variants predict a worse clinical outcome because average SDHB variants are, by chance, more biochemically severe. This suggests that SDHB loss may uniquely impact SDH biochemical function.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Somatic Mutations of GNA11 and GNAQ in CTNNB1-Mutant Aldosterone-Producing Adenomas Increases Aldosterone and Aldosterone Synthase (CYP11B2)

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Most aldosterone-producing adenomas (APA) have gain-of-function somatic mutations of ion channels or transporters. However, their frequency in aldosterone-producing cell-clusters of normal adrenals suggests the existence of co-driver mutations which influence the development or phenotype of APAs.

Gain-of-function mutations in both *CTNNB1* and *GNA11* were found by whole exome sequencing in 3 of 41 APAs from a UK/Irish cohort. Targeted sequencing for exon 3 mutations of *CTNNB1* and p.Gln209 mutations of either *GNA11* or closely homologous *GNAQ* confirmed these and 7 further double mutant APAs in this discovery cohort. The presence of *GNA11*/*Q* p.Gln209 mutations in *CTNNB1* mutant APAs were replicated in 2 cohorts from France (n=14) and Sweden (n=3). In total, 16 (59%) of the 27 CTNNB1 mutant APAs investigated had a mutation at p.Gln209 of *GNA11* (n=11) or *GNAQ* (n=5). Interestingly, *CTNNB1*-mutant APAs were more commonly present in women (23/27), and of these, those with *GNA11*/*Q* mutations were all women except for a pubertal boy. To also note, 9 of 10 of the UK/Irish double mutant APAs in the discovery cohort presented in puberty, pregnancy, or menopause.

Mutation of p.Gln209, or homologous p.Gln in *GNAS*, *GNA12-14*, impair hydrogen bonds between G-protein α and β subunits. Transfection of H295R cells, an immortalised adrenocortical cell line heterozygous for the p.Ser45Pro mutation of *CTNNB1* but wild-type for *GNA11-14/Q/S,* by each of the *GNA11/Q* mutations increased aldosterone secretion and *CYP11B2* expression (encoding aldosterone synthase) by 1.93-6.1-fold and 8.0-9.8-fold respectively, compared to vector or wild-type -transfected cells. In ZG, *GNA11/Q* mediate the aldosterone response to angiotensin II, via stimulation of intracellular Ca^{2+} release by inositol trisphosphate. In the mutant-transfected cells, the stimulatory effect of angiotensin II 10 nM was retained. In order to determine whether the p.Gln209 mutations stimulate aldosterone production even in the absence of CTNNB1 activation, the transfections of H295R cells were repeated after either 24-h treatment with the CTNNB1 inhibitor, ICG-001, or silencing of CTNNB1 using the ONTARGETplus SMARTpool SiRNAs (Dharmacon). Both interventions reduced the aldosterone production relative to vehicle/control-treated cells; however neither ICG-001 nor silencing of CTNNB1 blunted the fold-increase in aldosterone secretion seen in mutant-transfected cells compared to wild-type.

In summary, we report the discovery of gain-of-function mutations of the G-protein, *GNA11,* or its close homologue, *GNAQ*, in multiple APAs which majority presented during periods of high LH/HCG. To date, the mutation is always residue p.Gln209, and associated with a gain-of-function mutation of *CTNNB1*. These *GNA11/Q* p.Gln209 mutations increase aldosterone and CYP11B2 production both in the presence and in the absence of CTNNB1 activation.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

The Functional Glomerulosa Cells Define the Unicity of the Adult Human Adrenal Cortex

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The zonation of the human adrenal cortex has long been established morphologically and histologically as three distinct layers of cells. The outer zona glomerulosa (ZG) comprises densely packed cells arranged in clusters that produce aldosterone; the zona fasciculata (ZF) is composed of cells with large cytoplasm, containing lipid droplets arranged in radial columns that synthetize cortisol; and the zona reticularis is composed of compact and pigmented cells producing androgens. The main purpose of this work was to study the expression of aldosterone synthase (CYP11B2 which catalyzes the last steps of aldosterone synthesis) and 11β-hydroxylase (CYP11B1 which catalyzes the last step of cortisol synthesis) in normal adrenal glands to address issues regarding the zonation and