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Molecular diagnosis of Chinese patients with 21-hydroxylase deficiency and analysis of genotype–phenotype correlations

Bo Zhang[1], Lin Lu[2] and Zhaolin Lu[2]

Abstract

Objective: The spectrum of molecular defects in Chinese patients with 21-hydroxylase deficiency (21-OHD), and genotype–phenotype relationships are unknown.

Methods: We screened eight patients with non-classical (NC) 21-OHD and 35 with classical 21-OHD, and detected nine known mutations.

Results: The most frequent mutation among the 43 21-OHD cases was p.Ile172Asn (allele frequency, 36.0%), followed by c.290-13A/C > G (20.9%), Del (8.6%), p.Pro30Leu (7.0%), p.Gln318Ter (7.0%), p.Val281Leu (4.7%), p.Arg356Trp (2.3%), p.[Ile236Asn; Val237Glu; Met239Lys] (2.3%), and E3Δ8 bp (1.2%). The frequency spectrum of *CYP21A2* mutations in the Chinese population was similar to that in the Japanese population, except that p.Val281Leu was identified in Chinese NC21-OHD patients at a frequency of 25.0%, whereas it was absent in Japanese patients. We found that genotype could predict phenotype in 88.3% of patients.

Conclusion: Some characteristics appear to be unique to the Chinese population, but genotype was strongly predictive of phenotype.

Keywords

Congenital adrenal hyperplasia, 21-hydroxylase deficiency, steroid 21-monooxygenase, CYP21A2, genotype, phenotype

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[1]Department of Endocrinology, China - Japan Friendship Hospital, Beijing, China
[2]Department of Endocrinology, Peking Union Medical College Hospital, Beijing, China

The work was carried out at Department of Endocrinology, PUMC Hospital, No.1 Shuaifuyuan, Beijing, China.

Corresponding author:
Bo Zhang and Zhaolin Lu, Department of Endocrinology, China Japan Friendship Hospital, No.2 Yinghua Eastern Street, Beijing, China; Department of Endocrinology, PUMC Hospital, No.1 Shuaifuyuan, Beijing, China.
Emails: drbozhang@yahoo.com; luzhaolin39@163.com

Introduction

Congenital adrenal hyperplasia (CAH), caused by a deficiency of steroid 21-hydroxylase (CYP21A2), is one of the most common autosomal recessive diseases. Its incidence was reported to be around 1 in 28,000 in Taiwan,[1] but ranges from 1 in 10,000 to 1 in 15,000 live births in most other populations.[2] It causes ambiguous genitalia at birth in females and inefficient cortisol synthesis in both genders. Three major phenotypes of CAH from 21-hydroxylase deficiency (21-OHD) are described: the classical salt-wasting form (SW), the classical simple virilizing form at birth (SV), and the non-classical form with late-onset symptoms and diagnosis (NC21-OHD).

The molecular defects in Chinese patients with NC21-OHD have not been identified. Therefore, in the present study, we used a rapid mutation analysis method to ascertain the prevalence of nine common mutations in *CYP21A2* (complete allele deletion [Del]; c.328delGAGACTAC in exon 3 [E3Δ8bp]; p.Gln318Ter in exon 8; p.Arg356Trp in exon 8; the exon 6 p.[Ile236Asn; Val237Glu; Met239Lys] cluster; c.290-13A/C > G in intron 2; p.Ile172Asn in exon 4; p.Pro30Leu in exon 1; and p.Val281Leu in exon 7). We also analyzed genotype–phenotype correlations in Chinese patients with 21-OHD.[2–5]

Patients and methods

Study population

Eight female NC21-OHD patients aged 16.5 ± 4.5 years, and 35 classical 21-OHD patients (nine SW and 26 SV; 10 males and 25 females) aged 14.5 ± 10.0 years who were seen at the Department of Endocrinology, Peking Union Medical College Hospital were included in the study. The diagnosis was made according to clinical manifestations and the 17-hydroxyprogesterone (17-OHP) assay.[5] Polycystic ovary (PCO) was diagnosed by ultrasound examination as either 12 or more follicles measuring 2 ± 9 mm in diameter, or an increased ovarian volume ($>10\,cm^3$).[6] All subjects were unrelated Han Chinese who lived in northern China (provinces above the Yangtze River). All adult patients signed an informed consent form for DNA analysis. Parents/guardians signed an informed consent form for children. The study was approved by the Ethics Committee of the Peking Union Medical College Hospital.

