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Increased Risk of Autoimmune Disorders in 21-Hydroxylase Deficiency: A Swedish Population-Based National Cohort Study

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Context: The prevalence of autoimmune disorders in individuals with 21-hydroxylase deficiency (21OHD) is unclear. The gene responsible, *CYP21A2*, is located in a highly immunologically active region.

Objective: To study the prevalence of autoimmune disorders in individuals with 21OHD.

Design, Setting, and Participants: Patients with 21OHD (n = 714) were compared with controls matched for sex, year, and place of birth (n = 71,400). Data were derived by linking National Population-Based Registers. Subgroup analyses were performed regarding phenotype and *CYP21A2* genotype.

Main Outcome Measures: Number and type of autoimmune disorders.

Results: Mean age (± SD) was 29.8 ± 18.4 years. Individuals with 21OHD had more autoimmune disorders than did controls [7.4% vs 5.1%, $P < 0.01$; relative risk (RR) 1.47 (95% CI, 1.13 to 1.91)], especially male patients [6.8% vs 4.1%, $P < 0.05$; RR, 1.64 (95% CI, 1.08 to 2.49)], whereas it did not reach significance for female patients [7.9% vs 5.8%, $P = 0.068$; RR, 1.37 (95% CI, 0.98 to 1.92)]. Among the specific autoimmune groups and disorders, autoimmune endocrine disorders and autoimmune thyroid disorders, including Graves disease, were significantly increased in the entire cohort of patients and for male and female patients separately. Inflammatory bowel disease (IBD) and systemic connective tissue disorders did not reach significant levels for the entire cohort ($P = 0.075$ and 0.05, respectively), but male patients were more affected by IBD ($P = 0.022$). The groups with milder phenotypes and genotypes seemed to be more affected by autoimmune disorders.

Conclusions: 21OHD was associated with an increased prevalence of autoimmune disorders. The relatively young age of the patient cohort and possible protective effects by glucocorticoid treatment may have underestimated the risk.

Abbreviations: 21OHD, 21-hydroxylase deficiency; CAH, congenital adrenal hyperplasia; DSD, disorders of sex development; IBD, inflammatory bowel disease; NC, nonclassic; RR, relative risk; SV, simple virilizing; SW, salt-wasting.

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Autoimmune disorders are common, increase with age, and affect women more often than men [1]. Hormonal effects are thought to be an important explanation for this difference because estrogens have activating effects on humoral and cell-mediated immune responses [2, 3]. The effects of androgens are less well understood but have been reported to reduce natural killer cell activity and nuclear factor κB and to increase anti-inflammatory cytokines [4]. Our group found an increased risk for thyroid disorders included in a broad study of cardiovascular risk in congenital adrenal hyperplasia (CAH, Online Mendelian Influence in Man 201910) [5]. Autoimmune disorders in individuals with CAH have also been reported in a large study of individuals with different forms of disorders of sex development (DSD) [6]. An increased prevalence of autoimmune disorders has repeatedly been shown in patients with disorders that feature sex chromosome aberrations, such as Turner and Klinefelter syndromes [6–8], and several of the genes involved in immune responses and regulation are located on the X chromosome [4].

CAH is interesting from an immunological perspective in several ways. The most common variant of CAH, 21-hydroxylase deficiency (21OHD), accounts for 95% to 99% of all CAH cases and is caused by recessive mutations in the *CYP21A2* gene [9–12]. The enzyme deficiency results in deficient cortisol and aldosterone synthesis with increased androgen production. The *CYP21A2* gene is located in the HLA class III region in the major histocompatibility locus on chromosome 6 (p21.3) [12, 13]. A highly homologous pseudogene, *CYP21A1P*, is also present in this complex genomic region. *CYP21A2* and *CYP21A1P* are arranged in tandem repeat with the genes responsible for the fourth component of complement (*i.e.,* the *C4A* and *C4B* genes), forming *C4/CYP21* units flanked by a telomeric *RP* gene and a centromeric *TNX* gene forming the *RP-C4-CYP21-TNX* (RCCX) module [12, 14]. The modular repeated genomic structure with a highly homologous pseudogene results in genetic recombination during meiosis, such as deletions or duplications. Moreover, gene conversions occur where mutations from the pseudogene are transferred to the active gene. The region is highly active in the immune system, and a low *C4* copy number has been associated with autoimmune disorders [15].

