Absence of somatic mutations in natriuretic peptide receptor type A gene in human aldosterone-secreting adenomas

R Sarzani, F Salvi, F Pietrucci, L Buglia, C Candelaresi, B Balducci, M Francioni, F Fazioli, C Letizia¹, E D'Erasmo¹, P Dessì-Fulgheri and A Rappelli

Department of Internal Medicine, University of Ancona, Ancona, Italy

¹Department of Clinical Science, University of Rome 'La Sapienza', Rome, Italy

(Requests for offprints should be addressed to R Sarzani, Clinica di Medicina Interna, Polo Ospedaliero-Universitario, Via Conca, 60020 Ancona, Italy; Email: sarzani@unian.it)

Abstract

Somatic mutations of genes codifying for key regulatory proteins are the cause of different types of hormone-secreting adenomas. Natriuretic peptides (NP) are the strongest inhibitors of aldosterone secretion but aldosterone-secreting adenomas (aldosteronomas) are resistant to this inhibition and have reduced binding sites for NPs. The objective of this study was to sequence the entire coding region of the NP receptor type A (NPRA, codified by the *Npr1* gene) to find loss-of-function somatic mutations.

Total RNA was extracted from eight aldosteronomas and cDNA was synthesized. NPRA mRNA expression was evaluated by Northern blot analysis and compared with β -actin mRNA as the housekeeping gene. Twelve primer couples were designed on the basis of the *Npr1* gene organization to amplify, by PCR, all 22 coding exons of the gene. The two strands of amplified DNAs were purified and directly sequenced by automated capillary sequencer.

NPRA mRNA expression did not differ among aldosteronomas. *Npr1* open reading frame sequences obtained from eight aldosteronomas did not contain any mutation. The coding sequences of all 22 exons were identical in all samples and identical to published sequences. In the 3'-untranslated region (3'-UTR) a new length difference 3C/4C polymorphism was found at position 15 129 (three adenomas were 3C/4C and two were 3C/3C). Such a 3C/4C polymorphism was present in genomic DNA from 80 control subjects (25, 4C/4C; 40, 3C/4C; 15, 3C/3C).

Mutations in the coding exons of the *Npr1* gene do not appear to be a common cause of aldosteronomas. Moreover, the exons of *Npr1* encoding for the translated portion of mRNA do not appear to be prone to polymorphisms. The polymorphism identified in the 3'-UTR might affect mRNA stability resulting in lower receptor synthesis, but it is not likely to confer a predisposition to the development of aldosteronomas.

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Introduction

Aldosterone-secreting adenomas (aldosteronomas) are a common cause of primary aldosteronism in humans. These 'benign' endocrine tumours cause deadly cardiovascular complications due to hypertension and hypokalaemia, and arise from adrenal cortical cells by unknown molecular mechanisms.

It is well known that some hormone-secreting adenomas originate from somatic mutations of key regulatory proteins involved in the growth and function of endocrine cells. Activating mutations of the thyroid-stimulating hormone receptor in toxic thyroid adenomas (Parma *et al.* 1993), and gain-of-function mutations of G protein α_s subunit in both toxic thyroid adenomas (Parma *et al.* 1997) and growth hormone-secreting pituitary adenomas

Figure 1 (A) Genomic structure of the human *Npr1* gene; the exons are represented by black boxes and totalled 22. (B) Example of primer design: each primer pair was designed to amplify overlapping portions of coding sequences to obtain double-strand sequences of all amplified portions.

(Ballare *et al.* 1998) have been found. In these adenomas cAMP cascade permanent activation maintains cell differentiation, confers proliferative advantage and stimulates hormone secretion.

Following a similar reasoning, we (Sarzani *et al.* 1995) and others (Davies *et al.* 1997, Sachse *et al.* 1997) have analysed angiotensin II receptor 1 (AT1) gene expression and sequence in aldosteronomas according to the evidence that angiotensin II is the main stimulator of growth and function of normal aldosterone-secreting adrenal cells. The AT1 gene was expressed in aldosteronomas similarly to normal adrenals (Sarzani *et al.* 1995), explaining the angiotensin II responsiveness of some of these adenomas, but no somatic mutations were detected. Other authors have focused on the adrenocorticotrophin (ACTH) receptor gene because of the role of ACTH in the stimulation of aldosterone secretion but, again, no mutations were found (Latronico *et al.* 1995).