Molecular analysis

Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany).

CYP21A2 *Del detection*

PCR amplification of *CYP21A2* in several fragments was performed using oligonucleotide primers A1/A2, as shown in Table 1 and described below. PCR products were digested with *Taq* I to detect mutations, with the wild-type pattern showing 210 bp and 187 bp bands of equal intensity. The absence of one *CYP21A2* allele was indicated by a weak 210 bp band, whereas complete absence of this band indicated that both *CYP21A2* alleles were absent.

E3Δ8 bp detection

No PCR product could be amplified from patients with a homozygous whole-allele deletion or those with a deletion of *CYP21A2* exon 3. E3Δ8 bp could therefore be ascertained if whole-allele deletion had been excluded.

CYP21A2 *point mutation detection*

Upstream (fragment 1; exons 1–3) and downstream (fragment 2; exons 3–10)

Table 1. Primer sequences for *CYP21A2* amplification.

Primer	Nucleotide position	Sequence (5′–3′)
A1	−231 to −211	TGCATTTCCCTTCCTTGCTTC
A2	−42 to −22	*CTGAGGTGCCACTTATAGCTC
B1	−424 to −404	TTTTGTTCTTCAGGCGATTCA
B2	707–727	*TCCAGAGCAGGGAGTAGTCTC
C1	691–715	CCGGACCTGTCCTTGGGAGACTACT
C2	3232–3256	*CTGAGTGGCTGGGTGAAATGGAACA
Primer 7	524–547	TGGGGCATCCCCAATCCAGGTCCC
Primer 8	656–677	*ACCAGCTTGTCTGCAGGAGGA*T*
Primer 9	1000–1020	*TCTCCGAAGGTGAGGTAACA*T*

Note: The underlined sequence represents the 8-bp deletion.
*represents an antisense primer and italics indicate modified nucleotides.

Table 2. *CYP21A2* mutation-specific restriction fragments.

Mutation	Restriction enzyme	Fragment size (bp)	
		Wild-type	Mutant
p.Pro30Leu	*Aci* I	153/43	196
c.290-13A/C > G	*Sau*3A I	156	133/23
p.Ile172Asn	*Nde* I	330	307/23
p.[Ile236Asn; Val237Glu; Met239Lys]	*Dra* III	687/28	715
p.Val281Leu	*Apa*L I	994/376	1370
p.Gln318Ter	*Pst* I	567/298	865
p.Arg356Trp	*Aci* I	189/30	219

fragments of *CYP21A2* were amplified using primer pairs B1/B2 and C1/C2, respectively (Table 1).[3,4] PCR products were purified using the Wizard DNA clean-up system (Qiagen), then 5 µl was digested with 10–15 U restriction enzyme for 2–12 h. Mutations were detected in exon 1 (p.Pro30Leu) using *Aci* I, in exon 6 (p.[Ile236Asn; Val237Glu; Met239Lys]) using *Dra* III, in exon 8 (p.Gln318Ter) using *Pst* I, and in exon 8 (p.Arg356Trp) using *Aci* I. The digests were subjected to electrophoresis on 2.5%–4.0% agarose gels (Table 2).

