P1-D2-1

Allelic frequencies of *CYP21A2* variants and genotype-phenotype correlations in a cohort of 660 CAH patients from Germany and Austria

Stefan Riedl[1,2], Friedrich-Wilhelm Röhl[3], Susann Empting[4], Walter Bonfig[5], Helmuth-Günther Dörr[6], Reinhard W. Holl[7], Klaus Mohnike[4], and the AQUAPE Study Group

[1]St. Anna Children´s Hospital; and [2]Dept of Pediatric Pulmology, Allergology, and Endocrinology, Medical University of Vienna, Austria; [3]Institute of Biometry and Medical Informatics; and [4]Dept of Pediatrics, Otto-von-Guericke University Magdeburg; [5]Dept of Pediatrics, Technical University Munich; [6]Dept of Pediatrics and Adolescent Medicine, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen; [7]Institute of Epidemiology and Medical Biometry, University of Ulm, Germany

Context and Objective

Congenital adrenal hyperplasia (CAH) due to a *CYP21A2* defect leads to salt wasting (SW), simple virilizing (SV), or non-classical (NC) phenotypes depending on residual 21-hydroxylase (21-OH) function. Phenotype correlates with genotype in 80-90%.
We set out to test prediction of CAH phenotype based on genotype classification

Patients and Methods

Patient data from 37 centres were retrieved from a central data base as part of a German quality assurance program (AQUAPE*) within the German Association for Pediatric Endocrinology and Diabetes (DGKED). Allelic frequency and distribution of deletions/conversions (del/con) and the 11 most common *CYP21A2* mutations were analysed in 660 homozygous or compound heterozygous CAH patients (**Figure 1**). Associated clinical phenotypes (n=515) as classified by the treating physician were compared with predicted phenotypes from genotype classification according to magnitude of residual 21-OH function (group Null=0%; group A=0-2%; group B=2-5%; group C=20-60%). Moreover, patients were stratified according to time at diagnosis (pre-screening versus screening), including analysis of genital virilization (Prader stages) in girls.

Results

Allelic frequency of mutations was comparable to previous studies, with del/con (29.6%) and I2G (29.2%) being the most common, followed by I172N (13.1%) (**Table 1**). Severe genotypes (Null and A) correlated well with expected phenotypes (SW in 97% and 91%. respectively), whereas weaker genotypes (B and C; **Table 2**) showed poor correlation (SV in 45% and NC in 57%, respectively) with higher clinical severity than expected, specifically associated with I172N and P30L. In C genotypes, this was underlined by the degree of virilization (Prader Stage >1 in 28%; **Figure 2**). SW was ascertained in 90% of screened patients with classical CAH as compared to 74% of pre-screening patients, whereas Prader stages did not differ between these two groups (**Table 3**).

Figure 1. Breakdown of eligible patients according to genotype/phenotype availability and timepoint at diagnosis

