Cardiac Disorders And Structural Brain Abnormalities Are Commonly Associated With Hypospadias In Children With Neurodevelopmental Disorders

Running Head: Hypospadias and its comorbidities

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GG was supported by the Glasgow Children's Hospital Charity (grant number: YRSS/CRTF/2016/03). EST was supported by the MRC/EPSRC Molecular Pathology Node (MR/N005813/1). RM, EST and SFA are supported by a Scottish Executive Health Department grant (74250/1) for the Scottish Genomes Partnership. The DDD study is commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003], a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Sanger Institute [grant numberWT098051]

Conflict of interest statement: The authors declare no conflict of interest.

Abstract

Objective: To use an established cohort of boys to investigate common patterns of malformations in those with hypospadias.

Method: Retrospective review of the phenotype of participants in the Deciphering Developmental Disorders (DDD) Study with neurodevelopmental delay and an 'Abnormality of the genital system'. This group was divided into two subgroups: those with hypospadias and without hypospadias. Associated phenotypes of the two subgroups were compared and analysed.

Results: Of the 166 DDD participants with hypospadias and neurodevelopmental delay, 47 (28%) had cardiovascular (CVS) and 40 (24%) had structural brain abnormalities. The rate of CVS abnormalities in those with neurodevelopmental delay and genital abnormalities other than hypospadias (N=645) was lower at 19% (p=0.001). In addition, structural brain malformations were higher at 24% in the hypospadias group versus 15% in the group without hypospadias (p=0.002). The constellation of these features occured at a higher rate in the hypospadias group versus the no hypospadias group (p=0.038).

Conclusions: In summary, this is the first study to indicate that cardiovascular and brain abnormalities are frequently encountered in association with hypospadias in children with neurodevelopmental disorders. Not only do these associations provide insight into the underlying aetiology but they highlight the multisystem involvement in conditions with hypospadias.

Keywords – Brain, Cardiac, Disorders of sex development, DSD, Genitalia

Introduction

Disorders of sex development (DSD) are a group of conditions in which the chromosomal, gonadal and/ or phenotypic sex is atypical.(Lee et al., 2006) Hypospadias is one of the most common physical presentations of DSD(Ahmed et al., 2004) and while it may occur in isolation or in combination with other abnormalities of the external genitalia, in many cases, it may also be associated with several other developmental abnormalities.(Cox et al., 2014) Although the aetiology of hypospadias may be attributed to endocrine, environmental and genetic factors,(Yang et al., 2014) in the majority of cases, a single identifiable cause is not clear.(Fernandez et al., 2016) Over the last decade, many genetic conditions associated with DSD have been reported to be associated with extra-genital malformations.(Fernandez et al., 2016, Friedman et al., 2008, Latifoglu et al., 1998, Wu et al., 2002, Lin et al., 2014, Shima et al., 1979) A clear knowledge of common patterns of congenital malformations that could be associated with DSD has the potential to provide clues that may pinpoint the underlying aetiology. Between 2011 and 2015, the UK-wide Deciphering Developmental Disorders (DDD) study has relied on clinical geneticists to collect clinical information from over 13,000 undiagnosed patients presenting with a neurodevelopmental disorder in the UK. The primary aim of that study had been to identify the underlying molecular diagnosis, but it has also enabled the investigators to create a phenotypic database of the participants which could be used for complementary analyses.(Firth and Wright, 2011) Some phenotypic features of DSD can be relatively subjective such as small phallus, scrotal fusion and undescended testes and others, such as hypospadias, can be clearly identified. Given that hypospadias may be present in over three-quarters of cases of atypical genitalia that are associated with a DSD,(Ahmed et al., 2004) the aim of the current study was to study the range of

associated anomalies that may occur in children with neurodevelopmental anomalies and DSD, and within this group compare those with hypospadias to those without hypospadias. Analysis of these data would allow the identification of common constellations of congenital anomalies that may exist in such children.

