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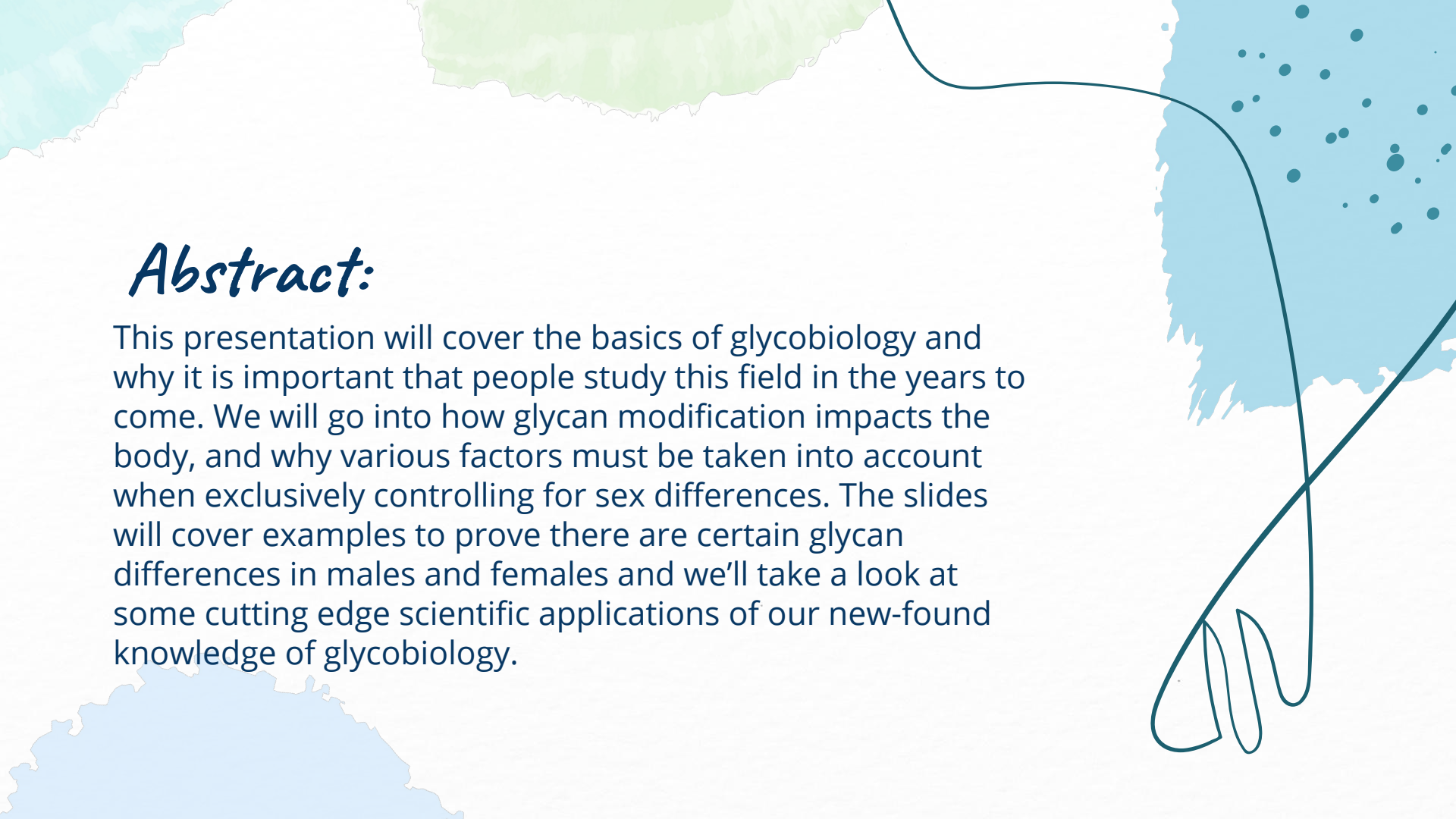
The Differential Effects of Glycans in Males and Females

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women in scholarship, engineering, science & technology



Abstract:

This presentation will cover the basics of glycobiology and why it is important that people study this field in the years to come. We will go into how glycan modification impacts the body, and why various factors must be taken into account when exclusively controlling for sex differences. The slides will cover examples to prove there are certain glycan differences in males and females and we'll take a look at some cutting edge scientific applications of our new-found knowledge of glycobiology.

A stylized world map in the background, with continents in light green and oceans in light blue. The map is centered on the Atlantic Ocean. In the top right corner, there are several small dark blue dots. In the bottom left corner, there are several light blue wavy lines.

