A German family with glucocorticoid-remediable aldosteronism

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Abstract

Background. The prevalence of primary hyperaldosteronism (PHA) in the hypertensive population has increased in recent years. Glucocorticoid-remediable aldosteronism (GRA) is a rare monogenic form of PHA. Here we report a German family with GRA. Since the phenotype of GRA varies widely, we asked whether recommended algorithms for PHA diagnosis distinguish GRA from other forms of PHA.

Methods. Plasma aldosterone (pg/ml) and renin (pg/ml) levels were determined in three hypertensive family members with GRA before and after sodium loading with 2 l of saline (0.9%), during posture and after 1 week of 2 mg dexamethasone daily. 24 h blood pressure and urinary excretion of aldosterone, cortisol precursors and metabolites were measured before and after dexamethasone. Southern blot hybridization and long-range PCR were performed to identify the chimeric gene.

Results. All three affected patients had normal potassium levels but markedly increased aldosterone/renin ratios of 472, 213 and $>322$ (normal range <50) indicating PHA. Sodium loading failed to lower plasma aldosterone below the threshold of 50 pg/ml in all patients. During posture, aldosterone increased in one but decreased in both other GRA patients. Elevated 18-hydroxycortisol, free aldosterone and its main metabolite aldosterone-18-glucuronid and tetrahydroaldosterone returned to normal range after 1 week dexamethasone in all patients, but blood pressure was reduced only in one patient. The chimeric gene was identified in affected family members by Southern blot and PCR.

Conclusions. The aldosterone/renin ratio is a valid screening and sodium loading a valid confirmation test in GRA. Determination of elevated urinary excretion of specific aldosterone metabolites and identification of the chimeric gene are mandatory since a lacking blood pressure response to dexamethasone can be misleading.

Keywords: 18-OH-cortisol; ambulatory blood pressure; eplerenone; glucocorticoid remediable aldosteronism; primary hyperaldosteronism; secondary hypertension

Introduction

The prevalence of primary hyperaldosteronism (PHA) has dramatically increased in recent years, mainly as a result of more liberal screening [1,2]. The aldosterone/renin ratio is the most valuable screening test, since most patients with stringent biochemical criteria for PHA are normokalaemic [3]. A recent survey of the literature, including 12 studies with 6649 patients from five continents, suggests an overall prevalence of PHA of about 6.5% of the hypertensive population [4]. In the case of positive screening, standardized proof of PHA is necessary. Confirmation of PHA by sodium loading or other suppression tests is followed by procedures to distinguish between aldosterone-producing adenoma (APA) or bilateral adrenal hyperplasia (BAH). This includes adrenal imaging, posture tests and adrenal venous sampling. In spite of clear recommendations, a differential diagnosis can be difficult in an individual patient [5]. Glucocorticoid-remediable aldosteronism (GRA) is a rare monogenic form of PHA [6,7]. Pedigrees have been described in the USA [8], China [9], Japan [10] and Italy [11], but to the authors’ knowledge not in Germany, suggesting that the disease may be less common in this country. Here we describe a German family with GRA. It is well known that the phenotype of affected patients varies widely [12]. We therefore focus in this report on the question whether recommended algorithms for PHA diagnosis differentiate between GRA and other forms of PHA. Furthermore, since eplerenone, a new aldosterone antagonist with less side effects [13] is now
available, we tested its blood-pressure-lowering effectiveness in GRA.

Subjects and methods

Index patient (case 1)

