0.099	0.074	0.04	0
0.8	D6S257	0.174	0.146	0.119	0.094	0.071	0.05	0.033	0.019	0.008	0.002	0
9	D6S254	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
16	D6S252	-99.999	-1.184	-0.672	-0.412	-0.254	-0.153	-0.087	-0.004	-0.018	-0.004	0
16	D6S474	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
13	D6S262	-99.999	-3.442	-2.285	-1.631	-1.183	-0.852	-0.595	-0.392	-0.229	-0.1	0
16	ATA1F08	-99.999	-3.721	-2.54	-1.861	-1.861	-1.028	-0.741	-0.506	-0.308	-0.142	0
22	D6S255	-99.999	-3.828	-2.655	-1.979	-1.979	-1.143	-0.848	-0.597	-0.377	-0.18	0
7	D6S305	-3.875	-0.897	-0.619	-0.465	-0.465	-0.283	-0.22	-0.163	-0.11	-0.056	0
11	D6S264	0.068	0.065	0.06	0.051	0.051	0.031	0.021	0.012	0.006	0.001	0
8	D6S503	-3.875	-0.897	-0.619	-0.465	-0.465	-0.283	0.22	-0.163	-0.11	-0.056	0

Table 3.11: Table of Lod scores for markers on chromosome 6 for family 2 in stage 2.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
2.3	D6S1650	0.202	0.183	0.154	0.122	0.093	0.067	0.044	0.025	0.011	0.003	0
1.3	D6S1669	1.08	0.932	0.862	0.732	0.559	0.465	0.333	0.209	0.101	0.027	0
4.8	D6S466	0.538	0.501	0.438	0.396	0.322	0.284	0.198	0.098	0.042	0.098	0
1.8	D6S1661	-0.01	-0.019	-0.026	0.067	0.109	0.122	0.117	0.099	0.074	0.04	0

Table 3.12: Table of two-point Lod scores for markers used to analyse chromosome 6 for family 1 and family 2 combined in stage 2 of the study. Additional markers added to the candidate region are in bold.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D6S477	-99.999	-0.27	-0.047	0.05	0.095	0.112	0.11	0.096	0.072	0.04	0
30	D6S259	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
5	D6S285	-99.999	-0.36	-0.145	-0.054	-0.011	0.007	0.011	0.009	0.005	0.001	0
39	D6S271	1.408	1.287	1.162	1.034	0.901	0.765	0.624	0.479	0.327	0.168	0
2.3	D6S1650	0.22	0.1487	0.1175	0.0979	0.0787	0.0603	0.0434	0.0285	0.016	0.0064	0
1.3	D6S1669	0.5842	0.5576	0.5495	0.5432	0.4841	0.3969	0.2949	0.1896	0.0942	0.0254	0
4.8	D6S466	0.4899	0.4789	0.4724	0.3851	0.3	0.2196	0.1467	0.0851	0.0384	0.0096	0
1.8	D6S1661	-0.1684	-0.1566	-0.1336	0.0062	0.0776	0.1089	0.114	0.101	0.0754	0.0409	0
0.8	D6S257	0.174	0.146	0.119	0.094	0.071	0.05	0.033	0.019	0.008	0.002	0
9	D6S254	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
16	D6S252	-99.999	-1.184	-0.672	-0.412	-0.254	-0.153	-0.087	-0.004	-0.018	-0.004	0
16	D6S474	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
13	D6S262	-99.999	-3.442	-2.285	-1.631	-1.183	-0.852	-0.595	-0.392	-0.229	-0.1	0
16	ATA1F08	-99.999	-3.721	-2.54	-1.861	-1.861	-1.028	-0.741	-0.506	-0.308	-0.142	0
22	D6S255	-99.999	-3.828	-2.655	-1.979	-1.979	-1.143	-0.848	-0.597	-0.377	-0.18	0
7	D6S305	-3.875	-0.897	-0.619	-0.465	-0.465	-0.283	-0.22	-0.163	-0.11	-0.056	0
11	D6S264	0.068	0.065	0.06	0.051	0.051	0.031	0.021	0.012	0.006	0.001	0
8	D6S503	-3.875	-0.897	-0.619	-0.465	-0.465	-0.283	0.22	-0.163	-0.11	-0.056	0

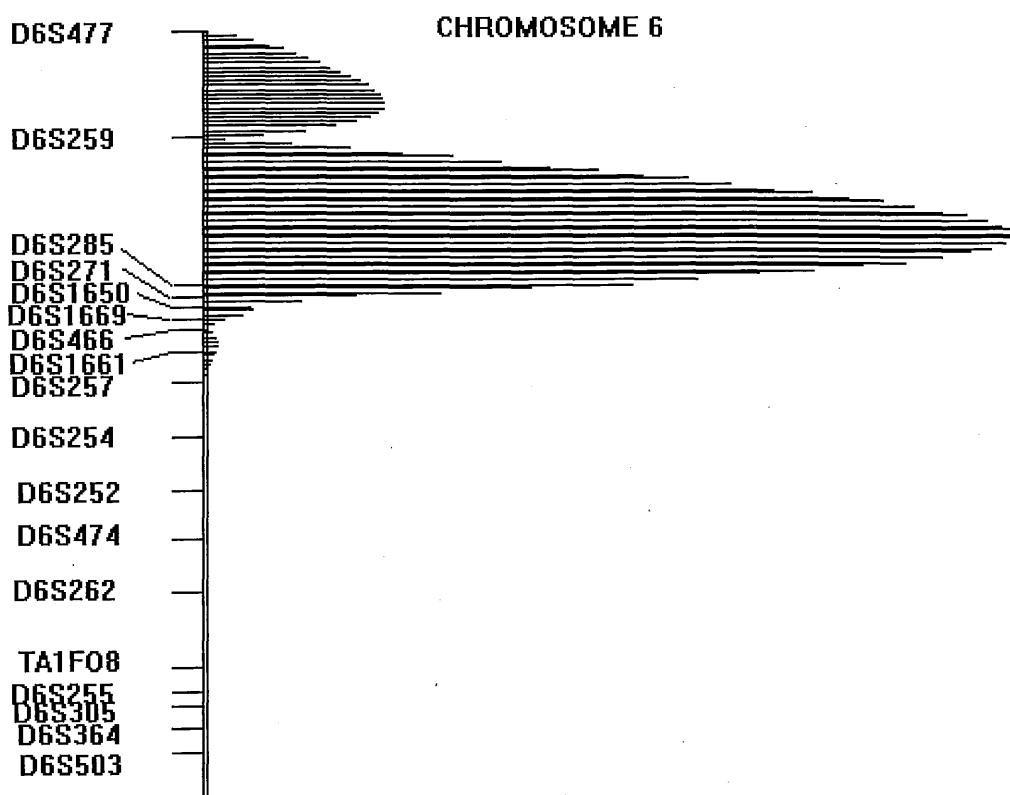


Figure 3.23: Exclusion map of chromosome 6 for stage 2 of the study.

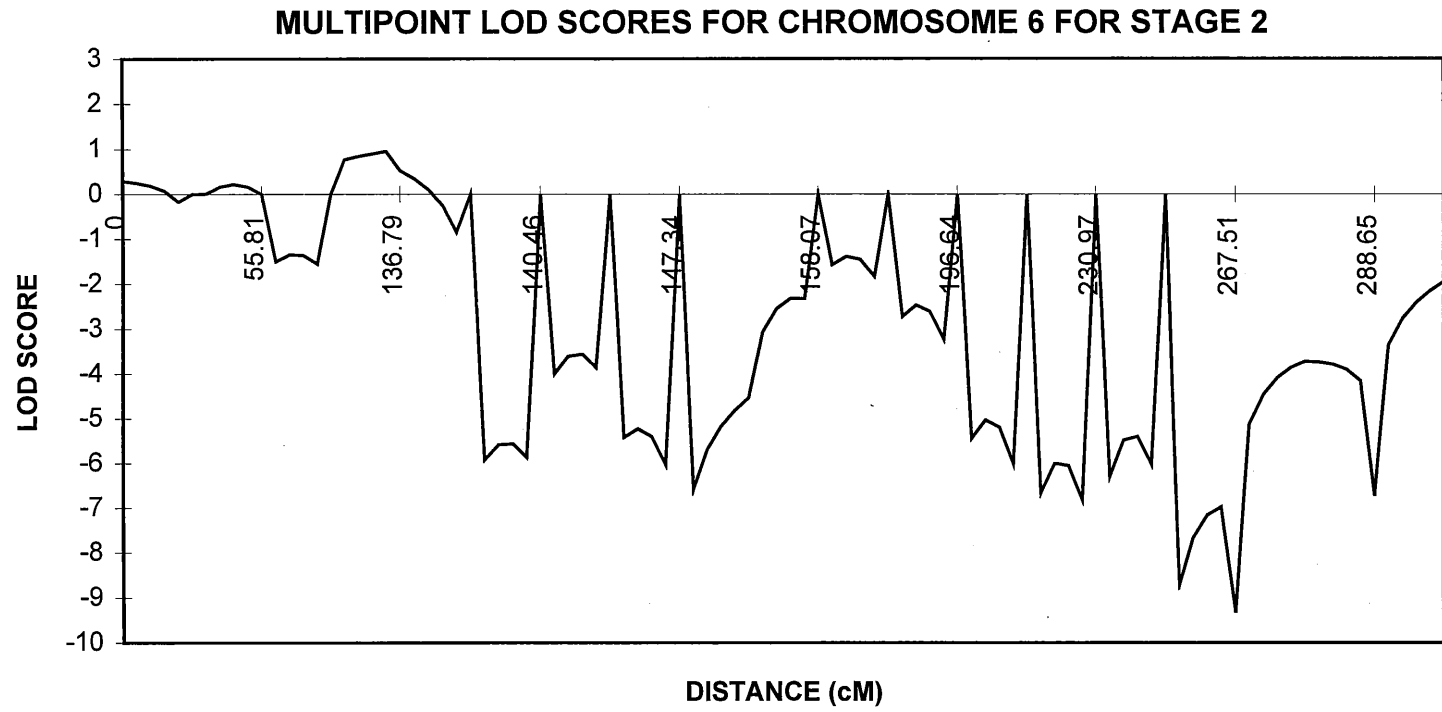


Figure 3.24: Graph of multipoint Lod scores for chromosome 6 for family 1 and family 2 combined.

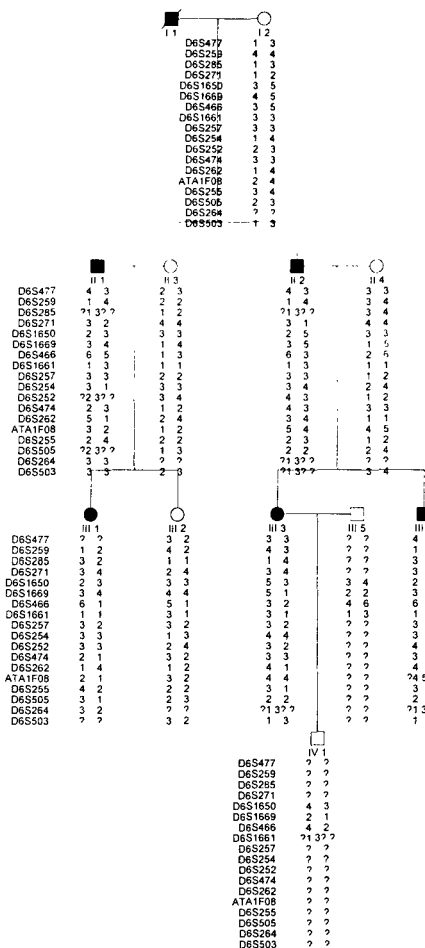


Figure 3.25: Haplotype of family 1 for all markers used in stage 2 to analyse chromosome 6.

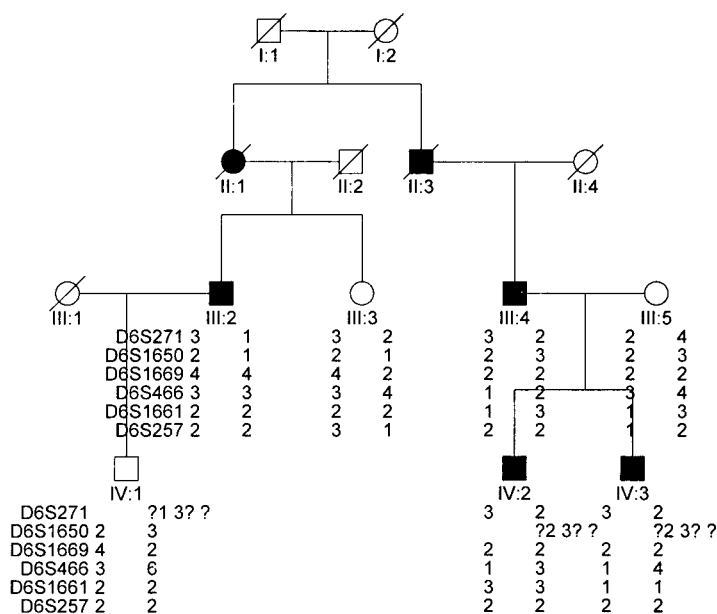


Figure 3.26: Haplotype of pedigree 2 for chromosome 6 for stage 2 of the study



### **3.7 Chromosome 7**

Sixteen markers at an average distance of 18 cM were used to analyse chromosome 7. Positive two-point Lod scores were obtained with 4 of these markers (table 3.13), located at different positions along the length of the chromosome and none of them adjacent. Slightly positive Lod score values (between 0.2 and 0.45) at recombination fraction zero were observed for D7S550 and D7S669 at positions 7q36.3 and 7q11.21 respectively. At the same theta value, the Lod score for D7S1808 was 0.75. The highest lod score for chromosome 7 was 1.057 at the marker D7S531 (position 7p22.3).

These two-point Lod scores are reflected in the map shown in figure 3.27, where multiple peaks of non-exclusion can be seen. In addition to the peaks expected on the basis of the linkage results described above, the map indicates that the region flanked by D7S657 at 7q21.13 and D7S630 at 7q21.11 cannot be excluded either. The latter peak results from the large 30 cM distance between the flanking markers, as well as by the two-point Lod scores in the range of 0.1 to 0.3 observed for marker D7S657 at recombination fraction values of 0.1 and over.

As indicated, the highest Lod score on this chromosome was obtained for D7S531, the most telomeric marker on 7p. The next marker D7S517, located at a distance of 6 cM, gave negative Lod scores (minus infinity at  $\theta = 0$  and -1.164 at 0.05).

Multipoint linkage analysis (figure 3.28) resulted in marginally positive Lod scores for the interval ranging from 0.12 to 0.2 with the

maximum of 0.2 obtained at D7S531. The Lod score for the flanking marker (D7S517) was negative thus excluding the position at this marker.

Haplotype analysis in family I (Fig. 3.29) demonstrates a crossover between D7S531 and D7S517 in individual III. Furthermore, the affected individuals in generation III have inherited the haplotypes of their unaffected grandmother. These results make it highly unlikely that the PKD3 gene is in this region.

Multipoint analysis also resulted in negative Lod scores for the intervals around D7S1808 and D7S669. The value of -1.16 for D7S1808 does not allow definite exclusion but places the interval in the group of regions which are highly unlikely to contain the gene. A multipoint score of -0.23 was obtained for D7S669 which thus remains a region which has not been excluded.

The two-point Lod score of 0.2 obtained for D7S550 was replicated in the multipoint linkage analysis. The closest marker to D7S550 is D7S640, at a distance of 44 cM. Given the large size of the interval and the slightly positive Lod scores (both two-point and multipoint) obtained for D7S550, the 7qter region has not been excluded.

Table 3.13: Two-point Lod scores for markers used to analyse chromosome 7.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D7S531	1 057	0 935	0 808	0 679	0 548	0 419	0 294	0 18	0 086	0 022	0
6	D7S517	-99 999	-1 184	-0 672	-0 412	-0 254	-0 153	-0 087	-0 445	-0 018	-0 004	0
9	D7S513	-99 999	0 033	0 229	0 298	0 313	0 298	0 263	0 214	0 153	0 081	0
19	D7S493	-99 999	-0 885	-0 377	-0 124	0 021	0 102	0 141	0 146	0 123	0 074	0
8	D7S1808	0 757	0 678	0 598	0 567	0 437	0 357	0 279	0 204	0 132	0 064	0
8	D7S516	-3 097	-0 163	0 067	0 168	0 214	0 227	0 216	0 187	0 141	0 078	0
10	D7S817	-99 999	-2 163	-1 331	-0 887	-0 581	-0 375	-0 227	-0 123	-0 053	-0 013	0
2	D7S484	-3 699	-0 721	-0 443	-0 292	-0 194	-0 125	-0 076	-0 041	-0 018	-0 004	0
7	D7S510	-99 999	-0 885	-0 377	-0 124	0 021	0 102	0 141	0 146	0 123	0 074	0
11	D7S519	-99 999	-1 442	-0 887	-0 585	-0 388	-0 25	-0 151	-0 082	-0 035	-0 009	0
11	D7S502	-99 999	-0 885	0 377	-0 124	0 021	0 102	0 141	0 146	0 123	0 074	0
11	D7S669	0 456	0 399	0 345	0 291	0 24	0 191	0 146	0 104	0 066	0 032	0
17	D7S657	-99 999	0 037	0 232	0 3	0 315	0 299	0 264	0 214	0 153	0 081	0
33	D7S530	-3 574	-0 599	-0 333	-0 197	-0 116	-0 066	-0 035	-0 017	-0 006	-0 001	0
5	D7S640	-99 999	-2 163	-1 331	-0 887	-0 581	-0 375	-0 227	-0 123	-0 053	-0 013	0
44	D7S550	0 204	0 172	0 141	0 112	0 085	0 061	0 04	0 023	0 01	0 003	0

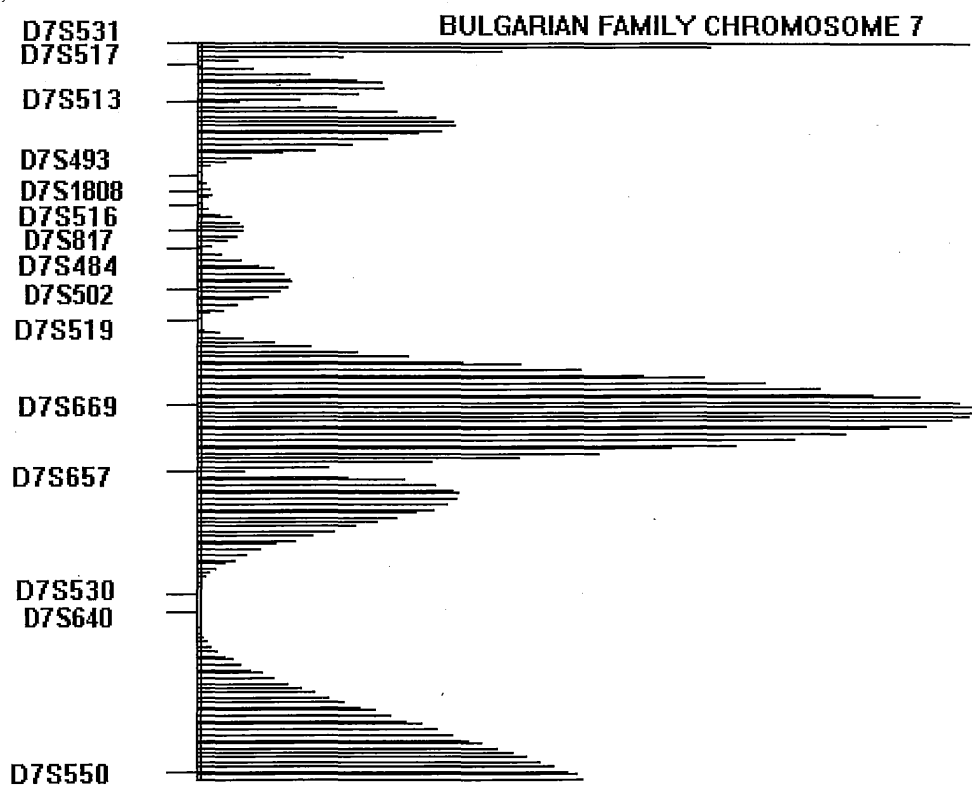


Figure 3.27: Exclusion map of chromosome 7.

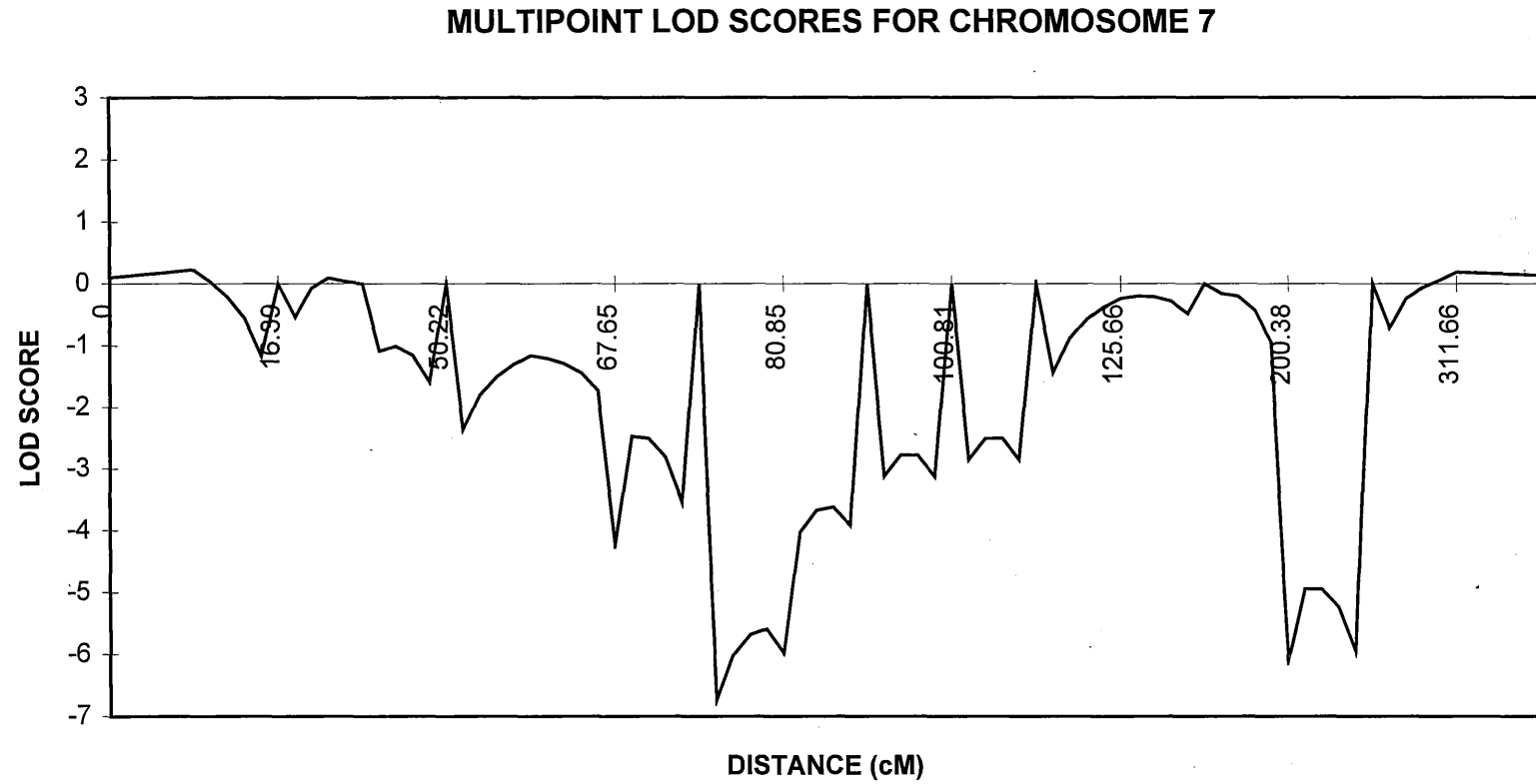


Figure 3.28: Graph of multipoint Lod scores for chromosome 7.



Figure 3.29: Haplotype of family 1 for chromosome 7.

### **3.8 Chromosome 8**

A total of 12 markers spaced 12cM apart were used to analyse chromosome 8. At theta zero, moderately positive two-point lod scores of 0.22 and 0.2 were obtained for D8S279 at 8q21.3 and D8S556 at 8q23.3 respectively (Table 3.14). All other polymorphic markers on chromosome 8 showed deeply negative Lod scores at this recombination fraction. A slightly positive Lod score of 0.3 was obtained for D8S284 (8q24.3) at recombination fraction 0.2 and over.

Figure 3.30 shows the exclusion map of chromosome 8 based on the two-point Lod scores. As can be seen on the map, the whole chromosome has been excluded apart from 8qter. These findings are replicated in the multipoint linkage analysis results where Lod scores well below -2 were obtained for most intervals, thus definitely excluding a very large proportion of chromosome 8. In the multipoint linkage calculations, the region telomeric to D8S284 remained slightly positive ( $Z = 0.2$ ), allowing us to classify this small interval as non-excluded but unlikely to contain PKD3.

From the haplotype in figure 3.32 it can be seen that none of the markers show a common allelic inheritance. A recombination occurred for marker D8S284 at individual III4, thus excluding the position at the marker itself. However because of the slightly positive Lod scores at the larger  $\theta$  values and the slightly positive multipoint results, the region telomeric to D8S284 could not be excluded.

Table 3.14: Table of two-point Lod scores for chromosome 8.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
9	D8S504	-99 999	-1 294	-0 766	-0 489	-0 315	-0 198	-0 118	-0 063	-0 027	-0 007	0
	D8S277	-99 999	-3 442	-2 285	-1 63	-1 183	-0 851	-0 595	-0 392	-0 229	-0 1	0
11	D8S550	-99 999	-3.442	-2 285	-1 63	-1.183	-0 851	-0 595	-0 392	-0 229	-0 1	0
20	D8S258	-99 999	-1.442	-0 887	-0 585	-0 388	-0 25	-0 151	-0 082	-0 035	-0 009	0
20	D8S283	-4 017	-1 016	-0 718	-0 547	-0 427	-0 335	-0 259	-0 191	-0 127	-0 065	0
12	D8S285	-3 942	-0 954	-0 666	-0 504	-0 393	-0 309	-0 239	-0 177	-0 119	-0 06	0
20	D8S279	0 222	0 187	0 154	0 123	0 093	0 067	0 044	0 025	0 011	0 003	0
12	D8S270	-99 999	-2 534	-1 687	-1.215	-0 896	-0 66	-0 475	-0 325	-0 2	-0 093	0
12	D8S556	0 204	0 172	0 141	0 112	0 085	0 061	0 04	0 023	0 01	0 003	0
12	D8S514	-99 999	-1 294	-0 766	-0 489	-0 315	-0 198	-0 118	-0 063	-0 027	-0 007	0
14	D8S284	-99 999	0 043	0 238	0 305	0 319	0 302	0 226	0 215	0 153	0 082	0



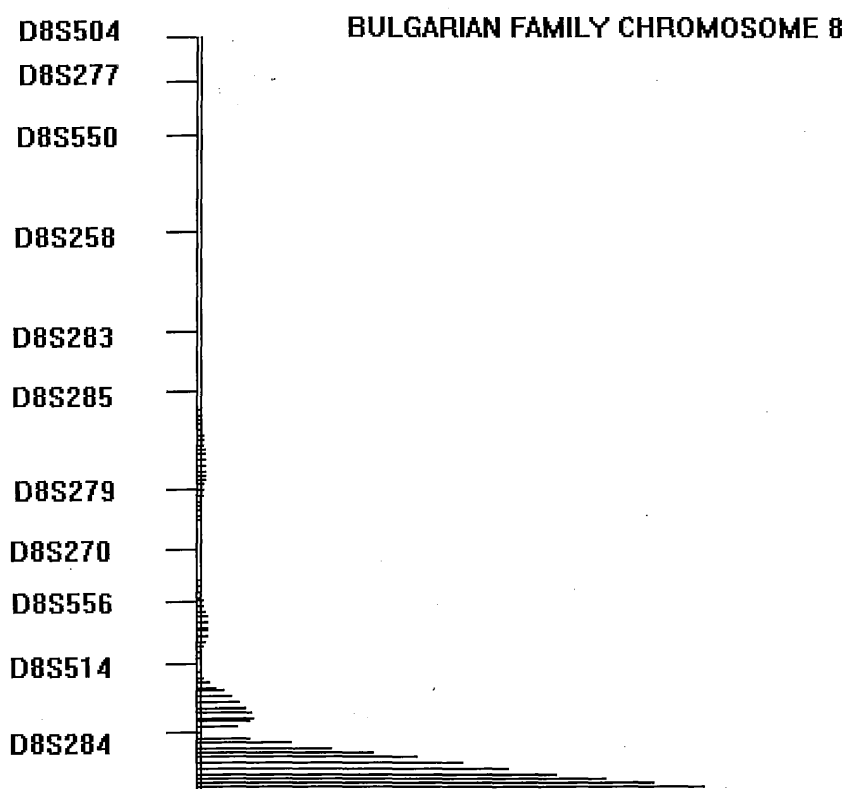


Figure 3.30: Exclusion map of chromosome 8.

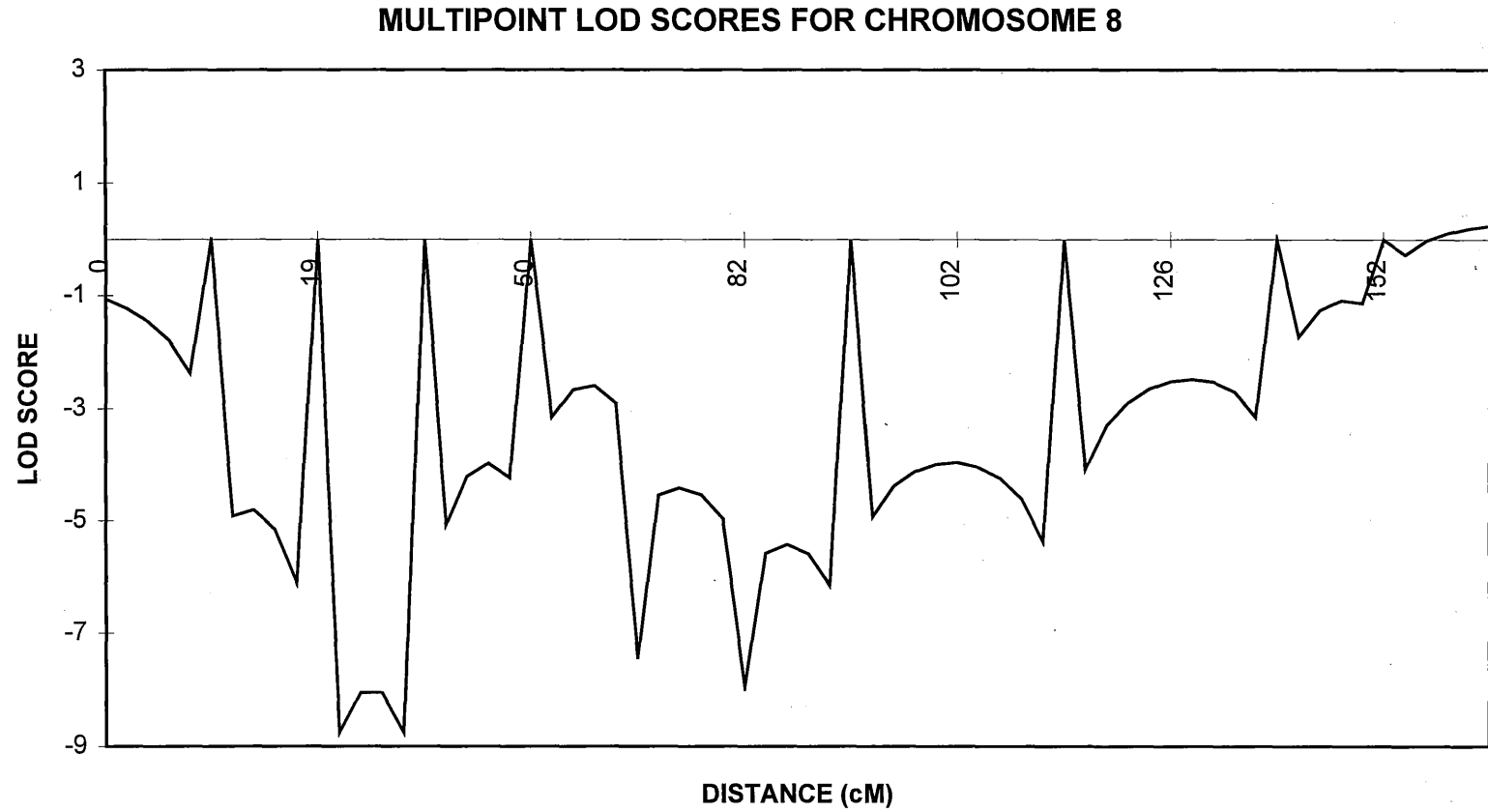


Figure 3.31: Graph of multipoint Lod scores for chromosome 8.

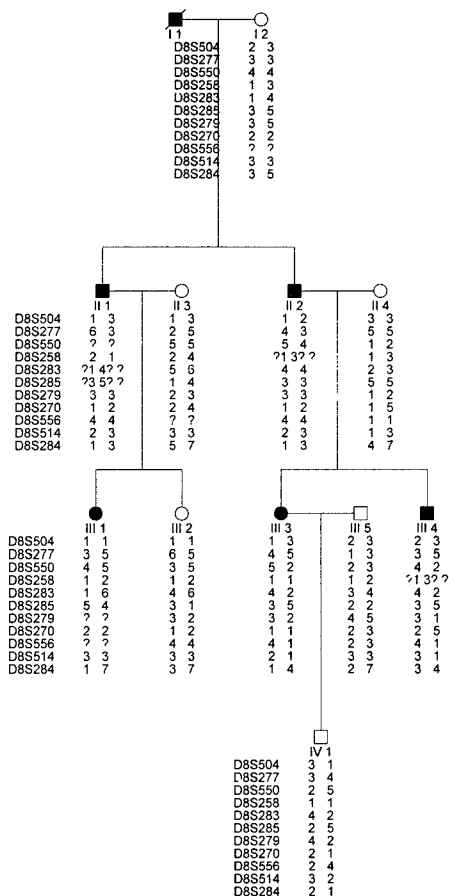


Figure 3.32: Haplotype of family 1 for chromosome 8.

### **3.9 Chromosome 9**

Chromosome 9 was analysed using 11 markers at an average distance of approximately 15cM. At  $\theta = 0$ , all except two markers gave negative two-point lod scores of less than -2.00. D9S741 (9q12) and D9S158 (9q34.3) gave slightly positive Lod scores of 0.177 and 0.125 respectively. These results are listed in table 3.15.

The exclusion map in figure 3.33 shows a broad peak on 9p and exclusion of the rest of the chromosome. The peak on 9p, between 9p24.2 and 9p21.3 at markers D9S178 and D9S741, is the result of the large distances between the markers in this interval (D9S178-22 cM-D9S156-18 cM-D9S741) and of the marginally positive two-point Lod scores at higher theta values for D9S178 and the Lod scores around 0.177 to 0.07 for all values of the recombination fraction for marker D9S741.

Further examination using haplotype analysis showed that for marker D9S741 no recombinations could be detected and the same allele was inherited by all affected individuals. Likewise for marker D9S158, a recombination could not be detected and all individuals inherited a common allele. Because of this lack of recombination, together with the weakly negative Lod score this region could not be excluded from the study.

Multipoint linkage analysis resulted in deeply negative values for a large proportion of chromosome 9. The three regions that could not be definitely excluded are defined by markers D9S741, D9S158 and D9S319 - D9S301 respectively. The region D9S319 (9p21.3) - D9S301 (9q12) is

10cM centromeric to D9S741 and produced Lod scores ranging from -1.32 to -1.98 thus allowing us to classify this region as being very unlikely to contain the PKD3 gene. The two other regions defined by markers D9S741 and D9S158 showed positive two-point Lod scores. With multipoint analysis they became negative (-0.67 for D9S741 and -1.15 for D9S158). Although such values do not allow the definite exclusion of these regions, they can be considered very unlikely to contain the PKD3 gene.

Table 3.15: Table of two-point Lod scores for markers used to analyse chromosome 9.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D9S178	-99.999	-0.339	-0.104	0.004	-0.059	0.086	0.093	0.067	0.038	0.009	0
22	D9S156	-99.999	-0.406	-0.184	-0.085	-0.036	-0.012	-0.002	-0.001	0	0	0
18	D9S741	0.177	0.148	0.121	0.096	0.072	0.051	0.034	0.019	0.009	0.002	0
10	D9S319	-99.999	0.008	-0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
11	D9S301	-99.999	-1.313	-0.782	-0.502	-0.325	-0.206	-0.123	-0.065	-0.028	-0.007	0
22	D9S303	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
7	D9S167	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.006	0
16	D9S197	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
11	D9S299	-99.999	-2.721	-1.841	-1.338	-0.989	0.727	-0.519	-0.351	-0.211	-0.096	0
36	D9S159	-99.999	-2.549	-1.7	-1.226	-0.905	-0.666	-0.48	-0.328	-0.201	-0.093	0
18	D9S158	0.125	0.104	0.084	0.066	0.049	0.035	0.023	0.013	0.006	0.001	0

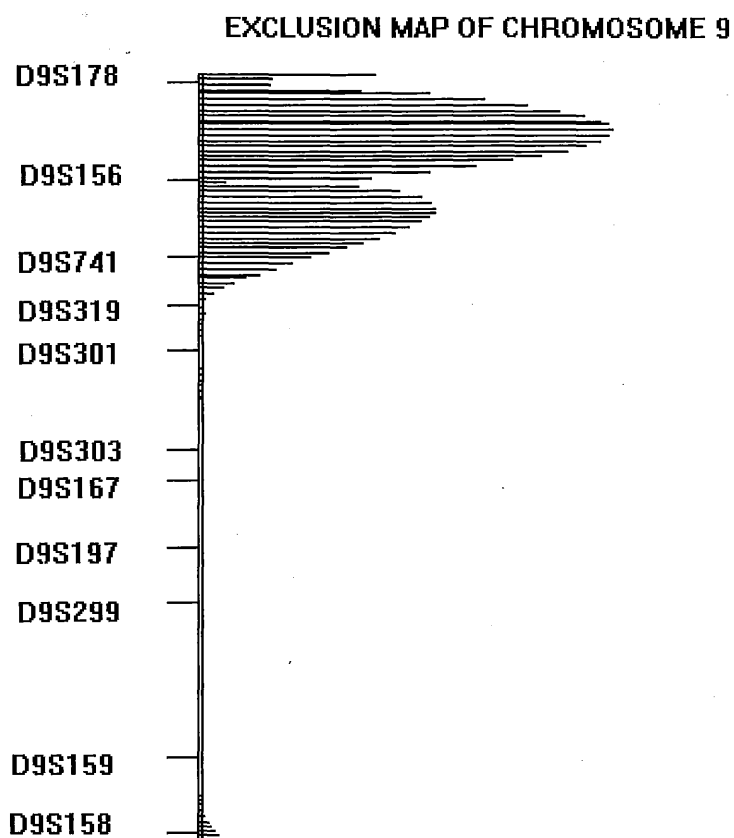


Figure 3.33: Exclusion map of chromosome 9.

## MULTIPOINT LOD SCORES FOR CHROMOSOME 9

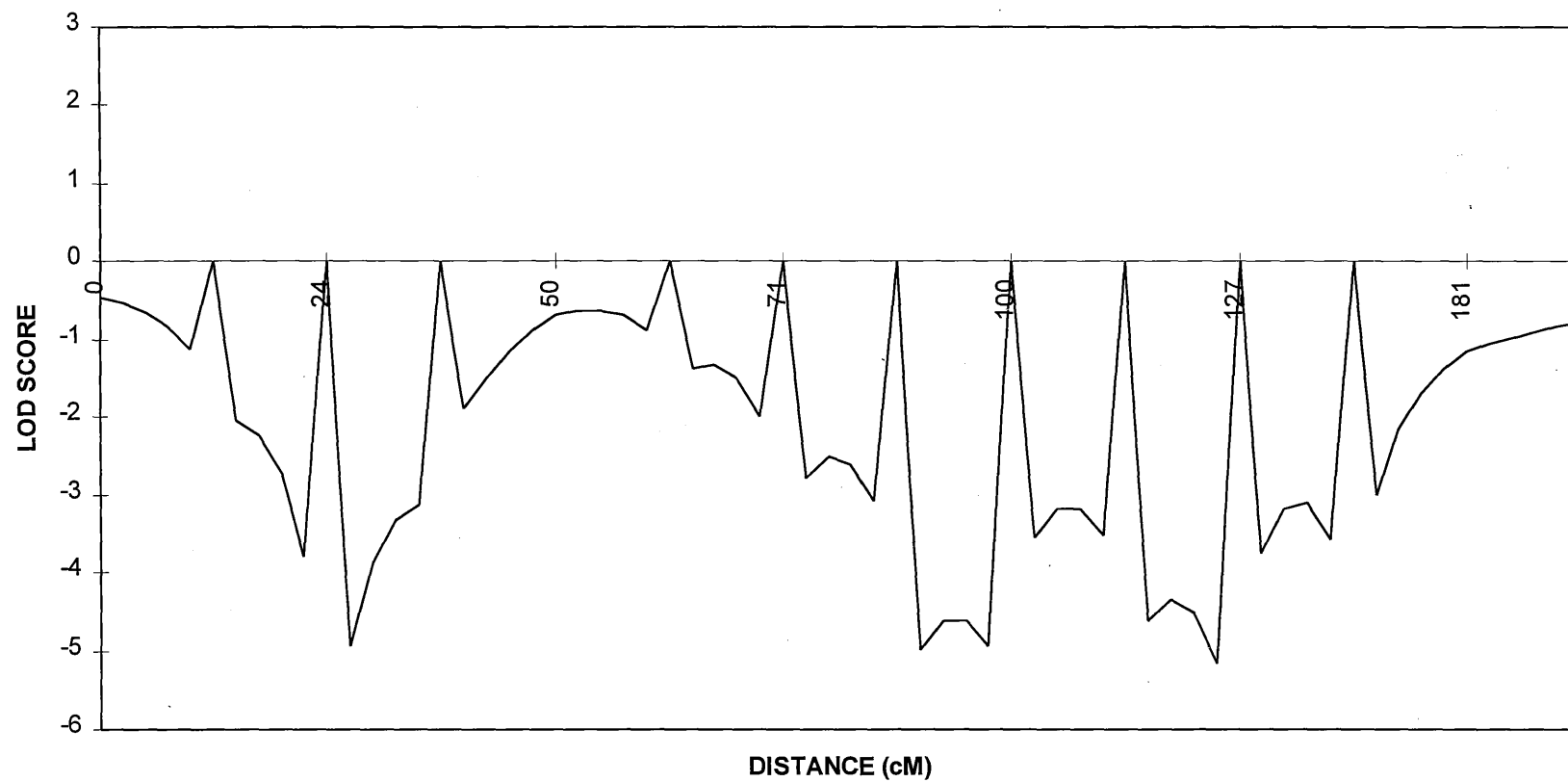


Figure 3.34: Graph of multipoint Lod scores for chromosome 9.



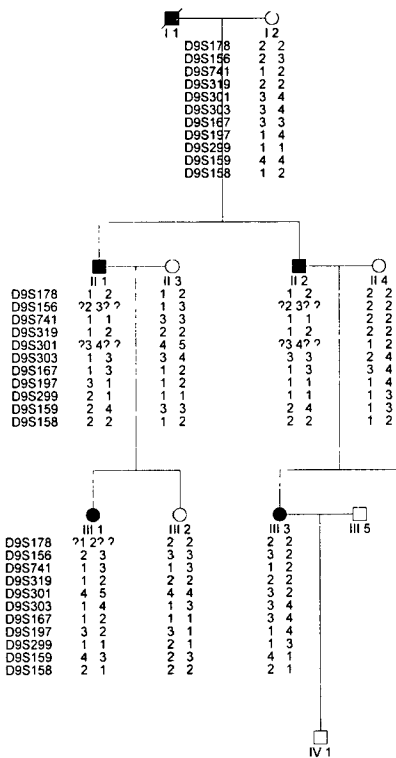


Figure 3.35: Haplotype of family I for chromosome 9.

### **3.10 Chromosome 10**

Chromosome 10 was analysed with a total of 19 markers spaced approximately 10cM apart.

From the two-point Lod scores in table 3.16 it can be seen that the only positive finding was at marker D10S187 (10q25.1) where a Lod score of 0.204 was obtained at  $\theta = 0$ . All other markers gave negative Lod scores below -2.00 at  $\theta = 0$  and below -1.00 at higher values of the recombination fraction, thus suggesting that the majority of chromosome 10 can be excluded. This is reflected in the exclusion map shown in figure 3.36.

Conclusive evidence that the entire length of chromosome 10 can be definitely excluded was provided by the multipoint linkage analysis (figure 3.37). All multipoint lod scores for chromosome 10 were below -2.00 except for a small region of 7cM near marker D10S249 at position 10p15.3, where the Lod scores ranged from -1.3 to -1.7. Haplotype analysis (figure 3.38) shows that for marker D10S187 (which gave a weakly positive two-point Lod score) it is not clear whether it is a common allele that has been inherited because the marker is uninformative. Multipoint Lod scores for this region did fall below -2.00, thus excluding this region as the location of the PKD3 gene.

Table 3.16: Table of two-point Lod scores for chromosome 10.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D10S249	-99.999	-0.885	-0.377	-0.124	0.021	0.102	0.141	0.146	0.123	0.074	0
13	D10S189	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
10	D10S465	-99.999	-1.294	-0.766	-0.489	-0.315	-0.198	-0.118	-0.063	-0.027	-0.007	0
11	D10S191	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
8	D10S466	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
10	D10S245	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
5	D10S89	-99.999	-1.405	-0.858	-0.562	-0.371	-0.238	-0.144	-0.078	-0.033	-0.008	0
11	D10S183	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
11	D10S220	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
10	D10S464	-99.999	-3.442	-2.285	-1.63	-1.183	-0.851	-0.595	-0.392	-0.229	-0.1	0
14	D10S676	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
10	D10S109	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
17	D10S677	-99.999	-1.271	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.006	0
11	D10S254	-99.999	-0.549	-0.303	-0.181	-0.109	-0.064	-0.036	-0.018	-0.007	-0.002	0
8	D10S187	0.204	0.172	0.141	0.112	0.085	0.061	0.04	0.023	0.01	0.003	0
5	D10S209	-99.999	-0.655	-0.39	-0.251	-0.163	-0.103	-0.062	-0.033	-0.014	-0.003	0
7	D10S1213	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
10	D10S186	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
15	D10S212	-99.999	-1.294	-0.766	-0.489	-0.315	-0.198	-0.118	-0.063	-0.027	-0.007	0

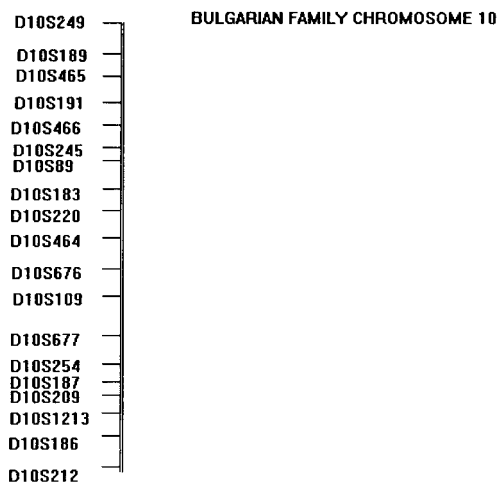


Figure 3.36: Exclusion map of chromosome 10.

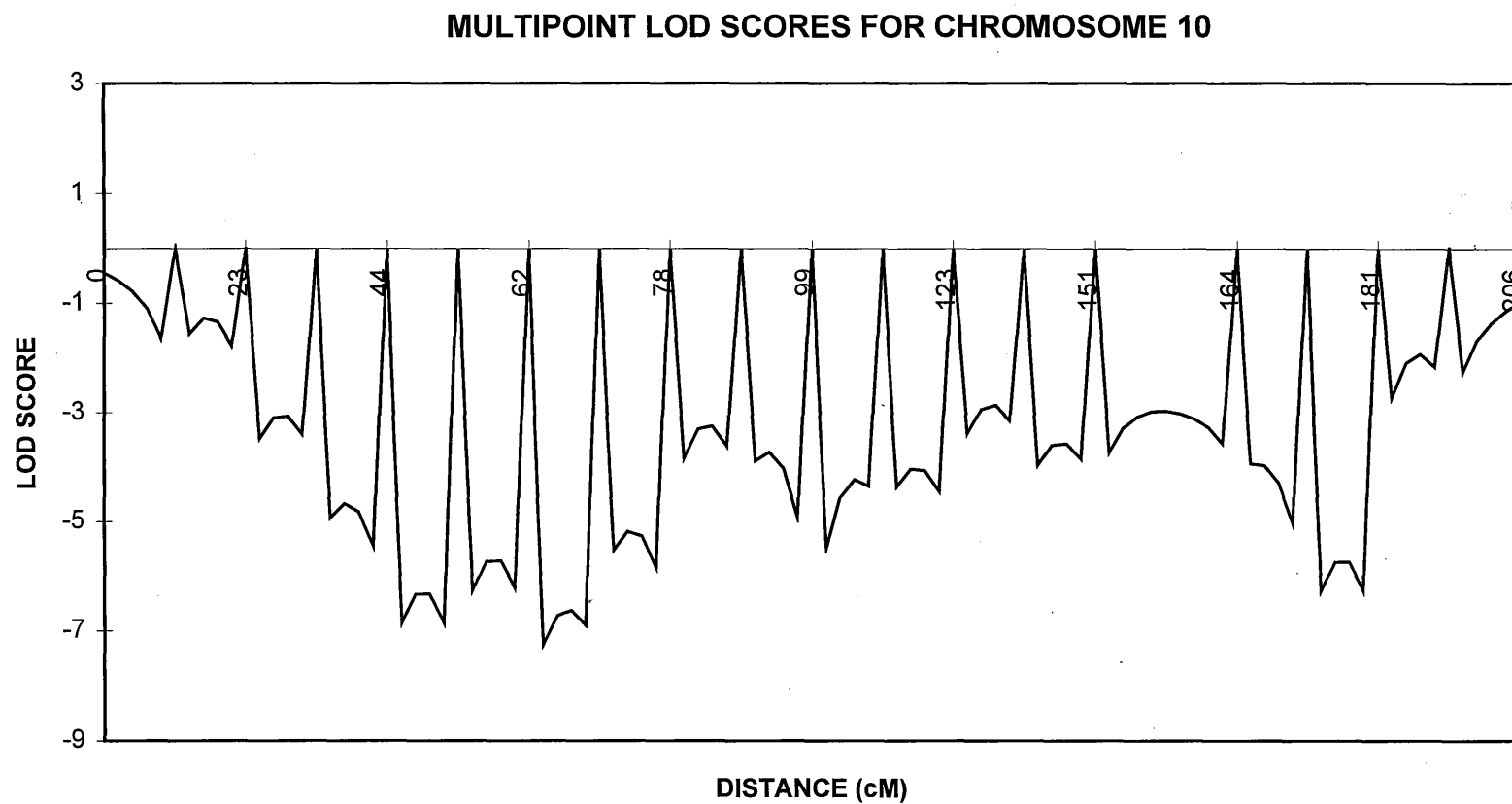


Figure 3.37: Graph of multipoint Lod scores for chromosome 10.

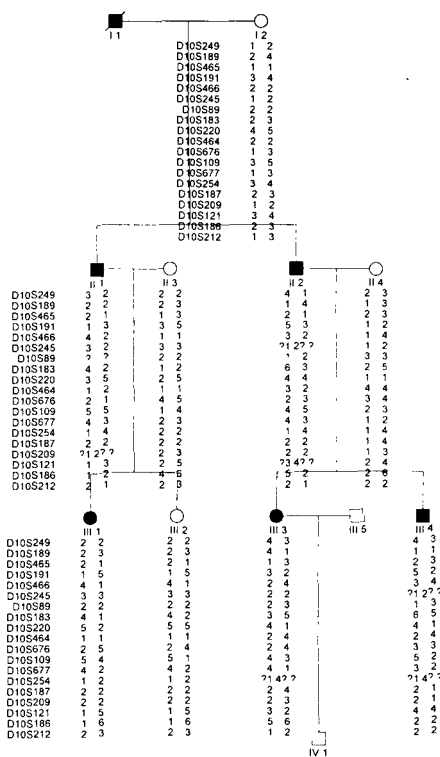


Figure 3.38: Haplotype of family 1 for chromosome 10.

### **3.11 Chromosome 11**

A total of 13 markers at average intervals of 12 cM were investigated on chromosome 11.

Two-point linkage analysis showed that for 10 of the markers, negative Lod scores of -2 or less were obtained at  $\theta = 0$ . For higher recombination fraction values, these markers gave Lod scores ranging between -0.9 and 0.6, thus making the exclusion of a large proportion of chromosome 10 inconclusive. Three adjacent markers spanning 18cM on the long short arm of chromosome 11 gave two-point Lod scores above 1.0. These included D11S875 at 11p15.3, D11S902 at 11p15.2 and D11S904 at 11p14.2 with Lod scores at  $\theta = 0$  respectively 1.1, 1.3, 1.4. The map in figure 3.39 illustrates these findings.

Multipoint analysis provided further support to the interval between D11S875 and D11S904 as a candidate PKD3 region. The highest multipoint lod score of 1.4 were obtained at D11S875 and D11S904 while D11S902 gave a Lod score of 1.3. The markers flanking this positive region, namely markers D11S988 at 11p15.5 and D11S1392 at 11p14.1, at distances D11S988-12 cM-D11S875 and D11S904-10 cM-D11S1392 produced negative lod scores.(figure 3.40). The markers D11S875, D11S902 and D11S904 and their corresponding positive lod scores are shown by the peak.

Investigating this region further using haplotype analysis it can be seen that a conserved region may exist between markers D11S988 and D11S904 among affected individuals. This is difficult to determine

however because the phase is unknown for marker D11S875. A recombination may have occurred between D11S1984 and D11S988 in individual III however this cannot be claimed for certain because the phase is unknown for marker D11S1984. This region corresponds to the positive region defined above by the markers D11S875, D11S902 and D11S904.

These findings require further investigation of chromosome 11, specifically of the D11S875-D11S904 interval as a candidate region for the PKD3 gene.



Table 3.17: Table of two-point Lod scores for markers used to analyse chromosome 11.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D11S1984	-2.495	0.394	0.577	0.629	0.622	0.579	0.508	0.414	0.298	0.161	0
4	D11S988	-3.096	-0.163	0.067	0.168	0.214	0.227	0.216	0.187	0.14	0.078	0
12	D11S875	1.145	1.321	1.191	1.057	0.92	0.779	0.633	0.484	0.329	0.169	0
4	D11S902	1.381	1.263	1.142	1.017	0.888	0.756	0.618	0.475	0.325	0.167	0
14	D11S904	1.408	1.287	1.162	1.033	0.901	0.765	0.624	0.478	0.327	0.168	0
10	D11S1392	-2.795	0.115	0.322	0.399	0.418	0.403	0.362	0.301	0.219	0.119	0
20	D11S1985	-2.495	0.394	0.577	0.629	0.622	0.579	0.508	0.414	0.298	0.161	0
16	D11S937	-2.495	0.394	0.577	0.629	0.622	0.579	0.508	0.414	0.298	0.161	0
6	D11S1396	-2.495	0.394	0.577	0.629	0.622	0.579	0.508	0.414	0.298	0.161	0
12	D11S876	-99.999	-0.884	-0.376	-0.124	0.02	0.102	0.14	0.145	0.122	0.074	0
8	D11S1391	-3.096	-0.163	0.067	0.168	0.214	0.227	0.216	0.187	0.14	0.078	0
10	D11S976	-99.999	-0.884	-0.376	-0.124	0.02	0.102	0.14	0.145	0.122	0.074	0
39	D11S968	-99.999	-0.617	-0.359	-0.227	-0.144	-0.09	-0.053	-0.028	-0.012	-0.003	0

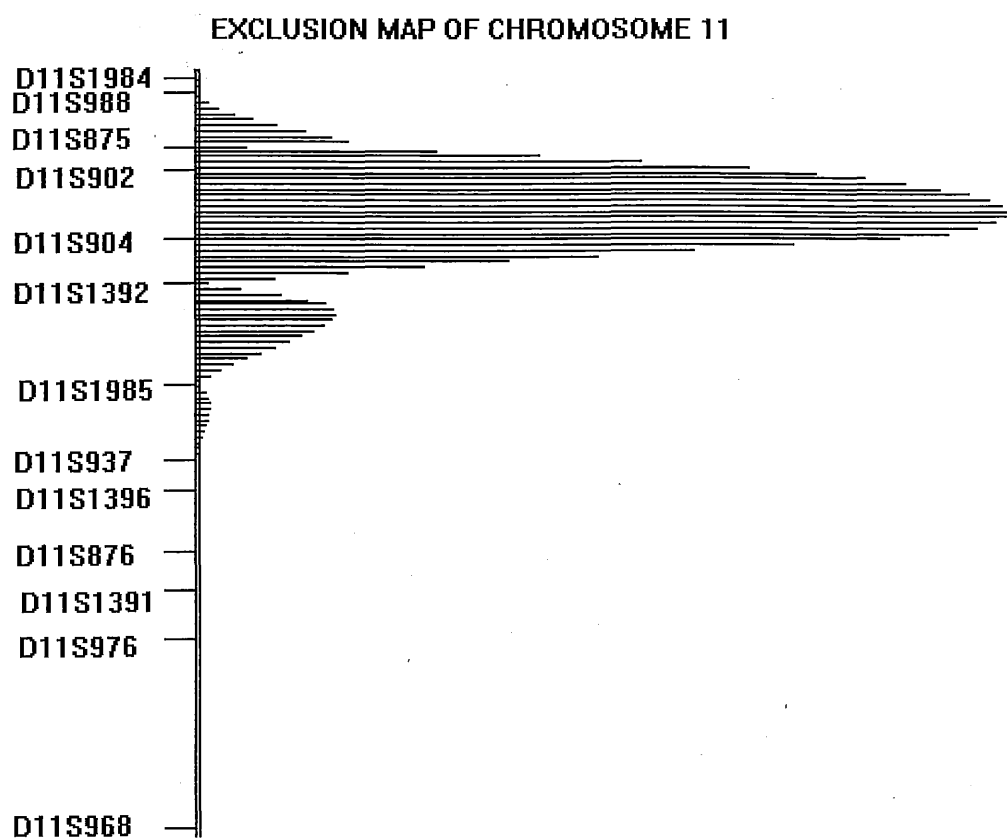


Figure 3.39: Exclusion map of chromosome 11.

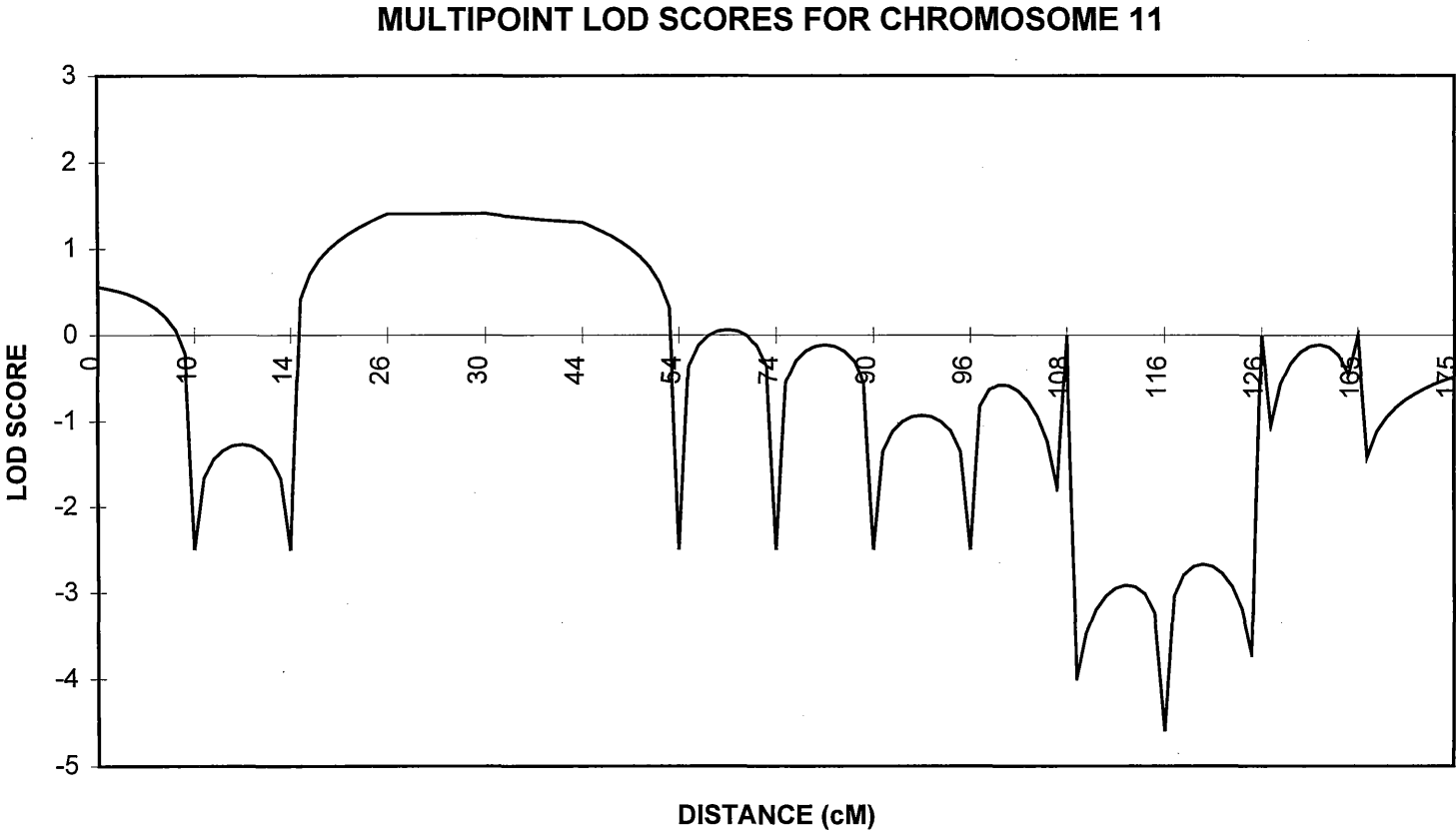


Figure 3.40: Graph of multipoint Lod scores for chromosome 11.

