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Genotype/phenotype correlations in 538 congenital adrenal hyperplasia patients from Germany and Austria: discordances in milder genotypes and in screened versus prescreening patients

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Abstract

Congenital adrenal hyperplasia (CAH) due to *CYP21A2* gene mutations is associated with a variety of clinical phenotypes (salt wasting, SW; simple virilizing, SV; nonclassical, NC) depending on residual 21-hydroxylase activity. Phenotypes and genotypes correlate well in 80–90% of cases. We set out to test the predictive value of CAH phenotype assignment based on genotype classification in a large multicenter cohort. A retrospective evaluation of genetic data from 538 CAH patients (195 screened) collected from 28 tertiary centers as part of a German quality control program was performed. Genotypes were classified according to residual 21-hydroxylase activity (null, A, B, C) and assigned clinical phenotypes correlated with predicted phenotypes, including analysis of Prader stages. Ultimately, concordance of genotypes with clinical phenotypes was compared in patients diagnosed before or after the introduction of nationwide CAH-newborn screening. Severe genotypes (null and A) correlated well with the expected phenotype (SW in 97 and 91%, respectively), whereas less severe genotypes (B and C) correlated poorly (SV in 45% and NC in 57%, respectively). This was underlined by a high degree of virilization in girls with C genotypes (Prader stage >1 in 28%). SW was diagnosed in 90% of screening-positive babies with classical CAH compared with 74% of prescreening patients. In our CAH series, assigned phenotypes were more severe than expected in milder genotypes and in screened vs prescreening patients. Diagnostic discrimination between phenotypes based on genotypes may prove overcome due to the overlap in their clinical presentations.

Key Words

- ▶ 21-hydroxylase
- ▶ *CYP21A2*
- ▶ genotype-phenotype
- ▶ congenital adrenal hyperplasia
- ▶ CAH

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Introduction

Congenital adrenal hyperplasia (CAH; incidence 1 in 10–15,000) due to 21-hydroxylase deficiency (21-OH) (*CYP21A2*; OMIM 201910) is an autosomal recessive disorder resulting in a deficient production of the steroid hormones cortisol and aldosterone. Consequently, the precursors of these hormones accumulate and are shunted in the androgen pathway causing androgen excess. A complete loss of 21-OH function results in salt-wasting (SW) phenotypes, while a minimal residual 21-OH production, which may be sufficient for aldosterone homeostasis, leads to simple virilizing (SV) or, less symptomatic, nonclassical CAH phenotypes (NC CAH). However, subclinical or clinical mineralocorticoid deficiency, particularly in small infants, leads to an overlap in clinical presentation and assignment among SW and SV forms (1). In a compound heterozygous state with two different mutations, phenotype is generally deemed to be determined by the less severely affected allele. Classic CAH is clinically often characterized by ambiguous genitalia in 46,XX individuals, whereas boys may show no signs at birth. Salt loss caused by aldosterone deficiency constitutes a life-threatening condition, particularly in early infancy when sodium intake is low and processing restricted due to physiological mineralocorticoid resistance (2). Without cortisone replacement, both sexes progress into precocious pseudopuberty. NC CAH has about 10-fold higher frequency than classic CAH, usually manifesting during childhood with early pubarche and signs of androgen excess, but often goes unnoticed, particularly in boys. This explains the relatively low numbers of diagnosed boys and the unequal sex distribution in various studies (3, 4, 5, 6, 7).

CYP21A2 lies on chromosome 6p in close proximity to its pseudogene (*CYP21A2P*), which is 96–98% homologous. Up to 75% of CAH mutations arise from the pseudogene by meiotic non-disjunction. Deletions (del) and *CYP21A2/CYP21A2P* chimeric genes account for the remainder mutations. Most mutations are inherited and only a small proportion are due to rare or *de novo* mutations (8).

Correlation between the genotype and clinical phenotype depends on the degree of residual 21-hydroxylase activity. Many studies have sought to establish genotype-phenotype associations, investigating large nation- or population-based patient cohorts all over the world (3, 4, 5, 6, 7, 9, 10, 11, 12). In general, severe mutations (residual 21-OH function 0–1%) have been found to correlate well with the clinical phenotype (SW) whereas milder mutations show a greater variability

with regard to individual clinical presentation of identical mutations.