An 18-year-old male student was admitted to our hospital for further evaluation of suspected PHA. He lived with his mother, who was divorced years back. There was no information about the blood pressure of his two brothers (22 and 26 years) and father (50 years), who lived in another city. At 15 years of age, a routine health examination had revealed a blood pressure of 150/90 mmHg. According to his mother, this had been dismissed by the primary physician. Three years later, the mother consulted a nephrologist who did a further work-up. At that time, a 24 h ambulatory blood pressure measurement (ABPM) revealed an average 24 h blood pressure of 156/98 mmHg with a heart rate of 65 bpm. There was no nocturnal dip. Plasma aldosterone concentration (PAC) was 580 pg/ml, renin 1.2 pg/ml, aldosterone/renin ratio (ARQ) 472; sodium 149 mmol/l, potassium 4.26 mmol/l; liver parameters as well as red and white blood cell counts were unremarkable. Renal parameters were normal, except for microalbuminuria of 61 mg/24 h (normal range <30 mg/24 h). Venous blood gas analysis was as follows: pH 7.344, pCO₂ 65.3 mmHg, pO₂ 27.5 mmHg, HCO₃ 34.8 mmol/l and base excess 6.3 mmol/l. Magnetic resonance imaging (MRI) of the abdomen revealed an enlarged left adrenal gland (Figure 1) with multiple small nodules (diameter 2-3 mm) which showed T2-weighted hyperintensity. The right adrenal was unremarkable. The patient was then admitted to our hospital for adrenal venous sampling (AVS), in order to prove suspected APA of the left adrenal. Hormone assays of AVS were as follows. Vena cava inferior: aldosterone (A) 572 pg/ml, cortisol (C) 29 µg/ml, A/C ratio 19.8; right adrenal vein: aldosterone (A) 588 pg/ml, cortisol (C) 25 µg/dl, A/C ratio 23.4; left adrenal vein: aldosterone (A) 52300 pg/ml, cortisol (C) 582 µg/dl, A/C ratio 89.9; vena cava superior: aldosterone (A) 1660 pg/ml, cortisol (C) 39 µg/dl, A/C ratio 42.5. The ratio of A/C left to right adrenal vein was 3.8, and that of left to vena cava inferior was 4.5. This was at first glance suggestive for an APA of the left adrenal; however, BAH could not be ruled out since the C/C ratio of right adrenal vein to vena cava inferior was below 1.1 (0.86), indicating that catheterization of the right adrenal was unsuccessful [14]. At the same time, the 22-year-old brother of the index patient was admitted to another hospital with an idiopathic paresis of the nervus facialis. Thereafter the father was contacted for further family history of hypertension (Figure 2).

Case 2

A 22-year-old male electronic engineer, the older brother of case 1, had a 4-year history of hypertension, but had never been treated. In December 2004, he suffered from idiopathic paresis of the nervus facialis during an episode of excessive blood pressure elevation. No stroke was detected by MRI, and antihypertensive treatment with urapidil, bisoprolol and benazepril was initiated without any blood-pressure-lowering effect. At admission to our hypertension unit, he had an average 24 h ABPM of 201/116 mmHg, and heart rate of 59 bpm without significant nocturnal dip (Figure 3). PAC was 166 pg/ml, renin <0.5 pg/ml, ARR >322; sodium 143 mmol/l, potassium 3.51 mmol/l; liver parameters as well as red and white blood cell counts were unremarkable. Renal parameters were normal except for microalbuminuria of 143 mg/24 h (normal range <30 mg/24 h). Venous blood gas analysis was as follows: pH 7.415, pCO₂ 44.5 mmHg, pO₂ 50.9 mmHg, HCO₃ 28 mmol/l and base excess 3.7 mmol/l. Computer tomography (CT) showed normal adrenals.

Case 3

A 50-year-old male, the father of cases 1 and 2, had a 10-year history of hypertension which had never been treated.
until December 2005. The patient reported office blood pressure levels of >200/100 mmHg. Except for a renal stone 20 years back, the patient’s history was unremarkable. On admission to our hypertension unit, he had an average 24 h ABPM of 183/113 mmHg and heart rate of 71 bpm. There was a significant nocturnal dip of 17%. PAC was 260 pg/ml, renin 1.22 pg/ml, ARR 213, sodium 144 mmol/l, potassium 4.01 mmol/l; liver parameters as well as red and white blood cell counts were unremarkable. Renal parameters were normal, except for microalbuminuria of 58 mg/24 h (normal range <30 mg/24 h). Venous blood gas analysis was as follows: pH 7.411, pCO2 47.2 mmHg, pO2 48.3 mmHg, HCO3 29.4 mmol/l and base excess 5.0 mmol/l. CT showed an enlarged medial portion of the left adrenal, otherwise normal size and appearance of both adrenals. Echocardiography showed concentric left ventricular hypertrophy with an interventricular septum thickness of 1.41 cm.