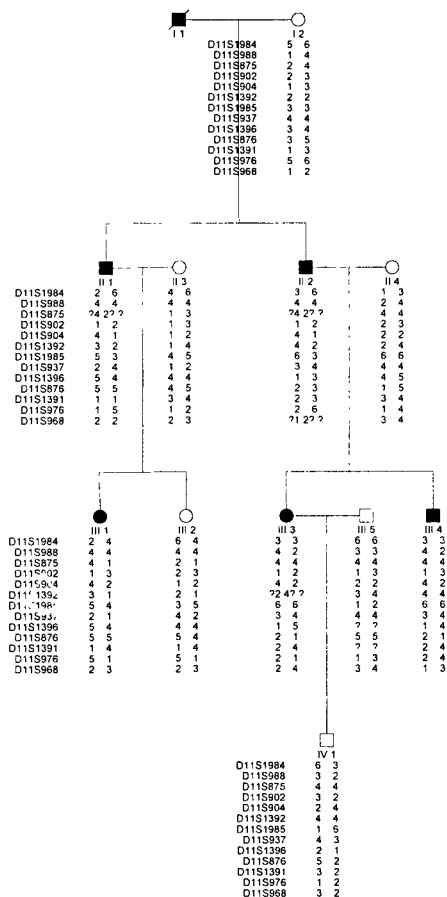


Figure 3.41: Haplotype of family I for chromosome 11.

### **3.12 Chromosome 12**

Chromosome 12 was analysed using a total of 11 markers spaced approximately 18cM apart.

Table 3.18 shows these markers, the distances between them and the two-point lod scores obtained. Two markers, namely D12S269 at 12p13.1 and D12S392 at 12q24.33, gave slightly positive lod scores of 0.204 at  $\theta = 0$ . All other markers gave deeply negative Lod scores at recombination distance 0 and less than -1 at higher theta values.

Figure 3.42 shows the graphical representation of these lod scores. The map presents a single peak of non-exclusion on the long arm of chromosome 12, near D12S392. This peak is the result of the large distance of 56cM between PAH and D12S392 as well as of the marginally positive Lod scores obtained at D12S392 with all theta values.

The examination of haplotypes in pedigree I (figure 3.44) shows that the two siblings III1 and III2, one affected and the other unaffected, have inherited the same haplotype from their affected father and that, in addition, this haplotype originates from the unaffected paternal grandmother. The other two affected sibs in generation III also do not share identical haplotypes.

In confirmation to these observations, multipoint linkage analysis results in deeply negative Lod scores for nearly the entire length of chromosome 12 (fig. 3.44), including one of the regions which gave a slightly positive two-point Lod score, namely that around marker D12S269. The second such region, near D12S392, showed multipoint Lod

scores ranging from -0.2 to -0.7 which do not allow the definite exclusion of this large interval but place it in the category of regions which are unlikely to contain the PKD3 gene.

Table 3.18: Table of two-point lod scores for markers used to analyse chromosome 12.

DISTANCE(cM)	MARKER	THETA VALUES										0.5
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	
	D12S372	-99 999	-1 271	-0 746	-0 473	-0 303	-0 189	-0 112	-0 059	-0 025	-0 006	0
7	D12S274	-99 999	-1 575	-1 024	-0 721	-0 521	-0 376	-0 265	-0 177	-0 106	-0 048	0
18	D12S391	-99 999	-1 239	-0 72	-0 451	-0 285	-0 176	-0 103	-0 054	-0 023	-0 005	0
17	D12S269	0 204	0 172	0 141	0 112	0 085	0 061	0 04	0 023	0 01	0 003	0
27	D12S61	-99 999	-2 163	-1 331	-0 877	-0 581	-0 375	-0 227	-0 123	-0 053	-0 013	0
3	GATA5A09	-99 999	-2 163	-1 331	-0 877	-0 581	-0 375	-0 227	-0 123	-0 053	-0 013	0
33	D12S375	-99 999	-3 442	-2 285	-1 63	-1 183	-0 851	-0 595	-0 392	-0 229	-0 1	0
10	D12S379	-99 999	-2 442	-1 586	-1 108	-0 786	-0 551	-0 373	-0 237	-0 132	-0 054	0
9	D12S101	-3 942	-0 954	-0 666	-0 504	-0 393	-0 309	-0 239	-0 177	-0 119	-0 06	0
9	PAH	-99 999	-2 549	-1 7	-1 226	-0 905	-0 666	-0 48	-0 328	-0 201	-0 093	0
56	D12S392	0 204	0 172	0 141	0 112	0 085	0 061	0 04	0 023	0 01	0 003	0

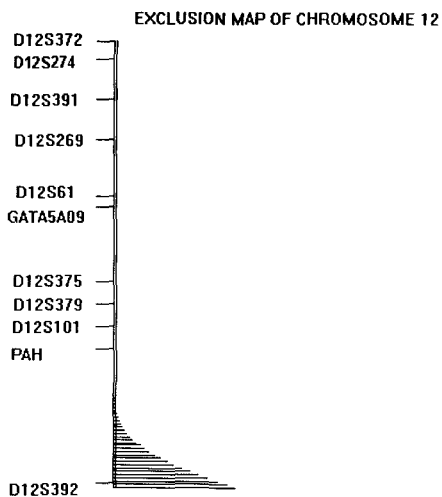


Figure 3.42: Exclusion map of chromosome 12.



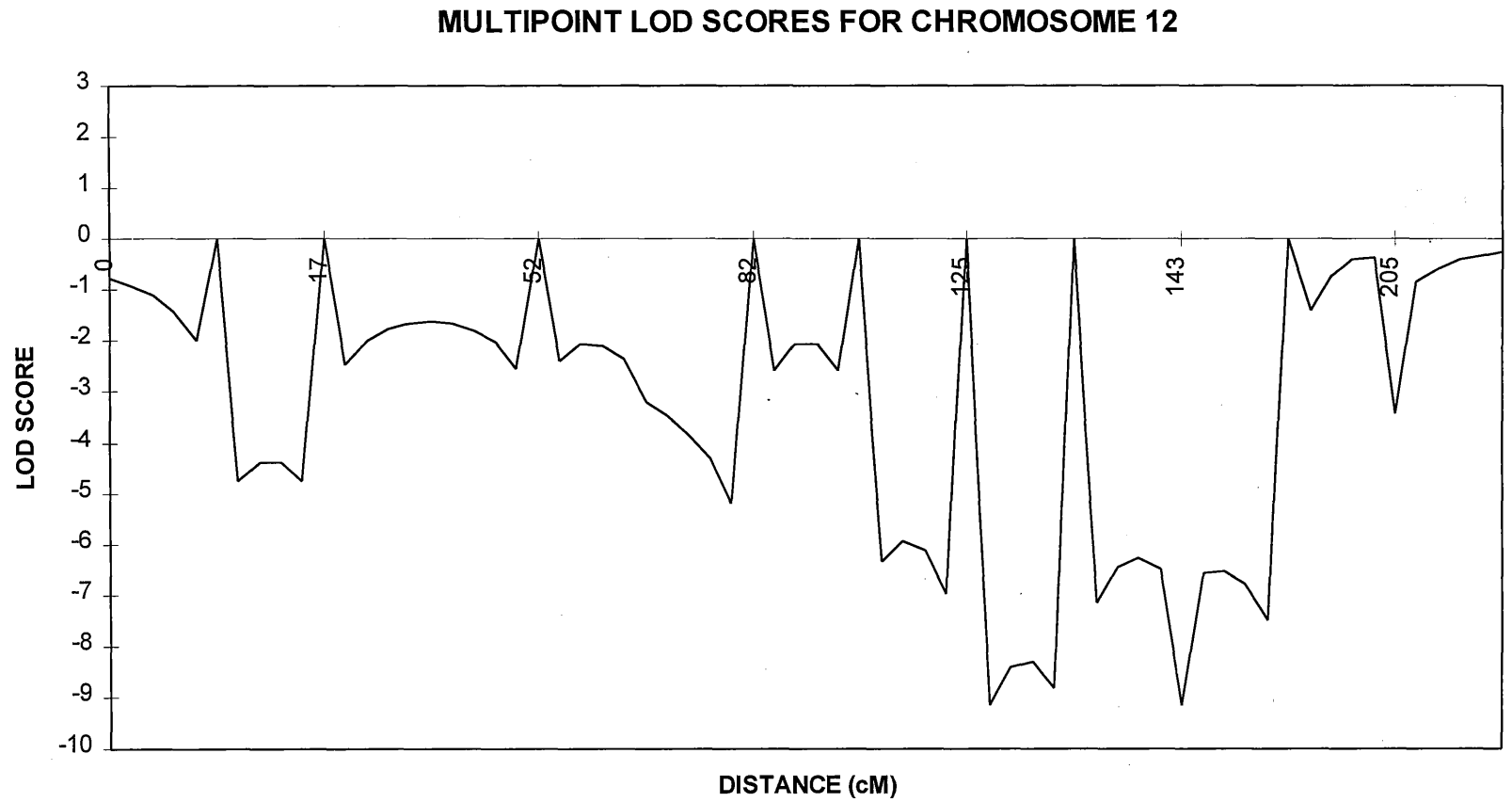


Figure 3.43: Graph of multipoint Lod scores for chromosome 12.

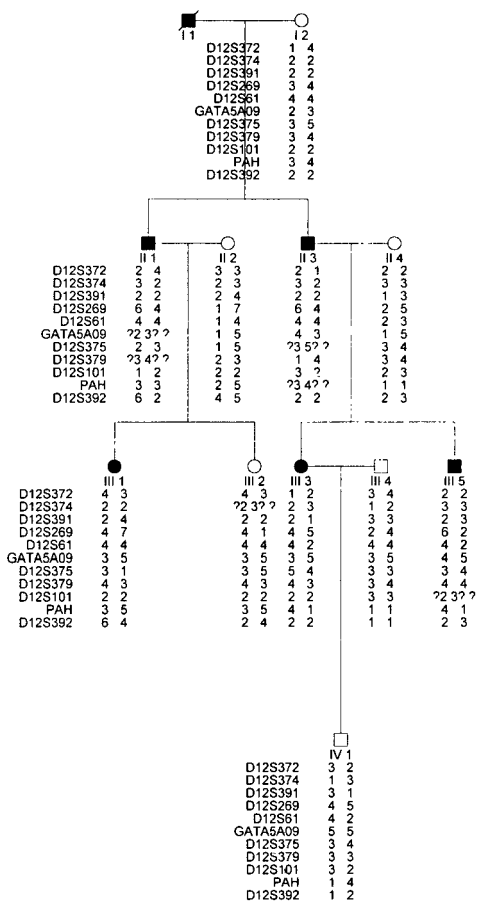


Figure 3.44: Haplotype of family 1 for chromosome 12.

### **3.13 Chromosome 13**

#### **3.13.1 Stage 1**

Stage 1 of the study on chromosome 13 involved the analysis of 6 markers spaced approximately 17 cM apart. Table 3.19 shows the two-point Lod scores for each of these markers. It can be seen that there is a large region where positive two-point Lod scores were obtained. The region is flanked centromerically by markers D13S325 at 13q14.11 and on the telomeric side and by D13S173 at 13q33.1. This region includes two more markers, namely D13S317 (13q34.1) and D13S225 (13q32.3). The two-point Lod scores for this region range from 0.7 to 1.4. This region is shown graphically as the large peak of non-exclusion in the map in figure 3.45.

The remaining markers on chromosome 13 gave deeply negative two-point Lod scores and allowed the exclusion of the rest of the chromosome.

All affected members of Family 1 have inherited identical haplotypes formed by the four markers from the positive region which span a distance of 57 cM (figure 3.45).

Multipoint linkage analysis confirmed these positive findings and produced a plateau on the graph (figure 3.46), with a Lod score of 1.4 for the entire length of the interval D13S317, D13S225 and D13S173. Marker D13S325, where a two-point Lod score of 0.7 was obtained, gave a multipoint score of 0.3. This region was therefore considered a possible candidate region and was investigated further.

Table 3.19: Two-point Lod scores for markers used to analyse chromosome 13 for family 1 in stage I.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D13S221	-99 999	-0 549	-0 303	-0 18	-0 109	-0 094	-0 036	-0 018	-0 007	-0 002	0
19	D13S220	-99 999	-2 573	-1 72	-1.242	-0 917	-0 675	-0 486	-0 331	-0 203	-0 094	0
8	D13S325	0 727	0 65	-0 573	0 495	0 417	0 34	0 265	0 193	0 124	0 06	0
47	D13S317	1 408	1 287	1 162	1 034	0 901	0 765	0 624	0 479	0 327	0 168	0
26	D13S225	1 107	1.008	-0 907	0 803	0 697	0 589	0 478	0 365	0 248	0 127	0
14	D13S173	1 426	1 302	1 175	1 044	0 91	0 771	0 628	0 481	0 328	0 168	0

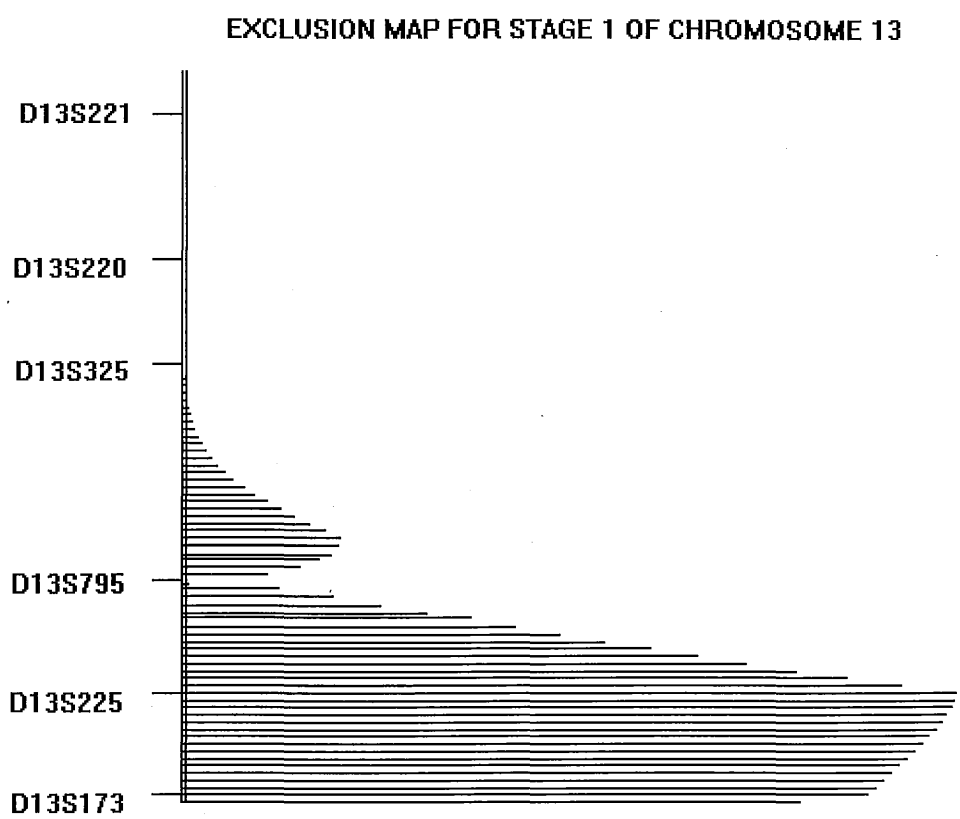


Figure 3.45: Exclusion map of chromosome 13 for stage 1 of the study.

MULTIPOINT LOD SCORES FOR CHROMOSOME 13 FOR STAGE 1

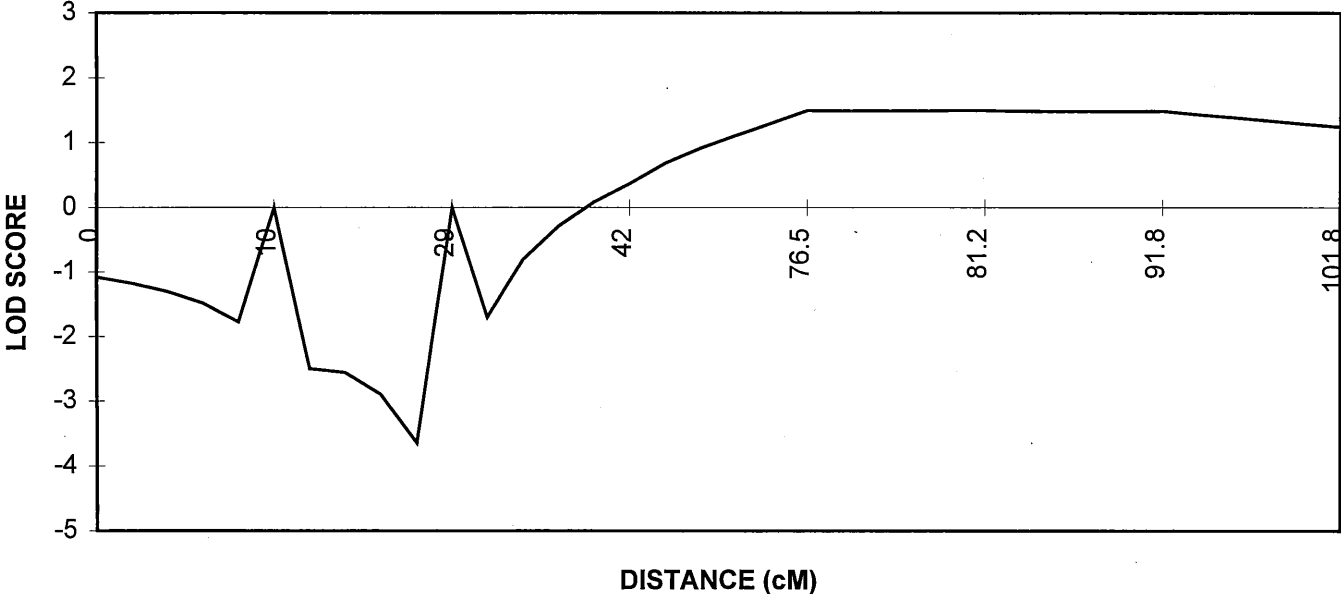


Figure 3.46: Graph of multipoint Lod scores on chromosome 13 for family 1.

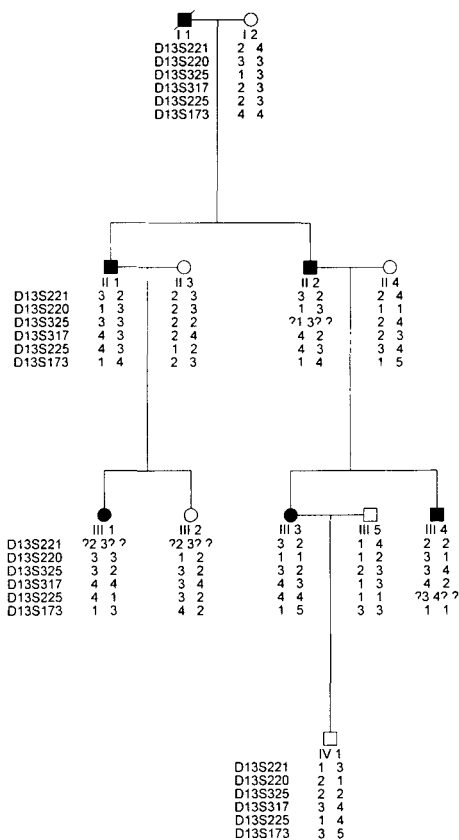


Figure 3.47: Haplotype of family 1 for markers used to analyse chromosome 13 in stage 1 of the study.

### 3.13.2 Stage 2

Stage 2 of the study of chromosome 13 was conducted by including the second Bulgarian PKD family and adding four new polymorphic markers to the positive region. This created a higher density map of the region with a total of 8 markers at an average distance of 9cM.

The four new markers, namely D13S321 at 13q14.3, D13S792 at 13q21.33, D13S795 at 13q31.1 and D13S797 at 13q33.1 all produced positive two-point results for family 1. The maximum Lod score of these new markers was 1.40 at  $\theta = 0$  for marker D13S797 while the other additional markers produced Lod scores ranging from 0.2 to 0.7 at  $\theta = 0$ .

Haplotype analysis of family 1 shows a recombination has occurred for marker D13S221 at individual III4 and that a conserved region for the rest of the markers may occur among affected individuals. This cannot be claimed for certain because of the uncertainty of inheritance of alleles for individual II2 for his parents. If the assumption is made that this individual inherits the correct alleles from the affected father then a common allele pattern can be determined.

Multipoint analysis of this region for family 1 yielded positive Lod scores ranging from 1.25 to 1.50. All markers between and including D13S792 and D13S173 produced Lod scores greater than 1.00, thus defining a positive region between 13q21.33 and 13q33.1 for family 1.

Two-point analysis of the second family with the newly added markers to this region produced negative Lod scores. These scores ranged



from -0.458 to -1.04 at  $\theta = 0$  thus suggesting exclusion of the region in family 2 as a candidate region.

Further examination of this region using haplotype analysis of family 2 shows the affected first cousins, individuals III2 and III4 have inherited different haplotypes. Individual III3, who is unaffected, has inherited part of the proximal and the distal haplotype which is identical to that of the affected cousin, individual III4. Finally, the two affected siblings, IV2 and IV3, have inherited different haplotypes thus excluding this region for family 2.

Multipoint analysis on this region for family 2 show that the Lod scores for this region are deeply negative. All new markers added to the region produced negative Lod scores ranging from .1.24 to -4.85, thus confirming the findings from haplotype analysis and showing that this region can be excluded for family 2.

If the two-point Lod scores for both families combined are examined it can be seen that only one of the new markers, D13S797 at 13q33.1, produced a positive Lod score, namely 0.75 at  $\theta = 0$ . The rest of the markers added to this region produced negative Lod scores in the range of -0.14 to -0.76. The two large peaks in figure 3.48 represent the positive Lod scores of 1.4 for the markers D13S317 and D13S173, these peaks however have been narrowed with the addition of the extra markers.

Multipoint Lod scores for both families combined show that the Lod scores obtained in stage 2 had reduced from what they were in stage 1 due to the addition of the second family. As mentioned above the

multipoint Lod scores for family 2 are deeply negative thus causing the overall Lod scores to reduce. The maximum combined Lod score for this region was 0.24 at D13S173 which is a reduction from the result of 1.4 achieved in stage 1 of the study. All other markers that showed positive findings in stage 1 of the study became negative due to the addition of the second family. These negative Lod scores ranged from -0.08 to -2.82.

Table 3.20: Two-point Lod scores for markers on chromosome 13 used to analyse family 1 in stage 2.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D13S221	-99.999	-0.549	-0.303	-0.18	-0.109	-0.094	-0.036	-0.018	-0.007	-0.002	0
19	D13S220	-99.999	-2.573	-1.72	-1.242	-0.917	-0.675	-0.486	-0.331	-0.203	-0.094	0
14.2	D13S325	0.727	0.65	-0.573	0.495	0.417	0.34	0.265	0.193	0.124	0.06	0
12.9	D13S321	0.14	0.145	0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
7.3	D13S792	0.721	0.634	0.59	0.489	0.388	0.291	0.2	0.12	0.056	0.014	0
12.6	D13S317	1.408	1.287	1.162	1.034	0.901	0.765	0.624	0.479	0.327	0.168	0
2.3	D13S795	1.08	0.845	0.75	0.628	0.505	0.385	0.269	0.165	0.08	0.022	0
24.3	D13S225	1.107	1.008	0.907	0.803	0.697	0.589	0.478	0.365	0.248	0.127	0
4.6	D13S797	1.408	1.346	1.14	1.016	0.887	0.755	0.617	0.475	0.325	0.168	0
6	D13S173	1.426	1.302	1.175	1.044	0.91	0.771	0.628	0.481	0.328	0.168	0

Table 3.21: Two-point Lod scores for markers on chromosome 13 used to analyse family 2 in stage 2.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D13S321	-0.905	-0.834	-0.735	-0.468	-0.303	-0.195	-0.122	-0.072	-0.039	-0.016	0
7.3	D13S792	-1.171	-0.869	-0.78	-0.497	-0.32	-0.2	-0.118	-0.061	-0.026	-0.006	0
14.9	D13S795	-1.223	-0.98	-0.88	-0.577	-0.38	-0.243	-0.146	-0.078	-0.034	-0.008	0
28.9	D13S797	-0.65	-0.641	-0.453	-0.296	-0.192	-0.117	-0.064	-0.027	-0.005	-0.004	0

Table 3.22: Shows the two point Lod scores for all markers used to analyse chromosome 13 for family 1 and family 2 in stage 2.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D13S221	-99 999	-0 549	-0 303	-0 18	-0 109	-0 094	-0 036	-0 018	-0 007	-0.002	0
19	D13S220	-99 999	-2 573	-1.72	-1 242	-0 917	-0 675	-0.486	-0 331	-0 203	-0.094	0
14.2	D13S325	0 727	0 65	-0 573	0 495	0 417	0 34	0 265	0 193	0 124	0 06	0
12.9	D13S321	-0 7658	-0.6897	-0.5269	-0.1873	-0 004	0 092	0 1344	0 1375	0 1123	0 1652	0
7.3	D13S792	-0 45	-0.2354	-0 1901	0 0089	0 0687	0 0914	0 0828	0 0584	0.0303	0 0083	0
12.6	D13S317	1 408	1 287	1 162	1.034	0.901	0 765	0 624	0 479	0 327	0 168	0
2.3	D13S795	-0 1432	-0 1355	-0.1299	0 0511	0 1255	0 1415	0 1233	0 087	0.0463	0 0139	0
24.3	D13S225	1 107	1 008	0 907	0 803	0 697	0 589	0 478	0 365	0 248	0 127	0
4.6	D13S797	0 758	0 7046	0 6861	0 719	0 6956	0 6375	0 5535	0 4473	0 3201	0 1717	0
6	D13S173	1 426	1.302	1.175	1 044	0 91	0 771	0 628	0 481	0 328	0 168	0

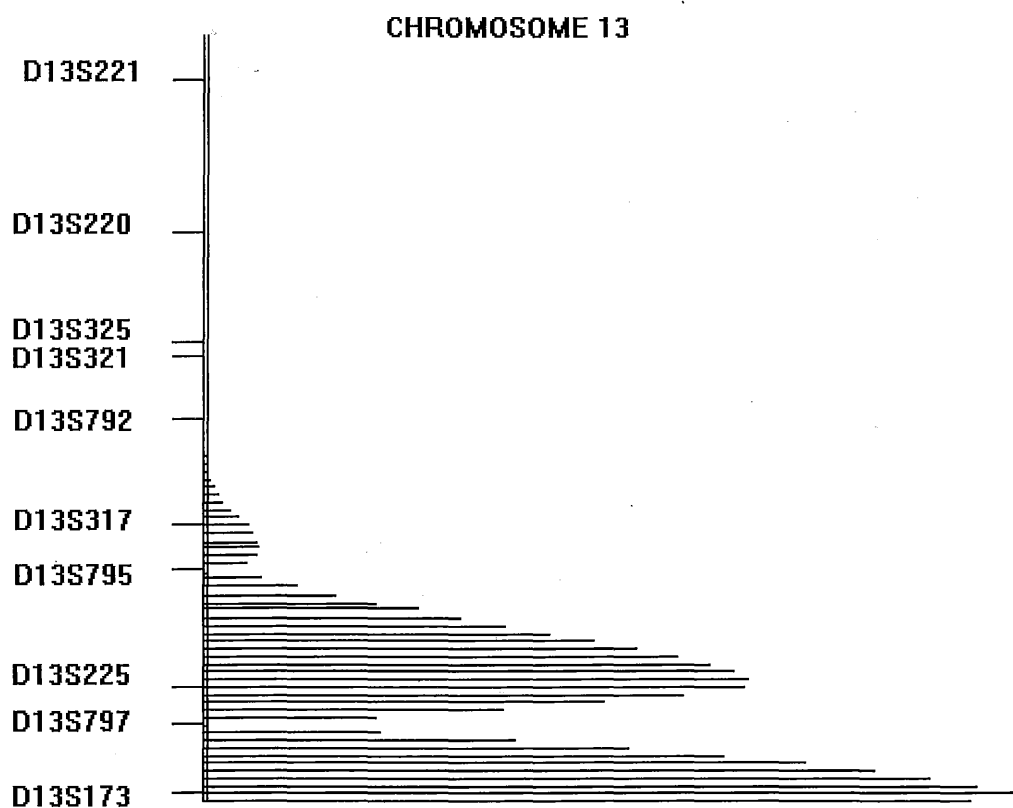


Figure 3.48: Exclusion map of chromosome 13 for stage2.

MULTIPOINT LOD SCORES OF CHROMOSOME 13 FOR STAGE 2

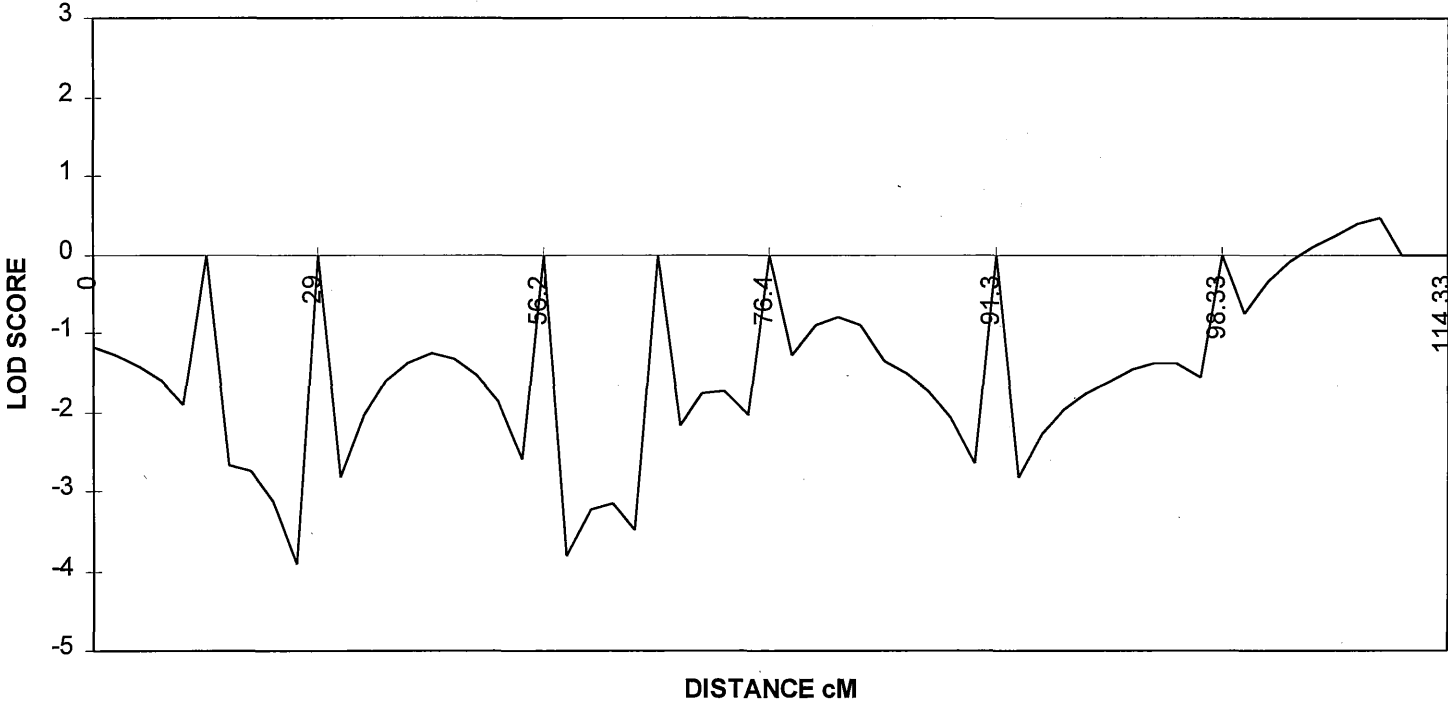


Figure 3.49: Graph of the multipoint Lod scores on chromosome 13 for family 1 and family 2 combined.

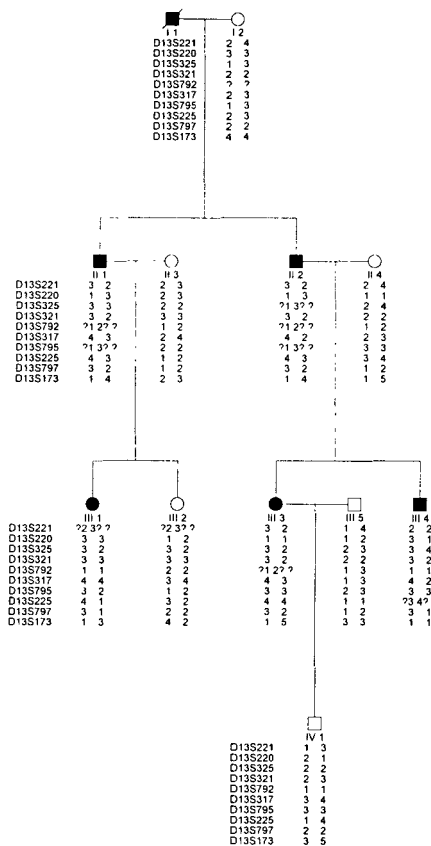


Figure 3.50: Haplotype of family 1 for all markers used to analyse chromosome 13 in stage 2 of the study.



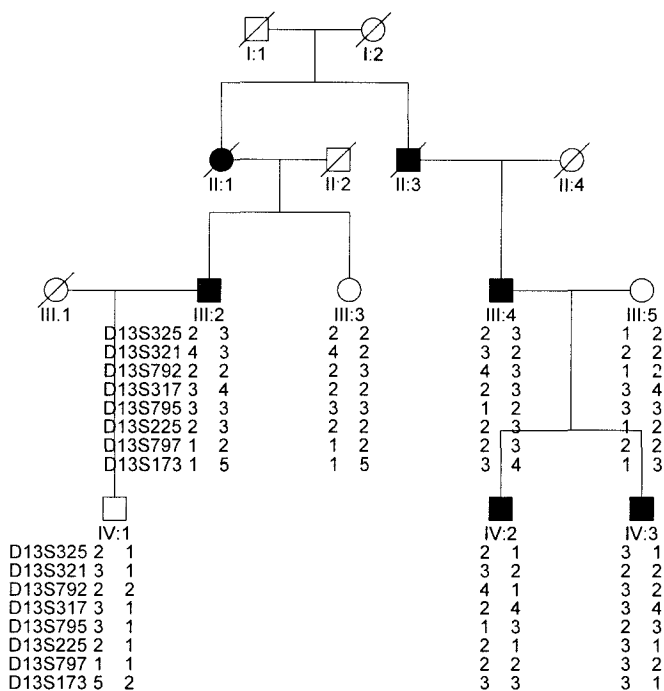


Figure 3.51: Haplotype of pedigree 2 showing markers run on chromosome 13 in stage 2.

### **3.14 Chromosome 14**

#### **3.14.1 Stage 1**

Stage 1 of the study on chromosome 14 included the analysis of 13 markers spaced approximately 10cm apart.

At recombination distance zero, deeply negative two-point Lod scores were obtained for most markers (table 3.23). The maximum two-point Lod score observed was 1.1 at  $\theta = 0$  for D14S118 at 14q32.31. The other positive two-point Lod score obtained on this chromosome was 0.7 at  $\theta = 0$  for D14S51 at 14q31.3.

The exclusion map (figure 3.52) shows a broad peak around the second marker, D14S51, resulting from the positive two-point Lod score at this marker and also from the slightly positive Lod scores obtained for the flanking markers (D14S81 [14q31.3] and D14S78 [14q32.2]) at higher recombination distances, which do not allow the exclusion of the interval. The second peak is higher and better defined, centering on marker D14S118 which gave the highest two-point Lod score on this chromosome, namely 1.156.

An examination of the inheritance of haplotypes in Family 1 reveals that, within the first positive interval, namely D14S81-D14S78, the two affected sibs in generation II do not share identical paternal haplotypes. Multipoint linkage analysis produced deeply negative Lod scores across this region, thus excluding it from further study (figure 3.53). By contrast, multipoint analysis of the telomeric region of 14q confirmed

the positive findings from the previous study, resulting in an increase in the Lod score at marker D14S118 to 1.4.

Table 3.23: Two-point Lod scores for the markers used to analyse chromosome 14 in stage 1 of the study.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D14S50	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.06	-0.025	-0.006	0
15	D14S90	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
4	D14S297	-99.999	-1.049	-0.529	-0.263	-0.104	-0.007	0.05	0.073	0.072	0.047	0
16	D14S306	-99.999	-2.573	-1.72	-1.242	-0.917	-0.675	-0.486	-0.331	-0.203	-0.094	0
12	GGAA10C09	-99.999	0.024	0.221	0.291	0.308	0.294	0.26	0.212	0.152	0.081	0
14	D14S63	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
11	D14S774	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
5	D14S53	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
11	D14S48	-3.097	-0.163	0.067	0.169	0.214	0.227	0.217	0.187	0.141	0.078	0
13	D14S81	-3.097	-0.163	0.067	0.169	0.214	0.227	0.217	0.187	0.141	0.078	0
6	D14S51	0.757	-0.678	0.598	0.518	0.437	0.357	0.279	0.204	0.132	0.064	0
12	D14S78	-3.097	-0.163	0.067	0.169	0.214	0.227	0.217	0.187	0.141	0.078	0
7	D14S118	1.156	1.051	0.943	0.833	0.721	0.606	0.49	0.372	0.251	0.128	0

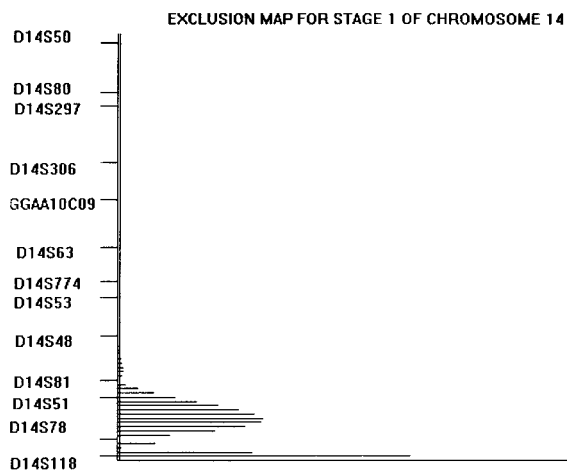


Figure 3.52: Exclusion map of chromosome 14 for stage 1.

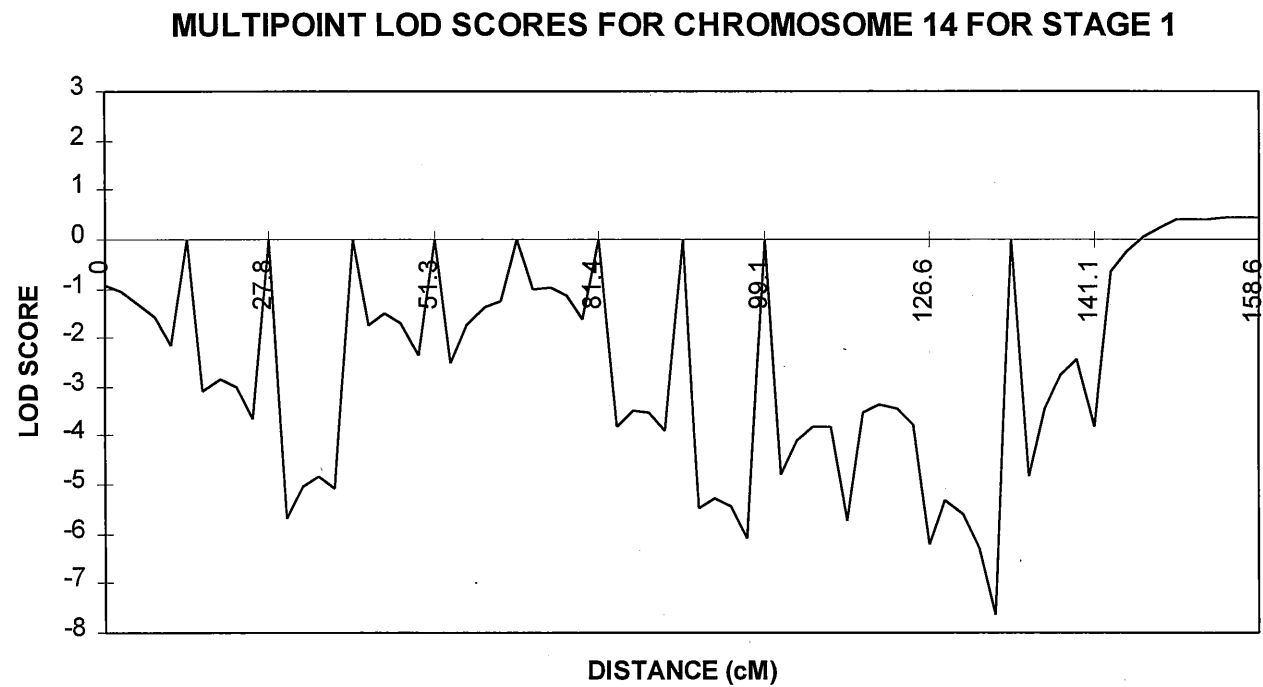


Figure 3.53: Graph of multipoint Lod scores on chromosome 14 for family 1 in stage 1.

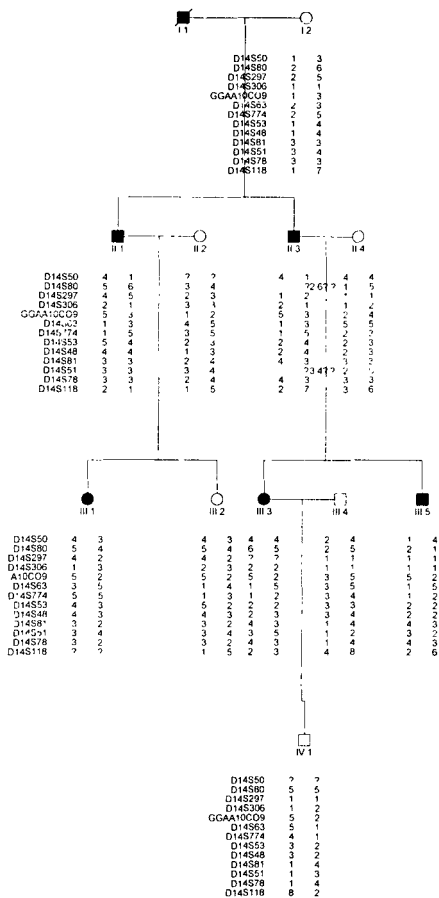


Figure 3.54: Haplotype of family I for markers used to analyse chromosome 14 in stage I.

### 3.14.2 Stage 2

In stage 2, six markers were added to the region near D14S118 to create a higher density map. The second pedigree was also added to the study in order to increase the sample size. All markers used to analyse chromosome 14 and the respective two point Lod scores are listed in table 3.24.

Of the six markers added, four (D14S265, D14S62, D14S65 and D14S267) fell within the D14S81-D14S78 interval. These markers gave negative two-point Lod scores below -2.00 thus suggesting exclusion of the interval.

The other two markers, D14S272 at 14q32.32 and D14S260 at 14q32.33, respectively at 1.2 and 3.4 cM telomeric of D14S118, gave positive two-point Lod scores of 1.3 and 0.7 respectively thus confirming 14qter as a possible PKD3 candidate region.

Haplotype analysis for family 1 reveals a recombination in individual II2 for marker D14S78 thus defining a possible conserved region at 14qter. Multi point analysis on the family 1 shows that the region between D14S81 and D14S267 has been excluded. The Lod scores for this region ranged from -1.51 to -8.06. Thus confirming the findings of the two-point analysis that the four additional markers that fell between D14S81 and D14S78 can be excluded for the first family. A positive region for family 1 was defined however between markers D14S118 and D14S260 where the Lod scores ranged from 1.21 to 1.47 with the



maximum Lod score of 1.47 being at D14S260. D14S118, which gave the maximum Lod score of 1.46 in stage 1 also produced this result in stage 2.

Haplotype analysis on family 2 shows that for the interval between D14S265 and D14S267 the affected cousins, individuals III2 and III4, have inherited different haplotypes. Furthermore, the affected siblings, individuals IV2 and IV3, have also inherited different haplotypes thus excluding the gene from this region for family 2. A recombination between D14S272 and D14S260 for individual IV3 places the gene above D14S260, thus marker D14S272 is the only marker that shows a common inheritance among affected individuals.

Multipoint analysis on family 2 supports the findings of the haplotype that the gene is not in this region for family 2. Negative Lod scores persist throughout the region. For the region between D14S265 and D14S78 the Lod scores are deeply negative, ranging from -2.00 to -5.00 thus excluding this region. Lod scores of -1.8 to -1.9 almost excludes the region between D14S118 and D14S260. The negative Lod scores obtained through multipoint analysis indicate that the region on chromosome 14 can be excluded for family 2.

The Lod scores for this region on chromosome 14 should not be pooled since the region has already been excluded for family 2.

Table 3.24: Lod scores for markers on chromosome 14 used to analyse family 1 in stage 2.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D14S50	-99 999	-1 27	-0 746	-0 473	-0 303	-0 189	-0 112	-0 06	-0 025	-0.006	0
15	D14S80	-99 999	-2 163	-1 331	-0.877	-0.581	-0 375	-0 227	-0 123	-0 053	-0.013	0
4	D14S297	-99 999	-1 049	-0.529	-0 263	-0 104	-0 007	0 05	0 073	0 072	0.047	0
16	D14S306	-99 999	-2 573	-1 72	-1 242	-0.917	-0 675	-0 486	-0 331	-0 203	-0.094	0
12	GGAA10C09	-99 999	0 024	0 221	0 291	0 308	0 294	0 26	0 212	0 152	0 081	0
14	D14S63	-99 999	-1 255	-0 733	-0 462	-0 294	-0 183	-0 107	-0 057	-0 024	-0.006	0
11	D14S774	-99 999	-1 255	-0 733	-0 462	-0 294	-0 183	-0 107	-0 057	-0 024	-0.006	0
5	D14S53	-99 999	-2.163	-1 331	-0.877	-0.581	-0 375	-0 227	-0 123	-0.053	-0.013	0
11	D14S48	-3 097	-0 163	0 067	0 169	0 214	0 227	0 217	0 187	0 141	0 078	0
13	D14S81	-3 097	-0 163	0 067	0 169	0 214	0 227	0 217	0 187	0 141	0 078	0
3 9	D14S265	-99 999	-3 345	-3 239	-2 384	-1.785	-1 329	-0 963	-0 66	-0 405	-0 187	0
12	D14S51	0 757	-0 678	0 598	0 518	0 437	0 357	0 279	0 204	0 132	0 064	0
1 1	D14S62	-99 999	0 348	0 221	0 291	0 308	0 294	0 26	0 212	0 152	0 081	0
2 3	D14S65	-99 999	0 513	0 598	0 517	0 437	0 357	0 279	0 204	0 132	0 064	0
4 7	D14S267	-99 999	0 43	0 249	0 315	0 326	0 308	0 269	0 218	0 155	0 082	0
3 6	D14S78	-3 097	-0.163	0 067	0 169	0 214	0 227	0 217	0 187	0 141	0 078	0
7	D14S118	1 156	1 051	0 943	0 833	0 721	0 606	0 49	0 372	0 251	0 128	0
12	D14S272	1 448	1 386	1.142	1 017	0 889	0 756	0 618	0 475	0 325	0 168	0
2 2	D14S260	1 067	0 795	0.573	0.495	0 417	0 339	0 265	0 193	0 124	0.059	0

Table 3.25: Lod scores for markers on chromosome 14 used to analyse family 2 in stage 2.

<b>DISTANCE (cM)</b>	<b>MARKER</b>	<b>0</b>	<b>0.05</b>	<b>0.1</b>	<b>0.15</b>	<b>0.2</b>	<b>0.25</b>	<b>0.3</b>	<b>0.35</b>	<b>0.4</b>	<b>0.45</b>	<b>0.5</b>
	D14S265	-0.484	-0.578	-0.632	-0.413	-0.27	-0.171	-0.102	-0.054	-0.023	-0.006	0
2.3	D14S62	-0.934	-0.774	-0.555	-0.364	-0.238	-0.151	-0.089	-0.048	-0.02	-0.005	0
2.3	D14S65	-0.934	-0.778	-0.732	-0.465	-0.3	-0.192	-0.118	-0.067	-0.032	-0.01	0
4.7	D14S267	-1.568	-1.328	-1.035	-0.707	-0.489	-0.334	-0.22	-0.135	-0.073	-0.028	0
11.8	D14S272	-0.106	-0.121	-0.022	-0.018	-0.015	-0.012	-0.008	-0.005	-0.002	-0.001	0
2.2	D14S260	-0.322	-0.227	-0.204	-0.106	-0.056	-0.029	-0.015	-0.007	-0.003	-0.001	0

Table 3.26: Table showing the two-point Lod scores for family 1 and family 2 combined for all markers used to analyse chromosome 14 in the second stage of the study.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D14S50	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.06	-0.025	-0.006	0
15	D14S80	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
4	D14S297	-99.999	-1.049	-0.529	-0.263	-0.104	-0.007	0.05	0.073	0.072	0.047	0
16	D14S306	-99.999	-2.573	-1.72	-1.242	-0.917	-0.675	-0.486	-0.331	-0.203	-0.094	0
12	GGAA10C09	-99.999	0.024	0.221	0.291	0.308	0.294	0.26	0.212	0.152	0.081	0
14	D14S63	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
11	D14S774	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
5	D14S53	-99.999	-2.163	-1.331	-0.877	-0.581	-0.375	-0.227	-0.123	-0.053	-0.013	0
11	D14S48	-3.097	-0.163	0.067	0.169	0.214	0.227	0.217	0.187	0.141	0.078	0
13	D14S81	-3.097	-0.163	0.067	0.169	0.214	0.227	0.217	0.187	0.141	0.078	0
3.9	D14S265	-99.999	-3.923	-3.871	-2.797	-2.055	-1.5	-1.065	-0.714	-0.428	-0.193	0
1.2	D14S51	0.757	-0.678	0.598	0.518	0.437	0.357	0.279	0.204	0.132	0.064	0
1.1	D14S62	-99.999	-0.4265	-0.333	-0.072	0.069	0.1432	0.1706	0.1644	0.1317	0.0764	0
2.3	D14S65	-99.999	-0.2653	-0.1335	0.052	0.1362	0.1652	0.1615	0.1371	0.099	0.053	0
4.7	D14S267	-99.999	-0.8977	-0.785	-0.392	-0.1628	-0.026	0.049	0.082	0.081	0.053	0
3.6	D14S78	-3.097	-0.163	0.067	0.169	0.214	0.227	0.217	0.187	0.141	0.078	0
7	D14S118	1.156	1.051	0.943	0.833	0.721	0.606	0.49	0.372	0.251	0.128	0
1.2	D14S272	1.342	1.265	1.119	0.998	0.873	0.744	0.609	0.469	0.322	0.167	0
2.2	D14S260	0.745	0.568	0.368	0.388	0.36	0.31	0.249	0.185	0.121	0.059	0

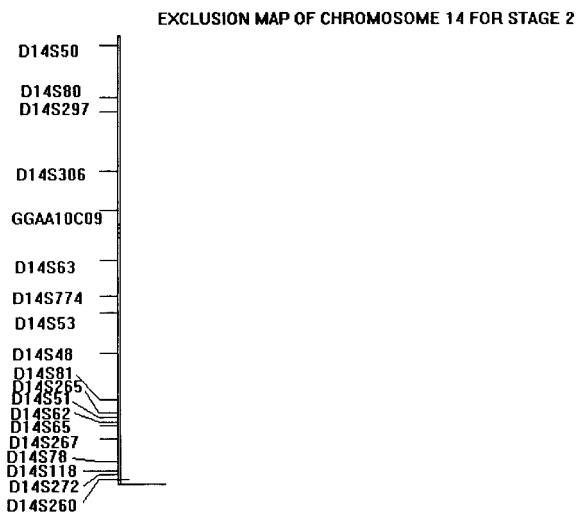


Figure 3.55: Exclusion map of chromosome 14 for stage 2.

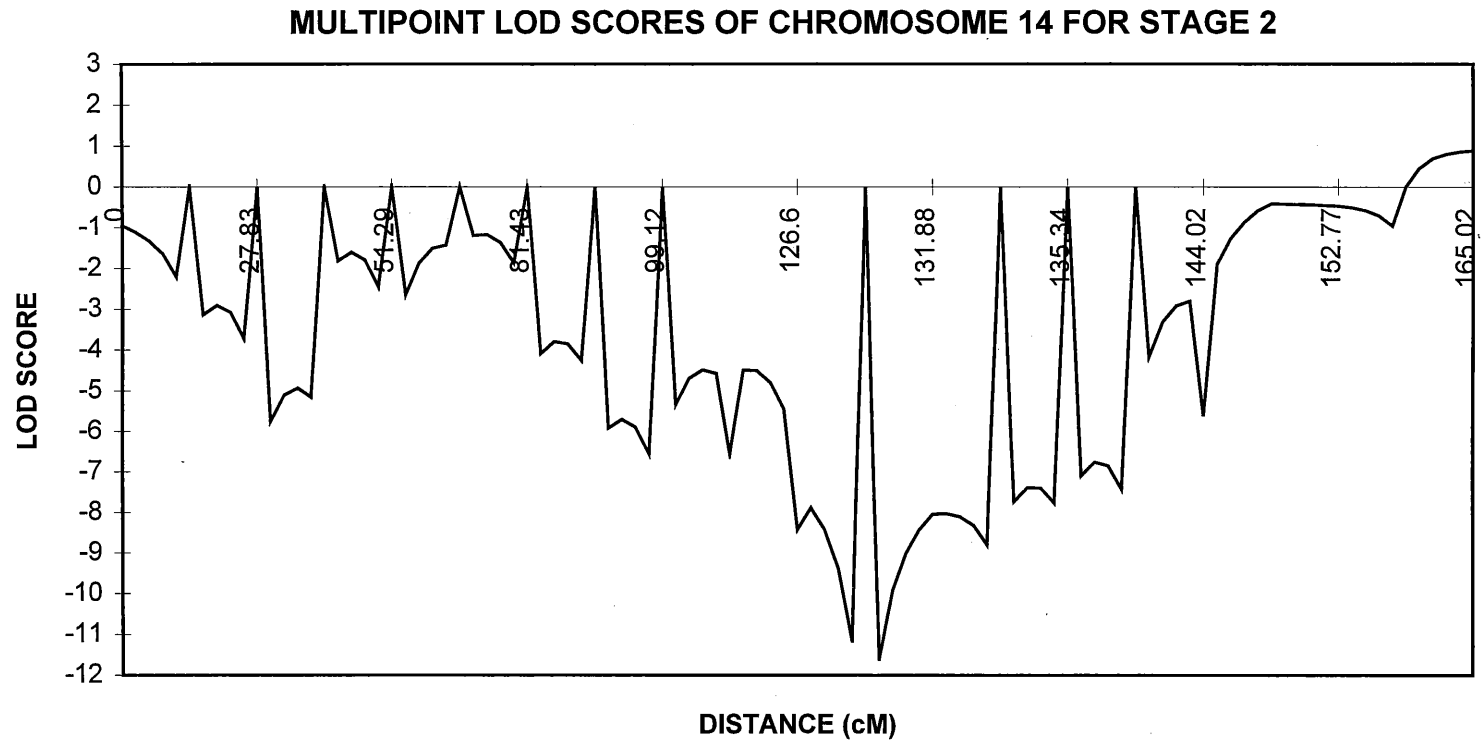


Figure 3.56: Graph of multipoint Lod scores for family 1 and family 2 combined for chromosome 14.

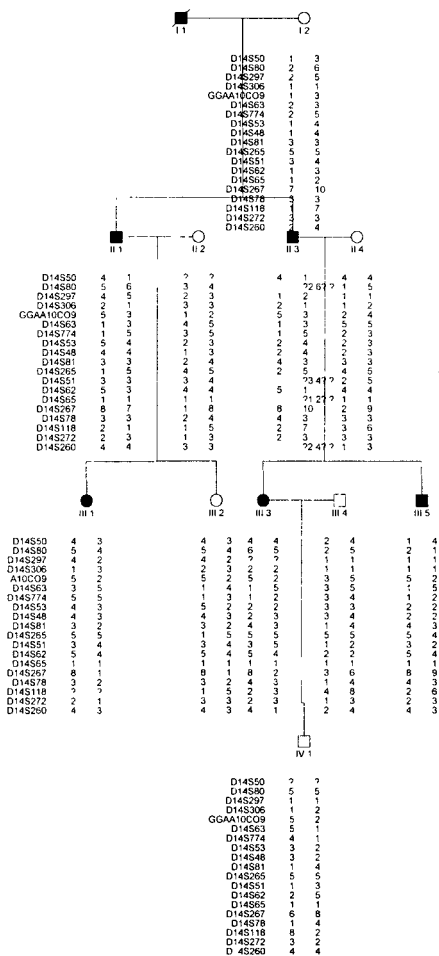


Figure 3.57: Haplotype of family 1 for all markers used to analyse chromosome 14 in stage 2 of the study.

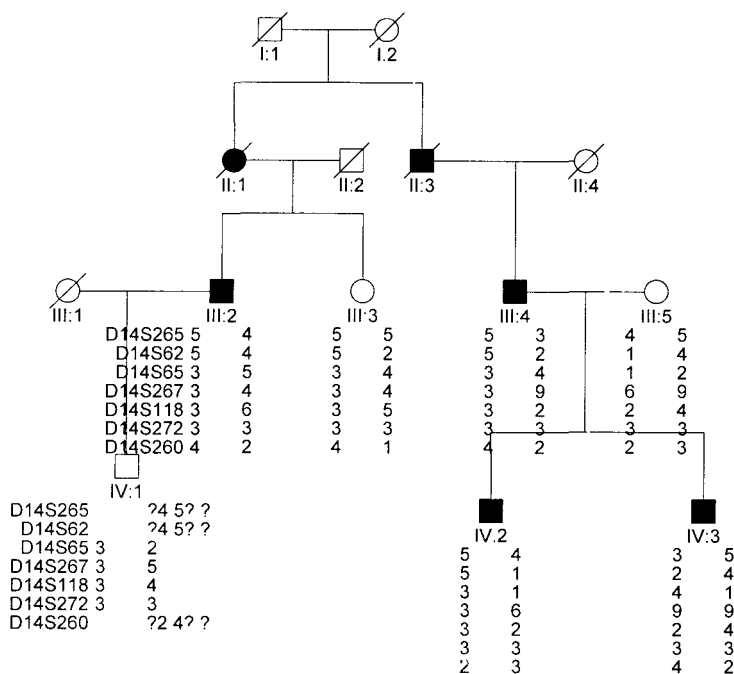


Figure 3.58. Haplotype of pedigree 2 for chromosome 14 for the markers added in stage 2



### **3.15 Chromosome 15**

Chromosome 15 was analysed using a total of 11 markers spaced approximately 9cM apart.

Two-point Lod score analysis demonstrated Lod score values below -2 for all chromosome 15 markers at recombination fraction = 0 (table 3.27). However, slightly positive two-point Lod scores were obtained for the first two markers, D15S122 and D15S165 on 15q11.1 and 15q13.3 respectively at higher recombination distances. The exclusion map in figure 3.59 shows that most of chromosome 15 can be excluded, with the exception of a moderate peak of non-exclusion defined by D15S122 and D15S165.

Multipoint linkage analysis confirms these findings: a large proportion of the chromosome is definitely excluded (figure 3.60), with Lod scores below -2. The region between D15S122 and D15S165 is negative on multipoint analysis, with Lod scores between these markers ranging from -0.5 to -0.8. Haplotype examination (figure 3.61) suggests the inheritance of different haplotypes by the two affected individuals in generation II from their affected father. Together, these findings suggest that even though this interval cannot be definitely excluded, it is unlikely to contain PKD3.

Table 3.27: Showing the two-point Lod scores for markers used to analyse chromosome 15.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D15S122	-2.8	-0.18	0.03	0.12	0.15	0.15	0.14	0.11	0.08	0.04	0
13.3	D15S165	-2.8	-0.16	0.07	0.17	0.22	0.23	0.22	0.19	0.14	0.1	0
25.2	D15S126	-2.62	-2.39	-1.79	-1.32	-0.98	-0.72	-0.52	-0.35	-0.21	-0.17	0
5.8	CYP19	-2.59	-0.27	-0.05	0.05	0.1	0.11	0.11	0.1	0.07	0.03	0
5	D15S117	-2.62	-1.77	-1.24	-0.93	-0.71	-0.54	-0.4	-0.29	-0.18	-0.09	0
4.3	D15S153	-2.62	-1.44	-0.92	-0.64	-0.46	-0.33	-0.23	-0.16	-0.1	-0.06	0
2.7	D15S131	-2.92	-0.46	-0.23	-0.12	-0.06	-0.03	-0.01	0	0	0	0
5.7	D15S175	-2.7	-0.46	-0.23	-0.12	-0.06	-0.03	-0.01	0	0	0	0
3.3	D15S116	-2.8	-2.29	-1.56	-1.1	-0.78	-0.55	-0.37	-0.24	-0.13	-0.05	0
11.1	D15S130	-2.62	-2.39	-1.79	-1.32	-0.98	-0.72	-0.52	-0.35	-0.21	-0.09	0
11	D15S120	-2.8	-1.5	-0.97	-0.68	-0.49	-0.35	-0.25	-0.17	-0.1	-0.06	0

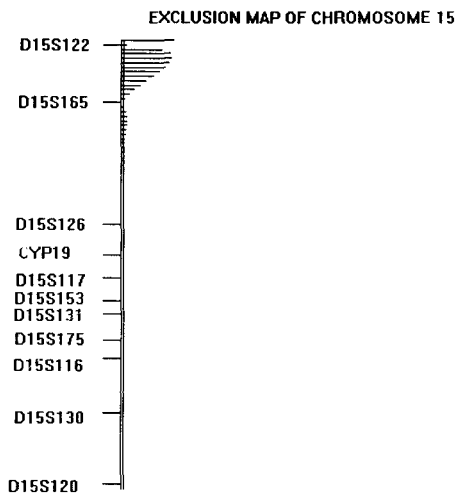


Figure 3.59: Exclusion map of chromosome 15.

