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Are carriers of *CYP21A2* mutations less vulnerable to psychological stress? A population-based national cohort study

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Abstract

Background Congenital adrenal hyperplasia (CAH) is one of the most common monogenic autosomal recessive disorders with an incidence of 1 in 15 000. About 1 in 70 individuals in the general population are carriers of a severe *CYP21A2* mutation. It has been suggested that this confers a survival advantage, perhaps as a result of increased activity in the hypothalamic–pituitary–adrenal axis. We investigated vulnerability to psychological stress in obligate carriers.

Method The Swedish CAH Registry encompasses more than 600 patients. Parents, i.e., obligate carriers of the *CYP21A2* mutation, were identified through the Multigeneration Register. The diagnosis of the child was used as the psychological stressor. Psychiatric diagnoses before and after the birth of a child with CAH were compared to those of controls derived from (i) the general population, (ii) parents of children with hypospadias, and (iii) parents of children with diabetes mellitus type 1 (T1DM).

Results Parents of children with CAH had less risk of being diagnosed with any psychiatric disorder (OR, 0.6), an affective disorder (OR, 0.5) or substance misuse (OR, 0.5) after the diagnosis of the child, compared to the general population. Their risk was also decreased compared to parents of a child with hypospadias (OR, 0.6, 0.4 and 0.2, respectively) and parents of a child with T1DM (OR 0.7, 0.6 and 0.2, respectively). The *CYP21A2* carriers had a lower risk of developing mood and stress-related disorders after the diagnosis of the child.

Conclusion Obligate *CYP21A2* carriers had a reduced risk of a psychiatric diagnosis and were less vulnerable to a psychologically stressful situation, at least with respect to receiving a psychiatric diagnosis. This indicates a better ability to cope with psychological stress among heterozygous carriers of severe *CYP21A2* mutations, which may contribute to the apparent survival advantage.

Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is one of the most common monogenic autosomal recessive disorders with an incidence of 1 in 10 000–20 000.¹ The incidence is even higher in some populations, e.g., 1/295 in Yupik Eskimos (classic CAH) and 1/27 in the Ashkenazi Jewish population (non-classic CAH).² About 1 in 50 individuals in the Swedish population are carriers of a mutation in the *CYP21A2* gene and 1 in 70 of a classic, more severe, *CYP21A2* mutation.³ It has been suggested that being a carrier confers a survival advantage that could explain the relatively high frequency of the disease in most worldwide populations, similarly to the situation with sickle cell anemia where carriers have a survival advantage due to resistance to malaria.^{4,5,6}

CAH results in varying degrees of cortisol and adrenalin deficiency. The severity of disease correlates with the severity of the *CYP21A2* mutation. Clinically, CAH has been divided into the salt-wasting (SW), simple virilising (SV) and non-classic (NCAH) forms.¹ Untreated, patients with the classic SW form develop lethal salt crises in the neonatal period while those with the SV form have a less pronounced risk of salt crisis. The cortisol deficiency leads to an increased pituitary ACTH production and results in increased synthesis of the steroid precursors prior to the enzyme block that are converted into androgens. Both SW and SV children with CAH result in virilisation of external genitalia at birth in children with 46,XX karyotype. The treatment consists of life long replacement therapy with hydrocortisone and in severe cases fludrocortisone. During periods of physical stress such as febrile illness the hydrocortisone dose must be adjusted. In contrast, patients with the less severe NCAH form escape salt crisis and affected females are not virilised at birth. Patients with NCAH often do not come to medical attention until they develop signs of androgen excess over time such as hirsutism or infertility. Men with NCAH remain largely undiagnosed.⁷

There is a strong correlation between disease severity and *CYP21A2* genotype based on the mildest allele.^{8,9} Deletions and mutations that completely abolish the enzyme activity in the homozygous form or in combination with the slightly less severe I2 splice mutation result in the most severe salt-wasting form of the disease. The I172N mutation is associated with the simple virilising form while V281L or P453S results in NCAH.¹⁰

Although carriers of *CYP21A2* mutations typically do not have clinical symptoms, increased androgen levels have been reported and their hypothalamic-pituitary-adrenal (HPA) axis is functionally affected.⁴ On ACTH stimulation, carriers respond with increased 17-hydroxyprogesterone (17-OHP) levels, the metabolite prior to the enzyme block.¹¹