Thus, it could be suspected that autoimmune disorders are more prevalent in 21OHD, but very little has been reported, and if mentioned, little or no details of the autoimmune disorders have been provided [6, 16, 17]. The aims of the current study were to investigate the prevalence of autoimmune disorders in all individuals with 21OHD in Sweden and to assess whether the outcomes differed between the sexes, among age groups, and for the different phenotypes and genotypes.

1. Patients and Methods

A. Patients

We identified individuals with 21OHD (with a complete personal identification number) born between 1910 and 2013 by using the National CAH Registry (n = 640) [10] and the National Patient Register by using the International Classification of Diseases (ICD), eighth, (255.01, 255.08), ninth (2552, 255C), and tenth (E25.0) editions (n = 74). If an individual had been registered three or more times with the ICD codes mentioned previously, they were further scrutinized by checking all their ICD codes to determine whether an alternative diagnosis was more likely. All patients found via the National Neonatal Screening Program,

late-diagnosed patients reported to the screening laboratory, all Swedish patients who underwent *CYP21A2* mutations analysis, and all patients known to our hospital through previous or current clinical contacts or studies (since the 1940s) had been included in the National CAH Registry. This procedure has been reported in detail before [5, 18–22]. In total, 714 patients with 21OHD were included in the study.

If possible, the patients were further divided into the three phenotype groups: salt-wasting (SW), simple virilizing (SV), and nonclassic (NC). In addition, patients were division into the five most common genotype groups: null, I2 splice, I172N, P30L, and V281L, according to *CYP21A2* mutation analysis, as previously described [10, 12]. The mildest mutation defines the genotype group in compound heterozygotes. Null is associated with the SW phenotype, I2 splice is usually associated with SW, I172N with SV, and V281L with NC [12]. The severity of P30L is in between SV and NC [12], but it was defined in this study as SV. Patients with unknown *CYP21A2* mutations were classified according to phenotype if possible by clinical data. The NC group consisted of patients with genetically verified or phenotypical NC disease.

B. Study Protocol

One hundred controls matched by birth year, sex, and place of birth for each 21OHD case were identified in the Total Population Register. Immigration to Sweden was also matched by using the Migration Records (Statistics Sweden), which contain all migrations since 1901. Because of the unique Swedish personal identification number, unambiguous linkage between the population-based registers was possible. The register holders de-identified all data prior to delivery. The National Patient Register (Swedish Board of Health and Welfare) has been used to identify all discharge diagnoses according to the ICD for both inpatient and outpatient care since 1964 and 2001, respectively. The outcome, an autoimmune diagnosis, was registered. The different ICD codes (Swedish version) used for the separate analyses are shown in Table 1.

The Regional Ethical Review Board in Stockholm, Sweden, approved the study. Informed consent was waived due to the epidemiological nature of the study.

C. Statistical Analysis

Means ± SD are reported for continuous variables, and absolute and relative frequencies are given for categorical outcomes. Categorical parameters were compared by using the Fisher exact test and relative risk (RR) calculations with 95% CIs for the composite outcome (any autoimmune disorder) in the entire cohort, in female patients and male patients, and in the different age groups. A P value <0.05 was considered indicate a statistically significant difference.

2. Results

A. Characteristics of Patients and Controls

The mean age of the 714 patients with 21OHD was 29.8 ± 18.4 years (range, 0 to 83 years). There were more female patients with 21OHD (n = 404; mean age, 30.5 ± 18.0 years) than male patients (n = 310; mean age, 28.7 ± 18.8 years) (Table 2). The severity of 21OHD could be established in 566 patients (79.3%). Details on the number of individuals and their mean age among the different phenotypes (SW, n = 288; SV, n = 188; NC, n = 90) and genotypes (null, n = 115; I2 splice, n = 155; I172N, n = 146; P30L, n = 29) are shown in Tables 3 and 4. Table 5 shows the number of individuals in the different age groups. Controls, matched for sex, year, and place of birth, were included from the Total Population Registry (n = 71,400). This cohort was updated through 2013 (previously 2009) [22], and hence more individuals have been included than in the cohort we reported in previous studies [5, 18–21].