Inactivating mutations of genes whose products mediate an inhibitory control on aldosterone secretion might also be involved in the molecular genesis of aldosteronomas. Cardiac natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are potent inhibitors of aldosterone secretion (Anderson *et al.* 1986, Holmes *et al.* 1993), being effective antagonists of angiotensin II. The effects of both ANP and BNP on aldosterone secretion are mediated by natriuretic peptide receptor type A (NPRA, codified by the *Npr1* gene), a membrane guanylyl cyclase with cGMP being the main intracellular messenger (Maack *et al.* 1996).

In patients with aldosteronomas, plasma levels of ANP (Yamaji *et al.* 1986, Opocher *et al.* 1992) and BNP (Mukoyama *et al.* 1990) are increased, but aldosterone secretion appears to be unaffected by these higher levels of natriuretic peptides. Moreover, ANP infusion in patients with aldosteronomas

Table 1 Clinical features and laboratory data of patients with aldosteronomas

Patient no.	Age (years)	Sex	K ⁺ (mEq/l)	Blood pressure at admission (mmHg)	Plasma renin activity (standing) (ng/ml per h)	Plasma aldosterone (standing) (ng/dl)	Urinary aldosterone (µg/24 h)
A	59	M	3.8	165/110	0.2	47	9.2
B	45	F	2.2	170/100	0.001	21.8	11.9
C	56	F	3.5	165/105	0.3	23.1	20.1
D	28	F	3.0	160/110	0.3	39.7	25.3
E	35	M	3.0	170/110	0.2	31.2	34
F	38	M	2.2	170/120	0.2	47	26.1
G	54	M	3.2	180/100	0.2	18	39.8
H	39	M	3.6	180/120	0.1	36.9	8.1

(Rocco *et al.* 1990) and ANP added to aldosteronoma cells *in vitro* (Higuchi *et al.* 1986a, Rocco *et al.* 1989) did not reduce aldosterone secretion, suggesting an unresponsiveness of aldosteronoma cells to natriuretic peptides.

The unresponsiveness of aldosterone-secreting adenomas to ANP might be due to reduced expression of NPRA and/or to its structural alterations. We have previously found that, in most cases, the genes for natriuretic peptide receptors are expressed in adrenals and in aldosteronomas without significant differences (Sarzan *et al.* 1999). Despite these findings, ANP binding was reduced in aldosteronomas in comparison with surrounding adrenal glomerulosa tissues (Sarzan *et al.* 1999).

We have therefore hypothesized that a somatic mutation of the NPRA gene might be responsible for the reduced ANP binding and/or reduced biological responsiveness, conferring a proliferative advantage and an increased aldosterone secretion to aldosteronoma cells.

The human *Npr1* gene is located on chromosome 1q21–22 and is composed of 22 exons spanning an area of 16 kb (Fig. 1A) (Takahashi *et al.* 1998). The primary objective of our project was to analyse the coding sequences of the NPRA-encoding gene in search of functional inactivating mutations.

Materials and methods

Patients and controls

Eight patients (five men and three women) with primary aldosteronism were found to have a secreting adrenal adenoma. The diagnosis of

aldosterone-secreting adenomas was based on the full clinical picture and follow-up, including endocrine tests. Table 1 shows some of the clinical and humoral characteristics of the patients studied and histology of the sections is shown in Fig. 2. After surgery, all adenomas were sectioned and examined by an expert pathologist and a portion of each adenoma was quickly frozen in liquid nitrogen and kept at -80°C until RNA extraction.

A healthy portion of a human kidney with a localized upper polar renal carcinoma was obtained at nephrectomy and used as control RNA/cDNA. We also obtained, after written informed consent, blood samples from 80 control subjects (40 males and 40 females) to verify, at the genomic level, the presence and the frequency of the gene variants identified.

