Supplemental information

Racial heterogeneity of IgA1 hinge-region

O-glycoforms in patients with IgA nephropathy

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Figure S1. IgA1 structure and schema of biosynthetic pathways of IgA1 O-glycosylation, related to Figure 2-4, Table 4-7, and a section of “Introduction”. (a) Structure of IgA1 and the amino acid sequence of its hinge region (HR). IgA1 contains two N-glycosylation sites at asparagine (N)263 and N459 in the constant region. The HR of IgA1 consists of a relatively long amino acid sequence that includes nine serine (S) and threonine (T) residues, the potential O-glycosylation sites. Up to six sites marked in red font can be O-glycosylated. C, constant domains; V, variable domains; H, heavy chains; L, light chains. (b) Schema showing the biosynthetic pathways of IgA1 O-glycosylation. N-acetylgalactosamine (GalNAc) residue(s) (indicated by yellow squares) is attached to the serine (S) or threonine (T) residues of IgA1 HR by polypeptide GalNAc-transferases (ppGalNAc-Ts). Galactose (Gal) residues (indicated by yellow circles) are attached to GalNAc(s) by core 1 β1,3-galactosyltransferase (C1GalT1) and its molecular chaperone, Cosmc. GalNAc or Gal residues are extended by N-acetylneuraminic acid (NeuAc) (indicated by purple diamonds) by α2,6 sialyltransferase-2 (ST6GalNAc-II) and α2,3-sialyltransferase-1 (ST3Gal-I), respectively. Galactosylation of GalNAc is less likely to occur when NeuAc binds to GalNAc prior to the binding of galactose (marked by *).
Figure S2. Analytical workflow for individual profiling of IgA1 HR O-glycosylation, related to STAR Methods and a section of “Introduction”.

IgA1 was purified from 100 µl serum using affinity chromatography with anti-human IgA. Five micrograms of purified IgA1 was treated with neuraminidase and subsequently reduced and digested using trypsin. Five hundred nanograms of desialylated and trypsin-digested IgA1 were injected in the high-resolution liquid chromatography-mass spectrometry (HR-LCMS) column, and the MS spectrum data were acquired. Acquired MS spectrum data were uploaded to Glycan Analyzer—an in-house automated program, and relative abundance of each glycopeptide was calculated.
Figure S3. Correlation between mean number of Gal per HR and Gd-IgA1 levels (mg/dl), related to Table 4-7

In each race of IgAN patients (Japanese IgAN patients [J-IgAN], n = 36; Greek IgAN patients [G-IgAN], n = 23), serum galactose-deficient IgA1 (Gd-IgA1) levels (measured using enzyme-linked immunosorbent assay [ELISA]) and the number of galactose (Gal) residues per hinge region (HR; calculated by mass spectrometry [MS] analysis) were negatively correlated ($r = -0.396$, $P = 0.017$; $r = -0.576$, $P = 0.004$). The results of MS analysis reflect the ELISA results.