Table 1. Autoimmune Disorders Based on ICD Diagnoses From the National Patient Registry, Including Both Inpatient and Outpatient Care

Diagnosis	ICD-8	ICD-9	ICD-10
Any autoimmune disorder	281.00, 281.09, 283.90, 283.91, 287.00, 287.11, 135, 242.00, 245.03, 340, 733.00, 563, 696, 704.00, 709.05, 712, 446	281A, 283A, 287A, 287E, 135, 242A, 245C, 258B, 340, 358A, 555, 556, 571G, 576B, 579A, 696, 704A, 714, 446, 710, 725, 720	D51.0, D59.0, D59.1, D68.6, D69.0, D69.3, D86, E05.0, E06.3, E10, E31.0, G35, G70.0, K50, K51, K74.3, K75.4, K83.0, K90.0, L40, L63, L80, M05, M06, M08, M30-M36, M45
Autoimmune endocrine	242,00 245,03	242A, 245C, 258B	E05.0, E06.3, E10, E31.0
Type 1 diabetes			E10
Autoimmune thyroid	242.00, 245.03	242A, 245C	E05.0, E06.3
Graves disease	242.00	242A	E05.0
Autoimmune thyroiditis	245.03	245C	E06.3
APS		258B	E31.0
Autoimmune GI disease	563	555, 556, 571G, 576B, 579A	K50, K51, K74.3, K75.4, K83.0, K90.0
IBD	563	555, 556	K50, K51
Primary biliary cholangitis		571G	K74.3
Autoimmune hepatitis			K75.4
Cholangitis		576B	K83.0
Celiac disease		579A	K90.0
MS and MG	340	340, 358A	G35, G70.0
Autoimmune skin	696, 709.05	696, 704A	L40
Psoriasis	696	696	L40
Alopecia areata		704A	
Vitiligo	709.05		
Sarcoidosis	135	135	D86
Rheumatic	712, 446	714, 446, 710, 725, 720	M05, M06, M08, M30-M36, M45
RA	712	714	M05 M06
Juvenile arthritis			M08
Systemic CTD		710, 725	M30-M36
Ankylosing spondylitis		720	M45

Abbreviations: APS, autoimmune polyglandular syndrome; CTD, connective tissue disorders; GI, gastrointestinal ICD-8, -9, -10, International Classification of Diseases, 8th, 9th, and 10th editions; MG, myasthenia gravis; MS, multiple sclerosis; RA, rheumatoid arthritis.

Table 2. Autoimmune Disorders in Patients With CAH Due to 21OHD Compared with Age- and Sex-Matched Controls (100 Controls per Case)

Variable	Patients With CAH (n = 714)	Controls (n = 71,400)	*P* Value	Female Patients With CAH (n = 404)	Female Controls (n = 40,400)	*P* Value	Male Patients With CAH (n = 310)	Male Controls (n = 31,000)	*P* Value
Any autoimmune disorder	53 (7.4)	3608 (5.1)	<0.01[a]	32 (7.9)	2329 (5.8)	0.068	21 (6.8)	1279 (4.1)	<0.05[a]
RR (95% CI)	1.47 (1.13–1.91)[a]			1.37 (0.98–1.92)[b]			1.64 (1.08–2.49)[a]		
Autoimmune endocrine disorder	21 (3.0)	896 (1.3)	<0.001[a]	13 (3.2)	568 (1.4)	<0.01[a]	8 (2.6)	328 (1.1)	<0.05[a]
Type 1 diabetes	9 (1.3)	614 (0.9)	0.22	5 (1.2)	322 (0.8)	0.26	4 (1.3)	292 (0.9)	0.54
Aautoimmune thyroid disorder	12 (1.7)	292 (0.4)	<0.001[a]	8 (2.0)	253 (0.6)	<0.01[a]	4 (1.3)	39 (0.1)	<0.001[a]
Graves disease	10 (1.4)	201 (0.3)	<0.001[a]	7 (1.7)	177 (0.4)	<0.01[a]	3 (1.0)	24 (0.1)	<0.01[a]
Autoimmune thyroiditis	3 (0.4)	103 (0.1)	0.088[b]	2 (0.5)	87 (0.2)	0.22	1 (0.3)	16 (0.1)	0.16
APS	0 (0)	3 (0)	1						
Autoimmune GI disorder	13 (1.8)	990 (1.4)	0.33	6 (1.5)	640 (1.6)	1	7 (2.3)	350 (1.1)	0.094[b]
IBD	10 (1.4)	535 (0.8)	0.075[b]	4 (1.0)	322 (0.8)	0.57	6 (2.0)	213 (0.7)	<0.05[a]
Primary biliary cholangitis	0 (0)	17 (0.02)	1						
Autoimmune hepatitis	0 (0)	15 (0.02)	1						
Cholangitis	1 (0.1)	51 (0.1)	0.40	1 (0.3)	26 (0.1)	0.24			
Celiac disease	2 (0.3)	411 (0.6)	0.45	1 (0.3)	284 (0.7)	0.54	1 (0.3)	127 (0.4)	1
MS and MG	0 (0)	138 (0.2)	0.65						
Autoimmune skin disorder	8 (1.1)	851 (1.2)	1	7 (1.7)	519 (1.2)	0.37	1 (0.3)	332 (1.1)	0.27
Psoriasis	8 (1.1)	664 (0.9)	0.55	7 (1.7)	397 (1.0)	0.13	1 (0.3)	267 (0.9)	0.53
Alopecia areata	0 (0)	102 (0.1)	0.63						
Vitiligo	0 (0)	87 (0.1)	1						
Sarcoidosis	1 (0.1)	94 (0.1)	0.61	1 (0.3)	431 (0.1)	0.35			
Rheumatic disease	14 (2.0)	881 (1.2)	0.087[b]	9 (2.2)	635 (1.6)	0.31	5 (1.6)	246 (0.8)	0.11
RA	4 (0.6)	332 (0.5)	0.58	3 (0.7)	256 (0.6)	0.75	1 (0.3)	76 (0.3)	0.54
Juvenile arthritis	0 (0)	120 (0.2)	0.64						
Systemic CTD	9 (1.3)	449 (0.6)	0.051[b]	6 (1.5)	336 (0.8)	0.16	3 (1.0)	113 (0.4)	0.11
Ankylosing spondylitis	2 (0.3)	82 (0.1)	0.20	1 (0.3)	42 (0.1)	0.35	1 (0.3)	40 (0.1)	0.34