Whereas in the past girls with classic CAH were generally detected at birth due to ambiguous genitalia, some affected boys were overlooked leading to unexplained death due to presumptive SW crises in several cases (13). For this reason, nationwide 17-hydroxyprogesterone (17-OHP) newborn screening was introduced in many countries throughout the world, including Germany (1999) and Austria (2001).

We report on allelic frequencies and genotype-phenotype correlations in a pediatric cohort of 538 CAH patients, the largest European cohort investigated to date. Furthermore, we analyzed the degree of virilization in affected girls with respect to genotype. Interestingly, we found considerable differences in genotype-phenotype correlations between prescreening and screened patients. To our knowledge, this is the first report of such data on screened CAH patients.

Subjects and methods

AQUAPE database

The AQUAPE (Working Group on Quality Assurance in Pediatric Endocrinology) CAH is an initiative prospectively recording clinical follow-up data from patients with CAH in a standardized database. This dataset is used for quality assurance and research. Altogether, 44 pediatric endocrine centers in Germany and Austria are participating in the initiative. Core data on phenotype, genetics and family history, as well as data on anthropometry, surgery, medication and biochemistry, collected during routine care at each clinical visit, are documented electronically at the individual center (<http://buster.zibmt.uni-ulm.de>). Twice a year, updated records are transferred in a pseudonymized form to the central data bank based in Ulm, Germany. Before processing for analysis, the data are verified and sent back to the original center for review if necessary. So far, data from 1298 patients, born between 1957 and 2013, comprising 22,552 outpatient appointments, have been recorded. Starting from Magdeburg, the AQUAPE CAH database was first approved by the data protection commissioner of the state of Saxony-Anhalt. Based on this central approval, each participating center attained institutional approval, complying with local ethical and data management guidelines. Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Clinical classification/phenotyping

In the prescreening era, SW was diagnosed if a patient presented with dehydration, hyponatremia and hyperkalemia during the first 8 weeks of life, genitalia were ambiguous in females, and basal 17-OHP and renin activity and/or concentration were markedly elevated. Treatment consisted of hydrocortisone, fludrocortisone and salt replacement. After the introduction of the nationwide screening program (from 1 January 1999 in Germany and from 1 April 2001 in Austria) measuring 17-OHP levels on filter paper by a fluoroimmunoassay (DELFLIA, Wallac Oy, Turku, Finland), virtually all SW patients and most SV patients were recognized by their elevated 17-OHP concentrations. SV was defined by the absence of SW, genital ambiguity in girls, pseudoprecocious development, growth and bone age acceleration and high 17-OHP levels at repeat sampling or after Synacthen stimulation. Treatment was confined to hydrocortisone replacement. NC CAH was diagnosed in girls when they had no or minimal genital virilization, precocious pubarche, hirsutism or other clinical signs of hyperandrogenemia, and high 17-OHP levels after Synacthen stimulation (>30 mmol/L), according to the nomogram by New (14). Similarly, NC CAH was diagnosed in boys if precocious pubarche/virilization developed beyond 4 years of age accompanied by high 17-OHP levels after Synacthen stimulation (1). Virilization in girls was graded according to Prader genital stages (PGS) with stages ranging from 1 (clitoromegaly) to 5 (complete virilization). In all patients, diagnosis of CAH was established or verified by a Pediatric Endocrinologist.

CYP21A2 analysis/genotyping

Different approaches and methods were used for genotype analysis. In general, Southern blot or, more recently, multiplex ligation-dependent probe amplification analysis (15) was used to detect gene deletions, chimeric genes and other gene alterations. Point mutations and small deletions as well as insertions were analyzed by Sanger sequencing, including the promoter region, from PCR-amplified genomic DNA. Sequencing was the primary approach in 83.2% of the samples analyzed. A few centers first screened for the ten most common mutations (P30L, I2G, G110_8bp, I172N, E6 cluster, V281L, F306+t, Q318X, R356W, P453S) employing allele-specific PCR (16) and performed Sanger sequencing only if these results were inconclusive. Overall, this led to a sequencing rate close to 90%. If available, parents and other family members

were tested to confirm homozygosity/compound heterozygosity of affected individuals, delineate the mode of transmission if a child had more than two mutations and screen for carrier status in clinically unaffected relatives.

