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ORIGINAL ARTICLE

ARMC5 mutation analysis in patients with primary aldosteronism and bilateral adrenal lesions

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Idiopathic hyperaldosteronism (IHA) due to bilateral adrenal hyperplasia is the most common subtype of primary aldosteronism (PA). The pathogenesis of IHA is still unknown, but the bilateral disease suggests a potential predisposing genetic alteration. Heterozygous germline mutations of *armadillo repeat containing 5* (*ARMC5*) have been shown to be associated with hypercortisolism due to sporadic primary bilateral macronodular adrenal hyperplasia and are also observed in African-American PA patients. We investigated the presence of germline *ARMC5* mutations in a group of PA patients who had bilateral computed tomography-detectable adrenal alterations. We sequenced the entire coding region of *ARMC5* and all intron/exon boundaries in 39 patients (37 Caucasians and 2 black Africans) with confirmed PA (8 unilateral, 27 bilateral and 4 undetermined subtype) and bilateral adrenal lesions. We identified 11 common variants, 5 rare variants with a minor allele frequency $< 1\%$ and 2 new variants not previously reported in public databases. We did not detect by *in silico* analysis any *ARMC5* sequence variations that were predicted to alter protein function. In conclusion, *ARMC5* mutations are not present in a fairly large series of Caucasian patients with PA associated to bilateral adrenal disease. Further studies are required to definitively clarify the role of *ARMC5* in the pathogenesis of adrenal nodules and aldosterone excess in patients with PA.

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INTRODUCTION

Idiopathic hyperaldosteronism (IHA) due to bilateral adrenal hyperplasia is the most common subtype of primary aldosteronism (PA), accounting for 50–70% of PA patients.[1,2] IHA is diagnosed by adrenal vein sampling (AVS) in confirmed PA patients who do not show lateralization of aldosterone secretion[2,3] and is treated by long-term therapy using mineralocorticoid receptor antagonists. The other common PA subtypes, aldosterone-producing adenoma (APA) and unilateral adrenal hyperplasia, display lateralization of aldosterone secretion at AVS and are treated by unilateral adrenalectomy. The past 4 years have witnessed important advances in understanding the pathogenesis of PA, with the demonstration that mutations in four different genes, namely *KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D*, explain the constitutive aldosterone production in ~50% of APAs.[4–8] Germline mutations in *KCNJ5* are also responsible for familial hyperaldosteronism type III, whereas type I is due to a recombination between *CYP11B1* and *CYP11B2* and type II is linked in some families to a so far unidentified gene in chromosome 7p22.[9,10] Recently, germline mutations in *CACNA1H* have been associated to a new familial form of PA.[11] The pathogenesis of IHA is still completely unknown, but the presence of bilateral alterations of the adrenals suggested a common genetic defect or predisposing alteration. Heterozygous germline mutations of *armadillo repeat containing 5* (*ARMC5*) have been shown to be associated to more than half of patients with hypercortisolism because of sporadic primary bilateral macronodular adrenal hyperplasia,[12,13] and biallelic germline inactivation of *ARMC5* has been consistently observed in bilateral macronodular adrenal hyperplasia familial cases with endogenous Cushing's syndrome. *ARMC5* inactivation has been demonstrated in such patients to promote cell survival and to decrease cortisol production *in vitro*, finally resulting in cortisol overproduction because of the formation of large adrenal masses *in vivo*. Heterozygous germline *ARMC5* mutations have also been observed in 22/56 (39.3%) PA patients, and 6 of these mutations (10.7%) were predicted to damage protein function by *in silico* modelling.[14] All six carriers were African Americans, an ethnic population generally characterized by a higher prevalence of multiple metabolic alterations, such as obesity or diabetes. Genetic alterations were not associated to a clinical or biochemical phenotype different to that found in the remaining cohort patients.

In the present study we investigated the presence of germline *ARMC5* mutations in a group of PA patients who had bilateral computed tomography (CT)-detectable adrenal alterations.