Extraction of RNA from adrenal tissue

Total RNA was extracted from tissue after homogenization in guanidine thiocyanate buffer and CsCl gradient as previously described (Sarzan *et al.* 1992). RNA was solubilized in RNase-free water and stored at -80°C . First-strand cDNA was synthesized from 1.25 µg total RNA which together with oligo-deoxythymidine primer (0.25 µg) and random primers (0.25 µg) were kept for 5 min at 72°C . Then, $5 \times$ reaction buffer (Tris-HCl, 5 mmol/l, pH 8.3; KCl, 7.5 mmol/l; MgCl₂, 150 µmol/l), deoxy(d)-NTP (1 mol/l), dithiothreitol (10 mmol/l), bovine serum albumin (2 µg), RNase inhibitor (15.7 U; Amersham Pharmacia Biotech, Milan, Italy) and Moloney murine leukaemia virus reverse transcriptase (400 U; Gibco-BRL, Life

Figure 2 (A) Macroscopic section of an aldosterone-secreting adrenal adenoma (patient H); the tumour is sharply demarcated and yellow-orange; the remaining adrenal gland is grossly normal. (B) Histology of the section shows tumour cells with a nesting pattern and lipid-rich cytoplasm (courtesy of M Scarpelli).

Technologies, Inc., Gaithersburg, MD, USA) were added to obtain a final volume of 25 μ l. Incubation was performed for 1 h at 38 °C.

Extraction of genomic DNA

Genomic DNA was isolated from the blood samples of 80 control subjects with a commercially available DNA extraction kit (QIAamp DNA Blood Midi kit; QIAGEN, Hilden, Germany). The quantity of DNA was evaluated by spectrophotometry and gel analysis.

Northern blot analysis

Aliquots of 15 μ g total RNA were resolved by electrophoresis on 1% agarose gel under denaturing conditions (formamide/formaldehyde) and triplicate blots were performed as previously described (Sarzani *et al.* 1999). In brief, nucleic acid was transferred to nylon membrane (Hybond-N⁺; Amersham Pharmacia Biotech), cross-linked by ultraviolet irradiation and baked at 80 °C. Membranes were hybridized with ³²P-labelled cDNA fragments corresponding to *Npr1* exons (2, 3 and 4,

Table 2 Primer pairs used for amplification of the human NPRA cDNA

Primer pair no.	Exons amplified	Position	Sequence	cDNA fragment size (bp)	Annealing (°C)
1	1 (5'-UTR)	719–739 1211–1185	5'-GCCTGATGCCTGGACCGGCC-3' 5'-CGGGACCCGCCAGTGC GCGGTGAAGCG-3'	493	62
2	1	807–833 1211–1185	5'-ATGCCGGGGCCCGCGCCCGCTGGCT-3' 5'-CGGGACCCGCCAGTGC GCGGTGAAGCG-3'	405	62
3	1	1162–1180 1527–1509	5'-TGACGCCCGCCGCCAGT-3' 5'-CTCGGCCTTTGCGCGGCAT-3'	366	70
4	2–3–4	1473–1493 4233–4211	5'-GACGACCTCAGCCACTACACC-3' 5'-ATCGCCACTGCTATCAATTTTCA-3'	543	64
5	5–6–7–8	3476–3499 7531–7508	5'-TGGGGGAACTGTTACTGATGGGA-3' 5'-TAGCAGGGAGCCGTAATTGAGCC-3'	523	60
6	9–10–11–12	6717–6740 8926–8903	5'-GGACGTTAGCCCCAGTAGCCTTGA-3' 5'-CAGATAGCCCCATTGTAGAAAC-3'	429	64
7	13–14–15	8755–8778 10 676–10 653	5'-GTACTCACTACCAATGACATCGT-3' 5'-CAGGTTGCCAGGATGTTGCTGCT-3'	541	60
8	16–17–18	9856–9879 11 537–11 514	5'-GGACCCACAGGAGAGGCCACCAT-3' 5'-CCCTGACACCACCTAGTAGGCATC-3'	439	60
9	19–20–21–22	11 185–11 208 15 124–15 101	5'-GCTGTTCATAGACAACTTTGATGTG-3' 5'-GGTGTGGAGGATAGGAGGAGGAGG-3'	487	60
10	22 (3'-UTR)	15 049–15 069 15 538–15 515	5'-CGGACCTACTGGCTCCTTGGG-3' 5'-TTGTGGGCCAGCCCCAGATGTGGG-3'	490	62
11	22 (3'-UTR)	15 427–15 447 15 711–15 691	5'-GCCTTGCTACCCCTGTGACTTA-3' 5'-TTCTGGCAACCCCTCTCTCCCT-3'	290	60
12	(3'-UTR)	15 622–15 639 15 810–15 793	5'-TGATGCCCTTGCTTCTTAC-3' 5'-GTTAAGAATCGAGAGTCT-3'	189	67

