Functional Characterization of a Large Deletion in the *AVPR2* Gene Causing Severe Nephrogenic Diabetes Insipidus in a Turkish Family

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OBJECTIVES

Diabetes insipidus (DI) is a rare disease and changes in arginine vasopressin receptor 2 gene (AVPR2) mostly cause of X-linked nephrogenic DI (NDI). NDI patients have problems about to concentrate urine and water reabsorption in response to the antidiuretic hormone vasopressin (AVP). We have identified a novel 388 bp deletion in AVPR2 gene in a Turkish family with NDI. In vitro functional characterization studies have importance about determining mutant receptor function. Therefore, in this study, we aim to characterize the deletion functionally and compare the clinical data of the NDI patient with the results from the in vitro studies.

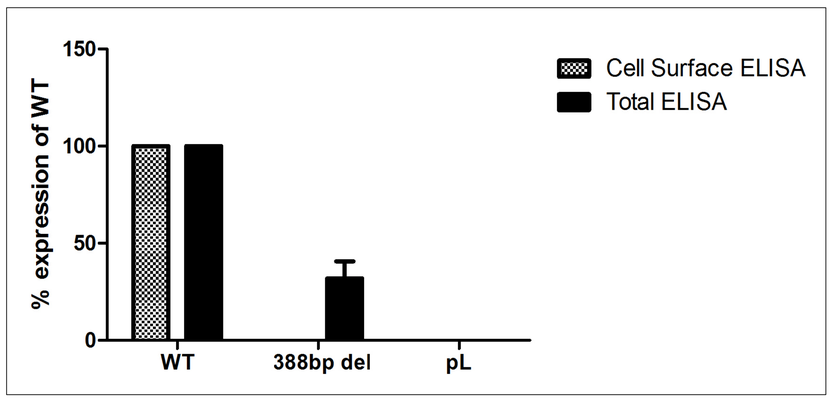

Figure 1. ELISA results, WT: Wild type of AVPR2, pL: Expression plasmid without AVPR2.

METHODS

Study Subject: NDI patient is a twenty five-year old male who was admitted to Gulhane Military Medical Academy, Department of Endocrinology and Metabolism in 2012 and he has under control for treatment. Detailed information about his family was described in our previous study (Saglar et al., 2014). Daily urination of patient was more than 20 liter (22 L/day) and he also had fatique, polydipsia since infancy. After treatment, his serum natrium level was 146 mmol/L, his glycemia level was 76 mg/dL, his potassium level was 3.1 mmol/L, his blood urea level was 18 mg/dL, his creatinine level was 0.94 mg/dL. Urine osmolality was as low as 72 mOsm/kg and it was stable at the end of the water deprivation-desmopressin test.

Mutant AVPR2 Construct: The 388 bp deletion was generated by using pLV2R (Dr. Angela Schulz, Leipzig University, Faculty of Medicine, Institute of Biochemistry) which is a human V2R expression plasmid. For generating construct, PCR based site-directed mutagenesis and restriction fragment replacement strategy. To allow receptor detection in immunological studies, both mutant and wild type AVPR2s were N- and C-terminal epitope-tagged (N-terminal: Hemagglutinin (HA)-tag; C-terminal: FLAG-tag). Mutant construct was verified by DNA sequencing.

Functional Characterization Studies: COS-7 cells were transfected with mutant and wild type AVPR2s. 72 hours after transfection, ELISA and cAMP accumulation assay were performed. Cell surface expression of mutant receptor which had a N-terminal HA-tag was estimated by indirect cellular ELISA. To measure total expression of double-tagged (N-terminal HA tag and C-terminal FLAG-tag) mutant receptor, sandwich ELISA was used. In addition, COS-7 cells were stimulated with various AVP concentrations ([Arg8]-vasopressin acetate salt, Sigma–Aldrich, Seelze, Germany) for 1 h at 37° C. The cAMP content of cell lysates was determined by a non-radioactive cAMP accumulation assay based on the ALPHAScreen technology according to the manufacturer's protocol (Perkin Elmer LAS, Rodgau-Jügesheim, Germany).

