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Abstract

A global rise in life expectancy comes with an increased burden of serious life-long health issues and the need for useful real-time measures of the aging processes. Studies have shown the value of biochemical signatures of IgG N-glycosylation as clinically relevant biomarkers to differentiate healthy from accelerated aging. Most human biological processes rely on glycosylation of proteins to regulate their function, but these events appear sensitive to environmental changes, age and the presence of disease. Specifically, variations in N-glycosylation of IgG can adversely affect inflammatory pathways underpinning unhealthy aging and chronic disease pathogenesis. This expert review highlights the discrepancies between an organism's age in years of life (chronological age) versus age in terms of health status (biological age). The paper examines and synthesizes the studies on IgG N-glycan profiles and the third alphabet of life, the sugar code, in relation to their relevance as dynamic indicators of aging, and to differentiate between normal and accelerated aging. The levels of N-glycan structures change with aging, suggesting that monitoring the alterations of serum glycan biosignatures with glycomics might allow real-time studies of human aging in the near future. Glycomics brings in yet another systems science technology platform to strengthen the emerging multi-omics studies of aging and age related diseases.

Introduction

Aging is a multifactorial interconnected biological process, which manifests as declining physiological functions and increasing susceptibility to chronic inflammation and to aging-related diseases (Kennedy et al., 2014; Lopez-Otin et al., 2013). Studies have suggested that healthier lifestyle such as healthy diet, continued physical exercise, restriction on alcohol drinking, reducing sitting time and smoking cessation may slow the appearance and/or progression of aging (Blair et al., 1989; Franceschi et al., 2018; Kovell et al., 2020; Reid et al., 2018; Vopat et al., 2014).

With these observations, extensive effort has been made during the past decades to identify biomarkers of aging which may predict or monitor age-associated physiological decline and disease. The American Federation for Aging Research (AFAR) has summarized nine "hallmarks of aging", including

- epigenetic alternations (e.g., methylation, phosphorylation, ubiquitination, glycosylation),
- loss of proteostasis,
- deregulated nutrient sensing,
- mitochondrial dysfunction,
- cellular senescence,
- stem cell exhaustion,
- altered intercellular communication,
- genomic instability, and
- telomere attrition (Lopez-Otin et al., 2013).

Among these hallmarks, recent advances in high-throughput technologies to study the sugar code, i.e., the third alphabet of life, converged into protein glycosylation as an essential biomarker of aging and aging related disease (Koga et al., 2011; Kristic et al., 2014; Moreno et al., 2020; Yu et al., 2016). According to the AFAR, "aging biomarkers" need to meet the following criteria (Butler et al., 2004; Johnson, 2006):

1) it must predict the physical and mental status of a person in their life span;

2) the effects of disease must be excluded;

3) it must be harmless and tested repeatably in humans;

4) it can be tested *in vivo* and *in vitro*.

No such marker has been identified to date that addresses all these dimensions although studies of glycans offer new insights. Thus, in this expert review, we focus on the role of protein glycosylation in aging progress (especially in inflammatory process) and aging related diseases.

Protein glycosylation is an enzymatic process responsible for the attachment of carbohydrates (glycans) to glycoproteins, predominantly via nitrogen (N) and oxygen (O) linkages. Glycosylation occurs in the endoplasmic reticulum/Golgi compartment of essentially all cells being mediated by the coordinated action of a portfolio of different glycosyltransferases and glycosidases enzymes (Alavi et al., 2006; Varki, 2006). The glycan is a significant part of nearly all cellular membrane and extracellular proteins, and of them, more than a half are glycoproteins (Apweiler et al., 1999; Ozdemir et al., 2020). The structure and/or composition of glycan

attached to protein backbones plays an important role in nearly all molecular interactions at the cell surface and in the intercellular space during the aging progress (Packer et al., 2008).