Unless otherwise noted, values are n (%). Autoimmune thyroiditis is often called Hashimoto thyroiditis. The numbers are not displayed if no female or male patient with 21OHD had the condition but analysis showed $P > 0.10$.

Abbreviations: APS, autoimmune polyglandular syndrome; CTD, connective tissue disorders; GI, gastrointestinal; MG, myasthenia gravis; MS, multiple sclerosis; RA, rheumatoid arthritis.

[a] $P < 0.05$.

[b] $P = 0.05$–0.09.

Table 3. Autoimmune Disorders in Patients With CAH Due to 21OHD Divided Into Phenotypes and Compared With Age- and Sex-Matched Controls (100 Controls Per Case)

	SW			SV			NC		
Variable	**All (n = 288)**	**Female Patients (n = 157)**	**Male Patients (n = 131)**	**All (n = 188)**	**Female Patients (n = 101)**	**Male Patients (n = 87)**	**All (n = 90)**	**Female Patients (n = 67)**	**Male Patients (n = 23)**
Mean age ± SD, y	24.5 ± 16.1	25.3 ± 15.6	23.6 ± 16.7	32.5 ± 19.3	32.2 ± 17.5	32.8 ± 21.3	29.3 ± 15.9	30.2 ± 15.7	26.7 ± 16.4
Any autoimmune disorder	12 (4.2)	9 (5.7)	3 (2.3)	17 (9.0)[a]	8 (7.9)	9 (10.3)[a]	10 (11.1)[a]	8 (11.9)[a]	2 (8.7)
Autoimmune endocrine disorder	4 (1.4)	2 (1.3)	2 (1.5)	4 (2.1)	2 (2.0)	2 (2.3)	6 (6.7)[a,b]	5 (7.5)[a,b]	1 (4.4)
Type 1 diabetes				4 (2.1)	2 (2.0)	2 (2.3)	2 (2.2)	2 (3.0)[a]	
Autoimmune thyroid disease	4 (1.4)[a,b]	2 (1.3)	2 (1.5)[a,b]				4 (4.4)[a,b]	3 (4.5)[a,b]	1 (4.4)[a]
Graves disease	3 (1.0)[a,b]	1 (0.6)	2 (1.5)[a,b]				4 (4.4)[a,b]	3 (4.5)[a,b]	1 (4.4)[a]
Autoimmune thyroiditis	1 (0.4)	1 (0.6)							
Autoimmune GI disorder	3 (1.0)	3 (1.9)		7 (3.7)[a]	2 (2.0)	5 (5.6)[a,b]			
IBD	3 (1.0)	3 (1.9)[c]		5 (2.7)[a]	1 (1.0)	4 (4.6)[a,b]			
Cholangitis				1 (0.5)[a]	1 (1.0)[a]				
Celiac disease				1 (0.5)		1 (1.2)			
Autoimmune skin disorder	3 (1.0)	3 (1.9)		3 (1.6)	3 (3.0)		1 (1.1)	1 (1.5)	
Psoriasis	3 (1.0)	3 (1.9)		3 (1.6)	3 (3.0)[c]		1 (1.1)	1 (1.5)	
Rheumatic disease	2 (0.7)	1 (1.2)	1 (0.8)	6 (3.2)[a]	4 (4.0)[c]	2 (2.3)	3 (3.3)[c]	2 (3.0)	1 (4.4)
RA				2 (1.1)	1 (1.0)	1 (1.2)	1 (1.1)	1 (1.5)	
Systemic CTD	1 (0.4)	1 (0.6)		4 (2.1)[a]	3 (3.0)[a]	1 (1.2)	2 (2.2)	1 (1.5)	1 (4.4)[c]
Ankylosing spondylitis	1 (0.4)		1 (0.8)[c]	1 (0.5)	1 (1.0)[c]				