PCR conditions

PCR was performed with approximately 200 ng of genomic DNA, 400 µmol/L of each primer, and 2.5 U of Taq in a volume of 50 µl. PCR conditions were 30 cycles of denaturation at 94°C for 20 s, and annealing and extension at 58°C for 90 s (B1/B2) or 68°C for 5 min (C1/C2). PCR conditions for A1/A2 were 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s.

c.290-13A/C > G and p.Ile172Asn were detected using two rounds of PCR. One microliter of the first-round PCR product (fragment 1 for c.290-13A/C > G, fragment 2 for p.Ile172Asn) was used as a template for the second round of PCR with appropriate primer pairs (7/8 for c.290-13 A/C > G and C1/9 for p.Ile172Asn). PCR conditions were 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C (c.290-13 A/C > G) or

60°C (p.Ile172Asn) for 30 s, and extension at 72°C for 30 s, in a volume of 50 μl. Five microliters of PCR product were digested with 10–15 U restriction enzyme for 2–12 h (c.290-13A/C > G using *Sau*3A I; p.Ile172Asn using *Ned* I) and the digests were subjected to electrophoresis on 3.0%–4.0% agarose gels to detect mutations (Table 2).

17-OHP assay

Serum 17-OHP levels were determined using a commercial radioimmunoassay (DPC, Los Angeles, CA) with an intra-assay coefficient of variation of 4.0%. The normal value for males was a mean of 1.5 μg/L (95% confidence interval, 0.4–3.5 μg/L). The normal value for females during the follicular stage was a mean of 0.8 μg/L (95% confidence interval, 0.2–2.6 μg/L).

Statistical analyses

Continuous data are presented as means ± standard deviation where appropriate. The variables without normal distributions are expressed as medians plus interquartile ranges. All analyses were carried out using SPSS v.22.0 software (IBM Corp., Armonk, NY).

Results

Mutation detection

Among the 43 patients with 21-OHD, we detected a mutation in 79/86 (91.9%) alleles. The most common mutation was p.Ile172Asn with an allele frequency of 36.0%, followed by c.290-13A/C > G (20.9%), and Del (18.6%). Together, these three mutations made up 75.6% of the total (65/86). The frequencies of the other mutations were p.Pro30Leu (7.0%), p.Gln318Ter (7.0%), p.Val281Leu (4.7%), p.Arg356Trp (2.3%), p.[Ile236Asn; Val237Glu; Met239Lys] (2.3%), and E3Δ8 bp (1.2%). No mutation was identified in seven alleles.

Genotype data are reported in Table 3 and Table 4. In total, 11 alleles (12.8%) in 10 patients carried two different mutations. Among 37 patients with mutations detected on both alleles, the homozygosity frequency was 10.8% (4/37), combined homozygosity was 2.7% (1/37), and compound heterozygosity was 86.5% (32/37). The zygosity status could not be classified for six patients (14.0%) because no mutation was identified on at least one of their alleles.

Table 3 and Table 4 show the patients classified by clinical phenotype. In the SW group ($n = 9$), the most common mutation was Del with an allele frequency of 44.4%, followed by c.290-13A/C > G (38.9%), p.Gln318Ter (5.6%), and E3Δ8 bp (5.6%).[7] These mutations, which are all predicted to severely compromise 21-hydroxylase enzymatic activity, made up 77.3% (17/22) of the mutant alleles in the SW group. In the SV group ($n = 26$), the p.Ile172Asn mutation, which moderately compromises 21-hydroxylase activity, was found in 44.2% of detected alleles, followed by c.290-13 A/C > G with an allele frequency of 17.3% and Del with an allele frequency of 17.3% also. In the NC21-OHD group ($n = 8$), the common mutations were p.Pro30Leu (allele frequency, 37.5%) and p.Val281Leu (25.0%), both of which only mildly compromise 21-hydroxylase enzymatic activity. All patients in the NC21-OHD group were homozygotes or compound heterozygotes with at least one allele carrying the p.Pro30Leu or p.Val281Leu mutation. Conversely, no SW patients carried p.Pro30Leu or p.Val281Leu, and only one SV patient carried the p.Pro30Leu/p.Arg356Trp genotype.

Genotype–phenotype correlation

We classified the genotypes into four groups (Null, Group A, Group B, and Group C)[2,7–11]

Table 3. Genotype frequency for the three subtypes of 21-OHD.