Interestingly, the cortisol response to ACTH stimulation has been shown to be more prompt and increased in carriers than in healthy controls.⁴ This increased efficiency of the HPA axis may be caused by an upregulated ACTH activity due to the relative deficiency in cortisol production and the resulting loss of negative feedback inhibition. One interpretation of these results would be that the carriers have fast and brisk response and a relatively quick down-regulation of the cortisol synthesis. It has been hypothesised that this increased cortisol response, in response to stress, could enable a more rapid return to homeostasis and hence be advantageous.⁴ This almost doubled capacity to synthesise cortisol on acute stimulation could explain the proposed evolutionary advantage of being a carrier of a *CYP21A2* mutation.

It has been shown that *CYP21A2* mutation carriers have a lower 24 hour urinary excretion of free cortisol.⁵ It has been hypothesized that the mild cortisol deficiency can result in a compensatory increase in hypothalamic CRH secretion. CRH is involved in the regulation of the HPA axis but also in other responses to stress such as the autonomic, immune, and

behavioral responses. CRH is important for mood and anxiety since it interacts with the amygdala and modulates the monoaminergic system.^{12,13} It is known that cortisol and CRH are increased in anxiety and depression.^{14,15,16} Thus, an increased CRH level could have negative effects. It was therefore suggested that increased CRH may result in vulnerability to anxiety and depression by Charmandari and co-workers who showed that CRH stimulation in *CYP21A2* carriers resulted in a higher peak ACTH. They report that a lower cortisol excretion and a higher ACTH response in 18 obligate *CYP21A2* carriers and 16 controls were associated with a predisposition to obsessive-compulsive behavior as assessed by psychometric questionnaires.⁵

The aim of this study was to investigate the vulnerability to psychological stress in obligate *CYP21A2* mutation carriers, i.e, parents of children with CAH. We used the birth or diagnosis of an affected child as the stressor, and investigated whether *CYP21A2* carriers had an increased risk for psychiatric morbidity *per se* and after this stressful event. Parents of children with hypospadias or diabetes mellitus type 1 (T1DM), both situations known to be stressful for the parents and having comparable aspects were used as controls.^{17,18}

Methods

Subjects

The Swedish National CAH Registry comprises more than 600 individuals and has been described in detail elsewhere.^{3,19} A complete personal identification number was known for 545 of these patients born between 1910 and 2009, and the *CYP21A2* genotype was known for more than 80%. An additional 43 patients were identified through the National Patient Register and validated as previously reported.¹⁹ Altogether, 588 patients with a 21-

hydroxylase deficiency were identified. All patients were anonymized by Statistics Sweden before the linkage to population based registers.

Parents of patients with CAH were identified through the Multigeneration Register. They were then divided into two groups based on the genotype or the clinical severity of the disease of the child. The parents of individuals with SW or SV CAH were assumed to carry a classic *CYP21A2* mutation. The other group comprised parents of patients with NCAH who may carry either a classic mutation or a mutation that would predict NCAH in addition to the parents of patients with CAH of unknown severity. A total of 610 carriers were identified and divided into two groups comprising 240 carriers of classic mutations and 370 carriers of a mixture of mutations and of undetermined severity. Three control groups of parents matched for age were used: parents from the general population (n=48,888), parents with a child diagnosed with hypospadias (n=22,294), and parents with a child diagnosed with T1DM (n=48,747).

Study protocol

The prevalence of psychiatric diagnoses in the different groups of parents previous to the birth of an affected child was investigated. The birth or diagnosis of the affected child was used as the psychological stressor. Psychiatric diagnoses of the parents before and after the birth of an affected child were analysed. The selected psychiatric diagnoses in the National Patient Register were mood disorders (ICD-8 codes 296.1, 296.3, 296.8, 300.4; ICD-9 codes 296, 300E, 311; ICD-10 codes F30–F39), anxiety, dissociative, stress-related, somatoform, phobias or unspecified non-psychotic psychiatric diagnoses (ICD-8 code 300 except 300.4, code 307, ICD-9 code 300 except 300E, codes 306, 308-309; ICD-10 codes F40-F45, F48), eating disorders (ICD-9 codes 307B and 307F; ICD-10 code F50), mental and behavioral

disorders due to psychoactive substance use (alcohol: ICD-8 codes 303; ICD-9 codes 303, 305A and 305X; ICD-10 codes F10; other drugs: ICD-8 codes 304; ICD-9 codes 304; ICD-10 codes F11–F19), and other behavioral emotional disorders with the onset usually occurring in childhood or adolescence (ICD-9 codes 312-313; ICD-10 codes F91-F98).