UTR, untranslated region. Position number according to GenBank accession number AF190631.

Figure 3 (A) Comparative Northern analysis of NPRA mRNA in our aldosteronomas; the control housekeeping gene (β -actin) expression is shown in the lower panel. (B) The histograms represent the NPRA expression normalized by β -actin in our adenomas; values are in ADU. The mean $\pm$ s.d. expression value was 0.67 ± 0.03 ADU ($\pm 4\%$ expression level).

amplified by primer pair number 4, see Table 2) under stringent conditions in a hybridization oven (HB-1 Hybridiser; Techne Ltd, Cambridge, Cambs, UK) at 68°C . The radioactive probes were prepared with a Rediprime DNA labelling kit (Amersham Pharmacia Biotech) and Redivue [α - ^{32}P]dCTP (10 mCi/ml, 3000 Ci/mmol; Amersham Pharmacia Biotech).

Membranes were washed and autoradiographed by standard procedures. Blots were exposed to Hyperfilm ECL (Amersham Pharmacia Biotech) for 4 h to 4 days at -80°C . Bound cDNA probes were removed by boiling for 10 min in 0.1% SDS, and the same membranes were re-hybridized with a control probe synthesized from a β -actin cDNA.

A semiquantitative analysis of NPRA expression was performed using arbitrary density units (ADU) of the housekeeping β -actin gene.

Amplification of *Npr1* cDNA

All 22 exons were amplified by PCR using the primers shown in Table 2. Primers were designed to amplify partially overlapping portions of NPRA cDNA (Fig. 1B). Each primer pair was used to amplify three or four exons, whereas exon 1, because of its length, was amplified with three primer pairs.

PCR was carried out in a total volume of 50 μl , from 50–100 ng cDNA or genomic DNA, $10 \times$ reaction buffer (KCl, 5 mmol/l; MgCl_2 , 150 $\mu\text{mol/l}$;

Figure 4 PCR products obtained from eight of the twelve primer pairs. PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. M.W.M., molecular weight markers ($\odot \times 174$ RF Hae III digest).

Tris-HCl, 1 mmol/l, pH 9), dNTPs (0.2 mmol/l each), Taq (2.5 U; Amersham Pharmacia Biotech), with 50 ng of each primer. Thirty-five amplification cycles were performed, consisting of denaturation at 95 °C for 1 min, annealing (see Table 2) and elongation at 72 °C for 1.5 min.

An initial denaturation step was carried out at 95 °C for 5 min and a final elongation was carried out at 72 °C for 7 min. These amplifications were performed using the DNA Thermal Cycler (Perkin Elmer Cetus) and PCR products were analysed by gel electrophoresis using 2% Seakem LE agarose (BioWhittaker Molecular Applications, Rockland, ME, USA).