MATERIALS AND METHODS

Patient selection

We retrospectively assessed 39 consecutive patients with PA (25 males and 14 females) at the Division of Internal Medicine and Hypertension Unit of the University of Torino between 2010 and 2014. All patients were unrelated and had bilateral alterations of the adrenals (bilateral nodules of at least 8 mm, bilateral adrenal limb thickness exceeding 5 mm and/or the body thickness exceeding 10 mm without clear nodularity or the combination of them) at CT scan. Screening and confirmation of PA were performed in agreement with the Endocrine Society guidelines[2]

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as described previously.[15] Briefly, patients were either in wash-out from all antihypertensive drugs (diuretics and spironolactone were stopped at least 6 and 8 weeks, respectively, and 3 weeks for the other antihypertensive agents) before aldosterone and plasma renin activity measurements or received an α-blocker (doxazosin) and/or a calcium channel blocker (verapamil or amlodipine) during the entire study period. After a positive screening, patients underwent the intravenous saline load as confirmatory test. Subtype diagnosis was performed by CT scanning with contrast and fine cuts of the adrenal and subsequent AVS. No patient had overt clinical features of Cushing's syndrome. Several days before testing for differential diagnosis of PA subtype, all patients were given 1 mg overnight dexamethasone as a screening suppression test for subclinical hypercortisolism, defined as post-dexamethasone cortisol to $< 1.8\ \mu g\ dl^{-1}$ ($50\ nmol\ l^{-1}$).[3,16]

In most cases AVS was performed under basal conditions ($n = 16$): continuous cosyntropin ($50\ \mu g\ h^{-1}$) infusion was used when the procedure was performed late in the morning or when a patient, at risk for an allergic reaction to the contrast, was pretreated with dexamethasone ($n = 10$). In some cases ($n = 10$) AVS was performed both under basal and after cosyntropin infusion. In three cases AVS was refused by the patients who requested to be treated with mineralocorticoid receptor antagonists. In 1/36 patients who underwent AVS, the procedure was not successful (incorrect cannulation of the right adrenal vein). The other 35 patients had a successful AVS (selectivity index > 3).[3] Eight patients displayed a lateralization index > 4, diagnostic for unilateral PA.[3] Of these eight patients, two are still awaiting adrenalectomy and six were operated, all with cure of hyperaldosteronism (normalization of aldosterone, plasma renin activity, ARR and potassium levels, normal suppressibility of aldosterone under saline load) and cure or significant reduction of hypertension.

Of the six adrenalectomized patients, one carried a G151R KCNJ5 mutation, one a L168R mutation and four were negative for mutations in KCNJ5, ATP1A1, ATP2B3 and CACNA1D[17] (Supplementary Table 1).

Hormonal assays were performed as described previously.[16] Serum aldosterone was assessed by solid-phase radioimmunoassay ALDOCTK-2 (DiaSorin, Saluggia, Italy). Plasma cortisol was measured by chemiluminescence (IMMULITE 2000, Erlangen, Germany).

All patients gave written informed consent for the study that was approved by our local ethics committee.

ARMC5 genetic analysis

Germline DNA was extracted from peripheral blood leukocytes by a standard procedure. Screening of the entire coding region of the *ARMC5* gene and all intron/exon boundaries was performed by bidirectional sequencing. Forward and reverse primers incorporated the -21M13F (5′-TGTAAAACGACGGCCAGT-3′) or M13R (5′-CAGGAAACAGCTATGAC-3′) extensions, respectively, at their 5′ ends. The sequences of all primers used in this study, and their annealing temperatures, are shown in Supplementary Table 2. Sequence reads were assembled with reference sequence using SeqScape Software v2.6 (Applied Biosystems, Foster City, CA, USA). The nomenclature of DNA sequence variants was made following the recommendation of the Human Genome Variation Society.[18] Sequence variants has been examined with Alamut Visual Software 2.5.0. (Interactive Biosoftware, Rouen, France) Computed missense predictions has been provided for Align GVGD, SIFT, Polyphen-2, MutationTaster; splicing predictions were based on SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder.

Statistical analysis

All data are expressed as mean ± s.d. for normally distributed variables and as median (25–75th percentile) for non-normally distributed variables.