Introduction

What is Glycobiology?

Glycobiology simply put is the study of structures and functions of glycans (Biolabs, 2021)!

The field of glycobiology, while continuously increasing in popularity, is still relatively new and understudied in comparison with other sciences. With the large role glycans play in human diseases it is interesting how little these concepts have been studied (Maverakis et al., 2015).

Hopefully this presentation shows why glycobiology is an important area of study, especially in the differing sexes.



Taken from (Storyset, 2019)

Glycans

Glycans, are chain-like structures composed of monosaccharides, or simple sugars. Some well known monosaccharides include glucose, fructose, and galactose, which chemically bind together in a variety of ways to create a “glycan” (GlyTech, Inc., 2018).

Glycans are one of the four most important molecules making up all living systems (Maverakis et al., 2015), and are found on the surface of all cells. Their role in the immune system is vital (*L. Willis, personal communication, July 2021*), which will be further examined throughout this presentation.

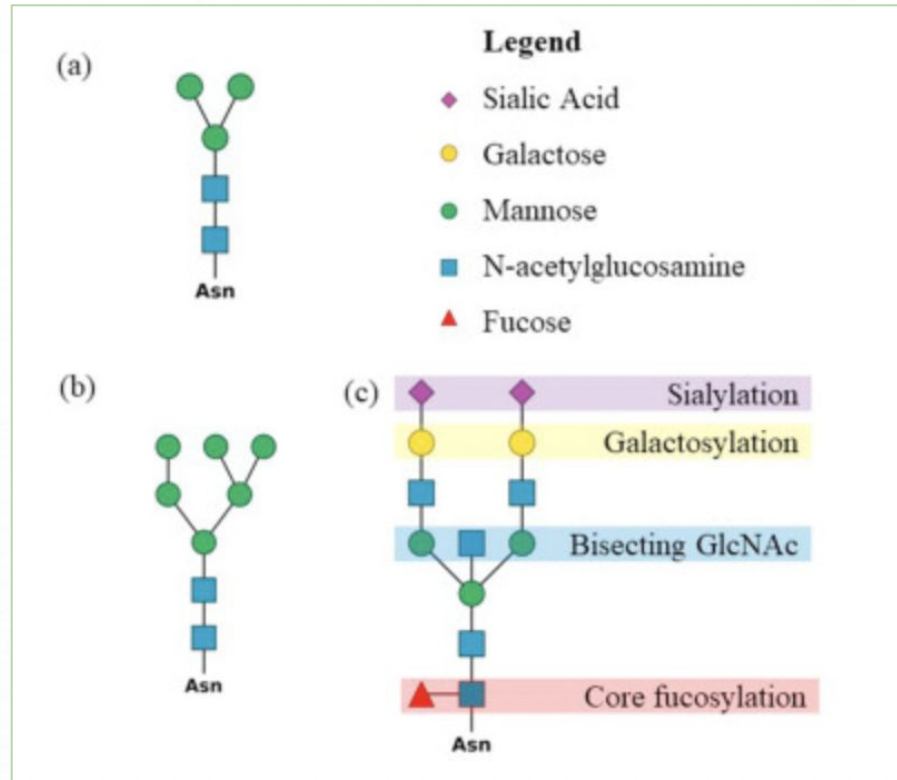
Figure 1:

a) shows a core N-glycan structure (Lim et al., 2020)

b) shows a high mannose glycan, where only mannose is added to the core structure (Lim et al., 2020)

c) The different glycosylation traits (Lim et al., 2020)

Later slides will go into more depth on glycosylation and these structures, but for now the diagram is to give a visual of glycan structures and their monosaccharide composition.



Taken from (Lim et al., 2020)



*Why is Glycobiology
Important?*

Historical Sex Based Research

Historically, females were not included in clinical drug trials. It was deemed unsafe for women of childbearing potential to be involved in such tests. Despite lifting the 1977 rule on fertile women in clinical trials, studies including women are not being conducted properly to include data on sex differences (Klein & Flanagan , 2016).

In a study of drugs between 1995 and 2000, substantial differences were found in how men absorb, metabolize, and excrete drugs, and there were no female dosage recommendations on the labels. This probably, in part, attributes to why women are 1.5 times more likely to develop adverse reactions to prescription drugs than men (Kim et al., 2010).

Despite these stark differences, immunology still ranks one of the the lowest 10 in biological disciplines, as of 2014, for reporting the sex of subjects in published papers (Klein & Flanagan , 2016).

Sex Based Research Cont'd.