Direct sequencing

The PCR products were purified by agarose gel electrophoresis and eluted using QIAquick Gel Extraction Kit (QIAGEN). Cycle sequencing (96 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min, for 25 cycles) was carried out on both strands using DNA sequencing kit Big Dye terminator Cycle sequencing Ready Reaction kit (Applied Biosystems, Warrington, Cheshire, UK) and the thermal cycler PCR Sprint (Hybaid). The reactions were purified by Centri-Sep Spin Columns (ABI

PRISM; Applied Biosystems, Foster City, CA, USA), dried, resuspended in TSR buffer (ABI PRISM; Applied Biosystems) and subjected to automated capillary sequencing (ABI PRISM 310 Genetic Analyzer; Applied Biosystems). Sequences were analysed on electropherograms with Sequencing Analysis (ABI PRISM 3.3 software; Applied Biosystems) and Sequence Navigator *f* (Applied Biosystems) software for Macintosh.

Results

The NPRA gene was expressed in all aldosteronomas analysed as shown by PCR (data not shown) and Northern blot analysis (Fig. 3A); we also evaluated, by Northern blot analysis, the NPRA expression levels which were similar among the adenomas studied (Fig. 3B). A representative PCR of eight different portions of the *Npr1* cDNA is shown in Fig. 4. We amplified and sequenced a total of 3988 *Npr1* cDNA bp from each aldosteronoma. *Npr1* open reading frame sequences obtained from the eight adenomas studied did not contain any mutation, being identical among all aldosteronomas, control kidney tissue and deposited sequence (GenBank, accession number

Figure 5 Direct sequencing of the PCR products in three patients around position 15 129 of the 3'-UTR of the NPRA cDNA showing (A) one homozygous 4C/4C, (B) one homozygous 3C/3C and (C) one heterozygous 3C/4C.

AF190631). Moreover, no mutations were detected with the first primers pair (Table 2) which spanned nucleotides -88 to 405 of the 5'-UTR encoded by exon 1.

A length difference 3C/4C polymorphism was detected with primer pair number 10 at position 15 129 in the 3'-UTR, encoded by exon 22, 30 bp downstream of the stop codon (Fig. 5). Sequencing analysis revealed that patients D and F were 3C/3C homozygous, whereas patients A, B, C, and the kidney sample were 3C/4C heterozygous and, finally, patients E, G and H were 4C/4C homozygous. To analyse whether this 3C/4C polymorphism was a somatic mutation in the adenomas or a polymorphic genomic site, we amplified and sequenced genomic DNA extracted from 80 healthy control subjects. This 3C/4C polymorphism was present in genomic DNA with a frequency of 50% for the heterozygous genotype, 31.25% for the homozygous (4C/4C) genotype and 18.75% for the homozygous (3C/3C) genotype,

according to the Hardy-Weinberg equilibrium, indicating the hereditary genomic nature of this polymorphism.

Discussion

Some endocrine tumours arise from gain-of-function mutations of key proteins (receptors or coupling proteins) that stimulate hormone secretion and cell growth. We hypothesized that, as a mirror image, aldosteronoma might arise from loss-of-function mutation(s) of key inhibitory proteins. Thus, we analysed eight human aldosteronomas searching for somatic mutation(s) in the *Npr1* gene. This gene is a likely candidate in the molecular pathogenesis of aldosteronomas because it mediates the potent ANP-dependent inhibition of glomerular adrenal cell secretion and growth (Higuchi *et al.* 1986b). The full-length direct double-strand sequence of the cDNAs synthesized from eight

aldosteronomas did not reveal any somatic mutation in *Npr1* exons. On the contrary, a remarkable stability of this sequence was found.

The only polymorphic site was a genomic length difference 3C/4C polymorphism at position 15 129 in the 3'-UTR (encoded by exon 22). The functional significance of this polymorphism in aldosteronomas is unknown and, because of its frequency in healthy subjects, it appears highly unlikely that it is a cause of aldosteronoma. It might affect mRNA stability (Misquitta *et al.* 2001) resulting in lower receptor synthesis (Knowles *et al.* 2003) but it is not likely that it confers a predisposition to the development of aldosteronomas (Pitzalis *et al.* 2003).