Different type of glycans can be synthesized with varying enzymes from varying number, order and type of monosaccharide units. The most abundant monosaccharides that can be found are: fucose (Fuc), galactose (Gal), glucose (Glu), mannose (Man), N-acetylgalactosamine (GalNAc), Nacetylglucosamine (GlcNAc), sialic acid (Sia) and xylose (Xyl) (Lauc et al., 2014). The extremely complex branched structures of glycan make the analysis of glycosylation significantly more challenging than the analysis of linear DNA and protein sequences. However, with the rapid development of biological techniques, such as HILIC (hydrophilic-interaction chromatography), HPLC (high performance liquid chromatography), CE (capillary electrophoresis), lectin affinity and MS (mass spectrometry), research on N-glycan of plasma/serum protein and immunoglobulin G (IgG) has experienced an unprecedented advance over recent years (Adamczyk et al., 2012; Wang, 2019; Wang et al., 2019).

Protein Glycosylation in Aging Progress

Diverse plasma glycans were shown to be closely related to aging over three decades ago (Parekh et al., 1988) and have also been replicated in recent studies in European (Valerie et al., 2008; Vanhooren et al., 2007; Vanhooren et al., 2009) and Chinese populations (Lu et al., 2011). In a human serum glycoproteins study, the level of the agalactosylated glycans are increased with age while the core alpha-1,6-fucosylated bi-galactosylated biantennary structure decreased, and the similar variation trend has been shown in the Werner syndrome (Vanhooren et al., 2007). These observations suggest that change of *N*-glycan level can provide a non-invasive surrogate marker of aging (Vanhooren et al., 2007). The levels of *N*-glycan structures are changed with

aging, indicating that examining alterations of serum glycan fingerprint might be suitable for monitoring the progress of aging in real time (Vanhooren et al., 2009).

The changes in plasmatic forms of two glycosyltransferases, Beta-1,4-galactosyltransferase 1 (B4GALT1) and Beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1), in aging progress also prove the strong association between glycosylation and aging, although the precise mechanism is still unknown (Dall'Olio, 2018). The selective combinations of IgG N-glycan structures associate with biological hallmarks of pre-chronic disease states when biological age precedes chronological age. The usefulness of IgG N-glycan profiles as indicators of aging can be achieved by profiling IgG N-glycosylation retro- and prospectively in real-time to longitudinally capture the influences of genetics, environment and time on glycome.

Immunoglobulins (Igs) are a group of plasma glycoproteins (IgG, IgM, IgA, IgE and IgD) with antibody activity accounting for approximately 20% of plasma proteins (Junqueira et al., 2003). Among them, IgG is the most abundant (over 80%) immunoglobulin and is the main component of antibody formation after a secondary immune response. IgG is considered as a perfect model glycoprotein because of its well-defined glycan structures and functions (Gornik et al., 2012). IgG molecules consist of two identical light chains and heavy chains, which are joined by covalent bonds to form a "Y-shaped" structure (Huber et al., 1976) (Fig. 1). The two domains of IgG that are responsible for their *in vivo* properties are the F_{ab} (antigen binding fragment) and the F_c (crystallizable fragment) (Schroeder et al., 2010). A single, *N*-linked glycosylation conservative site exists at the amino acid 297 (Asn297) in the heavy chain of all IgG subclasses

(Fig 1). On average, 2.8 glycans are linked to each IgG molecular and among them two glycans are located at Asn297 in the Fc region (Arnold et al., 2007).

Around 30% of IgG and some monoclonal antibodies contain N-linked glycosylation sites in F_{ab} and the hinge region, but they are not conserved (Jefferis, 2012; Wright et al., 1997). The F_c region of glycans generally contains a core heptasaccharide which is species-, tissue-, age- and sex- specific, and this specificity is conferred by the terminal oligosaccharides (Huhn et al., 2009). Normal structure of IgG N-glycans in the Fc region is complex fucosylated bi-antennary oligosaccharides, and the main sources of glycan heterogeneity are the galactosylation and/or sialylation in the residues. In the F_{ab} region, glycans are usually sialylated and with a bisecting GlcNAc residue (Wormald et al., 1997). Genetic recombination and/or somatic mutation can affect the process of IgG synthesis and result in the variation of N-linked glycosylation sites in the heavy and light chains of the IgG molecule. cDNA sequential detection shows that 9% of the variable regions of IgG contain potential N-linked glycosylation sites and the majority of the variable regions are located in the heavy chain of IgG (Zhu et al., 2002).