It's also interesting to note that females mount stronger innate and adaptive immune responses in comparison with males (Klein & Flanagan , 2016), and are 10 times more likely to develop an autoimmune disease. In contrast, males are twice as likely to die from non-reproductive cancers (Tajik et al., 2020).

If females are found to mount the same level of response as males, but to half the dose, would we not want to know the dose is too high for them (Cotton, 1993)?

Previously we have given men and women the same treatments for many medical conditions. But as glycobiology continues to uncover many disease processes in the body it it will be vital to include women in that research. This will be how we provide accurate treatments for both sexes.



Glycans Effects in the Differing Sexes

What is Glycosylation?

Glycosylation is the enzymatic process in which glycans (sugars) are added to proteins or other molecules (Qin et al., 2013).

There are two main types of glycosylation, N-glycosylation and O-glycosylation, which are differentiated by attachment. Glycan attachment to a nitrogen molecule is N-glycosylation, while attachment to the oxygen atom of serine or threonine is considered O-glycosylation.

Glycosylation has many differing modifications that contribute to protein diversity and overall, different body processes. Changes in **glycan bond, composition, structure, and length** all contribute to changes in body functions.

Glycosylation of Different Sugars

Here I will show an easy way to understand the different types of sialylations, or attachments of specific glycan structures to proteins.

The template is this:

Simple Sugar + Protein = ____ylated

Example 1: **Galactose** + Protein = **Galactosylated**

Example 2: **Sialic Acid** + Protein = **Sialylated**

Same goes for this:

No simple sugar + protein = **a**____ylated

Example 1: **No Galactose** + Protein = **Agalactosylated**

- Galactosylation is glycosylation with galactose. (*L. Willis, personal communication, August 10, 2021*)
- Sialylation is glycosylation with sialic acid. (*L. Willis, personal communication, August 10, 2021*)

Glycosylation and Glycan Variability in Human Functions

It is difficult to define a glycan's function, considering its structures and roles are fluid. (Chen et al., 2012). This is largely attributed to the modifications of glycan structure and glycosylation mentioned previously.

Glycan structural changes can be related to the idea of a light switch. Depending on the way you switch the light will determine whether the room is light or dark. Depending on whether the glycan structure is one way or another, will influence whether the response is pro- or anti-inflammatory. (Chen et al., 2012).

Understanding how glycan “switches” work is key in finding treatments, or even cures, to glycan initiated diseases.

IgG Glycosylation In Reference to Switches

A class of glycoproteins known as IgG contains an N-linked glycosylation site at a part called the Fc region. Its interaction with this region causes issues, and it is inferred that glycosylation of the region plays a crucial role in disease development (Chen et al., 2012).

Switch On:

When IgG is agalactosylated, its structures ending in the simple sugars GlcNAc or mannose, the inflammatory response is switched on. This structure correlates with autoimmune diseases.

Switch Off:

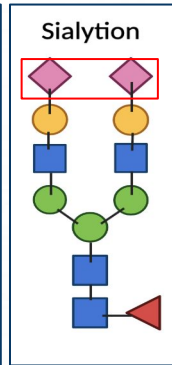
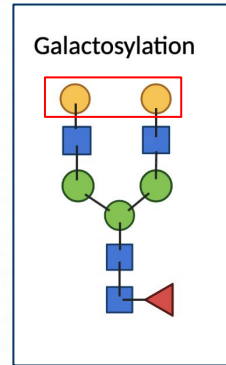
When the Fc region is sialylated, it is responsible for the anti-inflammatory response of IgG.

Figure 2:

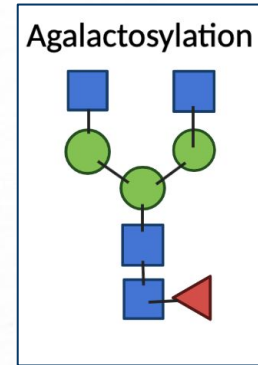
The picture to the right shows the structures of glycans involved in the **glycosylation of IgG**.

As discussed on the previous slide **agalactosylation of IgG initiates a pro-inflammatory response** while **sialylation of IgG initiates an anti-inflammatory response**.
(Chen et al., 2012; Singh, 2020)

Anti-Inflammatory:



Pro-Inflammatory:



Adapted from (Singh, S. 2020).

Determining Glycan's Effects on Males and Females

When looking into the effects of glycans on the human body **it cannot simply be broken down by sex**. Instead, we must consider that in addition to sex, variables such as age, health, and environment contribute to glycan structure and function just as much, if not more (Lim et al., 2020).

Knowing that these variables may alter glycan structure is important in conducting proper experiments, as not taking into account these underlying factors may result in inaccuracies.