Phenotype	Genotype	Number of patients
SW	Del/Del	1
	E3 D8bp/Del	1
	c.290-13A/C > G/Del	3
	p.Ile172Asn/Del	1
	[c.290-13A/C > G; p.Ile172Asn]/Del	1
	c.290-13A/C > G/p.[Ile172Asn; Gln318Ter]	1
	[c.290-13A/C > G; p.Ile172Asn]/[c.290-13A/C > G;p.Ile172Asn]	1
SV	Del/UD	4
	p.Ile172Asn/Del	5
	p.Ile172Asn/UD	1
	c.290-13A/C > G/p.Ile172Asn	3
	p.Ile172Asn/p.Ile172Asn	2
	p.Ile172Asn/[c.290-13A/C > G; p.Ile172Asn]	4
	p.Ile172Asn/p.Gln318Ter	2
	c.290-13A/C > G/p.Gln318Ter	2
	p.Pro30Leu/p.Arg356Trp	1
	p.[[Ile236Asn;Val237Glu;Met239Lys];Gln318Ter]/p.[Ile236Asn; Val237Glu; Met239Lys]	1
	UD/UD	1
NC	c.290-13A/C > G/p.Pro30Leu	1
	[c.290-13A/C > G; p.Pro30Leu]/p.Pro30Leu	1
	p.Pro30Leu/[c.290-13A/C > G; p.Ile172Asn]	1
	p.Pro30Leu/p.Ile172Asn	1
	p.Pro30Leu/p.Arg356Trp	1
	p.Val281Leu/p.Val281Leu	1
	p.Val281Leu/p.Gln318Ter	1
	p.Ile172Asn/p.Val281Leu	1

SW, salt-wasting form; SV, classical simple virilizing form at birth; NC, non-classical form with late-onset symptoms and diagnosis

according to the extent of 21-hydroxylase enzymatic compromise caused by the mutations (Table 5). The Null group comprised the 'severe' genotypes, homozygosity or compound heterozygosity for mutations (Del, E3Δ8 bp, p.Gln318Ter, p.Arg356Trp, p.[Ile236Asn; Val237Glu; Met239Lys]), that are predicted to cause a complete absence of 21-hydroxylase activity. Group A genotypes were those homozygous for c.290-13A/C > G, or compound heterozygous with a mutation in the Null group. Group B comprised p.Ile172Asn homozygotes or compound heterozygotes with a Null or Group A mutation. Group C comprised homozygotes or compound heterozygotes for mutations (p.Pro30Leu or p.Val281Leu) predicted to cause mild 21-hydroylase enzymatic compromise, or compound heterozygotes with Null, Group A, or Group B mutations. Six patients could not be classified in this way because no mutation was identified on one or both alleles. Nine of 11 patients in Group A were SW. Only five in 37 patients had an observed phenotype that was different from expected (one case in

Table 4. Allele frequency for the three subtypes of 21-OHD.

Phenotype	Type of mutation	Mutant allele no./ Total allele no.
SW	Del	8/18 (44.4%)
	c.290-13A/C > G	7/18 (38.9%)
	p.Ile172Asn	5/18 (27.8%)
	p.Gln318Ter	1/18 (5.6%)
	E3 Del8bp	1/18 (5.6%)
SV	p.Ile172Asn	23/52 (44.2%)
	c.290-13A/C > G	9/52 (17.3%)
	Del	9/52 (17.3%)
	p.Gln318Ter	5/52 (9.6%)
	p.[Ile236Asn;Val237Glu;Met239Lys]	2/52 (3.8%)
	p.Pro30Leu	1/52 (1.9%)
	p.Arg356Trp	1/52 (1.9%)
	UD	7/52 (13.5%)
NC	p.Pro30Leu	6/16 (37.5%)
	p.Val281Leu	4/16 (25.0%)
	p.Ile172Asn	3/16 (18.8%)
	c.290-13A/C > G	3/16 (18.8%)
	p.Gln318Ter	1/16 (6.25%)
	p.Arg356Trp	1/16 (6.25%)

SW, salt-wasting form; SV, classical simple virilizing form at birth; NC, non-classical form with late-onset symptoms and diagnosis

group Null, two in Group A, one in Group B, and one in Group C).