Fine changes in attached glycan composition can dramatically alter the conformation of the Fc domain with significant consequences for effector functions of IgG (Gornik et al., 2012). The addition of terminal sialic acid to this glycan reduces Fcγ receptors (FcγRs) binding and converts IgG antibodies from pro-inflammatory to anti-inflammatory mediators through the lectin receptor SIGN-R1 or DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin), which upregulates surface expression of the inhibitory F_c receptor, FcγRIIb, on inflammatory cells, thereby attenuating autoantibody-initiated inflammation

(Anthony et al., 2010). Studies have identified that decreased levels of bisecting GlcNAc glycoforms of IgG are associated with human longevity in Caucasians (Ruhaak et al., 2010); while the level of galactosylation of IgG decreased in aging process in Asian Japanese (Shikata et al., 1998). The aging-associated IgG glycosylation changes are often observed in the chronic inflammatory state (Dall'Olio, 2018), which makes IgG glycosylation an attractive biomarker in monitoring aging-related progress. The main domains of IgG glycan involved in the aging progress are demonstrated in Figure 1.

Protein Glycosylation and Aging-Related Diseases

A large variability of human plasma N-glycans was observed in a population-based study (Knezevic et al., 2009). However, the N-glycan structure are considerably stable within a short time-frame, indicating that changes of N-glycan profiles may the consequences of environmental determinants and/or pathophysiologic situations (Olga et al., 2009). A population-based study provided preliminary evidence of an association between the variation of plasma N-glycans and genetic and environmental factors by testing the whole plasma *N*-glycans (Knezevic et al., 2009; Zaytseva et al., 2020). Other studies with a population focus showed an association between several plasma N-glycan levels (trigalactosylated glycans, biantennary glycans, triantennary glycans, core-fucosylated glycans, monosialylated, disialylated and trisialylated glycans) and metabolic syndrome related risk factors (systolic blood pressure, diastolic blood pressure, body mass index, fasting plasma glucose, age and gender) (Lu et al., 2011). Therefore, glycosylation alterations are suggested to be associated with a number of aging related diseases (Alavi et al., 2008).

Malfunction of IgG glycosylation appears to be associated with the incidence and development of many aging related diseases, such as inflammatory bowel disease (IBD) (Akma I et al., 2015), hyperuricemia (Hou et al., 2019), type 2 diabetes (T2DM)(Liu et al., 2019), hypertension (Wang et al., 2016), rheumatoid arthritis (RA) (Parekh et al., 1985; Sebastian et al., 2016; Troelsen et al., 2012), systemic lupus erythematosus (SLE) (Huang et al., 2003; Sjowall et al., 2015; Vuckovic et al., 2015), Alzheimer's disease (AD) and progressive mild cognitive impairment (MCI) (Lundström et al., 2014), Parkinson's disease (PD) (Russell et al., 2017), dementia (Zhang et al., 2021) and cancer (Adamczyk et al., 2012; Lan et al., 2016, Wang, 2013).

