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

Kirsten Roomp1, Kamil Grzyb1, Christina Wolf1, Yara Rhayem2, Nuria Oliver3, Barbara Wardas4, Andreas Beck4, Antonio Miguel Pico Alfonso5, Veit Flockerzi4, Felix Beuschlein2, Alexander Skupin1*, Patrick May1*, Jochen G. Schneider1,4*

1 LCSB, University of Luxembourg, Luxembourg, 2 Klinikum der Universität München, Germany, 3 Telefonica Research and Development, Barcelona, Spain, 4 Internal Medicine II, University Medical Center of Saarland, Germany, 5 Departamento de Medicina Clínica, University Miguel Hernández, Alicante, Spain

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 hyperaldosteronism 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.

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-H285R cells were transfected with wildtype and mutant plasmid constructs and stimulated with 20 mM KCl; aldosterone synthesis and changes in 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 Ca2+ 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 CACNA1Hperissey, 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).

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 CACNA1HG1064R produced higher levels of aldosterone, elevated relative CYP11B2 mRNA (aldosterone synthase) expression levels and reduced relative CYP11B1 (11-beta-hydroxylase) expression levels (Fig. 4).

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.

Imaging studies of intercellular Ca2+ 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 Ca2+ influx into cells transfected with the mutant variant (Fig. 5).