Sodium suppression and posture tests
All patients received 2 l of saline (0.9%) over 4 h from 8.00 a.m. to 12.00 p.m. PAC, renin and aldosterone and renin ratio (ARR) were determined before and after sodium loading. On a second day PAC, renin and ARR were determined before and after posture between 8.00 a.m. and 12.00 p.m.

Urinary excretion of aldosterone and cortisol precursors and metabolites
All steroids analysed were determined in the steroid laboratory of the Department of Pharmacology, University of Heidelberg, by in-house radioimmunoassays (RIA) using tritiated steroids (Amersham Biosciences, Freiburg, Germany) and specific antibodies, raised and characterized in the steroid laboratory, as described elsewhere [15]. Prior to RIAs, steroids were extracted from plasma or urine (pre-treated with β-glucuronidase) with organic solvents and chromatographically purified using Celite columns (Celite 545 AW; Sigma Aldrich, Taufkirchen, Germany).

Southern blot hybridization and long-range PCR
Restriction digestion of genomic DNA using BamHI, Southern blotting and hybridization of exon 1, 3–4 of the CYP11B1/2 was performed to identify the chimeric gene. Long-range PCR primers for the chimeric gene were as follows: Primer pair for S1 product: forward (F) primer CYP11B2 5'UTR GTG ATA TGT TTC CAG AGC AGG TTC; reverse (R) primer CYP11B2: GAG TCC TCC AGC TGC CTC TCA ACC. Primer pair for S2 product: F-primer CYP11B1-F 5'TCA TGC ACC CCC AAT GAG TCC CTG; R-primer CYP11B2-R 5'GAG TCC TCC AGC TGC CTC TCA ACC according to Jonsson et al. [29]. Long-range PCR-primers for sequencing the break point region: CYP11B1 5'UTR F 5'-GACGTGAATCCTTCTGAG; CYP11B1 + 2: R 5'-CCCATGGTGTCCCTTCC.

Results

Sodium suppression and posture tests
After sodium loading there was a slight increase of PAC in the index patient (case 1) but a drop in both of the other cases (Table 1). Nevertheless, sufficient suppression (<50 pg/ml) was not achieved. Cases 2 and 3 showed a paradoxical decrease of PAC in the posture test, whereas in the index patient (case 1) PAC rose by 34%.
Effect of dexamethasone on plasma aldosterone concentration, urinary excretion of aldosterone and cortisol precursors and metabolites

All patients received 2 mg of dexamethasone per os for 1 week. PAC decreased to 12 pg/ml in cases 1 and 2 and to 39 pg/ml in case 3. Twenty-four-hour urinary excretion of aldosterone and cortisol precursors and metabolites before and after 1 week of dexamethasone are shown in Table 2. In all patients, the highly elevated excretion of 18-hydroxycortisol, the best marker of GRA, decreased during dexamethasone treatment. Moreover, the excretion of free aldosterone and its main metabolites aldosterone-18-glucuronid and tetrahydroaldosterone returned to normal range or even lower.

Diagnosis by Southern blot hybridization and long-range PCR

After restriction digestion of genomic DNA using BamHI, Southern blotting and hybridization of exon 1, 3-4 of the CYP11B1/2 genes evidenced a 6.5 kb fragment (Figure 4A) indicating a chimeric gene in all three cases, but not in the mother and the normotensive 26-year-old brother. Finally, a 3256 bp fragment was amplified by long-range PCR primers, both specific for CYP11B1 and the chimeric genes in order to identify the 84 bp region comprising the break point (Figure 4C). Long-range PCR products of the chimeric gene were shown only for affected family members (Figure 4B).

