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Hypogonadotropic Hypogonadism, Delayed Puberty and Risk for Neurodevelopmental Disorders

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Contributions and conflict of interest

VOG, PL, and KT designed the study. OS, LF, ES, SB, CA, HL, PL, and KT provided technical, administrative and material support. VOG conducted data analyses in collaboration with HL and PL. VOG and KT wrote the initial draft of the paper, and all authors provided interpretation of the data and critical revisions. All authors approved the final version of the paper for submission. The authors do not have any conflict of interest associated with this publication.

Data Availability Statement: We show here the summary statistics of data used for the analyses. All individual level data is available from the Swedish National Board of Health and Welfare (www.registerforskning.se) after required permits.

Abstract

Background: Hypogonadotropic hypogonadism (HH) is a rare disorder that manifests absent puberty and infertility. Genetic syndromes with hypogonadism, such as Klinefelter syndrome, are associated with an increased risk of neurodevelopmental disorders (NDDs). However, it is not clear if patients with HH or transient delayed puberty in general, have an increased risk of NDDs.
Methods: We performed a register-based study on a national cohort of 264 patients with HH and 7447 patients diagnosed with delayed puberty that was matched with 2640 and 74470 controls, respectively. The outcome was defined as having any of the following NDD diagnoses; (1) autism spectrum disorder (ASD), (2) attention deficit hyperactivity disorder (ADHD), or (3) intellectual disability (ID). Additional sensitivity analyses were performed to control for different parental and birth variables as well as diagnosed malformation syndromes and chromosomal anomalies (i.e., Down and Turner syndromes).
Results: Patients with HH had increased risk for being diagnosed with ASD (OR 5.7; 95% CI 2.6 - 12.6), ADHD (3.0; 1.8 - 5.1) and ID (18.0; 8.9 - 36.3) compared with controls. Patients with delayed puberty also had a significantly increased risk of being diagnosed with an NDD. These associations remained significant after adjustments.
Conclusions: This is the first study to demonstrate a significant association between HH, delayed puberty and NDDs in a population-based cohort. Clinicians should be aware of the overlap between these disorders. Further studies should explore the mechanisms behind these associations.

Keywords: sex hormones; ICD; autism spectrum disorder; intellectual disability; ADHD

Introduction

Gonadotropin-releasing hormone (GnRH) is a master hormone regulating the secretion of other hormones involved in reproduction. Deficiency in the production of GnRH will lead to delayed or absence of puberty and infertility, the two latter constituting the main symptoms of hypogonadotropic hypogonadism (HH; (1)). Delayed puberty is present if there is an absence of breast development in girls or testicular enlargement in boys at an age older than 2 to 2.5 standard deviations from the population mean age of puberty onset (2). It can be challenging to differentiate between transient delayed puberty and isolated permanent HH diagnoses during the early clinical evaluations of the adolescent (3). The incidence of HH is unknown, but estimates range from approximately 1 to 10 in every 100,000 live births, and the disorder has a 3 to 5:1 male: female

ratio (1, 4). The disorder often co-occurs with a range of other phenotypes such as cleft lip/palate, hearing loss, and skeletal anomalies. HH is considered a monogenic disorder; however research shows that rarely there is a clear genotype-phenotype correlation and complete penetrance among the mutation carriers (5). Currently, there are more than 30 genes implicated in the development of HH, including the *ANOS1*-gene (MIM: 308700) and genes involved in the Fibroblast Growth Factor (FGF) signaling pathways (1).

Neurodevelopmental disorders (NDDs) are a heterogeneous group of disabling childhood-onset behavioral conditions with an estimated prevalence of 10% (6). According to DSM-5 (APA, 2013), the umbrella concept of NDDs includes, among other diagnoses, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD) and intellectual disability (ID). ASD, ADHD, and ID frequently co-occur (7), and are associated with other conditions such as rare chromosomal syndromes, immune dysfunctions and gastrointestinal problems (8, 9). In addition to rare chromosomal syndromes, hundreds of genes have been implicated in the etiologies of NDDs demonstrating the complexity of genetic pathways underlying them (10, 11). Clinical genetic testing is recommended for individuals with ID and ASD (12). Currently, molecular diagnosis can be obtained for up to 50% of ID and ASD cases using chromosomal microarray and sequencing of the whole exome and genomes (13-15).