A matched control design was used employing three different control groups with parents matched for sex and year of birth: (i) parents of children in the general population before and after the birth of the child, (ii) parents of children with diabetes type 1 (T1DM) (250 in ICD-8/ICD-9 and E10 in ICD-10) before and after the diagnosis of the child, and (iii) parents of children with hypospadias (752.2 in ICD-8, 752.61 in ICD-9 and Q54 in ICD-10) before and after the birth of the child. Descriptive statistics are shown in Tables 1. Despite using the national registries it was not possible to identify matched controls for all parents resulting in a small variation in the number of parents in the different groups.

All calculations were performed with and without controlling for the highest level of education reached by the parents. The educational level was designated as one of four different levels: primary and lower secondary school, upper secondary school, post-secondary school, postgraduate, or unknown level of education.

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden.

Statistical analysis

The risk of psychiatric disorders was estimated by conditional logistic regression. Results were reported as Odds Ratios (ORs) and 95% Confidence Intervals (95%CI). The *CYP21A2* carriers were compared with three different control groups as described above.

Calculations were also performed as bivariate and multivariate analyses controlling for the level of education of the parents. All statistical analyses were conducted with SAS software (version 9.3; Cary, NC, USA).

Results

There was a decreased risk of receiving any type of psychiatric diagnosis when the total number of parents of children with CAH, regardless of severity, was analysed and compared with the controls groups before the birth or diagnosis of the child (Table 2). On breaking down the analysis into different diagnoses, a difference in mood and anxiety disorders persisted compared to the general population and to parents of a child with hypospadias. After adjusting for the educational level, the difference remained statistically significant for any type of psychiatric disorder compared to all three control groups and for mood disorder compared to the general population, as well as for anxiety disorders compared to parents with hypospadias. As seen in Table 3, after the birth of the child the risk of receiving any psychiatric diagnosis and of suicide, mood disorder, anxiety, substance misuse, or stress disorder was decreased for the *CYP21A2* carriers in all comparison groups except for anxiety compared to T1DM (OR 0.3–0.8).

The parents of children with classic CAH, i.e., obligate carriers of a classic, more severe, *CYP21A2* mutations, were also analysed separately. The difference in the risk between the groups receiving any psychiatric diagnosis before the birth of the child only reached statistical significance when compared to parents with a child with hypospadias (Table 4). After the diagnosis of a child, there were significant differences indicating a lower risk of receiving several psychiatric diagnoses for the carriers (Table 5). The carriers of classic *CYP21A2* mutations had a lesser risk of receiving any type of psychiatric diagnosis than parents in the

general population (OR 0.6, 95%CI 0.4–0.9, $p=0.014$) or compared to a child with T1DM (OR 0.6, 95%CI 0.4–0.9, $p=0.017$) or a child with hypospadias (OR 0.6, 95%CI 0.4–0.9, $p=0.007$). They were less likely to have a diagnosis of mood disorder/affective disorder after the diagnosis of the child, compared to the general population (OR 0.4, 95%CI 0.2–0.9, $p=0.0164$) or a child with hypospadias (OR 0.4, 95%CI 0.2–0.8, $p=0.013$). The carriers were less likely to develop substance misuse (including drugs and alcohol misuse) compared to all three control groups (OR 0.2–0.3, $p\leq 0.02$). When analysed separately, alcohol misuse showed a significant difference compared to that of the parents with a child with T1DM and a tendency towards that of the general population or hypospadias. The difference for anxiety disorder was significant compared to the general population, but did not reach significance for the T1DM and hypospadias parents on controlling for educational level. The difference for the risk of committing suicide was significant compared with that for the general population without adjusting for education, but in no other comparisons.

Comparisons between the parents of children with NCAH or CAH of unknown severity, i.e., a mixture of carriers of both severe and milder *CYP21A2* mutations, did not show significant differences in any of the parameters studied, only some tendencies, indicating that the severity of the mutation was important (data not shown).