Patients & Methods

Phenotypic Data Collection

The phenotypic data collated by the DDD study were entered into the study's secure website, DECIPHER, using Human Phenotype Ontology (HPO) terms to standardise the description of the observed features of the patients; hence, the choice of feature description was limited to the terms available within this ontological classification. Phenotypic data from cases recruited from April 2011 until May 2014 with at least one HPO term under 'Abnormality of the genital system' (HP:0000078) were selected. A total number of 324 HPO terms were identified within this category in 1092 DDD participants. The subset of this group with hypospadias was ascertained by selecting those with the HPO terms hypospadias (HP: 0000047), glandular hypospadias (HP:0000807), coronal hypospadias (HP:0008743), penile hypospadias (HP:0003244), penoscrotal hypospadias (HP:0000808) and perineal hypospadias (HP:0000051). All other participants with an abnormality of the genital system other than hypospadias were included in the 'no hypospadias' group. Given that the majority of the DDD cohort have a neurodevelopmental delay phenotype, to standardise the two sub-cohorts of hypospadias and no-hypospadias cases, only individuals with at least one of 20 HPO terms (Table 1) describing a neurodevelopmental delay phenotype were included in the current study. Ethics approval was granted by the Cambridge South REC (10/H0305/83) and the Republic of Ireland REC (GEN/284/12).

Data Analysis

P values of the two proportion test were calculated using the GraphPad QuickCalcs website: https://www.graphpad.com/quickcalcs/contingency1.cfm (accessed May 2018).

In addition, Minitab statistical software [Version 18, 2017, Minitab, Inc. (www.minitab.com) State College, PA] was used to perform two proportion test to determine how the proportions of individuals with a particular associated phenotype of the two groups (hypospadias and no hypospadias) differed from each other. P values derived from the Fisher exact and two proportion tests were calculated. The significance level (alpha) was set at 0.05 and $p<0.05$ was considered to be statistically significant. P values from the 2 proportion test were calculated only for those disease categories where p values from the Fisher exact test were significant ($p<0.05$).

Results

Cases of neurodevelopmental delay & hypospadias

Of the 13,632 DDD recruits, 1092 (8%) cases were recorded to have an 'Abnormality of the genital system' and were selected for inclusion. Of these 1092 participants, 245 (22%) were recorded to have hypospadias whilst 847 (78%) did not have any phenotype entries related to hypospadias. Of the 245 cases, 172 (70%) had isolated hypospadias whilst the others had a combination of the following genital anomalies: 41 (17%) had hypospadias and undescended testes, 18 (7%) had hypospadias combined with another genital malformation but not undescended testes and 14 (6%) had a combination of hypospadias, undescended testes and another form of DSD. Other DSD phenotypes included scrotal and penile abnormalities. 166 (68%) of 245 cases had a neurodevelopmental delay, and given that one of the inclusion criteria for the DDD study required the presence of a neurodevelopmental disorder, the high level of association of these cases of hypospadias with neurodevelopmental disorders represented the expected selection bias of the DDD cohort. Of the 166 cases with hypospadias and neurodevelopmental phenotype, 44 (26%) had more than one neurodevelopmental phenotype recorded (Figure 1). Of the 847 cases without hypospadias, 645 (76%) had a neurodevelopmental delay phenotype with 467 of these 645 (72%) having more than one neurodevelopmental phenotype entries (Figure 1).

Extra-genital malformations in cases with hypospadias & neurodevelopmental delay

In addition to hypospadias, cases of neurodevelopmental delay had several other congenital anomalies with ophthalmic and periorbital, skeletal and hand abnormalities being the most common associations (Figure 2). The disease category entitled 'Other' included various HPO phenotypes that were not possible to place in any other categories

and included disorders of limb, neck, voice, movement, EEG, biochemical, haematological, psychiatric abnormalities and others such as obesity or recurrent infections. However, comparison of cases with hypospadias and neurodevelopmental delay to cases without hypospadias but with another genital abnormality and a neurodevelopmental delay, revealed a strong association of the hypospadias cohort with cardiovascular (p=0.001) and structural brain abnormalities (p=0.002) (Table 2).

Cardiovascular & brain phenotypes in cases with hypospadias & neurodevelopmental delay

Of the 166 individuals with hypospadias and a neurodevelopmental delay phenotype, 47 (28%) had at least one cardiovascular phenotype, 40 (24%) had at least one structural brain abnormality and 12 (7%) exhibited both a cardiovascular and a structural brain abnormality in addition to hypospadias and neurodevelopment delay. In contrast, only 23 (3.6%) of the 645 individuals without hypospadias and a neurodevelopmental delay exhibited both a cardiovascular and a brain defect (p=0.038). The range of cardiovascular phenotypes encountered in the cases of neurodevelopmental delay with and without hypospadias were similar (Table 3). Likewise, no significant difference was observed in the range of structural brain phenotypes in the hypospadias compared to the no hypospadias group (Table 3).