Based on lack of research in this field it is difficult to conclusively state that any glycan alteration is solely based on sex, as a number of other factors must also be accounted for.

Age in Relation to Glycans

- Currently [age is the most studied area of glycan expression](#) (Lim et al., 2020).
- Glycans can be used to predict age and are better than any other biomarker (Lim et al., 2020). Biomarkers are substances in the body that indicate something, whether that be age, sex, or even disease (Strimbu & Tavel, 2010). Predictions of age are more accurate in females than males (Lim et al., 2020).
- IgG glycosylation and its relationship to inflammatory responses is the strongest marker (Lim et al., 2020).
- Glycans can be used to forecast disease progression (Lim et al., 2020).
- Glycans A2G2 and A2G2S1 were found to be associated with familial longevity (Lim et al., 2020).
- Another aspect that needs further research is the area of pathophysiology. In some cases it cannot be conclusively stated whether age or disease is the influencing factor on glycan function (Lim et al., 2020).

Sex in Relation to Glycans

- Sex differences within this field have been understudied (Lim et al., 2020).
- N-glycans are fucosylated differently in males and females. With women there are more core sialylated glycans, where as with men there are more branching fucosylated glycans (Lim et al., 2020). Branching glycans are associated with the inflammatory response (Knežević et al., 2010).
- Changes with age have also been found to be sex specific and there are characteristic glycosylation patterns in females. (Chen et al., 2012).
- In women, glycosylation of the IgG glycan is more pronounced at puberty and in menopause. (Chen et al., 2012).
- Sex differences are also associated with the levels of sex hormones. (Chen et al., 2012).
- Increases in sex hormones have shown to decrease agalatocsylation of IgG and therefore increase the production of galactosylated forms. (Chen et al., 2012).

Glycosylation Changes with Age and Sex, Case Study

The most pronounced evidence for sex specificity and the role of hormones, was shown during pregnancy. Patients with rheumatoid arthritis (RA) have increased agalactosylated IgG which results in increased inflammatory activity. Interestingly, during pregnancy RA patients go through disease remission. This is especially evident during the third trimester, when estrogen and progesterone levels are at their highest. This goes back to the “switch” analogy. The sex hormones play a role in changing the glycosylation of IgG. Instead of the agalactosylated IgG which results in inflammation, the Fc portion becomes sialylated which is responsible for the anti-inflammatory response (Chen et al., 2012). This has been further validated in other studies on mice and humans (Lim et al., 2020).

Further support for this occurs during menopause. When female sex hormones decrease and are at their lowest, incidences of arthritis increase along with inflammatory diseases in general (Chen et al., 2012; Lim et al., 2020).

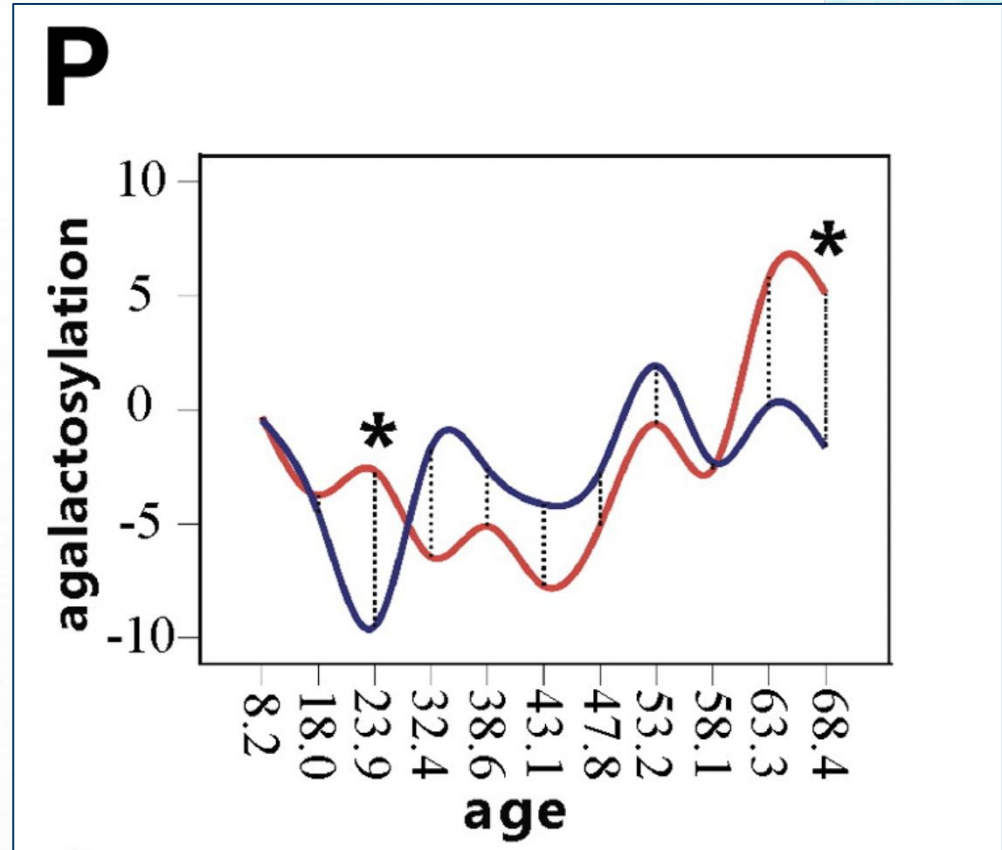
Figure 3:

