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Supplemental Data

Mutations in *AQP5*, Encoding a Water-Channel Protein, Cause Autosomal-Dominant Diffuse Nonepidermolytic Palmoplantar Keratoderma

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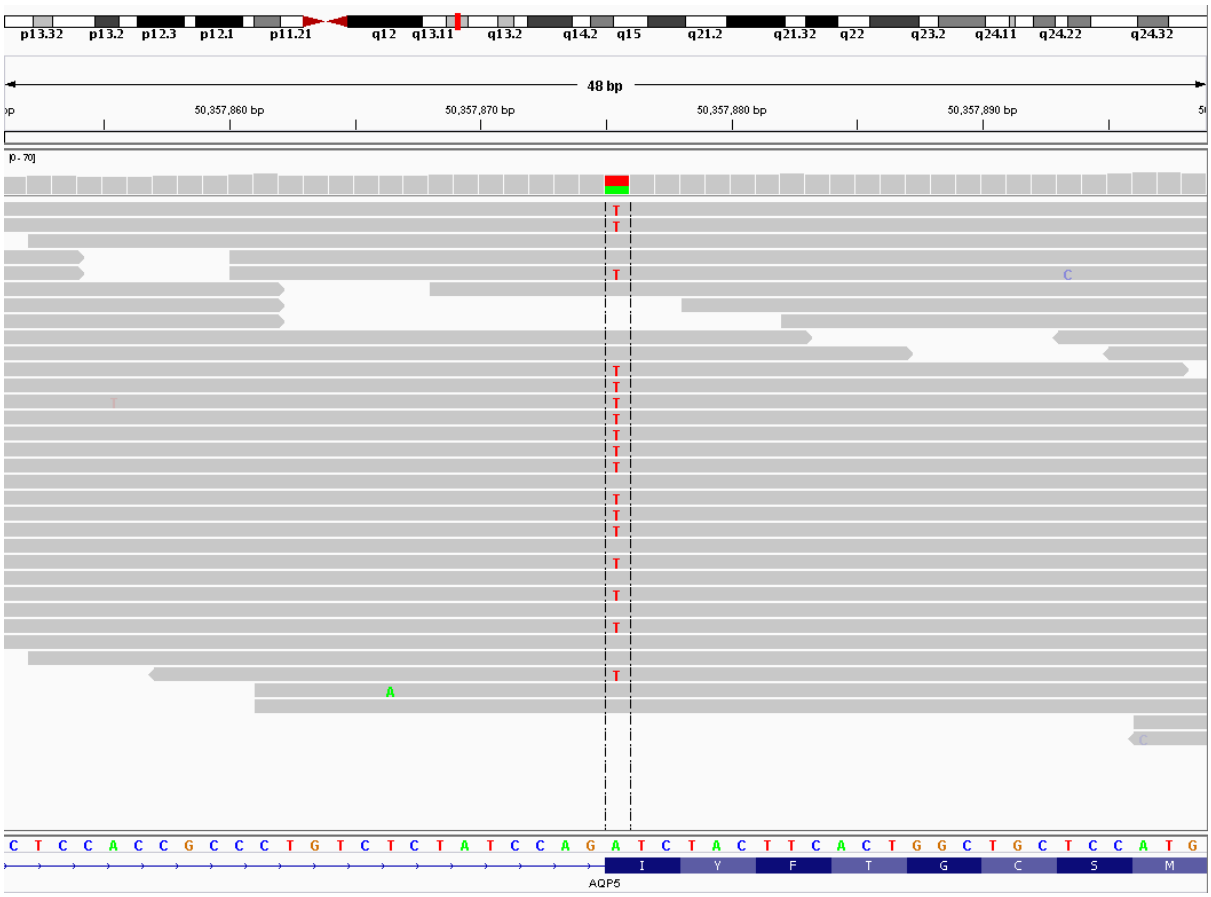
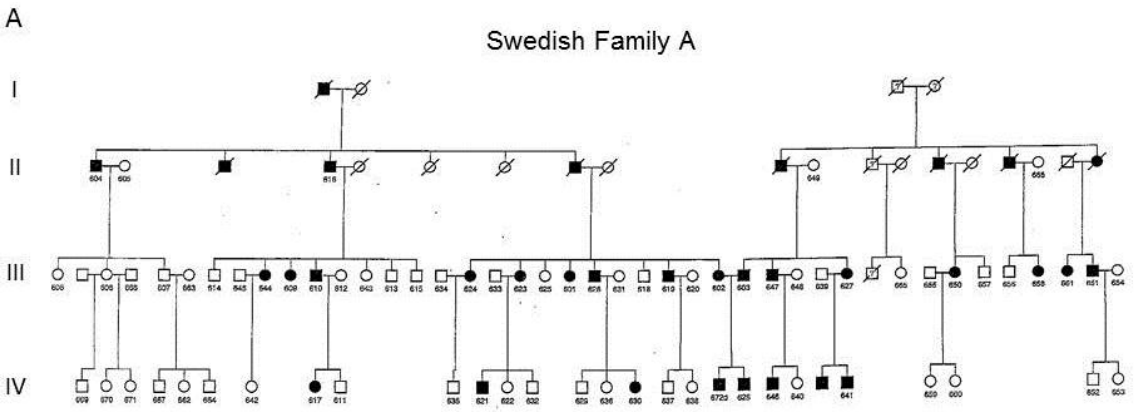


