

Online supplementary material

Novel *de novo* *AVPR2* Variant in a Patient with Congenital Nephrogenic Diabetes Insipidus

Shivani Joshi, Per Brandstrom, Niels Gregersen, Søren Rittig, and Jane Hvarregaard Christensen

Verification of the *de novo* c.800_879dup variant in *AVPR2*

To verify the presence of the 80bp duplication a tandem primer pair was designed (Table S1). This primer pair was designed to anneal in the 5' end of the 80bp duplication in such a way that it would make a 121bp PCR product only when the 80bp duplication is present, else there would be only primer dimers produced (Fig. S1). PCR products derived from the proband (II-3), his father (I-1), mother (I-2) and a healthy control (HC) were separated by polyacrylamide gel electrophoresis (PAGE) (Fig. S2). A PCR product of similar size as the 123bp DNA ladder was identified solely in the proband (II-3), thereby verifying the presence of 80bp duplication. Its absence in the father (I-1) and mother (I-2) further verifies that the 80bp duplication is a *de novo* variant.

Table S1. Primer sequences and PCR conditions

Forward primer	5'-AGACTGTGAGGATGACGCTA-3'
Reverse primer	5'-CACATAGACGACCACAATCAC-3'
PCR conditions	Pre-amplification: 95°C 15 min Cycling: 95 °C 30 sec 30 cycles 54 °C 30 sec 72 °C 1.5 min Post-amplification: 72 °C 2 min 10 °C forever

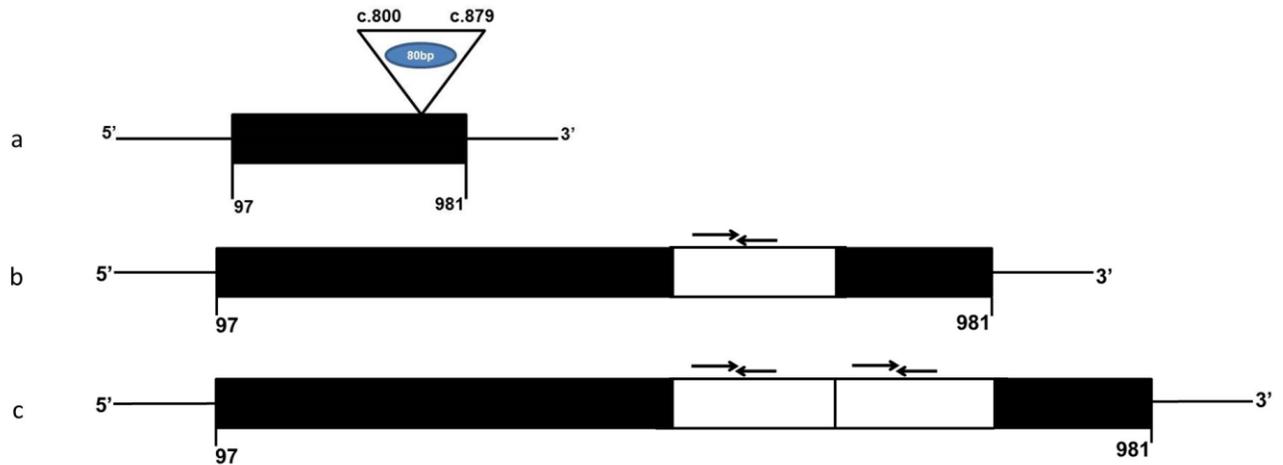


Fig. S1. Location of tandem primer pair designed to amplify the 80bp duplication. **a** Location of c.800_879dup in exon 2 of *AVPR2*. The black box represents exon 2 with cDNA numbering. The triangle indicates the location of the 80bp duplication. **b** Enlarged view of exon 2. The white box is the original sequence of 80bp that is duplicated. Arrows indicate forward and reverse primers designed in tandem within the 80bp region. **c** Enlarged view of exon 2 with white boxes to depict the duplication of 80bp identified in the patient. Arrows indicate the location of the tandem primer pair. Only in the presence of duplication, this tandem primer pair results in PCR amplification of a 121bp product.

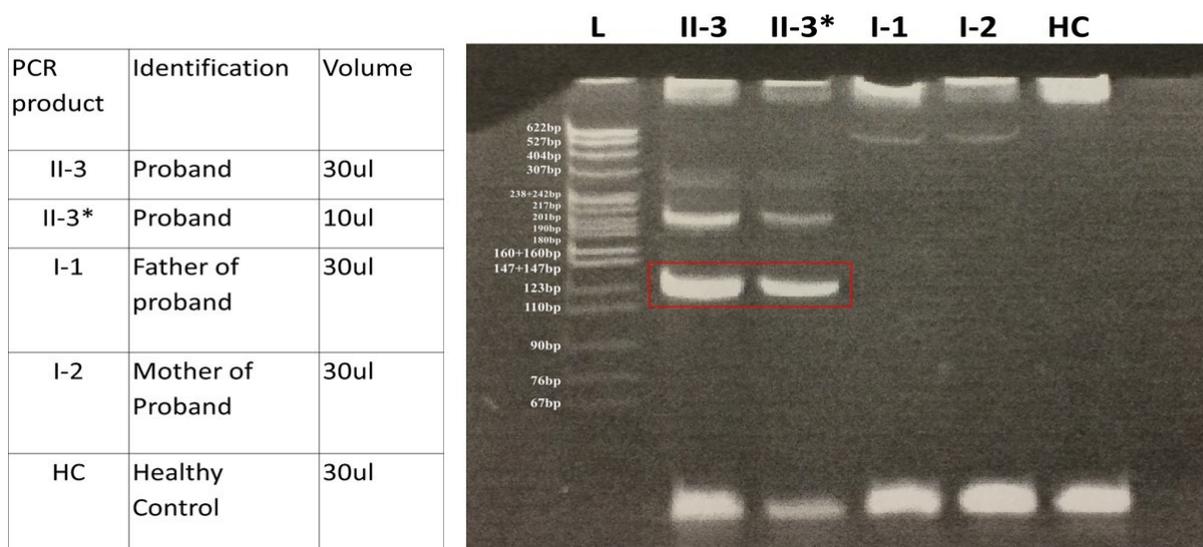


Fig. S2. Polyacrylamide gel electrophoresis (PAGE) analysis of PCR products. Polyacrylamide gel electrophoresis (PAGE) of PCR products derived from the proband (II-3), his father (I-1), mother (I-2) and a healthy control (HC). pBR322 DNA-MspI Digest (New England Biolabs, Ipswich, Massachusetts, United States) was loaded as the ladder (L). The numbers to the left of the ladder indicate sizes of the bands of the DNA ladder. The table states volumes of PCR products loaded for PAGE analysis.