A study found that the levels of glycosylated IgG are significantly higher in diabetic patients than that in normal controls, and such modification *in vivo* was associated with IgG functional alteration (Kaneshige, 1987). The glycation levels of IgG were increased in poorly controlled T2DM patients when compared to healthy subjects and also well controlled diabetic patients (Lapolla et al., 2000). In a multi-ethnic study (China, Croatia and Scotland), five glycans (IgG with digalactosylated glycans) significantly differed in participants with prehypertension or hypertension compared to those with normal blood pressure, while additional 17 glycan traits were only significantly differed in participants with hypertension compared to those of normal blood pressure (Wang et al., 2016). These glycans were also significant correlated with systolic blood pressure (SBP) or diastolic blood pressure (DBP) (Wang et al., 2016). IgG hypogalactosylation correlated to inflammation-associated molecules, such as interleukin-6 (IL-6), C-reactive protein (CRP) in RA patients (Troelsen et al., 2012).

Different distributions of asparagine-linked bi-antennary complex-type oligosaccharide structures of IgG were found between normal individuals and patients with RA (Troelsen et al., 2012) and patients with osteoarthritis (Parekh et al., 1985). A study on RA demonstrated that the agalactosylated IgG glycan due to its exposure of the mannose rich core glycan structure acquires the capacity to bind to mannose binding lectin (MBL), providing an explanation for this enhanced pro-inflammatory activity *in vivo* (Malhotra et al., 1995). The IgG differences between the normal individuals and the patients indicate that glycosylation is involved in the pathogenesis of the diseases (Parekh et al., 1985). In addition, disease activity of SLE patients correlated with higher exposure of Aleuria aurantia lectin-reactive fucosyl residues, an immobilized IgG complex (Sjowall et al., 2015). In another SLE study, the significant changes include decreased galactosylation and sialylation of IgG, as well as decreased core fucose but increased bisecting N-acetylglucosamine of IgG, indicating that changes in IgG glycosylation may be applied as a dynamic biomarker to the monitoring of SLE progress (Vuckovic et al., 2015).

A lower abundance of complex galactosylated and sialylated IgG glycan structures was found in AD patients (Lundström et al., 2014). Furthermore, IgG glycan profiling from AD patients with and normal controls suggested that there may be a reduced capacity for the IgG to inhibit Fc gamma-RIIIa binding, which would allow an increased ability for the IgG to cause antibody-dependent cell cytotoxicity (ADCC) and a possible state of low-grade inflammation in individuals with PD (Russell et al., 2017). In addition to the above diseases, glycosylation of whole plasma/serum protein is also found to be a potential biomarker for liver, pancreatic, prostate, ovarian, breast, lung and stomach cancers (Adamczyk et al., 2012; Wang et al., 2013).

And in most of these cancers, the fucosylation and sialylation expression are found to be significantly modified (Adamczyk et al., 2012; Wang et al., 2013).

These studies suggest that the individual variation in N-glycosylation of IgG contributes to the pathogenesis of aging and aging related disease, presumably via its effect on pro- and/or anti-inflammatory, ADCC and complement-dependent cytotoxicity (CDC) pathways. The addition of terminal sialic acid to glycan reduces Fcγ receptors (FcγRs) converts IgG antibodies from pro-inflammatory to anti-inflammatory mediators through the lectin receptor SIGN-R1 or DC-SIGN, which upregulates surface expression of the inhibitory Fc receptor FcγRIIb, on inflammatory cells, thereby attenuating autoantibody-initiated inflammation (Anthony et al., 2010). As the pervasive feature of aging tissues and nearly all age-related diseases are featured by chronic inflammation (Franceschi et al., 2014), the glycan profile could be a reliable biomarker in aging progress and even in the pathogenesis of aging related diseases.

Another important role which glycosylation plays in IgG is modulation of ADCC: the addition of fucose residues in the core of the glycan (core-fucose) modifies the conformation of the Fc domain in a way to significantly reduce its affinity for FcγRIIIa; therefore core-fucosylated IgGs are less efficient in activating ADCC (Anthony et al., 2010; Iida et al., 2006). Most of IgGs (over 95%) are core-fucosylated which can prevent them from wrong elicitation of ADCC (Scanlan et al., 2008). Decreased IgG Fc sialylation could reduce its CDC activity via C1q binding, which could lead to increase activation of the lectin-initiated complement pathway. These changes of the IgG N-glycan may make them possible converting from anti-inflammatory to proinflammatory effects upon antigenic challenge (Zhang et al., 2021). The main proposed roles

of IgG N-glycan involved in the pathogenesis of aging progress and aging related disease are demonstrated in Figure 2.

