New germline mutation in calcium channel CACNA1H causes late-onset primary aldosteronism

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Introduction

Primary aldosteronism (PA) is a very common secondary cause of hypertension. It occurs due to an excess secretion of aldosterone from the adrenal adrenal glands, resulting in high potassium, alkalosis, low renin levels and hypertension. Familial forms of hyper-aldosteronism are considered to be relatively rare, with only a small number of genes having been implicated so far.

The aim of the present study is to identify the cause of disease in a Spanish family suffering from late-onset PA (Fig. 1). Also, we aim to examine the disease mechanisms in an *in vitro* setting.

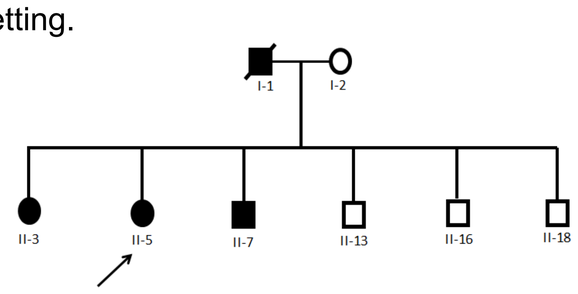


Figure 1. Pedigree of the PA family. Affected family members are shown in black. The black arrow depicts the index patient.

Methods

Comprehensive biochemical and clinical phenotyping, as well as genome-level sequencing (WGS) were employed to identify the molecular cause of disease. The identified mutation was confirmed using Sanger sequencing.

For *in vitro* validation, NCI-H295R cells were transfected with wildtype and mutant plasmid constructs and stimulated with 20 mM KCl; aldosterone synthesis and changes in *CYP11B1* and *CYP11B2* mRNA expression levels were measured. HEK293T cells were transfected with the same constructs for assessment of the production of electrophysiological recordings.

Finally, to further investigate the potential physiologic effects of the mutation, we measured cytosolic Ca^{2+} dynamics by fluorescent live cell imaging.

Results

A nonsynonymous single nucleotide variation was identified in *CACNA1H* (NM_001005407, exon16, c.G3190A, p.G1064R).This is the second PA causing mutation to be identified in this gene, the first being $CACNA1H^{M1549V}$, which causes early onset PA and is found in the conserved IIIS6 membrane-spanning helix (Scholl et al. 2015).

The *CACNA1H* gene encodes the low voltage-activated T-type calcium channel Cav3.2 (Fig. 2). The mutation is located in the cytosolic II-III linker region. Affected family members are heterozygous for the mutation. The position is conserved in all vertebrate orthologs that were compared (Fig. 3).

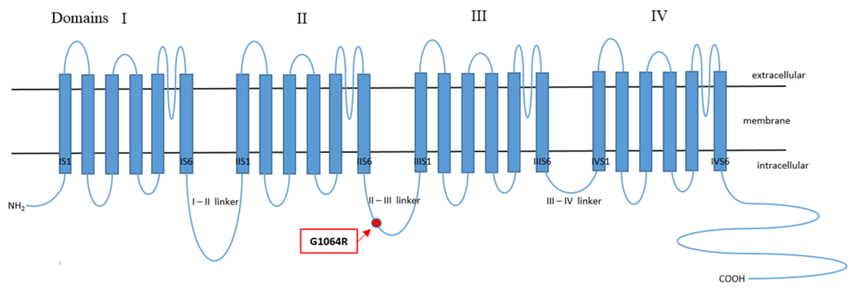


Figure 2. Transmembrane structure of Cav3.2, encoded by CACNA1H

Figure 3. Aligned orthologs of CACNA1H in vertebrates

Electrophysiological recordings (clamps) showed no significant differences between the wildtype and mutant.

NCI-H285R cells were treated with KCl to induce aldosterone production. Cells transfected with $CACNA1H^{G1064R}$ produced higher levels of aldosterone, elevated relative *CYP11B2* mRNA (aldosterone synthase) expression levels and reduced relative *CYP11B1* (11-beta-hydroxylase) expression levels (Fig. 4).