Table 1. PAC and aldosterone (pg/mlg) and renin (ng/l) ratio (ARR; normal range < 50) before and after sodium loading as well as before and after posture

<table>
<thead>
<tr>
<th>Patient</th>
<th>PAC (pg/ml)</th>
<th>ARR</th>
<th>PAC (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium loading</td>
<td></td>
<td>Sodium loading</td>
</tr>
<tr>
<td></td>
<td>Pre/Post</td>
<td>Pre/Post</td>
<td>Pre/Post</td>
</tr>
<tr>
<td>Case 1</td>
<td>189/208</td>
<td>332/416</td>
<td>199/267</td>
</tr>
<tr>
<td>Case 2</td>
<td>161/124</td>
<td>&gt;322/&gt;248</td>
<td>155/89</td>
</tr>
<tr>
<td>Case 3</td>
<td>260/115</td>
<td>213/75</td>
<td>157/102</td>
</tr>
</tbody>
</table>

Table 2. Urinary precursors and metabolites of aldosterone and cortisol before and after dexamethasone 2 mg p.o. for 1 week in three patients with GRA

<table>
<thead>
<tr>
<th>Precursor/metabolite</th>
<th>Case 1 Pre</th>
<th>Case 1 Post</th>
<th>Case 2 Pre</th>
<th>Case 2 Post</th>
<th>Case 3 Pre</th>
<th>Case 3 Post</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-hydroxycorticosterone</td>
<td>28.0</td>
<td>2.0</td>
<td>8.8</td>
<td>1.2</td>
<td>13.2</td>
<td>0.9</td>
<td>1.5–6.5 µg/24h</td>
</tr>
<tr>
<td>18-hydroxycortisol</td>
<td>183.0</td>
<td>70.0</td>
<td>785.0</td>
<td>50.0</td>
<td>541.0</td>
<td>38.0</td>
<td>40–145 µg/24h</td>
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<tr>
<td>Aldosterone-18-glucuronid</td>
<td>64.0</td>
<td>2.0</td>
<td>28.7</td>
<td>3.0</td>
<td>28.0</td>
<td>4.0</td>
<td>3.5–17.5 µg/24h</td>
</tr>
<tr>
<td>Tetrahydroaldosterone</td>
<td>184</td>
<td>&lt;10</td>
<td>113</td>
<td>10</td>
<td>126</td>
<td>12</td>
<td>10–70 µg/24h</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>1.7</td>
<td>&lt;0.1</td>
<td>1.0</td>
<td>0.1</td>
<td>0.7</td>
<td>0.12</td>
<td>0.1–0.4 µg/24h</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>0.3</td>
<td>&lt;0.1</td>
<td>0.3</td>
<td>&lt;0.1</td>
<td>0.6</td>
<td>0.1</td>
<td>0.1–0.4 µg/24h</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>6.1</td>
<td>1.0</td>
<td>1.1</td>
<td>0.2</td>
<td>1.5</td>
<td>0.2</td>
<td>0.1–2.5 µg/24h</td>
</tr>
<tr>
<td>18-hydroxydeoxycorticosterone</td>
<td>3.3</td>
<td>&lt;0.1</td>
<td>2.6</td>
<td>&lt;0.1</td>
<td>2.2</td>
<td>0.1</td>
<td>0.2–1.8 µg/24h</td>
</tr>
<tr>
<td>Tetrahydrocorticison</td>
<td>6.7</td>
<td>0.1</td>
<td>4.2</td>
<td>0.1</td>
<td>2.2</td>
<td>0.2</td>
<td>0.5–5.5 mg/24h</td>
</tr>
<tr>
<td>Tetrahydrocortisol</td>
<td>3.6</td>
<td>&lt;0.1</td>
<td>3.5</td>
<td>0.1</td>
<td>1.9</td>
<td>0.1</td>
<td>0.5–3.5 mg/24h</td>
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<tr>
<td>Cortisone</td>
<td>146</td>
<td>&lt;10</td>
<td>101</td>
<td>&lt;10</td>
<td>122</td>
<td>&lt;10</td>
<td>20–140 µg/24h</td>
</tr>
<tr>
<td>Cortisol</td>
<td>69</td>
<td>&lt;10</td>
<td>29</td>
<td>&lt;10</td>
<td>27</td>
<td>&lt;10</td>
<td>10–60 µg/24h</td>
</tr>
</tbody>
</table>