Genotype classification

Classification (null, A, B, C, D) was based on the scheme provided by Speiser (16). According to this grouping, the severity of mutations is classified by its residual enzymatic activity: 'null' (0% residual function; del/con, G110_8bp, E6 cluster, F306+t, Q318X, R356W). 'A' (0–1% residual function; I2G), 'B' (1–2% residual function; I172N) or 'C' (20–60% residual function; P30L, V281L, P453S). Since CAH is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations, the allele encoding for a protein with higher residual 21-OH activity is generally deemed to determine the severity of the overall genotype: 'null' if both alleles carried a 'null' mutation (null/null); 'A' if A/null or A/A; 'B' if B/null or B/A or B/B; or 'C' if C/null or C/A or C/B or C/C alleles were present. Novel mutations on at least one allele with lacking information on impairment of 21-OH function were genotyped as 'D' and excluded from analysis.

Results

Eligible patients

Information on genotype was complete for 660 (385 female/275 male; 58%/42%) among the 1298 patients recorded in the AQUAPE database. After exclusion of patients with incomplete phenotypes and group 'D' genotypes, 195 and 343 patients with complete datasets remained in the screened and prescreening groups, respectively (Fig. 1).

Screened patients (median age 9 years (0.5–14)) had the expected equal sex distribution in line with autosomal recessive inheritance (48% girls). However, the prescreening group (median age 23 years (12–56)) had a significantly higher proportion of females (64%). This was mainly due to the larger proportion of females with clinical diagnosis of NC CAH later in childhood.

Allelic frequencies of CYP21A2 mutations

Altogether, genetic data of 1320 alleles (Table 1) were available. The ten most common genetic variants were present in 96.8% of alleles, whereas rare or unknown

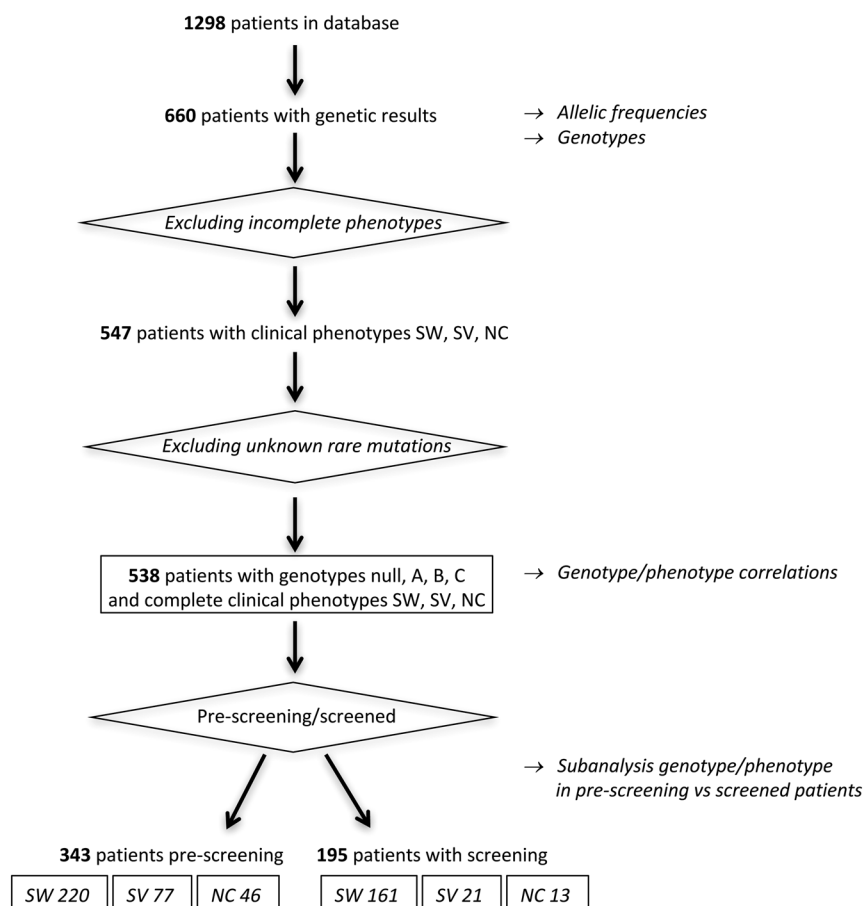


Figure 1

Flow chart of patient eligibility and data analysis. NC, nonclassical CAH; SV, simple virilizing CAH; SW, salt wasting CAH.