## MULTIPOINT LOD SCORES FOR CHROMOSOME 15

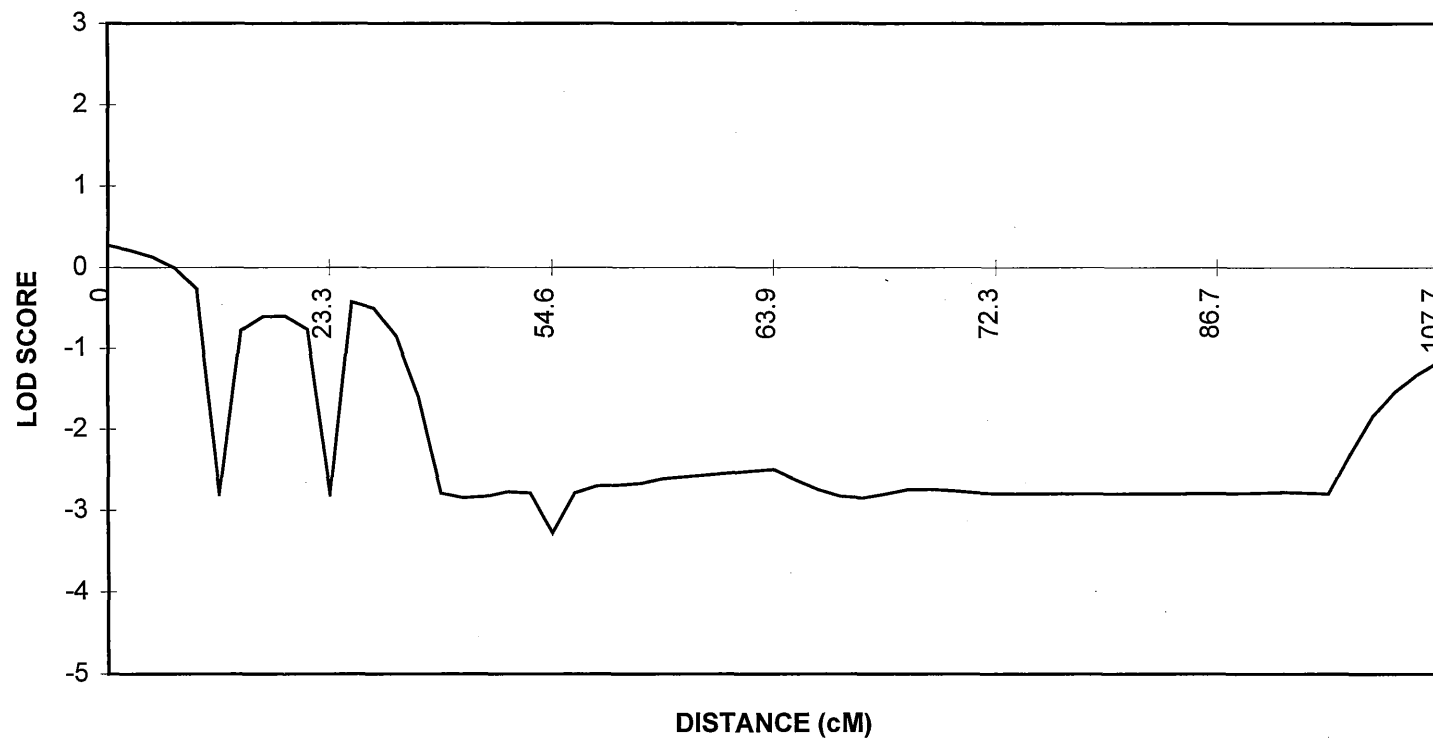


Figure 3.60: Graph of multipoint Lod scores for chromosome 15.

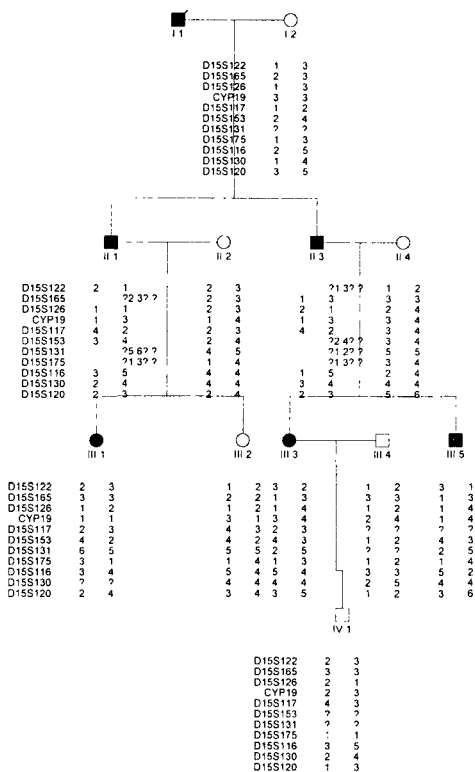


Figure 3.61: Haplotype of family 1 for chromosome 15.

### **3.16 Chromosome 16**

Chromosome 16 was analysed using a total of 7 markers spaced at approximately 18cM. These markers and the corresponding two-point Lod scores are listed in table 3.28.

Two markers on chromosome 16, D16S405 at 16p13.13 and D16S401 at 16p11.2 gave moderately positive Lod scores of 0.14 and 0.65 respectively. D16S420 at 16p11.2, the marker situated between the above two markers gave a negative two-point Lod score of -3.00 at recombination distance = 0 and moderately negative two-point Lod scores at higher values of the recombination fraction. The distance between D16S420 and D16S401 is less than 5 cM, however that between D16S420 and D16S405 is close to 17 cM.

Figure 3.62 presents the exclusion map for chromosome 16. The peak around D16S405 indicates the area of non-exclusion due to the reasons discussed in the previous paragraph. In addition, a large peak appears in the interval D16S415-D16S520 at positions 16q13 and 16q24.3. Even though two-point Lod scores less than -2 were obtained for both D16S415 and D16S520, the large distance between these two markers (56cM) does not allow the exclusion of the interval.

The results from the multipoint linkage analysis are shown in figure 3.63. The Lod scores around markers D16S405 and D16S401 became negative, at -0.6 and -0.07 respectively. The inconclusive values for both markers are partly due to incomplete genotyping data and partly to the low informativeness of the markers involved (which also precludes haplotype

examination). The multipoint values obtained for the region between markers D16S415 and D16S520 ranged between -0.6 and -1.5. Thus a substantial proportion of chromosome 16 has been ruled out as the location of PKD3, however several large regions remain which have not been definitely excluded even though our data suggests that they are unlikely to contain the PKD3 gene.

Table 3.28: This table shows the two point Lod scores for all the markers used to analyse chromosome 16.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D16D423	-2.8	-1.52	-0.99	-0.7	-0.5	-0.36	-0.26	-0.17	-0.1	-0.03	0
17.8	D16S405	0.14	0.11	0.08	0.05	0.03	0.01	0	-0.01	-0.01	-0.02	0
16.7	D16S420	-3	-0.72	-0.44	-0.29	-0.19	-0.12	-0.08	-0.04	-0.02	-0.01	0
4.8	D16S401	0.73	0.63	0.53	0.43	0.34	0.25	0.17	0.1	0.05	0.02	0
12.8	D16S411	-2.85	-1.59	-1.05	-0.75	-0.54	-0.39	-0.27	-0.18	-0.11	-0.06	0
11.5	D16S410	-2.98	-0.72	-0.44	-0.29	-0.19	-0.12	-0.08	-0.04	-0.02	-0.01	0
56	D16S520	-3.1	-2.35	-1.57	-1.1	-0.78	-0.55	-0.37	-0.24	-0.13	-0.08	0



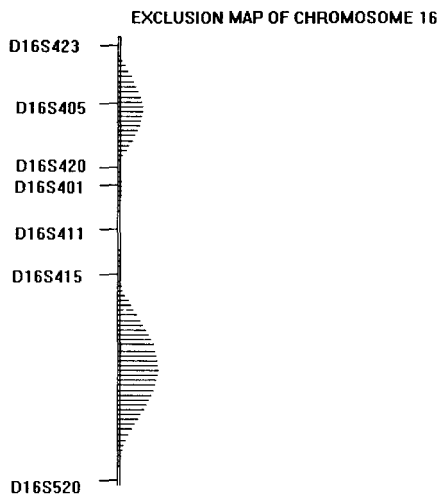


Figure 3.62: Exclusion map of chromosome 16.

MULTIPOINT LOD SCORES FOR CHROMOSOME 16

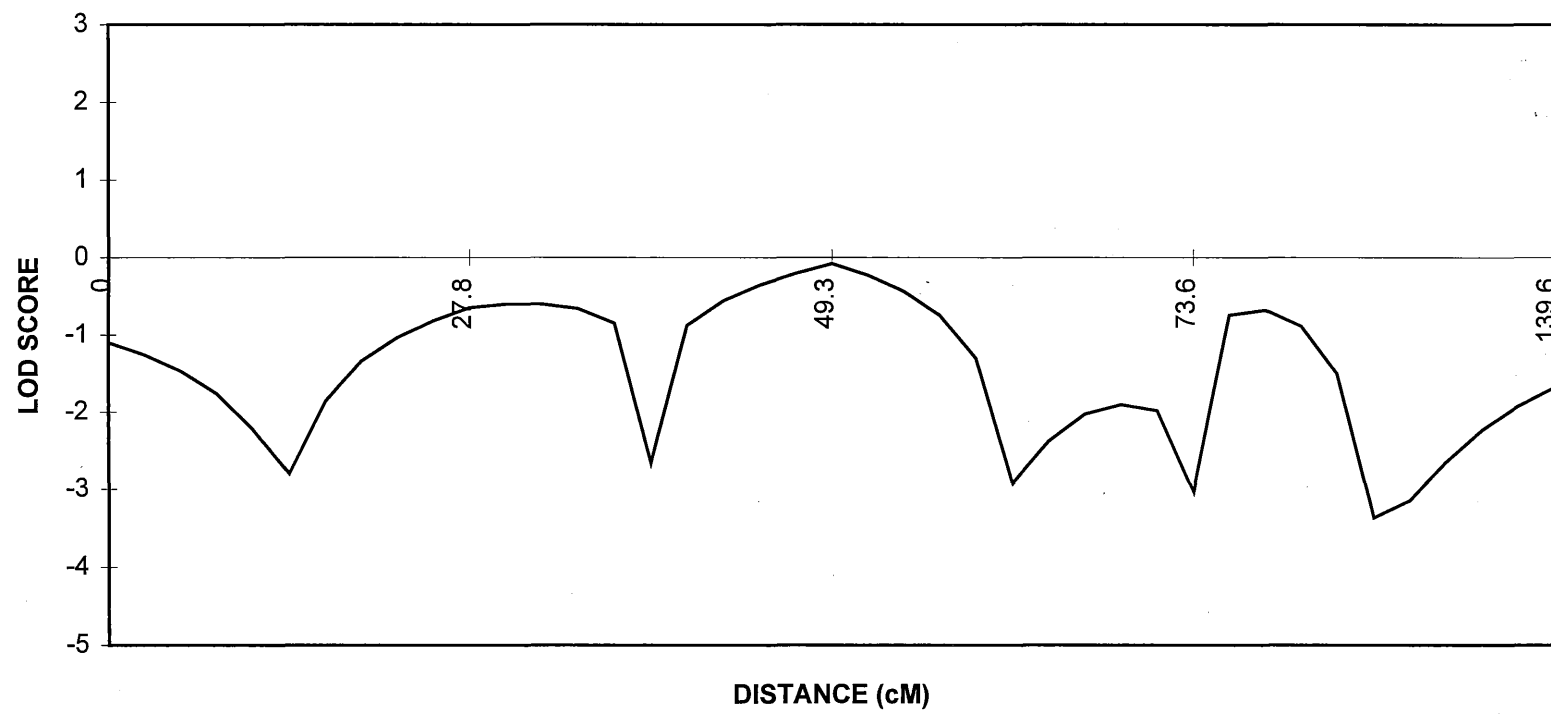


Figure 3.63: Graph of multipoint Lod scores for chromosome 16.

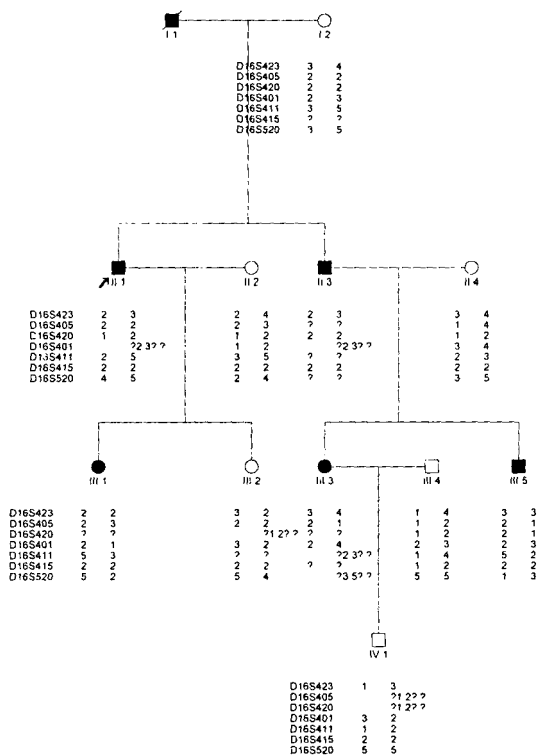


Figure 3.64: haplotype of family 1 for chromosome 16.

### **3.17 Chromosome 17**

#### **3.17.1 Stage 1**

Stage 1 of the analysis of chromosome 17 was carried out using a total of 9 markers spaced approximately 16cM apart.

Deeply negative two-point Lod scores were obtained for five of the markers at recombination fraction zero but at higher recombination fraction values most markers produced inconclusive results.

Positive two-point Lod score values were obtained for three markers: D17S849 (17p13.3) and the adjacent D17S938 at 17p13.2 (at a distance of 9 cM) showed Lod scores of 0.124 and 1.156 respectively at  $\theta = 0$ . Further centromeric on 17p, positive findings were obtained for marker D17S799 (position 17p12), with a Lod score of 0.456.

The combination of inconclusive two-point Lod scores for some markers, positive findings for other markers and some large intervals (24 cM between D17S122 and D17S579 and 43 cM between D17S579 and D17S785) resulted in the map shown in figure 3.65. As can be seen from the map, the entire length of chromosome 17 could not be excluded on the basis of our results.

The scoring of haplotypes for this region does suggest sharing between affected members of family I, however no firm conclusions can be drawn because of the missing genotyping results in individuals I.2, II.1 and III.4.

Multipoint analysis carried out on chromosome 17 confirmed the two-point Lod score at D17S938 which now increased slightly to 1.2. The

multipoint score for the immediately telomeric marker D17S849 increased substantially from 0.12 to 1.08. The negative two-point Lod score obtained for D17S945 at 17p13.1, became positive on multipoint analysis and increased to 1.2 thus defining as positive the region between D17S849 and D17S945 (spanning an overall distance of 16cM). The marginally positive two-point Lod score obtained for D17S799 became slightly negative (-0.08) after multipoint linkage analysis was carried out.

Thus from stage 1 it can be seen that a region exists where positive Lod scores ranging from 1.08 to 1.20 have been obtained. This region should be investigated further in stage 2 with the addition of extra markers and the second non PKD1/PKD2 family.

Table 3.29: Two point Lod scores for family 1 for the markers used to analysed chromosome 17 in stage 1 of the study.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D17S578	-99.999	0.008	-0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
21	D17S849	0.124	0.097	0.078	0.054	0.039	0.026	0.016	0.009	0.004	0.001	0
9	D17S938	1.156	0.945	0.812	0.638	0.478	0.337	0.218	0.126	0.061	0.021	0
7	D17S945	-0.824	-0.576	-0.316	-0.187	-0.111	-0.064	-0.034	-0.017	-0.006	-0.001	0
7	D17S796	-2.495	-0.394	-0.577	-0.629	-0.623	-0.579	-0.509	-0.415	-0.299	-0.161	0
19	D17S799	0.456	0.387	0.32	0.256	0.196	0.141	0.093	0.054	0.024	0.006	0
13	D17S122	-99.999	-1.184	-0.672	-0.411	-0.254	-0.153	-0.087	-0.044	-0.018	-0.004	0
24	D17S579	-3.875	-0.897	-0.619	-0.465	-0.361	-0.283	-0.219	-0.163	-0.11	-0.056	0
43	D17S785	-2.796	0.094	0.282	0.342	0.348	0.324	0.281	0.224	0.158	0.083	0

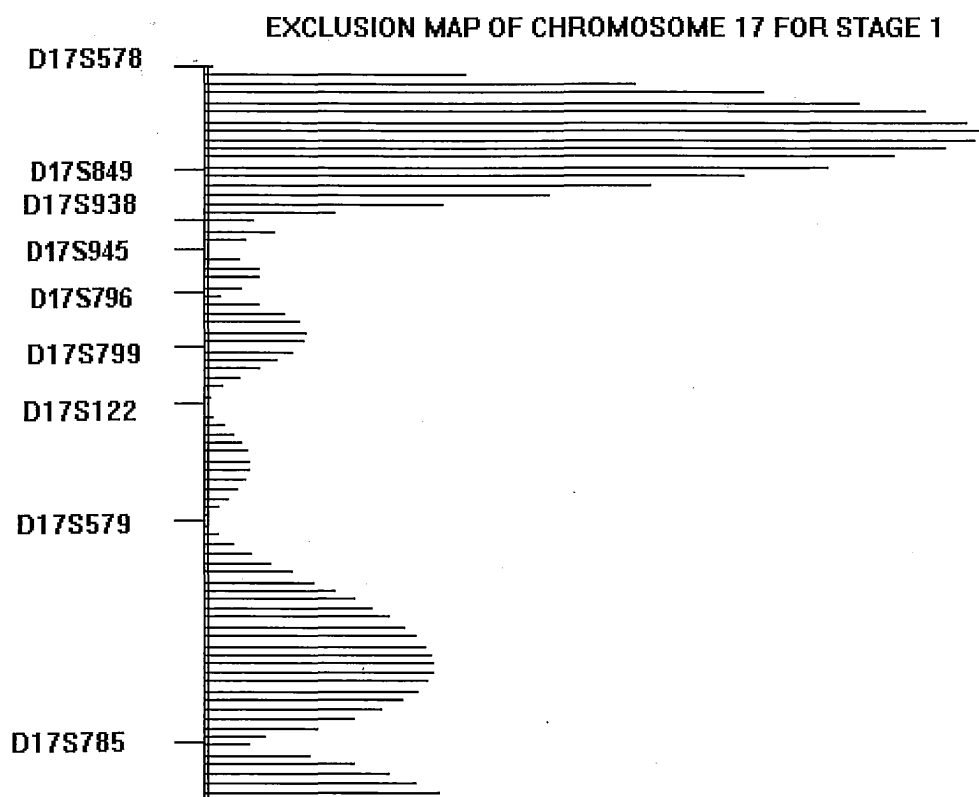


Figure 3.65: Exclusion map of chromosome 17 for stage 1.

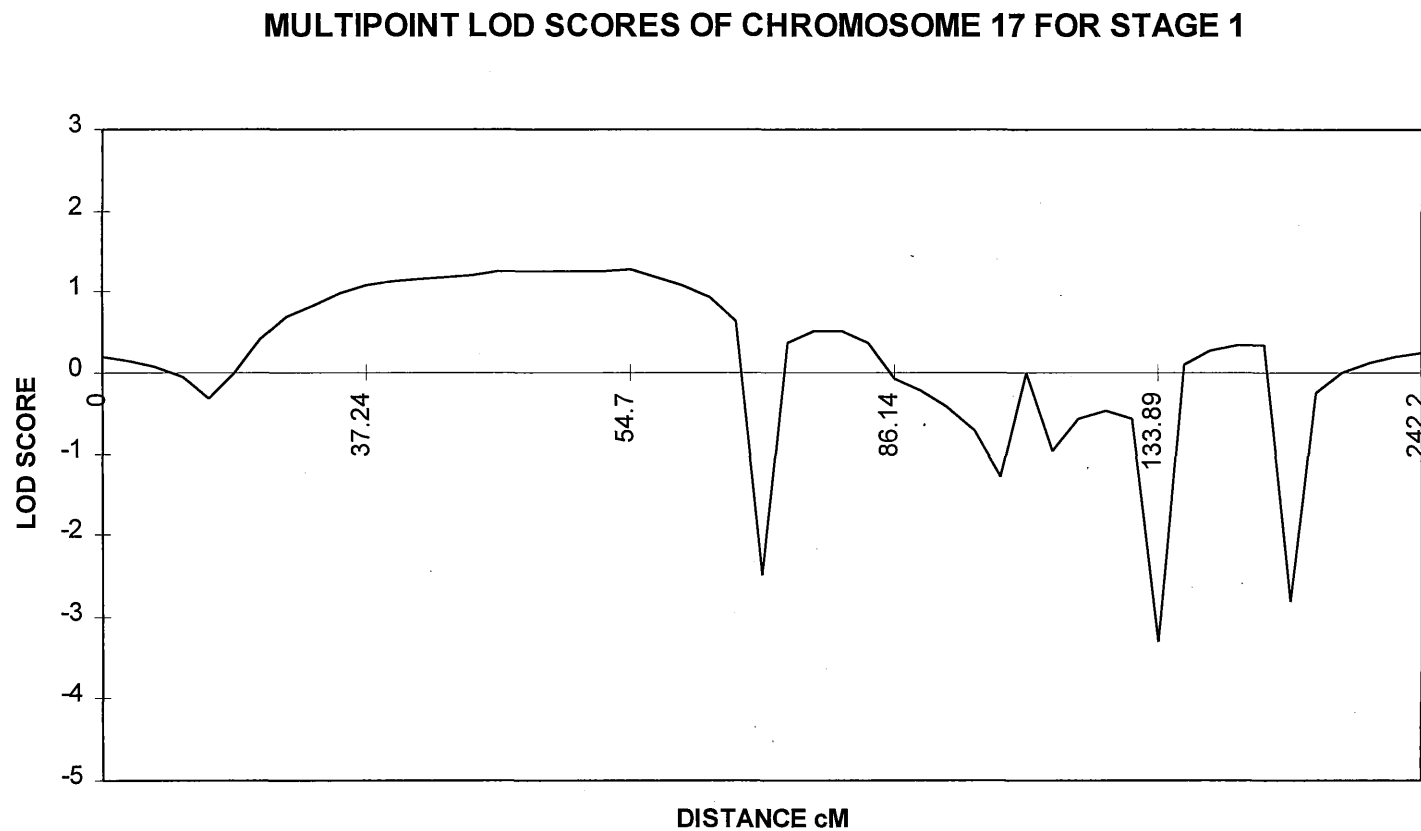


Figure 3.66: Graph of multipoint Lod scores for chromosome 17 for family 1.



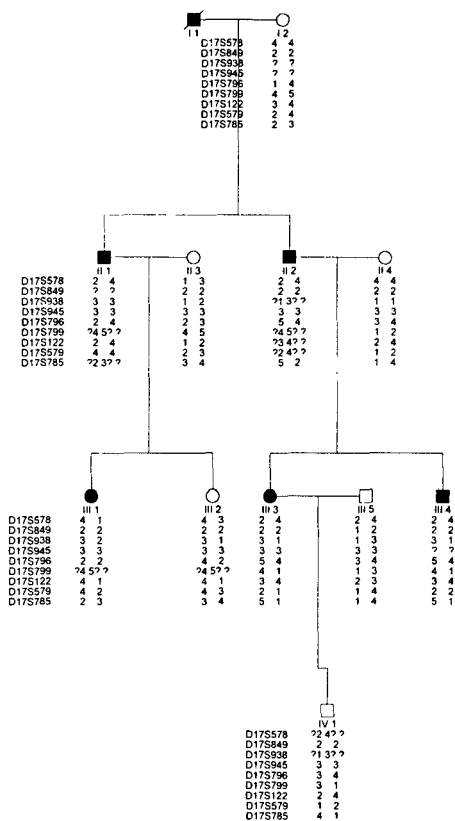


Figure 3.67: Haplotype of family 1 for chromosome 17 for stage 1 of the study.

### 3.17.2 Stage 2

Two markers were added to chromosome 17 in stage 2 of the study.

The first marker D17S1845 at 17p13.3, was between D17S849 and D17S938 to verify the positive Lod scores obtained in this region. The second marker, D17S1838 at 17q21.32, was between D17S579 at 17q21.1 and D17S785 at 17q24.3 to reduce the large distance of 43cM between these markers. The second non PKD1/PKD2 Bulgarian family was also added to the study.

The two point Lod score for D17S1845 placed between D17S849 and D17S938 was very negative (-2.533 at  $\theta = 0.05$ ). The Lod score for D17S1838 placed between D17S579 and D17S785 was also very negative with a result of -2.796 at  $\theta = 0$ . The two-point Lod scores suggest that from D17S122 to D17S785 can be excluded.

Haplotype analysis on family 1 shows a crossover occurring between markers D17S578 and D17S1845 at individual III1 individual III2. Allele sharing may exist for marker D17S945 however it is difficult to tell due to the lack of genotyping information at individual III4 and the fact that this marker is uninformative. Siblings III1 and III2 both inherited the same alleles although it cannot be determined which allele was inherited from which parent.

Haplotype analysis on family 2 shows that the affected cousins III2 and III4 inherit different haplotypes thus showing that this region can be excluded for family 2. Haplotype sharing is difficult to determine due to

the lack of informativeness of the markers and the missing genotyping information for the two affected siblings, individuals IV2 and IV3.

Multipoint Lod scores for family 1 from stage 1 of the study reduced with multipoint analysis and the addition of extra markers. Examining these multipoint Lod scores for family 1 it can be seen that a positive region does exist for this family between markers D17S938 and D17S796 and includes D17S945. However the Lod scores for this region range from 0.29 to 0.76 with the maximum Lod score being 0.76 at D17S945. Marker D17S796 produced a negative Lod score of -2.49 thus excluding this position.

Multipoint analysis on family 2 show that inconclusive Lod scores persist throughout the region due to the lack of informativeness of the markers for this family. Slightly positive Lod scores ranging from 0.06 to 0.3 were obtained between markers D17S578 and D17S938. Marker D17S945 was the only marker to be excluded with a Lod score of -3.64. All Lod scores obtained from D17S796 to D17S785 were negative however, only slightly.

Examining the multipoint Lod scores for both families combined, the positive two-point Lod scores obtained reduced with multipoint linkage analysis and the addition of the extra markers. The maximum Lod score achieved at D17S938 reduced to 0.4 at the position of this marker. The marker D17S945 produced the highest multipoint Lod score of 0.76. D17S849 which also gave a slightly positive two-point Lod score of 0.124

in stage 1, now produced a negative multipoint Lod score of -2.3 thus excluding this marker.

Table 3.30: Table of two-point Lod scores for chromosome 17 for pedigree 1 in stage 2

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D17S578	-99.999	0.008	-0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
21	D17S849	0.124	0.097	0.078	0.054	0.039	0.026	0.016	0.009	0.004	0.001	0
4	D17S1845	-99.999	-2.676	-1.781	-1.298	-0.972	-0.712	-0.523	-0.346	-0.213	-0.094	0
5	D17S938	1.156	0.945	0.812	0.638	0.478	0.337	0.218	0.126	0.061	0.021	0
7	D17S945	-0.824	-0.576	-0.316	-0.187	-0.111	-0.064	-0.034	-0.017	-0.006	-0.001	0
7	D17S796	-2.495	-0.394	-0.577	-0.629	-0.623	-0.579	-0.509	-0.415	-0.299	-0.161	0
19	D17S799	0.456	0.387	0.32	0.256	0.196	0.141	0.093	0.054	0.024	0.006	0
13	D17S122	-99.999	-1.184	-0.672	-0.411	-0.254	-0.153	-0.087	-0.044	-0.018	-0.004	0
24	D17S579	-3.875	-0.897	-0.619	-0.465	-0.361	-0.283	-0.219	-0.163	-0.11	-0.056	0
27.6	D17S1838	-2.92	-1.251	-0.707	-0.407	-0.222	-0.099	-0.021	0.041	0.048	0.033	0
15.4	D17S785	-2.796	0.094	0.282	0.342	0.348	0.324	0.281	0.224	0.158	0.083	0

Table 3.31: Table of two-point Lod scores for chromosome 17 for pedigree 2 in stage 2

	THETA VALUES										
MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
D17S1845	0.186	0.143	0.094	0.083	0.076	0.052	0.048	0.021	0.013	0.001	0
D17S1838	0.124	0.097	0.078	0.054	0.039	0.026	0.016	0.009	0.004	0.001	0

Table 3.32: Two point Lod scores for family 1 and family 2 for all markers used to analyse chromosome 17 in stage 2 of the study.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D17S578	-99.999	0.008	-0.208	0.28	0.299	0.288	0.256	0.21	0.151	0.081	0
21	D17S849	0.124	0.097	0.078	0.054	0.039	0.026	0.016	0.009	0.004	0.001	0
4	<b>D17S1845</b>	-99.999	-2.533	-1.687	-1.215	-0.896	-0.66	-0.475	-0.325	-0.2	-0.093	0
5	D17S938	1.156	0.945	0.812	0.638	0.478	0.337	0.218	0.126	0.061	0.021	0
7	D17S945	-0.824	-0.576	-0.316	-0.187	-0.111	-0.064	-0.034	-0.017	-0.006	-0.001	0
7	D17S796	-2.495	-0.394	-0.577	-0.629	-0.623	-0.579	-0.509	-0.415	-0.299	-0.161	0
19	D17S799	0.456	0.387	0.32	0.256	0.196	0.141	0.093	0.054	0.024	0.006	0
13	D17S122	-99.999	-1.184	-0.672	-0.411	-0.254	-0.153	-0.087	-0.044	-0.018	-0.004	0
24	D17S579	-3.875	-0.897	-0.619	-0.465	-0.361	-0.283	-0.219	-0.163	-0.11	-0.056	0
27.6	<b>D17S1838</b>	-2.796	-1.154	-0.629	-0.353	-0.183	-0.073	-0.005	0.032	0.044	0.032	0
15.4	D17S785	-2.796	0.094	0.282	0.342	0.348	0.324	0.281	0.224	0.158	0.083	0

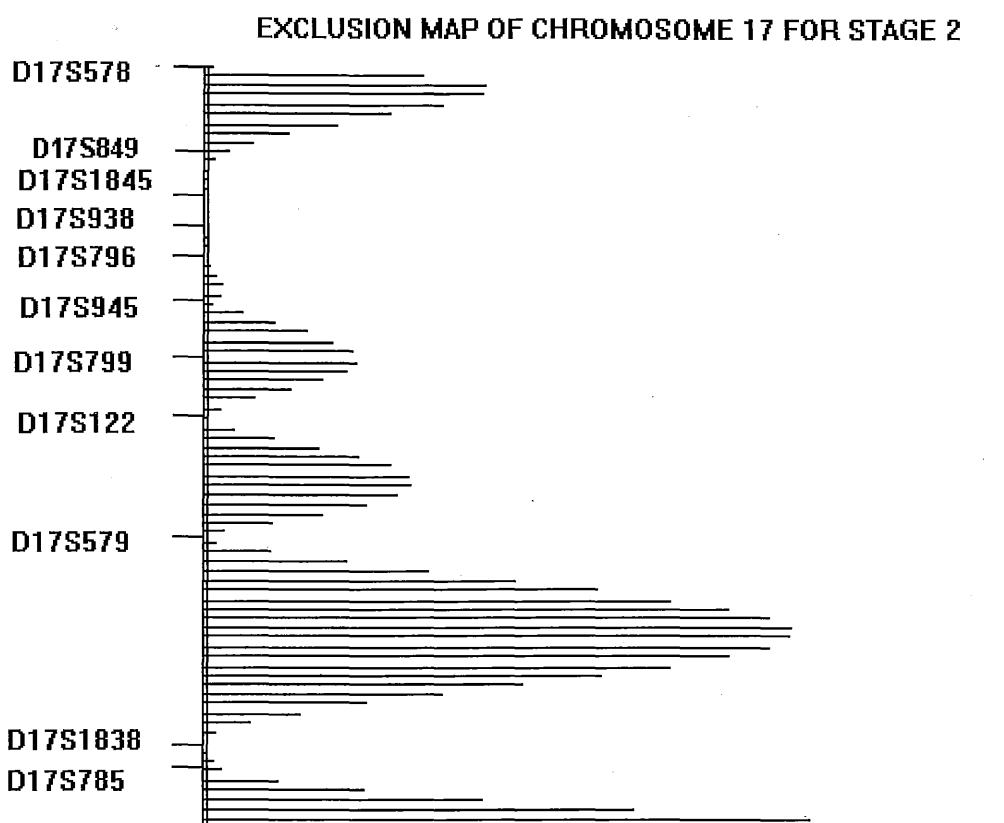


Figure 3.68: Exclusion map of chromosome 17 for stage 2.



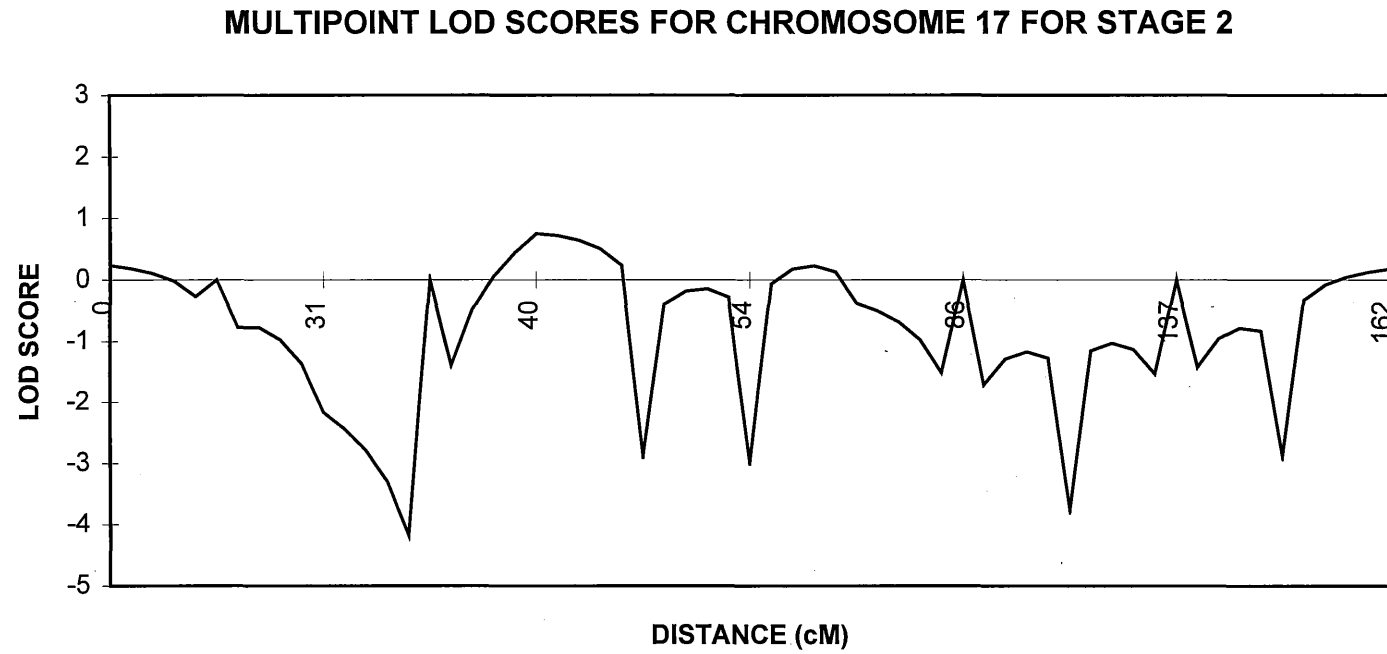


Figure 3.69: Graph of multipoint Lod score for chromosome 17 for family 1 and family 2 combined.

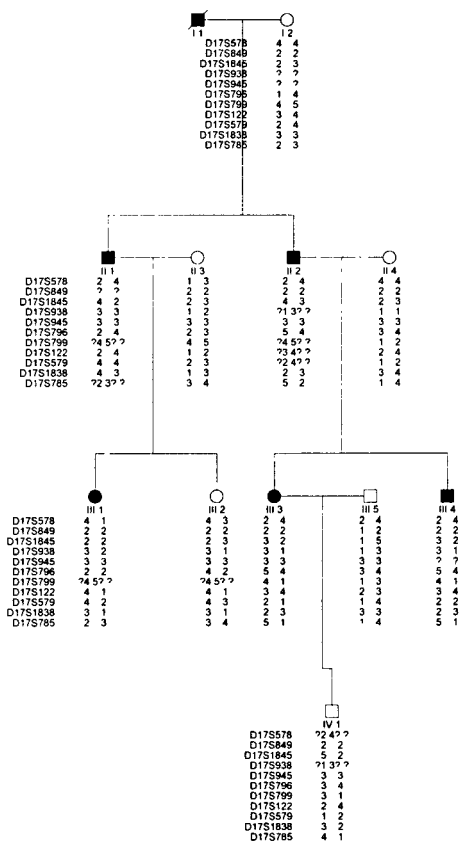


Figure 3.70: Haplotype of family 1 for all markers used to analyse chromosome 17 in stage 2.

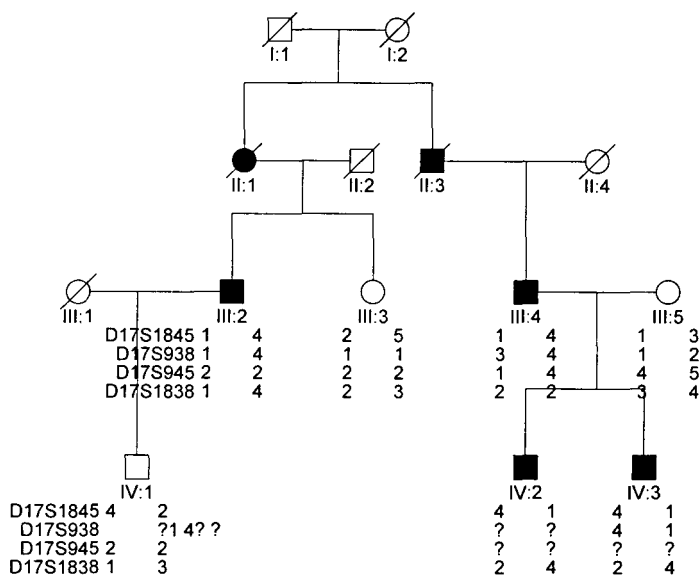


Figure 3.71: Haplotype of pedigree 2 for markers on chromosome 17 added in stage 2.

### **3.18 Chromosome 18**

Chromosome 18 was analysed using 8 markers spaced approximately 20cM apart.

The two-point Lod scores for these markers are listed in table 3.33. Six of the markers gave deeply negative results. Two markers, namely D18S63 18p11.32 and D18S363 at 18q21.1 gave marginally positive Lod scores of 0.222 and 0.236 respectively.

The exclusion map in figure 3.72 shows two small peaks around D18S64, the first resulting from the positive two-point Lod score at the flanking centromeric marker D18S363 and the 17 cM distance between the two markers and the second resulting from the low positive two-point Lod scores obtained for the telomeric flanking marker D18S543 (18q22.2) at higher recombination fraction values (0.1 and over), given the length of the interval bracketed by this marker and D18S64 (namely 15 cM). The largest peak of non-exclusion is telomeric to D18S543 and is due to the lack of markers in this large interval.

Multipoint analysis produced negative results for the region around D18S63, which gave a Lod score of -1.17. Negative findings, namely a Lod score of -0.7, were obtained for D18S363, the other marker which presented with a slightly positive two-point Lod score. The multipoint results for the region between D18S363 and D18S543 range between -1.00 and -2.00 thus placing this interval among the genomic regions which cannot be definitely excluded but which are highly unlikely to contain the disease gene.

Due to the large size of the interval, the area telomeric to D18S543 cannot be excluded, with multipoint Lod scores ranging from -0.2 to -0.9.

Table 3.33: Shows two-point Lod scores for markers used to analyse chromosome 18.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D18S59	-99.999	-0.885	-0.377	-0.124	0.021	0.102	0.141	0.146	0.123	0.074	0
6	D18S62	-3.699	-0.721	-0.443	-0.292	-0.194	-0.125	-0.076	-0.041	-0.018	-0.004	0
11	D18S63	0.222	0.187	0.154	0.123	0.093	0.067	0.044	0.025	0.011	0.003	0
10	D18S843	-99.999	-2.534	-1.687	-1.215	-0.896	-0.66	-0.475	-0.325	-0.2	-0.093	0
40	D18S535	-99.999	-1.27	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.006	0
9	D18S363	0.236	0.2	0.165	0.132	0.1	0.072	0.047	0.027	0.012	0.003	0
17	D18S64	-3.699	-0.721	-0.443	-0.292	-0.194	-0.125	-0.076	-0.041	-0.018	-0.004	0
15	D18S543	-99.999	-0.015	0.188	0.264	0.287	0.279	0.25	0.206	0.149	0.081	0

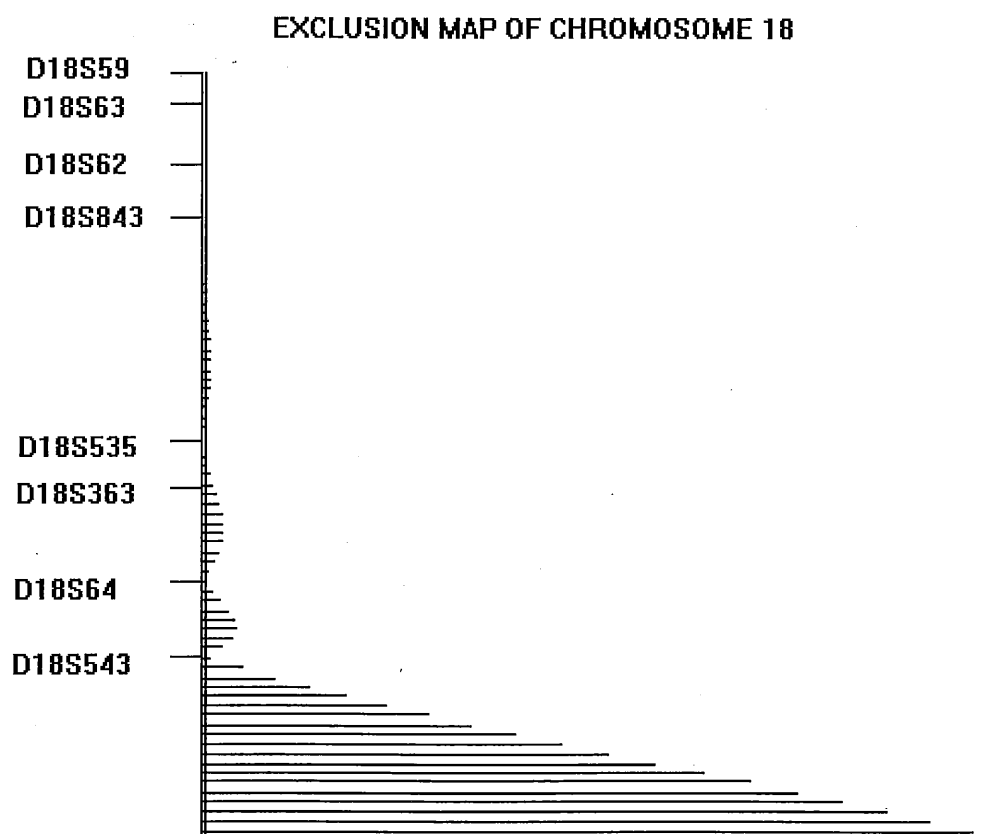


Figure 3.72: Exclusion map of chromosome 18.

## MULTIPOINT LOD SCORES FOR CHROMOSOME 18

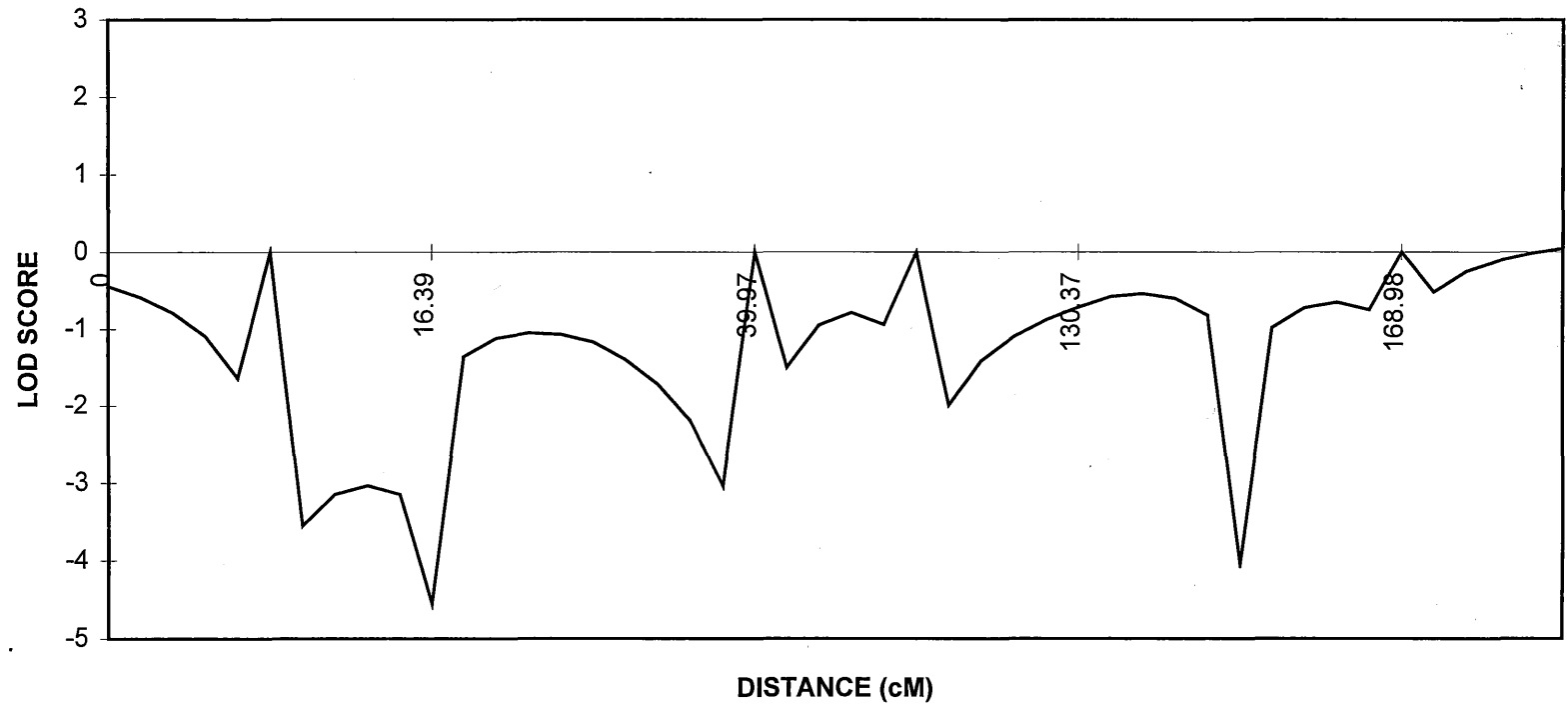


Figure 3.73: Multipoint Lod scores for markers analysed on chromosome 18.



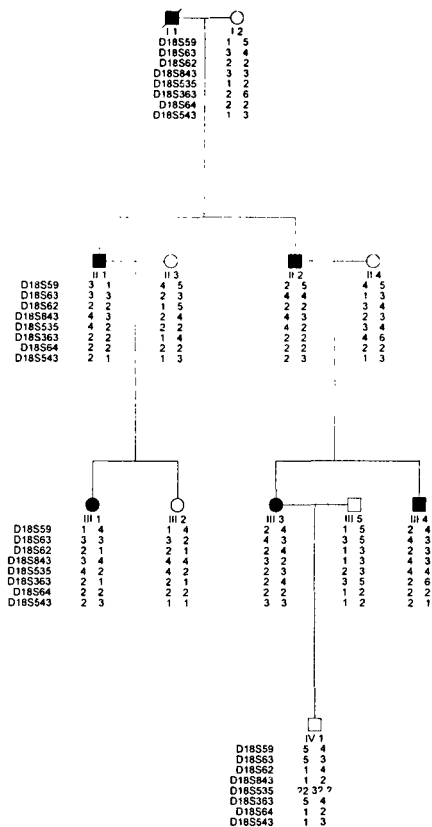


Figure 3.74: Haplotype of family 1 for chromosome 18.

### **3.19 Chromosome 19**

Chromosome 19 was analysed using 8 markers spaced approximately 13cM apart.

The two-point Lod scores in table 3.34 for this chromosome show that at  $\theta = 0$  all chromosome 19 markers gave Lod scores below -2.00. This is also shown in the exclusion map in figure 3.75 where no peaks are visible. Marker D19S294 at 19q12 gave a Lod score of negative infinity at  $\theta = 0$  however, as the recombination fraction increased, the two-point Lod scores became positive reaching a maximum value of 0.3 at  $\theta = 0.2$ .

Haplotype analysis of chromosome 19 shows a lack of haplotype sharing among affected individuals. The affected siblings III3 and III4 have inherited different haplotypes thus excluding the gene from this region. Furthermore, the unaffected sibling, individual III2, has inherited the same haplotype as her affected sibling, from marker D19S394 down to D19S224 thus providing further evidence that the gene is not in this region.

Multipoint analysis confirm that almost all of chromosome 19 can be excluded as the Lod scores are below -2.00. The region near D19S254 gave multipoint Lod scores ranging between -1.3 and 0.2, and therefore could not be excluded. Figure 3.76 shows a graphical representation of these Lod scores with the positive region near D19S254 represented as a slight rise in the graph.

Table 3.34: Table of two-point Lod scores for markers used to analys chromosome 19.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D19S247	-99.999	-1.549	-1.001	-0.703	-0.51	-0.365	-0.258	-0.173	-0.104	-0.047	0
23	D19S394	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
7	D19S253	-99.999	-1.271	-0.746	-0.473	-0.303	-0.189	-0.112	-0.059	-0.025	-0.006	0
13	D19S433	-99.999	-1.378	-0.836	-0.545	-0.358	-0.229	-0.138	-0.074	-0.032	-0.008	0
9	D19S224	-99.999	-1.255	-0.733	-0.462	-0.294	-0.183	-0.107	-0.057	-0.024	-0.006	0
20	D19S246	-3.942	-0.954	-0.666	-0.504	-0.393	-0.309	-0.239	-0.17	-0.119	-0.06	0
7	GGAA1B03	-99.999	-2.534	-1.687	-1.215	-0.896	-0.66	-0.475	-0.325	-0.2	-0.093	0
19	D19S294	-99.999	0.024	0.221	0.291	0.308	0.294	0.26	0.212	0.152	0.081	0

# EXCLUSION MAP OF CHROMOSOME 19

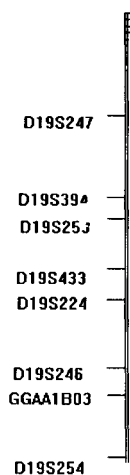


Figure 3.75: Exclusion map of chromosome 19.

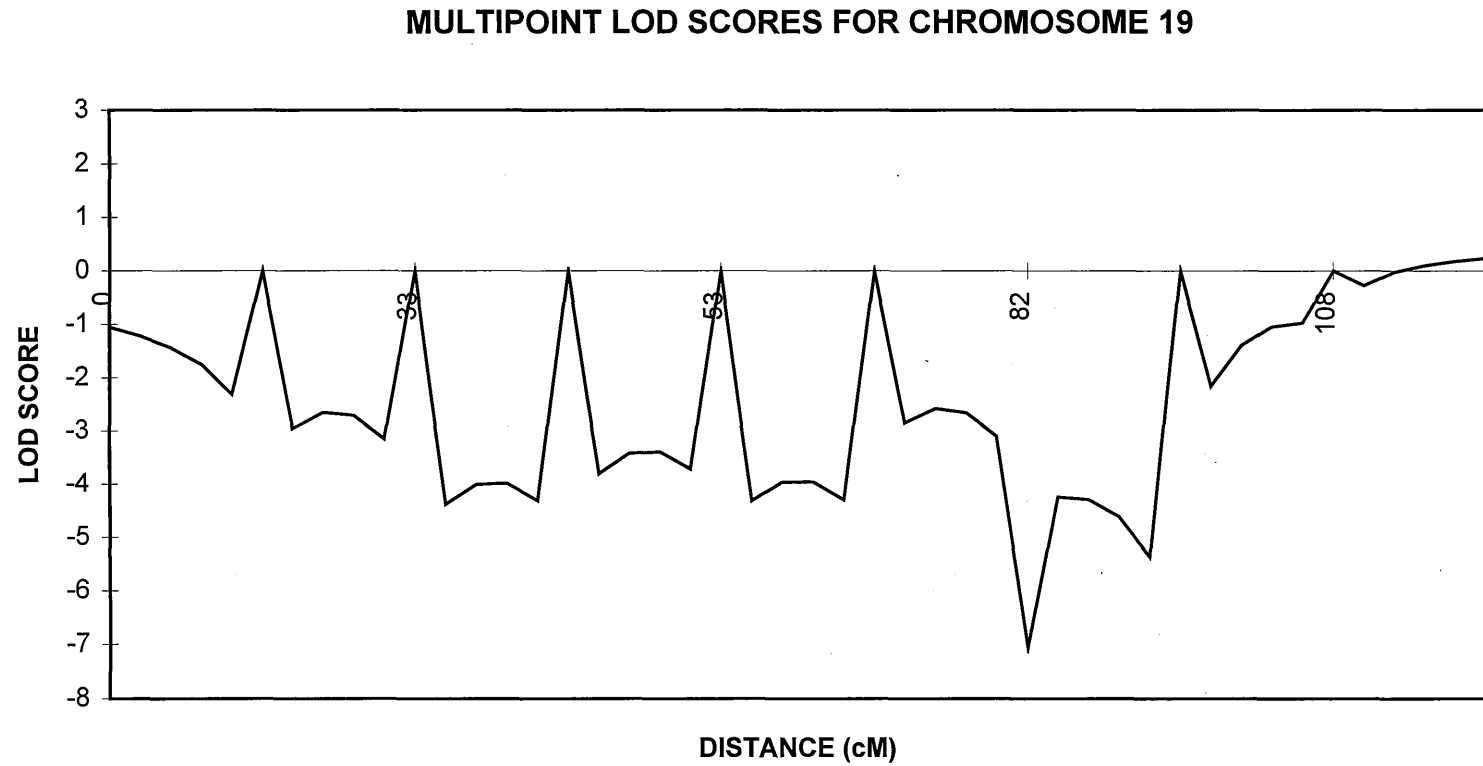


Figure 3.76: Graph of multipoint Lod scores for markers analysed on chromosome 19.

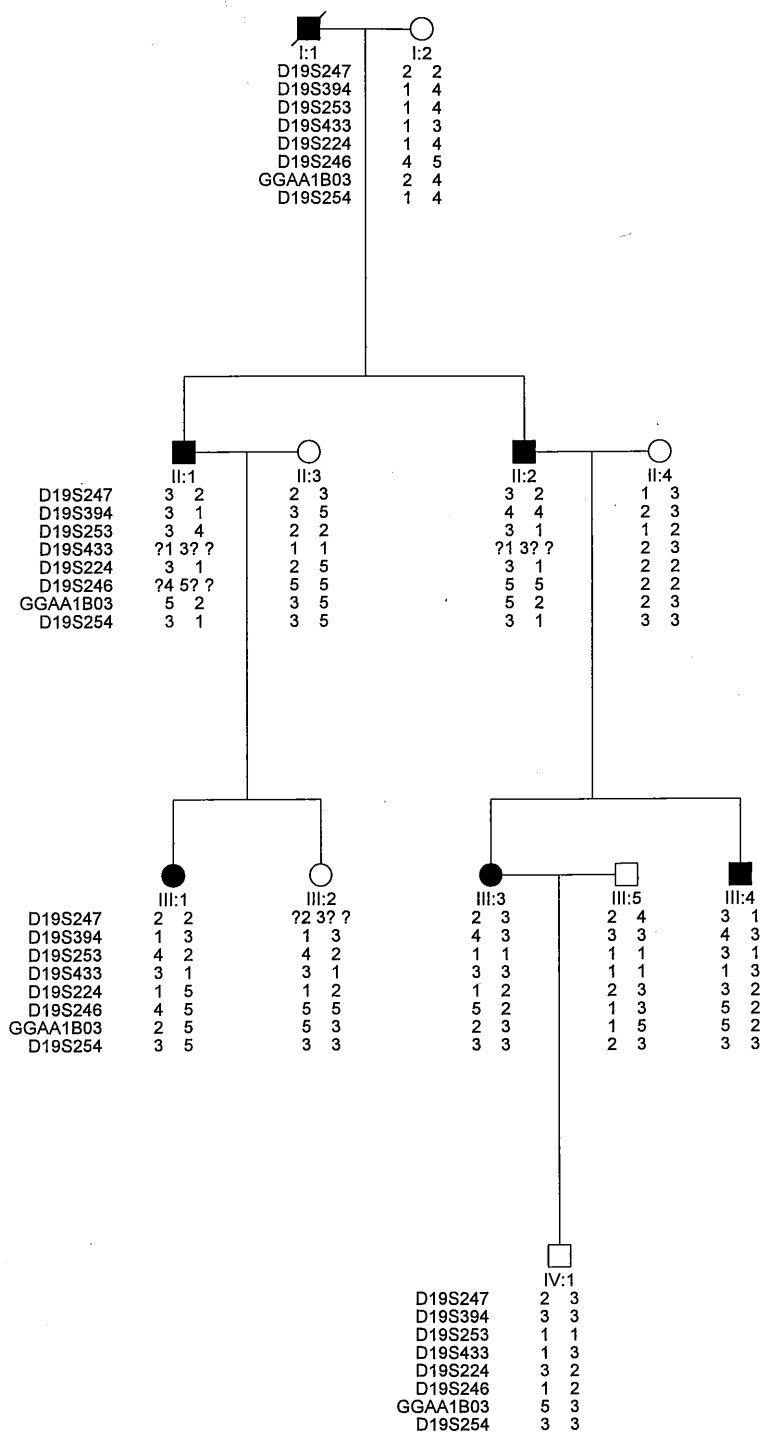


Figure 3.77: Haplotype of family 1 for chromosome 19.

### **3.20 Chromosome 20**

Chromosome 20 was analysed using 4 markers spaced approximately 20cM apart.

The two-point Lod scores for these markers are shown in table 3.35. D20S103 (20p13) is the only marker to give a positive Lod score of 0.177 at  $\theta = 0$ . The other 3 markers gave deeply negative Lod scores. This is shown graphically in the exclusion map in figure 3.78.

Multipoint analysis (figure 3.79) produced negative results for the region around D20S103. A multipoint Lod score of -1.46 was obtained at the position of this marker. For the entire interval from 20pter to D20S103, the Lod scores ranged between -0.98 and -1.46. These findings do not allow the definite exclusion of this interval, however they allow it to be classified as highly unlikely to contain PKD3. Similar conclusions can be drawn about the interval flanked by D20S115 at 20p13 and D20S120 at 20q13.2. Affected individuals in pedigree 1 do not share identical haplotypes for these two markers (figure 3.80) however the bracketed interval is 66 cM long. The results from the multipoint analysis, with Lod scores ranging from -1.43 to -3.73, also place this interval in the category of non-excluded but highly unlikely to contain the gene.

Table 3.35: Table of two-point Lod scores for the markers used to analyse chromosome 20.

		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D20S103	0.177	0.149	0.121	0.096	0.072	0.051	0.034	0.019	0.009	0.002	0
16	D20S95	-99.999	-3.442	-2.285	-1.63	-1.183	-0.851	-0.595	-0.392	-0.229	-0.1	0
6	D20S115	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
66	D20S120	-99.999	-2.534	-1.687	-1.215	-0.896	-0.66	-0.475	-0.325	-0.2	-0.093	0



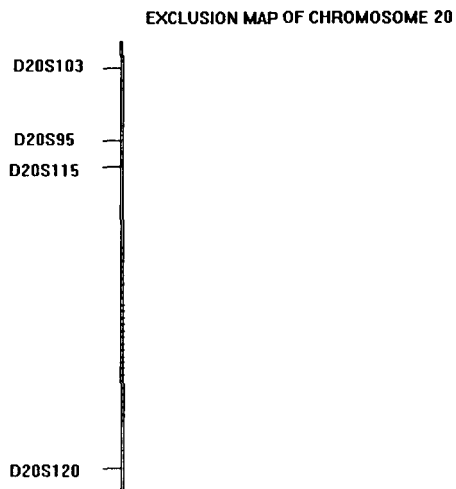


Figure 3.78: Exclusion map of chromosome 20.

