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N-Glycan profiling in plasma proteins of semi-supercentenarians using sialic acid linkage-specific derivatization and negative-ion MALDI-TOF MS

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0. Overview

- We compared plasma N-glycan profiles between semi-supercentenarians (SSCs) and healthy controls (70s, 80s, 90s).
- *N*-glycan preparation and measurement conditions are as follows:
 - Pooled plasma (one pool contains 4~5 individuals)
 - Major proteins (Albumin and IgG) were depleted
 - N-glycans were released with PNGaseF and purified with hydrazide beads (BlotGlyco[®])
 - Sialic acids were stabilized in linkage-specific manner (using SialoCapperTM-ID Kit)
 - Negative-ion MALDI-TOF MS after 2AA labeling
- Interestingly, an N-glycan with one $\alpha 2,3$ and two $\alpha 2,6$ -linked sialic acid residues was specifically increased in SSCs.

1. Introduction

Creating a society where individuals enjoy health and longevity is a common challenge the world over. Because human aging is modulated by various genetic and environmental factors, the full picture of aging currently remains unclear. Semi-supercentenarians (SSCs, older than 105 years) are a good example of human longevity, therefore physiological and biochemical analyses of SSCs can contribute to the understanding of healthy human aging. Glycosylation of plasma protein is a suitable analysis target, because glycosylated plasma proteins are derived from various different tissues and organs, and are highly susceptible to changes in the biological environment such as disease.

In previous studies, we performed glycome¹⁾ and glycoproteome²⁾ studies of healthy SSCs, and found high levels of tri-antennary and sialylated glycans on haptoglobin. In this study, focusing on the change in sialic acid linkages, we tried a comparative study of plasma protein N-glycan profiling in SSCs and younger control groups using sialic acid linkage specific derivatization followed by negative-ion MALDI mass spectrometry.

2. Materials & methods

2-1. Semi-supercentenarians (SSCs)

- A good example of healthy aging in humans.
- Proportion of SSCs in the population of Japan in 2020 is 0.005%.
- Inflammatory factors such as CRP and TNF-α were increased in SSCs.



Proportion of centenarians, semi-supercentenarians (SSCs), and supercentenarians in the population of Japan in 2020.



2-3. Mass spectrometry and multivariate analysis



Dual polarity linear bench-top MALDI-TOF MS Matrix: super-DHB

Negative-ion mass spectra Three spectra/pool

Easy statistical analysis software for direct ionization mass spectrometry data

3. Results & discussion

3-1. Typical *N***-glycan profiles**

• Albumin & IgG depleted plasma showed similar N-glycan profiles to those reported earlier in whole plasma, but specific glycoforms (so-called G0F, G1F, G2F etc., which derive from IgG) were relatively suppressed.



3-2. Multivariate analysis: Score plot

- Differences in N-glycan profiles between generations were successfully visualized in the score plot using eMSTAT solution.
- The score plot indicated that the *N*-glycan profiles in SSCs were particularly specific.



4. Conclusions

- By performing sialic acid linkage-specific derivatization followed by negative-ion MALDI MS and multivariate analysis, N-glycans that change with age could easily be specified.
- Interestingly, an N-glycan with one $\alpha 2,3$ and two $\alpha 2,6$ -linked sialic acid residues was specifically increased in SSCs. This change may play a role in anti-inflammatory responses against enhanced chronic inflammation.
- Sialic acid linkage-specific derivatization using the SialoCapper-ID Kit followed by negativeion MALDI-TOF MS provides researchers with the capability to handle large sample sets with high-throughput measurements, easy mass spectral interpretation of [M–H]⁻, and detailed analysis with sialic acid linkage type discrimination.





3-3. Multivariate analysis: Loading plot and box plot

- The N-glycans contributing to the separation in the score plot were analyzed further using loading plots and box plots.
- These plots highlighted the specific increase of an N-glycan with one α 2,3- and two α 2,6linked sialic acid residues (m/z 3243.4, averaged) in SSCs



5. References

- 1) Miura et al, *PLoS ONE* 2015, 10, e0142645.
- 2) Miura et al, *Biochim. Biophys. Acta Gen Subj*, 2018, 1862, pp. 1462-1471.
- 3) Nishikaze et al, Anal. Chem., 2017, 89, 2353-2360.

6. Acknowledgements

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