mutations were detected in 3.2%. Del/conversions resulting in chimeric genes (29.6%) and I2G (29.2%) were most frequent, followed by I172N (13.1%). Mutations with *in vitro* 21-OH activities between 10 and 60% (P30L, V281L, P453S and some rare mutations) constituted 12.2% of the alleles. Two non-adjacent mutations on one allele were present in 34 alleles (2.6%) and their genotypes classified according to the more severe mutation. Three or more adjacent mutations, comprising at least one severe mutation each, were classified as chimeric genes leading to a complete loss of 21-OH function. Among del/chimeric genes, 73% were caused by deletions, whereas chimeric genes accounted for the remainder. In general, frequencies were similar to published data with some differences, in particular affecting I172N and V281L.

Genotypes

Homozygous genotypes were present in 37% of the patients with more than two-thirds either del/del or I2G/I2G, whereas 63% of patients were compound heterozygous. The distribution of genotypes was equal

across sexes except for the null mutations that were found more frequently in males (30% vs 19% in females) and mutations, usually associated with a NC phenotype (P30L, V281L, P453S), that were more prevalent in females (25 vs 11% in males). The latter is likely caused by an ascertainment bias and corresponds to the significantly higher proportion of females with NC CAH in our cohort (87 females vs 13% males).

Genotype-phenotype correlations

In general, the frequency of the expected SW phenotype (Table 2) in patients with genotype null (97%) and A (91%) was similar to previously published studies. In contrast, the genotype-phenotype correlation was significantly lower for genotype B (46%) and C (58%) as compared to other studies. More than half of the patients (54%) with genotype B (I172N) were classified as SW. Among genotype C patients, 78% with a P30L mutation had classic CAH compared with 30% with V281L. Accordingly, genotype C with P30L constituted one-third (32.6%) of all patients with SV CAH. If virilization occurred in genotype C, PGS ranged from 1 to 5 in P30L, whereas V281L was

Table 1 Frequencies (%) of *CYP21A2* variants compared with published data.

Country	Germany + Austria present study	USA (11)	Argentina (7)	Netherlands (5)	Germany (4)	Sweden (9)
<i>n</i> (alleles)	1.320	3.005	866	370	310	400
Del/Kon	29.6	20.0	11.2	31.9	27.4	32.2
P30L	2.6	2.6	0.7	0.3	2.6	1.6
I2G	29.2	22.9	20.6	28.1	30.3	26.6
G110_8bp	2.5	2.1	0.8	4.3	1.6	1.1
I172N	13.1	8.2	8.2	12.4	19.7	19.8
E6 cluster	1.5	2.1	2.0	3.0	1.0	1.1
V281L	7.8	23.9	26.2	2.2	2.9	5.7
F306+t	0.3	–	–	0.3	0.3	<1.0
Q318X	4.6	3.5	6.7	3.5	4.8	2.4
R356W	4.1	3.6	4.2	8.4	4.5	3.0
P453S	1.4	–	–	0.5	0.3	<1.0
Other	3.2	–	–	2.6	4.6	<5.0

associated with PGS 1 and 2 at the most. After excluding NC phenotypes, the second allele was affected by a more severe mutation (null, A, B) in all patients except for one SW (P30L/V281L) and 5 SV patients with two C alleles (V281L/V281L, $n=3$; V281L/P453S, $n=1$; P453S/P453S, $n=1$) (Table 3). None of the patients with genotypes null, A and B had NC CAH. Analysis of relative frequencies of predicted phenotypes in relation to allelic combination of genotypes revealed that a more severe mutation on the second allele was associated with a more severe phenotype as compared to mutations displaying some residual function (Table 4).

