Frequency of CYP21 Gene Mutations in Czech Patients with Steroid 21-Hydroxylase Deficiency and Statistical Comparison with Other Populations

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Key Words

CYP21 gene · Frequency of mutations · 21-Hydroxylase deficiency · Genotype · Phenotype · Geographic distribution

Abstract

Objective: To undertake mutational analysis in patients with different forms of steroid 21-hydroxylase deficiency. **Subjects and Methods:** CYP21 gene molecular analysis was performed in 76 Czech patients diagnosed with steroid 21-hydroxylase deficiency. Eight of the most common point mutations (intron 2 splice, P30L, 8bp deletion in exon 3, I172N, V281L, Q318X, R356W, and P453S) were analyzed using an amplification-created restriction site method, and 5 additional mutations (intron 7 splice, F307insT, cluster in exon 6, R484P, and R484X) were analyzed using dot-blot hybridization with 5'-biotin-labeled oligonucleotides. Deletions and conversions were screened using a sequence-specific oligonucleotide hybridization method. Comparison of common mutation frequencies in CYP21 reported for different regions, both within Europe and worldwide (North and South America, Asia, and North Africa), was undertaken and the significance of the differences was determined by statistical

analysis (Fisher's F test, Student's t test, paired t test, and confidence intervals) using a value of $p < 0.05$. **Results:** The most frequent genetic defect found in this group of Czech patients was intron 2 splice mutation (46.7%). Comparison of mutation frequencies between Czech and other European populations showed that the Czech patients had a lower frequency of deletions/large gene conversions, R356W, and cluster mutations in exon 6, together with a higher frequency of intron 2 splice mutation, 8-bp deletion and F307insT compared with other populations. **Conclusion:** A high prevalence of P30L mutations, mostly associated with nonclassical forms of congenital adrenal hyperplasia, was found in Czech patients with classic simple virilizing forms of steroid 21-hydroxylase deficiency.

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Introduction

CYP21 is the structural gene for the adrenal microsomal cytochrome p-450 protein responsible for steroid 21-hydroxylation. This steroid 21-hydroxylase gene is located on the short arm of chromosome 6 in the HLA class III gene region, a location which shows a complex

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Table 1. Frequency (%) of mutation in patients with steroid 21-hydroxylase deficiency in different countries

Country	Alleles	Dele- tions ¹	Intron 2	P30L	8-bp dele- tions	I172N	V281L	Q318X	Cluster	F307insT	R356W	P453S	R483GG	R483X	Intron 7	Uniden- tified alleles	Refer- ences
Czech Rep.	152	9.2 (0.7)	46.7	4.6	5.9	12.5	6.5	5.3	0	3.9	3.3	2	0	3.3	0	–	present study
Hungary	306	35	43	0	–	23	9	7	1	7	8	–	–	–	–	14	9
Sweden	400	32.2	26.6	1.6	1.3	19.8	5.7	2.4	1.1	0.5	3	0.5	0.5	0.5	0.5	3.8	10
Finland	102	34.3 (8.8)	11.8	–	–	29.4	3	2	2	–	–	–	–	–	–	15.7	7
Denmark	136	36	33.8	2.2	–	10.3	4.4	8.8	1.5	0.7	2.2	–	–	–	–	–	13
Spain	58	16 (10)	26	2	5	2	17	3	–	–	3	–	–	–	–	11	11
Italy	146	26	20	3	–	6	11	8	–	–	–	–	–	–	–	26	14
Germany	310	29	30.3	2.6	1.6	19.7	2.9	4.8	1	0.3	4.5	0.3	–	–	–	1.3	8
France	258	19 (4)	20.5	–	2.7	8.9	16.7	3.9	5	1.1	–	0	5	–	–	11.2	15
UK	284	45	30.3	–	–	7	–	–	–	–	9.8	–	–	–	–	8	16
Egypt	64	6.3	42.2	–	–	17.2	14	9.4	–	3.1	–	–	–	–	–	12.5	12
Japan	68	12	26	1	0	12	0	9	1	0	18	–	–	–	–	18	18
India	46	16	27	2	–	32	–	22	–	–	–	–	–	–	–	–	17
China	188	–	36.7	–	–	19.7	–	2.1	–	–	12.8	–	–	–	–	28.7	19
Brazil	228	14.2	20.6	2.2	1.3	14	18	5.7	0.9	2.6	7	1.3	–	–	–	–	21
Argentina	72	18	18	–	2.7	15.3	–	13.8	0	–	5.5	–	–	–	–	26.7	22
Chile	126	22.9	19	0	–	7.1	8.3	10.5	2.4	0	12.7	0.5	–	–	–	12	23
Mexico	94	1	47.9	8.5	2.1	11.7	8.5	4.2	0	1	7.4	2.1	–	–	–	21.3	24
USA	394	26 (5)	31	2	3	10	9	4	–	1	3	–	–	–	–	3	25