Unless otherwise noted, values are n (%). The numbers are not displayed if no patient had the condition but analysis showed $P > 0.10$. Autoimmune thyroiditis is often called Hashimoto thyroiditis.

Abbreviations: CTD, connective tissue disorders; GI, gastrointestinal; RA, rheumatoid arthritis.

[a] $P < 0.05$.

[b] $P < 0.01$

[c] $P = 0.05$–0.09.

Table 4. Autoimmune Disorders in Individuals With CAH Constituting the Four Most Common *CYP21A2* Genotype Groups Compared With Age- and Sex-Matched Controls (100 Controls per Case)

	Null			I2 Splice			I172N			P30L		
Variable	**All (n = 115)**	**Female Patients (n = 63)**	**Male Patients (n = 52)**	**All (n = 155)**	**Female Patients (n = 85)**	**Male Patients (n = 70)**	**All (n = 146)**	**Female Patients (n = 79)**	**Male Patients (n = 67)**	**All (n = 29)**	**Female Patients (n = 15)**	**Male Patients (n = 14)**
Mean age ± SD, y	23.9 ± 14.8	24.7 ± 13.2	22.9 ± 16.7	23.8 ± 16.7	24.6 ± 17.0	22.7 ± 16.4	32.8 ± 20.4	32.8 ± 18.6	32.7 ± 22.4	25.6 ± 10.3	26.2 ± 10.4	24.9 ± 10.5
Any autoimmune disorder	3 (2.6)	1 (1.6)	2 (3.9)	6 (3.8)	6 (7.1)		13 (8.9)[a]	6 (7.6)	7 (10.5)[a]	3 (10.3)[b]	1 (6.7)	2 (14.2)[b]
Autoimmune endocrine disorder	2 (1.7)		2 (3.9)[a]	1 (0.7)	1 (1.1)		4 (2.7)	2 (2.5)	2 (3.0)			
Type 1 diabetes							4 (2.7)[a]	2 (2.5)[b]	2 (3.0)			
Graves disease	2 (1.7)[a,c]		2 (3.9)[a,d]	1 (0.7)	1 (1.1)							
Autoimmune thyroiditis												
Autoimmune GI disorders				3 (1.9)	3 (3.5)		5 (3.4)[a]	1 (1.3)	4 (6.0)[a,c]	1 (3.5)		1 (7.1)[b]
IBD				3 (1.9)[b]	3 (3.5)[a]		3 (2.1)[b]		3 (4.5)[a,c]	1 (3.5)		1 (7.1)[b]
Cholangitis							1 (0.7)[b]	1 (1.3)[a]				
Celiac diseases							1 (0.7)		1 (1.5)			
Autoimmune skin disorder	1 (0.9)	1 (1.6)		2 (1.3)	2 (2.4)		2 (1.4)	2 (2.5)		1 (3.5)	1 (6.7)	
Psoriasis	1 (0.9)	1 (1.6)		2 (1.3)	2 (2.4)		2 (1.4)	2 (2.5)		1 (3.5)	1 (6.7)[b]	
Rheumatic disease							5 (3.4)[a]	4 (5.1)[a]	1 (1.5)	1 (3.5)		1 (7.1)
RA							1 (0.7)	1 (1.3)		1 (3.5)[b]		1 (7.1)[a]
Systemic CTD							4 (2.7)[a]	3 (3.8)[a]	1 (1.5)			
Ankylosing spondylitis							1 (0.7)	1 (1.3)[a]				

Severity of the genotype ranging from left to right. Unless otherwise noted, values are n (%). Autoimmune thyroiditis is often called Hashimoto thyroiditis. The numbers are not displayed if no patient had the condition but analysis showed $P > 0.10$.

Abbreviations: CTD, connective tissue disorders; GI, gastrointestinal; RA, rheumatoid arthritis.