PGS in correlation with genotypes and phenotypes in 46,XX CAH patients

PGS information was available in 84% of 46,XX CAH (96% of screened and 79% of prescreening patients) showing virilization (PGS 1–5) in 95% and no virilization in 5%. Severe virilization (PGS 4–5) was common in the null (56%) and A (51%) groups, compared with 24% in genotype B and 6% in genotype C. At the extreme end, one B and C patient each presented with PGS 5 (2/10), whereas the remaining patients with PGS 5 had null (3/10) and A (5/10) genotypes. Twice as many patients with SW vs SV form (43 vs 22%) had PGS 4, whereas PGS 3 was similar in both groups (29 vs 31%) and mild virilization (PGS 1 and 2) occurred in considerably fewer patients with SW vs SV forms (24 vs 40%) (Table 5).

Genotype-phenotype correlations in prescreening vs screening patients

Both the prescreening and the screened group were analyzed separately. Genotype A (I2G) was 8% more frequent in screened than in prescreening patients

(40.4%). Conversely, genotype C was 8.4% more prevalent in prescreening patients. Notably, distribution of PGS 1–5 as well as median PGS (-3) in female CAH patients was similar in both groups (Table 6).

a. Genotype-phenotype correlations in prescreening patients ($n=334$)

Regarding classic CAH, SW occurred in 74%, correlating well with the literature. 4% of null and 15% of A had SV CAH. Accordance of B (SV) and C (NC) (56% and 61%, respectively), lay in a range found in some former studies (4, 5) and was pronouncedly higher than in screened patients (Table 2).

b. Genotype-phenotype correlations in screened patients ($n=204$)

SW was far above expected rates comprising 90% of patients with classic CAH. Null mutations (100%) and A (99%) showed a high degree of concordance with the SW phenotype, whereas correlation was very low in B (30%) and C genotypes (52%) (Table 2).

Discussion

Herein we present the largest European cohort of CAH patients reported to date including 538 patients with genotype-phenotype information. Allelic distribution of common mutations and the overall genotype distribution was largely similar to previously published cohorts (4, 5, 9) except for C genotypes influenced by ethnic factors and NC CAH proportion in different studies. However, clinical classification according to genotype groups revealed weak correlation with the predicted phenotype in less severe genotypes (B and C) (Table 2). Discordance was even more pronounced in patients diagnosed after screening had been introduced, who were classified by clinicians as clinically more severe (SW in 90% compared with SW in 74% of

Table 2 Concordance of null, A, B, C genotypes with assigned phenotypes as compared to published data. Prescreening and screened groups are listed also separately.

Genotype/predicted phenotype	Present study			Former studies			
	All (n = 538)	Prescreening (n = 334)	Screened (n = 204)	USA (11) (n = 1507)	Argentina (7) (n = 454)	Netherland (5) (n = 198)	Germany (4) (n = 155)
Null/SW%	97	96	100	≤100	100	97	100
A/SW%	91	85	99	79	84	96	90
B/SV%	46	56	30	76	87	53	74
C/NC%	58	61	52	>90	100	100	65

prescreening patients). This might in part be caused by the fact that genotype A was 8% more frequent in screened patients and genotype C overrepresented (+8.4%) in the prescreening group. However, PGS in female patients were overall identical in screened and prescreening patients (Table 6). Hence, relatively more patients of the screened cohort were treated with mineralocorticoids.

Deletions/chimeric genes and I2G comprised almost 30% each, followed by I172N in 13.1% (Table 1). C genotype mutations were found in 11.8%, led by V281L (65.6% of C) and P30L (25% of C). Some *CYP21A2* mutations such as I2G and V281L have strong ethnic distributions due to founder effects. For instance, V281L was found to be more prevalent in patients of Mediterranean descent, explaining the higher numbers in studies of such patients (7, 11). However, detailed information on ethnic distribution was not available in our cohort.