This graph comes from a 2012 study on the effects of age and sex on glycosylation.

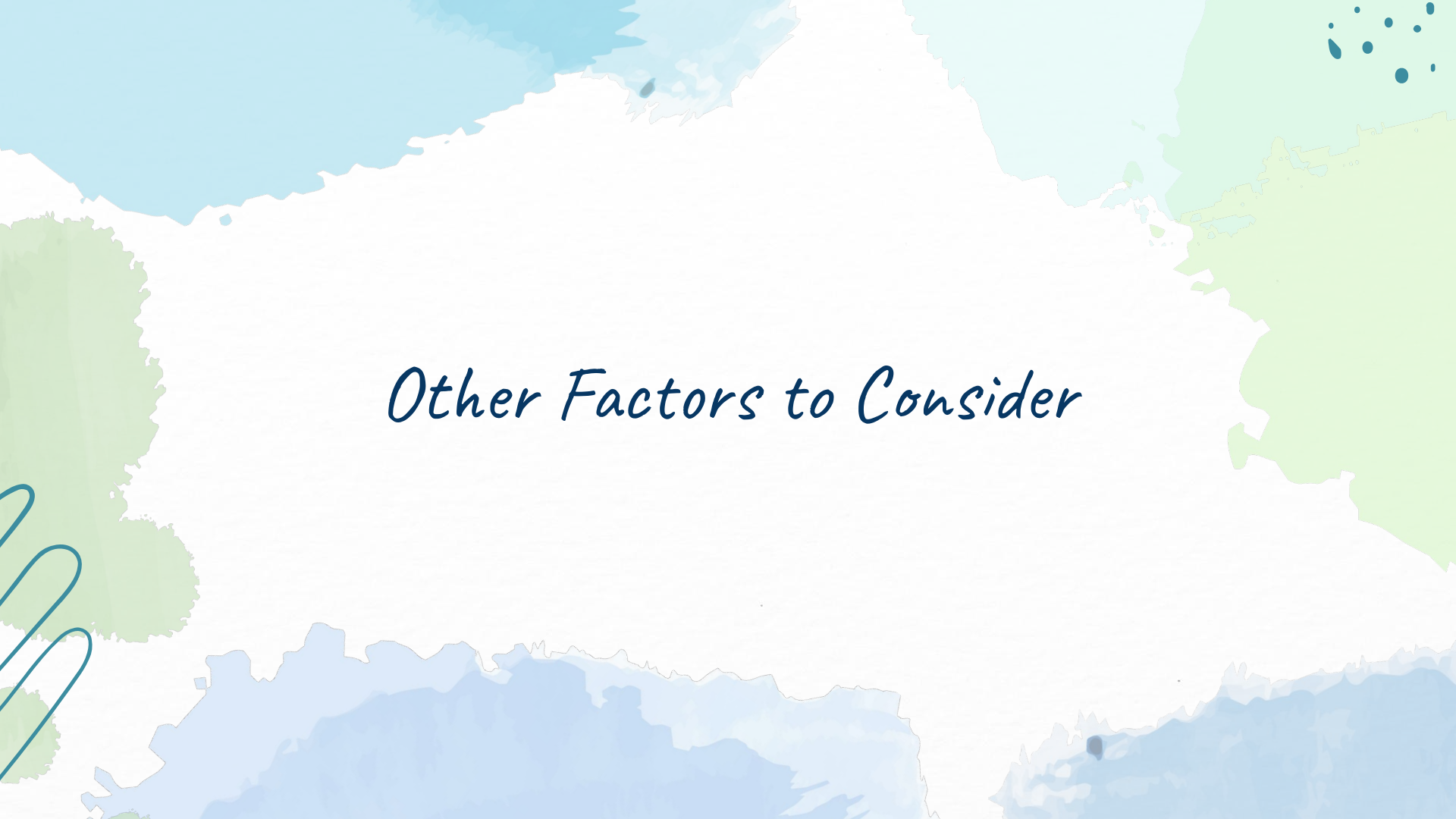
Here we see the levels of agalactosylation over the lifespan of males and females individually. (Chen et al., 2012).