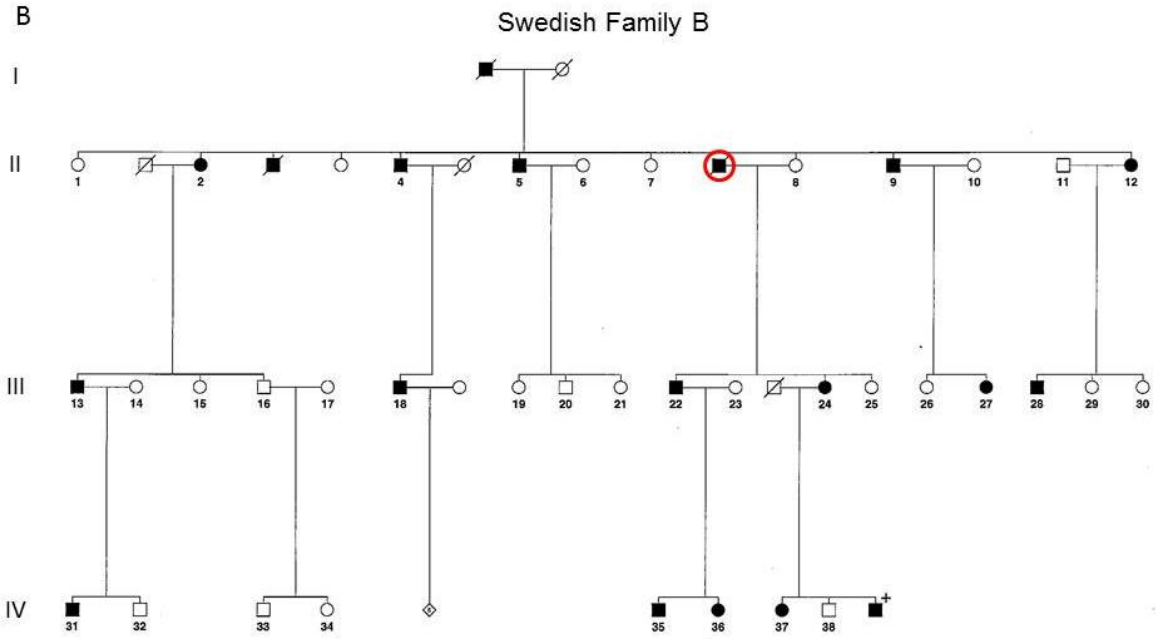
Figure S1. Exome Sequencing Identifies a Mutation in *AQP5* in an Individual with Autosomal-Dominant Diffuse NEPPK

Screen capture from integrative genomics viewer software[1] showing the A to T mutation (p.Ile177Phe) at the start of exon 3 in *AQP5* in approximately 50% of the reads from exome sequencing of a British individual affected with diffuse NEPPK.

