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Development of CRISPR/Cas9 therapeutics targeting A4GALT using patient derived kidney organoids model of Fabry disease

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Objectives: Deposition of globotriaosylceramide (Gb3) is a critical step for the progression of fabry disease nephropathy (FdN). In this study, we investigated whether knock-out (K/O) of *A4GALT* gene (Gb3 synthase gene) by CRISPR/Cas9 technology can reduce the deposition of Gb3; hence to appraise its potential as a therapeutic approach for FdN using kidney organoids.

Methods: First, we generated patient-specific hiPSC (human induced pluripotent stem cells) (CMC-Fb02) using PBMCs from a patient with FdN. Second, we generated disease-specific hiPSC (*GLA*-KO-WTC-11) by K/O of *GLA* gene in wild-type WTC-11-hiPSC. Third, we performed *A4GALT* K/O by CRISPR/Cas9 technology in both CMC-Fb02 and *GLA*-KO-WTC-11), so generated CMC-Fb02-Gb3-KO and GLA-Gb3-KO-WTC-11 hiPSCs. Lastly, we generated kidney organoids using wild-type-WTC-11, CMC-Fb02 (patient-specific), *GLA*-KO-WTC-11 (disease-specific), and their corresponding *A4GALT* K/O hiPSCs (CMC-Fb2-Gb3-KO, *GLA*-Gb3-KO-WTC-11). We compared alpha-galactosidase-A enzyme (α-GLA) activity, Gb-3 deposition by LC_MS/MS and immunofluorescent (IF) staining, zebra body formation under electron microscopy (EM).

Results: All of 5 hiPSCs showed typical pluripotency markers and normal karyotyping. All of generated kidney organoids from those hiPSCs showed the expression of typical nephron markers such as PODXL (Podocyte), LTL (Proximal tubule), and E-cad (distal tubule) under IF staining. In the kidney organoids derived from CMC-Fb02 and *GLA*-KO-WTC-11, α-GLA activity was significantly decreased compared to kidney organoids from wild-type WTC-11. In contrast, deposition of Gb3 measured by LC-MS/MS and IF staining was significantly increased in the CMC-Fb-2 and *GLA*-KO-WTC-11 kidney organoids. In both *A4GALT* KO kidney organoids (CMC-Fb2-Gb3-KO and *GLA*-Gb3-KO-WTC-11), Gb3 deposition by LC-MC/MC and IF significantly decreased in comparison with CMC-Fb2 and *GLA*-KO-WTC-11 kidney organoids respectively. In EM, typical zebra body formations were detected in CMC-Fb2 and *GLA*-KO-WTC-11 kidney organoids, but they disappeared in CMC-Fb2-Gb3-KO and *GLA*-Gb3-KO-WTC-11 kidney organoids

Conclusions: Our results suggest that *A4GALT* targeting therapy can be proposed as an innovative approach for the treatment of FdN.