MULTIPOINT LOD SCORES FOR CHROMOSOME 20

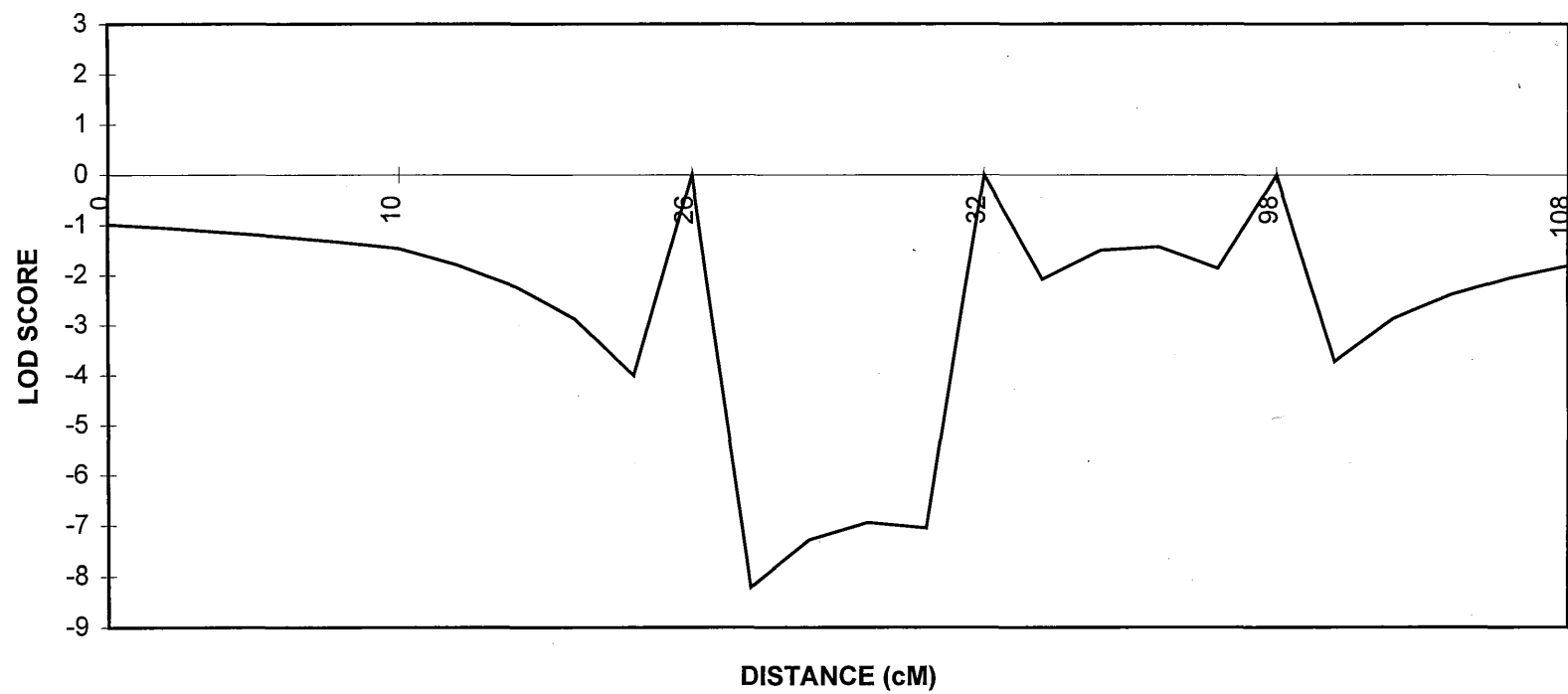


Figure 3.79: Graph of multipoint Lod scores for chromosome 20

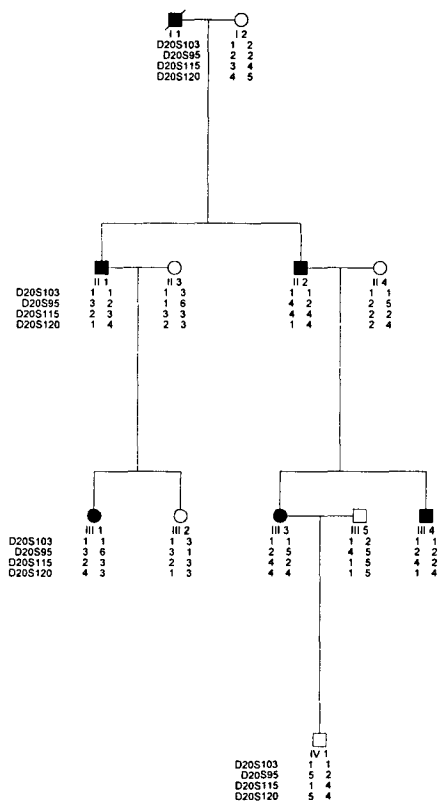


Figure 3.80: Haplotype of family I for chromosome 20.

### **3.21 Chromosome 21**

Chromosome 21 was analysed using 3 markers spaced approximately 13cM apart.

The two-point Lod scores for all three markers were negative infinity at  $\theta = 0$  (table 3.36). However for two of these markers, namely GGAA2E03 and D21S265 at positions 21q11 and 21q21.2 respectively, the Lod scores increased at higher recombination distances. The maximum Lod scores were reached at  $\theta = 0.2$  and were 0.299 for GGAA2E02 and 0.313 for D21S265. These findings and the large distance between D21S265 and the telomere resulted in the peaks of non-exclusion over chromosome 21, as shown on the map in figure 3.81.

Multipoint analysis of chromosome 21 (figure 3.82) also produced inconclusive evidence of exclusion, with Lod scores in the range -1.7 to 0.21.

Table 3.36: Table showing two-point Lod scores calculated for all markers used to analyse chromosome 21.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D21S258	-99.999	-1.442	-0.887	-0.585	-0.388	-0.25	-0.151	-0.082	-0.035	-0.009	0
17	GGAA2E02	-99.999	0.008	0.208	0.28	0.299	0.288	0.256	0.209	0.151	0.081	0
12	D21S265	-99.999	0.033	0.229	0.298	0.313	0.298	0.263	0.214	0.153	0.081	0

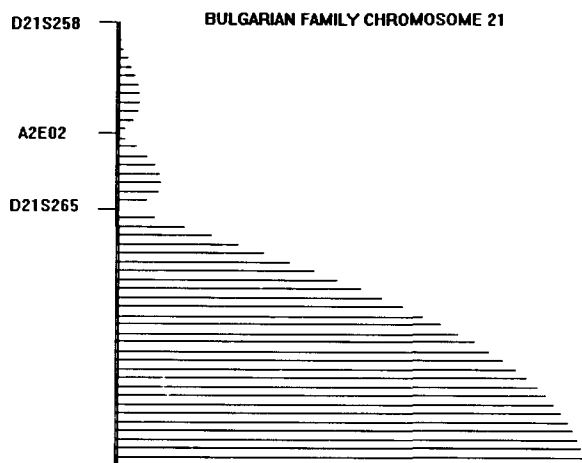


Figure 3.81: Exclusion map of chromosome 21.

### MULTIPOINT LOD SCORES FOR CHROMOSOME 21

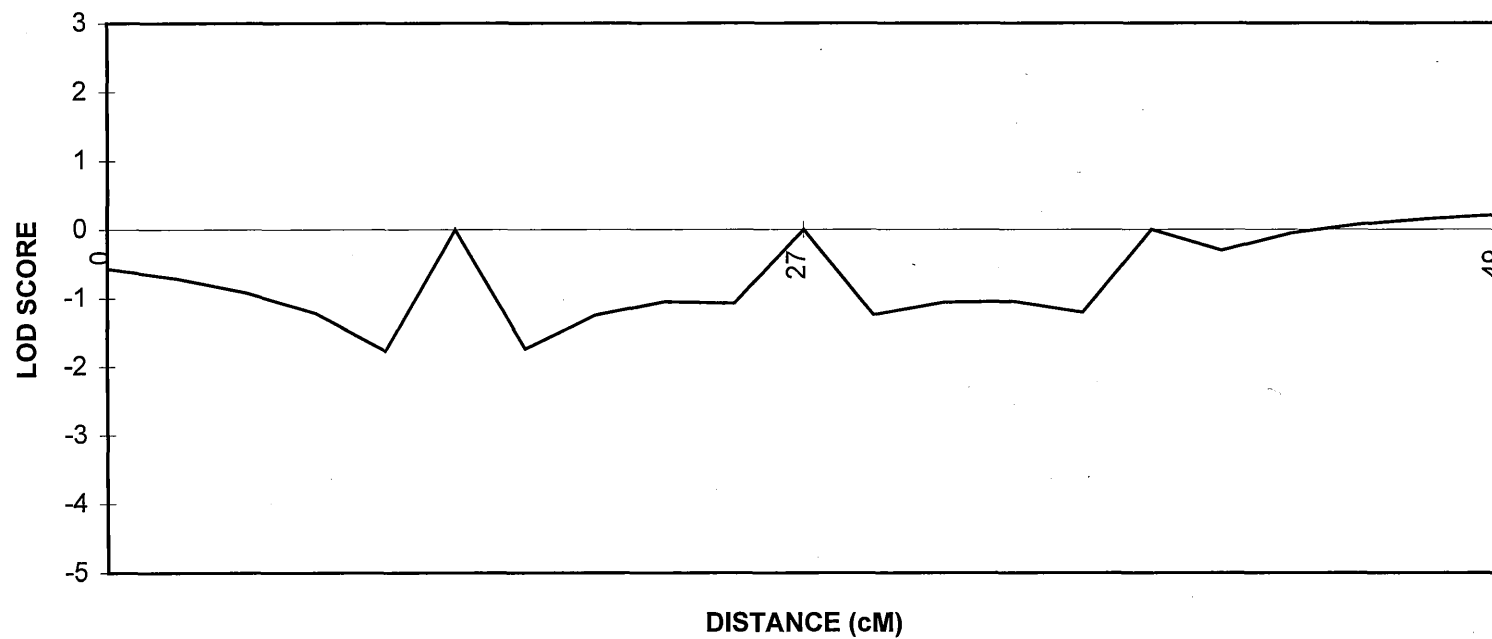


Figure 3.82: Graph of multipoint Lod scores for the markers used to analyse chromosome 21.

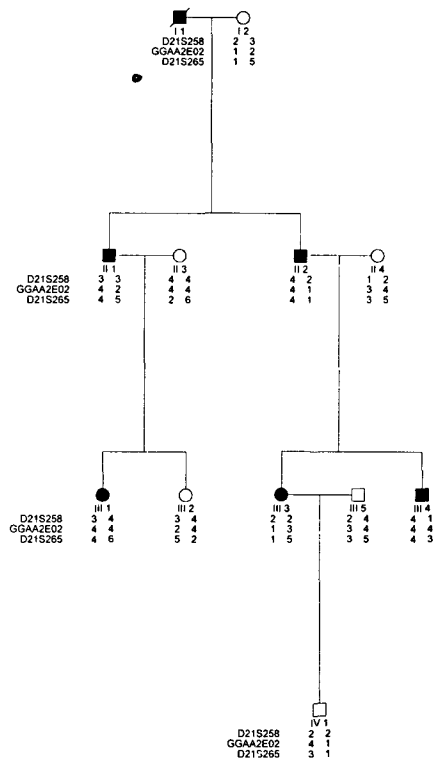


Figure 3.83: Haplotype of family 1 for chromosome 21.



### **3.22 Chromosome 22**

#### **3.22.1 Stage 1**

Chromosome 22q was studied using 4 markers spaced approximately 11 cM apart. Two point Lod scores for these markers are listed in table 3.37. Two-point Lod scores indicated a positive region of 11 cM including markers D22S264, D22S257 and D22S345. Lod score values of 1.4 were obtained for D22S264 at 22q11.21 and D22S345 at 22q11.23 and a slightly lower Lod score of 1.3 was obtained for D22S257 at 22q11.23. This positive region is represented in the exclusion map in figure 3.84 as a large peak of non-exclusion.

Multipoint analysis confirmed this region as positive, with all 3 markers mentioned above producing Lod scores of 1.4. The flanking marker D22S683 was excluded at its particular position, due to a recombination observed in unaffected individual III:2, however the region immediately adjacent was not definitely excluded even though the Lod scores were negative. These multipoint results are represented in figure 3.85.

Haplotype analysis (figure 3.86) of this region shows that haplotype sharing occurs among all affected individuals for markers D22S264, D22S257, D22S345, D22S270 and D22S284. As mentioned above, however, unaffected individual III:2 has inherited the same haplotype for markers D22S683, D22S270 and D22S684 as her affected sibling, thus placing the gene centromeric

to these markers. Because of the persisting positive Lod scores this region required further investigation.

Table 3.37: Table of two point Lod scores for the markers used to analyse chromosome 22 in stage 1.

DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D22S264	1 408	1.287	1.162	1.034	0.901	0.765	0 624	0 478	0 327	0.168	0
8	D22S257	1 329	1.219	1.105	0 987	0.866	0.739	0 607	0 469	0 322	0 167	0
3	D22S345	1 437	1.312	1.184	1 051	0.915	0.775	0 631	0 483	0 329	0 169	0
20	D22S683	-99.999	-0.592	-0.338	-0 21	-0.131	-0.081	-0 047	-0 024	-0 01	-0 002	0
18	D22S270	-99.999	0.008	0 208	0.28	0.3	0 288	0.256	0 21	0 151	0.081	0
6	D22S684	0.177	0.148	0.121	0 096	0.072	0 051	0 034	0.019	0.009	0 003	0

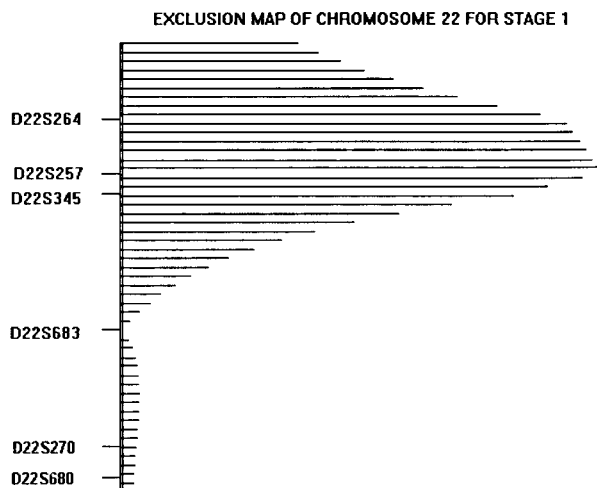


Figure 3.84: Exclusion map of chromosome 22 for stage 1.

MULTIPOINT LOD SCORES OF CHROMOSOME 22 FOR STAGE 1

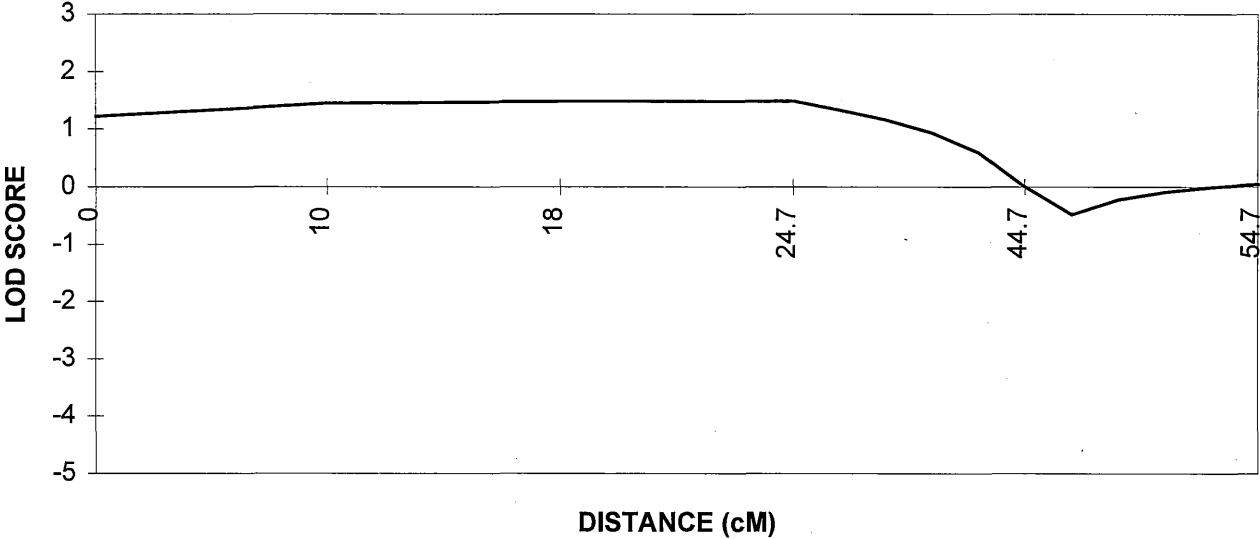


Figure 3.85: Graph of multipoint Lod scores on chromosome 22 for stage 1.

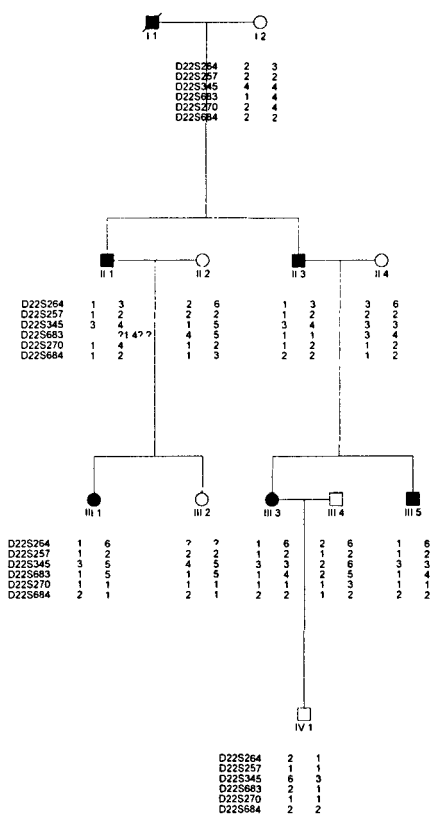


Figure 3.86: Haplotype of family I for chromosome 22 for stage 1.

### 3.22.2 Stage 2

During stage 2 of the study, 6 markers were added to the positive region mentioned above, creating a higher density map with an average of 2.2cM distance between markers. The second Bulgarian family was also included in the study and the new markers were used to analyse both families. To obtain a more conservative estimate, individuals IV1 in both families who are of relatively young age, were considered of unknown affection status or altogether excluded from the calculations. Two-point Lod scores for all markers used in stage 2 to analyse chromosome 22 are listed in table 3.38.

Two-point analysis showed that the positive region defined in stage 1 persisted for family 1. The two-point Lod scores for this family ranged from 0.204 to 1.44. The maximum Lod score was obtained at D22S446 and had a value of 1.44 at recombination distance zero. All markers in the positive region between D22S427 and D22S345 (except D22S425) produced Lod scores greater than 1.00. Marker D22S425 was uninformative in all affected individuals and gave a Lod score of 0.204 at  $\theta = 0$ .

Haplotype analysis shows sharing among all affected individuals.

Multipoint linkage analysis on family 1 confirmed the positive region bracketed by markers D22S427 and D22S345 with 4 other markers included in the interval. Multipoint Lod scores for

the entire interval ranged between 1.40 and 1.50. Marker D22S425 which is uninformative in this family and produced a relatively low two-point Lod score of 0.204 now showed a multipoint Lod score of 1.50 due to the addition of the extra markers which showed evidence of linkage.

As mentioned above, the second family was also analysed during stage 2. In this family, the two-point Lod scores ranged from -3.747 to 0.775 at D22S446. The maximum two-point Lod score was 0.775 at  $\theta = 0$  for marker D22S446, i.e at the same position where the highest two-point Lod score was obtained for family 1. The immediately adjacent (at 0.8 cM distance) marker D22S425 gave a two-point Lod score of 0.567 at  $\theta = 0$ . As mentioned above, this marker was uninformative in family 1 (hence the low two-point Lod score) but gave the peak value in multipoint analysis.

Haplotype analysis on family 2 shows haplotype sharing among affected individuals for markers between D22S446 and D22S345. The interval where haplotype sharing occurs is smaller than in family 1 and is narrowed down on the centromeric side by a recombination between markers D22S264 and D22S446 found in individual III:2. Examination of haplotypes also suggests sharing for marker D22S345 but, due to the lack of genotyping information for individual IV3, it is difficult to draw definite conclusions.



Multipoint analysis on family 2 contradicted the positive two-point results with all markers producing negative two-point results ranging from  $-0.48$  to  $-0.91$ . These inconclusive Lod scores were due to the predominantly uninformative markers in this region. Marker D22S264, which was informative produced a Lod score of  $-4.81$  thus excluding the marker at its position.

This region was however, investigated further due to several facts. These included (a) a positive Lod score of 1.50 was obtained for family 1; (b) the maximum two-point Lod score of 2.22 obtained at D22S446 at recombination frequency zero; (c) the markers in stage 2 for family 2 were uninformative thus no conclusions could be drawn.

Table 3.38: Table showing the Lod score for chromosome 22 markers used to analyse pedigree 1 in stage 2 of the study.

TWO-POINT LOD SCORES FOR STAGE 2 OF CHROMOSOME 22 FOR PEDIGREE 1												
		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D22S427	1.113	0.989	0.862	0.732	0.599	0.465	0.333	0.208	0.101	0.027	0
3.3	D22S264	1.139	1.036	0.931	0.823	0.712	0.6	0.485	0.369	0.249	0.127	0
2.5	D22S446	1.44	1.315	1.186	1.053	0.917	0.776	0.632	0.483	0.329	0.168	0
0.8	D22S425	0.204	0.171	0.141	0.111	0.084	0.06	0.039	0.022	0.1	0.002	0
4.7	D22S257	1.329	1.218	1.105	0.987	0.865	0.739	0.607	0.468	0.322	0.167	0
2	D22S345	1.436	1.312	1.183	1.051	0.915	0.775	0.631	0.482	0.328	0.168	0
20	D22S683	-99.999	-0.592	-0.338	-0.21	-0.131	-0.081	-0.047	-0.024	-0.01	-0.002	0
18	D22S270	-99.999	0.008	0.208	0.28	0.3	0.288	0.256	0.21	0.151	0.081	0
6	D22S684	0.177	0.148	0.121	0.096	0.072	0.051	0.034	0.019	0.009	0.003	0

Table 3.39: Table showing the Lod score for chromosome 22 markers used to analyse pedigree 2 in stage 2 of the study.

TWO-POINT LOD SCORES FOR STAGE 2 OF CHROMOSOME 22 FOR PEDIGREE 2												
		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D22S427	-0.184	-0.137	-0.102	-0.075	-0.054	-0.037	-0.024	-0.013	-0.006	-0.001	0
3.3	D22S264	-3.747	-0.76	-0.479	-0.329	-0.234	-0.168	-0.121	-0.085	-0.055	-0.027	0
2.5	D22S446	0.775	0.675	0.573	0.471	0.37	0.273	0.184	0.107	0.048	0.012	0
0.8	D22S425	0.567	0.48	0.396	0.314	0.238	0.169	0.109	0.061	0.027	0.006	0
4.7	D22S257	-0.096	-0.076	-0.059	-0.044	-0.032	-0.022	-0.014	-0.007	-0.003	0	0
2	D22S345	0.309	0.252	0.199	0.15	0.108	0.073	0.045	0.024	0.01	0.002	0

Table 3.40: Table of two-point Lod scores for family 1 and family 2 for markers used to analyse chromosome 22 in stage 2 of the study.

COMBINED TWO-POINT LOD SCORES FOR STAGE 2 OF SCREENING OF CHROMOSOME 22												
		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D22S427	0.93	0.85	0.76	0.66	0.54	0.43	0.31	0.19	0.1	0.02	0
3.3	D22S264	-2.61	0.47	0.61	0.62	0.57	0.51	0.42	0.32	0.22	0.11	0
2.5	D22S446	2.22	1.99	1.76	1.53	1.29	1.05	0.82	0.59	0.38	0.17	0
0.8	D22S425	0.77	0.65	0.54	0.43	0.32	0.23	0.15	0.08	0.04	0.02	0
4.7	D22S257	1.23	1.14	1.05	0.94	0.83	0.72	0.59	0.46	0.32	0.16	0
2	D22S345	1.75	1.56	1.38	1.2	1.02	0.85	0.68	0.51	0.34	0.17	0
20	D22S683	-99.999	-0.592	-0.338	-0.21	-0.131	-0.081	-0.047	-0.024	-0.01	-0.002	0
18	D22S270	-99.999	0.008	0.208	0.28	0.3	0.288	0.256	0.21	0.151	0.081	0
6	D22S684	0.177	0.148	0.121	0.096	0.072	0.051	0.034	0.019	0.009	0.003	0

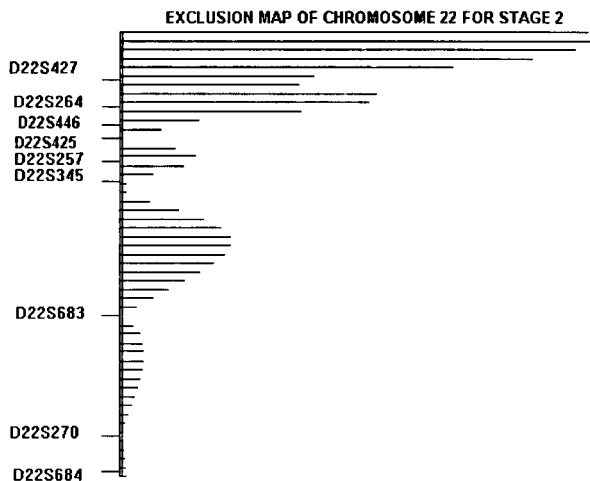


Figure 3.87: Exclusion map of chromosome 22 for stage 2 of the study.

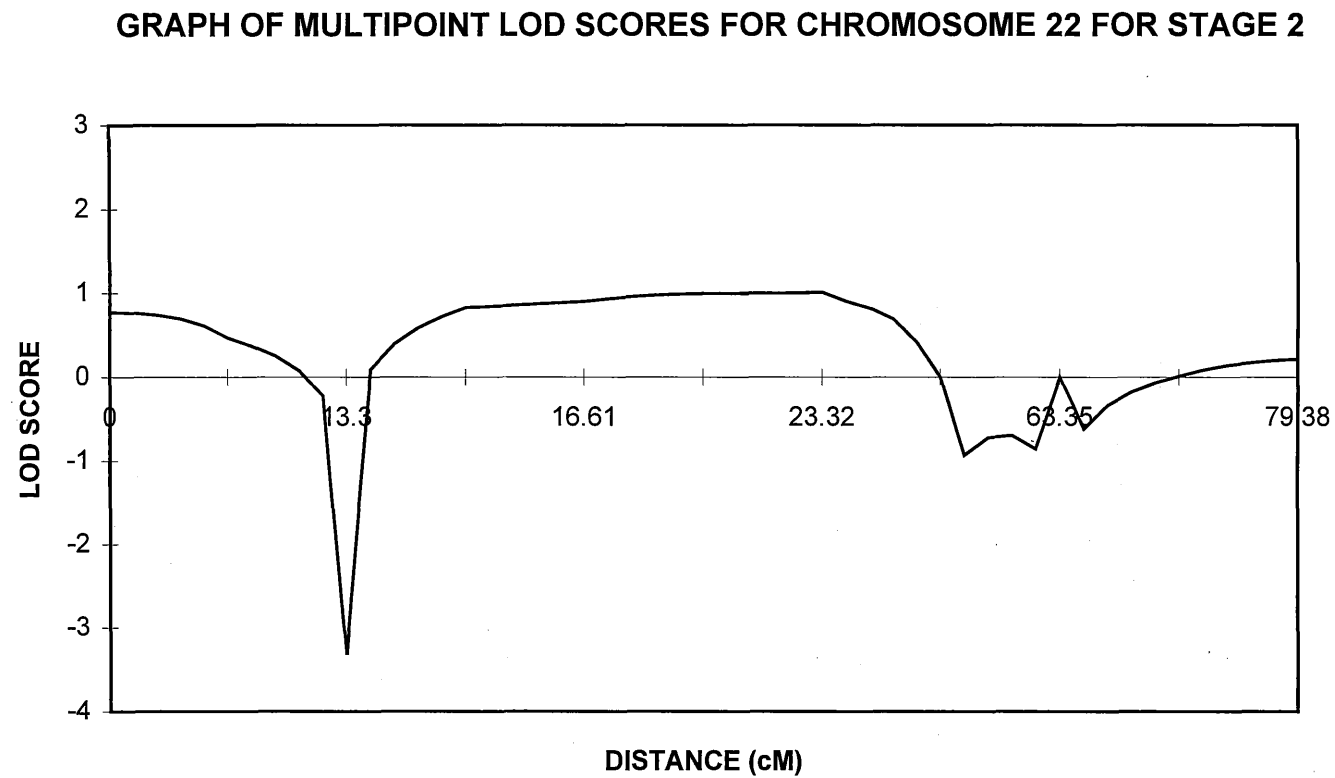


Figure 3.88: Graph of combined multipoint Lod scores for chromosome 22 for stage 2.

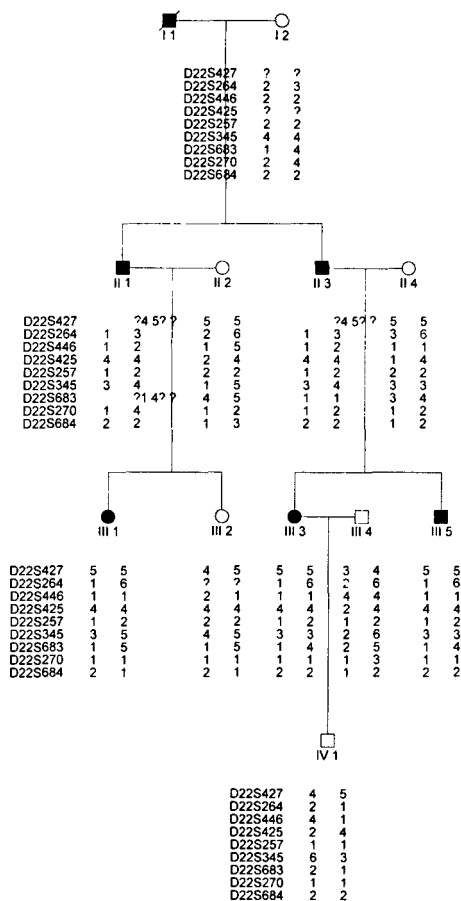


Figure 3.89: Haplotype of family 1 for chromosome 22 for stage 2 of the study.

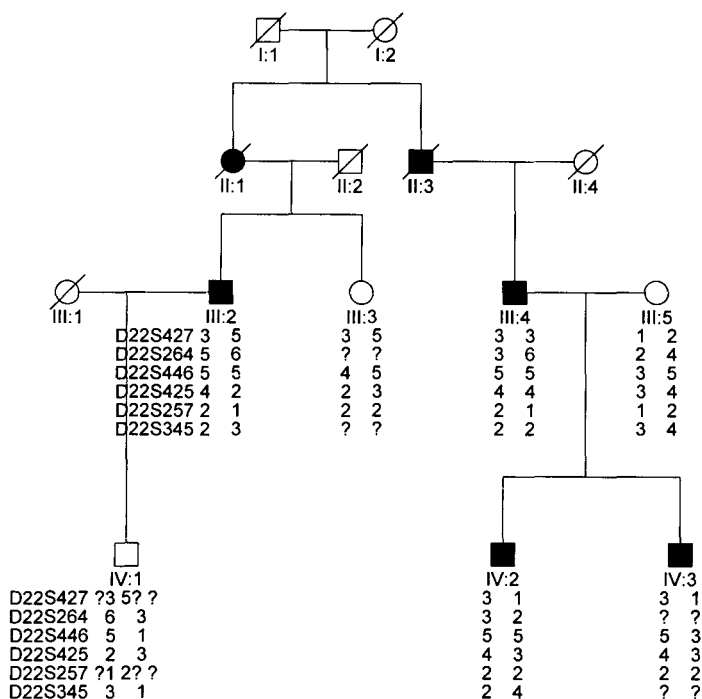


Figure 3.94: Haplotype of pedigree 2 for markers on chromosome 22 added in stage 2.



### 3.22.3 Stage 3

A total of 14 markers spaced approximately 1.3cM apart were used to analyse the positive region on chromosome 22 in stage 3 of the study. These markers and their respective two-point Lod scores are listed in table 3.41.

As can be seen from this table, the maximum combined two-point Lod score of 2.22 was still produced by D22S446 at recombination frequency zero. Two of the newly added markers, namely D22S1174 and D22S419 gave two-point Lod scores of greater than 1.00 while D22S1164 gave a slightly lower positive Lod score of 0.59 at  $\theta = 0$ . Marker D22S539, centromeric to D22S446 at zero distance also produced a relatively high positive Lod score of 1.81 at  $\theta = 0$ . The region which shows evidence of linkage in both families spans approximately 10 cM, from D22S539 on the centromeric side to D22S345 on the telomeric side. As discussed above, recombinations observed in both families have helped to narrow down the region as follows: the recombination observed in individual III:2 in family 1 places the gene centromeric to D22S683. Because of the large distance between D22S345 and D22S683 (20 cM), our data are not sufficient to define the telomeric boundary of the region. However, it is certain that marker D22S345 is within the linkage region. In family 2, the recombination in individual III:2 places the gene centromeric to this marker.

Haplotype analysis shows haplotype sharing between all affected individuals in the two families.

Multipoint analysis produced positive Lod scores over the entire interval. With the addition of the extra markers, the multipoint Lod scores in family 2 increased to above 1.00 thus dismissing any doubts as to whether this is a candidate region or not in the second family. The maximum multipoint Lod scores were 1.5 in family 1 and 1.45 in family 2. The maximum combined multipoint Lod scores of 2.95 were obtained at D22S1174 and D22S419. The multipoint results are shown in Figure 3.92.

Table 3.41: Table showing Lod scores for chromosome 22 markers used to analyse pedigree 1 in stage 3

TWO-POINT LOD SCORES FOR STAGE 3 OF CHROMOSOME 22 FOR PEDIGREE 1												
DISTANCE (cM)	MARKER	THETA VALUES										
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D22S427	1.113	0.989	0.862	0.732	0.599	0.465	0.333	0.208	0.101	0.027	0
3.3	D22S264	1.139	1.036	0.931	0.823	0.712	0.6	0.485	0.369	0.249	0.127	0
2.5	D22S539	1.135	1.033	0.928	0.82	0.711	0.599	0.485	0.368	0.249	0.127	0
0	D22S446	1.44	1.315	1.186	1.053	0.917	0.776	0.632	0.483	0.329	0.168	0
0.8	D22S425	0.204	0.171	0.141	0.111	0.084	0.06	0.039	0.022	0.1	0.002	0
4.7	D22S257	1.329	1.218	1.105	0.987	0.865	0.739	0.607	0.468	0.322	0.167	0
0.7	D22S1174	1.135	1.033	0.928	0.82	0.711	0.599	0.485	0.368	0.249	0.127	0
0	D22S419	1.135	1.033	0.928	0.82	0.711	0.599	0.485	0.368	0.249	0.127	0
0	D22S1164	0.221	0.187	0.154	0.122	0.093	0.066	0.044	0.025	0.011	0.002	0
1.3	D22S345	1.436	1.312	1.183	1.051	0.915	0.775	0.631	0.482	0.328	0.168	0
20	D22S683	-99.999	-0.592	-0.338	-0.21	-0.131	-0.081	-0.047	-0.024	-0.01	-0.002	0
18	D22S270	-99.999	0.008	0.208	0.28	0.3	0.288	0.256	0.21	0.151	0.081	0
6	D22S684	0.177	0.148	0.121	0.096	0.072	0.051	0.034	0.019	0.009	0.003	0

Table 3.42: Table showing Lod scores for chromosome 22 markers used to analyse pedigree 2 in stage 3

TWO-POINT LOD SCORES FOR STAGE 3 OF CHROMOSOME 22 FOR PEDIGREE 2												
		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D22S427	-0.184	-0.137	-0.102	-0.075	-0.054	-0.037	-0.024	-0.013	-0.006	-0.001	0
3.3	D22S264	-3.747	-0.76	-0.479	-0.329	-0.234	-0.168	-0.121	-0.085	-0.055	-0.027	0
2.5	D22S539	0.677	0.586	0.495	0.404	0.315	0.231	0.154	0.089	0.04	0.01	0
0	D22S446	0.775	0.675	0.573	0.471	0.37	0.273	0.184	0.107	0.048	0.012	0
0.8	D22S425	0.567	0.48	0.396	0.314	0.238	0.169	0.109	0.061	0.027	0.006	0
4.7	D22S257	-0.096	-0.076	-0.059	-0.044	-0.032	-0.022	-0.014	-0.007	-0.003	0	0
0.7	D22S1174	0.296	0.241	0.189	0.143	0.103	0.069	0.042	0.022	0.009	0.002	0
0	D22S419	0.42	0.343	0.274	0.212	0.16	0.166	0.081	0.054	0.032	0.014	0
0	D22S1164	0.371	0.3	0.237	0.182	0.136	0.099	0.069	0.046	0.028	0.013	0
1.3	D22S345	0.309	0.252	0.199	0.15	0.108	0.073	0.045	0.024	0.01	0.002	0

Table 3.43: Table of two point Lod score for family 1 and family 2 combined for all markers analysed on chromosome 22 in stage 3 of the study.

COMBINED TWO-POINT LOD SCORES FOR THE FINAL STAGE OF SCREENING OF CHROMOSOME 22												
		THETA VALUES										
DISTANCE (cM)	MARKER	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
	D22S427	0.93	0.85	0.76	0.66	0.54	0.43	0.31	0.19	0.1	0.02	0
3.3	D22S264	-2.61	0.47	0.61	0.62	0.57	0.51	0.42	0.32	0.22	0.11	0
2.5	D22S539	1.81	1.62	1.42	1.23	1.03	0.83	0.64	0.46	0.29	0.13	0
	D22S446	2.22	1.99	1.76	1.53	1.29	1.05	0.82	0.59	0.38	0.17	0
0.8	D22S425	0.77	0.65	0.54	0.43	0.32	0.23	0.15	0.08	0.04	0.02	0
4.7	D22S257	1.23	1.14	1.05	0.94	0.83	0.72	0.59	0.46	0.32	0.16	0
0.7	D22S1174	1.43	1.27	1.12	0.96	0.81	0.67	0.053	0.39	0.26	0.13	0
	D22S419	1.56	1.38	1.2	1.03	0.87	0.72	0.57	0.42	0.28	0.13	0
	D22S1164	0.59	0.49	0.39	0.31	0.23	0.17	0.11	0.07	0.04	0.02	0
1.3	D22S345	1.75	1.56	1.38	1.2	1.02	0.85	0.68	0.51	0.34	0.17	0
20	D22S683	-99.999	-0.592	-0.338	-0.21	-0.131	-0.081	-0.047	-0.024	-0.01	-0.002	0
18	D22S270	-99.999	0.008	0.208	0.28	0.3	0.288	0.256	0.21	0.151	0.081	0
6	D22S684	0.177	0.148	0.121	0.096	0.072	0.051	0.034	0.019	0.009	0.003	0

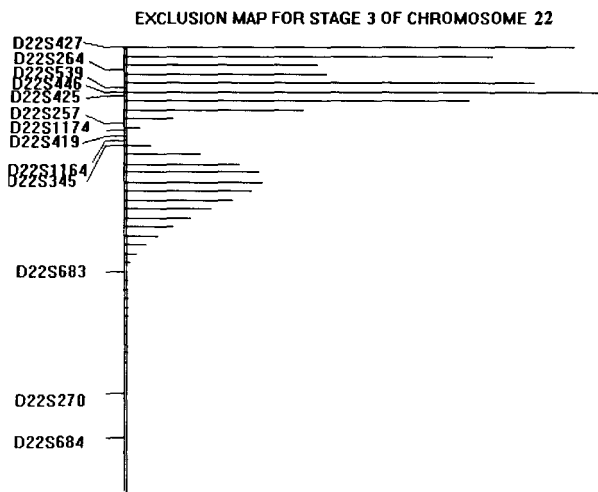


Figure 3.91: Exclusion map of chromosome 22 for stage 3 of the study.

GRAPH OF MULTIPOINT LOD SCORES FOR CHROMOSOME 22 FOR STAGE 3

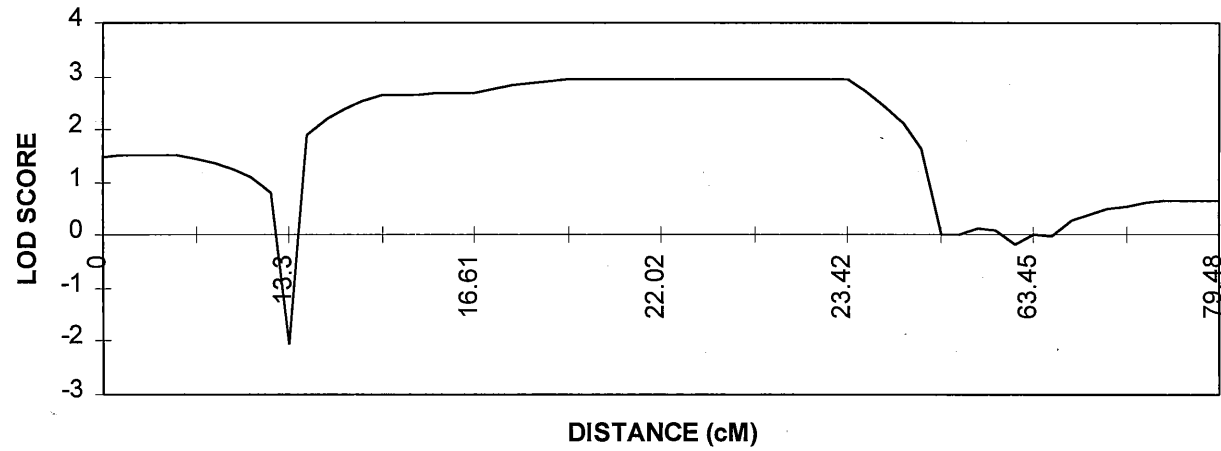


Figure 3.92: Graph of multipoint Lod scores on chromosome 22 for family 1 and family 2 combined in stage 3.

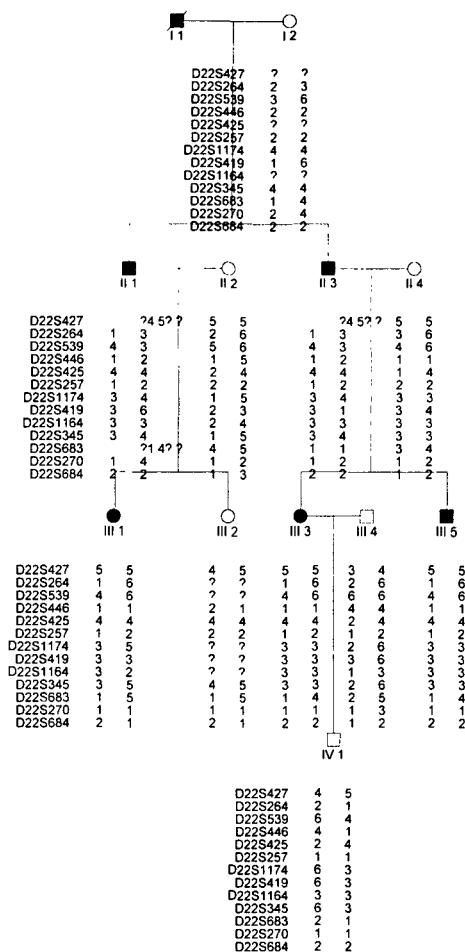


Figure 3.93: Haplotype of family 1 for all markers used to analyse chromosome 22 in stage 3 of the study.



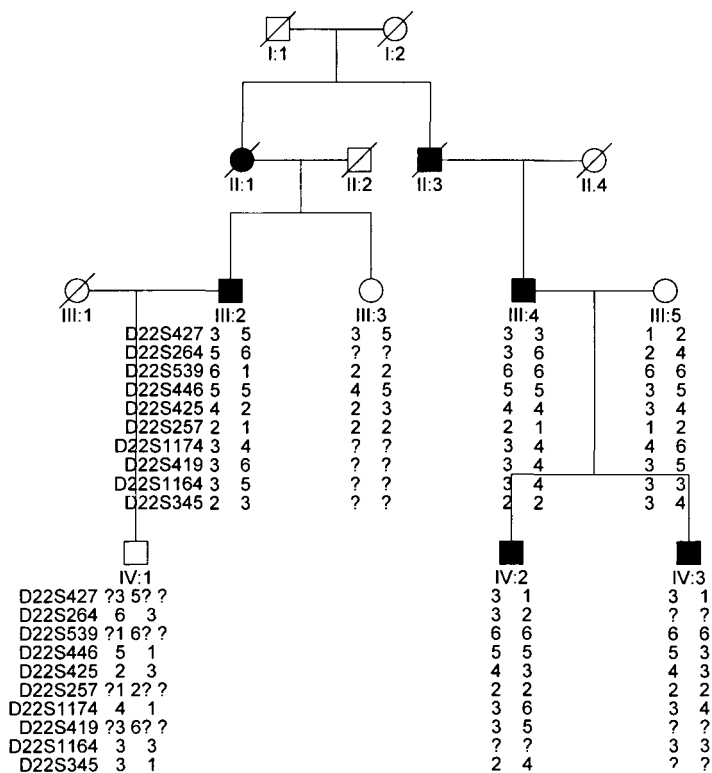


Figure 3.94: Haplotype of pedigree 2 for markers on chromosome 22 added in stage 3.

## **4. DISCUSSION**

### **4.1 Participating Families**

Two families, both of Bulgarian origin, participated in this study. The first family consisted of 11 individuals, of whom 5 were affected. The second family consisted of seven individuals with 4 affected. Simulations using the SLINK program (Ott., 1989, Weeks *et al.*, 1990) were carried out to determine the maximum Lod score obtainable for both families. Performing 1000 multipoint simulations under the assumption of linkage, using 2 markers and three alleles per locus, it was shown that the maximum Lod score expected for family 1 was 1.44 while the maximum Lod score expected for family 2 was 1.08. Thus a total of 2.56 was expected assuming both families are affected by mutations in the same gene.

The size of these two families does not allow the minimum Lod score suggestive of linkage ( $Z = 3.00$ ) to be reached. As a result, exclusion of the majority of the genome was the primary aim of the research. Simulations were carried out using the SLINK program (Ott., 1989, Weeks *et al.*, 1990) to determine the power of exclusion of the first family, on which stage 1 of the study is based. A total of 1000 multipoint simulations were performed under the assumption of no linkage between the gene and a given marker. The simulations showed that the minimum Lod score obtainable for family 1 at theta values 0.05 and 0.000 was - infinity while the average Lod score was also - infinity

and the maximum Lod score achieved from the simulations was 1.44. Simulations were also carried out to determine the probability of getting a Lod score of 1.00 or greater under the assumption of no linkage. Performing 1000 multipoint simulations we obtained a value of 3.4%, which is the probability of obtaining a false positive result i.e. a Lod score  $>1.00$  for a marker which in fact is not linked to the gene. On the basis of these results, we defined as candidate locations of the PKD3 gene, all regions showing Lod scores  $>1.00$ .

Under the assumption of linkage, 1000 simulations showed that the probability of getting a Lod score greater than 1.00 was 62.8% (Ott., 1989, Weeks *et al.*, 1990). Comparing this value of 62.8% to the value of 3.4% (percentage of false positives) it can be seen that proportion of false positives is quite small, thus justifying our minimum Lod score of 1.00 as a requirement of a candidate region. If a score of 0.00 was chosen as the minimum requirement of a candidate region then the proportion of false positives would be much larger. Simulations were again performed to justify this hypothesis. Again using 1000 simulations and under the assumption of no linkage the percentage of regions producing a Lod score of greater than 0.00 was 40.2% i.e. the percentage of false positives was 40.2%. This figure is substantially larger than that of 3.4% for a Lod score of 1.00, thus it would seem more financially efficient and less time consuming to use a Lod score of 1.00 as the minimum requirement of a candidate region.

## **4.2 Discussion of Methods**

The methods used to analyse the genotyping data were two-point analysis using MLINK of LINKAGE, exclusion mapping using the EXCLUDE program and multipoint analysis using the Genehunter program.

Two-point Lod scores are a numerical representation of the probability of one locus being linked to another locus. In the case of our study the two-point Lod scores represent the probability that the PKD3 gene is linked to any one of the markers analysed. MLINK of the LINKAGE program places the gene at various increments (recombination fraction values) from the marker and calculates the Lod scores at each of these increments. For the purpose of this study, Lod scores were calculated at recombination fractions ranging from  $\theta = 0.0$  to  $\theta = 0.45$ .

From the calculated two-point Lod scores, exclusion maps were constructed using the EXCLUDE program. These maps are graphical representations of the two-point Lod scores with regions of non-exclusion denoted by peaks on the graph and regions of exclusion denoted by flat areas.

Multipoint linkage analysis was carried out using the Genehunter program. Multipoint analysis produces Lod scores for a region between numerous markers with the gene being placed hypothetically at various increments between these markers. This type

of analysis takes into account the affect of flanking markers and the Lod scores obtained at these flanking markers. The distance to these flanking markers as well as the informativeness of the marker determines how negative or positive the Lod score will be. Genehunter is a package that analyses the haplotypes of the entire chromosome rather than three or four markers at one time (as is the case in most multipoint analyses).

#### **4.3 Strategy of the Study**

The aim of the study was to exclude a large proportion of the genome and thus outline possible locations of the PKD3 gene. An initial screening of the genome was performed in family 1 in the first stage of the study. The chromosomal regions were divided into 4 categories. The first category is defined by :

$$Z: Z \geq -2$$

where Z is the Lod score at  $\theta = 0$ . Regions of the genome that fall into this category were considered definitely excluded.

The second category was defined by :

$$Z: -2 \leq Z \leq -1$$

where Z is the multipoint Lod score at  $\theta = 0$ . This category included regions that could not be excluded with 100% confidence but were highly unlikely to contain the PKD3 gene.

The third category was defined by :

$$Z: -1 \leq Z \leq 1.$$

These are regions that could be not excluded but are unlikely locations of the gene.

The fourth category includes any genomic region that produces Lod scores greater than 1.00 and is defined by :

$$Z: Z \geq 1.00.$$

These regions were considered candidate regions for the possible location of the PKD3 gene and were investigated in greater detail. This threshold was chosen because simulations showed that the likelihood of obtaining a Lod score >1.00 for a linked marker was 62.8% whereas the rate of false positive results for this cut-off value was 3.4%.

A summary of the genomic regions that fall into these categories is shown in table 4.1. For a detailed list of the multipoint Lod scores obtained with the Genehunter package see appendix B.

GENOME REGIONS BY CATEGORY	OVERALL DISTANCE(cM)	% OF GENOME
Definitely excluded (Lod scores $\leq -2$ )	1829	45.6%
Probably excluded (Lod scores $-1$ to $-2$ )	882	22.1%
Not excluded Unlikely location (Lod scores $-1$ to $1$ )	1250	31.2%
Possible locations (Lod scores $>1$ )	45	1.1%

Table 4.1: A summary of the exclusion results from stage 1 of the study.

#### **4.4 Exclusion Categories**

##### **4.4.1 Definitely Excluded Regions**

The first category includes all regions of definite exclusion defined by Lod scores of  $-2.00$  or less. A two-point Lod score of  $-2.00$  corresponds to a likelihood ratio of 1:100 ie. for a marker that produces a Lod score of  $-2.00$  there is a 1:100 chance that the gene is at that position which is widely accepted as conclusive evidence against linkage.

For regions that fall into this category haplotype analysis shows numerous recombinations occur and lack of haplotype sharing among affected individuals.

As can be seen from table 4.1 this has been achieved for 45.6% of the human genome. Some regions that fall into this category cannot be excluded conclusively with two-point analysis but can be excluded as a result of multipoint analysis. An example is the region between D19S247 and D19S224 which includes 3 other markers. For all markers, conclusive evidence of linkage was obtained at  $\theta = 0$  but not at  $\theta$  values greater than 0, using two-point Lod analysis. Using multipoint analysis, the whole region was definitely excluded.

#### 4.4.2 Probably Excluded Regions

The second category of results in this study includes regions that gave multipoint Lod scores between -2.00 and -1.00 (table 4.2). These regions cannot be excluded definitely, however the negative Lod scores make it highly unlikely that the gene is located within these regions.



CHROMOSOMAL REGION	LENGTH (cM)
1p	47
2p	44
2q	91
3p	40
3q	25
4p	24
4q	87
5p	18
5q	10
6p	6
6q	19
7p	41
8q	14
9p	21
9q	21
10p	13
10q	6
11p	2
11q	20
12p	25
14p	25
14q	2
15p	5
15q	6
16p	14
17p	63
18p	17
18q	87
20p	6
20q	53
21cen	29
22q	1
TOTAL	882

Table 4.2: Genomic regions that were probably excluded with Lod scores between -2.00 and -1.00.

If the two-point Lod scores for these markers are examined, it can be seen that in most cases the Lod score is  $-99.999$  at  $\theta = 0$  thus excluding the position of the marker. However, as  $\theta$  increases, the Lod scores rise to greater than  $-2.00$ , indicating that even though the position at the marker can be excluded, at 5cM ( $\theta = 0.05$ ) from the marker, or higher, the region cannot be excluded with certainty.

Examining the haplotypes in these regions it can be seen that there is a lack of haplotype sharing.

Multipoint analysis of such regions also failed to decrease the Lod score to below  $-2.00$ .

These regions where no definite exclusion could be achieved are due to two main causes: large distances and uninformative genotyping data.

Given the size of the family used in stage 1 of the study, large distances between markers resulted in less conclusive Lod scores. This was shown by the multipoint simulations, where the minimum and average Lod scores reduced as the theta values increased. The largest minimum and average values ( $-0.40$  and  $-0.01$ ) were still negative, however as can be seen these Lod scores are inconclusive. This indicates that despite the negative Lod scores definite conclusions as to whether the gene is in these regions cannot be made. An example of this is a region on chromosome 2, around marker D2S111 which is 11cM telomeric to D2S110 and 36cM centromeric to D2S142. The two-point

Lod score at D2S111 is - infinity, thus excluding the position of this marker. However, as  $\theta$  increases to 0.05, the Lod score increases to - 0.362.

Informativeness of markers also affects Lod score values. In order to obtain conclusive Lod scores, it is important to have informative events. An informative event is one where the inheritance of alleles can be traced from one generation to the next without ambiguity within a family. Table 4.3 below shows that of the markers that fall into this category, 43% were uninformative.

LARGE DISTANCES		UNINFORMATIVENESS	
% large Distance (>10cM)	% Small Distance (>10cM)	% Uninformative Markers	% Informative Markers
71%	29%	80%	20%
	% U. % I.		
	87% 13%		

Table 4.3: Reasons for non-exclusion of markers in category 2 (22.1% of the genome). 'U' indicates the percentage of markers with small distances between them that were uninformative. 'I' indicates the percentage of markers with small distances between them that were informative.

As can be seen from table 4.3 the two main reasons for the non-exclusion of regions in this category were large distances between markers and

unformativeness of markers. The distance between markers in some regions was small ( $<10\text{cM}$ ) however as can be seen from the above table approximately 87% of these markers were uninformative. Furthermore, 80% of the total number of markers in this category were uninformative.

#### 4.4.3 Inconclusive Regions

The third category of results included all regions that produced Lod scores between -1.00 and 1.00. These regions cannot be excluded because in some cases the Lod scores are only slightly negative and in other cases the Lod scores are positive but not positive enough to be considered candidate regions.

CHROMOSOMAL REGION	LENGTH (cM)
1p	34
2q	45
3p	78
4p	33
4q	117
5p	10
5q	63
6p	123
7p	208
8q	10
9p	18
11p	40
11q	49
12q	55
13q	6
14q	20
15p	33
16q	72
17p	63
17q	22
18q	52
19q	18
21q	14
22q	67
TOTAL	1250

Table 4.4: Genomic regions with Lod scores between -1.00 and 1.00.

The regions that fall into this category can be subdivided into two categories; those regions that give Lod scores between -1.00 and 0.00, and those regions that give Lod scores between 0.00 and 1.00.

#### 4.4.3.1 Regions with Lod scores between -1.00 and 0.00

The two-point Lod scores for these regions are slightly positive, however if multipoint data is examined it can be seen that the Lod scores become negative (between 0 and -1.00). Large distances are the main

reason for these inconclusive results (84% of regions), followed by the lack of informativeness of the markers, which comprises of 65% of all markers in this category (table 4.5).

LARGE DISTANCES		UNINFORMATIVENESS	
% large Distance (>10cM)	% Small Distance (>10cM)	% Uninformative Markers	% Informative Markers
84%	16%	65%	35%
	% U. % I.		
	75% 25%		

Table 4.5: Table showing the main reasons for non exclusion of regions that produced Lod score between -1.00 and 0.00. 'U' indicates uninformativeness. 'I' indicates informativeness.

As can be seen from table 4.5, even though in 16% of cases the distances between markers was small, the majority of these markers (75%) were uninformative thus contributing to the inconclusive Lod scores.

The percentage of markers in this category that were uninformative was shown to be 65% however, while these markers were not completely uninformative (in which case a Lod score of 0 would be achieved) they lacked enough information to produce deeply negative results and thus exclude these regions.

A good example of a region such as this is on chromosome 12 at marker D12S392 which lies 56cM telomeric to PAH. The two-point Lod score for D12S392 at  $\theta = 0$  was 0.204. Multipoint analysis reduced this score to -3.43 thus excluding the position of the marker, however, the Lod scores for the large gap between P. H and D12S392 ranged from -0.35 to -1.40.

#### 4.4.3.2 Regions with Lod scores between 0.00 and 1.00

The second case where regions produced Lod scores between 0 and 1.00, deserves special attention. This region produced Lod scores that were positive but not positive enough to be considered candidate regions. Because of the positive Lod scores obtained in these regions, they certainly cannot be discounted as possible candidate regions. Table 4.6 shows the main reasons (large distances and uninformativeness) why the Lod scores in this sub category were inconclusive.

**LARGE DISTANCES****UNINFORMATIVENESS**

% large Distance (>10cM)	% Small Distance (>10cM)	% Uninformative Markers	% Informative Markers
79%	21%	60%	40%
	% U.    % I.		
	100%   0%		

Table 4.6: Table showing the main reasons for inconclusive Lod scores in this sub category. 'U' indicates uninformativeness and 'I' indicates informativeness.

The regions in this category carry a certain degree of importance since they represent the next group of regions to be investigated in the event of our candidate regions failing to show linkage to the gene.

Given the size of the family, it is quite possible that Lod scores between 0.00 and 1.00 will be obtained for a substantial proportion of the genome. Simulations carried out using SLINK (Ott,1989, Weeks *et al.*, 1990) showed that under the assumption of linkage, the probability of getting a Lod score of greater than 0.00 is 97.9% while the probability of getting a Lod score of greater than 1.00 is 62.8%. From these simulations it can be seen that the chances of obtaining a Lod score between 0.00 and 1.00 is quite high. However, simulations also showed that the probability of obtaining a Lod score of greater than 0.00 under



the assumption of an unlinked marker is 40.2% (Ott, 1989, Weeks *et al.*, 1990). Thus the probability of getting a false positive result is 40.2% indicating that a substantial proportion of the regions that produce Lod scores between 0.00 and 1.00 may prove to be negative following further investigation.

Further examination of these regions is definitely necessary in order to exclude them from the study or include them as candidate regions. Because of the large distances that these regions cover, the first step in investigating them further would be to add extra markers to these regions in order to create a higher density map. Due to financial and time limitations, this was not possible in the present study. Secondly, it would be ideal to increase the sample size of the study i.e. include more non-PKD1/PKD2 families in a large collaborative study.

#### **4.5 Candidate Regions**

The final category includes all regions that produced Lod scores of 1.00 or greater and are considered first rank candidate regions. A Lod score of 1.00 was chosen as the minimum score required for a region to be considered a candidate region. Because the maximum Lod score attainable for family 1 was 1.48, any region producing a Lod score of 1.00 or greater was thought to be a reliable indication as to the location of the gene. The probability of getting a Lod score of 1.00 or greater

with this family was quite high. Simulations showed this to be 62.8%. Furthermore, simulations also showed that under the assumption of non linkage the probability of getting a Lod score of 1.00 or greater was 3.4%, i.e. the probability of a false positive result was 3.4%. Therefore, considering the small proportion of false positives for this family, a cut-off point of 1.00 was justified. This cut-off score was also appropriate considering the time and financial limitations put on this study.

#### 4.5.1 Chromosome 4

The first region to be located in this study as a candidate region was on chromosome 4p between 4p16.1 and 4p15.33. The distance between these two loci is approximately 11cM. This region was further investigated by adding 4 additional markers and including the second Bulgarian family into the study. The multipoint Lod scores for both stages of the study for this region can be seen in figure 4.1, where the markers, the distances between them, and their respective Lod scores are shown.

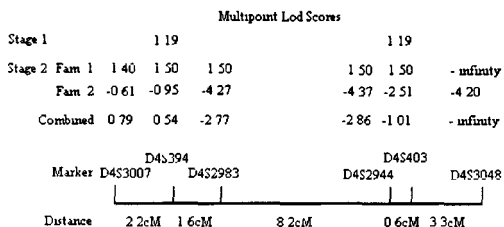


Figure 4.1: A map showing the relative positions of all markers in the positive region on chromosome 4.

A slight decrease from the two-point Lod scores of 1.42 and 1.40 for D4S394 and D4S403, to the multipoint results of 1.19 shown in figure 4.1, were still positive enough to consider this region as a candidate region for the location of the PKD3 gene.

Haplotype analysis revealed sharing among affected individuals for D4S394 and D4S403 while two recombinations place the gene centromeric to D4S412 and telomeric to D4S404 and within the region.

The positive two-point Lod score obtained in stage 1, persisted in stage 2 for family 1 with the introduction of the new markers. Two of these markers, namely D4S2983 and D4S2944, also give a Lod score greater than 1.00 (1.134 and 1.418 respectively). However, the two-point Lod scores for family 2 ranged from -0.117 to -0.619 at  $\theta = 0$ . As a result of this, the combined two-point Lod scores for the new markers were only slightly positive, ranging from 0.20 to 0.79 at  $\theta = 0$ .

The multipoint results for the higher density map (figure 4.1) in family 1 increased in stage 2 of the study. All markers except D4S3048, which produced a score of negative infinity, gave Lod scores of 1.50. The most telomeric marker in this region, namely D4S3007, produced a slightly lower Lod score of 1.40 however this is still significantly positive considering the size of the family. These results provide further indication that this region is a possible location for the PKD3 gene in family 1.

Family 2 showed quite different results. All markers that were analysed in the same region produced negative multipoint Lod scores ranging from -0.61 at D4S3007 to -4.37 at D4S2944, discounting this as a candidate region for the PKD3 gene in family 2.

Even though the Lod scores for the region between markers D4S3007 and D4S394 are not negative enough for definite exclusion, strongly results were obtained for the rest of the region

Haplotype analysis of family 2 revealed the affected siblings IV2 and IV3 inherit the same haplotype as the affected father, however the affected cousin, individual III2, presents a completely different haplotype thus excluding this region from the study for family 2.

Examining the combined multipoint Lod scores for both families for the region between D4S3007 and D4S394, it can be seen that the overall result was a decrease in the Lod scores.

This study was based on the assumption that both families share mutations in the same gene. Therefore the above results suggest that the region between 4p16.1 and 4p15.31 defined by D4S3007 and D4S3048 can no longer be considered a candidate region for the location of the PKD3 gene even though the Lod scores for the first family are close to the expected maximum.