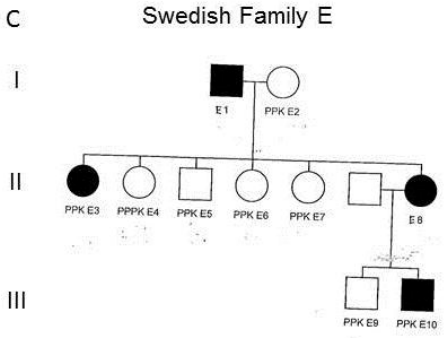
A


Swedish Family A
I
II
III
IV

B

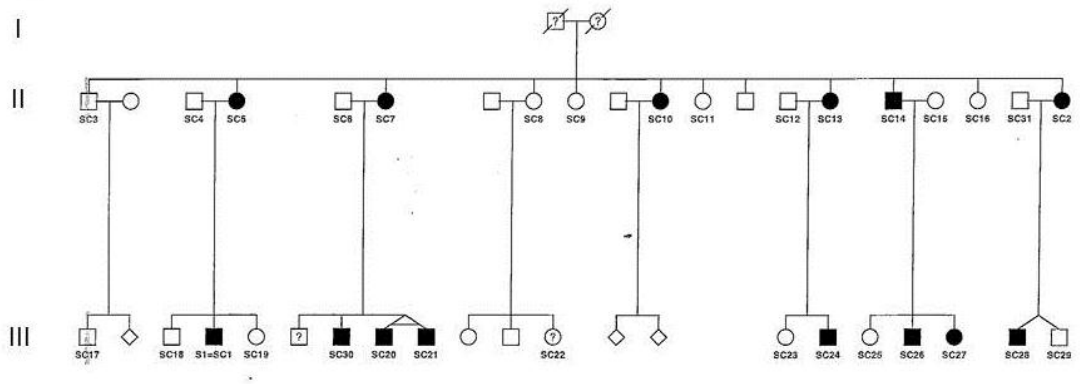

Swedish Family B
I
II
1
2
4
5
6
7
8
9
10
11
12
III
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
IV
31
32
33
34
35
36
37
38

C


Swedish Family E
I
E1
PPK E2
II
PPK E3
PPPK E4
PPK E5
PPK E6
PPK E7
E8
III
PPK E9
PPK E10