Statistical calculations for parents of girls and boys with CAH separately did not show significant differences between the different parental groups (data not shown), most probably due to small sample sizes. The same was true when mothers and fathers of children with severe mutations were assessed separately, whereby only a few parameters showed significant differences (data not shown), most likely due to sample size.

Discussion

This is the largest study investigating carriers of a *CYP21A2* mutation and is derived from a population-based cohort. We found that obligate carriers, i.e., parents of patients with known CAH, had a decreased risk of receiving a psychiatric diagnosis both *per se*, i.e., before and more markedly so after the birth of an affected child, regarded as a stressful event. The carriers were compared to three control groups: parents in the general population, parents of a child with T1DM, and parents of a child with hypospadias. These control groups were chosen to control for the type of stress that having a child with CAH may pose to the parents. The birth of a child with genital anomalies or even unclear sex at birth is a known psychological stressor, and posttraumatic stress disorders are increased among these parents.^{17,20}

Hypospadias and CAH affect genital development and may lead to uncertainty of gender assignment at birth.^{17,21} CAH and T1DM are both potentially life-threatening diseases^{21,22} that require daily treatment and extra attention from the parents, something that is well known to be stressful.

In order to investigate whether the severity of the mutation was important, we analysed the parents who were obligate carriers of classic and less severe *CYP21A2* mutations separately. Those who were carriers of a severe mutation had a lower risk of developing any psychiatric diagnosis after the birth of the affected child than parents from any of the three control groups. In addition, they had a decreased risk of being diagnosed with an affective disorder or substance misuse. Moreover, suicides were less prevalent in carriers, significantly so compared to general population. These effects were only seen for carriers of severe, i.e., classic *CYP21A2* mutations and not for the group with unknown and milder mutations.

We interpret our results to show the carriers to be less vulnerable to the psychologically stressful situation of having a child with a severe diagnosis, possibly because of an increased psychological resilience. It is not possible to decipher the mechanism for this in this type of epidemiological study, but it can be speculated that it is associated with adaptive changes in the HPA axis or mechanisms involving locus coeruleus, the monoaminergic system, or the amygdala via CRH effects.^{12,13}

Impairments in the function of the HPA axis, e.g., as a result of chronic stress, are known to be important in the development of depression owing to a hyperactive HPA axis with elevated basal cortisol levels, disrupted diurnal cortisol secretion, and dysregulation in the HPA negative feedback system.^{12,23} Carriers of severe *CYP21A2* mutations may be at decreased risk of developing affective disorders as a result of their prompt and more elevated response to ACTH.⁴ This may facilitate a more efficient return to homeostasis via a more effective feedback inhibition of cortisol on supra-hypothalamic, hypothalamic, and/or pituitary levels.⁴ The picture is complicated, however, by the positive feed-forward mechanism where glucocorticoids further enhance CRH activity and behavioral responses to stress²⁴ that would result in the opposite.

Interestingly, our findings are in contrast with the previous suggestion that *CYP21A2* carriers are at increased risk of affective and anxiety disorders. The study by Charmandari *et al.*⁵ was conducted on a small group of individuals but consisted of both psychological assessments and biochemical laboratory investigations including CRH stimulation. They saw no difference when the carriers and controls were compared on a group level, but correlations with the results of the different psychometric tests differed for the carriers and the healthy controls. An increased cortisol excretion correlated with more stress and anxiety in the controls, something

that could be expected in stressful circumstances. The study had a considerable exclusion rate of 50%. The carriers were excluded for somatic or psychiatric reasons. CRH stimulation gave an area under the curve for ACTH that was larger in carriers than in controls, but the cortisol response showed no difference. Not surprisingly, the carriers had a larger area under the curve for 17-OHP.

We used psychiatric diagnoses as a proxy or indicator for vulnerability to psychological stressors. By virtue of the study design, only associations can be identified, but not causal factors for the decreased risk for psychiatric morbidity. However, all comparisons point in the same direction, i.e., a lower risk of a psychiatric disorder whether related to stress, mood, anxiety, or suicide. Unfortunately, we were not able to specifically analyse obsessive-compulsive disorder, stress-related disorders (e.g., PTSD) because of the low number of patients with the diagnosis.²⁵ Our results point rather to a tendency towards a lower probability of receiving a diagnosis of anxiety in obligate carriers of a severe *CYP21A2* mutation. Unpredictable psychological stressors are more likely to result in depressive symptoms than predictable stressors.²⁶ Is it possible that the exaggerated increase in the ACTH response is of greater importance than a smaller increase in CRH over time.