Analysis of NC21-OHD patients

The seven cases with NC21-OHD presented mostly with menstrual disturbance, menopause, hirsutism, and acne. Case 8 was referred to hospital because her mother had SV 21-OHD. The age at first hospital visit for Cases 1–8 was 17.7 ± 3.2 years (range, 14–20 years). Epiphyseal arrest was noted at the first examination in Cases 1–7; their final height was therefore shorter (151.6 ± 7.1 cm) than average individuals of the same age. Physical examination findings included hirsutism, acne, and mild clitoromegaly. Seven patients underwent an $ACTH_{1\text{-}24}$ stimulation test. Their median basal 17-OHP level was 18.6 μg/L (range, 2.9–30.1 μg/L); after stimulation it was 82.0 μg/L (range, 11.9–139.1 μg/L). Four patients had PCO as detected by ultrasound examination (Table 6). Only two patients were homozygous for p.Val281Leu or p.Pro30Leu; the other six were compound heterozygotes for p.Pro30Leu or p.Val281Leu with a Null, Group A, or Group B mutation. It is possible that their offspring would be SW or SV. Case 8 had a peak 17-OHP level of 9.4 μg/L, which was below the usual cut-off (10.0 μg/L) for NC21-OHD diagnosis.

Discussion

CYP21A2 *mutations in Chinese 21-OHD patients*

The current protocol for the rapid genotyping of nine mutations causative of 21-OHD was revised from previous methods[3,4] and is suitable for clinical applications. The sensitivity of detection was 91.9%, in that 79/86

Table 5. Genotype–phenotype correlations in patients with 21-OHD.

Group	Genotype	Number of patients	Expected phenotype	Observed phenotype
Null	Del/Del	1	SW	SW
	E3 D8 bp/Del	1	SW	SW
	p.[[Ile236Asn;Val237Glu;Met239Lys];Gln318Ter]/ p.[Ile236Asn; Val237Glu; Met239Lys]	1	SW	SV
A	c.290-13A/C > G/Del	3	SV/SW	SW
	[c.290-13A/C > G; p.Ile172Asn]/Del	1	SV/SW	SW
	c.290-13A/C > G/p.[Ile172Asn; Gln318Ter]	1	SV/SW	SW
	[c.290-13A/C > G;p.Ile172Asn]/ [c.290-13A/C > G; p.Ile172Asn]	1	SV/SW	SW
	c.290-13A/C > G/p.Gln318Ter	2	SV/SW	SV
B	p.Ile172Asn/Del	6	SV	5 SV, 1 SW
	c.290-13A/C > G/p.Ile172Asn	3	SV	SV
	p.Ile172Asn/p.Ile172Asn	2	SV	SV
	p.Ile172Asn/[c.290-13A/C > G; p.Ile172Asn]	4	SV	SV
	p.Ile172Asn/p.Gln318Ter	2	SV	SV
C	p.Val281Leu/p.Val281Leu	1	NC	NC
	[c.290-13A/C > G; p.Pro30Leu]/p.Pro30Leu	1	NC	NC
	p.Pro30Leu/[c.290-13A/C > G; p.Ile172Asn]	1	NC	NC
	p.Pro30Leu/p.Ile172Asn	1	NC	NC
	c.290-13A/C > G/p.Pro30Leu	1	NC	NC
	p.Pro30Leu/p.Arg356Trp	2	NC	1 NC, 1 SV
	p.Val281Leu/p.Gln318Ter	1	NC	NC
	p.Ile172Asn/p.Val281Leu	1	NC	NC

SW, salt-wasting form; SV, classical simple virilizing form at birth; NC, non-classical form with late-onset symptoms and diagnosis

alleles were found to have at least one mutation. The most frequent mutation in Chinese patients with 21-OHD was p.Ile172Asn, with an allele frequency of 36%. However, if the patients were stratified by disease subtype, the most frequent mutations were Del in SW patients (allele frequency, 44.4%), p.Ile172Asn in SV patients (44.2%), and p.Pro30Leu in NC21-OHD patients (37.5%). As yet, the detailed molecular characterization of Chinese NC21-OHD patients has not been reported. The present study is therefore comprehensive because we detected all common mutations, and included all 21-OHD subtypes. This ensures an accurate reflection of the true spectrum of *CYP21A2* mutations and facilitates the analysis of the relationship between genotype and phenotype in these patients.