Outlook

Aging is one of the most important phenotypes in medicine and ecology. Aging biomarkers warrants more in-depth research. New post-genomic technologies such as glycomics offer prospects such as real-time or longitudinal analyses of aging. Current glycomics evidence shows that aging is closely correlated with IgG Fc monogalactosylation. IgG glycosylation has also been shown to change with both chronological and biological age of an individual. The decrease in levels of digalactosylation and increase in bisecting GlcNAc levels were observed in population-based aging studies. Age-related decline in IgG galactosylation is associated with increases in systemic inflammation in older people, which can be explained by higher levels of pro-inflammatory cytokines, such as TNF-α and IL-6, found in older individuals. Accordingly, decreased galactosylation also enhances the pro-inflammatory potential of IgG. The decrease in IgG galactosylation has been observed in numerous inflammatory and autoimmune diseases, such as IBD, RA and SLE. Population specific IgG N-glycosylation evidence suggests that persons in developing countries may be exposed to environmental factors that can cause low-grade chronic inflammation and an attendant apparent increase in biological age (Tambuk et al., 2020). Although we still do not fully know the exact mechanisms underlying the age-related changes in whole plasma/serum glycan and IgG glycan profiles, the cellular and humoral immunity pathways identified thus far, suggest various enzyme activities involved in glycosylation processes through modulation of the balance between pro- and anti-inflammation, and that this fine balance contributes to the process of both chronological and biological aging.

This expert review underscores the marked potential of glycan monitoring as an approach to better understand and unravel the biomarkers of aging progress, and aging related diseases. While new digital technologies such as three-dimensional facial-image analysis (Xia et al., 2020) and epigenetic approaches (Saul et al., 2021) offer comprehensive studies of temporal and spatial changes in aging related phenotypes, glycomics brings in yet another systems science technology platform to strengthen the emerging multi-omics studies of aging and age-related diseases.

Acknowledgment

References

Adamczyk, B., Tharmalingam, T., Rudd, P. M. (2012). Glycans as cancer biomarkers. Biochimica et Biophysica Acta 1820(9), 1347-1353.

Akma I, I. T., Ventham, N. T., Theodoratou, E., et al. (2015). Inflammatory bowel disease associates with proinflammatory potential of the immunoglobulin g glycome. Gastroenterology 148(4), 440.

Alavi, A., Axford, J. S. (2006). The pivotal nature of sugars in normal physiology and disease. Wien Med Wochenschr, 156(1-2), 19-33.

Alavi, A., Axford, J. S. (2008). Sweet and sour: The impact of sugars on disease. Rheumatology (Oxford), 47(6), 760-770.

Anthony, R. M., Ravetch, J. V. (2010). A novel role for the IgG Fc glycan: The anti-inflammatory activity of sialylated IgG Fcs. J Clin Immunol, 30 Suppl 1, S9-S14.

Apweiler, R., Hermjakob, H., Sharon, N. (1999). On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. Biochim Biophys Acta, 1473(1), 4-8.

Arnold, J. N., Wormald, M. R., Sim, R. B., et al. (2007). The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol, 25, 21-50.

Blair, S. N., Kohl, H. R., Paffenbarger, R. J., et al. (1989). Physical fitness and all-cause mortality. A prospective study of healthy men and women. JAMA, 262(17), 2395-2401.

Butler, R. N., Sprott, R., Warner, H., et al. (2004). Biomarkers of aging: From primitive organisms to humans. J Gerontol A Biol Sci Med Sci, 59(6), B560-B567.

Dall'Olio, F. (2018). Glycobiology of aging. Subcell Biochem, 90, 505-526.