The only coding sequences that were not analysed were the starting 21 bp of the published full-length sequence (GenBank, accession number AF190631) due to upstream primer annealing. Because NPRA was normally and equally expressed in aldosteronomas (Fig. 2), it is also unlikely that somatic mutations in the regulatory regions of the gene were present.

Our results suggest that NPRA gene somatic mutation is unlikely to be a common cause of aldosteronomas, even though the apparent lack of inhibition of aldosterone secretion by natriuretic peptides remains to be explained. Other genes involved in the post-receptor mediation of the biological effect of natriuretic peptides might be involved, and post-translational defects cannot be excluded.

Key proteins involved in steroidogenesis as well as the multiple endocrine neoplasia type 1 gene (Zenkert *et al.* 2000, Zwermann *et al.* 2000) have also been excluded as causes of aldosteronomas. One case of a somatic $G_s\alpha$ mutation of uncertain functional significance among 30 functional adenomas was recently reported (Kobayashi *et al.* 2000). Overall, the search for functional somatic mutations as a cause of aldosteronomas is still wide open.

References

- Anderson JV, Struthers AD, Payne NN, Slater JD & Bloom SR 1986 Atrial natriuretic peptide inhibits the aldosterone response to angiotensin II in man. *Clinical Science* **70** 507–512.
- Ballare E, Mantovani S, Lania A, Di Blasio AM, Vallar L & Spada A 1998 Activating mutations of the $G_s\alpha$ gene are associated with low levels of $G_s\alpha$ protein in growth hormone-secreting tumors. *Journal of Clinical Endocrinology and Metabolism* **83** 4386–4390.
- Davies E, Bonnardeaux A, Plouin PF, Corvol P & Clauser E 1997 Somatic mutations of the angiotensin II (AT1) receptor gene are not present in aldosterone-producing adenoma. *Journal of Clinical Endocrinology and Metabolism* **82** 611–615.
- Higuchi K, Nawata H, Kato K, Ibayashi H & Matsuo H 1986a Lack of inhibitory effect of alpha-human atrial natriuretic polypeptide on aldosteronogenesis in aldosterone-producing adenoma. *Journal of Clinical Endocrinology and Metabolism* **63** 192–196.
- Higuchi K, Nawata H, Kato K, Ibayashi H & Matsuo H 1986b Alpha-human atrial natriuretic polypeptide inhibits steroidogenesis in cultured human adrenal cells. *Journal of Clinical Endocrinology and Metabolism* **62** 941–944.
- Holmes SJ, Espiner EA, Richards AM, Yandle TG & Frampton C 1993 Renal, endocrine, and hemodynamic effects of human brain natriuretic peptide in normal man. *Journal of Clinical Endocrinology and Metabolism* **76** 91–96.
- Knowles JW, Erickson LM, Guy VK, Sigel CS, Wilder JC & Maeda N 2003 Common variations in noncoding regions of the human natriuretic peptide receptor A gene have quantitative effects. *Human Genetics* **112** 62–70.
- Kobayashi H, Usui T, Fukata J, Yoshimasa T, Oki Y & Nakao K 2000 Mutation analysis of $G_s\alpha$, adrenocorticotropin receptor and p53 genes in Japanese patients with adrenocortical neoplasms: including a case of $G_s\alpha$ mutation. *Endocrine Journal* **47** 461–466.
- Latronico AC, Reincke M, Mendonca BB, Arai K, Mora P, Allolio B, Wajchenberg BL, Chrousos GP & Tsigos C 1995 No evidence for oncogenic mutations in the adrenocorticotropin receptor gene in human adrenocortical neoplasms. *Journal of Clinical Endocrinology and Metabolism* **80** 875–877.
- Maack T, Nikonova LN, Friedman O & Cohen D 1996 Functional properties and dynamics of natriuretic peptide receptors. *Proceedings of the Society for Experimental Biology and Medicine* **213** 109–116.
- Misquitta CM, Iyer VR, Werstuijk ES & Grover AK 2001 The role of 3'-untranslated region (3'-UTR) mediated mRNA stability in cardiovascular pathophysiology. *Molecular and Cellular Biochemistry* **224** 53–67.
- Mukoyama M, Nakao K, Saito Y, Ogawa Y, Hosoda K, Suga S, Shirakami G, Jougasaki M & Imura H 1990 Human brain natriuretic peptide, a novel cardiac hormone. *Lancet* **335** 801–802.
- Opocher G, Rocco S, Carpena G, Vettoretti A, Cimolati M & Mantero F 1992 Usefulness of atrial natriuretic peptide assay in primary aldosteronism. *American Journal of Hypertension* **5** 811–816.
- Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J & Vassart G 1993 Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* **365** 649–651.
- Parma J, Duprez L, Van Sande J, Hermans J, Rocmans P, Van Vliet G, Costagliola S, Rodien P, Dumont JE & Vassart G 1997 Diversity and prevalence of somatic mutations in the thyrotropin receptor and $G_s\alpha$ genes as a cause of toxic thyroid adenomas. *Journal of Clinical Endocrinology and Metabolism* **82** 2695–2701.
- Pitzalis MV, Sarzani R, Dessi-Fulgheri P, Iacoviello M, Forleo C, Lucarelli K, Pietrucci F, Salvi F, Sorrentino S, Romito R, Guida P, Rappelli A & Rizzon P 2003 Allelic variants of natriuretic peptide receptor genes are associated with family history of hypertension and cardiovascular phenotype. *Journal of Hypertension* **21** 1491–1496.
- Rocco S, Opocher G, D'Agostino D, Leone L & Mantero F 1989 Lack of aldosterone inhibition by atrial natriuretic factor in primary aldosteronism: *in vitro* studies. *Journal of Endocrinological Investigation* **12** 13–17.
- Rocco S, Opocher G, Carpena G & Mantero F 1990 Atrial natriuretic peptide infusion in primary aldosteronism. Renal, hemodynamic and hormonal effects. *American Journal of Hypertension* **3** 668–673.