The strengths of the study are that it covers a population-based cohort, implying no selection bias. The CAH Registry covers virtually all patients in Sweden and the National Patient Register used for controls and for the psychiatric diagnosis has full coverage since 1964 for hospital care and from 2001 for out-patient care. The CAH diagnosis was validated in the Registry, which was the basis for this epidemiological registry study using de-identified data, thus it is unlikely that patients were misdiagnosed. Less than 1% of CAH patients have a

spontaneously occurring mutation, i.e., one of the parents is not a carrier, but since this is a rare event, and therefore unlikely to affect our results.

This study also provides interesting clues to our previous finding that psychiatric morbidity, more specifically substance misuse, anxiety, and mood disorders, is increased among patients, both men and women with CAH.^{27,28} Since there is high heritability for these psychiatric diseases, the finding that parents to patients with CAH have a decreased risk for psychiatric disorders suggests that the increased risk for psychiatric morbidity in patients with CAH is less likely to be genetically or socially determined. Hence, other factors related to the disease situation (e.g., psychological ones) are involved.

In conclusion, our findings imply that *CYP21A2* carriers have a decreased risk of any psychiatric diagnosis, especially affective and anxiety disorders. We compared parents of children with CAH, hypospadias, T1DM, and the general population and found that obligate *CYP21A2* carriers seemed to be less vulnerable to the psychologically stressful situation of having a child with a chronic disease. This indicates a better ability to cope with psychological stress among heterozygous carriers of *CYP21A2* mutations, which may contribute to a survival advantage and the relatively high incidence of *CYP21A2* carriers seen worldwide. A possible advantage with respect to somatic stress related disorders needs to be investigated.

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Table 1: Descriptive statistics for parents of children with CAH and their matched controls from general population, parents of children with type 1 diabetes or hypospadias.

	Comparison 1		Comparison 2		Comparison 3	
	Probands	Controls	Probands	Controls	Probands	Controls
Characteristic	Parents of children with CAH <i>N=610 n (%)</i>	Parents from general population <i>N=48,888 n (%)</i>	Parents of children with CAH <i>N=606 n (%)</i>	Parents of child with T1DM <i>N=48,747 n (%)</i>	Parents of children with CAH <i>N=577 n (%)</i>	Parents of child with hypospadias <i>N=22,294 n (%)</i>
Sex						
Male	297 (48.7)	10404 (21.3)	305 (50.3)	37658 (77.3)	281 (48.7)	10820 (48.5)
Female	313 (51.3)	38484 (78.7)	301 (49.7)	11089 (22.7)	296 (51.3)	11474 (51.5)
Age at child birth (yrs)						
<35	489 (80.2)	41810 (85.5)	485 (80.0)	38294 (78.6)	475 (82.3)	17387 (78.0)
≥35	121 (19.2)	7078 (14.5)	121 (20.0)	10453 (21.4)	102 (17.7)	4907 (22.0)
Education						
Primary and lower secondary	125 (20.5)	11292 (23.0)	123 (20.3)	11621 (23.8)	112 (19.4)	4769 (21.4)
Upper secondary	248 (40.7)	21300 (43.6)	247 (40.8)	21804 (44.7)	245 (42.5)	10052 (45.0)
Post-secondary	73 (12.0)	6006 (12.3)	73 (12.0)	5357 (11.0)	72 (12.5)	2777 (12.5)
Postgraduate	148 (24.2)	8984 (18.4)	148 (24.4)	8075 (16.5)	140 (24.2)	4315 (19.4)
Unknown	16 (2.6)	1306 (2.7)	15 (2.5)	1890 (3.9)	8 (1.4)	381 (1.7)

Table 2. Odds ratios (with 95% confidence interval) and p-values for receiving a psychiatric diagnosis before the childbirth in parents of children with all types of CAH compared to matched parents of a child from the general population, parents of a child with type 1 diabetes or parents of a child with hypospadias.

