Low prevalence of nonconservative mutations of serum and glucocorticoid-regulated kinase (SGK1) gene in hypertensive and renal patients

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Abstract

Background. The serum- and glucocorticoid-regulated kinase (SGK1) gene is an important mediator of aldosterone action, regulating the expression of the renal epithelial Na⁺ channel. In renal failure, blood pressure (BP) is markedly salt-dependent and increases with decreasing renal function. Mutations of the SGK1 gene affecting phosphorylation could be responsible for salt-mediated increases in BP and hypertension-related progression to end-stage renal disease (ESRD).

Methods. The SGK1 gene was analysed for mutations in the exons 4, 5, 8 and 10–12, because of potential phosphorylation sites, in 591 subjects, including 311 ESRD patients (either dialysis or transplanted). In addition, an intron 6 single-nucleotide polymorphism (SNP) described previously was also investigated in this study. Genotyping was performed either by using a strategy based on single strand conformation polymorphism analysis of polymerase chain reaction (PCR) products and subsequent direct sequencing of identified gel shift variants or by using high throughput 5' nuclease allelic discrimination assay.

Results. Two SNPs in coding regions of SGK1 potentially influencing the phosphorylation of Sgk1 were identified. Both SNPs were synonymous. The prevalence of the first variant, a previously reported SNP at codon 240 in exon 8, did not differ between ESRD patients (16.3%) and controls (15.7%). There was no association between the SNP in exon 8 and either BP within the control population or progression of renal disease in the ESRD population. The second SNP at codon 398 in exon 12 was identified in one patient only. Intron 6 and exon 8 SNPs were in strong linkage disequilibrium, but did not show any association with either BP or renal diseases.

Conclusions. Based on statistical analysis homozygosity for nonconservative mutations in the coding region of the SGK1 gene is estimated at <1/300 000 when a white Caucasian population is considered, arguing against an important role of mutations of this coding region in hypertension and hypertension-associated progression of renal disease.

Keywords: end-stage renal disease; genetics; glucocorticoids; hypertension; SGK1

Introduction

The epithelial sodium (Na⁺) channel (ENaC) located in the apical membrane of renal aldosterone-responsive epithelia, plays an essential role in controlling the Na⁺ balance of extracellular fluids and hence blood pressure (BP) [1,2]. The regulation of ENaC involves a variety of hormonal signals such as aldosterone, vasopressin and insulin [3]. Among regulatory proteins found in recent years the ubiquitin-protein ligase Nedd4-2 negatively controls ENaC cell surface expression, and the serum glucocorticoid-inducible kinase 1 (Sgk1) acts as an aldosterone- and insulin-dependent positive regulator of ENaC density at the plasma membrane [4].

Sgk1 was identified in 1993 as an immediate early gene whose mRNA levels increase dramatically within 30 min when cells are exposed to serum or glucocorticoids, or both [5]. The inactive protein Sgk1 is activated by phosphorylation in response to several agonists through a signalling cascade that includes PI3K and the protein kinases PDK1 and PDK2 [6,7]. Using in vitro mutagenesis to substitute serine for asparagine at codon 422 of SGK1, Park et al. [7] showed that Sgk1 becomes fully active under basal conditions. Since Sgk1 plays an important role in activating ion channels, this kinase is crucial in regulating processes.
such as cell survival, neuronal excitability and renal Na\(^+\) excretion [8]. Therefore, the SGK1 gene could be a candidate for abnormal BP regulation and possibly hypertension. Recently, Busjahn et al. [9] showed a significant association of the SGK1 gene locus to diastolic BP in twins. There was an association between two single-nucleotide polymorphisms (SNPs) in exon 8 and intron 6 and BP [9]. In patients with renal disease BP increases with decreasing glomerular filtration rate, regardless of the primary cause of the renal disease [10]. Hypertension in renal failure shows a marked salt-dependency and is one of the important contributors for the progressive increase in BP associated with the loss of renal function [11]. Therefore, a genetically determined predisposition to renal sodium retention could aggravate the sodium retention observed in renal failure and play an important role in patients suffering from end-stage renal disease (ESRD). The aim of the present genetic association study was to assess whether mutations of SGK1 affecting phosphorylation sites of the gene product can be identified in unselected patients with hypertension and patients with ESRD. We also analysed whether the reported association of exon 8 and intron 6 with BP in normotensive twins [9] is predictive of hypertension or renal failure in unrelated subjects.