The sex ratio of NDDs is skewed, with more boys being diagnosed than girls. While the reasons for the unbalanced sex-ratio remain unknown, it has been hypothesized that sex hormone dysregulation, especially elevated prenatal testosterone levels, increases the risk for ASD (16). Sex hormones have also been proposed to have a role in other NDDs (17). Additionally, association analyses of single nucleotide polymorphisms in sex steroid genes and cellular level functional analyses have linked the sex hormone pathways to NDDs (18-21).

Our earlier study has demonstrated an increased risk for NDDs in Klinefelter syndrome, which has hypogonadism as one of the clinical features (22, 23). NDDs and hypogonadism also co-occur in other defined genetic syndromes such as Turner, Prader-Willi, Bardet-Biedl, and CHARGE syndromes (24-27). Additionally, hypospadias, a common congenital malformation, which is sometimes caused by androgen insufficiency, increase the risk of an NDD (28).

Based on the earlier evidence, we hypothesized that patients with HH and diagnosed delayed puberty would also be at higher risk for NDDs. To explore this hypothesis, we conducted a registry study based on the total Swedish population to investigate whether HH and delayed puberty are associated with increased risk for ASD, ADHD, and ID.

Methods

Identification of cases, matched controls, outcomes, and covariates

We conducted a matched cohort study by linking several longitudinal population-based registers in Sweden, using a unique ten-digit personal identity number assigned to each resident. The registries utilized for this study, their coverage and content are summarized in **Table 1**. We identified individuals diagnosed with hypogonadism due to lack of production of gonadotropins (specific code used in Sweden E23.0E) or delayed puberty (E30.0), coded according to 10th version (used 1997 and onwards) of the International Classification of Diseases (ICD-10), from the National Patient Register (29). We constrained the cohort to individuals born between 1975 and 2000, to capture individuals that could have been diagnosed with the ICD-10 diagnoses for HH and delayed puberty. If a person had been given both diagnoses, it was included only in the HH group. Thus, 51 cases were excluded from the delayed puberty group. We matched approximately ten controls for each case. These were matched on birth year, sex, immigration status and birth county and were randomly selected from the Total Population Register. Cases could not be selected as controls.

To identify cases and controls diagnosed with the NDDs, we used the National Patient Register, the Child and Adolescent Psychiatry Register (PASTILL) (30), and the Habilitation Register (31) to obtain the ICD-10 diagnoses for ASD, ADHD, and ID (ICD-10 codes; F84.0, F84.1, F84.5, F84.8, F84.9, F90, and F70-F79). Additionally, we identified individuals diagnosed with ADHD using the Swedish Prescribed Drug Register containing data on pharmacotherapy with stimulant or non-stimulant medications (Anatomical Therapeutic Chemical code: N06BA01, N06BA02, N06BA04, N06BA09) (32) that are specifically used to treat ADHD symptoms (33).

To control for the potential influence of differences in parental socioeconomic status between cases and controls, we used information on education level. Parental education level was used rather than that of the cases and controls since both exposures and outcomes might have an effect on the attained education level. Familial relations were identified in the Multi-Generation Register (34). Data on the level of education that the parent had reached the same year the child turned 15 years of age was obtained from the Swedish Register of Education. In this register, the education level is coded from one to seven. One represents lower secondary school shorter than nine years, and seven represents Ph.D. studies. The parent's education was thereafter compared to each other, and the parent who had achieved the highest level of education was used in the

analysis. We also controlled for a range of variables that have been associated with an increased risk of NDDs. We calculated fathers age in years at the case or control's birth. Birth month was derived from the case or control's date of birth. Rural or urban living status was classified according to the recorded small area for market statistics (SAMS) code for the municipality where the individual was registered at the age of thirteen. SAMS-areas are given a code one to ten dependent on several characteristics of that area. We considered one to five to indicate urban residency and six to ten rural. Mothers that had been diagnosed with substance or alcohol abuse at any time (Substance abuse ICD-9: 304 or 305; ICD-10: F11-F19; Alcohol abuse ICD-9: 303, 305A; ICD-10: F10, excluding F10.5) were identified in the National Patient Register. Information on pregnancy length in weeks and if the child was small for gestational age were obtained from the Swedish Medical Birth Register.