RESULTS

Thirty-seven of our patients were Caucasians (36 Italians and 1 Rumanian) and 2 were blacks of African descent (1 from

Table 1. Clinical and biochemical parameters of APA patients included in the study

Parameters	*All patients*	*Bilateral nodules*	*Unilateral nodule(s)+contralateral hyperplasia*	*Bilateral hyperplasia*
N	39	14	12	13
Age (years)	51.6 ± 8	51.4 ± 6	52.4 ± 9	51.2 ± 10
Sex, N *(%)*				
Male	25 (64)	10 (71)	7 (58)	8 (61.5)
Female	14 (36)	4 (29)	5 (42)	5 (38.5)
BMI ($kg\ m^{-2}$)	26.3 ± 4	25.7 ± 5	26.6 ± 3	26.6 ± 4
Abdominal circ (cm)	97 (84–105)	97 (82–105)	99 (78–107)	97 (86–104)
Glucose ($mg\ dl^{-1}$)	101 ± 17	107 ± 19	102 ± 20	96 ± 11
Triglycerides ($mg\ dl^{-1}$)	134 ± 87	122 ± 54	159 ± 133	124 ± 59
HDL cholesterol ($mg\ dl^{-1}$)	53 ± 13	53 ± 12	51 ± 14	55 ± 14
Total cholesterol ($mg\ dl^{-1}$)	203 ± 39	207 ± 33	204 ± 51	197 ± 34
SBP (mm Hg)	160 ± 18	168 ± 25	155 ± 11	158 ± 14
DBP (mm Hg)	98 ± 11	102 ± 12	93 ± 10	99 ± 11
Potassium ($mEq\ l^{-1}$)	3.5 ± 0.5	3.4 ± 0.5	3.3 ± 0.6	3.8 ± 0.3
Metabolic syndrome, N *(%)*				
Yes	16 (41)	6 (43)	6 (50)	4 (30.8)
No	23 (59)	8 (57)	6 (50)	9 (69.2)
Aldosterone ($ng\ dl^{-1}$)	31 (19–41)	29 (20–39)	29 (21–43)	33 (19–42)
PRA ($ng\ ml^{-1}\ h^{-1}$)	0.3 (0.1–0.4)	0.3 (0.2–0.4)	0.35 (0.1–0.4)	0.3 (0.1–0.5)
ARR	125 (64–166)	113 (59–158)	127 (65–166)	103 (59–178)
Post-dex plasma cortisol ($\mu g\ dl^{-1}$)	1.2 (1–1.6)	1.2 (1–1.7)	1.3 (1–1.6)	1.1 (1–1.6)
Final diagnosis, N *(%)*				
Unilateral	8 (21)	3 (21.4)	3 (25)	2 (15)
Bilateral	27 (69)	8 (57.2)	9 (75)	10 (77)
Undetermined	4 (10)	3 (21.4)	0 (0)	1 (8)
Sporadic PA	36 (92)	13 (93)	11 (92)	12 (92)
Familial PA	3 (8)	1 (7)	1 (8)	1 (8)

Abbreviations: APA, aldosterone-producing adenoma; ARR, aldosterone/PRA ratio; BMI, body mass index; circ, circumference; DBP, diastolic blood pressure; dex, dexamethasone; HDL, high-density lipoprotein; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure. Column headings refer to computed tomography (CT) findings.

Cameroon and 1 from Nigeria). Thirty-six had a sporadic PA whereas three patients had FH-II, diagnosed because of the concomitant PA diagnosis in one or more first-degree relatives, as previously described.[19] At CT scanning, 14 patients displayed bilateral nodules >8 mm, 12 displayed unilateral nodule(s) and contralateral adrenal hyperplasia and 13 patients displayed bilateral adrenal hyperplasia without defined nodularity (both Africans displayed this last feature at CT scanning) (Table 1). No patient showed unsuppressed cortisol at 1 mg overnight dexamethasone test, excluding concurrent aldosterone and cortisol oversecretion. Sixteen patients (41%) displayed metabolic syndrome in accordance with ATP III clinical definition.[20]

We identified 18 sequence variations in comparison with the reference genome (GRCh37): 11 common variants, 5 rare variants with a minor allele frequency <1% and 2 new variants not previously reported in public databases (Table 2). Among the rare variants, one is located in the 5′-untranslated region (c.-117A>C), three in the coding region, two synonymous (c.438G>A p.Arg146= and c.1641G>A p.Ala547=) and one missense (c.1505G>A p.Arg502His) and one in the intronic region (c.1370 +50G>C). All these variations affected weakly conserved nucleotides.