(red representing females and dark blue representing males)

The data varies immensely, with women having the biggest peaks in their 20's and 60's (Chen et al., 2012).



Taken from (Chen et al., 2012).

A stylized world map serves as the background. The continents are depicted in light green and yellow, while the oceans are in various shades of blue. The map has a soft, painterly texture. In the top right corner, there are several small, dark blue dots. On the left side, there are some abstract, flowing blue lines that resemble a stylized coastline or perhaps a decorative element.

Other Factors to Consider

Environment as a Variable

A study was done in 2019 to further understand the influences of sex differences on respiratory inflammation (Ray & Holian, 2019).

Variables such as exposures to toxins and differences in quality of healthcare must be controlled for. (Ray & Holian, 2019)

Most exposures to the gas occur in male dominated fields such as mining or construction. In order to ensure that males increased respiratory inflammation in comparison to females was not attributed to increased exposure, male and female mice were used. By using mice, it was conclusively determined that males are indeed genetically more susceptible to adverse effects following exposure to cSiO₂, and that increased exposures in male-dominated fields had no relevance. (Ray & Holian, 2019).

Of course inaccuracies are always plausible, as the testing was not done on humans, but sometimes this is not ethically possible. Overall, the use of mice as a control was what determined that cSiO₂ respiratory inflammation is sex-linked (Ray & Holian, 2019).

Smoking in Relation to Glycans

A study in 2010 conducted an experiment to determine the effects of ageing, body mass index, and smoking on human N-glycans.

The test results indicated that the amount of cigarettes smoked is associated with increased branching N-glycan structures and decreased biantennary structures. This branching correlates with inflammation and also results in a decrease of IgG (Knežević et al., 2010).

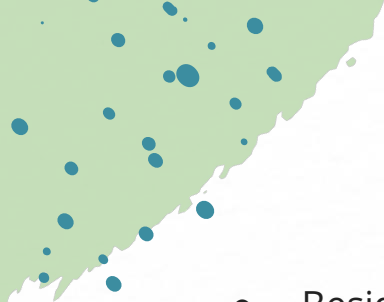
One of the most prominent smoking results is an increase in fucosylated- and -sialylated glycans (Lim et al., 2020).

Ethnicity in Relation to Glycans

- Little is known about the role ethnicity plays in glycosylation, as a study in 2018 is the one of the only studies on glycosylation differences in ethnic groups (Lim et al., 2020).
- The study looked at four different people groups: American origin, Japanese, Ethiopian, and Indians. Variation was shown among these ethnic groups, particularly in Ethiopians, who had higher levels of high mannose, core-fucosylated, branched and sialylated glycans compared to the other groups (Lim et al., 2020).
- But, a larger variety of studies, with larger sample sizes, must be conducted to fully understand the differing structures and functions of glycans in various ethnic groups (Lim et al., 2020).

A stylized world map with continents in light green and oceans in light blue. The map is centered on the Atlantic Ocean. In the top right corner, there are several small, dark blue dots of varying sizes. In the bottom left corner, there are several overlapping, light green, irregular shapes that look like stylized leaves or clouds.