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CONCLUSIONS

The 388 bp deletion were introduced into pLV2R with PCR based site-directed mutagenesis and restriction fragment replacement method. Transfection efficiency was determined by using pEGFP. According to the cAMP accumulation assay results, after stimulation of mutant AVPR2 with six different concentrations of AVP (ten fold dilutions starts with 10 µM), COS-7 cells expressing the mutant receptor showed no detectable cAMP formation. Cell surface expression and totally expression in the cell of the mutant receptor were estimated by performing ELISA experiments. Compared to the wild type receptor, mutant receptor did not show any expression on the cell surface (Figure 1). In addition, the truncated receptor showed reduced cellular expression in total (31.93% ± 8.8) compared to the wild type receptor (Figure 1). Also, we did western blotting for mutant receptor protein and totally expression in the cell of the truncated protein is similar with ELISA results but the data was not shown here. Functional characterization of 388 bp deletion in exon 2 of the *AVPR2* gene showed that mutant receptor most probably is retained in endoplasmic reticulum (ER) control mechanism because of the large deletion. To show that retention in ER, we have performed fluorescence studies (data were not shown).

In *AVPR2* gene, there are more than 16 gross deletions (The Human Gene Mutation Database, HMGD) and this study is the first functional characterization study of that kind of large deletion. In conclusion, we believe that our study will contribute to shedding light on mechanisms of molecular pathology of AVPR2 deletions.

References

H.B. Moeller, S. Rittig, R.A. Fenton.: Nephrogenic diabetes insipidus: essential insights into the molecular background and potential therapies for treatment. Endocr Rev. 34(2), 278-301 (2013)

N. Knoers, L.A. Monnens.: Nephrogenic diabetes insipidus: Clinical symptoms, pathogenesis, and treatment. Pediatr Nephrol. 6(5), 476-482 (1992)

E. Albertazzi, D. Zanchetta, P. Barbier, S. Faranda, A. Frattini, P. Vezzoni, M. Procaccio, A. Bettinelli, F. Guzzi, M. Parenti, B. Chini.: Nephrogenic diabetes insipidus: functional analysis of new AVPR2 mutations identified in Italian families. J Am Soc Nephrol. 11(6): 1033–1043 (2000)

D.G. Bichet. V2R mutations and nephrogenic diabetes insipidus. Prog. Mol. Biol. Transl. Sci. 89, 15–29 (2009)

D. Duzenli, E. Saglar, F. Deniz, O. Azal, B. Erdem, H. Mergen.: Mutations in the AVPR2, AVP-NPII and AQP2 genes in Turkish patients with diabetes insipidus. Endocrine 42(3), 664–669 (2012)

E. Saglar, F. Deniz, B. Erdem, T. Karaduman, A. Yönem, E. Cagiltay, H. Mergen.: A large deletion of the AVPR2 gene causing severe nephrogenic diabetes insipidus in a Turkish family. Endocrine. 46(1), 148-53 (2014)

T.M. Fujiwara, D.G. Bichet.: Molecular biology of hereditary diabetes insipidus. J. Am. Soc. Nephrol. 16, 2836–2846 (2005)

K. Pasel, A. Schulz, K. Timmermann, K. Linnemann, M. Hoeltzenbein, J. Jääskeläinen, A. Grüters, G. Filler, T. Schöneberg.: Functional characterization of the molecular defects causing nephrogenic diabetes insipidus in eight families. J Clin Endocrinol Metab. 85(4), 1703-10 (2000)

I. Böselt, D. Tramma, S. Kalamitsou, T. Niemeyer, P. Nykänen, K.J. Gräf, H. Krude, K.S. Marenzi, S. Di Candia, T. Schöneberg, A. Schulz.: Functional characterization of novel loss-of-function mutations in the vasopressin type 2 receptor gene causing nephrogenic diabetes insipidus. Nephrol Dial Transplant. 27(4), 1521-8 (2012)