In silico analysis for the missense p.Arg502His predicts a benign effect on protein, and it has been described only in two subjects among 4021 from the European-American population (ESP) (Table 2). We detected the p.Arg502His substitution in two patients and no information is available on its prevalence in large population with similar ancestry. We evaluated through *in silico* tools whether the rare and the newly identified sequence variants could affect normal pre-mRNA splicing via disruption or creation of splice site consensus sequences, without identifying a decrease in strength of wild-type splice sites or activation of cryptic splice sites (Table 3). Therefore, both rare and newly identified sequence variants identified in this study can be classified as unlikely pathogenic.

DISCUSSION

Considerable advances have been made over the past 4 years in the knowledge of the pathophysiology of familial and sporadic PA. Somatic mutations in different genes (*KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D*)[4–9] are associated with sporadic APA, resulting in an increase in intracellular Ca^{2+}concentration of *zona glomerulosa* cells leading to overexpression of *CYP11B2* and its transcriptional regulators NR4A2 and NR4A3,[21] and finally to aldosterone overproduction. All somatic mutations so far identified in APAs seem however to be ineffective on cell proliferation/apoptosis.[22–24] Germline mutations in *KCNJ5* are also responsible for familial hyperaldosteronism type III,[3,4,9] characterized by large bilateral adrenal masses. Much less is known about the aetiology of PA due to the presence of hyperplasia/nodules in both adrenal glands, mainly because of the difficulty in obtaining adrenal specimens adequate for pathological or molecular analysis. In a microarray study, a sample from a single patient with diffuse adrenal hyperplasia showed similar gene expression pattern to normal adrenals.[25] Studies on sporadic bilateral adrenal hyperplasia mainly focussed on germline genetic variants potentially increasing the susceptibility to develop aldosterone oversecretion. In this regard, several reports linked the *CYP11B2* gene locus to PA but this association was not fully explained by known polymorphisms.[26–28]

An intriguing study recently reported the consistent presence of germline heterozygous mutations of *ARMC5*, a gene associated with either familial or nonfamilial hypercortisolism due to bilateral macronodular adrenal hyperplasia, in African-American patients with sporadic PA.[14] *ARMC5* was found to be expressed in the *zona glomerulosa* and a primary aldosterone oversecretion from adrenal hyperplastic tissue, either unilateral or bilateral, was hypothesized in those patients. *ARMC5* seems in fact to induce apoptosis and dedifferentiation of adrenocortical cells, acting as a tumour suppressor gene in accordance with Knudson's two-hit model.[12] In case of sporadic disease, *ARMC5* should therefore bear a first germline mutation followed by a second somatic mutation[11]

Table 2. Molecular findings in the cohort of PA patients and their frequency in public database of genetic variants

ARMC5 cDNA nucleotide variation	*ARMC5 effect on protein*	*Gene region*	*rs (dbSNP)*	*Genetics of the studied PA patients*			*Population genetics1000 Genomes Project*[a]				*Population genetics NHLBI exome sequencing project*[b]			
				WT	*Het*	*Homo*	*WT*	*Het*	*Homo*	*MAF*	*WT*	*Het*	*Homo*	*MAF*
c.-306T>C		5'-UTR	rs3813002	5	21	13	86	426	580	T=0.274	—	—	—	—
c.-117A>C[c]		5'-UTR	rs76210462	36	3	0	1080	12	0	C=0.005	—	—	—	—
c.41T>A	p.Phe14Tyr	Exon 1	rs151069962	38	1	0	1030	61	1	A=0.029	5624	585	17	A=0.042
c.438G>A[c]	p.Arg146=	Exon 1	rs201280100	38	1	0	1079	13	0	A=0.006	6118	86	1	A=0.007
c.475+58A>G		Intron 1	rs9926717	26	11	2	534	409	149	G=0.324	—	—	—	—
c.508A>G	p.Ile170Val	Exon 2	rs35923277	38	1	0	1039	52	1	G=0.025	5866	399	12	G=0.034
c.583+26G>T		Intron 2	rs9921490	38	1	0	1015	72	5	T=0.038	5886	452	29	T=0.042
c.1370+50G>C[c]		Intron 3	rs139289075	36	3	0	1089	3	0	C=0.001	6121	10	0	C<0.001
c.1370+57G>A[d]		Intron 3		38	1	0	—	—	—	—	—	—	—	—
c.1505G>A[c]	p.Arg502His	Exon 4a	rs200054015	37	2	0	—	—	—	—	5948	2	0	A<0.001
c.1641G>A[c]	p.Ala547=	Exon 4a	rs61732352	38	1	0	1085	7	0	A=0.003	6160	66	0	A=0.005
c.1842C>G	p.Leu614=	Exon 4a	rs55800131	30	9	0	999	92	1	G=0.043	5501	788	36	G=0.068
c.1864+250C>T		Intron 4a	rs11150624	12	21	6	448	430	214	T=0.393	—	—	—	—
c.1865-256C>T		Intron 4a	rs28379274	38	1	0	1006	82	4	T=0.041	—	—	—	—
c.1865-476T>C		Intron 4a	rs111510548	33	6	0	1011	81	0	C=0.037	—	—	—	—
c.1865-109C>T[d]		Intron 4a		38	1	0	—	—	—	—	—	—	—	—
c.2058G>A	p.Ala686=	Exon 4c	rs11863886	38	1	0	986	101	5	A=0.051	5699	606	50	A=0.055
c.*234_*238dupGGCCT		3′-UTR	rs142544346	33	6	0	—	—	—	—	—	—	—	—