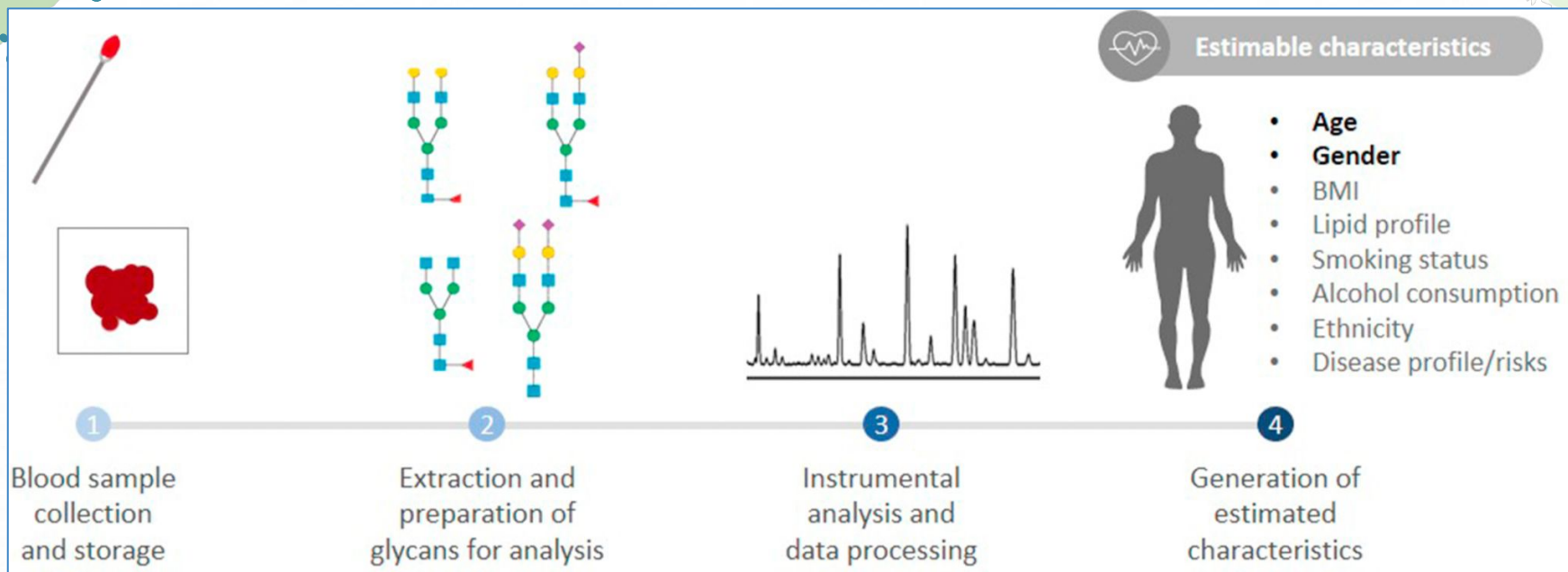
The future of Glycobiology



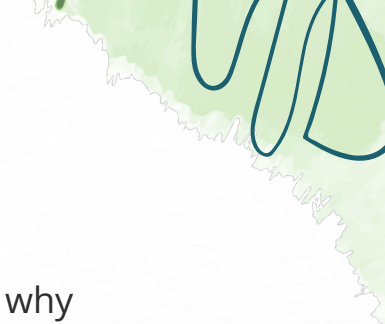
Forensic Sciences

- Besides glycan relevance in human diseases, there is huge potential for non-clinical applications, such as forensic sciences (Lim et al., 2020).
- While DNA analysis has been most commonly used to examine blood, it has limitations and thus, analysis of N-glycans is being looked into as an alternative (Lim et al., 2020).
- Recent studies in glycobiology show promise in the idea that analysis of glycans could provide information such as gender, ethnicity, chronological age, and biological age just by simple blood tests (Lim et al., 2020).
- While more testing and analysis must be done in confirming the accuracy of these biomarkers, the field of forensic science is well on its way to using glycans for forensic identification (Lim et al., 2020).

Figure 4:



Taken from (Lim et al., 2020)

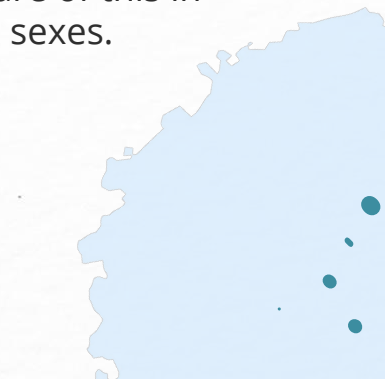


Disease Treatment and Possible Cures

All of the studies mentioned above are important in helping us fully understand why abnormal changes in IgG glycosylation are associated with the development of autoimmune diseases and cancers.

As we discussed earlier, IgG glycosylation plays a crucial role in disease development and inflammation. If we can better understand glycan structural changes and how they come about, we will be able to better understand how certain diseases form and how best to actively treat them.

As well, males and females have strikingly different disease pathways. Being aware of this in addition to studying this further, will allow us to find proper treatments for both sexes.





Conclusion

Conclusion:

Throughout the presentation we discovered that **glycobiology is a complex topic**, as many variables must be taken into account when analyzing glycan structure and function. We also went over **glycan's abilities as chemical switches** and how that can initiate anti- and pro-inflammatory responses. Overall, we can confirm that sex plays a huge role in glycan structure and function, but as glycobiology is relatively understudied, more research and analysis must be done to solidify, as well as add onto what has already been found. The study of glycobiology is booming, and as more experiments are properly done, separating male and female data, we may be able to see huge advancements in disease treatment, pharmaceuticals and other non-clinical applications.