Franceschi, C., Campisi, J. (2014). Chronic inflammation (Inflammaging) and its potential contribution to Age-Associated diseases. Journals of Gerontology, 69(Suppl 1), S4-S9.

Franceschi, C., Ostan, R., Santoro, A. (2018). Nutrition and inflammation: Are centenarians similar to individuals on Calorie-Restricted diets? Annu Rev Nutr, 38, 329-356.

Gornik, O., Pavic, T., Lauc, G. (2012). Alternative glycosylation modulates function of IgG and other proteins - implications on evolution and disease. Biochim Biophys Acta, 1820(9), 1318-1326.

Hou, H., Xu, X., Sun, F., et al. (2019). Hyperuricemia is associated with immunoglobulin g N-Glycosylation: A Community-Based study of glycan biomarkers. OMICS 23(12), 660-667.

Huang, Y. F., Wang, W., Han, J. Y., et al. (2003). Increased frequency of the mannose-binding lectin LX haplotype in Chinese systemic lupus erythematosus patients. Eur J Immunogenet, 30(2), 121-124.

Huber, R., Deisenhofer, J., Colman, P. M., et al. (1976). Crystallographic structure studies of an IgG molecule and an Fc fragment. Nature, 264(5585), 415-420.

Huhn, C., Selman, M. H., Ruhaak, L. R., et al. (2009). IgG glycosylation analysis. Proteomics, 9(4), 882-913.

Iida, S., Misaka, H., Inoue, M., et al. (2006). Nonfucosylated therapeutic IgG1 antibody can evade the inhibitory effect of serum immunoglobuling on Antibody-Dependent cellular cytotoxicity through its high binding to FcRIIIa. Clinical Cancer Research An Official Journal of the American Association for Cancer Research, 12(9), 2879-2887.

Jefferis, R. (2012). Isotype and glycoform selection for antibody therapeutics. Arch Biochem Biophys, 526(2), 159-166.

Johnson, T. E. (2006). Recent results: Biomarkers of aging. Exp Gerontol, 41(12), 1243-1246.

Junqueira, Carlos, L., Luiz, C. J., et al. (2003). Basic histology: Text & atlas: Lange Medical Books / McGraw-Hill, New York, NY.

Kaneshige, H. (1987). Nonenzymatic glycosylation of serum IgG and its effect on antibody activity in patients with diabetes mellitus. Diabetes 36(7), 822-828.

Kennedy, B. K., Berger, S. L., Brunet, A., et al. (2014). Geroscience: Linking aging to chronic disease. Cell, 159(4), 709-713.

Knezevic, A., Polasek, O., Gornik, O., et al. (2009). Variability, heritability and environmental determinants of human plasma N-glycome. J Proteome Res, 8(2), 694-701.

Koga, H., Kaushik, S., Cuervo, A. M. (2011). Protein homeostasis and aging: The importance of exquisite quality control. Ageing Res Rev, 10(2), 205-215.

Kovell, L. C., Yeung, E. H., Miller, E. R., et al. (2020). Healthy diet reduces markers of cardiac injury and inflammation regardless of macronutrients: Results from the OmniHeart trial. Int J Cardiol, 299, 282-288.

Kristic, J., Vuckovic, F., Menni, C., et al. (2014). Glycans are a novel biomarker of chronological and biological ages. J Gerontol A Biol Sci Med Sci, 69(7), 779-789.

Lan, Y., Hao, C., Zeng, X., et al. (2016). Serum glycoprotein-derived N- and O-linked glycans as cancer biomarkers. Am J Cancer Res, 6(11), 2390-2415.

Lapolla, A., Fedele, D., Garbeglio, M., et al. (2000). Matrix-assisted laser desorption/ionization mass spectrometry, enzymatic digestion, and molecular modeling in the study of nonenzymatic glycation of IgG. J Am Soc Mass Spectr, 11(2), 153-159.