Abbreviations: *ARMC5*, *armadillo repeat containing 5*; cDNA, complementary DNA; dbSNP, database single-nucleotide polymorphism; Het, heterozygous; Homo, homozygous; MAF, minor allele frequency; NHLBI, National Heart, Lung, and Blood Institute; PA, primary aldosteronism; rs, reference SNP; UTR, untranslated region; WT, wild-type. *ARMC5* cDNA nucleotide variation RefSeq NM_001105247.1; ARMC5 protein RefSeq NP_001098717.1. [a]1000 Genomes http://www.1000genomes.org/. [b]Exome Variant Server http://evs.gs.washington.edu/EVS/. [c]Rare sequence variants. [d]Newly described sequence variants.

Table 3. *In silico* analysis of newly described or rare sequence variations identified

Nucleotide variation	*Predicted effect on protein*	*Pathogenicity by in silico analysis*	
		Evolutionary conservation	*Deleteriousness prediction algorithms*
c.-117A>C		Weakly conserved nucleotide	
c.438G>A	p.Arg146=	Weakly conserved nucleotide	No effect on normal donor splice site
c.1370+50G>C		Weakly conserved nucleotide	No effect on normal donor splice site
c.1370+57G>A		Weakly conserved nucleotide	No effect on normal donor splice site
c.1505G>A	p.Arg502His	Weakly conserved nucleotide Moderately conserved amino acid	Missense prediction: tolerated variation No effect on splicing
c.1641G>A	p.Ala547=	Weakly conserved nucleotide	Cryptic donor site possibly activated
c.1865-109C>T		Weakly conserved nucleotide	No effect on normal acceptor splice site

The table is produced using the mutation interpretation software Alamut Visual v.2.5.0 (http://www.interactive-biosoftware.com/alamut-visual/). Evolutionary conservation is based on phastCons and phyloP values; missense predictions are automatically computed with Align-GVGD, SIFT and MutationTaster software; splicing prediction module integrates SpliceSiteFinder-like, MaxEntScan, NNSplice, GeneSplicer and Human Splicing Finder.

within the adrenal, leading to loss of its function and to tumour formation. In this regard, Zilbermint *et al.*[14] observed *ARMC5* immunoreactivity and western blot expression lower in APA than in the adjacent adrenal tissue of two unilaterally adrenalectomized patients with predicted germline *ARMC5* damaging variants. However, they did not demonstrate concurrent germline and somatic mutations or other somatic inactivating events, such as loss of heterozygosity, and therefore did not show the presence of a second hit. This raises doubt about the pathogenic role of the heterozygous *ARMC5* mutations in PA pathogenesis in these two patients. It should also be noted that the p.Leu156Phe substitution is present in 2–3% of African-American population (Exome Sequence Project and 1000 Genomes Project). Interestingly, *ARMC5* protein shares a repeating 42 amino acid motif with β-catenin, a protein with many important cellular and developmental functions.[29] WNT/β-catenin signaling was recently shown to be activated in APA,[30] and β-catenin structural alterations might affect adrenal growth and aldosterone production in this disease.