**Subjects and methods**

Study subjects were 311 patients with ESRD from our Division of Nephrology and Hypertension (University Hospital of Berne, Switzerland) and 280 control subjects without renal disease as described previously [12]. Ethnicity was homogeneous, with 88% subjects of Swiss German, 9% Mediterranean and 2% Asian origin. Of the control subjects, 167 were healthy normotensive individuals and 9% Mediterranean and 2% Asian origin. Of the control subjects, 167 were healthy normotensive individuals and 9% Mediterranean and 2% Asian origin. Of the control subjects, 167 were healthy normotensive individuals and 9% Mediterranean and 2% Asian origin. Of the control subjects, 167 were healthy normotensive individuals and 9% Mediterranean and 2% Asian origin. Of the control subjects, 167 were healthy normotensive individuals and 9% Mediterranean and 2% Asian origin. Of the control subjects, 167 were healthy normotensive individuals and 9% Mediterranean and 2% Asian origin. 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were analysed using SDS software (Applied Biosystems) and the genotype was determined. Amplification of exon 8 failed in one subject and amplification of intron 6 was not successful in five subjects. Thus, the numbers in Tables 3 and 4 do not add up to the expected 591 as predicted by the number of subjects investigated.

Statistical analysis

Differences between means were assessed by the unpaired t-test or ANOVA. For categorical variables the 2 × 2 contingency tables by the y2-test was used. The expected ‘disease’ frequency for the target population was calculated according to the Hardy–Weinberg equation and an estimate of the probability that, with a given prevalence, mutations are not detected was obtained by the Armitage equation: Z = (N.p – 0.5)/(N.p.(1 – p))2, where p = assumed prevalence, n = number of alleles, N.p. = expected number of mutations, as previously described [12].

Results

A total of two synonymous SNPs in coding regions of SGK1 potentially influencing the phosphorylation of Sgk1 were identified (Table 2). The first SNP was found in exon 8 and is identical with a previously reported SNP [9]. The frequency of this C → T transition at codon 240 in the different study groups is shown in Table 2. The overall prevalence of this SNP in exon 8 of the SGK1 gene was 15.9%, the frequency of the wild-type allele was 0.91 and the frequency of the mutated allele was 0.09.

The expected frequency of the SGK1 exon 8 genotype, under the assumption of the Hardy–Weinberg equilibrium, did not differ from observed frequencies in ESRD patients and control subjects (Table 2). This genetic polymorphism was also analysed for its relation to the rate of progression of renal disease in ESRD patients and to BP or the presence or absence of hypertension in the control population. Progression of renal disease in the ESRD population as a whole was not influenced by the SGK1 polymorphism in exon 8 (Table 3). There was no link between the SNP in exon 8 with either systolic or diastolic BP or the presence or absence of hypertension (χ2 = 0.346, P = 0.556) within the control population with normal renal function (Table 3). The intron 6 and exon 8 SNPs were in strong linkage disequilibrium, as shown in Table 4. We grouped both the ESRD and control population according to the two SNPs into subjects with exon 8 CT/CC+ intron 6 TT/CT, exon 8 CT/CC+ intron 6 CC, and exon 8 TT+ intron 6 CC genotypes as previously described [9]. This association analysis did not show any significant or suggestive association of the SGK1 gene locus to systolic and diastolic BP in the control population without ESRD, either with normal or increased BP (Table 5).

Table 2. Identified mutations in the coding and non-coding regions of the SGK1 gene in control subjects and patients with ESRD

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>ESRD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dialysis</td>
<td></td>
<td>Transplanted</td>
</tr>
<tr>
<td>N</td>
<td>591</td>
<td>96</td>
<td>215</td>
</tr>
<tr>
<td>Exon  4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Exon  5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Exon  8 C → T Asp240/Asp</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>81 (13.7%)</td>
<td>12 (12.5%)</td>
<td>31 (14.4%)</td>
</tr>
<tr>
<td>Homozygous</td>
<td>14 (2.4%)</td>
<td>1 (1%)</td>
<td>7 (3.2%)</td>
</tr>
<tr>
<td>Exon 10</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Exon 11</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Exon 12 T → C Ile398/Ile</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Intron 6 T → C</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>182 (30.8%)</td>
<td>28 (29.2%)</td>
<td>65 (30.2%)</td>
</tr>
<tr>
<td>Homozygous</td>
<td>33 (5.6%)</td>
<td>4 (4.2%)</td>
<td>17 (7.9%)</td>
</tr>
</tbody>
</table>
greater Na\(^+\) retention and plasma volume expansion. Although two different SNPs in the coding region of the \(SGK1\) gene were identified, they were both synonymous, thus not altering the amino acid sequence. Therefore, our data argue against an important role of nonconservative mutations of the \(SGK1\) gene in a white ESRD population.

Allele frequencies for the \(SGK1\) genotype in exon 8 did not differ significantly between control subjects and ESRD patients or between patients with hypertension and ESRD; moreover, the same polymorphism did not show any significant effect on the rate of progression to renal failure. The same applies for the intron 6/exon 8 SNPs combination, which showed a similar degree of linkage disequilibrium as previously reported [9], but had no reproducible effect on BP in subjects without renal failure. These linked SNPs were also not associated with a particular cause of renal disease in ESRD patients. These observations indicate that these polymorphisms do not have a significant effect on BP in unrelated subjects without renal diseases, do not account for the occurrence of renal diseases per se and are not a determinant of progressive renal failure.