Psychiatric disorder	Bivariate analysis						Multivariate analysis #					
	Control group						Control group					
	Parents from general population		Parents of child with T1DM		Parents of child with hypospadias		Parents from general population		Parents of child with T1DM		Parents of child with hypospadias	
	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p	Adj OR (95% CI)	p	Adj OR (95% CI)	p
Any psychiatric diagnosis	0.5 (0.3-0.9)	0.016	0.4 (0.2 -0.8)	0.015	0.4 (0.2-0.8)	0.006	0.5 (0.3-0.9)	0.020	0.5 (0.2-0.9)	0.019	0.4 (0.2-0.8)	0.008
Suicide	0.7 (0.3-1.6)	0.387	1.0 (0.4 -2.2)	0.943	0.7 (0.3-1.6)	0.422	0.7 (0.3-1.6)	0.406	1.0 (0.5-2.3)	0.918	0.8 (0.3-1.7)	0.491
Mood disorder	0.1 (0.0-0.9)	0.044	0.2 (0.0 -1.6)	0.131	<i>0.2 (0.0-1.1)</i>	<i>0.061</i>	0.1 (0.0-1.0)	0.046	0.2 (0.0-1.6)	0.130	<i>0.2 (0.0-1.1)</i>	<i>0.061</i>
Anxiety	<i>0.4 (0.1-1.0)</i>	<i>0.056</i>	<i>0.4 (0.1 -1.1)</i>	<i>0.084</i>	0.4 (0.1-1.0)	0.040	<i>0.4 (0.1-1.0)</i>	<i>0.058</i>	0.4 (0.1-1.2)	0.101	0.4 (0.1-1.0)	0.048
Substance misuse	0.6 (0.2-1.7)	0.363	0.4 (0.1 -1.4)	0.157	0.6 (0.2-1.5)	0.265	0.7 (0.2-1.8)	0.408	0.5 (0.2-1.5)	0.201	0.6 (0.2-1.6)	0.323
Drug Misuse	0.3 (0.0-2.5)	0.290	0.3 (0.0 -2.4)	0.271	0.4 (0.0-2.5)	0.301	0.4 (0.1-2.6)	0.312	0.4 (0.1-2.6)	0.310	†	
Alcohol misuse	0.7 (0.2-2.1)	0.479	0.4 (0.1 -1.7)	0.225	0.6 (0.2-1.9)	0.384	0.7 (0.2-2.2)	0.527	0.5 (0.1-1.9)	0.277	0.6 (0.2-2.0)	0.444
Stress disorder	0.4 (0.1-1.6)	0.187	0.2 (0.0 -1.4)	0.112	<i>0.3 (0.1-1.2)</i>	<i>0.084</i>	0.4 (0.1-1.6)	0.188	0.2 (0.0-1.6)	0.130	<i>0.3 (0.1-1.2)</i>	<i>0.089</i>

Adjustment for education.†-Values for this OR and 95% CI were not possible to calculate due to zero values. Bold values indicate P < 0 .05. Italic values indicate P = 0 .05–0.099

Table 3: Odds ratios (with 95% confidence interval) and p values for receiving a psychiatric diagnosis after the childbirth in parents of children with all types of CAH compared to matched parents of a child from the general population, parents of a child with type 1 diabetes or parents of a child with hypospadias.