As a bilateral adrenal disease rather than a single APA suggests a potential predisposing genetic alteration, we sequenced the entire *ARMC5* gene in 39 patients with confirmed PA and bilateral thickened/nodular adrenals at CT scan.

We identified 11 common variants in our PA patients cohort, 5 rare variants with a minor allele frequency $<1\%$ and 2 new variants previously unreported in public databases.

We were unable to detect any *ARMC5* sequence variations predicted to damage its function by *in silico* analysis. Limitations of our study are the relatively small number of patients investigated and the fact that our cohort included only two patients of African descent: therefore, we may need a much larger number of these patients to identify *ARMC5* mutations with a potentially damaging functional effect in this ethnicity, as reported by Zilbermint *et al.*[14] Concerning the clinical phenotype, the prevalence of metabolic syndrome was confirmed to be high in our PA population,[30] as previously observed.[31]

In conclusion, our study shows that *ARMC5* mutations affecting highly conserved nucleotides and/or influencing protein function are not present in a fairly large series of Caucasian patients with PA associated to bilateral adrenal alterations. Further studies are required to definitively clarify the role of *ARMC5* in the pathogenesis of adrenal nodules and aldosterone overproduction in patients with PA.

What is known about this topic?

- Heterozygous germline mutations of *armadillo repeat containing 5* (*ARMC5*) have been shown to be associated with hypercortisolism due to sporadic primary bilateral macronodular adrenal hyperplasia.
- Germline mutations of *ARMC5* have been shown to be also observed in African-American patients with primary aldosteronism.

What this study adds?

- In Caucasian patients with primary aldosteronism who had bilateral computed tomography (CT)-detectable adrenal alterations, we did not observe germline *ARMC5* mutations predicted to alter protein function by *in silico* analysis.
- Patients with primary aldosteronism and bilateral adrenal lesions do not display frequently germline mutations in *ARMC5*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L *et al.* Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. *J Clin Endocrinol Metab* 2004; **89**: 1045–1050.
2 Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M *et al.* Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008; **93**: 3266–3281.
3 Monticone S, Viola A, Rossato D, Veglio F, Reincke M, Gomez-Sanchez C *et al.* Adrenal vein sampling in primary aldosteronism: towards a standardised protocol. *Lancet Diabetes Endocrinol* 2015; **3**: 296–303.
4 Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C *et al.* K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science* 2011; **331**: 768–772.
5 Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD *et al.* Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet* 2013; **45**: 440–444 444e1-2.
6 Scholl UI, Goh G, Stölting G, de Oliveira RC, Choi M, Overton JD *et al.* Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet* 2013; **45**: 1050–1054.