In the last decade great progress has been made in human genetics with the identification of an enormous number of SNPs. However, the impact of these genetic polymorphisms on the understanding of polygenic multifactorial cardiovascular diseases like hypertension has yielded contrasting results [17]. Recently, Busjahn et al. [9] analysed two SNPs within the \(SGK1\) gene in monozygotic (126 pairs) and dizygotic (70 pairs) normotensive twin subjects and parents of dizygotic twins and found significant association of the \(SGK1\) gene locus to diastolic \((P<0.0002)\) and systolic \((P<0.04)\) BP. These authors concluded that the \(SGK1\) gene is relevant to BP regulation and probably to hypertension in man. The relevance of these new SNPs for the whole hypertensive population, however, is unclear. In fact the author did not test these two polymorphisms in hypertensive populations or subgroups of hypertensive patients.

In our control population we could not find any association of the \(SGK1\) polymorphic marker in intron 6/exon 8 and BP in normotensive or hypertensive patients. In the twin study an association between intron 6 and exon 8 SNPs and BP in an independent confirmation sample of 260 subjects was described [9]. However, this effect only applied for ~5% of subjects with the exon 8 CT/CC and intron 6 CC polymorphism compared to ~94% carriers of the exon 8 CT/CC and intron 6 TT/CT alleles. Genotype distribution was comparable in our population with 541 (92.7%) subjects having the intron 6 TT/CT and exon 8 CT/CC genotype and 31 (5.2%) with the intron 6 CC and exon 8 CT/CC genotype. This distribution was similar for the patients with or without renal failure. In the control population with the more prevalent genotype, BP values tended to be higher in normotensive but lower in hypertensive subjects, suggesting that these findings do not support the observation from the twin study. This view is also
supported by experiments using transgenic animals. Wulff et al. [18] showed with the Sgk1 knockout mouse, that the phenotype is far less dramatic than the one observed in the ENaC knockout [19] and the mineralocorticoid knockout mice [20]. This suggests that Sgk1 participates in, but does not fully account for the mineralocorticoid regulation of ENaC. The isoforms Sgk2 and Sgk3 might be able to compensate for the loss, suggesting that loss of function mutations of the Sgk1 gene may have no influence on the phenotype. Mutations with a gain of function could still lead to hypertension because of the positive regulation of ENaC density at the plasma membrane [4]. However, in our population no mutations with possible gain of function in the selected gene regions were identified.

The lack of association between the SNPs in Sgk1 and BP in our population could be due to the relatively small sample size, although this did not differ from the confirmation sample described in the former study [9] and illustrates the problems in determining genetic influences in complex traits.

The problem of genetic association studies is not just to test a given polymorphism in a case-control study, or in familial design, but also to determine the relevance of the regulatory transcriptional activity of the candidate gene with the appropriate assessment of all the confounding factors. The function of Sgk1 is not limited to regulation of renal ENaC activity, but is also relevant for other processes since it is expressed in all human tissues, including pancreas, liver, heart, lung, skeletal muscle, placenta, kidney and brain [21]. Therefore, it seems unlikely that a functionally relevant mutation in such a ubiquitous gene would affect BP regulation only, without showing additional features of functional significance.

The lack of detection of nonconservative mutations in our population could be due to the small sample size analysed. Nevertheless, according to the Armitage equation and for a probability <5% of missing any significant mutation causing an altered Sgk1 kinase with a total of 1182 alleles analysed, the estimated prevalence of mutated alleles would be 0.2%. Therefore, the sample size of this population is too small to detect significant mutations in the Sgk1 gene for an estimated prevalence of 0.15% or less. Thus, the expected ‘disease’ frequency of homozygous Sgk1 mutants for the target population, calculated according to the Hardy–Weinberg equation, would be <1/300,000, considering the frequency of a recessive allele of <1/550.

The findings of the present study argue against an important role of coding regions of Sgk1 in BP regulation and hypertension and in the hypertension-related progression of renal diseases. The rate of nonsynonymous, nonconservative coding SNPs of the Sgk1 altering the amino acid sequence and therefore generating a kinase with altered activity in this population is low. It seems more likely that abnormal regulation of the Sgk1 gene expression, rather than nonconservative mutations in the coding region of the gene itself, may play a role in mediating the effects of Sgk1 in vivo. Homozygosity for nonsynonymous, nonconservative mutations in the coding region of the Sgk1 gene of exons 4, 5, 8 and 10–12 is <1/300,000 when a white Caucasian population is considered, arguing against an important role of mutations of this coding region in hypertension and hypertension-associated progression of renal disease. Polymorphisms in regulatory regions may be more important than variations in coding regions for multifactorial diseases.

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