According to a previous study of 51 Chinese patients (with no NC21-OHD cases) in which the Del mutation was not detected at all,[12] the most common mutation was c.290-13 A/C > G in SW with an allele frequency of 43%. In contrast, in the present study, the most common mutation was Del with an allele frequency of 44.4%, followed by c.290-13A/C > G (38.9%). Previously, p.Ile172Asn (40.7%) was the most frequent mutation in SV patients, followed by c.290-13A/C > G (18.5%); these findings are similar to our own (p.Ile172Asn, 44.2%, c.290-13A/C > G, 17.3%). A study comprising eight SW and 11 SV patients and one NC patient from Taiwan showed that the most

Table 6. Clinical manifestation and genotype of eight female NC21-OHD patients.

Case no	Age at diagnosis (years)	Symptoms/ findings	Height (cm)	17-OHP basal (μg/L)	\17-OHP stimulated (μg/L)	Genotype
1	14.0	Menstrual disturbance; Hirsutism; Clitoromegaly; PCO	146.0	18.6	99.5	p.Val281Leu/p.Val281Leu
2	13.5	Menstrual disturbance; Clitoromegaly	154.0	82.4	249.0	[c.290-13A/C > G;p.Pro30Leu]/p.Pro30Leu
3	16.1	Secondary amenorrhoea; Hirsutism; Clitoromegaly	158.0	2.9	11.9	p.Pro30Leu/[c.290-13A/C > G;p.Ile172Asn]
4	18.0	Secondary amenorrhoea; Hirsutism; PCO	156.0	3.1	82.0	p.Pro30Leu/p.Ile172Asn
5	20.0	Menstrual disturbance; Hirsutism; Acne	145.0	30.1	139.1	c.290-13A/C > G/p.Pro30Leu
6	20.0	Amenorrhoea; Hirsutism; Clitoromegaly; PCO	142.0	21.9	unavailable	p.Pro30Leu/p.Arg356Trp
7	22.0	Amenorrhoea; Hirsutism; Acne; Clitoromegaly; PCO	160.0	27.4	52.2	p.Val281Leu/p.Gln318Ter
8	8.0	No symptoms and findings	122.0	2.5	9.4	p.Ile172Asn/p.Val281Leu

PCO, polycystic ovary

prevalent mutation was p.Ile172Asn (27.5%), followed by c.290-13A/C > G (25%), then Del (20%).[13] The genotype of the single NC21-OHD patient was p.Asp183Glu/undetected. In a report of 35 cases from Hong Kong, the most frequent mutations were Del (28.6%) and c.290-13A/C > G (28.6%), followed by p.Ile172Asn (18.5%).[14] Furthermore, 11 alleles (12.8%) in 10 patients carried two different mutations. This compound heterozygous state probably arose from large gene conversions or recurrent mutation events. The percentage of homozygous genotypes observed in the present study (10.8%) is lower than that reported in the USA (21%),[5] Germany (28%),[7] and Croatia (33%),[15] and much lower than in countries with a high rate of consanguinity such as Iran (50%)[16] and Iraq (50%).[17]

Among our Chinese NC21-OHD patients, the most frequent mutation was p.Pro30Leu, with an allele frequency of 37.5% (6/16). In contrast, among Caucasian NC21-OHD patients, the most common mutation was reported to be p.Val281Leu, with an allele frequency of 45%–60%.[18] The frequency of the p.Pro30Leu mutation is very low in parts of the world other than Eastern Asia: 3.6% in France,[19] 2.0% in Spain,[20] 2.2% in Israel,[21] and 1.7% in Brazil.[22] Japanese patients with NC21-OHD were similar to Chinese patients in that the most common mutation was also p.Pro30Leu with a reported allele frequency of 50.0% (7/14 alleles) in one study[23] and 37.5% (6/16 alleles) in another investigation of Japanese patients. [24] This suggests that the characteristics of *CYP21A2* mutations might differ between eastern Asian populations and others.