¹ Large conversions are indicated in parentheses.

structure and a high degree of variability [1]. Because of high homology between CYP21 and the nonfunctional pseudogene CYP21P (98% nucleotide identity in exons and 96% identity in introns), unequal homologous recombination occurs in a conversion-like manner. Approximately 80% of steroid 21-hydroxylase deficiency cases are caused by point mutations of the CYP21 gene, while deletions or gene conversions cause the remaining 20% of cases. CYP21 gene defects cause three different clinical phenotypes [salt wasting (SW), simple virilizing (SV) and nonclassical (NC)] of congenital adrenal hyperplasia (CAH) [1]. The most common mutations recorded in the CYP21 gene are P30L, A/C 655G, 8-bp deletion in exon 3, I172N, V281L, Q318X, R356W and P453S. This study was prompted by a lack of information concerning the frequencies of different types of CYP21 gene mutations in the Czech population. Knowledge of relative frequencies of mutations might be useful to delineate appropriate strategies for prenatal diagnosis in this population.

Materials and Methods

Seventy-six Czech patients (28 males, 48 females) with different forms of steroid 21-hydroxylase deficiency (previously diagnosed by biochemical testing) participated in the study.

Human DNA Preparation and PCR Analysis

Genomic DNA samples were prepared from white blood cells (collected into 5-ml tubes containing EDTA anticoagulant) by the guanidine hydrochloride method or phenol/chloroform extraction [2, 3]. A modification of the amplification-created restriction site method described previously [4] was used for detection of intron 2 splice, P30L, 8-bp deletion in exon 3, I172N, V281L, Q318X, R356W and P453S mutations. Five additional mutations (intron 7 splice, F307insT, cluster in exon 6, R484P, and R484X) were detected using dot-blot hybridization with biotin-labeled oligonucleotides, as described previously [5]. For deletion and conversion analysis, sequence-specific oligonucleotide hybridization was performed [6].

Statistical Analysis

Mutation frequencies in steroid 21-hydroxylase deficiency reported in studies from many different countries (table 1) were compared (ANOVA) using Fisher's F test, Student's t test, paired t test and confidence intervals. Statistical significance was assigned to a value of $p < 0.05$.

Table 2. Genotype prevalence in Czech patients with CAH

Genotype	SW	SV	NC
In2/In2	22	4	1
In2/In2+P453S/P453S	1	–	–
In2/normal+V281L/normal	–	1	–
In2/In2+8-bp del/8-bp del	1	–	–
In2/normal+P30L/normal+I172N/normal	–	1	–
In2/In2+V281L/V281L	1	–	–
In2/normal+I172N/normal	–	2	–
In2/normal+Q318X/normal	2	–	–
In2/In2+I172N/normal	–	1	–
In2/In2+P30L/P30L+prom konv	1	–	–
R356W/normal+F307insT/normal+R484X/normal+In2/normal	1	–	–
In2/normal+I172N/normal+V281L/normal	–	–	1
P30L/P30L	–	2	–
8-bp del/8-bp del	3	–	–
8-bp del/8-bp del+I172N/normal+V281L/normal+Q318X/normal+P453S/normal	–	–	1
I172N/normal	–	1	–
I172N/I172N	3	2	1
I172N/normal+Q318X/normal	1	1	–
V281L/V281L	1	–	–
V281L/normal	1	–	1
V281L/normal+F307insT/normal	–	–	1
Q318X/normal	1	–	–
Q318X/Q318X	1	–	–
R356W/R356W	2	–	–
Del/del	6	–	–
Del/normal	1	–	–
Del/normal+F307insT/normal+R483X/normal	–	–	1
R483X/normal	–	1	1
R483X/normal+F307insT/normal	–	–	1
F307insT/normal	1	–	1
Total	50	16	10

Results

Of the 76 patients with 21-hydroxylase deficiency in this study group, 50 (65.8%) presented with SW forms of CAH, 16 patients (21%) had SV forms, and 10 (13.2%) had NC forms. Genotypes determined for Czech CAH patients in this study are listed in table 2.

In the SW form, the most frequent mutation, intron 2 splice, was detected in 29 patients. Seven patients (14%) carried deletions or large gene conversions; 3 patients (6%) had 8-bp deletion in exon 3. In the SV form, the most frequent mutations were I172N (50% of patients with SV form), intron 2 splice (56% of patients with SV form), and P30L mutation (19% of patients with SV form). In the NC forms, the most frequent mutations were V281L (40%), I172N, A/C655G and R483X (30%). The

most common mutation in all forms of this disease in this study was intron 2 mutation (26% of all mutations).

Data from published reports on steroid 21-hydroxylase deficiency were collated (table 1) and analyzed with respect to populations in different geographic regions (tables 3, 4).