Psychiatric disorder	Bivariate analysis						Multivariate analysis #					
	Control group						Control group					
	Parents from general population		Parents of child with T1DM		Parents of child with hypospadias		Parents from general population		Parents of child with T1DM		Parents of child with hypospadias	
	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p	Adjusted OR (95% CI)	p	Adj OR (95% CI)	p
Any psychiatric diagnosis	0.7 (0.5-0.9)	0.017	0.8 (0.6-1.0)	0.043	0.7 (0.5-1.0)	0.024	0.7 (0.6-1.0)	0.034	<i>0.8 (0.6-1.0)</i>	<i>0.091</i>	0.7 (0.6-1.0)	0.042
Suicide	0.3 (0.1-0.8)	0.014	0.3 (0.1-0.9)	0.030	0.2 (0.0-0.7)	0.014	0.3 (0.1-0.8)	0.018	0.4 (0.1-1.0)	0.043	0.2 (0.0-0.7)	0.016
Mood disorder	0.6 (0.4-0.9)	0.022	0.8 (0.5-1.2)	0.321	0.5 (0.3-0.9)	0.016	0.6 (0.4-1.0)	0.031	0.8 (0.5-1.3)	0.381	0.5 (0.3-0.9)	0.020
Anxiety	0.6 (0.4-0.9)	0.016	0.7 (0.5-1.1)	0.173	0.6 (0.4-0.9)	0.021	0.6 (0.4-0.9)	0.024	0.8 (0.5-1.2)	0.228	0.6 (0.4-1.0)	0.034
Substance misuse	0.5 (0.2-0.9)	0.020	0.3 (0.2-0.7)	0.002	0.5 (0.2-0.9)	0.024	0.5 (0.2-0.9)	0.030	0.4 (0.2-0.7)	0.003	0.5 (0.3-0.9)	0.034
Drug Misuse	0.2 (0.1-1.0)	0.046	<i>0.3 (0.1-1.0)</i>	<i>0.056</i>	<i>0.3 (0.1-1.1)</i>	<i>0.059</i>	<i>0.3 (0.1-1.0)</i>	<i>0.056</i>	<i>0.3 (0.1-1.1)</i>	<i>0.070</i>	<i>0.3 (0.1-1.1)</i>	<i>0.067</i>
Alcohol misuse	<i>0.5 (0.3-1.1)</i>	<i>0.089</i>	0.4 (0.2-0.8)	0.007	0.6 (0.3-1.2)	0.146	0.6 (0.3-1.2)	0.121	0.4 (0.2-0.8)	0.013	0.6 (0.3-1.3)	0.186
Stress disorder	0.4 (0.2-0.9)	0.022	0.5 (0.2-1.0)	0.047	0.4 (0.2-0.8)	0.011	0.5 (0.2-0.9)	0.028	<i>0.5 (0.3-1.0)</i>	<i>0.062</i>	0.4 (0.2-0.8)	0.015

Adjustment for education.†-Values for this OR and 95% CI were not possible to calculate due to zero values. Bold values indicate P < 0 .05. Italic values indicate P = 0 .05–0.099.

Table 4: Odds ratios (with 95% confidence interval) and p values for receiving a psychiatric diagnosis before the childbirth in parents of children with classic form of CAH (i.e. carriers of a severe *CYP21A2* mutation) compared to matched parents of a child from the general population, parents of a child with type 1 diabetes or parents of a child with hypospadias.

Psychiatric disorder	Bivariate analysis						Multivariate analysis #					
	Control group						Control group					
	Parents from general population		Parents of child with T1DM		Parents of child with hypospadias		Parents from general population		Parents of child with T1DM		Parents of child with hypospadias	
	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Adj OR (95% CI)	p	Adj OR (95% CI)	p	Adj OR (95% CI)	p
Any psychiatric	<i>0.5 (0.3-1.1)</i>	<i>0.079</i>	<i>0.5 (0.2-1.0)</i>	<i>0.056</i>	0.5 (0.2-1.0)	0.039	0.5 (0.3-1.1)	0.088	<i>0.5 (0.2-1.1)</i>	<i>0.069</i>	0.5 (0.2-1.0)	0.047
Suicide	0.7 (0.3-1.9)	0.513	1.1 (0.4-2.9)	0.909	0.8 (0.3-2.0)	0.581	0.7 (0.3-2.0)	0.529	1.2 (0.4-3.1)	0.781	0.8 (0.3-2.2)	0.662
Mood disorder	0.2 (0.0-1.5)	0.113	0.3 (0.0-2.3)	0.253	0.2 (0.0-1.6)	0.131	0.2 (0.0-1.5)	0.118	0.3 (0.0-2.3)	0.251	0.2 (0.0-1.6)	0.132
Anxiety	0.5 (0.1-1.4)	0.181	0.4 (0.1-1.5)	0.172	0.4 (0.1-1.3)	0.116	0.5 (0.1-1.4)	0.185	0.4 (0.1-1.6)	0.206	0.4 (0.1-1.3)	0.141
Substance misuse	0.7 (0.2-2.3)	0.597	0.5 (0.1-1.8)	0.268	0.5 (0.2-1.7)	0.292	0.7 (0.2-2.3)	0.617	0.5 (0.1-2.0)	0.321	0.6 (0.2-1.8)	0.361
Drug misuse	0.5 (0.1-3.9)	0.545	0.5 (0.1-3.3)	0.441	0.4 (0.1-3.1)	0.410	0.6 (0.1-4.0)	0.559	0.5 (0.1-3.7)	0.509		
Alcohol misuse	0.7 (0.2-2.7)	0.574	0.4 (0.0-2.5)	0.300	0.5 (0.1-2.1)	0.349	0.7 (0.2-2.8)	0.597	0.4 (0.1-2.7)	0.336	0.6 (0.1-2.3)	0.414
Stress disorder	0.3 (0.0-2.3)	0.250		0.999	0.3 (0.0-1.8)	0.172	0.3 (0.0-2.3)	0.260	-	0.999	0.3 (0.0-1.9)	0.185