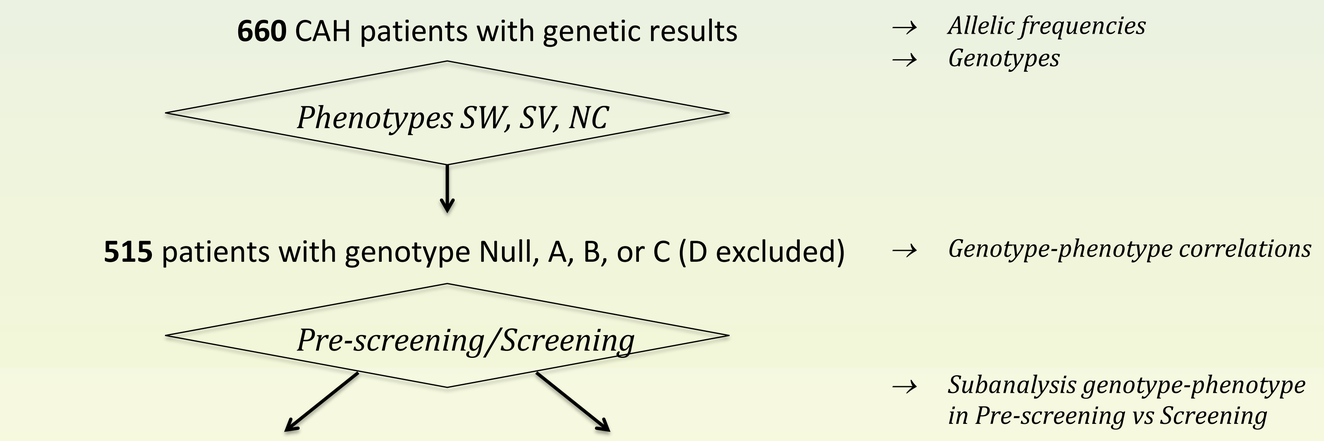

Table 1. Frequency (%) of *CYP21A2* variants in our population as compared to published data. Common mutations were present in 1285/1320 alleles (96.8%)

(Genotype defined by less affected allele)	*21-OH activity*	Expected *phenotype*	Country / Year	DE/AT **(Present study)**	US 2013[a]	AR 2011[b]	NL 2003[c]	AT 2001[d]	DE 2000[e]	SE 1998[f]
			n (alleles)	***1.320***	*3.005*	*866*	*370*	*158*	*310*	*400*
Genotype Null	0%	SW	Del/Kon	**29.6**	20.0	11.2	31.9	31.0	27.4	32.2
			G110_8bp	**2.5**	2.1	0.8	4.3	-	1.6	1.1
			E6 cluster	**1.5**	2.1	2.0	3.0	1.9	1.0	1.1
			F306+t	**0.3**	-	-	0.3	-	0.3	<1.0
			Q318X	**4.6**	3.5	6.7	3.5	2.5	4.8	2.4
			R356W	**4.1**	3.6	4.2	8.4	3.2	4.5	3.0
Genotype A	0-2%	SW	I2G	**29.2**	22.9	20.6	28.1	22.8	30.3	26.6
Genotype B	2-5%	SV	I172N	**13.1**	8.2	8.2	12.4	15.8	19.7	19.8
Genotype C	20-60%	NC	P30L	**2.6**	2.6	0.7	0.3	3.2	2.6	1.6
			V281L	**7.8**	23.9	26.2	2.2	12.0	2.9	5.7
			P453S	**1.4**	-	-	0.5	1.3	0.3	<1.0
Genotype D	variable	variable	Other	**3.2**	-	-	1.8	5.7	4.6	<5.0

Figure 2. Genital virilization (Prader stages) in CAH girls according to genotypes

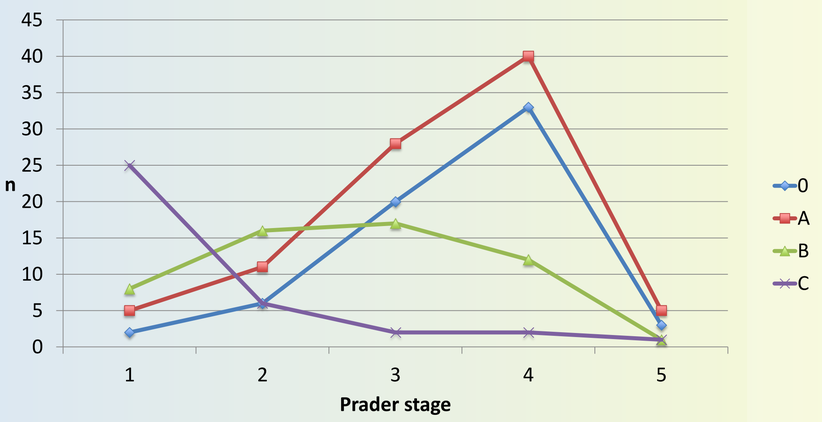

Table 2. CAH phenotype distribution according to genotype in pre-screening and screening patient groups

