Functional Analysis of Four Mutants of the V2 Receptor

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OBJECTIVES

Diabetes insipidus is a disorder characterized by severe liquid-imbalance because of the inability to concentrate urine. Inactivating mutations in either *AVPR2* (arginine vasopressin receptor type 2) or *AQP2* (aquaporin 2) gene can cause congenital Nephrogenic diabetes insipidus (NDI). AVPR2 is a G protein-coupled receptor (GPCR) and is mainly expressed at the basolateral side of the kidneys collecting duct principal cells. Activation of this receptor by vasopressin is responsible for elevation of cAMP levels resulting in insertion of AQP2 water channels in the cell membrane of the collecting duct cells.

In this study, four new mutations of *AVPR2* gene (R68W, R67_G69del/G107W, V162A and T273M) were found in patients and were functionally analyzed.

METHODS

A pLV2R, a mammalian expression vector containing the entire coding sequence of the human *AVPR2,* was used to generate all mutants with a PCR-based site-directed mutagenesis and restriction fragment replacement strategy. All constructs were N- and C-terminal epitope-tagged to allow receptor detection by ELISA studies (N-terminal: Haemagglutinin (HA)-tag; C-terminal: FLAG-tag). The correctness of all constructs was confirmed by DNA sequencing.

72 h after transfection of COS-7 cells, stimulation with various AVP concentrations ([Arg8]-vasopressin acetate salt, Sigma–Aldrich, Seelze, Germany) was performed for 1 h at 37° C. The cAMP content of cell extracts 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).

The cell surface expression of receptors carrying an N-terminal HA-tag was estimated by indirect cellular ELISA. As a further assay to measure the total expression of full-length double-tagged AVPR2s (N-terminal HA-tag, C- terminal FLAG-tag), a sandwich ELISA was used.

Table 1. Clinical features of the patients

Mutations	Sex	Age (Years)	Urine osmolality (mOsm/kg H_2O)	Plasma osmolality (mOsm/kg H_2O)	Urine volume (L/day)
R67_R69del/G107W	Male	20	107	301	12
R68W	Male	27	158	299	12,5
V162A	Male	27	99	302	12,1
T273M	Male	29	227	316	7,8

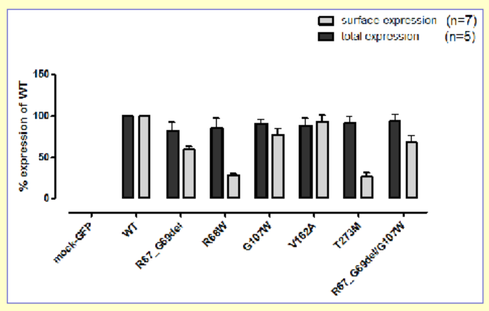

Figure 2. ELISA results of the mutants. WT (Wild type of V2R).

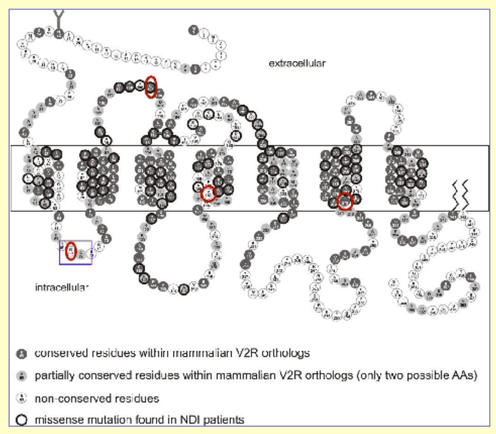

Figure 1. The amino acid sequence of the human V2 receptor (The figure is an excerpt from Böselt et al., 2009). Mutations which were analyzed in this study are shown in red circles. The purple square indicates the 9 bp deletion.

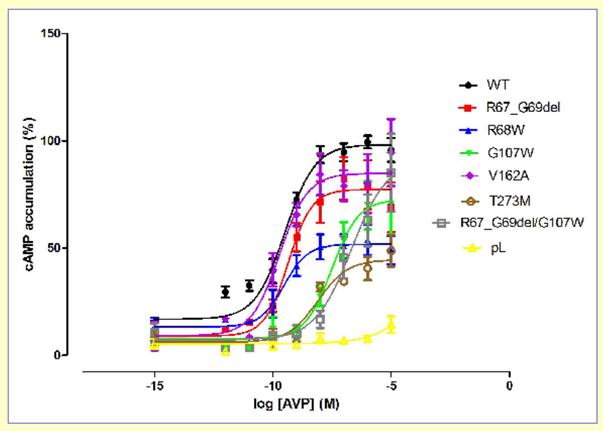

Figure 3. The graph of % response of the mutations

RESULTS & CONCLUSIONS

Five different mutations (Figure 1) were introduced into pLV2R with PCR-based site-directed mutagenesis and restriction fragment replacement method (R68W, R67_G69del/G107W, V162A and T273M). For the R67_G69del/G107W mutation (compound heterozygous), we also introduced and analyzed both mutations (R67_G69del and G107W) separately. The clinical characteristics of the patients who have the mutations are seen in Table 1. All patients show low urine osmolalities and large urine volumes, clearly indicating the symptoms of NDI.

According to total ELISA results (Figure 2), all mutant receptors were synthesized comparable to wild type receptor. However, cell surface expression was impaired for all mutants except of V162A.

cAMP measurement for mutant and wild type receptors revealed reduced E_{max} values for all mutants. For some mutants (R68W, R67_G69del/G107W and T273M) concentration response curves showed shifted EC_{50} values to higher vasopressin concentrations (Figure 3).

The in-depth characterization of the receptor function is important for our patients because mutations leading to only a slightly shifted EC_{50} values by one magnitude could be treated with higher amounts of AVP to reduce high urine volumes and to restore kidney function (R67_G69del/G107W mutant which can be seen on the graph).

In conclusion, we characterized four new *AVPR2* mutations found in Turkish patients (TUBITAK SBAG 112S513). Some mutations lead to shifted EC_{50} values by only one magnitude and treatment with higher amounts of AVP could be helpful for these patients to reduce high urine volumes and to restore kidney function.

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