Additionally, we carried out sensitivity analyses to investigate whether the potential associations between HH or delayed puberty and NDDs could be explained by other conditions affecting both the risk of HH and NDDs. Cases and controls diagnosed with other specified congenital malformation syndromes affecting multiple systems (Q87) or chromosomal abnormalities, not elsewhere classified (Q90-Q99) were identified in the National Patient Register.

Statistical analyses

Conditional logistic regression was performed to estimate the relative risk of NDD in individuals with HH or delayed puberty compared to matched controls. The relative risk was expressed as odds ratios (OR) with 95% confidence intervals (CI). To adjust for possible confounders, the parents' education level, the father's age, birth month, urban residency, small for gestational age, pregnancy length and the mother's substance or alcohol abuse were entered in the model as covariates and the analysis repeated. To explore if the relative risks were different in males and females, analyses were also performed stratified on sex. For sensitivity analyses, individuals diagnosed with congenital malformation syndromes or chromosomal abnormalities were excluded, and the analyses were repeated. All analyses were carried out in SAS version 9.4.

Ethical statement

The study and the use of Swedish registry data were approved by the Regional Ethics Committee in Stockholm.

Results

A total of 264 individuals (32% girls) with HH and 7447 with delayed puberty (23% girls) were included in the study. Descriptive statistics of the cohort are presented in **Table 2**.

Association between HH or delayed puberty and NDDs are shown in **Table 2**. The prevalence of ASD, ADHD and ID was 3.8%, 8.0% and 8.3% in HH cases and 0.7%, 2.8% and 0.5% in controls, respectively. Individuals with a diagnosis of HH were at increased risk of ASD (OR 5.7; 95% CI 2.6 - 12.6), ADHD (OR 3.0, 95% CI 1.8 - 5.1) and ID (OR 18.0, 95% CI 8.9 - 36.3) compared to matched controls (**Table 2, Figure 1**).

When comparing the cases diagnosed with delayed puberty with the matched controls, we found that 3% of the cases had received an ASD diagnosis, 8.2% were diagnosed with ADHD and 3.5% with ID and 2.1%, 5%, and 1.2%, respectively, of the controls (**Table 3, Figure 1**). Individuals with a diagnosis of delayed puberty were at increased risk of ASD (OR=1.5, 95% CI=1.3-1.7), ADHD (OR=1.7, 95 % CI = 1.6-1.9) and ID (OR=3.0, 95% CI=2.6-3.4).

Results were similar for analyses stratified by sex (**Table 2**) and adjusted for possible confounders (**Adjusted model, Table 3**). Excluding cases and controls with other conditions did not markedly affect the result of the analyses (**Table 4**).

Discussion

We found that patients with HH had a six times higher risk to be diagnosed with ASD, a three times increased risk of ADHD, and 18 times increased risk of ID. Also, the patients with delayed puberty had increased risk of the three NDDs. Even when we controlled for multiple variables and known malformation syndromes and chromosomal anomalies the associations remained significant. To the best of our knowledge, this is the first study reporting a significant association between HH, delayed puberty and NDD morbidity using a population-based design. Our results expand the current literature indicating neurodevelopmental problems in syndromes with hypogonadism as a clinical feature, such as Klinefelter (22, 23) and CHARGE syndromes (35).