During the second stage of screening, an additional marker was added at 4q22.1 between ATA2A03 (4p15.1) and D4S175 (4q21.1) in order to fill a large gap of 50cM. This marker, namely D4S1647 produced a combined Lod score of 1.08 on two-point analysis. Using multipoint analysis, an additive Lod score of 1.42 was obtained for both families. The flanking markers both produced negative Lod scores, however the results were positive for the bracketed region. Thus a positive region of approximately 16cM around D4S1647 was defined for both families during stage 2 of the study.

If the Lod scores obtained for each pedigree are examined individually it can be seen that for pedigree 1 the multipoint Lod score for D4S1647 is 1.40. This is close to the maximum Lod score that can be attained from family 1. The corresponding Lod score for pedigree 2 is 0.017, due to the lack of informativeness of the marker, which is only slightly positive and a great deal less than the maximum Lod score of 1.08 that can be attained with family 2.

The marker for which we obtained a Lod score of 1.4 for, namely D4S1647, is located on 4q23. The PKD2 gene has been localised to 4q21-q23 (Peters *et al.*, 1993, Kimberling *et al.*, 1993) thus our candidate region on chromosome 4 is very close to this region. In both families participating in this study, linkage to PKD2 has been excluded by Dr. N. Bogdanova, currently at the University of Muenster, Germany, with Lod scores of -2.697 (for family 1) and -2.389 (for family 2). Below is a map of the PKD2 region and the relative position of D4S1647.

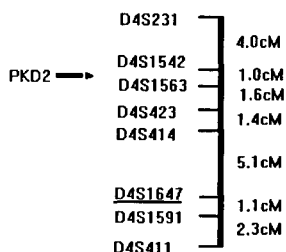


Figure 4.2: Map of the PKD2 region.

As can be seen from the map, D4S1647 is located approximately 8.1cM away from the actual location of the PKD2 gene. From the haplotype analysis of family 1, it can be seen that a common allele has been inherited by all affected individuals for D4S1647. The flanking marker, ATA2A03 which is located between D4S1647 and the

PKD2 region shows crossovers thus confirming that our results are not due to linkage to PKD2. In order to investigate this region further, a higher density map of this region needs to be created and the sample size needs to be increased. Additional markers should be added to this region and the inclusion of more non PKD1/PKD2 families as they arise would either increase the Lod score or exclude this region as a candidate for the third locus for ADPKD.

#### 4.5.2 Chromosome 6

The second candidate region to be investigated in this study was on chromosome 6p, defined by the markers D6S271 at 6p21.1 and D6S257 at 6p11.1. The distance between these markers was approximately 19cM. To create a denser map, four extra markers were added to this region. All markers are listed in figure 4.3 along with the distances between them and their respective multipoint Lod scores.

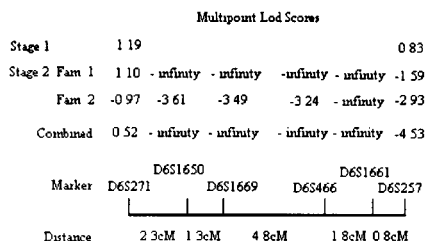


Figure 4.3: A map showing the relative positions of all markers in the positive region on chromosome 6.

In stage one, the multipoint Lod score for D6S271 was 1.10, a reduction from the two-point result (Lod score 1.40) due to the large distance to the flanking markers (D6S285 and D6S257) and the deeply negative two-point Lod score obtained at D6S285. For the second flanking marker, D6S257, the two-point Lod score was 0.174 and showed an increase in multipoint analysis to 0.83 as a result of the positive findings at D6S271.

The second stage of the study on chromosome 6 saw the introduction of four additional markers to the above defined region plus the addition of a second Bulgarian family. Multipoint analysis on family 1 showed that all of the new markers added to this region produced Lod scores of negative infinity. Marker D6S271 produced a multipoint Lod score of 1.10, a decrease from the previous value of 1.19 due to the



highly negative Lod scores obtained at the adjacent markers. Marker D6S257, which produced a positive Lod scores in stage 1, became negative with a Lod score of -1.59. This was also a result of the increased density of the map and the deeply negative Lod scores obtained for the markers added to this region.

Analysis on family 2 showed that the multipoint Lod scores were consistently negative for all markers throughout this region. Furthermore, the region between marker D6S1650 and D6S257 produced negative Lod scores below -2.00 thus excluding the region for this family. Marker D6S271, which produced a positive result for family 1, gave a negative Lod score of -0.97 for family 2 thus could not be excluded from the study but could certainly not be considered as a candidate region.

If both sets of Lod scores are combined for D6S271, then it can be seen that the overall Lod score for this marker is 0.13, due to the negative Lod score obtained for family 2.

Haplotype analysis of family 1 shows that a recombination has occurred between D6S271 and D6S1650 in individual III3, however all affected individuals have inherited the same allele of marker D6S271.

Haplotype analysis of the second family (figure 3.26), shows a recombination between D6S466 and D6S1661 in individual IV2, and inheritance of a different haplotype by the affected cousin, individual

III2, to that of the affected siblings. These results exclude 6p21.1 - 6p11.1 as the PKD3 region for family 2.

As is the case for chromosome 4, this region could not be ruled out as a candidate region in family 1. This region could not be conclusively excluded in family 2 either however, the low negative Lod score makes it an unlikely location. The small size of the positive segment and the deeply negative results at flanking markers make it an improbable PKD3 location which is likely to be definitely excluded by the addition of more markers at even smaller distances.

#### 4.5.3 Chromosome 11

The third region to be identified as a candidate region in this study was on chromosome 11p defined by the three markers D11S875, D11S902 and D11S904. Marker D11S875 at 11p15.3 is 4cM telomeric to D11S902 at 11p15.2 which is 14cM telomeric to D11S904 at 11p15.2. The total distance of this positive region is 18cM. These markers, their flanking markers, the distances and their respective Lod scores are listed in figure 4.4.

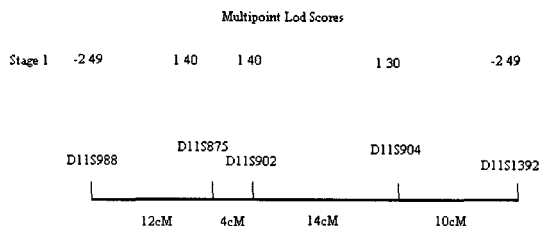


Figure 4.4: A map of the positive region on chromosome 11 showing the position of all markers in and flanking this region.

Two-point Lod scores defined a positive region between D11S875 and D11S904, where the maximum Lod score achieved was 1.40 at  $\theta = 0$  for D11S904. The other two markers in this region, D11S875 and D11S902 produced two-point Lod scores of 1.14 and 1.38 respectively. Marker D11S988, 12cM telomeric to this region, produced a Lod score of -3.09 thus excluding that position. The other flanking marker, D11S1392, 10cM centromeric to this region was also excluded, producing a Lod score of negative infinity.

The positive region defined by two-point analysis persisted with multipoint analysis. The Lod score for marker D11S904, which produced the maximum two-point Lod score of 1.40, reduced slightly to 1.30 due to the negative result obtained at the flanking marker.

D11S1392. The multipoint Lod score peaked at markers D11S875 and D11S902, with a maximum of 1.40.

Haplotype analysis on family 1 shows recombinations between D11S988 and D11S875 in individuals II2 and III1 and recombinations between D11S1392 and D11S904 in individuals II2, III3 and III4, placing the gene between D11S875 and D11S904. This coincides with the positive Lod scores obtained during two-point and multipoint analyses thus indicating that this region is a candidate region for the location of the PKD3 gene and requires further investigation in the future with the second Bulgarian family.

#### 4.5.4 Chromosome 13

The fourth region to be identified as a candidate region was on chromosome 13q, flanked by the markers D13S325 at 13q14.11 and D13S173 at 13q33.1. This region was quite large with a total distance of 57cM which included two other markers, D13S317 and D13S225 at 23cM and 20cM telomeric to D13S325 respectively. During the second stage of screening, four additional markers were added, thus creating a higher density map of the region. All markers, distances and multipoint Lod scores are shown in figure 4.5.

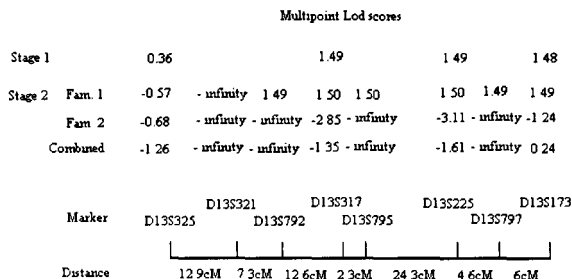


Figure 4.5: Figure showing the multipoint Lod scores for both stages of screening.

The positive region defined by the two-point analysis persisted with multipoint analysis for family 1. Looking at the multipoint Lod scores for family 1, it can be seen that positive results were obtained for the entire region between markers D13S792 and D13S173 with a maximum Lod score of 1.50 at markers D13S317, D13S795 and D13S225. The region was narrowed down during stage 2 as a result of D13S321, the addition of which gave a Lod score of -infinity. The newly added marker D13S795, which produced negative two-point Lod scores for values of  $\theta < 0.10$  and positive results for values of  $\theta > 0.15$ , became positive with multipoint analysis. This is due to the positive results obtained at the flanking markers. As can be seen from figure 4.5, a slight increase in the multipoint Lod scores from stage 1 to stage 2 is

evident. On the other hand, the addition of extra markers allowed the exclusion of part of the region centromeric to D13S321.

Haplotype analysis for family 1 indicates possible haplotype sharing among affected individuals however, because of the lack of genotyping for marker D13S792 and the lack of informativeness of D13S795, it is difficult to determine.

In family 2, all markers between D13S321 and D13S797 produced negative Lod scores below -2.00 thus excluding this region from the study for family 2. Marker D13S173 produced a negative Lod score of -1.24, which is not negative enough to exclude this marker but indicates that the gene is not in this region.

As was the case on chromosome 4, the region investigated on chromosome 13 also produced conflicting results in the two families analysed.

#### 4.5.5 Chromosome 14

The fifth candidate region was on chromosome 14q, defined by the markers D14S51 at 14q31.3 and D14S118 at 14q32.31 at a distance of 19cM from each other with D14S118 being the most telomeric marker. One marker (D14S78 at 14q32.2) was located within this region, 12cM telomeric to D14S51. As a result of the negative two-point Lod score obtained at D14S78, and the negative Lod score at marker D14S81, markers D14S51 produced deeply negative multipoint Lod

scores. Even though D14S51 produced deeply negative multipoint Lod scores, because of the positive two-point Lod score of 0.757 obtained at this marker it was investigated further with the addition of extra markers. The positive Lod scores obtained for D14S118 did persist and the maximum Lod score of 1.46 was reached. To investigate the regions around D14S51 and D14S118, six markers were added and the second Bulgarian family was introduced to the study. All markers, their relative distances and their respective multipoint Lod scores for both stages of the study are listed in figure 4.6.

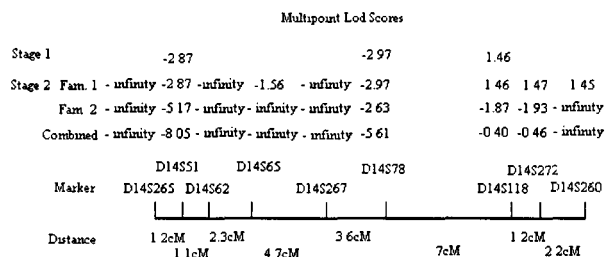


Figure 4.6: A map showing all markers used to investigate the positive region defined on chromosome 14 in stage 1 of the study .

The positive region defined by D14S118 in stage 1 persisted in stage 2 for family 1. Markers, D14S272 and D14S260, 1.2cM and 2.2cM telomeric to D14S118, produced positive Lod scores of 1.47 and

1.45 respectively. Thus the addition of extra markers defined a region telomeric to D14S118 in family 1 as a candidate region for the location of the PKD3 gene.

As can be seen from figure 4.6, consistently negative results were obtained in this region for family 2. Although markers D14S118 and D14S272 produced inconclusive Lod scores of -0.40 and -0.46 respectively, the region cannot be considered a likely candidate for the location of the PKD3 gene in family 2.

#### 4.5.6 Chromosome 17

The sixth region to be identified as a candidate region was on chromosome 17p, spanning 9cM between markers D17S849 at 17p13.3 and D17S938 at 17p13.2. Two-point analysis in stage 1 of the study defined this positive region with Lod scores of 1.12 at  $\theta = 0$  for D17S938 and 0.12 at  $\theta = 0$  for D17S849. The flanking marker D17S945 produced a slightly negative Lod score of -0.96 at  $\theta = 0$ .

This positive region persisted with multipoint analysis with a slight increase in the Lod score of D17S938 to 1.25. A notable increase was evident for D17S849, which increased from 0.12 to 1.08. This increase was due to the positive Lod scores produced by the flanking markers D17S938 (positive two-point Lod scores for all values of  $\theta$ ) and D17S578 (positive two-point Lod scores for values of  $\theta$  greater than 0.10). Furthermore, marker D17S945, which produced slightly negative



two-point Lod scores gave 1.25 in multipoint analysis. In order to investigate this region further, a single marker (D17S1845) was added between D17S849 and D17S938. All markers analysed in this region, the distances and respective Lod scores are listed in figure 4.7.

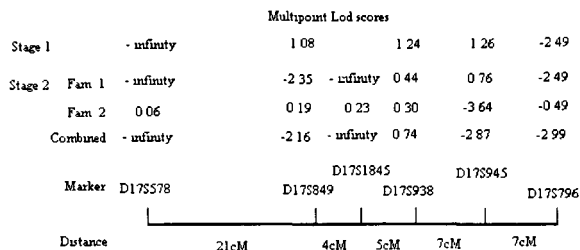


Figure 4.7: Shows the Lod scores for markers used to analyse the candidate region located on chromosome 17.

The newly added marker, D17S1845, produced a Lod score of negative infinity and the region between this marker and D17S849 was definitely excluded, with Lod scores less than -2.00. The other two markers, D17S938 and D17S945 remained positive, however due to the addition of the extra marker the Lod scores reduced to 0.44 and 0.76 respectively.

Moderately positive results were obtained for this region in family 2. There was a certain displacement of the positive findings in the two families (Figure 4.7) and the only marker producing positive Lod

scores in both families was D17S938. The Lod scores obtained for this marker however were only slightly positive. Thus 17p can no longer be considered an interesting region and further addition of markers may well exclude it for both families.

#### 4.5.7 Chromosome 22

The final positive region located during stage I was on chromosome 22q, defined by markers D22S264 at 22q11.21 and D22S345 at 22q11.23, spaced approximately 11cM apart. A third marker, D22S257, was within this region at position 22q11.23, 8cM telomeric to D22S264. All of these markers produced two-point Lod scores greater than 1.00 with the maximum Lod score of 1.44 at marker D22S345 for family 1.

Multipoint analysis confirmed this positive region with all three markers producing Lod scores greater than 1.00. The Lod score for marker D22S257 increased slightly from the two-point result of 1.32 to 1.50 as a result of the positive values obtained at the flanking markers and the relatively small distances to these markers (8cM and 3cM).

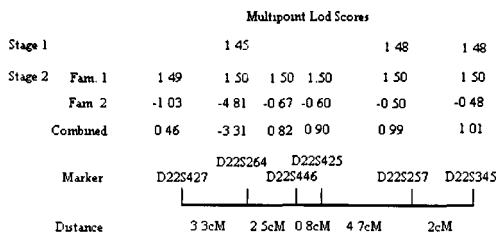


Figure 4.8: Multipoint Lod scores of markers in the positive region on chromosome 22 for both families.

In stages 2 and 3, both families were analysed for a total of ten markers spanning the entire interval. Unlike all other candidate regions where family 1 had produced some evidence of linkage during the first stage of the screening but the analysis of family 2 resulted in exclusion of the region, positive two-point results were obtained in both families for 22q11 in stage 2, while positive multipoint results were obtained for both families in stage 3.

Maximum two-point Lod score values were produced in both family 1 and family 2 at the same position, i.e. at D22S446 and recombination fraction zero. In family 1, the Lod score was 1.44 and in family 2 it was 0.775 thus, giving a maximum Lod score of 2.22 at recombination fraction zero.

For the remaining markers in the interval, family 1 produced two-point Lod scores ranging between 0.204 and 1.329 and family 2

gave values between -0.096 and 0.677. In some cases, the lower Lod scores result from the lack of informativeness of some markers. Examples include D22S425 and D22S1164 which are uninformative in family 1 and D22S446 in family 2. In other cases, the lower values were due to partial lack of genotyping information. In family 2 genotyping information is missing for some of the key individuals in the family who have not been investigated for some markers, eg. D22S1174, D22S419 and D22S1164.

The values discussed above need to be compared to the maximum obtainable two-point Lod scores shown by the computer simulations. These were 1.44 for family 1 and 1.08 for family 2. In making the comparison, one should take into account the fact that the maximum expected Lod scores were obtained under a number of assumptions which are not fulfilled in the real situation. These include: (a) the presence of individual IV:1 in family 2, which we have subsequently decided to exclude for this particular region and thus obtain a more conservative estimate of the results; (b) the simulations are based on genotyping information available for all individuals in each family whereas in actual fact information is missing for some family members and some markers and (c) in the simulations the maximum values are obtained for fully informative markers which is not the case for a number of the markers on 22q11. Given these

limitations, the Lod scores obtained in this study are reasonably close to the expected maximum Lod score.

Haplotype analysis and hence the multipoint Lod scores obtained using Genehunter provide even stronger evidence that 22q11 is an important PKD3 candidate region. Haplotype sharing was evident in both families over an interval spanning over 10 cM. Moreover, recombinations in each family helped to narrow down the critical interval. As mentioned in the Results section, a recombination in family 1 places the gene centromeric to D22S683, whereas a crossover in family 2 places PKD3 telomeric of D22S264. The multipoint Lod scores (Fig. 4.9) were positive over the entire interval. The highest values of 2.95 were obtained for a small region of less than 1cM, which can thus be considered as the most likely location of the gene. The Genthon map of this interval is as follows: D22S257 - 0.7 cM - D22S1174/D22S419 (placed at distance 0 cM) - 0.2 cM - D22S1164.

Although international collaboration is discussed further at the end of the thesis, one should mention here the results on this region obtained by other research groups. The information obtained in our study was made available to colleagues in the US and the Netherlands and was used to investigate additional families of North-Western European descent (Dutch, Belgian and French-Canadian) not linked to PKD1 or PKD2. None of these families produced evidence of linkage

to 22q11, suggesting the existence of further heterogeneity. Nevertheless, our results strongly suggest that this region is a very promising candidate. The inclusion of the missing genotyping information and the definitive clarification of the affection status of the individual in the youngest generation in family 2 should allow us to obtain a Lod score which is close to or even in excess of the threshold Lod score value of 3 and thus provide more conclusive evidence of the location of one of the minor PKD mutations on 22q11. Additional evidence will also be sought through the study of other non-PKD1/non-PKD2 families of South-Eastern European background where founder effect for the same rare mutation is more likely.

The positive region defined by multipoint analysis in family 1 during stage 2 was not so evident in family 2. The multipoint Lod scores for this family were inconclusive, ranging from -0.48 to -0.91, for all markers between D22S446 and D22S345 ie. the positive region defined in family 1. Marker D22S264 was excluded in this family with a negative Lod score of -3.77. Even though positive two-point results were obtained for this family, once multipoint analysis was carried out the Lod scores became negative, albeit inconclusive. A possible reason for this reduction in Lod scores from positive two-points to negative multipoint results is that D22S264 was informative and produced a negative Lod score of -4.81. Because of this deeply negative Lod score and the fact that the region is small (approximately

10cM) the Lod scores of other marker in this region may have been affected, resulting in slightly negative, inconclusive Lod scores. This theory seems further plausible given the fact that Genehunter calculates multipoint Lod scores using the haplotype of the entire chromosome and not just two or three markers at a time. This theory was tested by omitting D22S264 and performing the calculations without this marker. The resulting Lod scores were slightly positive ranging from 0.1 to 0.5. These results were inconclusive as expected, due to the uninformativeness of the markers in this region. However this indicates that the low negative Lod score obtained at D22S264 does have an effect on the Lod scores of other markers in this region, causing them to become negative.

The positive two-point Lod scores obtained for both families suggests the existence of a common positive region. However, due to the inconclusive multipoint results obtained in stage 2 for family 2, no real conclusions could be drawn about the region on 22q11. The inconclusive multipoint Lod scores and the positive two-point Lod scores obtained in family 2, coupled with the fact that this region was positive in the first family, make it difficult to determine if this is a candidate region for the location of the PKD3 gene in family 2. The multipoint Lod scores suggest this is a candidate region in family 1 however, because of the inconclusive multipoint Lod scores obtained in family 2 it is unclear whether this is a candidate region in this family.

Because of these inconclusive Lod scores and the fact that the combined two-point Lod scores (table 3.32) were positive for the interval D22S446-425-257-345, with a maximum two-point Lod score of 2.22 at D22S446, this region is considered the best candidate region, warranting further investigation.

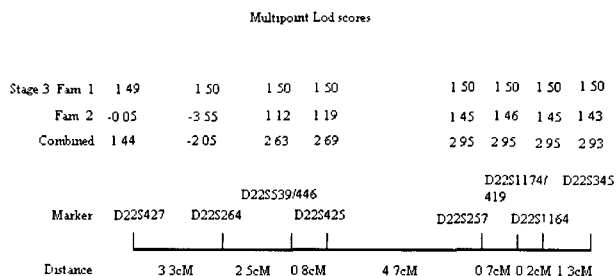


Figure 4.9: Multipoint Lod scores for all markers analysed in stage 3 of the study for both families.

As can be seen from figure 4.9, in stage 3 a positive region is defined in both families with the addition of extra markers in this region. The multipoint Lod score for all markers in this region for both families are listed in figure 4.9.

The Lod scores for family 1 ranged from 1.49 to 1.50 with all markers between D22S427 and D22S345 producing Lod scores of



greater than 1.00. This region is quite obviously a candidate region for the location of the PKD3 gene in family 1, given the fact that this positive region has been evident throughout 3 stages of screening. Analysing family 2 with the same markers it can be seen that the positive region defined in family 1 is also evident with the addition of the extra markers in family 2. Marker D22S264 produced a negative Lod score of -3.55, thus excluding the position of this marker. Furthermore, if the haplotype is examined it can be seen that a recombination between D22S427 and D22S264 in individual III2 places the gene below D22S264. All markers between D22S539 and D22S345 produced Lod scores of greater than 1.00 with a maximum Lod score of 1.45 being obtained at several markers between D22S257 and D22S1164. The negative Lod score at D22S264 therefore redefined the positive region in family 2 as being between D22S539 and D22S345 with the maximum Lod score in the region being 1.45. The Lod scores obtained in this stage greatly increased from the Lod scores obtained in stage 2 of the study, due to the addition of the extra markers and the subsequent increase in density of the genetic map. The positive region defined here for family 2 coincides with part of the positive region on family 1 where the Lod scores were 1.50 for all markers.

Concluding, a common positive region was defined in both families between D22S539 and D22S345 and included 6 other

markers. The maximum Lod score obtained in this region was 2.95 at markers D22S1174 and D22S419. As mentioned before, simulations showed that the maximum two-point Lod score obtainable for both families combined was 2.52. Our maximum two-point Lod score in this study was 2.22, which is very close to the maximum of 2.52 thus providing concrete evidence that the gene may be in this region. Furthermore, when multipoint analysis was performed, the maximum Lod score obtained in this study increased to 2.95 which is very close to the minimum Lod score requirement for linkage (3.00). This finding therefore provides the most convincing evidence yet that the gene may very well be in the region defined on chromosome 22. To examine this region further these markers need to be tested against other non PKD1/PKD2 families as they are discovered.

#### **4.6 Candidate Genes**

Gene homology between species is one method used to aid in the location of genes on the human genome. By locating the gene/genes for polycystic kidney disease in animals and finding the homologous region on the human genome an indication may be obtained as to where the gene may be located in humans.

Bihoreau *et al.*, (1997) located a gene for polycystic kidney disease in rats on chromosome 5. She showed that this gene (PKDr1), localised on rat chromosome 5 near marker D5Mit10 was not

homologous to the human PKD1 gene or the human PKD2 gene. Instead, the region this gene was localised to on the rat chromosome 5, was homologous to the region 8q21.3-q22.1 on the human genome. In the 8q21-q22 region, our Lod scores are deeply negative, thus excluding it as a candidate region for the PKD3 gene. The interval 8q24.13 to 8q24.3 defined by markers D8S514 and D8S284 was the only region not excluded on 8q where the Lod scores range from -1.7 to 0.24, with the highest Lod score being 0.24. Marker D8S284 is situated approximately 25cM from the telomere, consequently numerous genes may be located in that interval. It may well be that analysing markers further down chromosome 8q may reveal larger Lod scores and provide another candidate region for the location of the PKD3 gene.

Earlier in 1997, Janaswami *et al.* reported a new mouse PKD mutation *kat* localised to mouse chromosome 8. It was shown that this region of the mouse genome was homologous to chromosome 19p on the human genome. Our results however, showed no positive Lod scores for this region and multipoint analysis actually excluded chromosome 19p.

#### **4.7 Clinical heterogeneity**

Clinical heterogeneity is displayed by patients. It is thought that the clinical heterogeneity of ADPKD correlates with the genetic heterogeneity of the disease. In 1992 Ravine *et al.*, provided evidence

that non-PKD1 patients lived longer, had a lower risk of progression to renal failure and were less likely to develop cysts and hypertension. This evidence was supported by Peters *et al.*, (1993), Jeffery *et al.*, (1993) and the European Consortium (1994) where it was shown that PKD1 patients expressed a more severe clinical phenotype than that of non-PKD1 patients. In 1993 Wright *et al.*, reported a large three generation family from Northern Ireland unlinked to the PKD1 gene which showed a milder phenotype to that of the 'classic' phenotype shown by PKD1 patients. Further supporting this observation was the report by Jeffery *et al.*, (1993) that showed a family of Sicilian origin not linked to the PKD1 gene and showing a milder progression than families linked to PKD1.

The theory that non-PKD1 families show a milder phenotypic expression to that of PKD1 families does not apply to all families however. Some studies report no significant difference between PKD1 families and non-PKD1 families. In 1988 [unclear] *et al.* reported a non-PKD1 family with a clinical phenotype indistinguishable from that of other families previously studied that were linked to PKD1. In 1992 Wright *et al.* also described a non-PKD1 family which expressed the typical PKD1 phenotype. An obvious variation of phenotypic expression is evident within the group of families linked to the PKD1 gene. Some show a milder progression of ADPKD while other families display a similar phenotype to that shown by PKD1 families.

Furthermore, variation is also evident within families, with affected individuals in the same family expressing differences in severity of progression to renal failure, age at onset of renal ADPKD and presence of renal cysts (Parfrey *et al.*, 1990, Pound *et al.*, 1995).

Such phenotypic variation is also evident in the non-PKD1/non-PKD2 families. In 1995, Daoust *et al.* reported a three generation family of French-Canadian origin consisting of 6 affected individuals not linked to PKD1 or PKD2 showing a clinical phenotype similar to that of PKD1. Supporting this observation was deAlmeida *et al.* (1995) who presented a Portuguese family not linked to PKD1 or PKD2 which showed a similar clinical expression to that of PKD1. DeAlmeida also described variation within this family with dizygotic twins displaying clinical differences. One twin is aged 68, has pre-terminal renal failure and is hypertensive while the other twin has normal renal function and is normotensive. In 1996, Turco *et al.* described an Italian family displaying a milder clinical phenotype to that of other PKD3 families reported.

The two Bulgarian families in this study also show differences in severity of PKD. The first family shows a clinical progression similar to that of PKD1 families however, intrafamilial variation is also present. Three individuals in the first family showed normal kidney function into their 50's while two other individuals developed CRF in their early 50's. Conversely, the second family displayed a more severe form of the

disease with an earlier onset of CRF and a more rapid onset of end stage renal failure (ESRF). One individual showed an onset of CRF in the early 50's and ESRF within 5 years, which is considered quite severe given that the average time of progression from CRF to ESRF is usually about 10 years. A second individual in this family displayed an even more severe course of this disease reaching CRF in the 40's and ESRF within a space of 5 years.

As can be seen clinical heterogeneity is the rule rather than the exception in ADPKD. Although PKD1 patients in general tend to have a more severe course of the disease than non-PKD1 individuals, using phenotypic severity as a criterion in genetic classification is not possible. This study was based on the assumption of linkage homogeneity between the two families investigated and this was the only working assumption possible for the purposes of the study. Theoretically however, the disease in the two families may be caused by mutations in different genes. This being the case then additional nonPKD1/PKD2 families need to be added to the study in an attempt to conclusively find the gene. However, because of the small size of the families being studied the only working solution was to assume genetic homogeneity until proven otherwise. Indeed, conflicting evidence was obtained for most candidate regions identified in stage 1 of this study which were then discarded in most cases due to lack of linkage in family 2. The results of this project point to chromosome 22 as the best candidate

region in both families. However, these findings should be interpreted with caution, as possible further locus heterogeneity may be present. Completion of the haplotypes by filling in the missing genotyping information and re-evaluation of the individual in family 2 with unknown affection status should be carried out before any definite conclusions should be drawn. It must be reiterated that this study was based on the working assumption that no further locus heterogeneity existed. This being the case, the results of this study strongly indicate that the gene is in this region on chromosome 22q and that the addition of more non-PKD1/PKD2 as they arise would show linkage to this region.

#### **4.8 International Collaboration**

These regions need to be investigated further, however, due to the small size of the families in this study this investigation can go no further. Additional families need to be discovered and added to the studying order to increase the sample size. Once the sample size has increased, new markers need to be added to the above mentioned regions in order to create a higher density map of these regions and possibly map the PKD3 gene. Extensive international collaboration will make this possible.

As mentioned in the literature review various groups around the world have published ADPKD families not linked to the PKD1 or PKD2

gene. Once candidate regions had been located and confirmed with our families (completion of stage 2 of the study) correspondence was set up with these groups.

Contact was made with Dr. Dorien Peters, Department of Human Genetics, Sylvius Laboratory, Lieden University, Holland who has access to 3 families (1 Belgian and 2 Dutch) not linked to PKD1 or PKD2. Information on the positive regions were sent to Dr. Peters, who used them to analyse the Bulgarian and Dutch families. All three regions were excluded in these families. Correspondence was also made with a second group, at The Albert Einstein College of Medicine, Bronx, New York headed by Dr. Stefan Somlo. Dr. Somlo published a French-Canadian family with 11 members, of which 6 were affected, not linked to the PKD1 or PKD2 genes. Again primers for the markers which gave the highest Lod scores in our regions were sent to Dr. Somlo. These markers were used to analyse the French-Canadian family and were excluded in this family.

Results have also been exchanged with the Portuguese group in order for both groups to compare results and determine if any compatible regions exist between the two sets of results where the maximum Lod scores for each family has been obtained. Simulations were performed in Perth, Western Australia using SLINK (Ott, 1989, Weeks *et al.*, 1990) where the maximum Lod score obtainable for this family was found to be 2.69, under the assumption of linkage. Thus,



assuming no further heterogeneity exists, if the Lod scores for both Bulgarian families and the Portuguese family are combined then linkage of the gene is possible.

The analysis of the Portuguese family was carried out by Salome deAlmeida at the Clinica Harmonia in Portugal. The first part of this study involved the investigation of a Portuguese family with 10 members and 7 affected individuals, using 143 markers from the Research Genetics screening set version 5a. The second part of the study saw the extension of this family with the inclusion of two siblings, one of which was affected. The extended family was analysed with 113 markers from the Research Genetics screening set version 6. Below is a table of comparison, showing all regions where Lod scores of greater than 1.00 were obtained in either the Portuguese study or our study on the Bulgarian families.

<b>Two-point Lod scores</b>			
<b>Marker</b>	<b>Location</b>	<b>Portuguese</b>	<b>Bulgarian</b>
D1S518	1q25.2	1.60	-infinity
D4S1647	4q22.1	excluded	1.42
D7S517	7p22.3	1.60	-infinity
D11S902	11p15.2	-infinity	1.41
D17S796	17p13.2	1.57	-2.49
D19S254	19q13.43	1.54	-infinity
D20S103	20p13	1.13	-1.46
D22S446	22q11.21	Gap	2.22

Table 4.7: This table shows the comparison between the positive regions obtained for the Portuguese family and the Bulgarian families.

From the table it can be seen that no evidence of common regions of significance exists between the two studies. Marker D17S796 gave a positive Lod score of 1.57 for the Portuguese family, however analysing the Bulgarian families with this marker resulted in exclusion, with a multipoint Lod score of -2.49. It must be noted that this negative result was only obtained at the position of the marker. If the Lod scores for the regions immediately adjacent to this marker are examined it can be seen that they are positive ranging from 0.29 to 0.65. Furthermore, the marker D17S945 which is 7cM telomeric to D17S796 produced a positive Lod score of 0.76 for our families. Thus it can only be claimed

that the marker has been excluded and not the whole region. Further to this, because of the positive Lod scores obtained in this region (not necessarily at marker D17S796) it may be that further study of this region may reveal a common pattern of positive Lod scores between the Bulgarian family and the Portuguese family. Our study produced the highest two-point Lod score of 2.22 at marker D22S446 while the Portuguese study failed to cover this region. It would be interesting to see what Lod scores were obtained for this region using the same markers on the Portuguese family.

Dr. Alberto Turco at the Institute of Genetics, University Hospital Polyclinic "B.Roma", Verona, Italy is currently in the process of setting up a PKD3 consortium. So far several groups have been contacted by Dr. Turco including Dr. Stephan Somlo in New York who is willing to collaborate. Other groups contacted by Dr. Turco include Dr. Eliecer Coto at the Laboratory Immunologia, Hospital Covadonga, Oviedo, Spain who has sent DNA from a PKD3 family to Dr. Turco. Dr. J. Zhou in Harvard, Boston, USA, Dr. David Ravine, Cardiff, Wales, Dr. C. Deltas at the Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus and Dr. M. Martin Prata in Lisbon Portugal have also been contacted in the hope of expanding the network of collaboration. Detailed correspondence has been made with Dr. Turco who expressed his wish that we join the consortium in an effort to map the PKD3 gene. The results of this study have been sent to Dr. Turco along with DNA

for both Bulgarian families in an effort to expand this study. The results of Dr. Turco's findings once our results are combined with his results are eagerly awaited.

International collaboration is the next logical step in an attempt to map the PKD3 gene and also to determine whether or not further locus heterogeneity exists within the ADPKD population.

#### **4.9 Conclusion**

This study has identified 3 candidate regions that are possible locations of the PKD3 gene. The first is on 4q22.1 spanning a distance of 16cM with a maximum Lod score of 1.42 at marker D4S1647 and flanked by markers ATA2A03 - D4S175. The second is on 11p15.2 spanning a distance of 18cM with a maximum Lod score of 1.41 at D11S902 and flanked by the markers D11S875 and D11S904. The third candidate region is located on 22q11.21 spanning a distance of 7cM with a maximum two-point Lod score of 2.22 at marker D22S446 and flanked by marker D22S425. Furthermore, multipoint analysis on this region produced a Lod score of 2.95 at three markers in this region, namely D22S1174, D22S419 and D22S1164.

These regions need to be investigated further however, due to the small size of the families in this study this investigation can go no further. Additional families need to be discovered and added to the studying order to increase the sample size. Once the sample size has increased, new markers need to be added to the above mentioned regions

in order to create a higher density map of these regions and possibly map the PKD3 gene.

The international PKD3 consortium is likely to achieve this goal. However, as indicated previously in the discussion of our own findings, further locus heterogeneity is a possibility which deserves close attention. This possibility is enhanced by the fact that none of the families of North-West European descent showed linkage to any of our candidate regions. The mechanisms of regulation and signalling involved in cell proliferation are complex and can, theoretically, be affected by mutations in a large number of different genes. The best way to deal with the controversy would be for the international study to group together families from populations and ethnic groups which common gene pools such as the Mediterranean on one side and North-Western Europe on the other. A founder mutation is possible although not very likely, for the Bulgarian, Cypriot, Italian and perhaps Portuguese families on one hand and the French-Canadian, Belgian and Dutch on the other.

## **APPENDIX A**

### **PANEL 21 FAM MARKERS**

<u><b>Master Mix</b></u>	<u><b>1x</b></u>	<u><b>x</b></u>
10x Reaction Buffer (commercially supplied)	0.5ul	
dNTPs (5mM)	0.25ul	
MgCl <sub>2</sub> (25mM)	0.5ul	
Amplitaq GOLD	<u>0.05ul</u>	
	1.30ul	
<u><b>Multiplex Mix</b></u>	<u><b>1x</b></u>	<u><b>x</b></u>

H <sub>2</sub> O	0.19ul	
Master Mix	1.30ul	
Primers		
D16S405	0.48ul	
D16S401	0.32ul	
D16S411	0.32ul	
D15S130	0.63ul	
D16S515	<u>0.96ul</u>	
	4.20ul	
DNA (50ng/ul)	0.80ul	

### **PANEL 21 HEX MARKERS**

<u><b>Master Mix</b></u>	<u><b>1x</b></u>	<u><b>x</b></u>
10x Reaction Buffer (commercially supplied)	0.5ul	
dNTPs (5mM)	0.25ul	
MgCl <sub>2</sub> (25mM)	0.5ul	
Amplitaq GOLD	<u>0.05ul</u>	
	1.30ul	

<b><u>Multiplex Mix 1</u></b>	<b><u>1x</u></b>	<b><u>x</u></b>
H <sub>2</sub> O	1.62ul	
Master Mix	1.30ul	
Primers		
D16S520	0.32ul	
D15S165	0.48ul	
D15S131	<u>0.48ul</u>	
	4.20ul	

DNA (50ng/ul) 0.80ul

<b><u>Multiplex Mix 2</u></b>	<b><u>1x</u></b>	<b><u>x</u></b>
H <sub>2</sub> O	1.90ul	
Master Mix	1.30ul	
Primers		
D16S503	<u>1.0ul</u>	
	4.20ul	

DNA (50ng/ul) 0.80ul

## PANEL 21 TET MARKERS

<b><u>Master Mix</u></b>	<b><u>1x</u></b>	<b><u>x</u></b>
10x Reaction Buffer (commercially supplied)	0.5ul	
dNTPs (5mM)	0.25ul	
MgCl <sub>2</sub> (25mM)	0.5ul	
Amplitaq GOLD	<u>0.05ul</u>	
	1.30ul	

<b><u>Multiplex mix</u></b>	<b><u>1x</u></b>	<b><u>x</u></b>
H <sub>2</sub> O	1.62ul	
Master Mix	1.30ul	
Primers		
D15S127	0.32ul	

D16S511	0.32ul
D15S153	0.32ul
D15S117	<u>0.32ul</u>
	4.20ul
DNA (50ng/ul)	0.80ul

#### **PANEL 22 FAM MARKERS**

<b>Master Mix</b>	<b>1x</b>	<b>x</b>
10x Reaction Buffer (commercially supplied)	0.5ul	
dNTPs (5mM)	0.25ul	
MgCl <sub>2</sub> (25mM)	0.5ul	
Amplitaq GOLD	<u>0.05ul</u>	
	1.30ul	
<b>Multiplex Mix</b>	<b>1x</b>	<b>x</b>
H <sub>2</sub> O	1.78ul	
Master Mix	1.30ul	
Primers		
D15S205	0.48ul	
D16S415	0.16ul	
D16S420	<u>0.48ul</u>	
	4.20ul	
DNA (50ng/ul)	0.80ul	

#### **PANEL 22 HEX MARKERS**

<b>Master Mix</b>	<b>1x</b>	<b>x</b>
10x Reaction Buffer (commercially supplied)	0.5ul	
dNTPs (5mM)	0.25ul	
MgCl <sub>2</sub> (25mM)	0.5ul	
Amplitaq GOLD	<u>0.05ul</u>	
	1.30ul	



<b><u>Multiplex Mix 1</u></b>		<b><u>1x</u></b>	<b><u>x</u></b>
H <sub>2</sub> O		1.10ul	
Master Mix		1.30ul	
Primers			
	D16S423	0.90ul	
	D15S126	<u>0.90ul</u>	
		4.20ul	
DNA (50ng/ul)		0.80ul	
<b><u>Multiplex Mix 1</u></b>		<b><u>1x</u></b>	<b><u>x</u></b>
H <sub>2</sub> O		1.90ul	
Master Mix		1.30ul	
Primers			
	D16S407	<u>1.00ul</u>	
		4.20ul	
DNA (50ng/ul)		0.80ul	
<b><u>PANEL 22 TET MARKERS</u></b>			
<b><u>Master Mix</u></b>		<b><u>1x</u></b>	<b><u>x</u></b>
10x Reaction Buffer (commercially supplied)		0.5ul	
dNTPs (5mM)		0.25ul	
MgCl <sub>2</sub> (25mM)		0.5ul	
Amplitaq GOLD		<u>0.05ul</u>	
		1.30ul	
<b><u>Multiplex Mix</u></b>		<b><u>1x</u></b>	<b><u>x</u></b>
H <sub>2</sub> O		2.26ul	
Master Mix		1.30ul	
Primers			
	D15S120	0.16ul	
	D15S128	0.16ul	
	D16S516	<u>0.32ul</u>	
		4.20ul	
DNA (50ng/ul)		0.80ul	

### **8% Acrylamide**

1. Dissolve 420.5g Urea in H<sub>2</sub>O and make up to 600ml
2. Add 200ml Liqui-mix 19
3. Deionise for 30 minutes
4. Filter
5. Make up to 900ml
6. Add 100ml of 10X TBE
7. Degas for 30 minutes
8. Wrap bottle in foil and store at 4°C

### **6% Acrylamide**

1. Dissolve 420.5g in H<sub>2</sub>O and make up to 600ml
2. Add 150ml Liqui-mix 19
3. Deionise for 30 minutes
4. Filter
5. Make up to 900ml
6. Add 100ml of 10X TBE
7. Degas for 30 minutes
8. Wrap bottle in foil and store at 4°C

### **4% Acrylamide**

1. Dissolve 420.5g in H<sub>2</sub>O and make up to 600ml
2. Add 100ml Liqui-mix 19
3. Deionise for 30 minutes
4. Filter

5. Make up to 900ml
6. Add 100ml of 10X TBE
7. Degas for 30 minutes
8. Wrap bottle in foil and store at 4°C

**10X TBE (makes 1L)**

Trisma	108g
Boric Acid	55g
EDTA	9.3g
H <sub>2</sub> O	make up to 1L.

**1X TBE (makes 1L)**

1X TBE	100ml
H <sub>2</sub> O	900ml

## APPENDIX B

### GENEHUNTER - Complete Linkage Analysis (version 1.1)

<b>CHROMOSOME 1</b>					
analyzing pedigree 1 ..					
using non-originals 1 2 3 4 5 6 10					
	position	LOD score	NPL score	p-value	information
	-10.00	-1.066086	-0.281645	0.484375	0.317227
	-8.00	-1.236920	-0.321828	0.500000	0.364148
	-6.00	-1.464377	-0.366525	0.593750	0.420684
	-4.00	-1.794797	-0.416183	0.593750	0.490383
	-2.00	-2.375656	-0.471288	0.625000	0.580246
DIS243	0.00	-10000.000	-0.532358	0.671875	0.720021
	3.00	-2.635401	-0.472514	0.625000	0.597370
	6.00	-2.145533	-0.426774	0.593750	0.540215
	9.00	-1.917999	-0.393611	0.593750	0.513987
	12.00	-1.810233	-0.371833	0.593750	0.512425
DIS214	15.00	-1.783706	-0.360547	0.593750	0.538668
	17.80	-1.958169	-0.426940	0.593750	0.438998
	20.60	-2.225459	-0.498221	0.625000	0.427342
	23.40	-2.652157	-0.574338	0.703125	0.468081
	26.20	-3.457638	-0.655074	0.750000	0.566333
DIS228	29.00	-10000.000	-0.739971	0.843750	0.782733
	33.80	-2.711878	-0.588725	0.703125	0.488738
	38.60	-2.069312	-0.452832	0.625000	0.393415
	43.40	-1.894887	-0.353777	0.593750	0.402306
	48.20	-2.114807	-0.313948	0.500000	0.517808
DIS552	53.00	-10000.000	-0.360608	0.593750	0.839822
	55.60	-2.044329	0.046550	0.359375	0.681926
	58.20	-1.578092	0.439846	0.203125	0.620077
	60.80	-1.409370	0.826809	0.062500	0.607402
	63.40	-1.464438	1.214759	0.062500	0.641015
DIS233	66.00	-10000.000	1.611001	0.062500	0.752601
	67.60	0.061761	1.561744	0.062500	0.606268
	69.20	0.358203	1.521728	0.062500	0.541459
	70.80	0.531700	1.490462	0.062500	0.519870
	72.40	0.655723	1.467434	0.062500	0.540326
DIS186	74.00	0.753026	1.452095	0.062500	0.633200
	76.80	0.690943	0.854432	0.062500	0.450968
	79.60	0.546542	0.363211	0.250000	0.408601
	82.40	0.284965	-0.029158	0.406250	0.450236
	85.20	-0.235348	-0.326158	0.500000	0.581492
MYCL1	88.00	-10000.000	-0.527199	0.671875	0.891579
	89.40	-4.123922	-0.507122	0.625000	0.758806
	90.80	-3.807241	-0.489056	0.625000	0.701472
	92.20	-3.839574	-0.472640	0.625000	0.683113
	93.60	-4.221245	-0.457518	0.625000	0.701811
DIS162	95.00	-10000.000	-0.443337	0.625000	0.784077

	98.20	-1.778514	-0.202947	0.406250	0.563599
	101.40	-1.492091	0.000813	0.406250	0.479496
	104.60	-1.562007	0.188845	0.328125	0.470197
	107.80	-1.991469	0.382880	0.250000	0.533369
DIS550	111.00	-10000.000	0.606143	0.125000	0.720094
	113.60	-3.627760	0.242843	0.312500	0.547654
	116.20	-3.202567	-0.055911	0.406250	0.493876
	118.80	-3.133892	-0.296567	0.500000	0.504646
	121.40	-3.420315	-0.483395	0.625000	0.580745
DIS207	124.00	-10000.000	-0.619205	0.718750	0.778688
	126.80	-1.733384	-0.418724	0.593750	0.588180
	129.60	-1.212948	-0.222134	0.406250	0.502947
	132.40	-0.944194	-0.028841	0.406250	0.470694
	135.20	-0.780611	0.162035	0.328125	0.487680
DIS188	138.00	-0.677347	0.351559	0.250000	0.586562
	143.20	-0.698690	0.231959	0.312500	0.349208
	148.40	-0.858169	0.100262	0.359375	0.291836
	153.60	-1.199546	-0.041529	0.406250	0.343129
	158.80	-1.931276	-0.192522	0.406250	0.515453
DIS534	164.00	-10000.000	-0.353646	0.593750	0.951187
	165.80	-6.518702	-0.347016	0.500000	0.776533
	167.60	-6.118941	-0.400544	0.593750	0.720810
	169.40	-6.294798	-0.513668	0.625000	0.729738
	171.20	-7.120060	-0.686936	0.796875	0.803824
DIS305	173.00	-10000.000	-0.922008	0.968750	1.000000
	178.00	-4.248598	-0.865513	0.921875	0.702635
	183.00	-3.341634	-0.834981	0.906250	0.594628
	188.00	-3.029936	-0.824484	0.859375	0.580639
	193.00	-3.165991	-0.830071	0.906250	0.656506
DIS318	198.00	-10000.000	-0.848384	0.906250	0.898780
	199.80	-3.091454	-0.707842	0.796875	0.756711
	201.60	-2.736795	-0.583436	0.703125	0.701716
	203.40	-2.732742	-0.474522	0.625000	0.693974
	205.20	-3.079155	-0.380561	0.593750	0.733121
ATA4E02	207.00	-10000.000	-0.301109	0.500000	0.856412
	209.40	-3.513745	-0.354544	0.593750	0.738470
	211.80	-3.081802	-0.395144	0.593750	0.696577
	214.20	-3.011554	-0.423574	0.593750	0.702260
	216.60	-3.299301	-0.440237	0.625000	0.759203
DIS518	219.00	-10000.000	-0.445253	0.625000	0.914760
	223.40	-2.233746	-0.480487	0.625000	0.739442
	227.80	-1.890020	-0.510105	0.625000	0.675407
	232.20	-1.887584	-0.538497	0.671875	0.667425
	236.60	-2.226660	-0.568606	0.671875	0.709322
DIS249	241.00	-10000.000	-0.602244	0.718750	0.829540
	244.40	-2.700165	-0.551046	0.671875	0.569571
	247.80	-2.416924	-0.490761	0.625000	0.473665
	251.20	-2.494759	-0.420923	0.593750	0.461462
	254.60	-2.940435	-0.340155	0.500000	0.529770
DIS549	258.00	-10000.000	-0.246356	0.406250	0.738505
	259.80	-4.241495	-0.245355	0.406250	0.723010
	261.60	-4.011647	-0.245843	0.406250	0.730780
	263.40	-4.181210	-0.247492	0.406250	0.756294
	265.20	-4.823972	-0.249974	0.406250	0.802475

D1S103	267.00	-10000.000	-0.252961	0.484375	0.889998
	274.60	-2.595863	-0.172157	0.406250	0.563067
	282.20	-2.135903	-0.109725	0.406250	0.443135
	289.80	-2.190554	-0.047850	0.406250	0.422849
	297.40	-2.760620	0.033162	0.406250	0.507129
D1S102	305.00	-10000.000	0.159026	0.328125	0.841779
	307.00	-4.063831	0.106571	0.359375	0.667234
	309.00	-3.180612	0.060772	0.359375	0.558949
	311.00	-2.673184	0.020898	0.406250	0.477088
	313.00	-2.320233	-0.013702	0.406250	0.412063
	315.00	2.052252	-0.043608	0.406250	0.359003

## CHROMOSOME 2

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-0.829647	-0.043461	0.406250	0.386921
	-8.00	-0.981295	-0.041830	0.406250	0.433361
	-6.00	-1.187977	-0.040211	0.406250	0.487670
	-4.00	-1.495921	-0.038662	0.406250	0.552422
	-2.00	-2.052680	-0.037251	0.406250	0.632581
D2S207	0.00	-10000.000	-0.036059	0.406250	0.748909
	2.60	-3.741553	0.047570	0.359375	0.633325
	5.20	-3.460861	0.147925	0.359375	0.594356
	7.80	-3.496626	0.268588	0.265625	0.600211
	10.40	-3.746986	0.413667	0.250000	0.653405
D2S423	13.00	-10000.000	0.587941	0.125000	0.797069
	15.00	-4.725507	0.530453	0.187500	0.677356
	17.00	-4.787148	0.479385	0.203125	0.636276
	19.00	-5.110370	0.434437	0.203125	0.642976
	21.00	-5.847289	0.395406	0.250000	0.700350
D2S131	23.00	-10000.000	0.362190	0.250000	0.856227
	26.40	-3.578232	0.311397	0.265625	0.640072
	29.80	-2.661986	0.284603	0.265625	0.530225
	33.20	-2.138305	0.279232	0.265625	0.468541
	36.60	-1.780772	0.293392	0.265625	0.444188
D2S405	40.00	-1.517372	0.325782	0.265625	0.460440
	42.60	-1.394486	0.298998	0.265625	0.432142
	45.20	-1.293897	0.281607	0.265625	0.426685
	47.80	-1.212142	0.272742	0.265625	0.439001
	50.40	-1.147119	0.271694	0.265625	0.471374
D2S177	53.00	-1.097749	0.277891	0.265625	0.540013
	56.20	-1.080396	0.261551	0.265625	0.517696
	59.40	-1.086322	0.253037	0.312500	0.522164
	62.60	-1.120049	0.251420	0.312500	0.546609
	65.80	-1.190546	0.255882	0.312500	0.592796
D2S406	69.00	-1.315711	0.265671	0.265625	0.671952
	71.00	-1.451966	0.235725	0.312500	0.682449
	73.00	-1.644255	0.208095	0.312500	0.707042
	75.00	-1.938676	0.182416	0.328125	0.744760
	77.00	-2.482670	0.158337	0.328125	0.798800

D2S441	79.00	-10000.000	0.135503	0.359375	0.885493
	82.40	-3.203747	0.106459	0.359375	0.759793
	85.80	-2.855897	0.084359	0.359375	0.709506
	89.20	-2.853811	0.068635	0.359375	0.699447
	92.60	-3.197506	0.058827	0.359375	0.725742
D2S428	96.00	-10000.000	0.054563	0.359375	0.798585
	97.00	-4.161950	0.046935	0.359375	0.798112
	98.00	-3.621624	0.039794	0.359375	0.805031
	99.00	-3.335050	0.033126	0.406250	0.818264
	100.00	-3.155367	0.026914	0.406250	0.839127
D2S417	101.00	-3.037225	0.021143	0.406250	0.875453
	103.20	-2.919301	0.018866	0.406250	0.833783
	105.40	-2.939848	0.018621	0.406250	0.814066
	107.60	-3.108903	0.020425	0.406250	0.808336
	109.80	-3.556923	0.024304	0.406250	0.815566
D2S436	112.00	-10000.000	0.030293	0.406250	0.839510
	114.80	-2.852434	0.023010	0.406250	0.792601
	117.60	-2.367586	0.018889	0.406250	0.772310
	120.40	-2.142138	0.017939	0.406250	0.770569
	123.20	-2.032716	0.020190	0.406250	0.787364
D2S110	126.00	-1.999613	0.025701	0.406250	0.832195
	128.80	-2.064601	-0.135642	0.406250	0.723181
	131.60	-2.208243	-0.293193	0.500000	0.688039
	134.40	-2.470471	-0.448836	0.625000	0.696431
	137.20	-2.994924	-0.604338	0.718750	0.749761
D2S114	140.00	-10000.000	-0.761352	0.843750	0.886951
	141.20	-5.270773	-0.791635	0.859375	0.850713
	142.40	-5.043032	-0.823355	0.859375	0.836917
	143.60	-5.217934	-0.856405	0.921875	0.840492
	144.80	-5.869183	-0.890682	0.953125	0.863515
D2S222	146.00	-10000.000	-0.926083	0.968750	0.924105
	148.20	-5.694633	-0.918515	0.953125	0.864049
	150.40	-5.097510	-0.914882	0.953125	0.834131
	152.60	-4.980798	-0.915223	0.953125	0.821700
	154.80	-5.272824	-0.919566	0.953125	0.825844
D2S142	157.00	-10000.000	-0.927934	0.968750	0.854807
	159.20	-2.742052	-0.872472	0.953125	0.710136
	161.40	-2.220465	-0.818342	0.859375	0.628385
	163.60	-2.006585	-0.766355	0.843750	0.577806
	165.80	-2.024589	-0.717227	0.796875	0.554564
D2S111	168.00	-10000.000	-0.671612	0.781250	0.570556
	175.20	-1.185337	-0.607389	0.718750	0.348953
	182.40	-1.060554	-0.595289	0.718750	0.285966
	189.60	-1.171072	-0.632963	0.750000	0.302508
	196.80	-1.566142	-0.718210	0.796875	0.404547
D2S72	204.00	-10000.000	-0.847546	0.906250	0.714457
	205.00	-2.773961	-0.858241	0.921875	0.701460
	206.00	-2.612732	-0.870526	0.921875	0.701397
	207.00	-2.635122	-0.884350	0.953125	0.710524
	208.00	-2.841616	-0.899657	0.953125	0.729862
D2S155	209.00	-5.457116	-0.916388	0.953125	0.768465
	211.40	-3.347165	-0.906716	0.953125	0.712407
	213.80	-3.123141	-0.903428	0.953125	0.698064
	216.20	-3.167333	-0.906610	0.953125	0.707666

	218.60	-3.539918	-0.916267	0.953125	0.739893
D2S434	221.00	-10000.000	-0.932304	0.968750	0.808735
	222.40	-2.377573	-0.864144	0.921875	0.657449
	223.80	-1.780648	-0.784120	0.859375	0.579187
	225.20	-1.435309	-0.692271	0.796875	0.539513
	226.60	-1.194145	-0.588493	0.703125	0.537114
D2S126	228.00	-1.010783	-0.472542	0.625000	0.598539
	231.60	-1.084168	-0.497919	0.625000	0.458677
	235.20	-1.212179	-0.530877	0.671875	0.414392
	238.80	-1.449819	-0.571755	0.703125	0.421857
	242.40	-1.945783	-0.620959	0.718750	0.479295
D2S427	246.00	-10000.000	-0.678882	0.781250	0.625422
	250.80	-1.664442	-0.598012	0.718750	0.419807
	255.60	-1.192103	-0.532227	0.671875	0.339989
	260.40	-1.023174	-0.485225	0.625000	0.329462
	265.20	-1.082382	-0.460396	0.625000	0.393929
D2S125	270.00	-10000.000	-0.461396	0.625000	0.610147
	278.60	-1.021413	-0.371018	0.593750	0.241220
	287.20	-0.683151	-0.313711	0.500000	0.124162
	295.80	-0.563591	-0.290738	0.500000	0.097537
	304.40	-0.628190	-0.309769	0.500000	0.147208
D2S102	313.00	-10000.000	-0.389658	0.593750	0.369496
	315.00	-0.947895	-0.354558	0.593750	0.284930
	317.00	-0.677185	-0.323363	0.500000	0.231968
	319.00	-0.530352	-0.295593	0.500000	0.192094
	321.00	-0.433636	-0.270826	0.484375	0.160741
	323.00	-0.363941	-0.248700	0.406250	0.135535

### CHROMOSOME 3

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-0.444102	0.064933	0.359375	0.389390
	-8.00	-0.589392	0.004035	0.406250	0.440496
	-6.00	-0.789897	-0.069328	0.406250	0.501230
	-4.00	-1.091809	-0.157267	0.406250	0.575063
	-2.00	-1.642429	-0.262247	0.484375	0.668876
D3S1307	0.00	-10000.000	-0.387145	0.593750	0.812183
	3.86	-2.387499	-0.311274	0.500000	0.730822
	7.71	-2.049555	-0.276794	0.484375	0.702927
	11.57	-2.057389	-0.280076	0.484375	0.708000
	15.43	-2.410984	-0.319900	0.500000	0.747475
D3S1304	19.28	-10000.000	-0.397331	0.593750	0.847412
	24.06	-1.305841	0.265725	0.265625	0.609899
	28.84	-0.840428	0.897007	0.062500	0.530904
	33.62	-0.664798	1.536196	0.062500	0.531401
	38.40	-0.705062	2.223138	0.062500	0.609696
D3S1293	43.18	-10000.000	3.001542	0.062500	0.827525
	44.69	-1.578667	3.016750	0.062500	0.744059
	46.20	-1.399403	3.040879	0.062500	0.720254
	47.71	-1.394892	3.074086	0.062500	0.729797
	49.22	-1.565140	3.116588	0.062500	0.773469



D3S1266	50.73	-10000.000	3.168665	0.062500	0.880932
	53.21	-1.132944	3.150746	0.062500	0.766398
	55.70	-0.960337	3.159007	0.062500	0.724628
	58.18	-0.960032	3.193561	0.046875	0.723606
	60.66	-1.132031	3.255010	0.046875	0.763349
D3S1768	63.15	-10000.000	3.344455	0.046875	0.875916
	67.61	-0.644376	3.139072	0.062500	0.697748
	72.07	-0.480465	3.021653	0.062500	0.630212
	76.54	-0.480708	2.985472	0.062500	0.620881
	81.00	-0.645101	3.028682	0.062500	0.667398
D3S1766	85.46	-10000.000	3.154156	0.062500	0.811355
	88.75	-0.896867	3.104749	0.062500	0.695731
	92.03	-0.726590	3.100774	0.062500	0.659361
	95.32	-0.725822	3.141937	0.062500	0.668959
	98.60	-0.894567	3.229405	0.046875	0.726345
D3S1285	101.89	-10000.000	3.365858	0.015625	0.872882
	104.90	-0.953646	3.207687	0.046875	0.667678
	107.91	-0.794232	3.090997	0.062500	0.591487
	110.92	-0.805681	3.013153	0.062500	0.583264
	113.93	-0.987958	2.972627	0.062500	0.642125
D3S1284	116.94	-10000.000	2.968943	0.062500	0.825458
	119.69	-0.757367	2.350845	0.062500	0.691626
	122.43	-0.626112	1.777437	0.062500	0.636832
	125.18	-0.670234	1.234462	0.062500	0.627334
	127.92	-0.890385	0.708591	0.093750	0.664240
D3S1752	130.67	-4.175301	0.186959	0.328125	0.786881
	137.21	-1.326396	0.070193	0.359375	0.548592
	143.74	-1.154067	-0.065093	0.406250	0.476155
	150.28	-1.150234	-0.217066	0.406250	0.483542
	156.82	-1.292325	-0.386527	0.593750	0.568148
D3S1769	163.36	-4.199725	-0.573783	0.703125	0.797715
	164.64	-1.909487	-0.545508	0.671875	0.741068
	165.92	-1.644343	-0.512626	0.625000	0.712352
	167.20	-1.498564	-0.475621	0.625000	0.698191
	168.48	-1.400087	-0.434924	0.593750	0.697048
D3S1269	169.75	-1.327371	-0.390922	0.593750	0.714515
	172.50	-1.421188	-0.386568	0.593750	0.644527
	175.24	-1.564443	-0.397741	0.593750	0.621096
	177.99	-1.808150	-0.426864	0.593750	0.632166
	180.73	-2.302315	-0.476268	0.625000	0.682626
D3S1238	183.48	-10000.000	-0.548385	0.671875	0.810287
	184.98	-3.207423	-0.544234	0.671875	0.750821
	186.49	-2.772044	-0.544681	0.671875	0.714830
	188.00	-2.633818	-0.549442	0.671875	0.691561
	189.51	-2.719062	-0.558289	0.671875	0.679272
D3S1764	191.02	-4.230056	-0.571044	0.703125	0.679992
	193.50	-2.093311	-0.501123	0.625000	0.513626
	195.99	-1.899003	-0.441549	0.625000	0.445207
	198.47	-1.902560	-0.391028	0.593750	0.431714
	200.96	-2.101620	-0.348659	0.500000	0.473041
D3S1744	203.44	-4.192809	-0.313927	0.500000	0.608850
	205.42	-1.833733	-0.337868	0.500000	0.496739
	207.41	-1.884047	-0.374393	0.593750	0.489360
	209.39	-2.211481	-0.420752	0.593750	0.540591