Effect of dexamethasone and eplerenone on 24 h ABPM

Despite the marked reduction of PAC during 1 week of dexamethasone 2 mg per os, no substantial blood pressure reduction was observed in the 24 h ABPM in the index patient (case 1, Figure 5). Similarly, no effect on blood pressure was observed in case 3 (father) immediately after 1 week of treatment using dexamethasone (data not shown). In contrast, in case 2, after 1 week of dexamethasone, ABPM day time blood pressure declined by 44/22 mmHg. All patients were continued on 0.25 mg dexamethasone. The index patient showed significant blood pressure reduction after 8 weeks (Figure 5); however, the father’s 24 h ABPM remained at 170/108 mmHg after 8 weeks of oral dexamethasone 0.25 mg/day. The index patient was switched to eplerenone 50 mg o.d, which did not decrease blood pressure any further (Figure 5). Increasing the dose to 100 mg eplerenone normalized blood pressure of the index patient (case 1). Cases 2 and 3 required an addition of calcium antagonists to control blood pressure (data not shown).
Fig. 4. (A) Southern blot after restriction digest with BamHI of genomic DNA. A 6.5 kb fragment was detected in the index patient (case 1), Brother 1 (case 2), father (case 3), but not in the normotensive brother (Brother 2), the mother and controls (C1, C3). (B) Long-range PCR showed PCR products of the chimeric gene (lane S2) in the index patient, Brother 1 and the father, but not the other family members and the control. A PCR product for CYP11B2 (lane S1) was demonstrated in all subjects. (C) Sequencing data of PCR products to identify the break point of the chimeric gene. Nucleotide positions 108–162 CYP11B1, position 162–247 break point region, position 248–272 chimeric gene CYP11B1/2.
Discussion

Prevalence and screening

GRA, also termed familial hyperaldosteronism type I, was first described in 1966 [16] and is the consequence of unequal crossing-over of the genes encoding for aldosterone synthase and 11-β-hydroxylase [17]. The chimeric gene contains the promoter region of the 11-β-hydroxylase gene making the activity of the aldosterone synthase gene ACTH dependent. This autosomal dominant monogenic form of aldosteronism is thought to account for only 0.5–1.0% of primary aldosteronism [18]; however, the exact prevalence remains to be established. GRA patients have been identified worldwide. Many families in North America are of Celtic ancestry [6]. Cases have been reported for patients of Asian but not of African descent [9,10].

A recent genetic screening of a Polish population of 129 patients with PHA failed to detect GRA [19]. This suggests that random screening is not advisable [18,20]. Yet one can speculate that, in parallel to the observed increase of diagnosed PHA in all five continents [4], the prevalence of GRA may be higher than expected. Here we reported a German family with GRA of Silesian ancestry highlighting difficulties of differential diagnosis and treatment of PHA.

Differentiation of GRA from other forms of PHA

It is noteworthy to state that the diagnosis of PHA in the index patient was possible only because ARR screening was performed, although potassium levels were not indicative for PHA. Elevated ARR and PAC were followed by a structured work-up starting with a suppression test to confirm the diagnosis of PHA. As subsequently shown in all three cases, sodium loading did not lead to suppression of PAC below 50 pg/ml. In general, CT or MRI imaging is then performed to identify or exclude APA. However, selectivity and specificity of imaging is poor and adrenal vein sampling (AVS) is often required [14]. In our index patient, APA of the left adrenal was suspected; however, AVS was not selective. Insufficient catheterization occurs more often with the right than with the left adrenal vein, due to anatomical differences of the origin of the vessels. In our index patient, the suspected APA carrying left adrenal had an A/C left to right ratio of 3.8, comparable with that of A/C left to vena cava inferior (representative for peripheral venous blood) ratio of 4.5. This finding does not exclude the possibility of PHA due to BAH. In this situation, the posture test is recommended to achieve further clarification. In case of an APA, there should be a paradoxical decrease, which was not observed in the index patient. This was unexpected but lucky in the end, since the surgeon was already involved. Why is an increase of PAC in the posture test unexpected in GRA? The paradoxical decrease in APA is believed to result from ACTH responsiveness of aldosterone secretion from APA due to lower ACTH levels at 12.00 p.m. than at 8.00 a.m. This should be the same in GRA because aldosterone synthase activity in GRA is ACTH-dependent. We do not know why in our index patient there was a 34% increase of PAC during posture, whereas in cases 2 and 3 there was the expected decrease of PAC. There are no systematic investigations on the posture test in GRA.