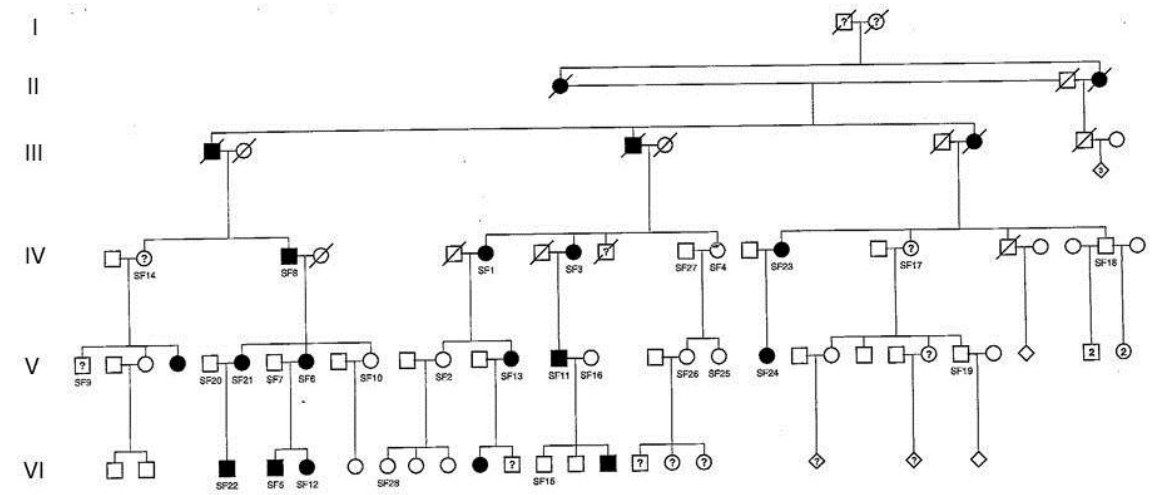
D

Swedish Family SC



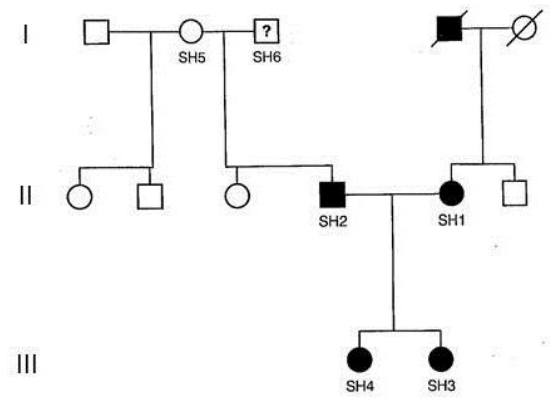
E

Swedish Family SF

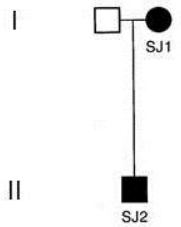


F

Swedish Family SH



G Swedish Family SJ



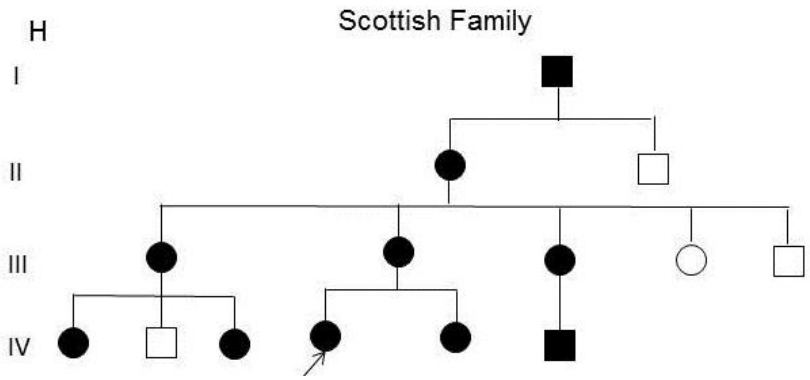


Figure S2. Pedigrees of Families Affected by Diffuse NEPPK

(A–G) Pedigrees of Swedish families in which most affected individuals carry the founder mutation c.113C>A (p.Ala38Glu), apart from family B (B), in which the majority of the affected individuals carry the *AQP5* mutation c.562C>T (p.Arg188Cys). However, one branch of family B carry the founder Swedish mutation c.113C>A (p.Ala38Glu) which they have inherited from an affected individual who married into this family (circled in red).

(H) Scottish family pedigree in which affected individuals carry the mutation c.367A>G (p.Asn123Asp).

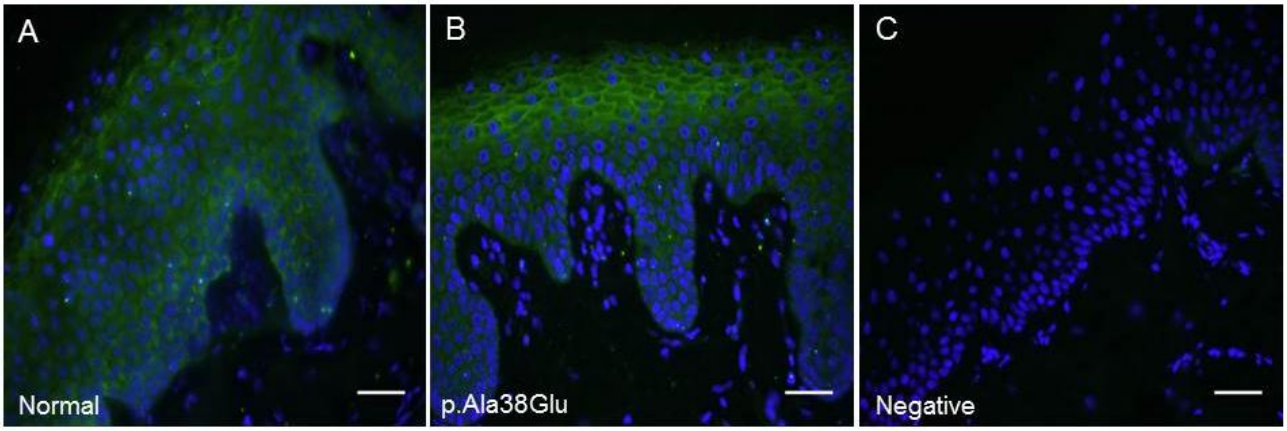


Figure S3. Immunofluorescent Staining of AQP5 in Palmar Epidermis from a Swedish Individual Affected by Diffuse NEPPK

Immunofluorescent staining of AQP5, using an antibody raised against the C-terminus of human AQP5 (green) in paraffin wax-embedded sections from unaffected, control, palm (A) and a Swedish individual affected with diffuse NEPPK (with the p.Ala38Glu variant) palm (B). Primary antibody is omitted in the negative control (C). Nuclei were counterstained with DAPI (blue). AQP5 appears to be present throughout the palmar epidermis with some localisation to the plasma membrane of the keratinocytes of the stratum granulosum in both the control and patient palm sections. Scale bar represents 40 μm.