Thank You's

I want to give a huge thank you to WISEST and its lovely coordinators. Their support and insight was what made this experience so amazing, and this presentation possible!

As well, a big thank you to my PI, Dr. Lisa Willis and The Willis Lab! I am so thankful I was able to be a part of the lab and learn from everyone there. It truly was a pleasure!

In addition, a massive thank you to all the WISEST supporters! Without you, the program would not have been possible, and for that I'm truly grateful!

And lastly, a thank you to Katie Walline! Working with you was so much fun! Thank you for all your support in the lab.

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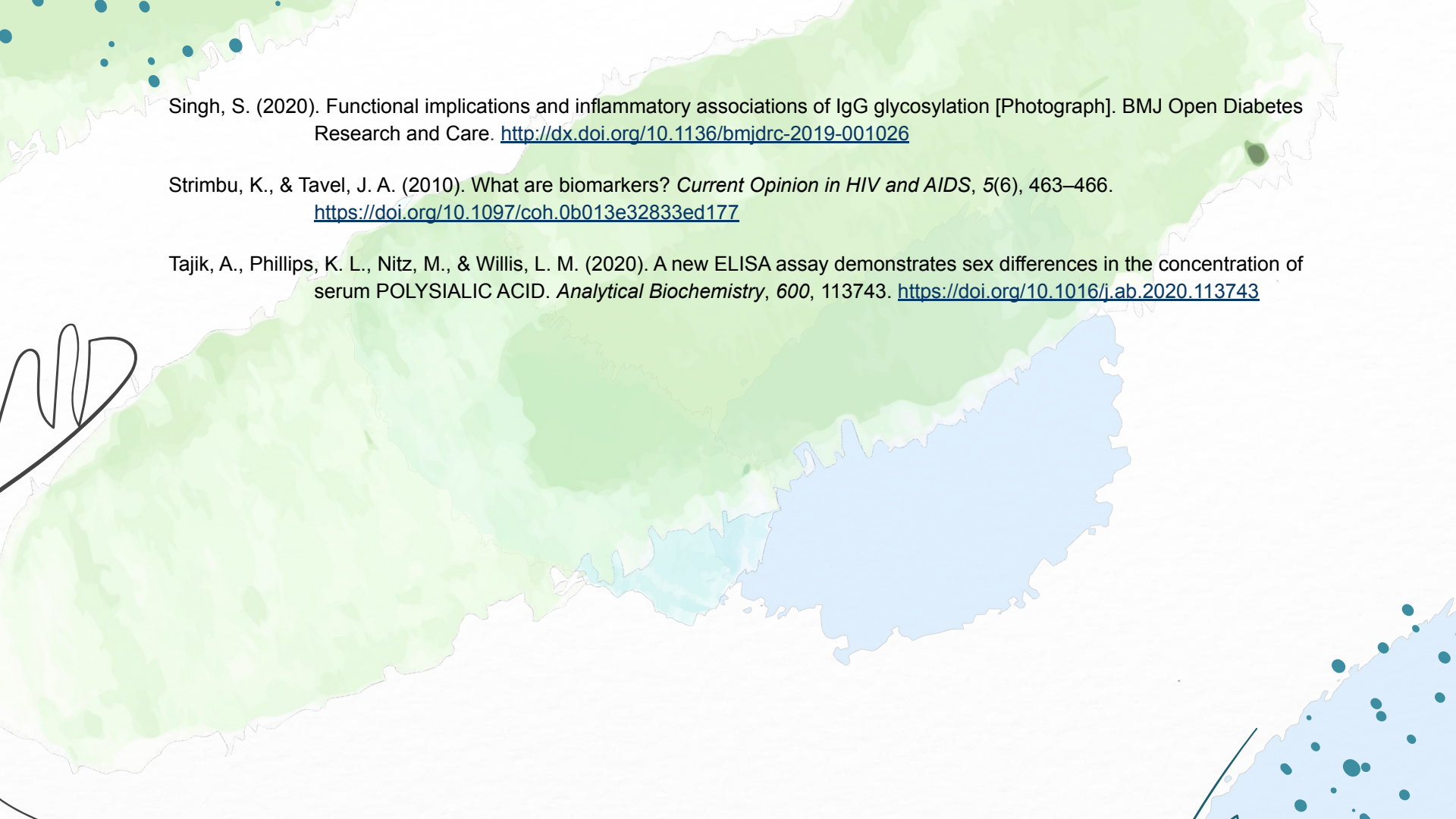
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