[a] $P < 0.05$.

[b] $P = 0.05$–0.09.

[c] $P < 0.01$.

[d] $P < 0.001$.

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Table 5. Any Autoimmune Disorders in Individuals With CAH Due to 21OHD Deficiency in Different Age Groups Compared With Age- and Sex-Matched Controls (100 Controls per Case)

	Age 0–18 y			Age 19–39 y			Age ≥40 y		
Variable	**Patients With CAH**	**Controls**	***P* Value**	**Patients With CAH**	**Controls**	***P* Value**	**Patients With CAH**	**Controls**	***P* Value**
All patients									
Patients, n	215	21 948		295	29 629		204	19 823	
Patients with any autoimmune disorder, n (%)	4 (1.9)	383 (1.7)	0.79	18 (6.1)	1497 (5.1)	0.42	21(6.8%)	1279(4.1%)	<0.01
RR (95% CI)	1.07 (0.40–2.83)			1.21 (0.77–1.89)			1.74 (1.26–2.42)		
Female patients									
Patients, n	108	11 142		180	18 011		116	11 247	
Patients with any autoimmune disorder, n (%)	2 (1.9)	206 (1.8)	1	9 (5.0)	1 016 (5.6)	0.89	21(18.1%)	1 107(9.8%)	<0.01
RR (95% CI)	1.00 (0.25–3.98)			0.89 (0.47–1.68)			1.84 (1.24–2.72)		
Male patients									
Patients, n	107	10 806		115	11 618		88	8 576	
Patients with any autoimmune disorder, n (%)	2 (1.9)	177 (1.6)	0.70	9 (7.8)	481 (4.1)	<0.05	10(11.4%)	621(7.2%)	0.15
RR (95% CI)	1.14 (0.29–4.54)			1.89 (1.00–3.56)			1.57 (0.87–2.83)		

B. Any Autoimmune Disorders

More patients with 21OHD than controls were affected by an autoimmune disorder (7.4% vs 5.1%, $P < 0.01$; RR, 1.47; 95% CI, 1.13 to 1.91), especially in male patients (6.8% vs 4.1%, $P < 0.05$; RR, 1.64; 95% CI, 1.08 to 2.49), but in female patients this did not reach significance (7.9% vs 5.8%, $P = 0.068$; RR, 1.37; 95% CI, 0.98 to 1.92) (Table 2). The SV and NC phenotype groups, especially male patients with SV and female patients with NC, had more autoimmune disorders than the controls (Table 3). The I172N genotype group, especially male patients, was significantly more affected, whereas the P30L group and male patients in that group only had a tendency to be more affected (Table 4). When assessed for the different age groups, autoimmune disorders were increased in all patients age 40 years and older, in female patients age 40 years and older, and in male patients age 19 to 39 years (Table 5).

C. Autoimmune Endocrine Disorders

Autoimmune endocrine disorders were increased in all individuals with 21OHD (3.0% vs 1.3%; $P < 0.01$) and in all female and male patients assessed separately (Table 2). The frequency of Graves disease was increased, but autoimmune thyroiditis was not significantly increased ($P = 0.088$). Men with SW had an increased risk for Graves disease, as did both women and men with the NC form (Table 3). Men with the null genotype also had an increased risk for Graves disease (Table 4). The risk for type 1 diabetes was increased in women with NC phenotype and all with the I172N genotype (Tables 3 and 4).

D. Autoimmune Gastrointestinal Disorders

Autoimmune gastrointestinal disorders were increased in male patients with the SV phenotype and I172N genotype (Table 3-4). Inflammatory bowel disease (IBD) was increased in male patients, male patients with the SV phenotype and I172N genotype, and female patients with the I2 splice genotype. Cholangitis was increased in female patients with the SV phenotype and I172N genotype. There was no difference between patients and controls concerning celiac disease, and no patients with 21OHD had primary biliary cholangitis or autoimmune hepatitis (Table 2).

E. Multiple Sclerosis, Myasthenia Gravis, Autoimmune Skin Disorders, and Sarcoidosis

Women with the SV phenotype and P30L genotype had a tendency for more psoriasis compared with controls, but in all other groups there was not even a tendency (Tables 2–4). There was no increased risk for sarcoidosis. No individuals with 21OHD had been diagnosed with multiple sclerosis, myasthenia gravis, alopecia areata, or vitiligo.