Our study shows that individuals with HH and delayed puberty have higher risk to be also diagnosed with NDDs; however determining putative causality and mechanisms underlying the associations will need series of follow-up investigations. One hypothesis is that HH and delayed puberty may share some common etiological factors with NDDs. Currently, more than 30 genes are implicated in the etiology of HH and more genes are identified as the use of exome and genome sequencing is increasing (1, 5). Mutations in some of these genes have also been described in ASD and other NDDs. Case examples of these co-incidences include a stop gain in

the *ANOS1* gene (OMIM#300836) that was discovered in an individual diagnosed with ASD (36). The *ANOS1* gene encodes a protein that is involved in the migration of GnRH neurons (37). Also, mutations in the *AXL* gene (OMIM#109135) were found in individuals diagnosed with NDDs (38, 39). The *AXL* gene encodes a tyrosine kinase receptor shown to affect reproductive function and GnRH neuron development in mice (40). A study investigating the overlap of developmental disorders and disorders of sex development also found novel genetic associations between the disorders, indicating the relevance to investigate the overlapping genetic causes (41). Further genomic studies of patients with HH and comorbid NDDs could indicate additional genes underlying these disorders. However, in both HH and NDDs, many identified genetic risk variants show incomplete penetrance and variable expressivity of phenotypes within and across families (42-46). This indicates that additional genetic and environmental factors and their interplay should be investigated to understand better which outcomes are associated with specific genetic alterations.

Our results give support to the hypothesis that sex hormones play a role in the development of ASD and other NDDs. However, it contradicts the notion that only elevated levels of prenatal sex hormone are associated with ASD (16). Earlier epidemiological studies have shown that children exposed to high levels of sex hormones *in utero* due to polycystic ovary syndrome in the mothers have an increased risk for NDDs (47, 48). Nonetheless, recent studies investigating the influence of elevated prenatal testosterone on autistic traits in typically developing children and girls with congenital adrenal hyperplasia showed no such association (49-51). We suggest that both high and low levels of sex hormones during early development are involved in NDDs, but more studies are needed to clarify to what extent. These studies should also include longitudinal follow-up to capture problems during mini puberty, puberty timing and fluctuation of NDD problems during early childhood until adulthood.

Strengths and limitations

This study is based on a nationwide population-based sample, minimizing the risk that the association is due to selection bias. Due to the unique rich data-sources from the Swedish registers, we were able to control for potentially confounding factors including important pre- and perinatal risk factors and parental variables as well as other disorders among the patients and controls.

Although using a large unbiased population-based sample to assess our research hypothesis, our study has several limitations. One clear limitation is that our study has only a small number of individuals diagnosed with HH. The latter was in part due to that we only used data on patients that had been diagnosed according to ICD-10 and not earlier versions of ICD. We aimed to use the more specific diagnostic codes for HH in ICD-10 (E23.0E) in comparison with ICD-9 as well as more recently defined diagnoses of NDDs. Additional limitations include the lack of validation use of the specific ICD-10 code for HH and the ability to identify specific causes of HH in the patient groups. It is possible that the association is stronger in some subgroups of HH which could be overrepresented in our sample due to unknown bias. For instance, this could be cases with CHARGE syndrome that were missed clinically or milder phenotypes associated with *CHD7* genetic variants(52). Further, the possibility of ascertainment bias, where patients have an increased possibility of receiving additional diagnoses since they already have contact with the health care system could explain part of the association observed. We, however, find it unlikely that ascertainment bias could account for the large relative risks observed in the present study.

Implications

Clinicians should be aware of the reported comorbidity between HH, delayed puberty and NDD and pursue needed clinical psychological investigations when adolescents with HH or delayed puberty report learning-, social- and attention difficulties. Furthermore, as genome-wide clinical genetic testing is becoming standard in NDDs, the children at genetic risk for HH in this population could be detected years before the delay in sexual maturation enabling earlier diagnosis and treatment of HH. Indeed, early androgen therapy in boys with Klinefelter syndrome has been shown to improve neurodevelopmental outcomes such as language development, cognitive functioning, and social skills in comparison with boys that did not receive the early treatment (53, 54).

We present here tje first population-based study to report an increased risk of NDDs in cases with HH and delayed puberty. This study gives new insights into comorbidity between HH, delayed puberty and NDDs, and further research should be directed at exploring the mechanisms of this overlap. Further studies should also include in more detail investigations of the attention, social and cognitive skills in patients with HH and delayed puberty. Further, as genetic screening is becoming more accessible, it might be possible to identify children with genetic risk

to develop HH and NDD early. More research should focus if early hormonal therapy could prevent adverse effects on neurodevelopment in critical postnatal periods.