Many studies have investigated genotype-phenotype relations in large national and multiethnic cohorts. In general, severe genotypes leading to SW uniformly showed a strong correlation with the clinical phenotypes. In our study, overall genotype-phenotype correlation was also high in severe mutations (null: 97%; A: 91%), but extraordinarily low in B (46%) and C (58%) phenotypes. In comparison (Table 2), former studies reported 97–100% concordance in null, 79–96% in A, 53–87% in B and 65–100% in C genotypes (4, 5, 7, 11). Regarding genotype C, 78% of our patients with a P30L mutation and 30% of V281L patients, respectively, had classical CAH (Table 3). PGS correlated with enzymatic loss in severe genotypes

Table 3 Genotype/phenotype correlations in C genotypes (P30L, V281L, P453S).

n (% of total)	SW	SV	NC	Total
P30L	7 (26) ^a	14 (52)	6 (22)	27 (100)
V281L	4 (6)	15 (24) ^a	45 (70)	64 (100)
P453S	–	1 (20)	4 (80)	5 (100)

^aOne patient each with P30L/V281L (SW) and V281L/P453S (SV) was assigned to the P30L and the V281L group, respectively, and counted only once.

(Table 5) but virilization was also common in genotype C, especially in female P30L patients. Variably, such results on metabolic and clinical phenotypes (PGS) in C genotypes have been observed before: Classical CAH was found in 11% (4) and 30% (17) of V281L patients and in two-thirds of patients with P30L mutations (4, 7, 17) associated with virilization in 24% of V281L and in 66% of P30L patients in the latter study (17).

The reasons for these observed discrepancies have not been clarified yet. Some residual activity, for example in I2G (splice site mutation) resulting in SV CAH may occur (up to 20% in 11) even though it is classified as a severe mutation usually leading to SW (18). Furthermore, effects from additional pathways that regulate the severity of enzymatic loss such as the androgen backdoor pathway (19) may play a critical role in individual patients causing more heterogeneous phenotypes. In addition, we observed increased clinical severity in compound heterozygous, who carried a more severe mutation on the other allele (Table 4). This would be supported by the observation of higher biochemical androgen response to ACTH stimulation in compound heterozygous NC CAH with one classical haplotype vs homozygotes with two mild mutations, although clinical effects have not been observed previously (20). From a functional perspective, ambiguity is reflected by variable degrees of residual *in vitro* activity reported in different studies, ranging between <2–5% in I172N (21, 22) and 10–60% in P30L (23, 24). Finally, particularly in patients with P30L mutations, additional mutations in the promotor or P30L mutations as part of minor gene conversions/chimeric genes may go unnoticed (25, 26). All these factors may contribute to the experience, that – apart from the null genotype, resulting in a complete loss of 21-OH function – clinical presentation of CAH shows a free flow depending on residual 21-OH function and impact of additional cofactors that are largely unknown.

Notably, assigned phenotypes were more severe in screened (90% SW) vs prescreening patients (74% SW) despite similar clinical characteristics (PGS; Table 6). In

Table 4 Relative frequencies of predicted phenotypes in relation to allelic combination of genotypes.

Genotype		n	Predicted phenotype	Observed phenotype			Relative frequency of predicted phenotype
Allele 1	Allele 2			SW	SV	NC	
Null	Null	150	SW	146	4	–	0.97
A	Null	123	SW	113	8	–	0.92
A	A	62	SW	53	9	–	0.85
B	Null	57	SV	36	21	–	0.37
B	A or B	45	SV	20	25	–	0.56
C	Null	42	NC	6	11	25	0.59
C	A or B	39	NC	4	16	19	0.49
C	C	20	NC	1	4	15	0.75