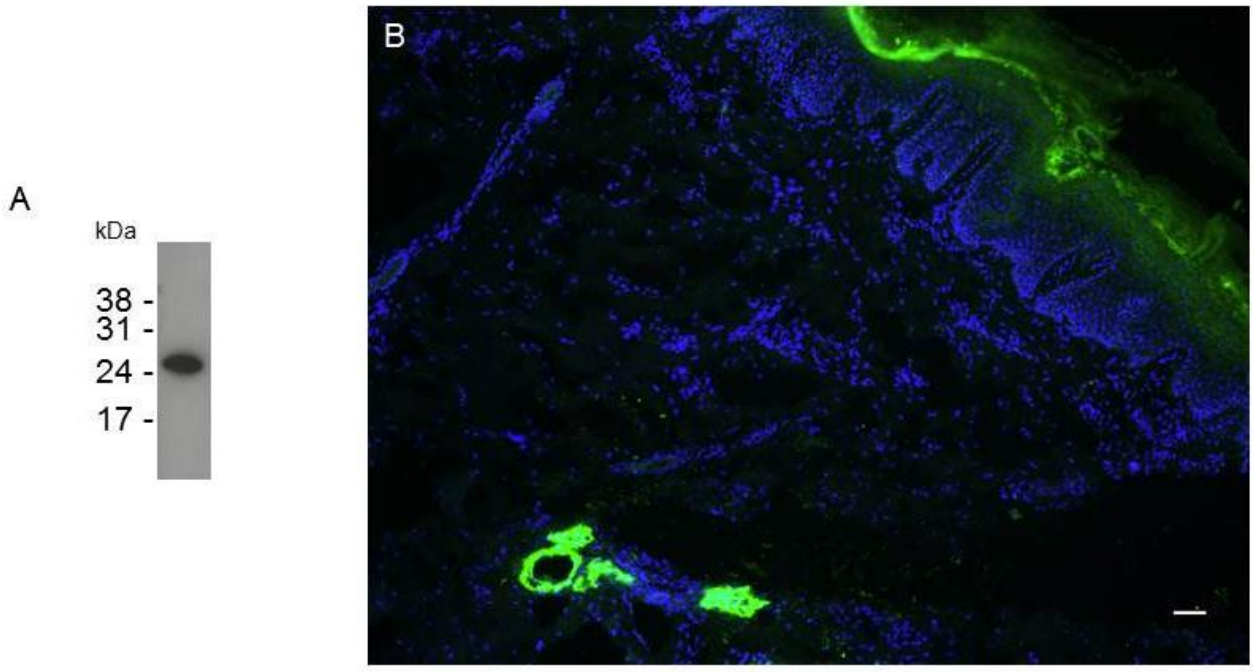


Figure S4. Specificity of Rabbit Monoclonal AQP5 Antibody (ab92320, Abcam)

(A) Immunoblot of cell lysate extracted from HEK293T cells transfected to over-express AQP5, illustrating that the rabbit monoclonal AQP5 antibody (ab92320) detects a protein product at the expected size of approximately 28 kDa.

(B) A lower magnification image of normal, frozen, palm skin stained with anti-AQP5 (ab92320, green) illustrating the lower levels of AQP5 expression in the epidermis in comparison to expression in the sweat gland cells. Nuclei were counterstained with DAPI (blue). Scale bar represents 50 µm.