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Figure legend

Figure 1. Risk of autism spectrum disorder (ASD), attention-deficit hyperactivity disorder (ADHD) and intellectual disability (ID) in a Swedish national sample of cases with hypogonadotropic hypogonadism and delayed puberty and matched controls. Estimates presented are odds ratios (ORs) and corresponding 95% confidence intervals (CIs).

Tables

Table 1. Overview of the registries used in the study.

Register	Coverage	Register Holder	Contents	Reference
National Patient Register	1964-2014	National Board of Health and Welfare	Inpatient and day surgery records since 1964 and outpatient records since 2001	Ludvigsson, Andersson et al. 2011
Multi-Generation Register	1961-2014	Statistics Sweden	Familial relationships	Ludvigsson et al. 2016
Habilitation register	1998-2015	Stockholm County	Habilitation records for patients in Stockholm County	Idring et al. 2012
Swedish Prescribed Drug Register	2005-2015	National Board of Health and Welfare	All drugs dispensed at Swedish pharmacies	Wettermark, Harnmar et al. 2007
Swedish Register of Education	1985-2013	Statistics Sweden	Highest achieved education	Statistics Sweden 2011
Total Population Register		Swedish Tax Agency	Place of birth, citizenship status, information about immigration and emigration, marital	

			status and place of residence	
Clinical Database for Child and Adolescent Psychiatry (PASTILL)	2001-2015	Stockholm County	Diagnoses from the child- and adolescent psychiatry in Stockholm County	Lundh et al. 2013
Small Area Market Statistics (SAMS)	1982-2013	Statistics Sweden	Classification of Swedish municipalities per year	
The Swedish Medical Birth Register	1973-2015	National Board of Health and Welfare	Information on prenatal, delivery and neonatal care	

Table 2. Association between hypogonadism, late puberty and neurodevelopmental disorders. *Descriptive statistics, odds ratios (OR) and 95% confidence intervals(CI). ASD = Autism spectrum disorder, ADHD = Attention deficits/hyperactivity disorder, ID = intellectual disability.*

	Hypogonadism					Delayed puberty				
	Cases		Controls		OR (95% CI)	Cases		Controls		OR (95 % CI)
ASD										
All	10	3.8%	19	0.7%	5.68 (2.56-12.59)	224	3.0%	1547	2.1%	1.47 (1.27-1.69)
Males	7	3.9%	13	0.7%	5.80 (2.23-15.08)	190	3.3%	1334	2.3%	1.44 (1.24-1.68)
Females	3	3.6%	6	0.7%	5.40 (1.27-22.89)	34	2.0%	213	1.3%	1.61 (1.12-2.33)
ADHD										
All	21	8.0%	75	2.8%	3.04 (1.82-5.07)	611	8.2%	3702	5.0%	1.72 (1.57-1.88)
Males	18	10%	53	2.9%	3.75 (2.13-6.63)	527	9.2%	3132	5.5%	1.76 (1.60-1.94)
Females	3	3.6%	22	2.6%	1.39 (0.40-4.87)	84	4.9%	570	3.3%	1.50 (1.19-1.90)
ID										
All	22	8.3%	13	0.5%	17.97 (8.88-36.34)	261	3.5%	909	1.2%	2.95 (2.56-3.39)
Males	14	7.8%	5	0.3%	28.00 (10.09-77.74)	197	3.4%	760	1.3%	2.65 (2.26-3.11)
Females	8	9.5%	8	1.0%	10.87 (3.93-30.12)	64	3.8%	149	0.9%	4.47 (3.31-6.03)

Table 3. **Association between hypogonadism, late puberty and neurodevelopmental disorders in population with information about possible confounders available.** *Descriptive statistics, odds ratios (OR) and 95% confidence intervals(CI). ASD = Autism spectrum disorder, ADHD = Attention deficits/hyperactivity disorder, ID = intellectual disability.*