Unfortunately, we did not record ACTH levels which might have explained the circumstances. Thus, it was more or less by chance that we were able to make the correct diagnosis of GRA because the brother (case 2) suffered from an idiopathic paresis of the nervus facialis during an episode of excessive blood pressure elevation, which led us to suspect inherited hypertension (Figure 2).

Dexamethasone suppression, aldosterone precursors and metabolites and genetic testing

The dexamethasone suppression test is the traditional screening test for GRA [21]; however, this test is not unequivocal, for several reasons. First, the blood pressure response may vary, as with the presented family. Although PAC decreased markedly in all cases, only in case 2 a significant blood pressure reduction was observed after high-dose dexamethasone for 1 week. Second, patients with BAH and APA may show a decrease of PAC during high-dose dexamethasone, provoking false positive diagnosis of GRA [22]. Litchfield et al. [21] suggested that a post-dexamethasone aldosterone level of <40 pg/ml will correctly diagnose GRA with high sensitivity.
and specificity. However, subsequent studies showed that a number of patients negative for the chimeric gene have a post-dexamethasone aldosterone of even <20 pg/ml [23,24]. As the dexamethasone suppression test cannot definitely distinguish APA from GRA, the diagnosis relies on the urinary excretion of abnormal steroid metabolites and genetic testing. We were able to show increased urinary excretion of 18-hydroxycortisol and other metabolites, the biochemical hallmarks of GRA. All abnormal values returned to normal range following high-dose dexamethasone. Although APA patients may have higher than normal excretion of 18-OH-cortisol and other abnormal metabolites, detection of high levels of 18-hydroxycortisol in the urine appears to be a specific test but requires 24-h urine collection. Recently, determination of plasma 18-hydroxycortisol was described as an alternative [22]. However, Southern blot hybridization or long-range PCR are indispensable to substantiate the definite diagnosis.

**Blood pressure and albuminuria: what is the best treatment?**

GRA is usually characterized by severe hypertension in early life [6]. Most of the chimeric gene carriers develop hypertension by the age of 13 years [25]. However, the penetrance of this phenotype is variable. Possible reasons include differences in sodium consumption and concomitant inheritance of genetic factors antagonizing high blood pressure. Subjects with GRA have two normal copies of the aldosterone synthase as well as of the 11-β-hydroxylase genes. The penetrance of the fusion gene may well be decreased in patients with lower blood pressure [26]. These differences are also illustrated in the presented pedigree. Both affected brothers had markedly different blood pressure levels as well as blood pressure response to dexamethasone. This confirms that a negligible blood pressure response during dexamethasone suppression test can be misleading. It is well known that after removal of APA, blood pressure may not begin to descend for months [1]. It is remarkable that all our patients had microalbuminuria. It is unlikely that this was solely a result of long-term damage by high blood pressure, since the magnitude of albuminuria was similar in the father, untreated for 50 years, and both sons, with substantially different blood pressure levels. Moreover, following therapy with eplerenone or dexamethasone, microalbuminuria disappeared in all patients. This indicates direct effects of aldosterone on glomerular permeselectivity [27]. Finally, the optimal therapy for GRA remains to be elucidated. Years ago, only spironolactone was available as an alternative to corticosteroid suppression of the hypothalamic–adrenal axis. Here we show that eplerenone as low as 100 mg/day may be sufficient to control blood pressure. However, it is unclear whether this monotherapy is indeed the best choice. Experimental evidence suggests that not all non-genomic deleterious cardiovascular effects of aldosterone are blocked by mineralocorticoid receptor blockers. For instance, aldosterone-induced constriction of rabbit renal arteries is not affected by spironolactone [28]. Perhaps a combination of both low-dose corticosteroids plus mineralocorticoid receptor blockers may be the optimal therapy in GRA. Nevertheless, the most important goal is to normalize blood pressure, which can often be achieved only with combination therapy using calcium antagonists [6].

*Conflict of interest statement.* None declared.

**References**


Received for publication: 9.8.06
Accepted in revised form: 16.10.06