F. Rheumatic Disorders

There was a tendency for more rheumatic disorders in all patients (Table 2); however, it was significantly increased only in those with SV phenotype and I172N genotype, including female patients with the I172N genotype (Tables 3 and 4). Rheumatoid arthritis was more common in male patients with the P30L genotype than controls. Systemic connective tissue disorders were diagnosed in 9 of the 14 patients with any kind of rheumatic disorder (Table 2) and significantly increased in all with the SV phenotype or I172N genotype and in female patients, when analyzed separately (Tables 3 and 4). Ankylosing spondylitis was increased in female patients with I172N genotype. No individual with 21OHD had been affected by juvenile arthritis.

3. Discussion

This study investigated autoimmune disorders in patients with 21OHD and evaluated the individual autoimmune disorders in detail. Moreover, all patients diagnosed with 21OHD in

Sweden were included. Autoimmune disorders in women and men, and the different phenotypes and genotypes, were also studied separately.

We found more autoimmune disorders in persons with 21OHD, especially in those age 40 years and older and in male patients in general. In addition, it was more common in patients with the SV and the NC phenotype and the I172N genotype. Autoimmune disorders have only very briefly been studied in 21OHD previously. They were studied as part of a larger study of patients with DSD, including women with CAH, and were then found to be increased compared with controls, but very few details were reported [6]. Another study of 127 patients with 21OHD reported autoimmune disorders in 4 of them (autoimmune thyroiditis, n = 2; IBD, n = 1; juvenile rheumatoid arthritis, n = 1) [16]. This latter study assessed the complement compound 4, *C4* copy number variation among the patients with different *CYP21A2* genotypes and found that 21OHD was associated with very low or very high *C4* copy number. Recently, this same group expanded the cohort to include 145 patients with 21OHD, of whom 5 had concurrent autoimmune disorders (autoimmune thyroid disorders, n = 2; IBD, n = 2; juvenile rheumatoid arthritis, n = 1) with no association with *C4* copy number or serum C4 levels [17].

It has been reported previously that autoimmune diseases, such as systemic lupus erythematosus, have been associated with lower *C4* copy number and lower serum C4 protein levels [23]. High levels of C4 would conversely lead to a lower susceptibility to autoimmune disorders. High *C4* was especially noted in patients with NC 21OHD (V281L), thereby indicating protection against autoimmunity [16]. This result is not congruent with our findings but may be partly explained by other factors discussed below.

Genes in the region where the *CYP21A2* gene is located are important in the immune system.

In the study by Chen *et al.* [16], V281L (NC phenotype) was associated with higher C4 levels, implicating a lower risk for autoimmune disorders. However, only 27 patients with NC phenotype were included compared with 90 in the current study. The SV phenotype/I172N genotype group had the most patients with an autoimmune disorder in our study. The null genotype groups (the SW phenotypes) are more likely to have a deletion, which could be expected to also encompass the complement region in some cases. However, our study did not indicate an increased incidence of autoimmunity in the SW genotype group. This may have been influenced by the fact that this was the youngest group. The null genotype group was also probably receiving higher glucocorticoid replacement doses and was more adherent compared with those with milder forms; thus, this group was less prone to autoimmune disorders (see below).

Patients with a sex chromosomal DSD, such as Turner and Klinefelter syndromes, have an increased risk of developing autoimmune disorders [6–8], possibly related to the X chromosome [24]. CAH results in increased exposure to androgens [25–27]. Women with CAH are exposed to higher levels of androgens than controls, which could have a “masculinizing" effect, or a less activating effect on the immune system. An androgen effect may be related to a lower risk of developing an autoimmune disease, which would counteract any other effect by, for example, CAH resulting in increasing autoimmunity [4, 28]. Hence, an increased autoimmunity would seem more likely among male patients with CAH rather than female patients compared to controls because the difference in androgen exposure is larger for female patients. Women with CAH in our study had more autoimmune disorders than men with CAH, as can be seen in Table 2 (7.9% vs. 6.8%). The difference between women with and without CAH did not reach statistical significance ($P = 0.068$); this finding, at least in part, can be explained by the higher frequency of autoimmune disorders in female controls. However, because women with CAH are exposed to higher levels of androgens than controls, this could have a negative effect, or a less activating effect on the immune system. The androgen effect may lower the risk of developing an autoimmune disease and thus counteract any other effect, resulting in increasing autoimmunity.