Discussion

The most frequent mutation in Czech patients with steroid 21-hydroxylase deficiency is aberrant splicing in intron 2 (A/C655G mutation; 46.7%). The second most frequent mutation in the Czech study group was I172N (12.5%), which is comparable with frequencies in other European countries. In the SW form, the most frequent

Table 3. Summary of mutation frequency analysis in different European regions ($p < 0.05$)

	Central Europe (Czech Republic, Hungary)	North Europe (Sweden, Finland, Denmark)	South Europe (Spain, Italy)	West Europe (Germany, France, Great Britain)
Central Europe (Czech Republic, Hungary)	–	P453S ↑, 8-bp del ↑, all other frequencies comparable	8-bp del ↓, all other frequencies comparable	intron 2 ↑, all other frequencies comparable
North Europe (Sweden, Finland, Denmark)	P453S ↓, 8-bp del ↓, all other frequencies comparable	–	cluster ↑, 8-bp del ↓, V281L ↓, all other frequencies comparable	all frequencies comparable
South Europe (Spain, Italy)	8-bp del ↑, all other frequencies comparable	cluster ↓, 8-bp del ↑, V281L ↑, all other frequencies comparable	–	all frequencies comparable
West Europe (Germany, France, Great Britain)	intron 2 ↓, all other frequencies comparable	all frequencies comparable	all frequencies comparable	–

Unless indicated, mutation frequencies did not show statistically significant differences.

Table 4. Summary of mutation frequency analysis through different continents ($p < 0.05$)

	Europe	Asia (China, India)	North America (USA, Mexico)	South America (Brazil, Argentina, Chile)	North Africa (Egypt)
Europe	–	8-bp del ↑, V281L ↑, F307insT ↑, Q318X ↓, all others comparable	P30L ↓, P453S ↓, all others comparable	intron 2 splice ↑, all others comparable	deletions ↑, V281L ↓, Q318X ↓, intron 2 splice ↓, all others comparable
Asia (China, India)	8-bp del ↓, V281L ↓, F307insT ↓, Q318X ↑, all others comparable	–	R356W ↑, P30L ↑, V281L ↑, 8-bp del ↑, all others comparable	all frequencies comparable	del ↑, V281L ↓, F307insT ↓, all others comparable
North America (USA, Mexico)	P30L ↑, P453S ↑, all others comparable	R356W ↓, P30L ↓, V281L ↓, 8-bp del ↓, all others comparable	–	all frequencies comparable	V281L ↓, Q318X ↓, all others comparable
South America (Brazil, Argentina, Chile)	intron 2 splice ↓, all others comparable	all frequencies comparable	all frequencies comparable	–	deletions ↑, intron 2 splice ↓, all others comparable
North Africa (Egypt)	deletions ↓, V281L ↑, Q318X ↑, intron 2 splice ↑, all others comparable	deletions ↓, V281L ↑, F307insT ↑, all others comparable	V281L ↑, Q318X ↑, all others comparable	deletions ↓, intron 2 splice ↑, all others comparable	–

Unless indicated, mutation frequencies did not show statistically significant differences.

mutation, intron 2 splice, was detected in 29 patients (58%), a frequency similar to results from Finland [7], Germany [8], Hungary [9], and Sweden [10].

The frequency of deletions or large gene conversions of R356W and cluster mutations in exon 6 found in our study was significantly lower than that found in some other populations, including other European countries (table 1). Frequency of intron 2 splice mutation, 8-bp deletion in exon 3 and F307insT was significantly higher than that reported in many other regions (tables 1, 3). Frequen-

cies of other mutations (P30L, V281L, I172N, Q318X, and P453S) in these Czech patients were broadly comparable with many reports on steroid 21-hydroxylase deficiency from different countries [11–25].

The V281L mutation is mostly associated with the NC form of CAH. Prevalence of V281L mutations is higher in South Europe (Spain and Italy) than in North Europe (Denmark, Sweden, and Finland) and correlates with a higher prevalence of NC forms in South Europe [11] and North Africa [12]. A higher prevalence of classical forms

associated with cluster mutations in exon 6 was found in North Europe [7, 10, 13] than in South Europe [11, 14] and a higher frequency of 8-bp deletions was found in South Europe [11, 14] than in Central Europe [present study, 9]. A higher frequency of intron 2 splice mutation was found in Central Europe [present study, 9] than in West Europe [8, 15, 16].

Mutation frequencies also vary in populations from different continents (table 4). A higher prevalence of classical forms of CAH, mostly associated with Q318X mutation, has been found in Asian populations (India, Japan, and China) [17–19] than in Europe, and a higher frequency of V281L was found in North Africa [12] than in Europe, Asia and North America.

Conclusions

The results of our study support the theory that specific mutations are involved in three different clinical forms of CAH and contribute in a complex manner to phenotypic variations in these conditions. Thus, mutations associated with milder forms of CAH may also occur in severe classical forms of CAH; in this study, a high prevalence of P30L mutations, mostly associated with NC forms of CAH, was found in Czech patients with classic SV forms of the disease.

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