Glucocorticoid-remediable aldosteronism (GRA), also known as familial hyperaldosteronism type I (FH-I, OMIM 103900), is a monogenic form of inherited hypertension first described in 1966 by Sutherland et al. (1966). This disease is characterized by high plasma aldosterone levels, suppressed plasma renin activity, and abnormally high production of two rare steroids: 18-hydroxycortisol (18OHF) and 18-oxocortisol (18oxoF). The synthesis of these steroids requires the simultaneous presence of a 17β-hydroxylase activity and the two C18 (18-hydroxylase and 18-oxidase) activities typical of the CYP11B2 (aldosterone synthase) enzyme (Lifton et al. 1992; Mulatero et al. 2002). In GRA, the secretion of aldosterone is primarily regulated by adrenocorticotropic hormone (ACTH) rather than angiotensin II; in fact, the symptoms are exacerbated by ACTH administration and normalized by glucocorticoid administration (Mulatero et al. 2002). Despite the state of hyperaldosteronism, hypokalaemia is not a common feature (Litchtfield et al. 1997). In affected families, there is an increased frequency of early death from stroke and an increased risk for exacerbation of hypertension during pregnancy (Litchfield et al. 1998; Wyckoff et al. 2000). However, the majority of affected family members have mild-to-moderate hy-

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**Abstract.** Glucocorticoid-remediable aldosteronism (GRA), also known as familial hyperaldosteronism type I (FH-I, OMIM 103900), is a monogenic form of inherited hypertension caused by the presence of a chimaeric gene originating from an unequal cross-over between the CYP11B1 (11β-hydroxylase) and CYP11B2 (aldosterone synthase) genes. The hybrid gene has the CYP11B1 sequence at the 5’ end, including the promoter, and the CYP11B2 sequence at the 3’ end. The aim of our study was to evaluate the prevalence of GRA in a Polish population of 129 patients with primary hyperaldosteronism (PHA) and 132 patients with essential hypertension (EH), through the use of a PCR-based test revealing the chimaeric gene. None of our PHA or EH patients was positive for the CYP11B1/CYP11B2 chimaeric gene. These data suggest that GRA is unlikely to be a common cause of hypertension in Polish subjects. However, the real prevalence of GRA in Poland, both in the high-risk group of individuals with primary hyperaldosteronism and in the general population, remains to be established.

**Key words:** hypertension, glucocorticoid-remediable aldosteronism (GRA), chimaeric gene, genetic screening, PCR.
pertension and normal biochemistry, and are clinically indistinguishable from patients with essential hypertension. It is therefore possible that this condition is underdiagnosed. This monogenic form of hypertension is noteworthy because it is frequently unresponsive to standard anti-hypertensive medication but successfully managed by treatment with amiloride, spironolactone or dexamethasone alone (Gates et al. 2001).

The GRA is inherited in an autosomal dominant fashion and is caused by the presence of a chimaeric gene originating from an unequal cross-over between the CYP11B1 (11β-hydroxylase) and CYP11B2 genes. The hybrid gene has the CYP11B1 sequence at the 5′ end, including the promoter, and the CYP11B2 sequence at the 3′ end (Lifton et al. 1992). The CYP11B1 promoter ensures the expression of the hybrid gene throughout the adrenal cortex, whereas the CYP11B2 sequence leads to the inappropriate synthesis of aldosterone, 18OHF, and 18oxoF. The exact position of the cross-over site, occurring between intron 2 and exon 4, does not seem to affect the phenotype (Mulatero et al. 2002). Aldosterone suppression by dexamethasone, and high 18OHF and 18oxoF levels are used to differentiate glucocorticoid-remediable aldosteronism from the other forms of primary aldosteronism (Mulatero et al. 1998). These methods are time-consuming and expensive. Therefore, they are not useful for a large epidemiological study. In addition, the dexamethasone suppression test has been shown to be nonspecific for diagnosis of GRA (Mulatero et al. 1998; Fardella et al. 2000). Definitive diagnosis can only be reached by identification of the CYP11B1/CYP11B2 chimaeric gene in genomic DNA using either Southern blotting (Lifton et al. 1992) or the long PCR technique (Jonsson et al. 1995; MacConnachie et al. 1998).

Therefore, the aim of our study was to evaluate the prevalence of GRA in a Polish population of 129 patients with primary hyperaldosteronism (PHA) and 132 patients with essential hypertension (EH), through the use of a PCR-based test to reveal the chimaeric gene.