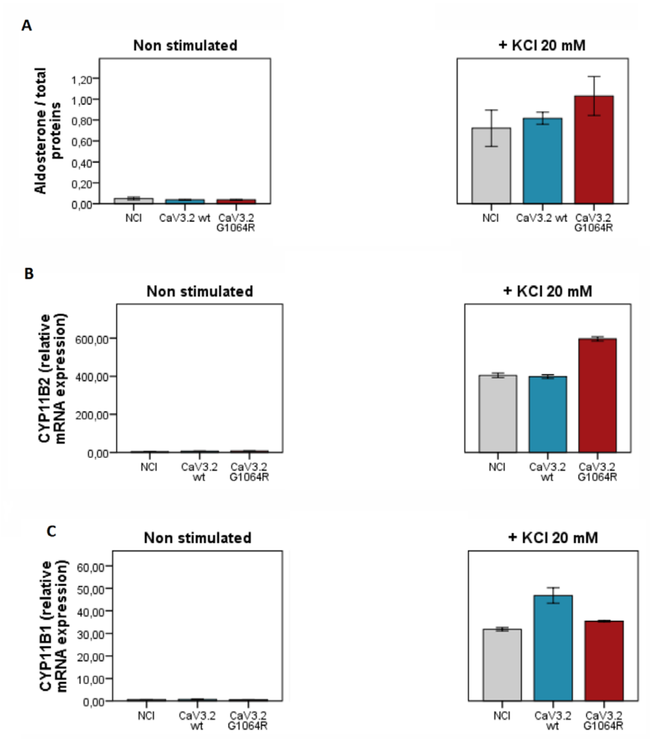
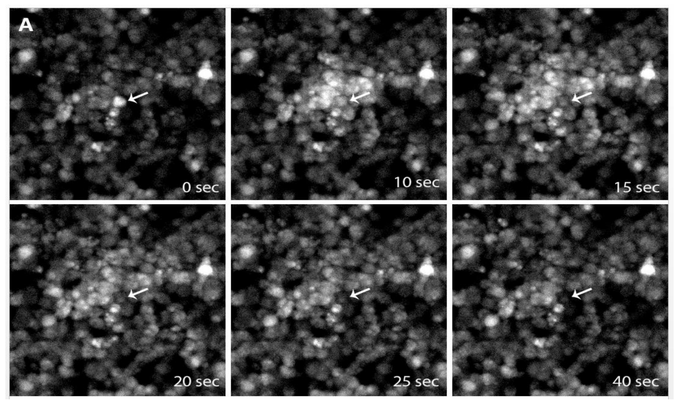


Figure 4. NCI-H285R cells transfected with $CACNA1H^{G1064R}$ (Cav3.2G1064R) and $CACNA1H^{WT}$ and stimulated with 20 mM KCl. (A) Aldosterone, (B) *CYP11B2* mRNA and (C) *CYP11B1* mRNA expression levels were measured.

Imaging studies of intercellular Ca^{2+} signaling showed that HEK293T cells transformed with the mutant variant had significant differences in the average period of spiking and average spike width, when compared to the wildtype variant. These findings were consistent with an increased Ca^{2+} influx into cells transfected with the mutant variant (Fig. 5).



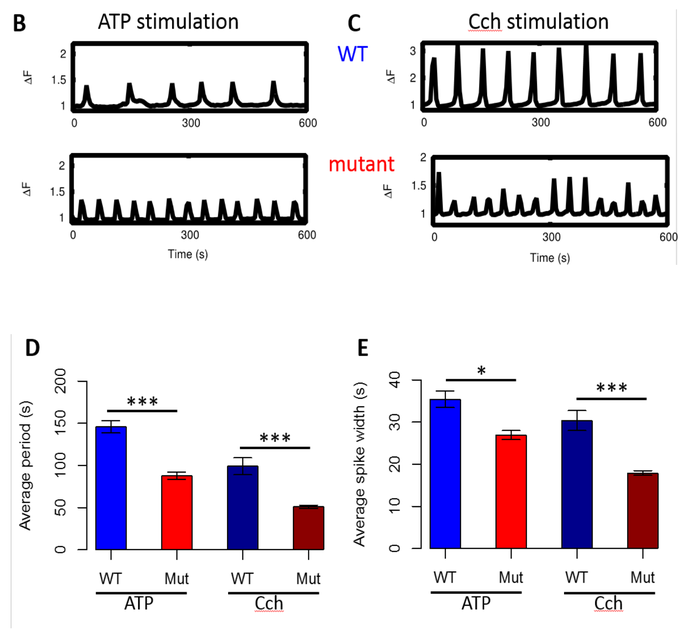


Figure 5. (A) Intercellular Ca2+ waves demonstrate the functionality of cloning in *CACNA1H* because normal HEK293T cells do not exhibit this behavior. (B, C) Representative examples of Ca^{2+} spiking in individual WT and mutant cells stimulated with ATP or Carbohol (Cch). (D, E) Statistically significant differences in the average period of spiking and average spike width for stimulations with ATP and Cch (n=45, 38, 42, 39 cells respectively). Comparisons only made for identical stimulations. The significance reached is indicated by * <= 0.05, ** <= 0.01, *** <= 0.001.

Conclusions

Our study further confirms the role of *CACNA1H* in the development of PA. Furthermore, the identified gain of function mutation is the first late-onset germline mutation implicated in PA in a calcium channel, and the first PA mutation located outside a pore-forming transmembrane helix in a calcium channel.

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