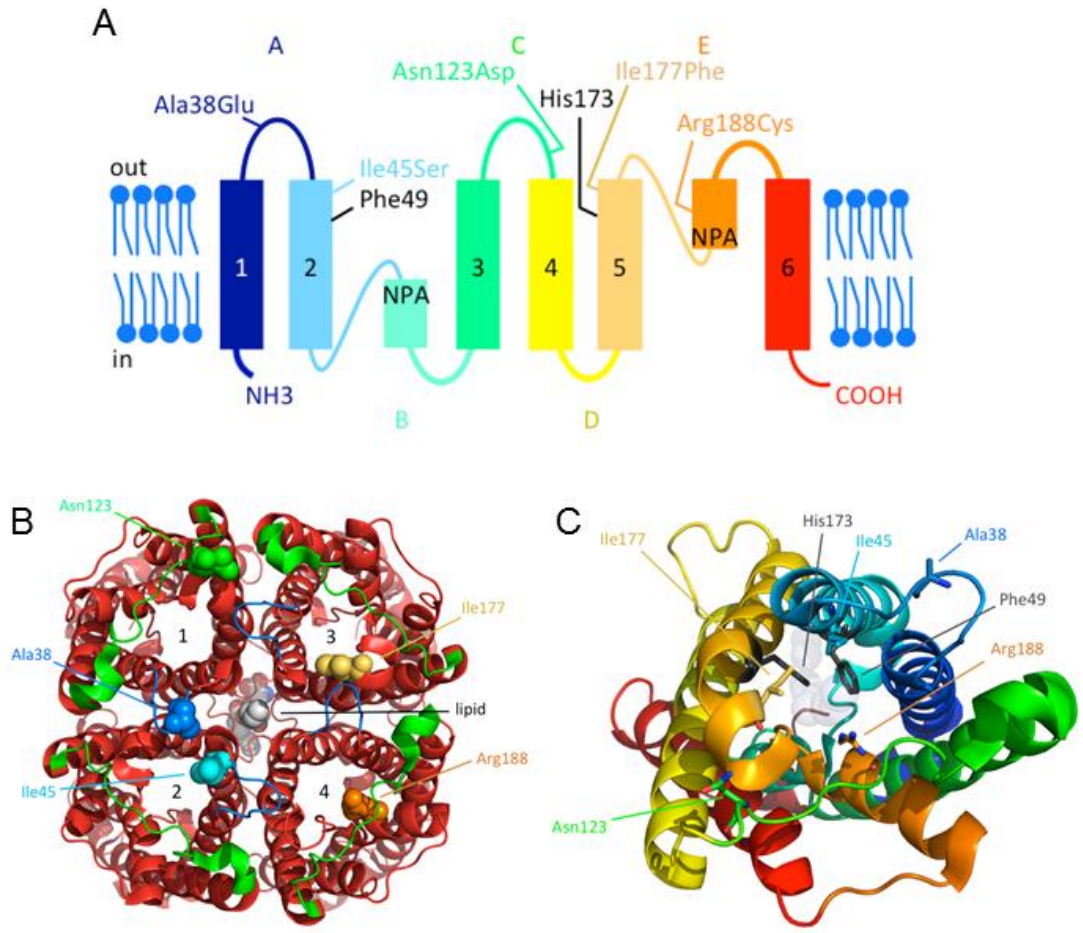


Figure S5. Structural Models of AQP5 Depicting the Positions of the Residues Identified as Substituted in Diffuse NEPPK

(A) Topological representation of AQP5 in the plasma membrane showing the positions of the NPA motifs in the two half-helices formed by loops B and E, the ar/R constriction point formed by Phe49, His173 (black) and Arg188 (dark orange). The relative positions of the variants identified in the diffuse NEPPK cases are indicated: p.Ala38Glu (dark blue), p.Ile45Ser (light blue), p.Asn123Asp (green), p.Ile177Phe (light orange) and p.Arg188Cys.

(B) The AQP5 tetramer shown in cartoon format and viewed from above the extracellular face. The protein chains are coloured red except for extracellular loops A and C which are shown in blue and green, respectively. The four water channels (one per monomer) are numbered and the residues substituted in diffuse NEPPK are shown in space-fill for Ala38 (blue in monomer 1), Ile45 (cyan in monomer 2), Asn123 (green in monomer 1), Ile177 (gold in monomer 3) and Arg188 (orange in monomer 4). The central pore of the AQP5 tetramer is blocked by a lipid molecule.

(C) Human AQP5 monomer (pdb 3D9S) in cartoon format viewed from the extracellular surface with helices and loops colour-coded as per (A). Water molecules co-crystallized in the channel are represented by light blue spheres. The side-chains of the residues substituted in diffuse NEPPK and the ar/R constriction point are shown in stick format. Residues Ile45, Ile177 and Arg188 line the water channel. Structural models were generated in MacPyMOL (Delano Scientific). Despite the AQP5 variants displaying a wide distribution the primary sequence of the AQP5 protein, they are clustered in the tertiary structure of the channel protein, either lining the extracellular end of the water channel, or situated in extracellular loops important for stabilising the tertiary structure of AQP5.

Reference

1. Robinson, J.T., Thorvaldsdottir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., and Mesirov, J.P. (2011). Integrative genomics viewer. Nat Biotechnol 29, 24-26.