The mutational distribution was shown to be very similar between Chinese and Japanese populations.[23,24] Interestingly, however, the p.Val281Leu mutation has not been detected in Japanese patients.[23,24] One Japanese NC21-OHD patient was heterozygous for p.Val281Leu, but the girl was born in Japan to Brazilian parents.[25] Some investigators have even speculated that NC21-OHD does not occur in Asian populations, or was incorrectly diagnosed, because they had not observed p.Val281Leu.[26] Our finding of p.Val281Leu at a frequency of 25% in Chinese NC21-OHD patients provides evidence to refute this, and suggests a crucial difference between Chinese and Japanese cases that most likely reflects the ethnic differences between these populations. These observations should be confirmed by the molecular analysis of additional Chinese NC21-OHD patients.

The relationship between phenotype and genotype

21-OHD may increase in severity with a rise in the extent of 21-hydroxylase compromise.[7–11] Mutations causing the complete loss of 21-hydroxylase activity are Del, E3Δ8 bp, p.[Ile236Asn; Val237Glu; Met239Lys], p.Gln318Ter, and p.Arg356Trp. c.290-13A/C > G leads to almost no enzymatic activity, while p.Ile172Asn causes a moderate degree of compromise (1%–2% of wild-type activity), and p.Pro30Leu and p.Val281Leu are mild mutations that retain 20%–50% of the wild-type activity.[12,27]

To determine whether genotype can predict phenotype, we classified the genotypes into four groups according to severity. Because 21-OHD is an autosomal recessive disorder, the allele with the less severe mutation has the greater influence on phenotype. We found that our stratification method had a positive predictive value (PPV) of 81.8% (9/11) for predicting SW 21-OHD and a negative predictive value (NPV) of 96.2% (25/26). The PPV for SV was 94.1% (16/17) and the NPV was 80.0% (16/20), while the PPV for NC21-OHD was 88.9% (8/9) and the NPV was 100% (28/28). The average PPV was high (up to 88.3%) and the overall NPV was 92.1%. This suggests that genotype is strongly correlated with phenotype in Chinese 21-OHD

patients. Other investigators have found that the concordance rate between genotype and phenotype varies among the three forms of 21-OHD.[5,28] Speiser et al.[29] showed that the prediction of phenotype from genotype became more difficult in patients who were compound heterozygotes or in those carrying mutations of intermediate severity. Others found the same results in different populations.[30–32] New et al.[33] reported that 98% of 21-OHD patients had a genotype that corresponded to the hormonal phenotype.

In the present study, we found that the genotype could not be used to predict the phenotype in only five patients. This genotype/phenotype discrepancy may be explained in a number of ways. In the two p.Gln318Ter/c.290-13A/C > G patients, alternative splicing causing variable levels of normal 21-hydroxylase activity might explain why the phenotype was SV not SW.[34] Furthermore, the p.Ile172Asn mutation, which retains 1%–2% of 21-hydroxylase activity, does not always give rise to SV so could explain why the single Del/p.Ile172Asn case manifested as SW not SV. In the p.Pro30Leu/p.Arg356Trp case, compound heterozygosity may lead to a level of enzymatic activity intermediate between that of p.Pro30Leu and p.Arg356Trp homozygotes, possibly explaining why the case presented as SV not NC21-OHD. Additionally, the presence of unidentified rare mutations modulating the phenotype cannot be excluded.[35] Nevertheless, these genotype–phenotype discrepancies indicate that great caution must be exercised when predicting phenotypes after a prenatal diagnosis using fetal DNA.[36]

In conclusion, the genotyping method presented here is practical, suitable for clinical applications, and clearly illustrates the close correlation between genotype and phenotype. We identified many similarities in the mutational spectrum between Chinese and Japanese NC21-OHD patients, but disparity was also noted. Thus, the characterization of NC21-OHD genotypes in different ethnic populations deserves further study.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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