particular, genotype B, suggesting SV CAH, was present in 70 vs 44% of screened vs prescreening SW CAH patients, respectively (Table 2). Since absolute 17-OHP levels at newborn screening may not discriminate between SW and SV forms and confounding factors such as prenatal glucocorticoid treatment or postnatal stress interfere, CAH consensus guidelines recommend treating newborns with classic CAH with glucocorticoids, fludrocortisone and salt supplements, starting from diagnosis by neonatal 17-OHP screening (27). Clinical or subclinical SW is considerably more frequent than SV and may not be obvious before the end of the first week of life or even until several weeks after birth. However, because of the non-SW CAH likelihood of around 25%, the current guidelines recommend critically reevaluating the further need for mineralocorticoid treatment after early infancy assessing blood pressure, plasma renin activity, and the aldosterone-to-renin ratio and adjusting treatment accordingly (27). Therefore, the current approach to provide for potential SW in all patients with elevated 17-OHP at screening by adding a standard dose of fludrocortisone and salt supplements will maximize safety. On the other hand, fludrocortisone overtreatment – theoretically in one-fourth of patients who will rather have SV CAH – might lead to hypertension that could go unnoticed for months or even years with potential long-term effects in later life. Additionally, blood pressure measurements in infants/children in an ambulatory setting may be unreliable if the child is not calm and therefore easily relativized or disregarded

if high. In a recent study from the same working group (AQUAPE database), elevated blood pressure was found in 12.5% of 3- to 18-year-old CAH patients ($n=716$), with an even higher proportion in the younger age group (18.5 vs 4.9% in adolescents) (28). Generally, elevated blood pressure together with suppressed renin levels should always prompt the physician to reduce or stop fludrocortisone treatment. However, even if this decision is theoretically evident, it may be precarious to make in an individual clinical setting, particularly in very young children. Neonatal management and clinical guidance may be optimized by second-tier tests which are nowadays available in many institutions, such as tandem mass spectrometry or rapid genetic tests, for example screening for the most common mutations and deletions (29).

Our study has several strengths. We have investigated the so far largest European cohort of CAH patients arising from 28 centers. Furthermore, the quality of genetic data was superior, with a sequencing rate close to 90%, far exceeding other large-scale studies (11). In addition, this is the first report of genotype-phenotype correlations in a large young patient cohort diagnosed after the introduction of screening as compared to the prescreening era. As to limitations, despite clear diagnostic criteria and thorough data editing we cannot exclude arbitrary overestimation of severity of clinical phenotypes. If in doubt from a clinical perspective, the presence of mineralocorticoid treatment might have influenced classification of an individual patient as SW, causing a bias toward higher numbers of

Table 5 Prader genital stages (%) stratified according to genotypes (null, A, B, C, D) and according to clinical phenotypes (SW, SV, NC).

%	Genotypes				Phenotypes		
	Null (n = 66)	A (n = 90)	B (n = 58)	C (n = 50)	SW (n = 183)	SV (n = 49)	NC (n = 32)
No virilization	–	–	2	24	–	2	38
Prader							
1	3	6	17	54	8	20	62
2	9	12	28	12	16	20	–
3	32	31	29	4	29	31	–
4	51	45	22	4	43	22	–
5	5	6	2	2	4	4	–

Table 6 Prader stages (%) in prescreening and screened 46,XX individuals with CAH.

Prader stage	Prescreening % (n = 174)	Screened % (n = 90)
No virilization	3	9
1	15	19
2	17	12
3	26	26
4	36	30
5	3	4
Prader stage (1–5) median	3	3

severe phenotypes, particularly in patients diagnosed after screening had been introduced. Not least, shortcomings of molecular genetic techniques, in particular if sequencing was not carried out (~10% in our series), might have resulted in additional allelic mutations being overlooked.

In conclusion, in this the largest European study reported to date, *CYP21A2* phenotype prediction was reasonably conclusive in severe genotypes but unreliable in weaker genotypes. Current management guidelines recommending to treat all screening-positive babies as SW CAH might result in overrating of the clinical phenotype and mineralocorticoid overtreatment in a small but significant proportion of patients. Since CAH has to be regarded as a continuum with fluent boundaries between SW and SV phenotypes, subclassification should be rather driven by clinical and biochemical markers than by genotype.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

F-W Röhl, K Mohnike, R W Holl carried out study design and project management. Data collection was done by S Riedl, W Bonfig, J Brämshwig, A Richter-Unruh, S Fricke-Otto, M Bettendorf, F Riepe, E Schönau, G Even, B Hauffa and H-G Dörr. Data analysis was done by F-W Röhl, K Mohnike, S Riedl and G Kriegshäuser. Preparation of the manuscript was done by S Riedl. Editing and final approval of the manuscript were done by all authors.

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