- Sachse R, Shao XJ, Rico A, Finckh U, Rolfs A, Reincke M & Hensen J 1997 Absence of angiotensin II type 1 receptor gene mutations in human adrenal tumors. *European Journal of Endocrinology* **137** 262–266.
- Sarzani R, Fallo F, Dessì-Fulgheri P, Pistorello M, Lanari A, Paci VM, Mantero F & Rappelli A 1992 Local renin–angiotensin system in human adrenals and aldosteronomas. *Hypertension* **19** 702–707.
- Sarzani R, Opocher G, Dessì-Fulgheri P, Paci V, Cola G, Rocco S, Vianello B, Mantero F & Rappelli A 1995 Expression of type 1 angiotensin II receptors in human aldosteronomas. *Endocrine Research* **21** 189–195.
- Sarzani R, Opocher G, Paci MV, Belloni AS, Mantero F, Dessì-Fulgheri P & Rappelli A 1999 Natriuretic peptides receptors in human aldosterone-secreting adenomas. *Journal of Endocrinological Investigation* **22** 514–518.
- Takahashi Y, Nakayama T, Soma M, Izumi Y & Kanmatsuse K 1998 Organization of the human natriuretic peptide receptor A gene. *Biochemical and Biophysical Research Communication* **246** 736–739.
- Yamaji T, Ishibashi M, Sekihara H, Takaku F, Nakaoka H & Fujii J 1986 Plasma levels of atrial natriuretic peptide in primary aldosteronism and essential hypertension. *Journal of Clinical Endocrinology and Metabolism* **63** 815–818.
- Zenkert S, Schubert B, Fassnacht M, Beuschlein F, Allolio B & Reincke M 2000 Steroidogenic acute regulatory protein mRNA expression in adrenal tumours. *European Journal of Endocrinology* **142** 294–299.
- Zwermann O, Beuschlein F, Mora P, Weber G, Allolio B & Reincke M 2000 Multiple endocrine neoplasia type 1 gene expression is normal in sporadic adrenocortical tumors. *European Journal of Endocrinology* **142** 689–695.

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