

## KSN 2017 Abstract

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### Urinary metabolomic study in patients with membranous nephropathy

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**Objectives** : Membranous nephropathy (MN) is a leading cause of adult-onset nephrotic syndrome. Primary MN is an autoimmune disease caused by autoantibodies such as phospholipase A2 receptor antibody (PLA2RAb) and thrombospondin type 1 domain-containing 7A antibody against the podocyte antigen. However, the treatment response varies among individuals, and adverse reaction to immunosuppressive agent (ISA) is significant. The treatment response should be optimized and the adverse reaction to ISA treatment should be minimized. Thus, we performed a urine metabolomic study to identify the predictive biomarker of the prognosis and treatment response in patients with MN.

**Methods** : We used urine samples from patients with biopsy-proven primary membranous nephropathy that were stored at the time of kidney biopsy in Seoul National University Hospital Biobank to find differences in urine metabolites between the MN (n = 79), minimal-change-disease (MCD, n = 74), and control groups (n=82). The 800-MHz nuclear magnetic resonance-based metabolomic method was used. We investigated the urine metabolites specific to MN after excluding outliers and matching factors such as age, sex, and presence of diabetes mellitus (DM). Serum PLA2RAb level was examined using an enzyme-linked immunosorbent assay. Hard outcome was defined as initiation of dialysis, a 50% decrease in the estimated glomerular filtration rates, and doubling of serum creatinine levels. We reviewed the association of urine metabolites with each patient's ISA response and the presence of hard outcome. Immunohistochemistry (IHC) and immunofluorescence (IF) studies were performed to find the differences in expression patterns of NADPH oxidase 4 (NOX4) and fumarate hydratase (FH) in biopsy specimens from patients with MN, those with MCD, and healthy controls. In vitro assay using primary cultured human podocytes treated with immunoglobulin G (IgG) purified from primary MN patient's serum showed a high serum PLA2RAb level.

**Results** : After excluding outliers and matching for age, sex, and presence of DM, urinary fumarate level was significantly higher in the patients with steroid-resistant MN than that measured in the urine sample from the steroid-responsive patients. In this study, the patients with a hard outcome during the follow-up period showed urine fumarate levels 2.32-fold higher than those of

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the patients with a non-hard outcome. We investigated the role of NOX4, a known inhibitor of FH hydrolyzing fumarate. IHC of the biopsy specimens revealed that the NOX4 expression level was higher in the MN group than in the MCD and control groups. IF of the biopsy specimens revealed that the FH expression level was lower in the MN group than in the MCD and control groups. Purified IgG-treated primary cultured podocytes showed higher NOX4 expression levels but lower FH expression levels than the IgG-treated podocytes of the healthy controls.

**Conclusions** : Fumarate is a reliable biomarker for predicting the prognosis and treatment response of patients with MN. In vitro assay to validate the results of this metabolomic study showed that the FH expression inhibited by NOX4 was reduced in the primary cultured human podocytes in the IgG-treated sera from MN patients.

**Keywords** : Membranous nephropathy, Fumarate, NADPH oxidase 4