Adjustment for education.†-Values for this OR and 95% CI were not possible to calculate due to zero values. Bold values indicate $P < 0.05$. Italic values indicate $P = 0.05-0.099$.

Table 5: Odds ratios (with 95% confidence interval) and p values for receiving a psychiatric diagnosis after the childbirth in parents of children with classic form of CAH (i.e. carriers of a severe CYP21A2 mutation) compared to matched parents of a child from the general population, parents of a child with type 1 diabetes or parents of a child with hypospadias.

Psychiatric disorder	Bivariate analysis						Multivariate analysis #					
	Control group						Control group					
	Parents from general population		Parents of child with T1DM		Parents of child with hypospadias		Parents from general population		Parents of child with T1DM		Parents of child with hypospadias	
	Crude OR (95% CI)	P	Crude OR (95% CI)	P	Crude OR (95% CI)	P	Adj OR (95% CI)	P	Adj OR (95% CI)	P	Adj OR (95% CI)	P
Any psychiatric diagnosis	0.6 (0.4-0.9)	0.014	0.6 (0.4-0.9)	0.017	0.6 (0.4-0.9)	0.007	0.6(0.4-0.9)	0.017	0.7(0.4-1.0)	0.030	0.6(0.4-0.9)	0.009
Suicide	0.2 (0.1-1.0)	0.048	<i>0.3 (0.1-1.1)</i>	<i>0.076</i>	<i>0.3 (0.1-1.1)</i>	<i>0.067</i>	<i>0.2(0.1-1.0)</i>	<i>0.051</i>	<i>0.3(0.1-1.2)</i>	<i>0.095</i>	<i>0.3(0.1-1.1)</i>	<i>0.075</i>
Mood disorder	0.4 (0.2-0.9)	0.016	<i>0.6 (0.3-1.1)</i>	<i>0.085</i>	0.4 (0.2-0.8)	0.013	0.5(0.2-0.9)	0.019	<i>0.6(0.3-1.1)</i>	<i>0.101</i>	0.4(0.2-0.8)	0.014
Anxiety	0.5 (0.3-0.9)	0.022	0.6 (0.3-1.1)	<i>0.105</i>	0.5 (0.3-0.9)	0.026	0.5(0.3-0.9)	0.027	<i>0.6(0.3-1.2)</i>	<i>0.135</i>	0.4(0.1-1.1)	0.078
Substance misuse	0.3 (0.1-0.8)	0.020	0.2 (0.1-0.6)	0.005	0.2 (0.1-0.7)	0.014	0.3(0.1-0.8)	0.022	0.2(0.1-0.7)	0.007	0.2(0.1-0.8)	0.016
Drug misuse	-	0.999	-	0.999	-	0.999	-	0.999	-	0.999	-	0.999
Alcohol misuse	<i>0.4 (0.1-1.1)</i>	<i>0.075</i>	0.3 (0.1-0.8)	0.018	<i>0.3 (0.1-1.1)</i>	<i>0.067</i>	<i>0.4(0.1-1.1)</i>	<i>0.082</i>	0.3(0.1-0.8)	0.024	0.4(0.1-1.1)	0.078
Stress disorder	0.3 (0.1-0.8)	0.023	0.3 (0.1-0.9)	0.039	0.3 (0.1-0.8)	0.018	0.3(0.1-0.9)	0.027	0.3(0.1-1.0)	0.048	0.3(0.1-0.8)	0.020

Adjustment for education.†-Values for this OR and 95% CI were not possible to calculate due to zero values. Bold values indicate $P < 0.05$. Italic values indicate $P = 0.05-0.099$.