	211.38	-2.963296	-0.474109	0.625000	0.651981
D3S1763	213.36	-10000.000	-0.531431	0.671875	0.878449
	214.87	-5.018481	-0.505062	0.625000	0.832125
	216.38	-4.207825	-0.480734	0.625000	0.806055
	217.89	-3.777479	-0.458412	0.625000	0.790818
	219.40	-3.508058	-0.438060	0.593750	0.784444
D3S1282	220.90	-3.332286	-0.419643	0.593750	0.786838
	222.89	-3.330615	-0.437033	0.593750	0.768820
	224.87	-3.429466	-0.457717	0.625000	0.771655
	226.86	-3.663467	-0.481752	0.625000	0.790073
	228.84	-4.173871	-0.509204	0.625000	0.825889
D3S1754	230.83	-10000.000	-0.540142	0.671875	0.893874
	233.06	-5.076740	-0.547901	0.671875	0.762135
	235.29	-4.804211	-0.560353	0.671875	0.719959
	237.52	-4.935997	-0.577542	0.703125	0.734702
	239.75	-5.545083	-0.599551	0.718750	0.809711
D3S1262	241.98	-10000.000	-0.626493	0.750000	1.000000
	243.73	-6.005596	-0.649902	0.750000	0.818032
	245.47	-5.346315	-0.677141	0.781250	0.738369
	247.21	-5.130342	-0.708032	0.796875	0.709805
	248.96	-5.219775	-0.742415	0.843750	0.727848
GATA6G12	250.70	-10000.000	-0.780139	0.859375	0.818446
	251.53	-6.012442	-0.777991	0.859375	0.792200
	252.37	-6.034369	-0.776673	0.859375	0.783898
	253.20	-6.337273	-0.776196	0.859375	0.786832
	254.04	-7.067079	-0.776571	0.859375	0.801659
D3S1314	254.87	-10000.000	-0.777810	0.859375	0.838621
	257.61	-4.559241	-0.599062	0.718750	0.649275
	260.36	-4.040416	-0.446448	0.625000	0.575202
	263.10	-4.046646	-0.318312	0.500000	0.568150
	265.85	-4.577978	-0.213263	0.406250	0.628338
D3S1311	268.59	-10000.000	-0.130318	0.406250	0.808974
	270.59	-3.872871	-0.125512	0.406250	0.666059
	272.59	-3.007958	-0.121464	0.406250	0.572584
	274.59	-2.517243	-0.117998	0.406250	0.499042
	276.59	-2.179384	-0.114978	0.406250	0.438562
	278.59	-1.925025	-0.112290	0.406250	0.387676

#### CHROMOSOME 4 STAGE 1

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
D4S412	-10.00	-0.021516	0.501296	0.203125	0.435616
	-8.00	-0.080816	0.523833	0.187500	0.491788
	-6.00	-0.166933	0.545835	0.187500	0.558217
	-4.00	-0.302756	0.566842	0.125000	0.638409
	-2.00	-0.560952	0.586274	0.125000	0.739103
	0.00	-2.509458	0.603407	0.125000	0.887725
	2.16	0.476027	1.131816	0.062500	0.778710
	4.32	0.777299	1.668027	0.062500	0.743235
	6.48	0.956362	2.220732	0.062500	0.749995
	8.64	1.086881	2.798893	0.062500	0.800119
D4S394	10.80	1.191944	3.411962	0.015625	0.929443

	12.88	1.186459	3.321799	0.046875	0.772842
	14.96	1.183533	3.252141	0.046875	0.704955
	17.04	1.183173	3.202387	0.046875	0.682792
	19.12	1.185379	3.172202	0.062500	0.703256
D4S403	21.20	1.190147	3.161518	0.062500	0.793586
	23.52	1.045400	2.315961	0.062500	0.471772
	25.84	0.872853	1.587571	0.062500	0.347551
	28.16	0.648828	0.958383	0.062500	0.329175
	30.48	0.298869	0.413726	0.203125	0.416464
D4S404	32.80	-2.624464	-0.058235	0.406250	0.700961
	36.40	0.145792	0.188091	0.328125	0.429937
	40.00	0.321965	0.338595	0.265625	0.350250
	43.60	0.330003	0.400105	0.250000	0.378924
	47.20	0.169810	0.371350	0.250000	0.521035
D4S174	50.80	-2.502562	0.242513	0.312500	0.886401
	51.80	-2.026875	0.241804	0.312500	0.870390
	52.80	-1.902621	0.241311	0.312500	0.860964
	53.80	-1.902733	0.241035	0.312500	0.855739
	54.80	-2.027212	0.240975	0.312500	0.854379
D4S1627	55.80	-2.503124	0.241129	0.312500	0.857662
	60.00	-0.970322	0.231409	0.312500	0.755506
	64.20	-0.809959	0.224869	0.312500	0.712409
	68.40	-0.812457	0.221933	0.312500	0.704922
	72.60	-0.977790	0.222657	0.312500	0.731461
D4S392	76.80	-2.515487	0.226753	0.312500	0.809838
	79.80	-1.346396	0.044371	0.359375	0.689804
	82.80	-1.308236	-0.133432	0.406250	0.649619
	85.80	-1.485045	-0.310168	0.500000	0.656355
	88.80	-1.950287	-0.489432	0.625000	0.711293
ATA2A03	91.80	-6.208200	-0.674873	0.781250	0.853588
	94.40	-0.237608	-0.261152	0.484375	0.550118
	97.00	0.346235	0.266390	0.265625	0.431141
	99.60	0.683627	0.904325	0.062500	0.407737
	102.20	0.921943	1.654335	0.062500	0.477268
D4S175	104.80	1.107410	2.522762	0.062500	0.717536
	112.20	0.825063	1.227471	0.062500	0.403541
	119.60	0.494427	0.369821	0.250000	0.290454
	127.00	0.061129	-0.212714	0.406250	0.277079
	134.40	-0.638200	-0.616502	0.718750	0.359656
D4S1625	141.80	-4.731530	-0.902421	0.953125	0.620849
	144.00	-2.320677	-0.832861	0.906250	0.555192
	146.20	-1.966866	-0.766538	0.843750	0.558243
	148.40	-1.947460	-0.704295	0.796875	0.604301
	150.60	-2.224574	-0.646105	0.750000	0.698231
D4S1090	152.80	-2.796466	-0.591031	0.718750	0.889067
	156.00	-2.004428	-0.533108	0.671875	0.677378
	159.20	-1.702424	-0.514886	0.625000	0.594537
	162.40	-1.713456	-0.537538	0.671875	0.577778
	165.60	-2.060066	-0.602533	0.718750	0.622491
D4S243	168.80	-6.243569	-0.711791	0.796875	0.763920
	171.40	-2.128794	-0.539792	0.671875	0.588941
	174.00	-1.658044	-0.369365	0.593750	0.534134
	176.60	-1.473747	-0.198466	0.406250	0.545109
	179.20	-1.502828	-0.025079	0.406250	0.623192

D4S415	181.80	-2.523768	0.152776	0.359375	0.828223
	184.20	-1.473102	0.131775	0.359375	0.711759
	186.60	-1.325039	0.110600	0.359375	0.666645
	189.00	-1.338042	0.089256	0.359375	0.661039
	191.40	-1.511500	0.067713	0.359375	0.694615
D4S408	193.80	-2.553882	0.045919	0.359375	0.798500
	195.80	-1.225226	-0.069536	0.406250	0.661872
	197.80	-1.007452	-0.181467	0.406250	0.592444
	199.80	-0.899375	-0.289719	0.500000	0.565530
	201.80	-0.825970	-0.394089	0.593750	0.583257
D4S1652	203.80	-0.759297	-0.494299	0.625000	0.678905
	207.00	-0.859158	-0.431583	0.593750	0.477160
	210.20	-0.944583	-0.376888	0.593750	0.399854
	213.40	-1.054913	-0.330959	0.500000	0.387383
	216.60	-1.275115	-0.294464	0.500000	0.436958
	219.80	-2.793279	-0.268144	0.484375	0.597694

## CHROMOSOME 4 STAGE 2

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	L.O.D score	NPL score	p-value	information
	-10.00	0.243417	0.513626	0.187500	0.439796
	-8.00	0.192092	0.537004	0.187500	0.496811
	-6.00	0.113979	0.559871	0.187500	0.564293
	-4.00	-0.014035	0.581757	0.125000	0.645832
	-2.00	-0.265726	0.602070	0.125000	0.748314
D4S412	0.00	-10000.000	0.620068	0.125000	0.899507
	1.89	0.692132	1.057134	0.062500	0.815761
	3.77	0.993605	1.501422	0.062500	0.787524
	5.66	1.172433	1.958486	0.062500	0.790200
	7.55	1.302395	2.434044	0.062500	0.822183
D4S3007	9.44	1.406603	2.934080	0.062500	0.892728
	9.89	1.427514	3.045518	0.062500	0.905186
	10.34	1.447659	3.158047	0.062500	0.920496
	10.79	1.467114	3.271751	0.046875	0.939206
	11.24	1.485947	3.386713	0.015625	0.962652
D4S394	11.69	1.504217	3.503017	0.015625	0.997118
	12.01	1.504050	3.500634	0.015625	0.989244
	12.34	1.503957	3.498774	0.015625	0.983619
	12.66	1.503938	3.497435	0.015625	0.979126
	12.99	1.503992	3.496617	0.015625	0.975546
D4S2983	13.31	1.504119	3.496321	0.015625	0.972893
	15.10	1.499788	3.434131	0.015625	0.886670
	16.90	1.497669	3.387459	0.015625	0.852834
	18.69	1.497766	3.356054	0.015625	0.853287
	20.48	1.500079	3.339819	0.046875	0.888644
D4S2944	22.27	1.504604	3.338806	0.046875	0.986745
	22.39	1.504578	3.338663	0.046875	0.986650
	22.51	1.504563	3.338588	0.046875	0.986665
	22.63	1.504558	3.338582	0.046875	0.986770
	22.75	1.504562	3.338645	0.046875	0.986964
D4S403	22.87	1.504577	3.338776	0.046875	0.987259

	23.56	1.395983	2.747971	0.062500	0.868746
	24.24	1.259386	2.160485	0.062500	0.818928
	24.92	1.071648	1.575367	0.062500	0.807099
	25.60	0.758967	0.991673	0.062500	0.834534
D4S3048	26.29	-10000.000	0.408461	0.250000	0.935587
	28.10	-1.052135	0.236648	0.312500	0.595635
	29.92	-1.037517	0.069601	0.359375	0.472946
	31.73	-1.249557	-0.094153	0.406250	0.458621
	33.55	-1.762284	-0.256180	0.484375	0.551460
D4S404	35.36	-10000.000	-0.418130	0.593750	0.847096
	39.83	-1.571364	-0.279496	0.484375	0.549390
	44.29	-0.977767	-0.150199	0.406250	0.448967
	48.75	-0.702035	-0.024003	0.406250	0.455538
	53.21	-0.659979	0.104569	0.359375	0.571217
D4S174	57.68	-10000.000	0.240011	0.312500	0.898228
	58.73	-1.857623	0.238877	0.312500	0.878950
	59.78	-1.682500	0.237978	0.312500	0.867240
	60.84	-1.682771	0.237314	0.312500	0.860242
	61.89	-1.858435	0.236885	0.312500	0.857529
D4S1627	62.95	-10000.000	0.236689	0.312500	0.859933
	68.39	-0.478601	0.222512	0.312500	0.711806
	73.84	-0.323371	0.213044	0.312500	0.652222
	79.29	-0.326720	0.209337	0.312500	0.642311
	84.73	-0.488595	0.211508	0.312500	0.679477
D4S392	90.18	-10000.000	0.218818	0.312500	0.793298
	93.75	-0.929853	0.039514	0.359375	0.662223
	97.32	-0.885627	-0.134650	0.406250	0.619256
	100.88	-1.061364	-0.308500	0.500000	0.628350
	104.45	-1.530852	-0.487032	0.625000	0.691485
ATA2A03	108.02	-10000.000	-0.675379	0.781250	0.853838
	111.03	0.059114	-0.260884	0.484375	0.536150
	114.04	0.640310	0.263037	0.265625	0.411136
	117.05	0.976679	0.895764	0.062500	0.384908
	120.06	1.215829	1.643486	0.062500	0.454030
D4S1647	123.07	1.404029	2.518705	0.062500	0.697504
	136.54	1.043008	1.103466	0.062500	0.272396
	150.01	0.688618	0.337924	0.265625	0.150779
	163.48	0.278421	-0.154657	0.406250	0.139454
	176.95	-0.366505	-0.542811	0.671875	0.231604
D4S175	190.42	-10000.000	-0.905974	0.953125	0.580619
	192.91	-2.108591	-0.835745	0.906250	0.516800
	195.39	-1.740881	-0.768757	0.843750	0.524223
	197.88	-1.721713	-0.705768	0.796875	0.576824
	200.36	-2.051099	-0.646793	0.750000	0.680460
D4S1625	202.85	-10000.000	-0.591031	0.703125	0.888958
	206.70	-1.600542	-0.525419	0.640625	0.656753
	210.56	-1.261497	-0.502001	0.625000	0.567491
	214.42	-1.267515	-0.522473	0.640625	0.550673
	218.27	-1.618568	-0.588950	0.703125	0.601135
D4S1090	222.13	-10000.000	-0.704287	0.796875	0.758875
	225.14	-1.729860	-0.528686	0.671875	0.578572
	228.15	-1.249575	-0.356734	0.593750	0.522538
	231.16	-1.065617	-0.185619	0.406250	0.534794
	234.17	-1.104216	-0.012598	0.406250	0.617123

D4S243	237.19	-10000.000	0.164993	0.328125	0.832795
	239.93	-1.090959	0.143783	0.359375	0.708357
	242.67	-0.931961	0.122441	0.359375	0.660206
	245.42	-0.945276	0.100985	0.359375	0.654303
	248.16	-1.130906	0.079376	0.359375	0.690200
D4S415	250.91	-10000.000	0.057528	0.359375	0.800939
	253.14	-0.896084	-0.056006	0.406250	0.653701
	255.37	-0.773829	-0.165537	0.406250	0.578630
	257.60	-0.829475	-0.270820	0.484375	0.548790
	259.83	-1.064053	-0.371544	0.593750	0.566611
D4S408	262.07	-10000.000	-0.467282	0.625000	0.668302
	265.92	-1.578358	-0.405877	0.593750	0.447942
	269.78	-1.203329	-0.354195	0.593750	0.365540
	273.63	-1.100122	-0.313703	0.500000	0.353004
	277.49	-1.213444	-0.285699	0.484375	0.406930
D4S1652	281.35	-10000.000	-0.271638	0.484375	0.581914
	283.35	-0.684837	-0.243058	0.406250	0.468039
	285.35	-0.416383	-0.217496	0.406250	0.394784
	287.35	-0.272115	-0.194613	0.406250	0.338110
	289.35	-0.178260	-0.174113	0.406250	0.292290
	291.35	-0.111710	-0.155734	0.406250	0.254392

analyzing pedigree 2...

using non-originals: 22 1 24 2 3 4 6 5

	position	LOD score	NPL score	p-value	information
	-10.00	-0.055869	-0.084335	0.281250	0.041472
	-8.00	-0.073485	-0.093261	0.312500	0.049947
	-6.00	-0.094772	-0.103263	0.343750	0.060342
	-4.00	-0.120629	-0.114489	0.343750	0.073150
	-2.00	-0.152262	-0.127105	0.343750	0.089013
D4S412	0.00	-0.191348	-0.141305	0.343750	0.108791
	1.89	-0.237241	-0.156358	0.343750	0.132114
	3.77	-0.294999	-0.173234	0.343750	0.161419
	5.66	-0.369539	-0.192180	0.375000	0.198937
	7.55	-0.469546	-0.213482	0.406250	0.248761
D4S3007	9.44	-0.612886	-0.237466	0.421875	0.325948
	9.89	-0.660152	-0.255864	0.421875	0.325380
	10.34	-0.715024	-0.274457	0.437500	0.326619
	10.79	-0.779986	-0.293258	0.437500	0.329111
	11.24	-0.858994	-0.312281	0.468750	0.332793
D4S394	11.69	-0.958935	-0.331542	0.500000	0.337712
	12.01	-1.051413	-0.345620	0.500000	0.342111
	12.34	-1.171915	-0.359835	0.531250	0.347324
	12.66	-1.343515	-0.374194	0.531250	0.353525
	12.99	-1.639750	-0.388703	0.531250	0.361087
D4S2983	13.31	-4.274774	-0.403368	0.562500	0.371752
	15.10	-1.807375	-0.387103	0.531250	0.362966
	16.90	-1.619185	-0.374798	0.531250	0.365661
	18.69	-1.608455	-0.366507	0.531250	0.377657
	20.48	-1.775352	-0.362346	0.531250	0.400279
D4S2944	22.27	-4.370730	-0.362505	0.531250	0.442061
	22.39	-3.139265	-0.362532	0.531250	0.440871
	22.51	-2.863806	-0.362578	0.531250	0.439911
	22.63	-2.705443	-0.362642	0.531250	0.439072

	22.75	-2.596591	-0.362724	0.531250	0.438329
D4S403	22.87	-2.515422	-0.362824	0.531250	0.437670
	23.56	-2.282854	-0.363737	0.531250	0.435255
	24.24	-2.220052	-0.365236	0.531250	0.434810
	24.92	-2.263210	-0.367322	0.531250	0.436265
	25.60	-2.458931	-0.369999	0.531250	0.439830
D4S3048	26.29	-4.206352	-0.373271	0.531250	0.446766
	28.10	-0.887693	-0.332750	0.500000	0.340168
	29.92	-0.601876	-0.297058	0.437500	0.273220
	31.73	-0.442995	-0.265574	0.437500	0.223021
	33.55	-0.336793	-0.237763	0.421875	0.183769
D4S404	35.36	-0.259752	-0.213163	0.406250	0.152419
	39.83	-0.137888	-0.163920	0.343750	0.098103
	44.29	-0.067810	-0.127081	0.343750	0.064398
	48.75	-0.025178	-0.099303	0.312500	0.042913
	53.21	0.001223	-0.078197	0.281250	0.028962
D4S174	57.68	0.017460	-0.062041	0.281250	0.019771
	58.73	0.020234	-0.058805	0.281250	0.018095
	59.78	0.022683	-0.055761	0.281250	0.016571
	60.84	0.024836	-0.052896	0.281250	0.015183
	61.89	0.026722	-0.050198	0.281250	0.013920
D4S1627	62.95	0.028366	-0.047656	0.281250	0.012769
	68.39	0.033784	-0.036659	0.265625	0.008243
	73.84	0.035603	-0.028491	0.265625	0.005396
	79.29	0.035289	-0.022364	0.265625	0.003580
	84.73	0.033754	-0.017724	0.265625	0.002408
D4S392	90.18	0.031569	-0.014177	0.265625	0.001641
	93.75	0.029962	-0.012308	0.265625	0.001286
	97.32	0.028296	-0.010724	0.265625	0.001014
	100.88	0.026620	-0.009378	0.265625	0.000804
	104.45	0.024966	-0.008229	0.265625	0.000641
ATA2A03	108.02	0.023358	-0.007245	0.265625	0.000514
	111.03	0.022047	-0.006522	0.265625	0.000429
	114.04	0.020786	-0.005885	0.265625	0.000359
	117.05	0.019579	-0.005321	0.265625	0.000302
	120.06	0.018428	-0.004821	0.265625	0.000254
D4S1647	123.07	0.017333	-0.004376	0.265625	0.000215
	136.54	0.013115	-0.002902	0.265625	0.000107
	150.01	0.009887	-0.001985	0.265625	0.000056
	163.48	0.007452	-0.001392	0.265625	0.000030
	176.95	0.005623	-0.000996	0.265625	0.000017
D4S175	190.42	0.004250	-0.000723	0.265625	0.000010
	192.91	0.004037	-0.000683	0.265625	0.000009
	195.39	0.003835	-0.000645	0.265625	0.000008
	197.88	0.003643	-0.000609	0.265625	0.000007
	200.36	0.003461	-0.000575	0.265625	0.000006
D4S1625	202.85	0.003288	-0.000544	0.265625	0.000006
	206.70	0.003037	-0.000499	0.265625	0.000005
	210.56	0.002806	-0.000458	0.265625	0.000004
	214.42	0.002593	-0.000420	0.265625	0.000004
	218.27	0.002396	-0.000386	0.265625	0.000003
D4S1090	222.13	0.002214	-0.000355	0.265625	0.000003
	225.14	0.002082	-0.000333	0.265625	0.000002
	228.15	0.001958	-0.000312	0.265625	0.000002

	231.16	0.001841	-0.000292	0.265625	0.000002
	234.17	0.001732	-0.000274	0.265625	0.000002
D4S243	237.19	0.001629	-0.000257	0.265625	0.000001
	239.93	0.001541	-0.000242	0.265625	0.000001
	242.67	0.001457	-0.000229	0.265625	0.000001
	245.42	0.001378	-0.000216	0.265625	0.000001
	248.16	0.001304	-0.000204	0.265625	0.000001
D4S415	250.91	0.001233	-0.000192	0.265625	0.000001
	253.14	0.001179	-0.000183	0.265625	0.000001
	255.37	0.001127	-0.000175	0.265625	0.000001
	257.60	0.001077	-0.000167	0.265625	0.000001
	259.83	0.001029	-0.000160	0.265625	0.000001
D4S408	262.07	0.000984	-0.000152	0.265625	0.000001
	265.92	0.000910	-0.000141	0.265625	0.000000
	269.78	0.000842	-0.000130	0.265625	0.000000
	273.63	0.000779	-0.000120	0.265625	0.000000
	277.49	0.000721	-0.000111	0.265625	0.000000
D4S1652	281.35	0.000667	-0.000102	0.265625	0.000000
	283.35	0.000640	-0.000098	0.265625	0.000000
	285.35	0.000615	-0.000094	0.265625	0.000000
	287.35	0.000591	-0.000090	0.265625	0.000000
	289.35	0.000568	-0.000087	0.265625	0.000000
	291.35	0.000545	-0.000083	0.265625	0.000000

Totalling pedigrees: ..

	position	LOD_score	NPL_score	p-value	information
	-10.00	0.187548	0.30355	0.211670	0.240634
	-8.00	0.118607	0.31377	0.211182	0.273379
	-6.00	0.019206	0.32287	0.210693	0.312318
	-4.00	-0.134664	0.33041	0.208252	0.359491
	-2.00	-0.417988	0.33585	0.204346	0.418664
D4S412	0.00	-INFINITY	0.33854	0.202393	0.504149
	1.89	0.454891	0.63694	0.161865	0.473937
	3.77	0.698607	0.93917	0.128174	0.474472
	5.66	0.802894	1.24897	0.121094	0.494568
	7.55	0.832849	1.57017	0.121094	0.535472
D4S3007	9.44	0.793717	1.90679	0.097412	0.609338
	9.89	0.767362	1.97258	0.088135	0.615283
	10.34	0.732635	2.03901	0.076660	0.623557
	10.79	0.687129	2.10611	0.068115	0.634158
	11.24	0.626953	2.17395	0.059326	0.647723
D4S394	11.69	0.545282	2.24257	0.049561	0.667415
	12.01	0.452637	2.23093	0.051025	0.665678
	12.34	0.332042	2.21956	0.053223	0.665472
	12.66	0.160423	2.20846	0.053711	0.666325
	12.99	-0.135758	2.19763	0.054199	0.668316
D4S2983	13.31	-2.770655	2.18705	0.055176	0.672323
	15.10	-0.307587	2.15457	0.060059	0.624818
	16.90	-0.121516	2.13027	0.064453	0.609248
	18.69	-0.110689	2.11393	0.067627	0.615472
	20.48	-0.275273	2.10539	0.068115	0.644462
D4S2944	22.27	-2.866126	2.10456	0.068115	0.714403
	22.39	-1.634686	2.10444	0.068115	0.713760
	22.51	-1.359243	2.10436	0.068115	0.713288



	22.63	-1.200385	2.10431	0.068115	0.712921
	22.75	-1.092029	2.10429	0.068359	0.712647
D4S403	22.87	-1.010845	2.10432	0.068115	0.712465
	23.56	-0.886871	1.68591	0.120361	0.652001
	24.24	-0.960666	1.26943	0.121674	0.626869
	24.92	-1.191562	0.85422	0.137207	0.621682
	25.60	-1.699964	0.43959	0.186279	0.637182
D4S3048	26.29	-INFINITY	0.02488	0.344482	0.691177
	28.10	-1.939828	-0.06795	0.400146	0.467901
	29.92	-1.639393	-0.16084	0.458008	0.373083
	31.73	-1.692552	-0.25437	0.510498	0.340821
	33.55	-2.099077	-0.34927	0.556885	0.367614
D4S404	35.36	-INFINITY	-0.44639	0.604736	0.499757
	39.83	-1.709252	-0.31354	0.538330	0.323746
	44.29	-1.045577	-0.19607	0.477051	0.256683
	48.75	-0.727214	-0.08719	0.413818	0.249226
	53.21	-0.658757	0.01865	0.344727	0.300089
D4S174	57.68	-INFINITY	0.12584	0.284424	0.459000
	58.73	-1.837388	0.12733	0.284424	0.448522
	59.78	-1.659817	0.12885	0.283691	0.441905
	60.84	-1.657934	0.13040	0.283203	0.437713
	61.89	-1.831713	0.13201	0.283203	0.435724
D4S1627	62.95	-INFINITY	0.13367	0.283203	0.436351
	68.39	-0.444817	0.13142	0.283203	0.360025
	73.84	-0.287768	0.13050	0.283203	0.328809
	79.29	-0.291431	0.13221	0.283203	0.322946
	84.73	-0.454841	0.13703	0.282227	0.340942
D4S392	90.18	-INFINITY	0.14470	0.278809	0.397469
	93.75	-0.899892	0.01924	0.344727	0.331754
	97.32	-0.857330	-0.10279	0.422852	0.310135
	100.88	-1.034745	-0.22477	0.495605	0.314577
	104.45	-1.505886	-0.35020	0.557373	0.346063
ATA2A03	108.02	-INFINITY	-0.48269	0.620605	0.427176
	111.03	0.081161	-0.18908	0.474854	0.268290
	114.04	0.661096	0.18183	0.254395	0.205748
	117.05	0.996258	0.62964	0.165527	0.192605
	120.06	1.234257	1.15871	0.121094	0.227142
D4S1647	123.07	1.421363	1.77790	0.115967	0.348860
	136.54	1.056123	0.77822	0.147217	0.136251
	150.01	0.698505	0.23754	0.233887	0.075417
	163.48	0.285672	-0.11034	0.434082	0.069742
	176.95	-0.360883	-0.38453	0.572021	0.115810
D4S175	190.42	-INFINITY	-0.64113	0.702393	0.290314
	192.91	-2.104555	-0.59144	0.678955	0.258404
	195.39	-1.737046	-0.54405	0.654297	0.262115
	197.88	-1.718070	-0.49948	0.632324	0.288416
	200.36	-2.047639	-0.45776	0.608643	0.340233
D4S1625	202.85	-INFINITY	-0.41831	0.587891	0.444482
	206.70	-1.597505	-0.37188	0.566895	0.328379
	210.56	-1.258691	-0.35529	0.557617	0.283747
	214.42	-1.264922	-0.36974	0.566895	0.275338
	218.27	-1.616172	-0.41672	0.587891	0.300569
D4S1090	222.13	-INFINITY	-0.49826	0.631592	0.379439
	225.14	-1.727778	-0.37407	0.567871	0.289287

	228.15	-1.247617	-0.25247	0.509033	0.261270
	231.16	-1.063775	-0.13146	0.443848	0.267398
	234.17	-1.102484	-0.00910	0.364990	0.308562
D4S243	237.19	-INFINITY	0.11649	0.289795	0.416398
	239.93	-1.089418	0.10150	0.298828	0.354179
	242.67	-0.930504	0.08642	0.307861	0.330103
	245.42	-0.943898	0.07125	0.313965	0.327152
	248.16	-1.129602	0.05598	0.322754	0.345101
D4S415	250.91	-INFINITY	0.04054	0.335693	0.400470
	253.14	-0.894905	-0.03973	0.385498	0.326851
	255.37	-0.772702	-0.11718	0.436279	0.289315
	257.60	-0.828399	-0.19162	0.474854	0.274395
	259.83	-1.063023	-0.26283	0.512695	0.283306
D4S408	262.07	-INFINITY	-0.33053	0.552979	0.334151
	265.92	-1.577447	-0.28710	0.529541	0.223971
	269.78	-1.202487	-0.25055	0.505127	0.182770
	273.63	-1.099343	-0.22191	0.494141	0.176502
	277.49	-1.212723	-0.20210	0.477051	0.203465
D4S1652	281.35	-INFINITY	-0.19215	0.474854	0.290957
	283.35	-0.684196	-0.17194	0.462646	0.234020
	285.35	-0.415768	-0.15386	0.457275	0.197392
	287.35	-0.271524	-0.13768	0.446777	0.169055
	289.35	-0.177692	-0.12318	0.442139	0.146145
	291.35	-0.111165	-0.11018	0.434082	0.127196

# **CHROMOSOME 5**

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6

	position	LOD score	NPL score	p-value	information
	-10.00	-0.564842	-0.344323	0.500000	0.205321
	-8.00	-0.637160	-0.366747	0.593750	0.242022
	-6.00	-0.736467	-0.390545	0.593750	0.286926
	-4.00	-0.885926	-0.415777	0.593750	0.343015
	-2.00	-1.159380	-0.442501	0.625000	0.416008
D5S392	0.00	-10000.000	-0.470771	0.625000	0.529108
	2.00	-0.981991	-0.522153	0.625000	0.499512
	4.00	-0.812951	-0.579608	0.703125	0.504331
	6.00	-0.816966	-0.643344	0.750000	0.537060
	8.00	-0.993914	-0.713618	0.796875	0.604138
D5S406	10.00	-3.857541	-0.790725	0.859375	0.744020
	12.00	-1.788896	-0.735837	0.796875	0.528195
	14.00	-1.484190	0.686703	0.796875	0.457018
	16.00	-1.408247	-0.642841	0.750000	0.457429
	18.00	-1.538846	-0.603532	0.718750	0.522134
D5S807	20.00	-3.327224	-0.567801	0.671875	0.688057
	22.40	-1.459437	-0.569378	0.671875	0.667513
	24.80	-1.402610	-0.560063	0.671875	0.684627
	27.20	-1.567906	-0.565779	0.671875	0.734844
	29.60	-2.029210	-0.576466	0.703125	0.825415
D5S416	32.00	-10000.000	-0.591031	0.718750	1.000000
	37.00	-2.072782	-0.564510	0.671875	0.532337
	42.00	-1.870553	-0.500424	0.625000	0.359986

	47.00	-2.039690	-0.385512	0.593750	0.327144
	52.00	-2.594115	-0.201811	0.406250	0.425950
D5S426	57.00	-10000.000	0.075188	0.359375	0.767510
	59.60	-4.152021	0.118069	0.359375	0.657727
	62.20	-4.046968	0.170878	0.328125	0.639908
	64.80	-4.392753	0.235966	0.312500	0.678536
	67.40	-5.336956	0.315941	0.265625	0.778440
D5S407	70.00	-10000.000	0.413722	0.250000	1.000000
	74.00	-5.848403	0.397420	0.250000	0.826486
	78.00	-5.032174	0.402938	0.250000	0.761056
	82.00	-4.862864	0.428937	0.203125	0.752633
	86.00	-5.266644	0.475160	0.203125	0.797887
D5S424	90.00	-10000.000	0.542433	0.187500	0.923984
	92.40	-0.747293	0.352343	0.250000	0.377647
	94.80	0.039080	0.475355	0.203125	0.195937
	97.20	0.402281	0.971659	0.062500	0.205778
	99.60	0.489864	1.918843	0.062500	0.408146
D5S428	102.00	-10000.000	3.416022	0.015625	0.978811
	103.20	0.504520	2.032014	0.062500	0.609133
	104.40	0.431214	1.123410	0.062500	0.481998
	105.60	0.080331	0.631877	0.125000	0.483673
	106.80	-0.695702	0.502129	0.203125	0.614236
D5S815	108.00	-10000.000	0.681044	0.125000	0.987607
	109.40	-4.847779	0.577563	0.125000	0.787190
	110.80	-4.183446	0.478796	0.203125	0.715499
	112.20	-3.962958	0.383587	0.250000	0.713394
	113.60	-4.047332	0.290802	0.265625	0.781000
D5S409	115.00	-10000.000	0.199319	0.328125	0.977784
	116.20	-4.095298	0.261166	0.265625	0.700221
	117.40	-4.041920	0.327765	0.265625	0.605672
	118.60	-4.284766	0.399847	0.250000	0.608241
	119.80	-4.966352	0.478188	0.203125	0.707909
ATA40107	121.00	-10000.000	0.563613	0.187500	0.990453
	122.40	-7.821135	0.557609	0.187500	0.970259
	123.80	-7.294850	0.554447	0.187500	0.960880
	125.20	-7.295835	0.554109	0.187500	0.959308
	126.60	-7.824087	0.556596	0.187500	0.965434
D5S421	128.00	-10000.000	0.551926	0.187500	0.981980
	130.20	-6.655100	0.300227	0.265625	0.775238
	132.40	-6.131097	0.042143	0.359375	0.701732
	134.60	-6.132933	-0.215714	0.406250	0.699436
	136.80	-6.660601	-0.476783	0.625000	0.768116
D5S818	139.00	-10000.000	-0.744612	0.843750	0.969198
	140.80	-6.836680	-0.729880	0.796875	0.841397
	142.60	-6.239701	-0.718824	0.796875	0.788369
	144.40	-6.125102	-0.711312	0.796875	0.775224
	146.20	-6.421206	-0.707256	0.796875	0.800614
D5S816	148.00	-10000.000	-0.706609	0.796875	0.893488
	149.80	-4.341585	-0.631257	0.750000	0.695692
	151.60	-4.060121	-0.560731	0.671875	0.626772
	153.40	-4.189763	-0.493516	0.625000	0.628922
	155.20	-4.802182	-0.428083	0.593750	0.702960
D5S210	157.00	-10000.000	-0.362868	0.593750	0.911437
	159.80	-2.871486	-0.382698	0.593750	0.542957

	162.60	-2.281486	-0.448989	0.625000	0.397298
	165.40	-2.175418	-0.551293	0.671875	0.371147
	168.20	-2.483502	-0.678212	0.781250	0.461039
D5S820	171.00	-10000.000	-0.817096	0.859375	0.754939
	173.00	-2.039847	-0.762680	0.843750	0.566380
	175.00	-1.638959	-0.712401	0.796875	0.521740
	177.00	-1.591890	-0.667398	0.781250	0.561738
	179.00	-1.897610	-0.627465	0.750000	0.691803
D5S415	181.00	-10000.000	-0.591031	0.718750	1.000000
	182.00	-3.222551	-0.540177	0.671875	0.924913
	183.00	-2.750049	-0.489727	0.625000	0.878561
	184.00	-2.577745	-0.439681	0.593750	0.844993
	185.00	-2.632107	-0.390035	0.593750	0.820992
GATA11A11	186.00	-10000.000	-0.340784	0.500000	0.806123
	195.40	-0.614758	-0.297732	0.500000	0.568581
	204.80	-0.469913	-0.299219	0.500000	0.499921
	214.20	-0.543541	-0.347748	0.500000	0.517010
	223.60	-0.900724	-0.445426	0.625000	0.633188
GABRA1	233.00	-10000.000	-0.591031	0.718750	1.000000
	235.00	-1.642429	-0.555351	0.671875	0.812840
	237.00	-1.091809	-0.519489	0.625000	0.692242
	239.00	-0.789897	-0.483997	0.625000	0.598304
	241.00	-0.589392	-0.449292	0.625000	0.521728
	243.00	-0.444102	-0.415684	0.593750	0.457826

# **CHROMOSOME 6 STAGE 1**

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-0.123588	0.364388	0.250000	0.276687
	-8.00	-0.185492	0.384795	0.250000	0.311484
	-6.00	-0.274222	0.405721	0.250000	0.352439
	-4.00	-0.412635	0.427014	0.203125	0.401589
	-2.00	-0.673267	0.448478	0.203125	0.462799
D6S477	0.00	-2.576071	0.469856	0.203125	0.551483
	6.00	-0.793642	0.459013	0.203125	0.482619
	12.00	-0.609519	0.473411	0.203125	0.474545
	18.00	-0.582811	0.514481	0.187500	0.509520
	24.00	-0.713760	0.584355	0.125000	0.597107
D6S259	30.00	-2.505506	0.685842	0.093750	0.790450
	31.00	-2.020215	0.657213	0.125000	0.718814
	32.00	-1.896222	0.629426	0.125000	0.703314
	33.00	-1.897678	0.602404	0.125000	0.719114
	34.00	-2.023798	0.576069	0.125000	0.767326
D6S285	35.00	-2.500867	0.550343	0.187500	0.878084
	35.78	0.498630	1.042870	0.062500	0.702166
	36.56	0.798700	1.546474	0.062500	0.636225
	37.34	0.974325	2.062259	0.062500	0.628061
	38.12	1.099181	2.591346	0.062500	0.677168
D6S271	38.90	1.196373	3.134882	0.062500	0.833330
	40.94	1.137154	2.756977	0.062500	0.736048
	42.98	1.074014	2.405827	0.062500	0.681029

	45.02	1.005174	2.076499	0.062500	0.647859
	47.06	0.927932	1.764426	0.062500	0.632331
D6S257	49.10	0.837907	1.465322	0.062500	0.634460
	50.90	0.730290	1.162017	0.062500	0.624604
	52.70	0.596126	0.873977	0.062500	0.629358
	54.50	0.412341	0.598432	0.125000	0.648600
	56.30	0.105379	0.332814	0.265625	0.685480
D6S254	58.10	-2.576635	0.074711	0.359375	0.757567
	61.30	-1.349109	-0.070799	0.406250	0.620826
	64.50	-1.304493	-0.223848	0.406250	0.579039
	67.70	-1.475866	-0.385406	0.593750	0.596762
	70.90	-1.936937	-0.556865	0.671875	0.678663
D6S252	74.10	-6.191799	-0.739921	0.796875	0.888722
	77.30	-2.927574	-0.711882	0.796875	0.701897
	80.50	-2.685971	-0.695560	0.796875	0.623726
	83.70	-2.718970	-0.687717	0.796875	0.601128
	86.90	-2.824718	-0.685074	0.796875	0.629693
D6S474	90.10	-2.796479	-0.684275	0.796875	0.741591
	92.70	-2.878439	-0.707746	0.796875	0.632078
	95.30	-2.984735	-0.738625	0.796875	0.595918
	97.90	-3.139560	-0.778825	0.859375	0.602472
	100.50	-3.416400	-0.830434	0.906250	0.652782
D6S262	103.10	-6.267426	-0.895857	0.953125	0.782328
	106.30	-4.244923	-0.877173	0.953125	0.653719
	109.50	-4.072893	-0.870561	0.921875	0.614235
	112.70	-4.073498	-0.875651	0.953125	0.627548
	115.90	-4.247221	-0.892393	0.953125	0.696822
ATA1F08	119.10	-6.322226	-0.921042	0.953125	0.873517
	123.50	-3.972026	-0.861535	0.921875	0.701918
	127.90	-3.770319	-0.822905	0.859375	0.638999
	132.30	-3.759346	-0.805323	0.859375	0.631113
	136.70	-3.954645	-0.810134	0.859375	0.673995
D6S255	141.10	-6.319390	-0.839933	0.906250	0.792453
	142.50	-4.916652	-0.816567	0.859375	0.707202
	143.90	-4.723378	-0.795446	0.859375	0.681283
	145.30	-4.692917	-0.776528	0.859375	0.687978
	146.70	-4.822974	-0.759784	0.843750	0.727713
D6S305	148.10	-5.810237	-0.745203	0.843750	0.828928
	150.30	-3.728991	-0.730890	0.796875	0.713362
	152.50	-3.412472	-0.722456	0.796875	0.644244
	154.70	-3.239829	-0.719583	0.796875	0.596270
	156.90	-3.140109	-0.722061	0.796875	0.564128
D6S264	159.10	-3.092699	-0.729773	0.796875	0.547700
	160.70	-3.120129	-0.733374	0.796875	0.518490
	162.30	-3.170114	-0.739992	0.843750	0.507305
	163.90	-3.250273	-0.749580	0.843750	0.513088
	165.50	-3.377073	-0.762122	0.843750	0.538972
D6S503	167.10	-3.593530	-0.777627	0.859375	0.605261
	169.10	-2.834324	-0.717771	0.796875	0.501659
	171.10	-2.346932	-0.664283	0.781250	0.430938
	173.10	-2.031287	-0.616406	0.718750	0.374637
	175.10	-1.801664	-0.573475	0.703125	0.328123
	177.10	-1.622926	-0.534903	0.671875	0.288943

# CHROMOSOME 6 STAGE 2

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	0.088746	0.310003	0.265625	0.261882
	-8.00	0.032944	0.327418	0.265625	0.294660
	-6.00	-0.049704	0.345237	0.265625	0.333246
	-4.00	-0.182307	0.363323	0.250000	0.379584
	-2.00	-0.438636	0.381500	0.250000	0.437397
D6S477	0.00	-10000.000	0.399539	0.250000	0.521838
	9.16	-0.243577	0.352171	0.250000	0.386288
	18.33	-0.074155	0.348705	0.250000	0.355285
	27.49	-0.039844	0.391066	0.250000	0.384773
	36.65	-0.140554	0.483636	0.203125	0.488691
D6S259	45.81	-10000.000	0.633060	0.125000	0.764059
	46.87	-1.869244	0.602763	0.125000	0.692332
	47.92	-1.695928	0.573376	0.125000	0.676956
	48.98	-1.698010	0.544810	0.187500	0.693092
	50.03	-1.875490	0.516975	0.187500	0.741971
D6S285	51.08	-10000.000	0.489783	0.203125	0.854455
	66.22	0.625527	0.614234	0.125000	0.332752
	81.37	0.807581	0.754004	0.062500	0.211893
	96.51	0.972565	1.053345	0.062500	0.220932
	111.65	1.195325	1.703493	0.062500	0.362075
D6S271	126.79	1.499434	3.153974	0.062500	0.891326
	127.26	1.403143	2.578002	0.062500	0.821201
	127.73	1.278969	2.001126	0.062500	0.805076
	128.20	1.103787	1.422906	0.062500	0.818126
	128.67	0.803812	0.842898	0.062500	0.860659
D6S1650	129.14	-10000.000	0.260653	0.265625	0.958608
	129.41	-3.052720	0.260070	0.265625	0.964261
	129.67	-2.876604	0.259505	0.312500	0.970685
	129.93	-2.876534	0.258955	0.312500	0.977987
	130.20	-3.052512	0.258423	0.312500	0.986486
D6S1669	130.46	-10000.000	0.257907	0.312500	0.997713
	131.47	-1.887396	0.257305	0.312500	0.987418
	132.48	-1.712000	0.256943	0.312500	0.982954
	133.49	-1.712048	0.256823	0.312500	0.982859
	134.50	-1.887541	0.256944	0.312500	0.987123
D6S466	135.51	-10000.000	0.257304	0.312500	0.997171
	135.87	-2.765901	0.257157	0.312500	0.978591
	136.24	-2.590123	0.257043	0.312500	0.964834
	136.61	-2.590351	0.256959	0.312500	0.953195
	136.97	-2.766586	0.256908	0.312500	0.943092
D6S1661	137.34	-10000.000	0.256888	0.312500	0.934291
	137.50	-2.267801	0.256372	0.312500	0.928908
	137.66	-1.974039	0.255862	0.312500	0.923893
	137.82	-1.805304	0.255357	0.312500	0.919138
	137.99	-1.687813	0.254858	0.312500	0.914618
D6S257	138.15	-1.598446	0.254365	0.312500	0.910339
	140.13	-1.170866	0.227367	0.312500	0.847649
	142.12	-1.075112	0.201070	0.312500	0.807008
	144.10	-1.110716	0.175419	0.328125	0.781898
	146.08	-1.308261	0.150338	0.359375	0.770653

D6S254	148.07	-10000.000	0.125732	0.359375	0.776213
	151.93	-0.889295	-0.026889	0.406250	0.604415
	155.78	-0.839867	-0.187966	0.406250	0.547023
	159.64	-1.010059	-0.358962	0.593750	0.562132
	163.50	-1.473684	-0.542043	0.671875	0.656193
D6S252	167.35	-10000.000	-0.739830	0.796875	0.912073
	171.21	-2.475210	-0.702393	0.796875	0.700477
	175.07	-2.253069	-0.681851	0.781250	0.612417
	178.92	-2.428063	-0.673556	0.781250	0.586794
	182.78	-3.073983	-0.672777	0.781250	0.618160
D6S474	186.64	-10000.000	-0.674636	0.781250	0.742383
	189.65	-5.332687	-0.693546	0.796875	0.625150
	192.66	-4.932595	-0.722426	0.796875	0.586222
	195.67	-5.108031	-0.763693	0.843750	0.593031
	198.68	-5.932785	-0.820065	0.859375	0.646957
D6S262	201.69	-10000.000	-0.894818	0.953125	0.786978
	205.55	-6.602553	-0.866147	0.921875	0.637274
	209.40	-5.954459	-0.854802	0.921875	0.588729
	213.26	-6.013272	-0.860017	0.921875	0.599274
	217.12	-6.781206	-0.881680	0.953125	0.672513
ATA1F08	220.97	-10000.000	-0.920305	0.953125	0.867582
	226.77	-6.266238	-0.832961	0.906250	0.649197
	232.57	-5.465434	-0.780313	0.859375	0.574674
	238.37	-5.383748	-0.761482	0.843750	0.568974
	244.17	-6.011774	-0.778833	0.859375	0.627271
D6S255	249.96	-10000.000	-0.838245	0.906250	0.791060
	251.47	-8.706051	-0.814164	0.859375	0.704250
	252.98	-7.655049	-0.792724	0.859375	0.677770
	254.49	-7.151449	-0.773868	0.859375	0.684587
	256.00	-6.973488	-0.757558	0.843750	0.725230
D6S305	257.51	-9.315551	-0.743781	0.843750	0.828939
	259.99	-5.118379	-0.725689	0.796875	0.702392
	262.48	-4.454701	-0.715086	0.796875	0.628125
	264.96	-4.085150	-0.711500	0.796875	0.577770
	267.44	-3.856639	-0.714625	0.796875	0.545342
D6S264	269.93	-3.722652	-0.724313	0.796875	0.531048
	271.67	-3.734062	-0.727031	0.796875	0.502009
	273.42	-3.785162	-0.733257	0.796875	0.491449
	275.16	-3.898327	-0.742935	0.843750	0.498142
	276.90	-4.147482	-0.756045	0.843750	0.525268
D6S503	278.65	-6.713688	-0.772606	0.859375	0.593396
	280.65	-3.346180	-0.713270	0.796875	0.491192
	282.65	-2.761867	-0.660223	0.781250	0.421662
	284.65	-2.404950	-0.612721	0.718750	0.366388
	286.65	-2.146899	-0.570108	0.703125	0.320768
	288.65	-1.945128	-0.531807	0.671875	0.282371

analyzing pedigree 2...

using non-originals: 22 1 24 2 3 4 5

position	LOD score	NPL score	p-value	information
-10.00	0.202891	0.173271	0.250000	0.037754
-8.00	0.217528	0.188519	0.250000	0.044708
-6.00	0.233069	0.205174	0.250000	0.053054
-4.00	0.249556	0.223378	0.250000	0.063132

	-2.00	0.267027	0.243288	0.250000	0.075405
D6S477	0.00	0.285524	0.265079	0.250000	0.090554
	9.16	0.250644	0.234432	0.250000	0.047620
	18.33	0.240602	0.239047	0.250000	0.031164
	27.49	0.258244	0.283589	0.250000	0.028346
	36.65	0.306756	0.381766	0.250000	0.037841
D6S259	45.81	0.388970	0.561555	0.203125	0.068568
	46.87	0.372887	0.530366	0.203125	0.061965
	47.92	0.357241	0.500961	0.203125	0.056060
	48.98	0.342026	0.473222	0.250000	0.050766
	50.03	0.327233	0.447044	0.250000	0.046007
D6S285	51.08	0.312856	0.422324	0.250000	0.041723
	66.22	0.146323	0.182438	0.250000	0.010521
	81.37	0.030819	0.059339	0.250000	0.002663
	96.51	-0.075377	-0.027760	0.265625	0.002032
	111.65	-0.244187	-0.130039	0.343750	0.012095
D6S271	126.79	-0.971415	-0.310735	0.437500	0.117289
	127.26	-1.062897	-0.318703	0.468750	0.127587
	127.73	-1.182202	-0.326864	0.468750	0.139192
	128.20	-1.352221	-0.335223	0.500000	0.152480
	128.67	-1.645586	-0.343786	0.500000	0.168177
D6S1650	129.14	-3.613525	-0.352558	0.531250	0.189341
	129.41	-2.868445	-0.356526	0.531250	0.173428
	129.67	-2.701530	-0.360713	0.531250	0.167793
	129.93	-2.682731	-0.365123	0.531250	0.167293
	130.20	-2.810963	-0.369759	0.531250	0.171466
D6S1669	130.46	-3.492423	-0.374627	0.531250	0.183036
	131.47	-2.114723	-0.371101	0.531250	0.171353
	132.48	-1.893307	-0.369621	0.531250	0.181415
	133.49	-1.844708	-0.370358	0.531250	0.210967
	134.50	-1.968198	-0.373496	0.531250	0.263186
D6S466	135.51	-3.248301	-0.379233	0.531250	0.361672
	135.87	-2.651095	-0.422115	0.609375	0.275447
	136.24	-2.633101	-0.465293	0.609375	0.241206
	136.61	-2.812499	-0.508713	0.671875	0.233888
	136.97	-3.263846	-0.552318	0.671875	0.252941
D6S1661	137.34	-10000.000	-0.596050	0.734375	0.320622
	137.50	-4.308309	-0.592613	0.734375	0.309962
	137.66	-3.716869	-0.589189	0.734375	0.301343
	137.82	-3.370393	-0.585779	0.734375	0.293550
	137.99	-3.124974	-0.582382	0.734375	0.286317
D6S257	138.15	-2.935108	-0.578998	0.718750	0.279515
	140.13	-1.897885	-0.538504	0.671875	0.215477
	142.12	-1.469941	-0.500156	0.656250	0.171234
	144.10	-1.205708	-0.463990	0.609375	0.138158
	146.08	-1.018956	-0.429999	0.609375	0.112595
D6S254	148.07	-0.877382	-0.398143	0.562500	0.092444
	151.93	-0.678708	-0.342107	0.500000	0.064011
	155.78	-0.540388	-0.293311	0.437500	0.045005
	159.64	-0.438427	-0.251058	0.421875	0.031997
	163.50	-0.360472	-0.214628	0.406250	0.022941
D6S252	167.35	-0.299344	-0.183319	0.343750	0.016557
	171.21	-0.250519	-0.156476	0.343750	0.012010
	175.07	-0.210968	-0.133506	0.343750	0.008748



	178.92	-0.178576	-0.113875	0.343750	0.006394
	182.78	-0.151813	-0.097116	0.312500	0.004686
D6S474	186.64	-0.129542	-0.082818	0.281250	0.003443
	189.65	-0.114711	-0.073135	0.281250	0.002710
	192.66	-0.101762	-0.064587	0.281250	0.002137
	195.67	-0.090425	-0.057041	0.281250	0.001686
	198.68	-0.080475	-0.050381	0.281250	0.001333
D6S262	201.69	-0.071722	-0.044502	0.281250	0.001054
	205.55	-0.062011	-0.037969	0.265625	0.000783
	209.40	-0.053727	-0.032401	0.265625	0.000582
	213.26	-0.046642	-0.027654	0.265625	0.000434
	217.12	-0.040568	-0.023608	0.265625	0.000325
ATA1F08	220.97	-0.035350	-0.020158	0.265625	0.000243
	226.77	-0.028838	-0.015902	0.265625	0.000159
	232.57	-0.023617	-0.012550	0.265625	0.000105
	238.37	-0.019417	-0.009908	0.265625	0.000069
	244.17	-0.016024	-0.007826	0.265625	0.000047
D6S255	249.96	-0.013274	-0.006184	0.265625	0.000032
	251.47	-0.012648	-0.005817	0.265625	0.000029
	252.98	-0.012054	-0.005471	0.265625	0.000026
	254.49	-0.011490	-0.005147	0.265625	0.000024
	256.00	-0.010956	-0.004841	0.265625	0.000021
D6S305	257.51	-0.010450	-0.004554	0.265625	0.000019
	259.99	-0.009671	-0.004118	0.265625	0.000017
	262.48	-0.008956	-0.003724	0.265625	0.000014
	264.96	-0.008300	-0.003368	0.265625	0.000012
	267.44	-0.007697	-0.003046	0.265625	0.000011
D6S264	269.93	-0.007142	-0.002755	0.265625	0.000009
	271.67	-0.006780	-0.002567	0.265625	0.000008
	273.42	-0.006438	-0.002393	0.265625	0.000008
	275.16	-0.006115	-0.002230	0.265625	0.000007
	276.90	-0.005811	-0.002079	0.265625	0.000006
D6S503	278.65	-0.005523	-0.001937	0.265625	0.000006
	280.65	-0.005213	-0.001787	0.265625	0.000005
	282.65	-0.004922	-0.001649	0.265625	0.000005
	284.65	-0.004649	-0.001521	0.265625	0.000004
	286.65	-0.004393	-0.001403	0.265625	0.000004
	288.65	-0.004153	-0.001294	0.265625	0.000003

Totalling pedigrees: ..

	position	LOD_score	NPL_score	p-value	information
	-10.00	0.291638	0.34173	0.201416	0.149818
	-8.00	0.250472	0.36482	0.199707	0.169684
	-6.00	0.183365	0.38920	0.191895	0.193150
	-4.00	0.067249	0.41486	0.190674	0.221358
	-2.00	-0.171609	0.44179	0.186279	0.256401
D6S477	0.00	-INFINITY	0.46996	0.181152	0.306196
	9.16	0.007067	0.41479	0.190674	0.216954
	18.33	0.166447	0.41560	0.190674	0.193225
	27.49	0.218400	0.47705	0.179688	0.206560
	36.65	0.166201	0.61193	0.166504	0.263266
D6S259	45.81	-INFINITY	0.84472	0.139160	0.416313
	46.87	-1.496357	0.80124	0.144043	0.377149
	47.92	-1.338687	0.75967	0.148193	0.366508

	48.98	-1.355984	0.71986	0.154297	0.371929
	50.03	-1.548257	0.68166	0.159424	0.393989
D6S285	51.08	-INFINITY	0.64496	0.161865	0.448089
	66.22	0.771850	0.56333	0.170654	0.171636
	81.37	0.838400	0.57512	0.169922	0.107278
	96.51	0.897188	0.72520	0.154297	0.111482
	111.65	0.951138	1.11260	0.121582	0.187085
D6S271	126.79	0.528019	2.01047	0.084473	0.504308
	127.26	0.340246	1.59757	0.121094	0.474394
	127.73	0.096767	1.18388	0.121094	0.472134
	128.20	-0.248434	0.76911	0.147461	0.485303
	128.67	-0.841774	0.35293	0.200195	0.514418
D6S1650	129.14	-INFINITY	-0.06499	0.400146	0.573975
	129.41	-5.921165	-0.06820	0.400146	0.568845
	129.67	-5.578134	-0.07156	0.408447	0.569239
	129.93	-5.559265	-0.07507	0.409912	0.572640
	130.20	-5.863475	-0.07873	0.412598	0.578976
D6S1669	130.46	-INFINITY	-0.08253	0.413818	0.590375
	131.47	-4.002119	-0.08047	0.413086	0.579386
	132.48	-3.605307	-0.07967	0.413086	0.582184
	133.49	-3.556756	-0.08028	0.413086	0.596913
	134.50	-3.855739	-0.08241	0.413818	0.625154
D6S466	135.51	-INFINITY	-0.08622	0.413818	0.679422
	135.87	-5.416996	-0.11664	0.435791	0.627019
	136.24	-5.223224	-0.14726	0.453369	0.603020
	136.61	-5.402850	-0.17802	0.468018	0.593542
	136.97	-6.030432	-0.20889	0.480957	0.598016
D6S1661	137.34	-INFINITY	-0.23982	0.498535	0.627457
	137.50	-6.576110	-0.23776	0.498535	0.619435
	137.66	-5.690908	-0.23570	0.498535	0.612618
	137.82	-5.175697	-0.23364	0.498535	0.606344
	137.99	-4.812788	-0.23159	0.498535	0.600467
D6S257	138.15	-4.533554	-0.22955	0.498291	0.594927
	140.13	-3.068751	-0.22001	0.492676	0.531563
	142.12	-2.545053	-0.21149	0.485352	0.489121
	144.10	-2.316424	-0.20405	0.478516	0.460028
	146.08	-2.327217	-0.19775	0.477051	0.441614
D6S254	148.07	-INFINITY	-0.19262	0.474854	0.434328
	151.93	-1.568003	-0.26092	0.511963	0.334213
	155.78	-1.380255	-0.34031	0.556396	0.296014
	159.64	-1.448485	-0.43135	0.591797	0.297064
	163.50	-1.834155	-0.53505	0.652100	0.339567
D6S252	167.35	-INFINITY	-0.65276	0.723389	0.464315
	171.21	-2.725728	-0.60731	0.690186	0.356244
	175.07	-2.464036	-0.57654	0.668457	0.310583
	178.92	-2.606638	-0.55680	0.661621	0.296594
	182.78	-3.225796	-0.54440	0.654297	0.311423
D6S474	186.64	-INFINITY	-0.53560	0.652100	0.372913
	189.65	-5.447398	-0.54213	0.653809	0.313930
	192.66	-5.034357	-0.55650	0.661621	0.294179
	195.67	-5.198456	-0.58035	0.668945	0.297359
	198.68	-6.013260	-0.61550	0.694336	0.324145
D6S262	201.69	-INFINITY	-0.66420	0.730957	0.394016
	205.55	-6.664563	-0.63931	0.702393	0.319028

	209.40	-6.008186	-0.62735	0.698730	0.294656
	213.26	-6.059914	-0.62768	0.698730	0.299854
	217.12	-6.821774	-0.64014	0.702393	0.336419
ATA1F08	220.97	-INFINITY	-0.66501	0.730957	0.433913
	226.77	-6.295075	-0.60024	0.684570	0.324678
	232.57	-5.489052	-0.56064	0.662598	0.287389
	238.37	-5.403165	-0.54546	0.654785	0.284522
	244.17	-6.027798	-0.55625	0.660645	0.313659
D6S255	249.96	-INFINITY	-0.59710	0.684570	0.395546
	251.47	-8.718699	-0.57981	0.668945	0.352139
	252.98	-7.667103	-0.56441	0.663330	0.338898
	254.49	-7.162940	-0.55085	0.658936	0.342306
	256.00	-6.984444	-0.53910	0.653809	0.362626
D6S305	257.51	-9.326001	-0.52915	0.645996	0.414479
	259.99	-5.128049	-0.51605	0.641357	0.351204
	262.48	-4.463657	-0.50828	0.635010	0.314070
	264.96	-4.093450	-0.50549	0.634033	0.288891
	267.44	-3.864335	-0.50747	0.635010	0.272676
D6S264	269.93	-3.729794	-0.51411	0.639404	0.265529
	271.67	-3.740842	-0.51590	0.641357	0.251009
	273.42	-3.791600	-0.52018	0.641357	0.245728
	275.16	-3.904442	-0.52691	0.642090	0.249074
	276.90	-4.153292	-0.53607	0.652100	0.262637
D6S503	278.65	-6.719211	-0.54768	0.658447	0.296701
	280.65	-3.351393	-0.50562	0.634033	0.245599
	282.65	-2.766789	-0.46801	0.612549	0.210833
	284.65	-2.409599	-0.43433	0.593018	0.183196
	286.65	-2.151292	-0.40412	0.585449	0.160386
	288.65	-1.949281	-0.37696	0.567871	0.141187

# **CHROMOSOME 7**

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	0.104777	-0.128747	0.406250	0.329721
	-8.00	0.126250	-0.135500	0.406250	0.383281
	-6.00	0.149070	-0.141449	0.406250	0.448958
	-4.00	0.172963	-0.146235	0.406250	0.531372
	-2.00	0.197568	-0.149412	0.406250	0.639506
D7S531	0.00	0.222421	-0.150431	0.406250	0.810650
	1.28	0.028669	-0.216196	0.406250	0.632458
	2.56	-0.219990	-0.270723	0.484375	0.581972
	3.84	-0.569744	-0.314162	0.500000	0.602404
	5.11	-1.167993	-0.346520	0.500000	0.693742
D7S517	6.39	-10000.000	-0.367660	0.593750	0.915070
	8.38	-0.541153	-0.435725	0.593750	0.604761
	10.36	-0.073494	-0.448545	0.625000	0.499950
	12.35	0.095320	-0.437571	0.593750	0.502121
	14.33	0.038947	-0.431824	0.593750	0.607160
D7S513	16.31	-10000.000	-0.459010	0.625000	0.897372
	21.09	-1.091354	-0.494265	0.625000	0.791691
	25.87	-1.013959	-0.547578	0.671875	0.759981
	30.66	-1.155247	-0.620014	0.718750	0.774380

	35.44	-1.588997	-0.712877	0.796875	0.838953
D7S493	40.22	-10000.000	-0.827443	0.906250	1.000000
	41.96	-2.357880	-0.791050	0.859375	0.949798
	43.70	-1.805240	-0.757667	0.843750	0.921774
	45.45	-1.503213	-0.727269	0.796875	0.906197
	47.19	-1.304910	-0.699824	0.796875	0.901612
D7S1808	48.93	-1.164572	-0.675293	0.781250	0.910847
	50.68	-1.207427	-0.695723	0.796875	0.888460
	52.42	-1.293653	-0.719389	0.796875	0.881485
	54.16	-1.443920	-0.746368	0.843750	0.885269
	55.91	-1.730800	-0.776740	0.859375	0.900238
D7S516	57.65	-4.228576	-0.810587	0.859375	0.933147
	59.88	-2.471599	-0.801751	0.859375	0.829973
	62.11	-2.499824	-0.798787	0.859375	0.798065
	64.35	-2.807292	-0.801622	0.859375	0.807613
	66.58	-3.540774	-0.810182	0.859375	0.859537
D7S817	68.81	-10000.000	-0.824393	0.859375	0.987395
	69.22	-6.728563	-0.824511	0.859375	0.957693
	69.62	-6.012116	-0.824824	0.859375	0.939155
	70.03	-5.681101	-0.825333	0.859375	0.925543
	70.44	-5.588040	-0.826037	0.859375	0.915669
D7S484	70.85	-5.981478	-0.826937	0.859375	0.909561
	72.36	-4.029731	-0.804472	0.859375	0.716130
	73.87	-3.675571	-0.784529	0.859375	0.652189
	75.37	-3.624779	-0.767122	0.843750	0.664384
	76.88	-3.917331	-0.752265	0.843750	0.755729
D7S510	78.39	-10000.000	-0.739971	0.843750	1.000000
	80.88	-3.121861	-0.706709	0.796875	0.901029
	83.36	-2.776274	-0.679397	0.781250	0.853152
	85.84	-2.778910	-0.658096	0.781250	0.831875
	88.33	-3.129768	-0.642880	0.750000	0.833532
D7S519	90.81	-10000.000	-0.633845	0.750000	0.863870
	93.30	-2.855597	-0.609312	0.718750	0.737560
	95.78	-2.504739	-0.591468	0.718750	0.683547
	98.27	-2.502102	-0.580471	0.703125	0.668397
	100.75	-2.847690	-0.576484	0.703125	0.689536
D7S502	103.24	-10000.000	-0.579670	0.703125	0.766208
	105.72	-1.434938	-0.452977	0.625000	0.598749
	108.21	-0.881974	-0.335661	0.500000	0.543841
	110.69	-0.579032	-0.226505	0.406250	0.553146
	113.18	-0.379272	-0.124518	0.406250	0.628836
D7S669	115.66	-0.237360	-0.028942	0.406250	0.828264
	119.82	-0.201432	0.000765	0.406250	0.758460
	123.97	-0.213281	0.029157	0.406250	0.742230
	128.13	-0.285669	0.056415	0.359375	0.760505
	132.28	-0.486878	0.082422	0.359375	0.815476
D7S657	136.44	-10000.000	0.106628	0.359375	0.934771
	147.22	-0.159452	-0.011170	0.406250	0.422207
	158.01	-0.199585	-0.107022	0.406250	0.270243
	168.80	-0.435145	-0.177508	0.406250	0.263310
	179.59	-0.951174	-0.219625	0.406250	0.394509
D7S530	190.38	-6.106240	-0.223378	0.406250	0.839773
	191.43	-4.941560	-0.225840	0.406250	0.770650
	192.48	-4.947571	-0.230062	0.406250	0.762110

	193.54	-5.235459	-0.236003	0.406250	0.789569
	194.59	-5.952402	-0.243640	0.406250	0.855849
D7S640	195.64	-10000.000	-0.252961	0.484375	1.000000
	216.85	-0.715821	-0.203128	0.406250	0.246252
	238.05	-0.240158	-0.126470	0.406250	0.086459
	259.25	-0.057453	-0.050711	0.406250	0.038783
	280.45	0.058500	0.040031	0.359375	0.048202
D7S550	301.66	0.188958	0.193829	0.328125	0.257898
	303.66	0.177355	0.178972	0.328125	0.200511
	305.66	0.166311	0.165148	0.328125	0.163892
	307.66	0.155815	0.152297	0.359375	0.135917
	309.66	0.145857	0.140359	0.359375	0.113636
	311.66	0.136424	0.129279	0.359375	0.095518

# **CHROMOSOME 8**

analyzing pedigree 1....

