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Oral Communication Abstract
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Successful gene correction of Na-Cl cotransporter mutation using CRISPR-Cas9 in kidney organoid generated from Gitelman's syndrome patient-derived iPSC

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Objectives: Gitelman's syndrome (GIT) is an autosomal recessive disorder characterized by hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria that has recently been reported to be linked to thiazide-sensitive Na-Cl cotransporter gene (NCC or SLC12A3) mutation. Until now, in vitro modeling is limited using patient-derived samples for treating the disease. Recently, organoid derived from human pluripotent stem cells are a potential tool for the representing human kidney organs. In this study, we explored the possibility of iPSC-derived kidney organoid for disease modeling.

Methods: To model GIT in human cells, we used the iPSC line (CMC-GIT-001) from PBMC of 29-year-old male with GIT caused by the mutation of SLC12A3 gene. Using CRISPR-Cas9 genome editing system, we corrected mutated genes of CMC-GIT-001 line (CMC-GIT-001^{corr}). Each iPSCs were differentiated into kidney organoids and analyzed the NCC expression.

Results: Both iPSCs of each group differentiated into kidney organoids, but the number of matured kidney organoid from CMC-GIT-001^{corr} group were significantly higher in 3.3-fold than CMC-GIT-001 group (12.2 ± 0.7/cm^2 vs. 3.7 ± 0.2/cm^2, $P < 0.05$ vs. CMC-GIT-001 group). qRT-PCR was performed using harvested kidney organoid and found that relative NCC mRNA level was increased in CMC-GIT-001^{corr} group compared with CMC-GIT-001 group (4.1 ± 0.8 vs. 2.5 ± 0.2, $P < 0.05$ vs. CMC-GIT-001 group). Consistently, immunoblot analysis revealed that increased NCC protein amount was also detected in CMC-GIT-001^{corr} group compared with CMC-GIT-001 group (4.1 ± 0.8 vs. 2.5 ± 0.2, $P < 0.05$ vs. CMC-GIT-001 group). In addition, we found that increased immunoreactivity of NCC in CMC-GIT-001^{corr} group was colocalized with e-cadherin (distal tubule marker) using confocal microscopy.

Conclusions: Kidney organoid from GIT patient-derive iPSC recapitulate the Gitelman syndrome phenotype, and we showed the potential platform as in vitro disease modeling. The result from gene correction of NCC mutation using CRISPR-Cas9 technology provide the evidence for the therapeutic possibilities.