	Present study			Former studies			
Genotype / *predicted phenotype*	**All patients** ***n=515***	Pre-Screening (before 1999) *n=327*	Screening (from 1999) *n=188*	US 2013[a] *n=1507*	AR 2011[b] *n=454*	NL 2003[c] *n=198*	DE 2000[e] *n=155*
Null / *SW*	**97**	96	100	≤100	100	97	100
A / *SW*	**91**	85	99	79	84	96	90
B / *SV*	**46**	54	31	76	87	53	74
C / *NC*	**57**	61	48	>90	100	100	65

Table 3. Prader stages in pre-screening and screening female CAH patients

Prader stage	**Pre-Screening** *n*	*%*	**Screening** *n*	*%*
1	26	15.5	13	17.3
2	29	17.2	11	14.7
3	45	26.8	22	29.3
4	62	36.9	25	33.3
5	6	3.6	4	5.3
All (1-5)	168	100	75	100
Median	3		3	
Mean	2.96 ± 1.14		2.95 ± 1.18	

Conclusions

- ✓ In this largest European CAH series reported to date, allelic frequencies of CYP21A2 mutations were comparable to former studies.
- ✓ Prediction was accurate, i.e. in line with expected phenotype, in del/con and severe mutations (genotypes Null and A), but unreliable in weaker genotypes (genotypes B and C).
- ✓ By treating all screening-positive babies as SW-CAH from the start, current management strategies[g] might result in overrating of clinical phenotype.
- ✓ CAH severity should be regarded as a continuum requiring ongoing reevaluation of phenotype and flexibility in clinical management.

*AQUAPE Study Group

Aue Kinderklinik, Berlin Charite Universitäts-Kinderklinik, Bochum Endokrinologikum Ruhr, Bremen Zentralkrankenhaus Nord, Chemnitz Kinderklinik, Cottbus Kinderarztpraxis, Datteln Kinderklinik, Dresden Gemeinschaftspraxis, Dresden Universitäts-Kinderklinik, Erlangen Universitätsklinik für Kinder und Jugendliche, Essen Universitäts-Kinderklinik, Frankfurt Universitäts-Kinderklinik, Halle/S. Universitäts-Kinderklinik, Hamburg MVZ Dr. Commenz, Hannover Medizinische Hochschule, Hannover Endokrinologikum, Hannover Kinderkrankenhaus, Heidelberg Universitäts-Kinderklinik, Hildesheim St. Bernward Krankenhaus, Homburg Universitäts-Kinderklinik, Jena Universitäts-Kinderklinik, Kiel Universitäts-Kinderklinik, Krefeld Kinderklinik, Köln Städtische Kinderklinik, Köln Universitäts-Kinderklinik, Leipzig Universitäts-Kinderklinik, Lübeck Universitäts-Kinderklinik, Magdeburg Universitäts-Kinderklinik, München-Gauting Kinderarztpraxis, Münster Universitäts-Kinderklinik, Nürnberg Cnopfsche Kinderklinik, Stade Elbekliniken Kinderklinik, Tübingen Universitäts-Kinderklinik, Ulm Endokrinologikum, Wien Universitäts-Kinderklinik

AQUAPE is supported by NOVO Nordisk Germany

References

[a]**New** et al.: Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency, PNAS 2013
[b]**Marino** et al.: Steroid 21-hydroxylase gene mutational spectrum in 454 Argentinean patients..., Clin Endocrinol 2011
[c]**Stikkelbroeck** et al.: CYP21 gene mutation analysis in 198 patients with 21-hydroxylase deficiency in The Netherlands..., JCEM 2003
[d]**Baumgartner-Parzer** et al.: Mutational spectrum of the steroid 21-hydroxylase gene in Austria: identification of a novel missense mutation, JCEM 2001
[e]**Krone** et al.: Predicting phenotype in steroid 21-hydroxylase deficiency? Comprehensive genotyping in 155 unrelated, well defined patients from southern Germany, JCEM 2000
[f]**Wedell**: Molecular genetics of congenital adrenal hyperplasia (21-hydroxylase deficiency): implications for diagnosis, prognosis and treatment, Acta Paediatr 1998
[g]**Speiser** et al.: Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline, JCEM 2010