	Hypogonadism						**Delayed puberty**					
	Cases *n*=192		Controls *n*=1752		OR (95% CI)		Cases *n*=6398		Controls *n*=58109		OR (95% CI)	
					Unadjusted	Adjusted[1]					Unadjusted	Adjusted[1]
ASD	6	3.1%	12	0.7%	4.51 (1.69-12.03)	4.25 (1.39-13.00)	189	3.0%	1243	2.1%	1.39 (1.19-1.62)	1.36 (1.16-1.59)
ADHD	14	7.3%	55	3.1%	2.51 (1.34-4.68)	2.73 (1.41-5.28)	518	8.1%	3074	5.3%	1.58 (1.43-1.74)	1.58 (1.43-1.75)
ID	14	7.3%	12	0.7%	10.45 (4.83-22.61)	11.94 (4.73-30.16)	174	2.74%	651	1.1%	2.45 (2.07-2.91)	2.50 (2.09-2.98)

[1]*Analyses adjusted for parents' highest achieved education, father's age, urban residency at age 13, birth month, length of pregnancy in weeks, small for gestational age, mother diagnosed with alcohol or drug abuse.*

Table 4. Sensitivity analyses excluding individuals with the following ICD-10 conditions: other specified congenital malformation syndromes affecting multiple systems (Q87) or chromosomal abnormalities, not elsewhere classified (Q90-Q99).

	Hypogonadism					**Delayed puberty**				
	Cases *n=245*		Controls *n*=2446		OR (95 % CI)	Cases *n=7311*		Controls *n*=72829		OR (95 % CI)
ASD	9	3.7%	17	0.7%	5.59 (1.90-15.26)	213	2.9%	1484	2.0%	1.45 (1.25-1.67)
ADHD	18	7.3%	70	2.9%	2.76 (1.29-5.02)	592	8.1%	3611	5.0%	1.70 (1.55-1.86)
ID	16	6.5%	12	0.5%	13.33 (6.57-37.42)	217	3.0%	806	1.1%	2.74 (2.35-3.19)

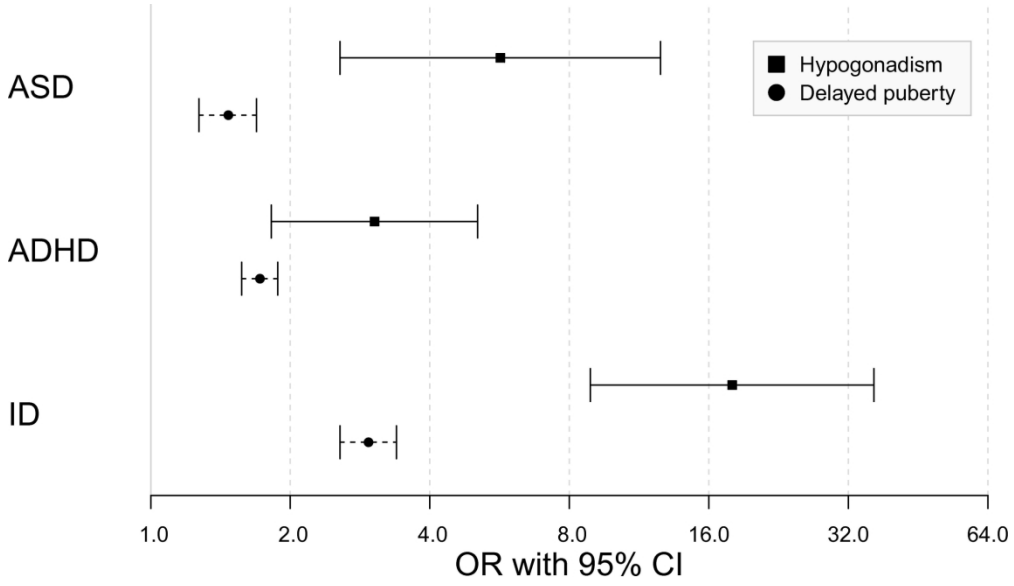

Figure 1. Risk of autism spectrum disorder (ASD), attention-deficit hyperactivity disorder (ADHD) and intellectual disability (ID) in a Swedish national sample of cases with hypogonadotropic hypogonadism and delayed puberty and matched controls. Estimates presented are odds ratios (ORs) and corresponding 95% confidence intervals (CIs).