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A large consanguineous family with a mild and transient form of autosomal recessive Pseudohypoaldosteronism type 1 (PHA1) caused by a novel mutation in the SCNN1A gene: functional studies

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We have nothing to disclose

BACKGROUND AND OBJECTIVE

Pseudohypoaldosteronism type 1 (PHA1) is a rare inherited disease characterized by resistance to aldosterone action and distinguished in two forms: the autosomal dominant renal form caused by mutations of the *NR3C2* gene (MR) and the autosomal recessive systemic form caused by mutations of the subunit genes *SCNN1A, SCNN1B, SCNN1G* of the epithelial sodium channel (ENaC). The classic phenotype of the autosomal recessive form of PHA1 is usually severe, lifelong, and expressed with multiorgan symptoms, whereas the autosomal dominant form is milder, transient and restricted to the kidneys. A large consanguineous family with a mild and transient form of autosomal recessive PHA1 due to a novel homozygous mutation in the SCNN1A gene has been previously described.

In this study we investigate the effect of the p.F226C SCNN1A gene mutation on the PHA1 phenotype by functional studies..

PATIENTS AND METHODS

Human αENaC wt, or αF226C or αF226S, together with human β and γ ENaC from synthetic cRNAs, were expressed in Xenopus laevis oocytes. F226S was generated to have a conservative substitution of the Cys226 lacking the reactive -SH side chain. ENaC activity was measured as amiloride-sensitive currents (INa) with the addition of trypsin during concurrent recording. The expression of α ENaC wt and mutants was determined by Western blot.

RESULTS

Patients were diagnosed between 5 and 60 days of age presenting with failure to thrive or during the course of a respiratory illness, with hyperkalemia, hyponatremia, elevated renin and aldosterone levels and a positive sweat test. All patients responded well to sodium supplementation with decreasing requirements with age until discontinuation of treatment.

All patients were found to be homozygotes, whereas their parents were heterozygotes for the mutation p.F226C of the SCNN1A gene except of two mothers who were homozygous without any medical history and with normal biochemical findings. Additionally two siblings were found to be homozygous, one with a short history of sodium supplementation in infancy and the other with no symptoms, while both of them have normal clinical and laboratory findings.

ENaC activity measured as amiloride-sensitive currents (INa) in the absence of trypsin was significantly reduced for the F226C (83% reduction, n= 40) and the F226S (94 % reduction, n= 13). Both mutants responded to trypsin by a robust increase in ENaC current, but under trypsin, the magnitude of ENaC current remained lower for the mutants than the wt. [Figure 1]

However, the trypsin-induced increase in ENaC current relative to baseline was larger for the ENaC mutants than for the wt. Specifically the effect of trypsin was 2 fold greater for F226C and 4.7 fold greater for F226S compared to wt, consistent with a 2 fold lower channel open probability for F226C (50% reduction in channel activity) and a 4-5 fold lower open probability for F226S. [Figure 2]

Western blot revealed that a reduced amount of ENaC protein was expressed under both the full-length and the cleaved αENaC mutants F226C or F226S, compared to wt. [Figure 3] Therefore the lower ENaC current expressed by F226C and F226S under trypsin treatment was due to a reduced amount of aENaC protein made in the oocytes.

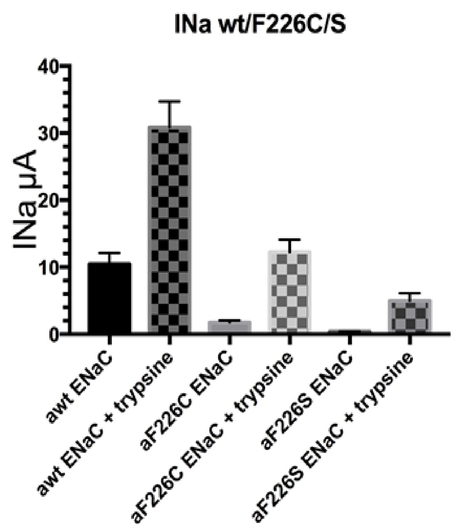

Figure 1: The amiloride-sensitive current(INa) with the addition of trypsin measured for aENaC: wt, F266C and F226S co-expressed with β and γ subunits

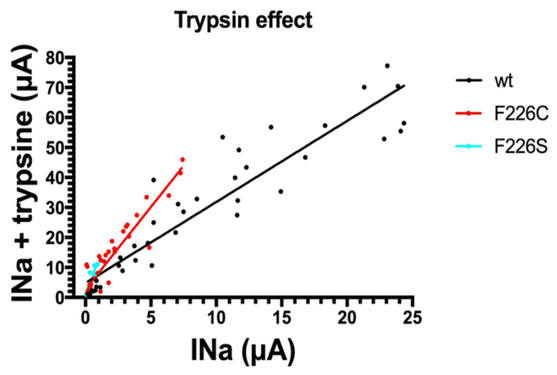

Figure 2: The values of amiloride-sensitive current(INa) measured without trypsin versus Ina with trypsin for aENaC: wt, F266C and F226S

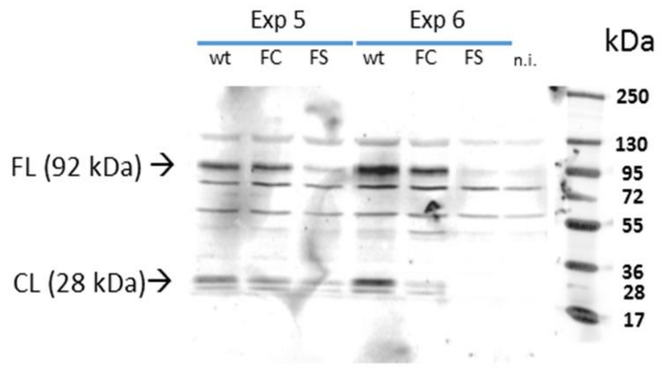

Figure 3: Anti-haENaC Western blot for the analysis of soluble fractions of human ENaC: wt, F226C and F226S mutant exressed in oocytes or in non-injected. FL: full-length (uncleaved) aENaC, CL: cleaved (active) aENaC

CONCLUSIONS

We present a large family with a mild and transient form of autosomal recessive PHA1 due to a novel homozygous mutation in the *SCNN1A* gene. Functional studies showed that the p.F226C SCNN1A gene mutation leads to a partial loss of function resulting mainly from a 50% decrease in the intrinsic ENaC activity, probably due to a lower channel open probability and secondly from a 60% reduction in channel expression at the protein level. These findings could explain the mild phenotype of the patients carrying the F226C mutation demonstrating that there is a great variability in the phenotype of a usually severe disease.

REFERENCES

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