Lauc, G., Kristic, J., Zoldos, V. (2014). Glycans - the third revolution in evolution. Front Genet, 5, 145.

Liu, J., Dolikun, M., Stambuk, J., et al. (2019). Glycomics for type 2 diabetes biomarker discovery: Promise of immunoglobuling Subclass-Specific fragment crystallizable n-glycosylation in the uyghur population. OMICS 23(12), 640-648.

Lopez-Otin, C., Blasco, M. A., Partridge, L., et al. (2013). The hallmarks of aging. Cell, 153(6), 1194-1217.

Lu, J. P., Knezevic, A., Wang, Y. X., et al. (2011). Screening novel biomarkers for metabolic syndrome by profiling human plasma N-glycans in Chinese Han and Croatian populations. J Proteome Res, 10(11), 4959-4969.

Lundström, S. L., Yang, H., Lyutvinskiy, Y., et al. (2014). Blood plasma IgG Fc glycans are significantly altered in Alzheimer's disease and progressive mild cognitive impairment. J Alzheimers Dis, 38(3).

Malhotra, R., Wormald, M. R., Rudd, P. M., et al. (1995). Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med, 1(3), 237-243.

Moreno, D. F., Aldea, M. (2020). Proteostatic stress as a nodal hallmark of replicative aging. Exp Cell Res, 394(2), 112163.

Olga, G., Jasenka, W., Maja, P., et al. (2009). Stability of N-glycan profiles in human plasma. Glycobiology, (12), 1547-1553.

Ozdemir, V., Arga, Y., Aziz, R., et al. (2020). Digging deeper into Precision/Personalized medicine: Cracking the sugar code, the third alphabet of life, and sociomateriality of the cell. OMICS 24(2), 62-68.

Packer, N. H., von der Lieth, C. W., Aoki-Kinoshita, K. F., et al. (2008). Frontiers in glycomics: Bioinformatics and biomarkers in disease. An NIH white paper prepared from discussions by the focus groups at a workshop on the NIH campus, Bethesda MD (September 11-13, 2006). Proteomics, 8(1), 8-20.

Parekh, R. B., Dwek, R. A., Sutton, B. J., et al. (1985). Association of rheumatoid arthritis and

primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature, 316(6027), 452-457.

Parekh, R., Roitt, I., Isenberg, D., et al. (1988). Age-related galactosylation of the N-linked oligosaccharides of human serum IgG. J Exp Med, 167(5), 1731-1736.

Reid, N., Healy, G. N., Gianoudis, J., et al. (2018). Association of sitting time and breaks in sitting with muscle mass, strength, function, and inflammation in community-dwelling older adults. Osteoporos Int, 29(6), 1341-1350.

Ruhaak, L. R., Uh, H. W., Beekman, M., et al. (2010). Decreased levels of bisecting GlcNAc glycoforms of IgG are associated with human longevity. Plos One, 5(9), e12566.

Russell, A. C., Simurina, M., Garcia, M. T., et al. (2017). The N-glycosylation of immunoglobulin G as a novel biomarker of Parkinson's disease. Glycobiology, 27(5), 501-510.

Saul, D., Kosinsky, R. L. (2021). Epigenetics of aging and Aging-Associated diseases. Int J Mol Sci, 22(1).

Scanlan, C. N., Burton, D. R., Dwek, R. A. (2008). Making autoantibodies safe. P Natl Acad Sci USA, 105(11), 4081-4082.

Schroeder, H. J., Cavacini, L. (2010). Structure and function of immunoglobulins. J Allergy Clin Immunol, 125(2 Suppl 2), S41-S52.

Sebastian, A., Alzain, M. A., Asweto, C. O., et al. (2016). Glycan biomarkers for rheumatoid arthritis and its remission status in Han Chinese patients. OMICS 20(6), 343-351.

Shikata, K., Yasuda, T., Takeuchi, F., et al. (1998). Structural changes in the oligosaccharide moiety of human IgG with aging. Glycoconj J, 15(7), 683-689.