The PHA group consisted of adult Caucasians with primary hyperaldosteronism diagnosed in 1990-2003 at the Department of Hypertension, National Institute of Cardiology, Warszawa (n = 67) (Prejblisz et al. 2003) or at the Department of Internal Medicine & Hypertension, Medical University, Warszawa (n = 62) (Ignatowska-Świtalska et al. 1997). Of 129 PHA patients, 82 were identified as having aldosterone-produc-

ing adenoma and 47 as having idiopathic hyperaldosteronism. The patients with essential hypertension (EH) were recruited among subjects who had the pattern of salt-sensitivity of blood pressure previously evaluated at the Department of Endocrinology, Hypertension & Metabolic Diseases, Pomeranian Medical University, Szczecin, Poland (Ciechanowicz et al. 2001). Informed consent was obtained before enrolment and the study protocol was approved by local ethics committees.

Genomic DNA was prepared from EDTA-treated blood, as described previously (Ciechanowicz et al. 2001). The presence of the CYP11B1/CYP11B2 chimaeric gene was studied using the long PCR technique introduced by Jonsson et al. (1995). Briefly, each DNA sample was subjected to two concurrent amplification reactions with sense primers specific for the 5′-untranslated regions of genes encoding aldosterone synthase (5′-TCCTTCATCTCTACTCTTGGCCTGGG-3′) or 11β-hydroxylase (5′-TCATGCACCCCCAATGAGTCCCTCCTCCGAGGC-3′). The antisense primer (5′-GAGTCCCTCCAGCTG CCTCTCAACC-3′) was specific for intron E of the aldosterone synthase gene. The DNA (100 ng) was amplified in a Mastercycler Gradient Thermal Cycler (Eppendorf, Germany) in a 50 μL reaction volume, and the “hot start” technique was employed. The PCR mixture, apart from genomic DNA and 15 pM of each of the appropriate primers, contained 1 × Expand High Fidelity Buffer with 1.5 mM MgCl₂ (Roche Applied Science), 200 μM each dNTP (dATP, dCTP, dGTP and dTTP) (MBI Fermentas), and 2.6 U of the Expand High Fidelity PCR System Enzyme Mix (Roche Applied Science). The Expand High Fidelity PCR System Enzyme Mix is an enzyme blend containing Taq DNA polymerase and a polymerase with proofreading activity. Each PCR run included DNA samples from both a young female already identified as a GRA patient (positive control) and her GRA-negative father (negative control) (Litwin et al. 2002). Both samples were kindly provided by Dr Mieczysław Litwin (Children’s Memorial Health Institute, Warszawa, Poland). The conditions used in both amplification reactions were a denaturation step at 95°C for 3 min, followed by 12 cycles of denaturation at 95°C for 1 min and annealing/extension at 68°C for 5 min, then 18 cycles of denaturation at 95°C for 1 min, and annealing/extension at 68°C for 5 min with the annealing/extension time increasing by 30 s for each cycle. Following amplification, PCR products (20 μL) were loaded into 0.7%
agarose gel (Agarose Type I, SIGMA) containing 1 mg mL\(^{-1}\) ethidium bromide. Gel was electrophoresed for approximately 1 h at 60 V and photographed with UV transillumination (Transilluminator 4000, Stratagene) using DS34 Polaroid Direct Screen Instant Camera.

DNA from all subjects (positive control, negative control, patients with PHA or EH) produced a 3.9-kb fragment when amplified with the pair of primers specific for the aldosterone synthase gene (CYP11B2). The CYP11B2 amplification for each individual served as a control for the integrity of each DNA sample. Only DNA from the GRA patient (positive control) produced a 3.9-kb fragment when the sense primer for the 11\(-\)hydroxylase gene and the antisense primer for intron E of the aldosterone synthase gene were used for amplification. None of our PHA or EH patients was positive for the CYP11B1/CYP11B2 chimaeric gene (Figure 1).

Mulatero et al. (1998) reported no carriers of the GRA mutation among 117 Italian patients with PHA. On the other hand, Fardella et al. (2000) found 3 subjects harbouring the CYP11B1/CYP11B2 gene among 32 Chileans with PHA.

Taking into consideration these conflicting results, we conclude that the long PCR technique is a reliable and simple test to at least exclude GRA among Poles with PHA. Such a direct genetic approach for the diagnosis of glucocorticoid-remediable aldosteronism could replace the inconvenient and low-specific dexamethasone-suppression test. Therefore, genetic testing should be recommended for the relatives of GRA-affected patients.

Our sample of 132 EH patients (264 chromosomes) would have a probability of approximately 80% to detect the GRA mutation even if it was present at a frequency as low as 0.005. These data suggest that as in other Caucasian populations (Fardella et al. 2000; Gates et al. 2001), GRA is unlikely to be a common cause of hypertension in Polish subjects. However, the real prevalence of GRA in Poland, both in the high-risk group of individuals with primary hyperaldosteronism and in the general population, remains to be established.

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