7 Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A *et al.* Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet* 2013; **45**: 1055–1060.
8 Monticone S, Else T, Mulatero P, Williams TA, Rainey WE. Understanding primary aldosteronism: impact of next generation sequencing and expression profiling. *Mol Cell Endocrinol* 2015; **399**: 311–320.
9 Mulatero P, Monticone S, Rainey WE, Veglio F, Williams TA. Role of KCNJ5 in familial and sporadic primary aldosteronism. *Nat Rev Endocrinol* 2013; **9**: 104–112.
10 Sukor N, Mulatero P, Gordon RD, So A, Duffy D, Bertello C *et al.* Further evidence for linkage of familial hyperaldosteronism type II at chromosome 7p22 in Italian as well as Australian and South American families. *J Hypertens* 2008; **26**: 1577–1582.
11 Scholl UI, Stölting G, Nelson-Williams C, Vichot AA, Choi M, Loring E *et al.* Recurrent gain of function mutation in calcium channel CACNA1H causes early-onset hypertension with primary aldosteronism. *Elife* 2015; **4**: e06315.
12 Assié G, Libé R, Espiard S, Rizk-Rabin M, Guimier A, Luscap W *et al.* ARMC5 mutations in macronodular adrenal hyperplasia with Cushing's syndrome. *N Engl J Med* 2013; **369**: 2105–2114.
13 Faucz FR, Zilbermint M, Lodish MB, Szarek E, Trivellin G, Sinaii N *et al.* Macronodular adrenal hyperplasia due to mutations in an armadillo repeat containing 5 (ARMC5) gene: a clinical and genetic investigation. *J Clin Endocrinol Metab* 2014; **99**: E1113–E1119.
14 Zilbermint M, Xekouki P, Faucz FR, Berthon A, Gkourogianni A, Helene Schernthaner-Reiter M *et al.* Primary aldosteronism and ARMC5 variants. *J Clin Endocrinol Metab* 2015; **100**: E900–E909.
15 Williams TA, Monticone S, Schack VR, Stindl J, Burrello J, Buffolo F *et al.* Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. *Hypertension* 2014; **63**: 188–195.
16 Fallo F, Bertello C, Tizzani D, Fassina A, Boulkroun S, Sonino N *et al.* Concurrent primary aldosteronism and subclinical cortisol hypersecretion: a prospective study. *J Hypertens* 2011; **29**: 1773–1777.
17 Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S *et al.* Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension* 2014; **64**: 354–361.
18 den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 2000; **15**: 7–12.
19 Mulatero P, Tizzani D, Viola A, Bertello C, Monticone S, Mengozzi G *et al.* Prevalence and characteristics of familial hyperaldosteronism: the PATOGEN study (Primary Aldosteronism in TOrino-GENetic forms). *Hypertension* 2011; **58**: 797–803.
20 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486–2497.
21 Monticone S, Hattangady NG, Nishimoto K, Mantero F, Rubin B, Cicala MV *et al.* Effect of KCNJ5 mutations on gene expression in aldosterone-producing adenomas and adrenocortical cells. *J Clin Endocrinol Metab* 2012; **97**: E1567–E1572.
22 Oki K, Plonczynski MW, Lam ML, Gomez-Sanchez EP, Gomez-Sanchez CE. Potassium channel mutant KCNJ5 T158A expression in HAC-15 cells increases aldosterone synthesis. *Endocrinology* 2012; **153**: 1774–1782.
23 Gomez-Sanchez CE. Channels and pumps in aldosterone-producing adenomas. *J Clin Endocrinol Metab* 2014; **99**: 1152–1156.
24 Gomez-Sanchez CE, Kuppusamy M, Gomez-Sanchez EP. Somatic mutations of the ATP1A1 gene and aldosterone-producing adenomas. *Mol Cell Endocrinol* 2015; **408**: 213–219.
25 Williams TA, Monticone S, Morello F, Liew CC, Mengozzi G, Pilon C *et al.* Teratocarcinoma-derived growth factor-1 is upregulated in aldosterone-producing adenomas and increases aldosterone secretion and inhibits apoptosis in vitro. *Hypertension* 2010; **55**: 1468–1475.
26 Connell JM, Fraser R, MacKenzie SM, Friel EC, Ingram MC, Holloway CD *et al.* The impact of polymorphisms in the gene encoding aldosterone synthase (CYP11B2) on steroid synthesis and blood pressure regulation. *Mol Cell Endocrinol* 2004; **217**: 243–247.
27 Mulatero P, Schiavone D, Fallo F, Rabbia F, Pilon C, Chiandussi L *et al.* CYP11B2 gene polymorphisms in idiopathic hyperaldosteronism. *Hypertension* 2000; **35**: 694–698.
28 McManus F, Sands W, Diver L, MacKenzie SM, Fraser R, Davies E *et al.* APEX1 regulation of aldosterone synthase gene transcription is disrupted by a common polymorphism in humans. *Circ Res* 2012; **111**: 212–219.
29 Tewari R, Bailes E, Bunting KA, Coates JC. Armadillo-repeat protein functions: questions for little creatures. *Trends Cell Biol* 2010; **20**: 470–481.
30 Berthon A, Drelon C, Ragazzon B, Boulkroun S, Tissier F, Amar L *et al.* WNT/β-catenin signalling is activated in aldosterone-producing adenomas and controls aldosterone production. *Hum Mol Genet* 2014; **23**: 889–905.
31 Fallo F, Veglio F, Bertello C, Sonino N, Della Mea P, Ermani M *et al.* Prevalence and characteristics of the metabolic syndrome in primary aldosteronism. *J Clin Endocrinol Metab* 2006; **91**: 454–459.

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