Patients with classic 21OHD require glucocorticoid supplementation for survival [11, 25, 26], and it is plausible to assume that most patients in our cohort were receiving long-term

glucocorticoid replacement, with the exception of some with the milder mutations, such as P30L and V281L, the latter typically consistent with NC 21OHD [29]. It could be speculated that the long-term glucocorticoid replacement contributes helps protect against autoimmune disorders. Before the introduction of neonatal screening, especially boys but also some girls with SV 21OHD were identified later during childhood and thus were not treated with glucocorticoids from the neonatal period [30, 31]. In some cases, they were not diagnosed and treated for several years but were exposed to prolonged periods of elevated levels of androgens instead. Only rarely have patients, mostly men, presented with adrenal incidentalomas at age older than 50 years and subsequently been diagnosed with SV 21OHD, both in Sweden [32] and in other countries [33]. Most often, patients with NC 21OHD present in young adulthood or later, and many are not commenced on glucocorticoids because this treatment is not necessary for survival [29, 34]. Moreover, symptoms of androgen excess can be treated, if necessary, with other drugs, such as oral contraceptive pills in female patients.

Furthermore, it is known that both women and men with 21OHD treated with glucocorticoids usually have decreased androgens compared with matched controls [30, 35]. Sex hormones, as well as genes, affect autoimmunity [28, 36]. Whether the mild androgen deficiency found in some or the elevated androgens found in others with untreated or poorly controlled 21OHD modify the prevalence of autoimmune disorders remains unclear.

An alternative effect of glucocorticoids could be through imprinting or differential methylation of genes related to the immune system. Our group showed an association between DNA methylation and exposure to dexamethasone during the first trimester in otherwise healthy individuals [37]. Effects were seen for T-cell DNA methylation, which could affect immune function, inflammation, and immunity and possibly contribute to the development of immune-related disorders; effects differed for male and female patients. In mice, prenatal glucocorticoid exposure increases susceptibility to autoimmunity, which is potentially caused by epigenetically programmed glucocorticoid receptor expression and glucocorticoid response [38]. Hence, the glucocorticoid influence is complex, with a putative interaction between a glucocorticoid enhancing effect on immunity via DNA methylation and repressing effect via anti-inflammatory activity. In addition, the mineralocorticoid receptor may have immune modulating effects, which may play a role in individuals with CAH. Inhibition of the mineralocorticoid receptor has an anti-inflammatory effect by decreasing proinflammatory cytokines [39].

We found that the risk for autoimmune disorders increased with age in the total cohort of patients with CAH but was only significantly increased in those age 40 years and older. Women with CAH also showed an increased risk in the oldest age group, whereas in men the only significant increase was in those age 19 to 39 years. In the older men, assessed as a subgroup, this was not significant, possibly because of the lower number of men in this age group.

The current study has important clinical implications. Because autoimmune disorders are more prevalent in 21OHD, clinicians caring for adult patients need to be vigilant for symptoms and signs that may indicate autoimmune disorders. Some conditions, such as autoimmune thyroid disorder, may be screened for regularly by using thyroid function tests.

The major limitations of the current study were that all outcome data were derived from national registries; hence, we did not have data on treatment, hormone levels, autoantibodies, or complement factors and their gene expression. Moreover, the mean age of included patients was low, and the risk of being affected by an autoimmune disorder increases with age in other disorders associated with autoimmunity, such as Turner syndrome [40]. If we were to repeat the study in a few decades, the associations may be more pronounced. Because a prerequisite for obtaining ethical approval was that all participants included were anonymized to protect their privacy, we could not compare the study results with information from medical files. Furthermore, even though this is a large CAH cohort, the number of patients in the different phenotype and genotype groups was low, and we could not study the patients with a deletion of the gene separately. Hence, the results from the subgroup analyses must be interpreted with caution. In addition, we studied only 21OHD and no other variants of CAH,

such as 11β-hydroxylase deficiency or 3β-hydroxysteroid dehydrogenase type 2 deficiency (variants that have hardly been studied at all) [26, 41, 42]. However, if the results of the current study could be applied to other variants of CAH, it would mean that results are less likely to be related to the complement factor. In contrast, the strength of this study is the unique national CAH registry with the very high coverage of all patients diagnosed in Sweden, with both genotype and phenotype available for most patients. By including the patients identified via the National Patient Register, we obtained almost complete coverage. The inclusion of 100 matched controls for each 21OHD case made the analyses robust.

In conclusion, theoretically there are factors indicating that autoimmunity could be affected in 21OHD. We found that, in particular, patients age 40 years old and older and men in general with 21OHD had a higher risk of developing autoimmune disorders, as did patients with the SV and NC phenotypes as well as the I172N genotype. The relatively young age of the patients and possible protective effects of glucocorticoid treatment may have led to underestimates in the lifetime risks for autoimmune disorders.

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