Sjowall, C., Zapf, J., von Lohneysen, S., et al. (2015). Altered glycosylation of complexed native IgG molecules is associated with disease activity of systemic lupus erythematosus. Lupus, 24(6),

569-581.

Tambuk, J., Naki, N., Vukovi, F., et al. (2020). Global variability of the human IgG glycome. Aging, 12(15).

Troelsen, L. N., Jacobsen, S., Abrahams, J. L., et al. (2012). IgG glycosylation changes and MBL2 polymorphisms: Associations with markers of systemic inflammation and joint destruction in rheumatoid arthritis. J Rheumatol, 39(3), 463-469.

Valerie, Vanhooren, Wouter, et al. (2008). N-Glycan profiling in the study of human aging. Biogerontology, 9, 351-356.

Vanhooren, V., Desmyter, L., Liu, X. E., et al. (2007). N-glycomic changes in serum proteins during human aging. Rejuvenation Res, 10(4), 521-531.

Vanhooren, V., Liu, X. E., Franceschi, C., et al. (2009). N-glycan profiles as tools in diagnosis of hepatocellular carcinoma and prediction of healthy human ageing. Mech Ageing Dev, 130(1-2), 92-97.

Varki, A. (2006). Nothing in glycobiology makes sense, except in the light of evolution. Cell, 126(5), 841-845.

Vopat, B. G., Klinge, S. A., McClure, P. K., et al. (2014). The effects of fitness on the aging process. J Am Acad Orthop Surg, 22(9), 576-585.

Vuckovic, F., Kristic, J., Gudelj, I., et al. (2015). Association of systemic lupus erythematosus with decreased immunosuppressive potential of the IgG glycome. Arthritis Rheumatol, 67(11), 2978-2989.

Wang, W. (2013). Validation and development of N-glycan as biomarker in cancer diagnosis. Curr Pharmacogenomics Person Med, 11, 53-58.

Wang, W. (2019). Glycomics research in china: The current state of the art. OMICS 23(12), 601-602.

Wang, W., and Ozdemir, V. (2019). Special issue: Glycomics and personalized glycomedicine. OMICS 23(12), 599-600.

Wang, Y., Klaric, L., Yu, X., et al. (2016). The association between glycosylation of immunoglobulin g and hypertension: A multiple ethnic Cross-Sectional study. Medicine (Baltimore), 95(17), e3379.

Wormald, M. R., Rudd, P. M., Harvey, D. J., et al. (1997). Variations in oligosaccharide-protein interactions in immunoglobulin G determine the site-specific glycosylation profiles and modulate the dynamic motion of the Fc oligosaccharides. Biochemistry-US, 36(6), 1370-1380.

Wright, A., Morrison, S. L. (1997). Effect of glycosylation on antibody function: Implications for genetic engineering. Trends Biotechnol, 15(1), 26-32.

Xia, X., Chen, X., Wu, G., et al. (2020). Three-dimensional facial-image analysis to predict heterogeneity of the human ageing rate and the impact of lifestyle. Nature Metabolism, 2(9), 946-957.

Yu, X., Wang, Y., Kristic, J., et al. (2016). Profiling IgG N-glycans as potential biomarker of chronological and biological ages: A community-based study in a Han Chinese population. Medicine (Baltimore), 95(28), e4112.

Zaytseva, O. O., Freidin, M. B., Keser, T., et al. (2020). Heritability of human plasma N-Glycome. J Proteome Res, 19(1), 85-91.

Zhang, X., Yuan, H., Lyu, J., et al. (2021). Association of dementia with immunoglobulin G N-glycans in a Chinese Han Population. npj Aging and Mechanisms of Disease, 7(1), 3.

Zhu, D., McCarthy, H., Ottensmeier, C. H., et al. (2002). Acquisition of potential N-

glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. Blood, 99(7), 2562-2568.