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-1.060936	-0.220905	0.406250	0.401243
	-8.00	-1.231254	-0.245817	0.406250	0.450907
	-6.00	-1.458154	-0.272708	0.484375	0.509437
	-4.00	-1.788016	-0.301665	0.500000	0.579946
	-2.00	-2.368571	-0.332767	0.500000	0.668590
D8S504	0.00	-10000.000	-0.366077	0.593750	0.801830
	1.80	-4.917562	-0.505279	0.625000	0.703663
	3.60	-4.810388	-0.621452	0.718750	0.675545
	5.40	-5.156335	-0.714436	0.796875	0.687933
	7.20	-6.102844	-0.783891	0.859375	0.742401
D8S277	9.00	-10000.000	-0.829292	0.906250	0.876421
	11.20	-8.754543	-0.815515	0.859375	0.843825
	13.40	-8.053456	-0.805048	0.859375	0.832319
	15.60	-8.053456	-0.797868	0.859375	0.835273
	17.80	-8.754543	-0.793946	0.859375	0.853212
D8S550	20.00	-10000.000	-0.793244	0.859375	0.895649
	24.00	-5.073037	-0.785922	0.859375	0.701710
	28.00	-4.215938	-0.789074	0.859375	0.628981
	32.00	-3.978798	-0.803070	0.859375	0.623829
	36.00	-4.230942	-0.828232	0.906250	0.687051
D8S258	40.00	-10000.000	-0.864833	0.921875	0.874432
	44.00	-3.156672	-0.787784	0.859375	0.636898
	48.00	-2.669713	-0.718814	0.796875	0.557013
	52.00	-2.598235	-0.659078	0.781250	0.561826
	56.00	-2.912548	-0.609785	0.718750	0.650668
D8S283	60.00	-7.442337	-0.572426	0.703125	0.886818
	62.40	-4.544798	-0.562948	0.671875	0.780311
	64.80	-4.419233	-0.556977	0.671875	0.745538
	67.20	-4.540687	-0.554500	0.671875	0.750643
	69.60	-4.970895	-0.555568	0.671875	0.795616
D8S285	72.00	-7.943771	-0.560301	0.671875	0.910723
	73.80	-5.583655	-0.559107	0.671875	0.899160
	75.60	-5.425924	-0.559938	0.671875	0.900617
	77.40	-5.587875	-0.562783	0.671875	0.912502
	79.20	-6.150757	-0.567653	0.671875	0.935852

D8S260	81.00	-10000.000	-0.574581	0.703125	0.977893
	83.20	-4.933044	-0.442753	0.625000	0.741464
	85.40	-4.388371	-0.315701	0.500000	0.646365
	87.60	-4.125936	-0.192085	0.406250	0.622994
	89.80	-3.998531	-0.070670	0.406250	0.667723
D8S279	92.00	-3.964892	0.049690	0.359375	0.831613
	94.40	-4.054084	0.030364	0.406250	0.750090
	96.80	-4.249047	0.013552	0.406250	0.718617
	99.20	-4.614410	-0.000914	0.406250	0.718291
	101.60	-5.367933	-0.013186	0.406250	0.749341
D8S270	104.00	-10000.000	-0.023408	0.406250	0.831743
	106.40	-4.092103	-0.009126	0.406250	0.684983
	108.80	-3.298740	0.007258	0.406250	0.614483
	111.20	-2.890799	0.025964	0.406250	0.583868
	113.60	-2.651148	0.047223	0.359375	0.588182
D8S556	116.00	-2.516976	0.071285	0.359375	0.638851
	118.40	-2.478474	0.066779	0.359375	0.627902
	120.80	-2.529689	0.065099	0.359375	0.639956
	123.20	-2.707018	0.066268	0.359375	0.676188
	125.60	-3.151682	0.070331	0.359375	0.742834
D8S514	128.00	-10000.000	0.077350	0.359375	0.872520
	130.80	-1.725017	0.192116	0.328125	0.767816
	133.60	-1.253962	0.307660	0.265625	0.737862
	136.40	-1.079363	0.425073	0.203125	0.753275
	139.20	-1.127428	0.545419	0.187500	0.816583
D8S284	142.00	-10000.000	0.669710	0.125000	0.968964
	144.00	-0.265977	0.637974	0.125000	0.798411
	146.00	-0.014275	0.606515	0.125000	0.685340
	148.00	0.113749	0.575631	0.125000	0.596251
	150.00	0.191873	0.545544	0.187500	0.523023
	152.00	0.243209	0.516417	0.187500	0.461489

# **CHROMOSOME 9**

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6

	position	LOD score	NPL score	p-value	information
	-10.00	-0.451631	-0.378420	0.593750	0.276585
	-8.00	-0.539732	-0.404451	0.593750	0.312713
	-6.00	-0.656786	-0.432111	0.593750	0.355919
	-4.00	-0.826072	-0.461464	0.625000	0.408790
	-2.00	-1.121558	-0.492568	0.625000	0.476321
D9S178	0.00	-10000.000	-0.525471	0.640625	0.579018
	2.80	-2.049289	-0.594189	0.718750	0.431451
	5.60	-2.233332	-0.662928	0.781250	0.421989
	8.40	-2.743748	-0.731485	0.796875	0.492569
	11.20	-3.801828	-0.799636	0.859375	0.650841
D9S168	14.00	-10000.000	-0.867123	0.921875	0.991202
	15.60	-4.938280	-0.850609	0.906250	0.909855
	17.20	-3.863868	-0.835493	0.906250	0.866427
	18.80	-3.339063	-0.821798	0.859375	0.845880
	20.40	-3.142607	-0.809542	0.859375	0.846637
D9S156	22.00	-10000.000	-0.798745	0.859375	0.877331

	25.60	-1.907231	-0.605341	0.718750	0.539007
	29.20	-1.491325	-0.398943	0.593750	0.399278
	32.80	-1.147032	-0.178195	0.406250	0.351829
	36.40	-0.870382	0.058898	0.359375	0.380934
D9S741	40.00	-0.673569	0.314935	0.265625	0.513453
	42.00	-0.636595	0.349720	0.250000	0.531879
	44.00	-0.635604	0.389238	0.250000	0.568684
	46.00	-0.692460	0.433459	0.203125	0.623946
	48.00	-0.879893	0.482369	0.203125	0.703203
D9S319	50.00	-10000.000	0.535958	0.187500	0.833741
	52.20	-1.374300	0.524358	0.187500	0.647270
	54.40	-1.329881	0.516212	0.187500	0.589469
	56.60	-1.510139	0.511505	0.203125	0.602626
	58.80	-1.988856	0.510226	0.203125	0.688786
D9S301	61.00	-10000.000	0.512372	0.203125	0.915382
	65.40	-2.774750	0.307293	0.265625	0.774976
	69.80	-2.509482	0.107427	0.359375	0.723664
	74.20	-2.608848	-0.092833	0.406250	0.729166
	78.60	-3.086885	-0.299521	0.500000	0.794329
D9S303	83.00	-10000.000	-0.519139	0.625000	0.972027
	84.40	-4.995164	-0.481799	0.625000	0.928785
	85.80	-4.627139	-0.446529	0.625000	0.904954
	87.20	-4.611578	-0.413195	0.593750	0.891509
	88.60	-4.948663	-0.381673	0.593750	0.887035
D9S167	90.00	-10000.000	-0.351849	0.500000	0.893733
	93.20	-3.553596	-0.362361	0.593750	0.825840
	96.40	-3.192589	-0.381085	0.593750	0.799538
	99.60	-3.184847	-0.408641	0.593750	0.800844
	102.80	-3.529842	-0.445869	0.625000	0.831441
D9S197	106.00	-10000.000	-0.493865	0.625000	0.913254
	108.20	-4.605103	-0.455560	0.625000	0.660730
	110.40	-4.355545	-0.428422	0.593750	0.556431
	112.60	-4.512189	-0.409412	0.593750	0.527875
	114.80	-5.148006	-0.395659	0.593750	0.571872
D9S299	117.00	-10000.000	-0.384421	0.593750	0.746177
	124.20	-3.753555	-0.327753	0.500000	0.498464
	131.40	-3.173256	-0.269145	0.484375	0.419720
	138.60	-3.116736	-0.209044	0.406250	0.421415
	145.80	-3.579514	-0.146592	0.406250	0.499715
D9S159	153.00	-10000.000	-0.079940	0.406250	0.721923
	156.60	-3.007629	-0.022319	0.406250	0.605841
	160.20	-2.170811	0.038456	0.359375	0.548082
	163.80	-1.704722	0.103446	0.359375	0.522680
	167.40	-1.388011	0.173892	0.328125	0.529501
D9S158	171.00	-1.151311	0.251225	0.312500	0.592687
	173.00	-1.056850	0.226016	0.312500	0.484304
	175.00	-0.974542	0.202950	0.312500	0.411757
	177.00	-0.902124	0.181851	0.328125	0.354550
	179.00	-0.837884	0.162561	0.328125	0.307661
	181.00	-0.780497	0.144932	0.359375	0.268464

# CHROMOSOME 10

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analyzing pedigree 1...
using non-origins: 1 2 3 4 5 6
position LOD score NPL score p-value information
-10.00 -0.444102 -0.362809 0.593750 0.428272
-8.00 -0.589392 -0.393347 0.593750 0.486114
-6.00 -0.789897 -0.424924 0.593750 0.555045
-4.00 -1.091809 -0.457251 0.625000 0.639045
-2.00 -1.642429 -0.489938 0.625000 0.745946
D10S249 0.00 -10000.000 -0.522471 0.640625 0.909078
2.60 -1.560653 -0.561311 0.671875 0.664114
5.20 -1.272566 -0.606206 0.718750 0.565418
7.80 -1.342143 -0.660722 0.781250 0.542518
10.40 -1.772913 -0.725362 0.796875 0.590789
D10S189 13.00 -10000.000 -0.797606 0.859375 0.753024
15.00 -3.478017 -0.725895 0.796875 0.666848
17.00 -3.095117 -0.660042 0.781250 0.639769
19.00 -3.069473 -0.600314 0.718750 0.649411
21.00 -3.400308 -0.547024 0.671875 0.696800
D10S465 23.00 -10000.000 -0.500543 0.625000 0.810969
25.20 -4.933965 -0.496978 0.625000 0.793115
27.40 -4.678790 -0.497622 0.625000 0.800633
29.60 -4.826580 -0.502692 0.625000 0.830445
31.80 -5.450637 -0.512449 0.625000 0.887482
D10S191 34.00 -10000.000 -0.527199 0.671875 1.000000
35.60 -6.858708 -0.523808 0.640625 0.937091
37.20 -6.334816 -0.522770 0.640625 0.901675
38.80 -6.334816 -0.524071 0.640625 0.880328
40.40 -6.858708 -0.527709 0.671875 0.870626
D10S466 42.00 -10000.000 -0.533692 0.671875 0.874106
44.00 -6.265975 -0.525096 0.640625 0.841586
46.00 -5.735534 -0.520149 0.625000 0.827231
48.00 -5.724095 -0.518830 0.625000 0.826583
50.00 -6.216046 -0.521144 0.625000 0.839990
D10S245 52.00 -10000.000 -0.527123 0.640625 0.874163
53.00 -7.252067 -0.538814 0.671875 0.818142
54.00 -6.735354 -0.551520 0.671875 0.785075
55.00 -6.629790 -0.565225 0.671875 0.762644
56.00 -6.903699 -0.579914 0.703125 0.748334
D10S89 57.00 -10000.000 -0.595574 0.718750 0.741540
59.20 -5.526937 -0.614761 0.718750 0.722498
61.40 -5.186979 -0.638913 0.750000 0.733059
63.60 -5.271211 -0.667900 0.781250 0.772422
65.80 -5.844890 -0.701618 0.796875 0.847885
D10S183 68.00 -10000.000 -0.739971 0.796875 1.000000
70.20 -3.842517 -0.756396 0.843750 0.683293
72.40 -3.301106 -0.779900 0.859375 0.550377
74.60 -3.248791 -0.808993 0.859375 0.510477
76.80 -3.616910 -0.843211 0.906250 0.557502
D10S220 79.00 -10000.000 -0.883162 0.953125 0.750505
81.00 -3.892923 -0.839058 0.906250 0.532476
83.00 -3.730449 -0.799977 0.859375 0.459060
85.00 -4.026723 -0.766348 0.843750 0.467146
87.00 -4.927581 -0.737855 0.796875 0.558294
D10S464 89.00 -10000.000 -0.713432 0.796875 0.805334

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	91.80	-5.486259	-0.706654	0.796875	0.672382
	94.60	-4.558413	-0.689531	0.796875	0.632428
	97.40	-4.230357	-0.664225	0.781250	0.647599
	100.20	-4.354717	-0.632689	0.750000	0.721082
D10S676	103.00	-10000.000	-0.596699	0.718750	0.906797
	105.00	-4.365545	-0.592632	0.718750	0.796798
	107.00	-4.039972	-0.592857	0.718750	0.763559
	109.00	-4.068372	-0.597616	0.718750	0.773833
	111.00	-4.451201	-0.607202	0.718750	0.829090
D10S109	113.00	-10000.000	-0.621966	0.718750	0.969665
	116.40	-3.382239	-0.427069	0.593750	0.753751
	119.80	-2.941027	-0.239067	0.406250	0.675850
	123.20	-2.864914	-0.055301	0.406250	0.669262
	126.60	-3.148226	0.126806	0.359375	0.730867
D10S677	130.00	-10000.000	0.309695	0.265625	0.906300
	132.20	-3.973235	0.330209	0.265625	0.866916
	134.40	-3.602986	0.352479	0.250000	0.850976
	136.60	-3.573490	0.376664	0.250000	0.849922
	138.80	-3.860337	0.402905	0.250000	0.863204
D10S254	141.00	-10000.000	0.431332	0.203125	0.896129
	142.60	-3.734529	0.423225	0.203125	0.870897
	144.20	-3.287326	0.416309	0.203125	0.857494
	145.80	-3.076717	0.410594	0.250000	0.852104
	147.40	-2.981344	0.406084	0.250000	0.854313
D10S187	149.00	-2.969997	0.402778	0.250000	0.865901
	150.00	-3.021358	0.307308	0.265625	0.767868
	151.00	-3.114894	0.213511	0.312500	0.733153
	152.00	-3.272595	0.121281	0.359375	0.733227
	153.00	-3.567889	0.030524	0.406250	0.768404
D10S209	154.00	-10000.000	-0.058849	0.406250	0.869086
	155.40	-3.943070	-0.211929	0.406250	0.844536
	156.80	-3.975997	-0.365110	0.593750	0.842899
	158.20	-4.289662	-0.518608	0.625000	0.862130
	159.60	-5.031060	-0.672631	0.781250	0.906038
D10S1213	161.00	-10000.000	-0.827369	0.859375	0.999410
	163.00	-6.269479	-0.778657	0.859375	0.879676
	165.00	-5.744854	-0.734021	0.796875	0.837132
	167.00	-5.744854	-0.693651	0.796875	0.837264
	169.00	-6.269479	-0.657739	0.781250	0.880063
D10S186	171.00	-10000.000	-0.626493	0.750000	1.000000
	174.00	-2.727500	-0.384840	0.593750	0.685376
	177.00	-2.088881	-0.168551	0.406250	0.557391
	180.00	-1.923449	-0.015452	0.406250	0.523222
	183.00	-2.157455	0.039590	0.359375	0.576481
D10S1212	186.00	-10000.000	-0.038428	0.406250	0.779068
	188.00	-2.254036	-0.019221	0.406250	0.646164
	190.00	-1.683994	-0.002517	0.406250	0.558111
	192.00	-1.363531	0.011944	0.406250	0.488351
	194.00	-1.145062	0.024396	0.406250	0.430670
	196.00	-0.982327	0.035055	0.406250	0.381918

## CHROMOSOME 11

analyzing pedigree 1...					
using non-origins: 1 2 3 4 5 6 10					
	position	LOD score	NPL score	p-value	information
	-10.00	0.557343	0.174502	0.328125	0.458580
	-8.00	0.508443	0.118792	0.359375	0.520443
	-6.00	0.432472	0.050561	0.359375	0.594205
	-4.00	0.306362	-0.032372	0.406250	0.684169
	-2.00	0.056599	-0.132554	0.406250	0.798796
DI1S1984	0.00	-2.494893	-0.252961	0.484375	0.973878
	0.80	-1.446414	-0.249465	0.406250	0.968939
	1.60	-1.284547	-0.247723	0.406250	0.968567
	2.40	-1.285374	-0.247728	0.406250	0.971601
	3.20	-1.448897	-0.249473	0.406250	0.978195
DI1S988	4.00	-2.499031	-0.252961	0.484375	0.990331
	6.40	0.705175	0.324396	0.265625	0.812431
	8.80	1.000676	0.885225	0.062500	0.745524
	11.20	1.174609	1.439248	0.062500	0.736549
	13.60	1.300709	1.995867	0.062500	0.782616
DI1S875	16.00	1.402096	2.564395	0.062500	0.917363
	16.80	1.402836	2.570658	0.062500	0.912046
	17.60	1.403958	2.578868	0.062500	0.910109
	18.40	1.405462	2.589040	0.062500	0.910707
	19.20	1.407349	2.601194	0.062500	0.913842
DI1S902	20.00	1.409616	2.615350	0.062500	0.920367
	22.80	1.382033	2.449082	0.062500	0.780304
	25.60	1.357939	2.306359	0.062500	0.723925
	28.40	1.337231	2.183995	0.062500	0.714055
	31.20	1.319785	2.079233	0.062500	0.749434
DI1S904	34.00	1.305454	1.989657	0.062500	0.862101
	36.00	1.207076	1.513577	0.062500	0.809365
	38.00	1.082975	1.044482	0.062500	0.790851
	40.00	0.910035	0.577120	0.125000	0.796727
	42.00	0.614541	0.106238	0.359375	0.829139
DI1S1392	44.00	-2.494974	-0.373495	0.593750	0.910937
	48.00	-0.121952	-0.293347	0.500000	0.735446
	52.00	0.046225	-0.257448	0.484375	0.665390
	56.00	0.046241	-0.262683	0.484375	0.651219
	60.00	-0.121903	-0.308740	0.500000	0.690052
DI1S1985	64.00	-2.494893	-0.398082	0.593750	0.818959
	67.20	-0.301667	-0.350592	0.500000	0.786981
	70.40	-0.131197	-0.331782	0.500000	0.788755
	73.60	-0.131197	-0.340309	0.500000	0.817111
	76.80	-0.301667	-0.375978	0.593750	0.877150
DI1S937	80.00	-2.494893	-0.439727	0.625000	1.000000
	81.20	-1.117310	-0.431548	0.593750	0.981055
	82.40	-0.947978	-0.427470	0.593750	0.971402
	83.60	-0.947978	-0.427470	0.593750	0.967849
	84.80	-1.117310	-0.431548	0.593750	0.970092
DI1S1396	86.00	-2.494893	-0.439727	0.625000	0.979894
	88.40	-0.635309	-0.426077	0.593750	0.790245
	90.80	-0.587820	-0.434569	0.593750	0.725195
	93.20	-0.763495	-0.458044	0.625000	0.728318
	95.60	-1.236121	-0.489636	0.625000	0.800173
DI1S876	98.00	-10000.000	-0.522471	0.640625	1.000000

	99.60	-3.464360	-0.518375	0.625000	0.897260
	101.20	-3.037268	-0.516164	0.625000	0.859203
	102.80	-2.911784	-0.515871	0.625000	0.856570
	104.40	-3.015445	-0.517519	0.625000	0.889154
D11S1391	106.00	-4.601056	-0.521128	0.625000	0.985226
	108.00	-2.796129	-0.528824	0.671875	0.880648
	110.00	-2.668753	-0.539708	0.671875	0.844755
	112.00	-2.776977	-0.553742	0.671875	0.847312
	114.00	-3.191310	-0.570873	0.703125	0.888573
D11S976	116.00	-10000.000	-0.591031	0.718750	1.000000
	123.80	-0.562490	-0.474253	0.625000	0.580499
	131.60	-0.198045	-0.392458	0.593750	0.417903
	139.40	-0.114019	-0.365206	0.593750	0.365001
	147.20	-0.235722	-0.405130	0.593750	0.413425
D11S968	155.00	-10000.000	-0.529130	0.671875	0.664037
	157.00	-1.125967	-0.491565	0.625000	0.531028
	159.00	-0.839773	-0.456826	0.625000	0.445452
	161.00	-0.678651	-0.424697	0.593750	0.379410
	163.00	0.568756	-0.394975	0.593750	0.326215
	165.00	-0.486916	-0.367475	0.593750	0.282420

## CHROMOSOME 12

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-0.765064	-0.440872	0.625000	0.405788
	-8.00	-0.913137	-0.475623	0.625000	0.455966
	-6.00	-1.116148	-0.513536	0.625000	0.514894
	-4.00	-1.420332	-0.554882	0.671875	0.585435
	-2.00	-1.973252	-0.599940	0.718750	0.673014
D12S372	0.00	-10000.000	-0.648997	0.750000	0.799195
	1.40	-4.739552	-0.620895	0.718750	0.773925
	2.80	-4.388030	-0.594104	0.718750	0.763785
	4.20	-4.387609	-0.568522	0.671875	0.762453
	5.60	-4.738288	-0.544056	0.671875	0.769630
D12S274	7.00	-10000.000	-0.520617	0.625000	0.789850
	10.60	-2.471462	-0.453114	0.625000	0.714286
	14.20	-1.988744	-0.391345	0.593750	0.681381
	17.80	-1.763596	-0.333709	0.500000	0.676939
	21.40	-1.653184	-0.278790	0.484375	0.701179
D12S391	25.00	-1.618785	-0.225324	0.406250	0.770477
	28.40	-1.670493	-0.183340	0.406250	0.737484
	31.80	-1.794346	-0.140746	0.406250	0.738619
	35.20	-2.030835	-0.096488	0.406250	0.767240
	38.60	-2.523761	-0.049549	0.406250	0.828189
D12S269	42.00	-10000.000	0.001087	0.406250	0.952002
	47.40	-2.374124	-0.041935	0.406250	0.664428
	52.80	-2.070409	-0.077971	0.406250	0.559114
	58.20	-2.091221	-0.106930	0.406250	0.544717
	63.60	-2.370640	-0.128362	0.406250	0.616855
D12S61	69.00	-3.199780	-0.141366	0.406250	0.845041
	69.60	-3.469072	-0.164651	0.406250	0.860192

	70.20	-3.822674	-0.187714	0.406250	0.880488
	70.80	-4.329906	-0.210561	0.406250	0.906722
	71.40	-5.211936	-0.233195	0.406250	0.941140
GATA5A09	72.00	-10000.000	-0.255622	0.484375	0.994565
	78.60	-2.562031	-0.263780	0.484375	0.627514
	85.20	-2.048785	-0.297337	0.500000	0.491463
	91.80	-2.048785	-0.358865	0.593750	0.466076
	98.40	-2.562031	-0.452103	0.625000	0.554987
D12S375	105.00	-10000.000	-0.581893	0.703125	0.889198
	107.00	-6.354433	-0.443621	0.625000	0.598115
	109.00	-5.954343	-0.277173	0.484375	0.513811
	111.00	-6.130145	-0.080731	0.406250	0.538354
	113.00	-6.955626	0.148227	0.359375	0.669541
D12S379	115.00	-10000.000	0.412958	0.250000	0.999138
	116.80	-9.166150	0.273375	0.265625	0.865415
	118.60	-8.407029	0.137793	0.359375	0.795736
	120.40	-8.309393	0.005085	0.406250	0.756306
	122.20	-8.804766	-0.125839	0.406250	0.740990
D12S101	124.00	-10000.000	-0.256042	0.484375	0.750794
	125.80	-7.142794	-0.360721	0.593750	0.626027
	127.60	6.458222	-0.466916	0.625000	0.585037
	129.40	-6.274311	-0.575811	0.703125	0.592531
	131.20	-6.506864	-0.688633	0.796875	0.647999
PAH1	133.00	-9.139379	-0.806662	0.859375	0.781243
	134.20	-6.556151	-0.766393	0.843750	0.680850
	135.40	-6.530474	-0.727544	0.796875	0.648105
	136.60	-6.790985	-0.689997	0.796875	0.653568
	137.80	-7.483998	-0.653639	0.750000	0.698601
D12S392	139.00	-10000.000	-0.618363	0.718750	0.820008
	150.20	-1.408265	-0.425863	0.593750	0.344855
	161.40	-0.718556	-0.342891	0.500000	0.185815
	172.60	-0.414491	-0.333078	0.500000	0.149674
	183.80	-0.351381	-0.409314	0.593750	0.225987
	195.00	-3.439994	-0.628515	0.750000	0.580309

# **CHROMOSOME 13 STAGE 1**

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-1.077939	0.084946	0.359375	0.397331
	-8.00	-1.179332	0.096543	0.359375	0.450798
	-6.00	-1.306579	0.109107	0.359375	0.514918
	-4.00	-1.481612	0.122716	0.359375	0.593813
	-2.00	-1.776719	0.137455	0.359375	0.695642
D13S221	0.00	-10000.000	0.153424	0.359375	0.854182
	3.80	-2.500002	0.045819	0.359375	0.628094
	7.60	-2.562764	-0.055707	0.406250	0.540577
	11.40	-2.895598	-0.153963	0.406250	0.515865
	15.20	-3.646243	-0.251715	0.406250	0.542229
D13S220	19.00	-10000.000	-0.351764	0.500000	0.633190
	21.60	-1.702364	-0.201132	0.406250	0.454348
	24.20	-0.811259	0.004340	0.406250	0.390927
	26.80	-0.291157	0.266140	0.265625	0.393815

	29.40	0.079307	0.587884	0.125000	0.465607
D13S325	32.00	0.369500	0.975357	0.062500	0.668389
	38.90	0.684010	1.221671	0.062500	0.421380
	45.80	0.914248	1.502653	0.062500	0.351464
	52.70	1.112441	1.864799	0.062500	0.390003
	59.60	1.301068	2.364346	0.062500	0.550556
D13S317	66.50	1.491528	3.079777	0.062500	0.977965
	67.44	1.490867	3.107004	0.062500	0.776398
	68.38	1.490809	3.138029	0.062500	0.697115
	69.32	1.491356	3.172868	0.062500	0.680926
	70.26	1.492506	3.211550	0.046875	0.726063
D13S225	71.20	1.494258	3.254112	0.046875	0.882388
	73.32	1.485832	3.190794	0.062500	0.815468
	75.44	1.480414	3.147134	0.062500	0.787021
	77.56	1.478018	3.122612	0.062500	0.786222
	79.68	1.478650	3.116967	0.062500	0.814458
D13S173	81.80	1.482312	3.130189	0.062500	0.893861
	83.80	1.431828	2.855468	0.062500	0.743793
	85.80	1.382727	2.609389	0.062500	0.642136
	87.80	1.335002	2.388569	0.062500	0.561239
	89.80	1.288644	2.190062	0.062500	0.494269
	91.80	1.243639	2.011297	0.062500	0.437671

## CHROMOSOME 13 STAGE 2

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-1.076393	0.086494	0.359375	0.397848
	-8.00	-1.177704	0.098170	0.359375	0.451418
	-6.00	-1.304868	0.110815	0.359375	0.515666
	-4.00	-1.479815	0.124505	0.359375	0.594723
	-2.00	-1.774836	0.139328	0.359375	0.696761
D13S221	0.00	-10000.00	0.155383	0.328125	0.855585
	3.80	-2.497340	0.047902	0.359375	0.629693
	7.60	-2.559365	-0.053535	0.406250	0.542621
	11.40	-2.891425	-0.151758	0.406250	0.518605
	15.20	-3.641264	-0.249554	0.406250	0.546036
D13S220	19.00	-10000.00	-0.349762	0.500000	0.638731
	21.60	-2.439768	-0.150408	0.406250	0.491000
	24.20	-1.589867	0.138310	0.359375	0.450025
	26.80	-1.117157	0.519052	0.187500	0.473140
	29.40	-0.802047	0.998060	0.062500	0.564523
D13S325	46.20	-10000.00	3.115485	0.062500	0.832543
	48.78	-1.090643	3.054376	0.062500	0.815154
	51.36	-0.919637	3.021359	0.062500	0.815225
	53.94	-0.920598	3.015748	0.062500	0.830372
	56.52	-1.093526	3.037429	0.062500	0.862735
D13S321	59.10	-10000.00	3.086856	0.062500	0.923407
	60.56	0.791278	3.083301	0.062500	0.821815
	62.02	1.092393	3.088765	0.062500	0.786360
	63.48	1.269969	3.103271	0.062500	0.787215
	64.94	1.397791	3.126904	0.062500	0.824369

D13S792	66.40	1.498981	3.159807	0.062500	0.927520
	68.92	1.491006	3.107756	0.062500	0.823989
	71.44	1.487371	3.082972	0.062500	0.792856
	73.96	1.488089	3.085011	0.062500	0.803838
	76.48	1.493161	3.113962	0.062500	0.858512
D13S317	79.00	1.502574	3.170453	0.062500	0.994273
	79.46	1.502444	3.200653	0.046875	0.829727
	79.92	1.502461	3.231805	0.046875	0.753348
	80.38	1.502625	3.263914	0.046875	0.719875
	80.84	1.502934	3.296985	0.046875	0.725231
D13S795	81.30	1.503391	3.331024	0.046875	0.792706
	81.79	1.503028	3.328484	0.046875	0.785504
	82.27	1.502828	3.327027	0.046875	0.788429
	82.76	1.502793	3.326653	0.046875	0.802180
	83.24	1.502920	3.327360	0.046875	0.829411
D13S225	83.73	1.503212	3.329150	0.046875	0.884403
	84.65	1.501382	3.313282	0.046875	0.865892
	85.57	1.500135	3.301282	0.046875	0.862058
	86.49	1.499472	3.293127	0.046875	0.870369
	87.41	1.499392	3.288800	0.046875	0.892822
D13S797	88.33	1.499897	3.288297	0.046875	0.943386
	89.53	1.496288	3.259096	0.046875	0.923207
	90.73	1.493656	3.236358	0.046875	0.910249
	91.93	1.492005	3.219997	0.046875	0.902683
	93.13	1.491335	3.209954	0.046875	0.900276
D13S173	94.33	1.491648	3.206198	0.046875	0.904331
	96.33	1.440776	2.922776	0.062500	0.751417
	98.33	1.391289	2.669057	0.062500	0.648065
	100.33	1.343182	2.441522	0.062500	0.565987
	102.33	1.296446	2.237104	0.062500	0.498135
	104.33	1.251070	2.053131	0.062500	0.440852

analyzing pedigree 2...

using non-originals: 22 1 24 2 3 4 6 5

	position	LOD score	NPL score	p-value	information
	-10.00	-0.097641	-0.036275	0.265625	0.008385
	-8.00	-0.105401	-0.039352	0.281250	0.009274
	-6.00	-0.113889	-0.042693	0.281250	0.010271
	-4.00	-0.123187	-0.046322	0.281250	0.011391
	-2.00	-0.133389	-0.050263	0.281250	0.012654
D13S221	0.00	-0.144599	-0.054545	0.281250	0.014079
	3.80	-0.169128	-0.063726	0.281250	0.017326
	7.60	-0.198784	-0.074479	0.281250	0.021466
	11.40	-0.234944	-0.087076	0.281250	0.026791
	15.20	-0.279492	-0.101839	0.312500	0.033703
D13S220	19.00	-0.335082	-0.119140	0.343750	0.042766
	21.60	-0.381415	-0.132663	0.343750	0.050605
	24.20	-0.436458	-0.147734	0.343750	0.060161
	26.80	-0.502645	-0.164524	0.343750	0.071879
	29.40	-0.583476	-0.183221	0.343750	0.086347
D13S325	46.20	-1.0000.00	-0.364335	0.531250	0.365637
	48.78	-2.714744	-0.361310	0.531250	0.347663
	51.36	-2.317582	-0.362567	0.531250	0.355169
	53.94	-2.231125	-0.368117	0.531250	0.381478

	56.52	-2.387649	-0.378016	0.531250	0.429456
D13S321	59.10	-4.854395	-0.392372	0.531250	0.520018
	60.56	-2.948880	-0.394508	0.562500	0.444002
	62.02	-2.858866	-0.399680	0.562500	0.402327
	63.48	-2.997867	-0.407814	0.593750	0.378207
	64.94	-3.439074	-0.418844	0.593750	0.369412
D13S792	66.40	-10000.00	-0.432705	0.609375	0.379631
	68.92	-2.772431	-0.417808	0.593750	0.279355
	71.44	-2.374032	-0.414387	0.593750	0.228114
	73.96	-2.283677	-0.422317	0.609375	0.202276
	76.48	-2.404299	-0.441499	0.609375	0.197643
D13S317	79.00	-2.857948	-0.471866	0.609375	0.215305
	79.46	-3.021369	-0.478612	0.656250	0.221481
	79.92	-3.241775	-0.485729	0.656250	0.228849
	80.38	-3.565107	-0.493216	0.656250	0.237671
	80.84	-4.137705	-0.501074	0.656250	0.248496
D13S795	81.30	-10000.00	-0.509301	0.671875	0.263824
	81.79	-4.331817	-0.506439	0.671875	0.258738
	82.27	-3.771636	-0.503795	0.671875	0.255036
	82.76	-3.462852	-0.501368	0.656250	0.252062
	83.24	-3.259313	-0.499159	0.656250	0.249660
D13S225	83.73	-3.115622	-0.497171	0.656250	0.247758
	84.65	-2.945634	-0.494012	0.656250	0.245388
	85.57	-2.871439	-0.491651	0.656250	0.244531
	86.49	-2.889382	-0.490095	0.656250	0.245185
	87.41	-3.059711	-0.489353	0.656250	0.247512
D13S797	88.33	-10000.00	-0.489433	0.656250	0.252386
	89.53	-2.246284	-0.467939	0.609375	0.217496
	90.73	-1.834718	-0.447181	0.609375	0.192935
	91.93	-1.578160	-0.427164	0.609375	0.172790
	93.13	-1.391414	-0.407887	0.593750	0.155659
D13S173	94.33	-1.245396	-0.389345	0.531250	0.140817
	96.33	-1.057883	-0.360048	0.531250	0.119996
	98.33	-0.914913	-0.332707	0.500000	0.102949
	100.33	-0.801031	-0.307246	0.437500	0.088800
	102.33	-0.707634	-0.283580	0.437500	0.076945
	104.33	-0.629424	-0.261617	0.421875	0.066935

Totalling pedigrees: ..

	position	LOD score	(alpha, HLOD)	NPL score	p-value	information
	-10.00	-1.174034	(0.0000, 0.0000)	0.03551	0.336670	0.203117
	-8.00	-1.283105	(0.0001, 0.0001)	0.04159	0.334229	0.230346
	-6.00	-1.418756	(0.0000, 0.0000)	0.04817	0.325195	0.262968
	-4.00	-1.603002	(0.0000, 0.0000)	0.05528	0.322754	0.303057
	-2.00	-1.908225	(0.0000, 0.0001)	0.06298	0.319580	0.354708
D13S221	0.00	-INFINITY	(0.0000, 0.0000)	0.07130	0.313965	0.434832
	3.80	-2.666468	(0.0000, 0.0000)	-0.01119	0.366699	0.323509
	7.60	-2.758149	(0.0000, 0.0000)	-0.09052	0.415283	0.282044
	11.40	-3.126369	(0.0000, 0.0000)	-0.16888	0.459961	0.272698
	15.20	-3.920756	(0.0000, 0.0000)	-0.24847	0.505127	0.289869
D13S220	19.00	-INFINITY	(0.0000, 0.0000)	-0.33156	0.554932	0.340749
	21.60	-2.821183	(0.0000, 0.0000)	-0.20016	0.477051	0.270802
	24.20	-2.026325	(0.0000, 0.0000)	-0.00666	0.364990	0.255093
	26.80	-1.619802	(0.0000, 0.0000)	0.25069	0.229492	0.272510

	29.40	-1.385523	(0.0000, 0.0000)	0.57618 0.169922	0.325435
D13S325	46.20	-INFINITY	(0.0000, 0.0000)	1.94536 0.093750	0.599090
	48.78	-3.805387	(0.0000, 0.0000)	1.90429 0.098877	0.581409
	51.36	-3.237220	(0.0000, 0.0000)	1.88005 0.104248	0.585197
	53.94	-3.151724	(0.0000, 0.0000)	1.87216 0.104736	0.605925
	56.52	-3.481175	(0.0000, 0.0000)	1.88049 0.104248	0.646096
D13S321	59.10	-INFINITY	(0.0000, 0.0000)	1.90529 0.098877	0.721712
	60.56	-2.157602	(0.4050, 0.2661)	1.90126 0.098877	0.632908
	62.02	-1.766473	(0.4569, 0.5274)	1.90147 0.098877	0.594344
	63.48	-1.727899	(0.4722, 0.6923)	1.90598 0.098877	0.582711
	64.94	-2.041284	(0.4794, 0.8136)	1.91489 0.096680	0.596891
D13S792	66.40	-INFINITY	(0.4836, 0.9109)	1.92835 0.094971	0.653575
	68.92	-1.281425	(0.4842, 0.9039)	1.90208 0.098877	0.551672
	71.44	-0.886661	(0.4853, 0.9014)	1.88697 0.103271	0.510485
	73.96	-0.795588	(0.4858, 0.9025)	1.88281 0.103760	0.503057
	76.48	-0.911138	(0.4854, 0.9069)	1.88972 0.103027	0.528078
D13S317	79.00	-1.355374	(0.4845, 0.9150)	1.90819 0.096680	0.604789
	79.46	-1.518925	(0.4842, 0.9146)	1.92477 0.094971	0.525604
	79.92	-1.739314	(0.4841, 0.9115)	1.94177 0.093750	0.491098
	80.38	-2.062483	(0.4839, 0.9145)	1.95018 0.091309	0.478773
	80.84	-2.634771	(0.4838, 0.9148)	1.97701 0.086670	0.486863
D13S795	81.30	-INFINITY	(0.4838, 0.9152)	1.99526 0.085205	0.528265
	81.79	-2.828790	(0.4838, 0.9148)	1.99549 0.085205	0.522121
	82.27	-2.268808	(0.4839, 0.9147)	1.99633 0.085205	0.521733
	82.76	-1.960060	(0.4840, 0.9147)	1.99778 0.084961	0.527121
	83.24	-1.756393	(0.4841, 0.9149)	1.99984 0.084961	0.539536
D13S225	83.73	-1.612409	(0.4842, 0.9153)	2.00251 0.084961	0.566081
	84.65	-1.444252	(0.4843, 0.9137)	1.99352 0.085205	0.555640
	85.57	-1.371304	(0.4844, 0.9126)	1.98671 0.085205	0.553294
	86.49	-1.389911	(0.4843, 0.9119)	1.98204 0.086182	0.557777
	87.41	-1.560319	(0.4841, 0.9117)	1.97951 0.086182	0.570167
D13S797	88.33	-INFINITY	(0.4837, 0.9118)	1.97910 0.086182	0.597886
	89.53	-0.749996	(0.4864, 0.9106)	1.97365 0.086670	0.570351
	90.73	-0.341062	(0.4909, 0.9118)	1.97225 0.088135	0.551592
	91.93	-0.086155	(0.4970, 0.9150)	1.97483 0.086670	0.537737
	93.13	0.099921	(0.5044, 0.9204)	1.98136 0.086182	0.527967
D13S173	94.33	0.246253	(0.5134, 0.9276)	1.99182 0.085205	0.522574
	96.33	0.382893	(0.5288, 0.8918)	1.81212 0.112793	0.435707
	98.33	0.476376	(0.5478, 0.8593)	1.65205 0.121094	0.375507
	100.33	0.542151	(0.5691, 0.8298)	1.50916 0.121094	0.327394
	102.33	0.588811	(0.5941, 0.8030)	1.38135 0.121094	0.287540
	104.33	0.621646	(0.6221, 0.7787)	1.26679 0.121094	0.253894

## CHROMOSOME 14 STAGE 1

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

position	LOD score	NPL score	p-value	information
-10.00	-0.856072	0.130302	0.359375	0.572248
-8.00	-1.023426	0.127107	0.359375	0.602846
-6.00	-1.247688	0.122323	0.359375	0.638188
-4.00	-1.575184	0.115815	0.359375	0.679583



	-2.00	-2.153559	0.107463	0.359375	0.729591
D14S50	0.00	-10000.000000	0.097165	0.359375	0.799329
	3.10	-3.204168	-0.076057	0.406250	0.742090
	6.19	-2.973237	-0.262759	0.484375	0.733846
	9.29	-3.150951	-0.454680	0.625000	0.762528
	12.38	-3.810921	-0.643000	0.750000	0.833367
D14S80	15.48	-10000.000000	-0.819908	0.859375	0.983522
	16.28	-5.725289	-0.745362	0.843750	0.921843
	17.08	-5.080858	-0.669212	0.781250	0.897370
	17.88	-4.902084	-0.593524	0.718750	0.893725
	18.68	-5.115180	-0.520416	0.625000	0.910537
D14S297	19.48	-10000.000000	-0.451932	0.625000	0.962610
	22.80	-1.850036	-0.140204	0.406250	0.758296
	26.12	-1.630431	-0.044833	0.406250	0.669618
	29.43	-1.822113	-0.109278	0.406250	0.649505
	32.75	-2.498958	-0.238114	0.406250	0.693895
D14S306	36.07	-10000.000000	-0.341916	0.500000	0.827007
	38.51	-2.655462	-0.000851	0.406250	0.683751
	40.96	-1.845451	0.509751	0.203125	0.642026
	43.41	-1.458139	1.213952	0.062500	0.666020
	45.86	-1.346151	2.107041	0.062500	0.758381
GGAA10C09	48.31	-10000.000000	3.153107	0.062500	0.967643
	51.18	-1.090988	2.599219	0.062500	0.786087
	54.06	-1.061897	2.046276	0.062500	0.713426
	56.94	-1.251532	1.510702	0.062500	0.708115
	59.81	-1.733589	1.010238	0.062500	0.769334
D14S63	62.69	-10000.000000	0.559997	0.187500	0.935041
	64.93	-3.877614	0.510212	0.203125	0.908800
	67.16	-3.548562	0.460798	0.203125	0.896853
	69.40	-3.579694	0.412413	0.250000	0.895509
	71.64	-3.970981	0.365662	0.250000	0.905069
D14S774	73.87	-10000.000000	0.321082	0.265625	0.931234
	74.88	-5.552296	0.206309	0.312500	0.921945
	75.88	-5.326537	0.089960	0.359375	0.922437
	76.88	-5.505898	-0.026409	0.406250	0.932315
	77.89	-6.164064	-0.141222	0.406250	0.953290
D14S53	78.89	-10000.000000	-0.252961	0.484375	0.995978
	81.13	-3.462395	-0.265669	0.484375	0.847718
	83.36	-2.687775	-0.281924	0.484375	0.782673
	85.60	-2.331414	-0.301764	0.500000	0.765892
	87.84	-2.245752	-0.325181	0.500000	0.796161
D14S48	90.07	-3.981386	-0.352254	0.593750	0.901646
	92.73	-1.716267	-0.364874	0.593750	0.821563
	95.39	-1.400718	-0.385825	0.593750	0.781928
	98.06	-1.280731	-0.414559	0.593750	0.765805
	100.72	-1.352788	-0.450546	0.625000	0.770869
D14S81	103.38	-3.363128	-0.493476	0.625000	0.800418
	104.58	-1.593783	-0.498134	0.625000	0.802571
	105.79	-1.312840	-0.504238	0.625000	0.811043
	106.99	-1.162367	-0.511708	0.625000	0.825310
	108.20	-1.072148	-0.520472	0.625000	0.846520
D14S51	109.41	-1.022100	-0.530463	0.671875	0.881273
	109.65	-1.110634	-0.534602	0.671875	0.881697
	109.89	-1.226853	-0.538814	0.671875	0.882623

	110.13	-1.393197	-0.543099	0.671875	0.883906
	110.37	-1.680088	-0.547457	0.671875	0.885571
D14S78	110.61	-3.180795	-0.551890	0.671875	0.887902
	112.02	-0.115889	-0.380616	0.593750	0.839984
	113.43	0.219998	-0.200826	0.406250	0.817066
	114.83	0.428445	-0.014647	0.406250	0.807543
	116.24	0.583112	0.175565	0.328125	0.809935
D14S118	117.65	1.464545	2.868589	0.062500	0.885815
	119.65	1.466067	2.880249	0.062500	0.894811
	121.65	1.467623	2.892136	0.062500	0.905490
	123.65	1.469213	2.904253	0.062500	0.918127
	125.65	1.460838	2.916602	0.062500	0.933532
	127.65	1.462496	2.929185	0.062500	0.955640

## CHROMOSOME 14 STAGE 2

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-0.920681	0.129151	0.359375	0.353683
	-8.00	-1.078431	0.125950	0.359375	0.396590
	-6.00	-1.291524	0.121396	0.359375	0.446835
	-4.00	-1.606189	0.115261	0.359375	0.506806
	-2.00	-2.169982	0.107284	0.359375	0.581044
D14S50	0.00	-10000.000	0.097165	0.359375	0.687845
	3.57	-3.090434	-0.076464	0.406250	0.589684
	7.13	-2.857899	-0.254728	0.484375	0.575439
	10.70	-3.023122	-0.438050	0.593750	0.617043
	14.27	-3.659910	-0.626591	0.750000	0.724658
D14S80	17.83	-10000.000	-0.819908	0.859375	0.974368
	18.67	-5.681729	-0.743876	0.843750	0.877073
	19.50	-5.041639	-0.668345	0.781250	0.839558
	20.34	-4.866276	-0.594033	0.718750	0.833922
	21.17	-5.081853	-0.521656	0.625000	0.859566
D14S297	22.00	-10000.000	-0.451932	0.625000	0.941837
	25.86	-1.734967	-0.121060	0.406250	0.603990
	29.72	-1.516904	-0.033500	0.406250	0.473040
	33.57	-1.695572	-0.092758	0.406250	0.444830
	37.43	-2.344736	-0.219657	0.406250	0.511006
D14S306	41.29	-10000.000	-0.341916	0.500000	0.730899
	44.03	-2.526509	0.015660	0.406250	0.498002
	46.77	-1.736469	0.516203	0.187500	0.437135
	49.52	-1.369241	1.183725	0.062500	0.472886
	52.26	-1.277237	2.049393	0.062500	0.610560
GGAA10C0955.01	-10000.000		3.153107	0.062500	0.949667
	58.29	-1.017731	2.554169	0.062500	0.653101
	61.58	-0.974479	2.013950	0.062500	0.545701
	64.86	-1.152509	1.513232	0.062500	0.537759
	68.15	-1.625595	1.034309	0.062500	0.627788
D14S63	71.43	-10000.000	0.559997	0.187500	0.898952
	73.92	-3.803653	0.506766	0.203125	0.853965
	76.40	-3.483023	0.456431	0.203125	0.834002
	78.89	-3.513802	0.408837	0.250000	0.832047
	81.37	-3.896310	0.363791	0.250000	0.848398
D14S774	83.86	-10000.000	0.321082	0.265625	0.893031

	84.91	-5.510252	0.206368	0.312500	0.878150
	85.96	-5.282567	0.091695	0.359375	0.878583
	87.02	-5.457677	-0.023027	0.406250	0.893460
	88.07	-6.109280	-0.137883	0.406250	0.925784
D14S53	89.12	-10000.000	-0.252961	0.484375	0.993744
	91.61	-4.821480	-0.266413	0.484375	0.795265
	94.09	-4.125963	-0.282655	0.484375	0.723126
	96.58	-3.860425	-0.302103	0.500000	0.718043
	99.06	-3.878958	-0.325165	0.500000	0.779590
D14S48	101.55	-5.778638	-0.352254	0.593750	0.965124
	104.56	-3.592799	-0.372694	0.593750	0.846202
	107.57	-3.445119	-0.400218	0.593750	0.797099
	110.58	-3.540029	-0.434462	0.593750	0.784980
	113.59	-3.905291	-0.475082	0.625000	0.805891
D14S81	116.60	-6.431113	-0.521701	0.625000	0.868560
	117.41	-5.731334	-0.619045	0.718750	0.716531
	118.23	-6.042610	-0.693296	0.796875	0.673587
	119.04	-6.722332	-0.748098	0.843750	0.689847
	119.85	-8.065607	-0.787290	0.859375	0.766027
D14S265	120.66	-10000.000	-0.814937	0.859375	0.954571
	120.91	-6.100027	-0.800364	0.859375	0.689167
	121.15	-4.648991	-0.686493	0.796875	0.581354
	121.39	-3.830130	-0.422113	0.593750	0.552766
	121.63	-3.277129	0.061009	0.359375	0.598607
D14S51	121.88	-2.878417	0.848156	0.062500	0.767729
	122.10	-2.813767	1.066690	0.062500	0.766453
	122.32	-2.798556	1.300598	0.062500	0.776488
	122.54	-2.850404	1.549870	0.062500	0.797452
	122.77	-3.039409	1.814757	0.062500	0.832063
D14S62	122.99	-10000.000	2.095763	0.062500	0.894888
	123.46	-2.140567	2.122582	0.062500	0.891836
	123.93	-1.866100	2.149890	0.062500	0.890045
	124.40	-1.718909	2.177703	0.062500	0.889212
	124.87	-1.625607	2.206038	0.062500	0.889279
D14S65	125.34	-1.563579	2.234914	0.062500	0.890315
	126.33	-1.517328	2.181427	0.062500	0.884278
	127.32	-1.538434	2.130320	0.062500	0.882020
	128.31	-1.633567	2.081456	0.062500	0.883146
	129.29	-1.868568	2.034699	0.062500	0.887939
D14S267	130.28	-10000.000	1.989921	0.062500	0.898238
	131.03	0.056024	1.503178	0.062500	0.840106
	131.77	0.231670	1.017747	0.062500	0.812835
	132.52	0.231557	0.532868	0.187500	0.806240
	133.27	0.055766	0.047779	0.359375	0.821233
D14S78	134.02	-2.975671	-0.438281	0.593750	0.875182
	135.52	0.433809	0.224661	0.312500	0.710140
	137.03	0.842490	0.882602	0.062500	0.653651
	138.54	1.105331	1.539763	0.062500	0.659137
	140.05	1.303562	2.200350	0.062500	0.725909
D14S118	141.56	1.464545	2.868589	0.062500	0.885815
	141.80	1.466067	2.880249	0.062500	0.894811
	142.04	1.467623	2.892136	0.062500	0.905490
	142.29	1.469213	2.904253	0.062500	0.918127
	142.53	1.470838	2.916602	0.062500	0.933532

D14S272	142.77	1.472496	2.929185	0.062500	0.955640
	143.22	1.468464	2.916057	0.062500	0.937579
	143.67	1.464549	2.903718	0.062500	0.924307
	144.12	1.460751	2.892158	0.062500	0.913263
	144.57	1.457071	2.881370	0.062500	0.903940
D14S260	145.02	1.453509	2.871347	0.062500	0.896323
	147.02	1.403818	2.615085	0.062500	0.749083
	149.02	1.355507	2.385796	0.062500	0.646254
	151.02	1.308566	2.180301	0.062500	0.564029
	153.02	1.262985	1.995827	0.062500	0.495894
	155.02	1.218750	1.829953	0.062500	0.438338

analyzing pedigree 2...

using non-originals 22 1 24 2 3 4 6 5

	position	LOD score	NPL score	p-value	information
	-10.00	-0.032878	0.001707	0.265625	0.001086
	-8.00	-0.034399	0.001856	0.265625	0.001177
	-6.00	-0.035997	0.002018	0.265625	0.001276
	-4.00	-0.037676	0.002194	0.265625	0.001384
	-2.00	-0.039441	0.002386	0.265625	0.001501
D14S50	0.00	-0.041297	0.002595	0.265625	0.001628
	3.57	-0.044851	0.003015	0.265625	0.001882
	7.13	-0.048744	0.003505	0.265625	0.002177
	10.70	-0.053015	0.004077	0.265625	0.002518
	14.27	-0.057705	0.004744	0.265625	0.002915
D14S80	17.83	-0.062863	0.005524	0.265625	0.003376
	18.67	-0.064141	0.005725	0.265625	0.003494
	19.50	-0.065449	0.005933	0.265625	0.003616
	20.34	-0.066787	0.006148	0.265625	0.003743
	21.17	-0.068155	0.006372	0.265625	0.003875
D14S297	22.00	-0.069555	0.006604	0.265625	0.004011
	25.86	-0.076465	0.007796	0.265625	0.004708
	29.72	-0.084160	0.009209	0.265625	0.005532
	33.57	-0.092743	0.010887	0.265625	0.006508
	37.43	-0.102336	0.012880	0.265625	0.007665
D14S306	41.29	-0.113080	0.015249	0.265625	0.009041
	44.03	-0.121518	0.017204	0.265625	0.010179
	46.77	-0.130692	0.019417	0.265625	0.011470
	49.52	-0.140678	0.021923	0.265625	0.012938
	52.26	-0.151566	0.024763	0.265625	0.014610
GAA10C09	55.01	-0.163456	0.027981	0.265625	0.016519
	58.29	-0.179167	0.032403	0.265625	0.019166
	61.58	-0.196709	0.037542	0.265625	0.022285
	64.86	-0.216353	0.043517	0.265625	0.025969
	68.15	-0.238430	0.050464	0.265625	0.030339
D14S63	71.43	-0.263338	0.058540	0.250000	0.035540
	73.92	-0.284352	0.065508	0.250000	0.040138
	76.40	-0.307520	0.073313	0.250000	0.045411
	78.89	-0.333145	0.082053	0.250000	0.051476
	81.37	-0.361591	0.091834	0.250000	0.058468
D14S774	83.86	-0.393298	0.102773	0.250000	0.066554
	84.91	-0.407854	0.107791	0.250000	0.070359
	85.96	-0.423140	0.113051	0.250000	0.074413
	87.02	-0.439206	0.118561	0.250000	0.078734

	88.07	-0.456110	0.124334	0.250000	0.083342
D14S53	89.12	-0.473914	0.130379	0.250000	0.088260
	91.61	-0.519875	0.145780	0.250000	0.101223
	94.09	-0.572329	0.162909	0.250000	0.116406
	96.58	-0.632679	0.181919	0.250000	0.134254
	99.06	-0.702783	0.202958	0.250000	0.155321
D14S48	101.55	-0.785153	0.226163	0.250000	0.180302
	104.56	-0.906561	0.257352	0.250000	0.217107
	107.57	-1.060807	0.292017	0.250000	0.263083
	110.58	-1.264302	0.330146	0.250000	0.321247
	113.59	-1.548949	0.371500	0.250000	0.396214
D14S81	116.60	-1.992599	0.415489	0.250000	0.495984
	117.41	-2.165064	0.427675	0.250000	0.528996
	118.23	-2.379415	0.439945	0.250000	0.565592
	119.04	-2.663790	0.452263	0.250000	0.606764
	119.85	-3.100507	0.464591	0.250000	0.654398
D14S265	120.66	-10000.000	0.476882	0.250000	0.715732
	120.91	-5.499429	0.479025	0.250000	0.717297
	121.15	-5.273688	0.481218	0.250000	0.719250
	121.39	-5.182476	0.483464	0.203125	0.721492
	121.63	-5.154754	0.485761	0.203125	0.724006
D14S51	121.88	-5.171702	0.488110	0.203125	0.726789
	122.10	-5.223717	0.490306	0.203125	0.729577
	122.32	-5.316961	0.492547	0.203125	0.732604
	122.54	-5.472304	0.494832	0.203125	0.735889
	122.77	-5.762289	0.497161	0.203125	0.739464
D14S62	122.99	-10000.000	0.499534	0.203125	0.743447
	123.46	-5.603486	0.530278	0.203125	0.672896
	123.93	-5.531622	0.561233	0.203125	0.650530
	124.40	-5.688137	0.592424	0.156250	0.653889
	124.87	-6.146064	0.623875	0.156250	0.683108
D14S65	125.34	-10000.000	0.655611	0.125000	0.761319
	126.33	-5.582764	0.621093	0.156250	0.629021
	127.32	-5.227426	0.587801	0.156250	0.572490
	128.31	-5.223276	0.555624	0.203125	0.554209
	129.29	-5.570553	0.524458	0.203125	0.571393
D14S267	130.28	-10000.000	0.494196	0.203125	0.646045
	131.03	-4.223661	0.483974	0.203125	0.604526
	131.77	-3.547720	0.473840	0.250000	0.571853
	132.52	-3.143960	0.463814	0.250000	0.544077
	133.27	-2.857899	0.453909	0.250000	0.520098
D14S78	134.02	-2.639426	0.444139	0.250000	0.499277
	135.52	-2.321802	0.424860	0.250000	0.465435
	137.03	-2.108162	0.406202	0.250000	0.441194
	138.54	-1.966266	0.388163	0.250000	0.425782
	140.05	-1.885826	0.370701	0.250000	0.419080
D14S118	141.56	-1.873730	0.353740	0.250000	0.421569
	141.80	-1.880047	0.351048	0.250000	0.422901
	142.04	-1.889322	0.348365	0.250000	0.424510
	142.29	-1.901955	0.345691	0.250000	0.426405
	142.53	-1.918468	0.343025	0.250000	0.428597
D14S272	142.77	-1.939548	0.340367	0.250000	0.431099
	143.22	-1.972801	0.321623	0.250000	0.436579
	143.67	-2.037791	0.303415	0.250000	0.446266

	144.12	-2.157168	0.285733	0.250000	0.461086
	144.57	-2.404333	0.268567	0.250000	0.483043
D14S260	145.02	-10000.000	0.251909	0.250000	0.521539
	147.02	-0.951676	0.237889	0.250000	0.416193
	149.02	-0.666394	0.223359	0.250000	0.346948
	151.02	-0.509154	0.208726	0.250000	0.293851
	153.02	-0.405288	0.194287	0.250000	0.251472
	155.02	-0.331011	0.180256	0.250000	0.216891

Totalling pedigrees: ..

	position	L.O.D. score	NPL score	p-value	information
	-10.00	-0.953559	0.09253	0.303955	0.177384
	-8.00	-1.112830	0.09037	0.304932	0.198883
	-6.00	-1.327520	0.08727	0.307861	0.224056
	-4.00	-1.643865	0.08305	0.309082	0.254095
	-2.00	-2.209423	0.07755	0.310059	0.291272
D14S50	0.00	-INFINITY	0.07054	0.313965	0.344737
	3.57	-3.135284	-0.05194	0.393311	0.295783
	7.13	-2.906643	-0.17764	0.468018	0.288808
	10.70	-3.076137	-0.30687	0.530029	0.309781
	14.27	-3.717615	-0.43971	0.595947	0.363786
D14S80	17.83	-INFINITY	-0.57586	0.668457	0.488872
	18.67	-5.745870	-0.52195	0.641357	0.440284
	19.50	-5.107088	-0.46840	0.612549	0.421587
	20.34	-4.933063	-0.41570	0.587891	0.418832
	21.17	-5.150008	-0.36436	0.562012	0.431720
D14S297	22.00	-INFINITY	-0.31489	0.538330	0.472924
	25.86	-1.811432	-0.08009	0.413086	0.304349
	29.72	-1.601064	-0.01718	0.368408	0.239286
	33.57	-1.788315	-0.05789	0.393555	0.225669
	37.43	-2.447072	-0.14621	0.451904	0.259335
D14S306	41.29	-INFINITY	-0.23099	0.498535	0.369970
	44.03	-2.648027	0.02324	0.344727	0.254091
	46.77	-1.867161	0.37874	0.194336	0.224303
	49.52	-1.509919	0.85252	0.137207	0.242912
	52.26	-1.428803	1.46665	0.121094	0.312585
GAA10C09	55.01	-INFINITY	2.24937	0.049316	0.483093
	58.29	-1.196899	1.82898	0.111328	0.336134
	61.58	-1.171188	1.45062	0.121094	0.283993
	64.86	-1.368862	1.10079	0.122070	0.281864
	68.15	-1.864025	0.76705	0.147461	0.329063
D14S63	71.43	-INFINITY	0.43737	0.187256	0.467246
	73.92	-4.088005	0.40466	0.191650	0.447051
	76.40	-3.790543	0.37459	0.194336	0.439706
	78.89	-3.846947	0.34711	0.201172	0.441761
	81.37	-4.257901	0.32218	0.210693	0.453433
D14S774	83.86	-INFINITY	0.29971	0.212158	0.479792
	84.91	-5.918106	0.22214	0.242188	0.474255
	85.96	-5.705707	0.14478	0.278809	0.476498
	87.02	-5.896883	0.06755	0.315430	0.486097
	88.07	-6.565390	-0.00958	0.364990	0.504563
D14S53	89.12	-INFINITY	-0.08668	0.413818	0.541002
	91.61	-5.341355	-0.08530	0.413818	0.448244
	94.09	-4.698291	-0.08467	0.413818	0.419766

	96.58	-4.493104	-0.08498	0.413818	0.426149
	99.06	-4.581741	-0.08641	0.413818	0.467456
D14S48	101.55	-6.563791	-0.08916	0.414551	0.572713
	104.56	-4.499359	-0.08156	0.413086	0.531655
	107.57	-4.505926	-0.07651	0.410645	0.530091
	110.58	-4.804331	-0.07376	0.409912	0.553114
	113.59	-5.454241	-0.07324	0.409912	0.601052
D14S81	116.60	-8.423712	-0.07510	0.409912	0.682272
	117.41	-7.896397	-0.13532	0.445312	0.622763
	118.23	-8.422026	-0.17915	0.469971	0.619589
	119.04	-9.386123	-0.20919	0.481689	0.648306
	119.85	-11.166114	-0.22818	0.498291	0.710212
D14S265	120.66	-INFINITY	-0.23904	0.498535	0.835152
	120.91	-11.599457	-0.22722	0.497559	0.703232
	121.15	-9.922679	-0.14515	0.451904	0.650302
	121.39	-9.012606	0.04338	0.332520	0.637129
	121.63	-8.431883	0.38662	0.191895	0.661306
D14S51	121.88	-8.050119	0.94488	0.128174	0.747259
	122.10	-8.037484	1.10096	0.122070	0.748015
	122.32	-8.115516	1.26794	0.121094	0.754546
	122.54	-8.322708	1.44582	0.121094	0.766671
	122.77	-8.801697	1.63477	0.121094	0.785764
D14S62	122.99	-INFINITY	1.83515	0.110840	0.819167
	123.46	-7.744053	1.87586	0.104736	0.782366
	123.93	-7.397722	1.91705	0.096680	0.770287
	124.40	-7.407046	1.95878	0.091309	0.771551
	124.87	-7.771670	2.00105	0.084961	0.786193
D14S65	125.34	-INFINITY	2.04391	0.076660	0.825817
	126.33	-7.100092	1.98168	0.086182	0.756650
	127.32	-6.765861	1.92200	0.095947	0.727255
	128.31	-6.856843	1.86470	0.104736	0.718677
	129.29	-7.439121	1.80960	0.112793	0.729666
D14S267	130.28	-INFINITY	1.75654	0.117432	0.772142
	131.03	-4.167637	1.40513	0.121094	0.722316
	131.77	-3.316050	1.05471	0.122559	0.692334
	132.52	-2.912403	0.70476	0.154297	0.675159
	133.27	-2.802133	0.35475	0.200195	0.670666
D14S78	134.02	-5.615097	0.00414	0.359131	0.687230
	135.52	-1.887993	0.45928	0.181152	0.587787
	137.03	-1.265672	0.91132	0.133301	0.547422
	138.54	-0.860936	1.36325	0.121094	0.542459
	140.05	-0.582263	1.81801	0.111816	0.572495
D14S118	141.56	-0.409185	2.27853	0.046143	0.653692
	141.80	-0.413980	2.28487	0.046143	0.658856
	142.04	-0.421699	2.29138	0.046143	0.665000
	142.29	-0.432742	2.29806	0.043213	0.672266
	142.53	-0.447631	2.30490	0.042236	0.681065
D14S272	142.77	-0.467052	2.31192	0.042236	0.693370
	143.22	-0.504337	2.28939	0.046143	0.687079
	143.67	-0.573242	2.26779	0.046143	0.685286
	144.12	-0.696417	2.24711	0.049561	0.687175
	144.57	-0.947262	2.22734	0.051758	0.693492
D14S260	145.02	-INFINITY	2.20848	0.053711	0.708931
	147.02	0.452142	2.01736	0.081055	0.582638

149.02	0.689113	1.84495	0.109131	0.496601
151.02	0.799412	1.68930	0.120361	0.428940
153.02	0.857697	1.54864	0.121094	0.373683
155.02	0.887739	1.42143	0.121094	0.327614

# **CHROMOSOME 15**

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	0.270187	0.058603	0.359375	0.440249
	-8.00	0.213278	-0.000336	0.406250	0.501566
	-6.00	0.129148	-0.071252	0.406250	0.575200
	-4.00	-0.005265	-0.156151	0.406250	0.665730
	-2.00	-0.201459	-0.257366	0.484375	0.782219
D15S122	0.00	-2.796469	-0.377617	0.593750	0.963349
	2.66	-0.781043	-0.349776	0.500000	0.720533
	5.32	-0.606704	-0.339119	0.500000	0.630520
	7.98	-0.602244	-0.344958	0.500000	0.619878
	10.64	-0.767800	-0.367427	0.593750	0.685259
D15S165	13.30	-2.796450	-0.407487	0.593750	0.885276
	18.40	-0.423463	-0.404273	0.593750	0.425558
	23.50	-0.502056	-0.434169	0.593750	0.276895
	28.60	-0.849238	-0.440773	0.625000	0.288451
	33.70	-1.598077	-0.385257	0.593750	0.463938
D15S126	38.80	-2.789393	-0.240455	0.406250	0.973299
	39.96	-2.846373	-0.279558	0.484375	0.861563
	41.12	-2.830347	-0.306738	0.500000	0.816864
	42.28	-2.772247	-0.322555	0.500000	0.807502
	43.44	-2.789013	-0.327526	0.500000	0.832437
CYP19	44.60	-3.277243	-0.322132	0.500000	0.918196
	45.60	-2.786651	-0.269682	0.484375	0.617562
	46.60	-2.701339	-0.192037	0.406250	0.504172
	47.60	-2.687232	-0.088427	0.406250	0.487730
	48.60	-2.667335	0.041986	0.359375	0.565464
D15S117	49.60	-2.610179	0.200119	0.328125	0.812174
	50.46	-2.585574	0.257597	0.312500	0.701044
	51.32	-2.561976	0.317052	0.265625	0.668922
	52.18	-2.539327	0.378709	0.250000	0.682360
	53.04	-2.517479	0.442791	0.203125	0.743101
D15S153	53.90	-2.496233	0.509528	0.203125	0.893740
	54.44	-2.625848	0.116061	0.359375	0.588469
	54.98	-2.744181	-0.202213	0.406250	0.605711
	55.52	-2.827133	-0.445878	0.625000	0.586429
	56.06	-2.847529	-0.615394	0.718750	0.628736
D15S131	56.60	-2.798146	-0.711104	0.796875	0.781821
	57.74	-2.744065	-0.634822	0.750000	0.606710
	58.88	-2.739062	-0.559797	0.671875	0.559513
	60.02	-2.757230	-0.485939	0.625000	0.589335
	61.16	-2.781423	-0.413163	0.593750	0.701462
D15S175	62.30	-2.796731	-0.341391	0.500000	0.977495
	62.96	-2.795827	-0.343448	0.500000	0.956615
	63.62	-2.795935	-0.345567	0.500000	0.944734



	64.28	-2.796304	-0.347742	0.500000	0.937475
	64.94	-2.796512	-0.349973	0.500000	0.934198
D15S116	65.60	-2.796466	-0.352254	0.593750	0.935976
	67.82	-2.796102	-0.348620	0.500000	0.870677
	70.04	-2.795114	-0.345474	0.500000	0.835030
	72.26	-2.794062	-0.342783	0.500000	0.818396
	74.48	-2.792962	-0.340518	0.500000	0.818866
D15S13076	76.70	-2.791299	-0.338605	0.500000	0.839926
	78.90	-2.788794	-0.296982	0.500000	0.774896
	81.10	-2.781535	-0.255077	0.484375	0.756074
	83.30	-2.777487	-0.213053	0.406250	0.765539
	85.50	-2.784232	-0.171069	0.406250	0.803906
D15S120	87.70	-2.796441	-0.129297	0.406250	0.890702
	89.70	-2.292939	-0.124503	0.406250	0.734722
	91.70	-1.837982	-0.120473	0.406250	0.632787
	93.70	-1.542201	-0.117031	0.406250	0.552533
	95.70	-1.331404	-0.114037	0.406250	0.486458
	97.70	-1.170824	-0.111381	0.406250	0.430789

# **CHROMOSOME 16**

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-1.102862	-0.062313	0.406250	0.436868
	-8.00	-1.259440	-0.063321	0.406250	0.493613
	-6.00	-1.466840	-0.064835	0.406250	0.560958
	-4.00	-1.761690	-0.067016	0.406250	0.642714
	-2.00	-2.228630	-0.070061	0.406250	0.746429
D16S423	0.00	-2.796435	-0.074206	0.406250	0.904521
	3.56	-1.858723	-0.053336	0.406250	0.587765
	7.12	-1.341102	-0.034644	0.406250	0.443892
	10.68	-1.033308	-0.015326	0.406250	0.382524
	14.24	-0.815731	0.007109	0.406250	0.395768
D16S405	17.80	-0.646370	0.035026	0.406250	0.535286
	21.14	-0.602498	-0.003163	0.406250	0.430371
	24.48	-0.601623	-0.037459	0.406250	0.394660
	27.82	-0.661094	-0.068569	0.406250	0.402980
	31.16	-0.850087	-0.097369	0.406250	0.457680
D16S420	34.50	-2.655908	-0.124990	0.406250	0.582638
	35.46	-0.879835	-0.072170	0.406250	0.581461
	36.42	-0.554883	-0.016461	0.406250	0.600706
	37.38	-0.352636	0.042411	0.359375	0.634434
	38.34	-0.201213	0.104735	0.359375	0.684887
D16S401	39.30	-0.077849	0.170825	0.328125	0.768630
	41.86	-0.228254	0.054131	0.359375	0.474728
	44.42	-0.436924	-0.047485	0.406250	0.360353
	46.98	-0.748066	-0.138517	0.406250	0.340099
	49.54	-1.302709	-0.222525	0.406250	0.406974
D16S411	52.10	-2.921780	-0.302197	0.500000	0.615769
	54.40	-2.380979	-0.325687	0.500000	0.391808
	56.70	-2.027433	-0.356135	0.593750	0.314873
	59.00	-1.903680	-0.393033	0.593750	0.323463

	61.30	-1.985222	-0.435863	0.593750	0.421686
D16S415	63.60	-3.030492	-0.484070	0.625000	0.688873
	74.80	-0.748012	-0.397177	0.593750	0.269473
	86.00	-0.679509	-0.391412	0.593750	0.176835
	97.20	-0.888768	-0.457092	0.625000	0.205844
	108.40	-1.506415	-0.582945	0.703125	0.362564
D16S520	119.60	-3.362550	-0.754177	0.843750	0.906274
	121.60	-3.143149	-0.720722	0.796875	0.726265
	123.60	-2.653374	-0.687893	0.796875	0.612231
	125.60	-2.241144	-0.655799	0.750000	0.524840
	127.60	-1.930873	-0.624530	0.718750	0.454677
	129.60	-1.690806	-0.594162	0.718750	0.396945

# CHROMOSOME 17 STAGE I

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	0.204993	0.282329	0.265625	0.332034
	-8.00	0.151644	0.291065	0.265625	0.371664
	-6.00	0.071463	0.298854	0.265625	0.417997
	-4.00	-0.058657	0.305326	0.265625	0.473238
	-2.00	-0.312487	0.310024	0.265625	0.541654
D17S578	0.00	-10000.000	0.312381	0.265625	0.641075
	5.45	0.415513	0.501777	0.203125	0.513429
	10.89	0.683558	0.688939	0.093750	0.465526
	16.34	0.844111	0.894797	0.062500	0.462215
	21.79	0.971081	1.140339	0.062500	0.503649
D17S849	27.24	1.087580	1.449728	0.062500	0.618937
	29.22	1.117170	1.529876	0.062500	0.617120
	31.21	1.148018	1.620807	0.062500	0.628926
	33.19	1.180159	1.723637	0.062500	0.652288
	35.17	1.213623	1.839643	0.062500	0.689329
D17S938	37.16	1.248437	1.970283	0.062500	0.751977
	38.67	1.251763	1.990134	0.062500	0.591988
	40.18	1.255268	2.009603	0.062500	0.533109
	41.68	1.258989	2.028724	0.062500	0.526852
	43.19	1.262952	2.047506	0.062500	0.570385
D17S945	44.70	1.267176	2.065928	0.062500	0.692080
	46.21	1.188880	1.654876	0.062500	0.697526
	47.72	1.083296	1.220811	0.062500	0.725339
	49.22	0.927348	0.760569	0.062500	0.776050
	50.73	0.647351	0.270623	0.265625	0.856005
D17S796	52.24	-2.494893	-0.252961	0.484375	1.000000
	57.02	0.376652	0.031986	0.406250	0.738787
	61.80	0.520238	0.183338	0.328125	0.626978
	66.58	0.510468	0.239286	0.312500	0.587724
	71.36	0.377508	0.223600	0.312500	0.615029
D17S799	76.14	-0.084036	0.149289	0.359375	0.762452
	79.15	-0.228166	0.050517	0.359375	0.715204
	82.17	-0.427779	-0.042188	0.406250	0.717491
	85.18	-0.728443	-0.129721	0.406250	0.752069
	88.19	-1.277113	-0.212715	0.406250	0.822262

D17S122	91.20	-10000.000	-0.291513	0.500000	0.961789
	97.74	-0.970665	-0.257882	0.484375	0.513050
	104.28	-0.578278	-0.242903	0.406250	0.347263
	110.82	-0.471241	-0.256725	0.484375	0.310354
	117.36	-0.575717	-0.309863	0.500000	0.397707
D17S579	123.89	-3.296493	-0.416790	0.593750	0.738973
	143.56	0.106974	-0.063406	0.406250	0.171351
	163.22	0.259532	0.073730	0.359375	0.077436
	182.88	0.331812	0.121833	0.359375	0.085031
	202.54	0.349274	0.069808	0.359375	0.205473
D17S785	222.20	-2.801908	-0.301928	0.500000	0.888904
	224.20	-0.250237	-0.231901	0.406250	0.708436
	226.20	0.000060	-0.171597	0.406250	0.593597
	228.20	0.127057	-0.119776	0.406250	0.505355
	230.20	0.204263	-0.075355	0.406250	0.434418
	232.20	0.254739	-0.037384	0.406250	0.376050

# **CHROMOSOME 17 STAGE 2**

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	0.185752	0.235621	0.312500	0.338715
	-8.00	0.131315	0.241529	0.312500	0.378996
	-6.00	0.050016	0.246466	0.312500	0.426033
	-4.00	-0.081252	0.250105	0.312500	0.482021
	-2.00	-0.336256	0.252039	0.312500	0.551207
D17S578	0.00	-10000.000	0.251768	0.312500	0.651381
	4.20	-0.862214	0.012202	0.406250	0.477048
	8.40	-0.880435	-0.188222	0.406250	0.421286
	12.60	-1.102425	-0.357212	0.593750	0.431240
	16.80	-1.525925	-0.504250	0.625000	0.506208
D17S849	21.00	-2.359388	-0.641156	0.750000	0.677070
	21.80	-2.636839	-0.668718	0.781250	0.703679
	22.60	-2.998521	-0.696529	0.796875	0.735844
	23.40	-3.513785	-0.724720	0.796875	0.774657
	24.20	-4.403976	-0.753428	0.843750	0.822910
D17S1845	25.00	-10000.000	-0.782801	0.859375	0.893754
	26.00	-1.639433	-0.611817	0.718750	0.725410
	27.00	-0.740051	-0.419426	0.593750	0.642280
	28.00	-0.214908	-0.198932	0.406250	0.606109
	29.00	0.157353	0.056286	0.359375	0.615266
D17S938	30.00	0.446106	0.352861	0.250000	0.696314
	31.40	0.535572	0.376984	0.250000	0.501096
	32.80	0.605513	0.404257	0.250000	0.419378
	34.20	0.664027	0.437337	0.203125	0.396814
	35.60	0.716105	0.478769	0.203125	0.428326
D17S945	37.00	0.765079	0.530975	0.187500	0.539520
	38.40	0.727021	0.413727	0.203125	0.565953
	39.80	0.659399	0.281643	0.265625	0.617183
	41.20	0.539949	0.130280	0.359375	0.695171
	42.60	0.295605	-0.045757	0.406250	0.808217
D17S796	44.00	-2.494893	-0.252961	0.484375	1.000000

	47.80	0.287249	-0.018466	0.406250	0.790250
	51.60	0.440309	0.112172	0.359375	0.699623
	55.40	0.432922	0.161194	0.328125	0.669082
	59.20	0.290156	0.144138	0.359375	0.694066
D17S799	63.00	-0.255410	0.071236	0.359375	0.815385
	65.60	-0.397174	0.000150	0.406250	0.785931
	68.20	-0.595737	-0.067928	0.406250	0.792982
	70.80	-0.896725	-0.133196	0.406250	0.824908
	73.40	-1.447107	-0.195685	0.406250	0.884289
D17S122	76.00	-10000.000	-0.255236	0.484375	0.996008
	80.80	-1.673512	-0.251486	0.406250	0.654710
	85.60	-1.261465	-0.257248	0.484375	0.532747
	90.40	-1.146743	-0.274879	0.484375	0.520472
	95.20	-1.257197	-0.306739	0.500000	0.616212
D17S579	100.00	-3.757027	-0.355517	0.593750	0.921696
	105.40	-1.150202	-0.313498	0.500000	0.649655
	110.80	-1.029309	-0.294306	0.500000	0.560343
	116.20	-1.134286	-0.294577	0.500000	0.559560
	121.60	-1.536269	-0.311560	0.500000	0.644607
D17S1838	127.00	-10000.000	-0.342539	0.500000	0.898074
	130.00	-1.430809	-0.324140	0.500000	0.605767
	133.00	-0.963102	-0.305658	0.500000	0.497316
	136.00	-0.790450	-0.291444	0.500000	0.490176
	139.00	-0.839026	-0.285840	0.484375	0.588746
D17S785	142.00	-2.884228	-0.293473	0.500000	0.891618
	144.00	-0.332483	-0.231619	0.406250	0.711130
	146.00	-0.081967	-0.177864	0.406250	0.596240
	148.00	0.045395	-0.131245	0.406250	0.507924
	150.00	0.123106	-0.090907	0.406250	0.436898
	152.00	0.174223	-0.056095	0.406250	0.378431

analyzing pedigree 2 .

using non-originals: 22 1 24 2 4 6 5

	position	LOD score	NPL score	p-value	information
	-10.00	0.045518	-0.018205	0.375000	0.001350
	-8.00	0.049326	-0.020873	0.375000	0.001602
	-6.00	0.053557	-0.023815	0.375000	0.001911
	-4.00	0.058267	-0.027054	0.375000	0.002291
	-2.00	0.063514	-0.030617	0.375000	0.002763
D17S578	0.00	0.069368	-0.034532	0.375000	0.003351
	4.20	0.083984	-0.044033	0.375000	0.005126
	8.40	0.102453	-0.055526	0.375000	0.008070
	12.60	0.125797	-0.069351	0.375000	0.013096
	16.80	0.155228	-0.085881	0.375000	0.021947
D17S849	21.00	0.192127	-0.105504	0.375000	0.038108
	21.80	0.200124	-0.109624	0.375000	0.042528
	22.60	0.208455	-0.113873	0.375000	0.047543
	23.40	0.217133	-0.118253	0.375000	0.053249
	24.20	0.226165	-0.122766	0.375000	0.059766
D17S184	25.00	0.235563	-0.127415	0.375000	0.067241
	26.00	0.247840	-0.133419	0.375000	0.078225
	27.00	0.260722	-0.139642	0.375000	0.091519
	28.00	0.274228	-0.146087	0.375000	0.107926
	29.00	0.288375	-0.152756	0.375000	0.128964

D17S938	30.00	0.303180	-0.159651	0.375000	0.160691
	31.40	0.183269	-0.222242	0.437500	0.112231
	32.80	0.036030	-0.283247	0.437500	0.092831
	34.20	-0.161678	-0.343250	0.500000	0.087105
	35.60	-0.483598	-0.402809	0.500000	0.092599
D17S945	37.00	-3.644186	-0.462462	0.500000	0.112593
	38.40	-1.126802	-0.428361	0.500000	0.089803
	39.80	-0.844431	-0.396855	0.500000	0.074974
	41.20	-0.686169	-0.367736	0.500000	0.063537
	42.60	-0.578562	-0.340812	0.500000	0.054340
D17S796	44.00	-0.498563	-0.315909	0.500000	0.046779
	47.80	-0.354257	-0.257280	0.437500	0.031863
	51.60	-0.264870	-0.209681	0.437500	0.022201
	55.40	-0.203720	-0.170950	0.375000	0.015710
	59.20	-0.159477	-0.139371	0.375000	0.011245
D17S799	63.00	-0.126298	-0.113578	0.375000	0.008121
	65.60	-0.108167	-0.098691	0.375000	0.006527
	68.20	-0.092888	-0.085707	0.375000	0.005262
	70.80	-0.079921	-0.074376	0.375000	0.004253
	73.40	-0.068855	-0.064483	0.375000	0.003446
D17S122	76.00	-0.059365	-0.055843	0.375000	0.002798
	80.80	-0.045161	-0.042656	0.375000	0.001915
	85.60	-0.034285	-0.032373	0.375000	0.001319
	90.40	-0.025897	-0.024355	0.375000	0.000915
	95.20	-0.019400	-0.018107	0.375000	0.000638
D17S579	100.00	-0.014354	-0.013245	0.375000	0.000448
	105.40	-0.010007	-0.009064	0.375000	0.000303
	110.80	-0.006738	-0.005932	0.375000	0.000207
	116.20	-0.004287	-0.003600	0.375000	0.000143
	121.60	-0.002461	-0.001879	0.375000	0.000100
D17S1838	127.00	-0.001112	-0.000624	0.375000	0.000071
	130.00	-0.000527	-0.000085	0.375000	0.000058
	133.00	-0.000038	0.000361	0.375000	0.000049
	136.00	0.000367	0.000726	0.375000	0.000040
	139.00	0.000701	0.001023	0.375000	0.000034
D17S785	142.00	0.000973	0.001261	0.375000	0.000028
	144.00	0.001125	0.001392	0.375000	0.000025
	146.00	0.001255	0.001503	0.375000	0.000023
	148.00	0.001366	0.001595	0.375000	0.000020
	150.00	0.001461	0.001672	0.375000	0.000018
	152.00	0.001539	0.001734	0.375000	0.000016

Totalling pedigrees: ..

	position	LOD_score	NPL_score	p-value	information
	-10.00	0.231270	0.15374	0.291992	0.170033
	-8.00	0.180641	0.15603	0.291992	0.190299
	-6.00	0.103573	0.15744	0.289062	0.213972
	-4.00	-0.022985	0.15772	0.289062	0.242156
	-2.00	-0.272741	0.15657	0.291992	0.276985
D17S578	0.00	-INFINITY	0.15361	0.291992	0.327366
	4.20	-0.778229	-0.02251	0.356445	0.241087
	8.40	-0.777982	-0.17236	0.451172	0.214678
	12.60	-0.976628	-0.30163	0.524414	0.222168
	16.80	-1.370697	-0.41729	0.589844	0.264078

D17S849	21.00	-2.167261	-0.52797 0.647461	0.357589
	21.80	-2.436716	-0.55037 0.651367	0.373103
	22.60	-2.790065	-0.57304 0.666992	0.391693
	23.40	-3.296653	-0.59607 0.677734	0.413953
	24.20	-4.177811	-0.61956 0.689453	0.441338
D17S184	25.00	-INFINITY	-0.64362 0.711914	0.480498
	26.00	-1.391593	-0.52696 0.647461	0.401818
	27.00	-0.479328	-0.39532 0.584961	0.366900
	28.00	0.059320	-0.24397 0.490234	0.357018
	29.00	0.445728	-0.06821 0.381836	0.372115
D17S938	30.00	0.749286	0.13662 0.295898	0.428503
	31.40	0.718842	0.10942 0.308594	0.306664
	32.80	0.641543	0.08557 0.313477	0.256104
	34.20	0.502349	0.06653 0.315430	0.241959
	35.60	0.232508	0.05371 0.319336	0.260463
D17S945	37.00	-2.879106	0.04845 0.319336	0.326056
	38.40	-0.399781	-0.01035 0.350586	0.327878
	39.80	-0.185032	-0.08147 0.398438	0.346079
	41.20	-0.146220	-0.16791 0.449219	0.379354
	42.60	-0.282956	-0.27335 0.510742	0.431278
D17S796	44.00	-2.993456	-0.40225 0.586914	0.523390
	47.80	-0.067007	-0.19498 0.463867	0.411057
	51.60	0.175440	-0.06895 0.381836	0.360912
	55.40	0.229202	-0.00690 0.346680	0.342396
	59.20	0.130679	0.00337 0.345703	0.352656
D17S799	63.00	-0.381708	-0.02994 0.360352	0.411753
	65.60	-0.505341	-0.06968 0.381836	0.396229
	68.20	-0.688625	-0.10864 0.406250	0.399122
	70.80	-0.976646	-0.14678 0.429688	0.414580
	73.40	-1.515962	-0.18397 0.458008	0.443867
D17S122	76.00	-INFINITY	-0.21997 0.475586	0.499403
	80.80	-1.718674	-0.20799 0.466797	0.328313
	85.60	-1.295750	-0.20479 0.466797	0.267033
	90.40	-1.172640	-0.21159 0.472656	0.260693
	95.20	-1.276597	-0.22970 0.486328	0.308425
D17S579	100.00	-3.771381	-0.26075 0.496094	0.461072
	105.40	-1.160209	-0.22809 0.478516	0.324979
	110.80	-1.036047	-0.21230 0.474609	0.280275
	116.20	-1.138573	-0.21084 0.472656	0.279852
	121.60	-1.538730	-0.22164 0.475586	0.322354
D17S1838	127.00	-INFINITY	-0.24265 0.490234	0.449072
	130.00	-1.431336	-0.22926 0.486328	0.302913
	133.00	-0.963140	-0.21588 0.475586	0.248682
	136.00	-0.790083	-0.20557 0.466797	0.245108
	139.00	-0.838325	-0.20140 0.466797	0.294390
D17S785	142.00	-2.883255	-0.20663 0.466797	0.445823
	144.00	-0.331359	-0.16280 0.442383	0.355578
	146.00	-0.080711	-0.12471 0.418945	0.298131
	148.00	0.046761	-0.09168 0.398438	0.253972
	150.00	0.124567	-0.06310 0.381836	0.218458
	152.00	0.175762	-0.03844 0.362305	0.189224

# CHROMOSOME 18

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-0.444102	-0.362809	0.593750	0.469336
	-8.00	-0.589392	-0.393347	0.593750	0.532601
	-6.00	-0.789897	-0.424924	0.593750	0.608098
	-4.00	-1.091809	-0.457251	0.625000	0.700315
	-2.00	-1.642429	-0.489938	0.625000	0.818167
D18S59	0.00	-10000.000	-0.522471	0.640625	1.000000
	1.28	-3.553113	-0.535592	0.671875	0.844924
	2.56	-3.143666	-0.549857	0.671875	0.770634
	3.84	-3.032128	-0.565285	0.671875	0.736949
	5.11	-3.144999	-0.581886	0.703125	0.741003
D18S62	6.39	-4.549501	-0.599666	0.718750	0.808285
	8.88	-1.362979	-0.510597	0.625000	0.511004
	11.36	-1.123594	-0.436828	0.593750	0.386732
	13.85	-1.050987	-0.378408	0.593750	0.352014
	16.33	-1.072226	-0.335421	0.500000	0.401055
D1863	18.81	-1.172798	-0.308022	0.500000	0.586277
	21.05	-1.401597	-0.339057	0.500000	0.585281
	23.28	-1.717251	-0.371415	0.593750	0.606105
	25.51	-2.188232	-0.405360	0.593750	0.647424
	27.74	-3.035229	-0.441138	0.625000	0.714240
D18S843	29.97	-10000.000	-0.478977	0.625000	0.833598
	46.07	-1.502472	-0.241592	0.406250	0.355606
	62.16	-0.951347	-0.083447	0.406250	0.234889
	78.26	-0.790281	0.058328	0.359375	0.233580
	94.35	-0.944140	0.223605	0.312500	0.353230
D18S535	110.44	-10000.000	0.426102	0.203125	0.836459
	112.43	-1.989102	0.426734	0.203125	0.781095
	114.41	-1.421462	0.428855	0.203125	0.753933
	116.40	-1.102498	0.432636	0.203125	0.743129
	118.38	-0.884708	0.438229	0.203125	0.748151
D18S363	120.37	-0.721932	0.445772	0.203125	0.778031
	124.52	-0.580414	0.191042	0.328125	0.514828
	128.68	-0.544328	-0.057615	0.406250	0.418081
	132.83	-0.605871	-0.304899	0.500000	0.416971
	136.99	-0.825852	-0.555166	0.671875	0.513747
D18S64	141.14	-4.043326	-0.812210	0.859375	0.798846
	144.71	-0.984986	-0.679723	0.781250	0.621791
	148.28	-0.730448	-0.560836	0.671875	0.564206
	151.84	-0.654115	-0.454197	0.625000	0.570005
	155.41	-0.754699	-0.358735	0.593750	0.638370
D18S543	158.98	-10000.000	-0.273761	0.484375	0.821156
	160.98	-0.522042	-0.244310	0.406250	0.677209
	162.98	-0.255442	-0.217473	0.406250	0.582806
	164.98	-0.113160	-0.193019	0.406250	0.508426
	166.98	-0.021414	-0.170738	0.406250	0.447185
	168.98	0.042914	-0.150444	0.406250	0.395607

# CHROMOSOME 19

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analyzing pedigree 1...
using non-origins: 1 2 3 4 5 6 10
position LOD score NPL score p-value information
-10.00 -1.054120 -0.414667 0.593750 0.328558
-8.00 -1.214183 -0.440921 0.625000 0.371509
-6.00 -1.429524 -0.468951 0.625000 0.422943
-4.00 -1.746367 -0.498858 0.625000 0.486071
-2.00 -2.312260 -0.530741 0.671875 0.567235
D19S247 0.00 -10000.000 -0.564693 0.671875 0.693326
4.60 -2.960480 -0.599270 0.718750 0.508166
9.20 -2.651138 -0.646739 0.750000 0.471504
13.80 -2.710305 -0.707042 0.796875 0.516504
18.40 -3.152409 -0.780311 0.859375 0.650328
D19S394 23.00 -10000.000 -0.866716 0.921875 0.974296
24.40 -4.383467 -0.826626 0.859375 0.870635
25.80 -4.007858 -0.788099 0.859375 0.817989
27.20 -3.984707 -0.751240 0.843750 0.789358
28.60 -4.313967 -0.716156 0.796875 0.780046
D19S253 30.00 -10000.000 -0.682960 0.781250 0.791174
32.60 -3.809858 -0.526296 0.640625 0.679735
35.20 -3.428000 -0.370416 0.593750 0.642985
37.80 -3.397503 -0.213619 0.406250 0.650233
40.40 -3.718065 -0.054121 0.406250 0.702113
D19S433 43.00 -10000.000 0.109972 0.359375 0.830836
44.80 -4.318880 0.104759 0.359375 0.797131
46.60 -3.962622 0.101503 0.359375 0.788936
48.40 -3.956851 0.100155 0.359375 0.797054
50.20 -4.301574 0.100680 0.359375 0.822173
D19S224 52.00 -10000.000 0.103048 0.359375 0.876411
56.00 -2.852015 -0.048249 0.406250 0.641506
60.00 -2.581659 -0.193123 0.406250 0.555572
64.00 -2.667650 -0.336172 0.500000 0.550854
68.00 -3.102826 -0.481981 0.625000 0.626411
D19S246 72.00 -7.051283 -0.635306 0.750000 0.843023
73.40 -4.246320 -0.500157 0.625000 0.772920
74.80 -4.291923 -0.366967 0.593750 0.762403
76.20 -4.616543 -0.235220 0.406250 0.786670
77.60 -5.367656 -0.104413 0.406250 0.847882
GGAA1B03 79.00 -10000.000 0.025943 0.406250 0.981524
82.80 -2.159065 0.296855 0.265625 0.694097
86.60 -1.395435 0.728984 0.093750 0.576725
90.40 -1.049956 1.326511 0.062500 0.546897
94.20 -0.975009 2.108896 0.062500 0.599476
D19S294 98.00 -10000.000 3.111761 0.062500 0.794577
100.00 -0.277655 2.842024 0.062500 0.662088
102.00 -0.025439 2.600151 0.062500 0.572131
104.00 0.103094 2.382864 0.062500 0.500392
106.00 0.181718 2.187309 0.062500 0.440910
108.00 0.233545 2.010995 0.062500 0.390578

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**CHROMOSOME 20**  
analyzing pedigree 1...



using non-origins: 1 2 3 4 5 6 10					
	position	LOD score	NPL score	p-value	information
	-10.00	-0.982892	-0.037503	0.406250	0.240448
	-8.00	-1.057519	-0.034407	0.406250	0.271436
	-6.00	-1.140957	-0.030903	0.406250	0.307834
	-4.00	-1.234944	-0.026980	0.406250	0.351319
	-2.00	-1.341767	-0.022631	0.406250	0.405013
D20S103	0.00	-1.464521	-0.017860	0.406250	0.481241
	3.20	-1.793237	-0.166572	0.406250	0.448598
	6.40	-2.231075	-0.304234	0.500000	0.478664
	9.60	-2.870581	-0.431472	0.593750	0.551970
	12.80	-4.006881	-0.548325	0.671875	0.676383
D20S95	16.00	-10000.000	-0.654196	0.750000	0.905189
	17.20	-8.210054	-0.676360	0.781250	0.848582
	18.40	-7.273936	-0.698528	0.796875	0.816961
	19.60	-6.933481	-0.720789	0.796875	0.798824
	20.80	-7.041121	-0.743232	0.843750	0.793351
D20S115	22.00	-10000.000	-0.765941	0.843750	0.808225
	35.20	-2.081854	-0.523854	0.640625	0.304311
	48.40	-1.503961	-0.307744	0.500000	0.160234
	61.60	-1.436262	-0.141968	0.406250	0.155482
	74.80	-1.865956	-0.016844	0.406250	0.290855
D20S120	88.00	-10000.000	0.077433	0.359375	0.817326
	90.00	-3.729564	0.063675	0.359375	0.671280
	92.00	-2.873087	0.051260	0.359375	0.575365
	94.00	-2.390449	0.040055	0.359375	0.499927
	96.00	-2.060307	0.029945	0.406250	0.437982
	98.00	-1.813309	0.020829	0.406250	0.385975

## CHROMOSOME 21

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	-0.982892	-0.037503	0.406250	0.240448
	-8.00	-1.057519	-0.034407	0.406250	0.271436
	-6.00	-1.140957	-0.030903	0.406250	0.307834
	-4.00	-1.234944	-0.026980	0.406250	0.351319
	-2.00	-1.341767	-0.022631	0.406250	0.405013
D21S258	0.00	-1.464521	-0.017860	0.406250	0.481241
	3.20	-1.793237	-0.166572	0.406250	0.448598
	6.40	-2.231075	-0.304234	0.500000	0.478664
	9.60	-2.870581	-0.431472	0.593750	0.551970
	12.80	-4.006881	-0.548325	0.671875	0.676383
GGA2E02	16.00	-10000.000	-0.654196	0.750000	0.905189
	17.20	-8.210054	-0.676360	0.781250	0.848582
	18.40	-7.273936	-0.698528	0.796875	0.816961
	19.60	-6.933481	-0.720789	0.796875	0.798824
	20.80	-7.041121	-0.743232	0.843750	0.793351
D21S265	22.00	-10000.000	-0.765941	0.843750	0.808225
	35.20	-2.081854	-0.523854	0.640625	0.304311
	48.40	-1.503961	-0.307744	0.500000	0.160234
	61.60	-1.436262	-0.141968	0.406250	0.155482

74.80 -1.865956 -0.016844 0.406250 0.290855

# **CHROMOSOME 22 STAGE 1**

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6 10

	position	1.OD score	NPL score	p-value	information
	-10.00	1.216622	2.147726	0.062500	0.414952
	-8.00	1.261088	2.339519	0.062500	0.468557
	-6.00	1.306909	2.552503	0.062500	0.531923
	-4.00	1.354095	2.789445	0.062500	0.608353
	-2.00	1.402656	3.053512	0.062500	0.704140
D22S264	0.00	1.452600	3.348345	0.015625	0.844306
	1.60	1.456447	3.318790	0.046875	0.801820
	3.20	1.461915	3.300976	0.046875	0.781588
	4.80	1.469005	3.294805	0.046875	0.773114
	6.40	1.477717	3.300268	0.046875	0.774785
D22S257	8.00	1.488049	3.317448	0.046875	0.788830
	9.34	1.485908	3.297370	0.046875	0.778309
	10.68	1.484987	3.285472	0.046875	0.774628
	12.02	1.485286	3.281709	0.046875	0.776943
	13.36	1.486805	3.286081	0.046875	0.785894
D22S345	14.70	1.489542	3.298632	0.046875	0.805827
	18.70	1.332434	2.841434	0.062500	0.612991
	22.70	1.155036	2.493233	0.062500	0.530538
	26.70	0.934307	2.234378	0.062500	0.514559
	30.70	0.596507	2.051074	0.062500	0.569757
D22S683	34.50	-10000.000	2.881240	0.062500	0.937274
	38.27	-0.712087	2.816293	0.062500	0.872756
	43.04	-0.515290	2.808885	0.062500	0.847245
	45.81	-0.504105	2.860869	0.062500	0.845896
	49.58	-0.678498	2.974761	0.062500	0.868730
D22S270	53.35	-10000.000	3.153753	0.062500	0.930427
	54.55	-0.453348	3.032974	0.062500	0.885303
	55.76	-0.170031	2.918626	0.062500	0.853241
	56.97	-0.012709	2.810519	0.062500	0.827126
	58.17	0.092426	2.708453	0.062500	0.805551
D22S684	59.38	0.168521	2.612225	0.062500	0.788452
	61.38	0.239656	2.378129	0.062500	0.720161
	63.38	0.287357	2.160984	0.062500	0.668653
	65.38	0.319500	1.960384	0.062500	0.625751
	67.38	0.340591	1.775782	0.062500	0.589241
	69.38	0.353480	1.606516	0.062500	0.557875

# **CHROMOSOME 22 STAGE 2**

analyzing pedigree 1...

using non-originals: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	1.234274	2.105797	0.062500	0.617260
	-8.00	1.287390	2.329641	0.062500	0.656601
	-6.00	1.340543	2.574087	0.062500	0.702425
	-4.00	1.393546	2.840119	0.062500	0.756482
	-2.00	1.446215	3.128571	0.062500	0.821995
D22S427	0.00	1.498371	3.440100	0.015625	0.910135
	0.66	1.499851	3.448166	0.015625	0.919491
	1.32	1.501113	3.458256	0.015625	0.930396
	1.98	1.502154	3.470379	0.015625	0.943015
	2.64	1.502975	3.484547	0.015625	0.957878
D22S264	3.30	1.503576	3.500770	0.015625	0.977309
	3.81	1.504262	3.498710	0.015625	0.976019
	4.31	1.504712	3.497888	0.015625	0.976443
	4.81	1.504925	3.498303	0.015625	0.978083
	5.31	1.504903	3.499957	0.015625	0.981027
D22S446	5.81	1.504644	3.502851	0.015625	0.986213
	5.97	1.504541	3.501497	0.015625	0.985291
	6.13	1.504405	3.500265	0.015625	0.984512
	6.29	1.504236	3.499155	0.015625	0.983838
	6.45	1.504033	3.498168	0.015625	0.983265
D22S425	6.61	1.503797	3.497302	0.015625	0.982814
	7.55	1.504490	3.478296	0.015625	0.962565
	8.49	1.504659	3.463625	0.015625	0.947971
	9.44	1.504303	3.453287	0.015625	0.936947
	10.38	1.503421	3.447286	0.015625	0.928914
D22S257	11.32	1.502016	3.445632	0.015625	0.923871
	11.72	1.502072	3.440476	0.015625	0.920020
	12.12	1.502029	3.436060	0.015625	0.916788
	12.52	1.501889	3.432383	0.015625	0.914047
	12.92	1.501651	3.429447	0.015625	0.911795
D22S345	13.32	1.501315	3.427252	0.015625	0.910155
	17.56	1.382561	3.145826	0.062500	0.789932
	21.79	1.232630	2.954310	0.062500	0.741344
	26.03	1.028969	2.848171	0.062500	0.743381
	30.27	0.698172	2.824389	0.062500	0.797773
D22S683	34.50	-10000.000	2.881240	0.062500	0.937274
	38.27	-0.712087	2.816293	0.062500	0.872756
	42.04	-0.515290	2.808885	0.062500	0.847245
	45.81	-0.504105	2.860869	0.062500	0.845896
	49.58	-0.678498	2.974761	0.062500	0.868730
D22S270	53.35	-10000.000	3.153753	0.062500	0.930427
	54.55	-0.453348	3.032974	0.062500	0.885303
	55.76	-0.170031	2.918626	0.062500	0.853241
	56.97	-0.012709	2.810519	0.062500	0.827126
	58.17	0.092426	2.708453	0.062500	0.805551
D22S684	59.38	0.168521	2.612225	0.062500	0.788452
	61.38	0.239656	2.378129	0.062500	0.720161
	63.38	0.287357	2.160984	0.062500	0.668653
	65.38	0.319500	1.960384	0.062500	0.625751
	67.38	0.340591	1.775782	0.062500	0.589241
	69.38	0.353480	1.606516	0.062500	0.557875

analyzing pedigree 2...

using non-originals: 22 1 24 2 3 4 6

	position	LOD score	NPL score	p-value	information
	-10.00	-0.462345	-0.032422	0.265625	0.482665
	-8.00	-0.524240	-0.018323	0.265625	0.505636
	-6.00	-0.602147	-0.003059	0.265625	0.532908
	-4.00	-0.702582	0.013306	0.265625	0.565546
	-2.00	-0.837381	0.030695	0.265625	0.605336
D22S427	0.00	-1.032676	0.049028	0.265625	0.657665
	0.66	-1.127083	0.031070	0.265625	0.632912
	1.32	-1.249757	0.012954	0.265625	0.626762
	1.98	-1.423851	-0.005228	0.265625	0.630013
	2.64	-1.723130	-0.023378	0.265625	0.641252
D22S264	3.30	-4.818010	-0.041394	0.281250	0.660779
	3.81	-1.415450	-0.050657	0.281250	0.672193
	4.31	-1.105491	-0.059806	0.281250	0.688308
	4.81	-0.919695	-0.068784	0.281250	0.709758
	5.31	-0.784320	-0.077535	0.281250	0.738366
D22S446	5.81	-0.676256	-0.086002	0.281250	0.783342
	5.97	-0.661730	-0.056548	0.281250	0.732148
	6.13	-0.647065	-0.026924	0.265625	0.714614
	6.29	-0.632235	0.002759	0.265625	0.715040
	6.45	-0.617216	0.032387	0.265625	0.733441
D22S425	6.61	-0.601990	0.061849	0.250000	0.785598
	7.55	-0.569911	0.054070	0.265625	0.780527
	8.49	-0.545440	0.046901	0.265625	0.778202
	9.44	-0.526932	0.040371	0.265625	0.778116
	10.38	-0.513158	0.034510	0.265625	0.780415
D22S257	11.32	-0.503156	0.029352	0.265625	0.786615
	11.72	-0.502827	0.031289	0.265625	0.769603
	12.12	-0.501222	0.033367	0.265625	0.756025
	12.52	-0.498388	0.035586	0.265625	0.743861
	12.92	-0.494382	0.037947	0.265625	0.732669
D22S345	13.32	-0.489271	0.040451	0.265625	0.722233
	17.56	-0.476911	0.000436	0.265625	0.608434
	21.79	-0.411060	-0.033922	0.265625	0.533545
	26.03	-0.340879	-0.062356	0.281250	0.481128
	30.27	-0.283944	-0.084714	0.281250	0.444084
D22S683	34.50	-0.242175	-0.101055	0.312500	0.417962
	38.27	-0.221849	-0.108581	0.343750	0.405343
	42.04	-0.205051	-0.114012	0.343750	0.394995
	45.81	-0.191133	-0.117285	0.343750	0.386634
	49.58	-0.179355	-0.118434	0.343750	0.379969
D22S270	53.35	-0.168993	-0.117592	0.343750	0.374718
	54.55	-0.166676	-0.117122	0.343750	0.373653
	55.76	-0.164367	-0.116547	0.343750	0.372637
	56.97	-0.162061	-0.115869	0.343750	0.371669
	58.17	-0.159754	-0.115087	0.343750	0.370749
D22S684	59.38	-0.157439	-0.114203	0.343750	0.369875
	61.38	-0.153976	-0.112703	0.343750	0.368660
	63.38	-0.150445	-0.110973	0.343750	0.367532

65.38	-0.146836	-0.109021	0.343750	0.366491
67.38	-0.143140	-0.106854	0.343750	0.365532
69.38	-0.139350	-0.104485	0.343750	0.364654

Totalling pedigrees: ..

	position	LOD_score	NPL_score	p-value	information
	-10.00	0.771930	1.46610	0.121094	0.549963
	-8.00	0.763150	1.63435	0.121094	0.581119
	-6.00	0.738396	1.81799	0.111816	0.617666
	-4.00	0.690964	2.01768	0.081055	0.661014
	-2.00	0.608834	2.23394	0.050049	0.713666
D22S427	0.00	0.465695	2.46719	0.034668	0.783900
	0.66	0.372768	2.46019	0.034668	0.776201
	1.32	0.251355	2.45452	0.036377	0.778579
	1.98	0.078304	2.45023	0.036377	0.786514
	2.64	-0.220155	2.44742	0.036377	0.799565
D22S264	3.30	-3.314434	2.44615	0.036377	0.819044
	3.81	0.088812	2.43814	0.036377	0.824106
	4.31	0.399221	2.43109	0.036865	0.832376
	4.81	0.585230	2.42504	0.036865	0.843921
	5.31	0.720582	2.42002	0.036865	0.859697
D22S446	5.81	0.828388	2.41608	0.037354	0.884777
	5.97	0.842811	2.43595	0.036377	0.858719
	6.13	0.857340	2.45602	0.034668	0.849563
	6.29	0.872001	2.47623	0.034424	0.849439
	6.45	0.886816	2.49648	0.034424	0.858353
D22S425	6.61	0.901807	2.51670	0.033691	0.884206
	7.55	0.934580	2.49776	0.034424	0.871546
	8.49	0.959219	2.48232	0.034424	0.863087
	9.44	0.977371	2.47039	0.034424	0.857531
	10.38	0.990263	2.46200	0.034668	0.854665
D22S257	11.32	0.998861	2.45719	0.034668	0.855243
	11.72	0.999244	2.45491	0.034668	0.844811
	12.12	1.000807	2.45326	0.036377	0.836407
	12.52	1.003501	2.45222	0.036377	0.828954
	12.92	1.007268	2.45182	0.036377	0.822232
D22S345	13.32	1.012044	2.45204	0.036377	0.816194
	17.56	0.905650	2.22474	0.052246	0.699183
	21.79	0.821571	2.06503	0.074463	0.637445
	26.03	0.688090	1.96987	0.090088	0.612254
	30.27	0.414228	1.93724	0.094971	0.620928
D22S683	34.50	-INFINITY	1.96589	0.090088	0.677618
	38.27	-0.933936	1.91464	0.096680	0.639050
	42.04	-0.720340	1.90556	0.098877	0.621120
	45.81	-0.695238	1.94001	0.094727	0.616265
	49.58	-0.857853	2.01973	0.080322	0.624350
D22S270	53.35	-INFINITY	2.14689	0.060791	0.652573
	54.55	-0.620024	2.06182	0.074463	0.629478
	55.76	-0.334398	1.98137	0.086182	0.612939
	56.97	-0.174770	1.90540	0.098877	0.599398
	58.17	-0.067328	1.83379	0.110840	0.588150

D22S684	59.38	0.011082	1.76637	0.116455	0.579163
	61.38	0.085680	1.60190	0.121094	0.544411
	63.38	0.136912	1.44958	0.121094	0.518093
	65.38	0.172664	1.30911	0.121094	0.496121
	67.38	0.197451	1.18011	0.121094	0.477387
	69.38	0.214130	1.06210	0.122559	0.461265

# **CHROMOSOME 22 STAGE 3**

analyzing pedigree 1...

using non-origins: 1 2 3 4 5 6 10

	position	LOD score	NPL score	p-value	information
	-10.00	1.234383	2.106410	0.062500	0.617291
	-8.00	1.287505	2.330345	0.062500	0.656640
	-6.00	1.340665	2.574894	0.062500	0.702474
	-4.00	1.393674	2.841039	0.062500	0.756546
	-2.00	1.446348	3.129618	0.062500	0.822082
D22S427	0.00	1.498510	3.441287	0.015625	0.910271
	0.66	1.500000	3.449435	0.015625	0.919641
	1.32	1.501272	3.459610	0.015625	0.930563
	1.98	1.502323	3.471820	0.015625	0.943203
	2.64	1.503154	3.486077	0.015625	0.958095
D22S264	3.30	1.503764	3.502392	0.015625	0.977577
	3.81	1.504580	3.501475	0.015625	0.979498
	4.31	1.505118	3.501807	0.015625	0.982540
	4.81	1.505377	3.503391	0.015625	0.986650
	5.31	1.505358	3.506227	0.015625	0.992049
D22S539/	5.81	1.505060	3.510316	0.015625	0.999887
446	5.97	1.504927	3.507971	0.015625	0.994951
	6.13	1.504774	3.505746	0.015625	0.99171
	6.29	1.504603	3.503642	0.015625	0.987972
	6.45	1.504412	3.501658	0.015625	0.984915
D22S425	6.61	1.504202	3.499795	0.015625	0.982240
	7.55	1.501400	3.463903	0.015625	0.940976
	8.49	1.500033	3.431714	0.015625	0.922207
	9.44	1.500147	3.403704	0.015625	0.918695
	10.38	1.501744	3.380374	0.015625	0.929602
D22S2257	11.32	1.504775	3.362217	0.015625	0.957476
	11.46	1.504766	3.358584	0.015625	0.962683
	11.60	1.504788	3.355050	0.015625	0.968704
	11.74	1.504842	3.351616	0.015625	0.975716
	11.88	1.504926	3.348283	0.015625	0.984172
D22S1174/	12.02	1.505042	3.345054	0.046875	0.996241
419	12.04	1.505035	3.345126	0.046875	0.995211
	12.06	1.505027	3.345200	0.046875	0.994353
	12.08	1.505019	3.345276	0.015625	0.993560
	12.10	1.505011	3.345354	0.015625	0.992809
D22S1164	12.12	1.505003	3.345434	0.015625	0.992091

	12.38	1.504951	3.343061	0.046875	0.988522
	12.64	1.504827	3.341003	0.046875	0.985513
	12.90	1.504629	3.339257	0.046875	0.982901
	13.16	1.504358	3.337825	0.046875	0.980637
D22S345	13.42	1.504014	3.336705	0.046875	0.978770
	17.66	1.400671	3.074162	0.062500	0.851819
	21.89	1.258671	2.903359	0.062500	0.797892
	26.13	1.054642	2.818172	0.062500	0.790655
	30.37	0.715181	2.813958	0.062500	0.829255
D22S683	34.60	-10000.000	2.887616	0.062500	0.938324
	38.37	-0.712871	2.821308	0.062500	0.873719
	42.14	-0.517707	2.812832	0.062500	0.848019
	45.91	-0.508215	2.863904	0.062500	0.846469
	49.68	-0.684434	2.976911	0.062500	0.869098
D22S270	53.45	-10000.000	3.154904	0.062500	0.930521
	54.65	-0.453213	3.034018	0.062500	0.885386
	55.86	-0.169905	2.919571	0.062500	0.853315
	57.07	-0.012591	2.811372	0.062500	0.827193
	58.27	0.092534	2.709221	0.062500	0.805612
D22S684	59.48	0.168621	2.612915	0.062500	0.788506
	61.48	0.239751	2.378740	0.062500	0.720204
	63.48	0.287447	2.161523	0.062500	0.668686
	65.48	0.319586	1.960858	0.062500	0.625777
	67.48	0.340672	1.776198	0.062500	0.589263
	69.48	0.353556	1.606880	0.062500	0.557892

analyzing pedigree 2...

	using non-origins:	22	1	24	2	3	4	6
	position	LOD score	NPL score	p-value	information			
	-10.00	0.227334	0.371893	0.250000	0.445041			
	-8.00	0.209913	0.404380	0.250000	0.462234			
	-6.00	0.180684	0.437765	0.250000	0.482931			
	-4.00	0.134346	0.471692	0.250000	0.508098			
	-2.00	0.061319	0.505746	0.203125	0.539385			
D22S427	0.00	-0.059001	0.539459	0.203125	0.581922			
	0.66	-0.139256	0.515375	0.203125	0.558195			
	1.32	-0.246417	0.490993	0.203125	0.553398			
	1.98	-0.403557	0.466401	0.250000	0.558404			
	2.64	-0.684313	0.441688	0.250000	0.571926			
D22S264	3.30	-3.555160	0.416945	0.250000	0.594623			
	3.81	0.378901	0.652055	0.125000	0.593237			
	4.31	0.690705	0.894209	0.062500	0.609731			
	4.81	0.878265	1.142534	0.062500	0.638158			
	5.31	1.015356	1.396107	0.062500	0.679865			
D22S539/	5.81	1.125096	1.653970	0.062500	0.747605			
446	5.97	1.139423	1.694489	0.062500	0.743241			
	6.13	1.153359	1.735154	0.062500	0.740007			
	6.29	1.166926	1.775957	0.062500	0.737289			
	6.45	1.180144	1.816890	0.062500	0.734961			
D22S425	6.61	1.193031	1.857946	0.062500	0.732968			
	7.55	1.258443	2.105176	0.062500	0.719809			
	8.49	1.315841	2.359119	0.062500	0.715468			
	9.44	1.366873	2.618142	0.062500	0.718809			

	10.38	1.412694	2.880565	0.062500	0.731063
DSS2257	11.32	1.454146	3.144734	0.062500	0.760508
	11.46	1.454438	3.150640	0.062500	0.757890
	11.60	1.454741	3.156693	0.062500	0.756052
	11.74	1.455056	3.162897	0.062500	0.754609
	11.88	1.455382	3.169250	0.062500	0.753518
D22S1174/	12.02	1.455721	3.175755	0.062500	0.752937
419	12.04	1.455524	3.175043	0.062500	0.752437
	12.06	1.455327	3.174333	0.062500	0.751969
	12.08	1.455130	3.173624	0.062500	0.751516
	12.10	1.454933	3.172918	0.062500	0.751074
D22S1164	12.12	1.454737	3.172213	0.062500	0.750646
	12.38	1.449960	3.148343	0.062500	0.741737
	12.64	1.445180	3.124856	0.062500	0.733792
	12.90	1.440396	3.101749	0.062500	0.726386
	13.16	1.435609	3.079016	0.062500	0.719391
D22S345	13.42	1.430818	3.056655	0.062500	0.712733
	17.66	1.303161	2.409200	0.062500	0.599096
	21.89	1.174017	1.887366	0.062500	0.526257
	26.13	1.045706	1.473656	0.062500	0.475715
	30.37	0.920672	1.149886	0.062500	0.440188
D22S683	34.60	0.801316	0.898824	0.062500	0.415220
	38.37	0.727950	0.767508	0.093750	0.403178
	42.14	0.655743	0.652703	0.125000	0.393310
	45.91	0.585821	0.553402	0.203125	0.385340
	49.68	0.519221	0.468273	0.250000	0.378987
D22S270	53.45	0.456819	0.395809	0.250000	0.373978
	54.65	0.442736	0.380337	0.250000	0.372961
	55.86	0.428765	0.365286	0.250000	0.371992
	57.07	0.414924	0.350662	0.250000	0.371068
	58.27	0.401235	0.336467	0.250000	0.370189
D22S684	59.48	0.387717	0.322705	0.250000	0.369355
	61.48	0.367968	0.303038	0.250000	0.368194
	63.48	0.348540	0.284178	0.250000	0.367116
	65.48	0.329508	0.266146	0.250000	0.366120
	67.48	0.310938	0.248956	0.250000	0.365203
	69.48	0.292891	0.232611	0.250000	0.364363

Totalling pedigrees: ..

	position	LOD_score	NPL_score	p-value	information
	-10.00	1.461717	1.75242	0.117432	0.531166
	-8.00	1.497418	1.93374	0.094971	0.559437
	-6.00	1.521349	2.13027	0.064453	0.592702
	-4.00	1.528020	2.34246	0.040771	0.632322
	-2.00	1.507668	2.57059	0.031738	0.680733
D22S427	0.00	1.439510	2.81481	0.016357	0.746097
	0.66	1.360744	2.80354	0.018066	0.738918
	1.32	1.254854	2.79350	0.018066	0.741980
	1.98	1.098765	2.78474	0.018799	0.750803
	2.64	0.818840	2.77735	0.018799	0.765010
D22S264	3.30	-2.051396	2.77139	0.018799	0.786100
	3.81	1.883481	2.93699	0.008301	0.786368



	4.31	2.195823	3.10845	0.004150	0.796135
	4.81	2.383643	3.28516	0.003906	0.812404
	5.31	2.520714	3.46647	0.003906	0.835957
D22S539/	5.81	2.630156	3.65170	0.003906	0.873746
446	5.97	2.644349	3.67869	0.003906	0.869096
	6.13	2.658133	3.70588	0.003906	0.865589
	6.29	2.671529	3.73324	0.003906	0.862581
	6.45	2.684556	3.76078	0.003906	0.859938
D22S425	6.61	2.697232	3.78850	0.003906	0.857604
	7.55	2.759843	3.93793	0.003906	0.830392
	8.49	2.815874	4.09474	0.003906	0.818837
	9.44	2.867020	4.25809	0.003906	0.818752
	10.38	2.914438	4.42715	0.003906	0.830332
D5S2257	11.32	2.958920	4.60111	0.003662	0.858992
	11.46	2.959204	4.60272	0.003662	0.860286
	11.60	2.959529	4.60450	0.003662	0.862378
	11.74	2.959898	4.60646	0.003662	0.865163
	11.88	2.960309	4.60859	0.003662	0.868845
D22S1174/	12.02	2.960763	4.61091	0.003662	0.874589
419	12.04	2.960558	4.61046	0.003662	0.873824
	12.06	2.960354	4.61001	0.003662	0.873161
	12.08	2.960149	4.60956	0.003662	0.872538
	12.10	2.959945	4.60911	0.003662	0.871942
D22S1164	12.12	2.959740	4.60867	0.003662	0.871369
	12.38	2.954912	4.59012	0.003662	0.865129
	12.64	2.950007	4.57205	0.003906	0.859652
	12.90	2.945025	4.55448	0.003906	0.854644
	13.16	2.939967	4.53739	0.003906	0.850014
D22S345	13.42	2.934832	4.52079	0.003906	0.845752
	17.66	2.703832	3.87732	0.003906	0.725458
	21.89	2.432688	3.38755	0.003906	0.662075
	26.13	2.100349	3.03478	0.004639	0.633185
	30.37	1.635852	2.80286	0.018066	0.634721
D22S683	34.60	-INFINITY	2.67742	0.026367	0.676772
	38.37	0.015079	2.53768	0.033447	0.638449
	42.14	0.138036	2.45050	0.036777	0.620665
	45.91	0.077606	2.41640	0.037354	0.615905
	49.68	-0.165212	2.43611	0.036377	0.624042
D22S270	53.45	-INFINITY	2.51073	0.033691	0.652249
	54.65	-0.010477	2.41431	0.037354	0.629174
	55.86	0.258860	2.32274	0.042236	0.612653
	57.07	0.402333	2.23590	0.050049	0.599131
	58.27	0.493769	2.15363	0.060059	0.587901
D22S684	59.48	0.556337	2.07580	0.073486	0.578931
	61.48	0.607719	1.89630	0.099121	0.544199
	63.48	0.635988	1.72937	0.119629	0.517901
	65.48	0.649094	1.57473	0.121094	0.495949
	67.48	0.651610	1.43200	0.121094	0.477233
	69.48	0.646447	1.30072	0.121094	0.461127

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