Discussion

This retrospective review of associated features of DDD participants with hypospadias and neurodevelopmental delay not only confirms the previous observation that conditions such as hypospadias are often associated with additional malformations(Cox et al., 2014) but it also revealed that the coexistence of hypospadias and neurodevelopmental delay with cardiovascular and structural brain abnormalities represented a distinct constellation. In this study we selected DDD participants with DSD and neurodevelopmental delay and compared associated phenotypes of those with hypospadias to those without hypospadias. Since the description of some of the genital abnormalities such as penile length, scrotal abnormalities or undescended testes can be subjective, our aim was to select a DSD phenotype with the greatest level of reporter certainty. Hypospadias represents a distinct phenotype and can be clearly identified by clinicians and, therefore, we selected this subset of DDD participants as our study group. Although the HPO classification system provides a standardised method to describe phenotype abnormalities in humans and allows a comprehensive capture of human phenotype(Kohler et al., 2017), the overlap that exists between HPO classes may have given rise to a degree of inter-reporter variation in the choice of HPO terms when describing some abnormalities such as those affecting the brain.

Reduced androgen exposure in utero as well as other environmental and genetic causes are well established contributing factors to the development of isolated hypospadias in humans, (Bouty et al., 2015) but hypospadias has also been linked to a large number of potentially causative developmental genes, causing a combination of congenital abnormalities. Indeed, the Online Mendelian Inheritance in Man (OMIM) database lists

283 conditions with hypospadias.(Amberger et al., 2015) The constellation of hypospadias, neurodevelopmental delay, cardiovascular abnormalities and brain malformations returned 79 different syndromes when entered in combination into the London Medical Database (v.1.0.30) while performing a search for 'syndromes on features'.(Suri, 2002) There are several examples of these associations existing together as part of well-defined syndromes including Rubinstein-Taybi syndrome (RSTS).(Hennekam, 2006, Agarwal et al., 2002, Bonioli et al., 1989), FG syndrome,(Fitzky et al., 1998) Mowat-Wilson syndrome(Garavelli and Mainardi, 2007) and Lin-Gettig syndrome.(Bashir et al., 2017, Doyon et al., 2006). The genes that are associated with pathogenic alterations in these conditions have roles in regulating gene expression through mediating or modulating gene transcription (*MED12, ZEB2, KAT6B, EP300, CREBBP*) and epigenetic regulation (*KAT6B, EP300, CREBBP*) and thus have the potential to influence the expression of numerous other genes that may play key roles in normal human development. The lack of more detailed clinical information such as results of biochemical tests and other endocrine data limited the current study from exploring whether the hypospadias was associated with a disorder of gonadal development, androgen synthesis or androgen action. However, previous studies suggest that associated anomalies are more likely to be associated with a disorder of gonadal development.(Cox et al., 2014)

Whether there is a common pathway responsible for the development of hypospadias, neurodevelopmental delay, cardiovascular and structural brain abnormalities together is yet to be established and further studies will be required to investigate the association of these features.

In summary, cardiovascular and structural brain abnormalities are frequently described in association with hypospadias in children with developmental disorders. In a substantial number of cases, hypospadias, neurodevelopmental, cardiovascular and structural brain abnormalities exist as a constellation. While the recognition of phenotypic associations with hypospadias might alert clinicians to the possible presence of undetected additional clinical problems and thus further improve clinical management, these findings also highlight the importance of a multidisciplinary team approach in these complex cases. In addition, recognition of these associations may also aid with identifying the underlying genetic aetiology.

Acknowledgements

We would like to thank the families participating in the DDD study and also the DDD Study team for their contribution. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003], a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Sanger Institute [grant numberWT098051]. The views expressed in this publication are those of the author(s) and not necessarily those of the Wellcome Trust or the Department of Health. The research team acknowledges the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network. This study makes use of DECIPHER (http://decipher.sanger.ac.uk), which is funded by the Wellcome Trust.

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HPO code	HPO term
HP:0001263	Global developmental delay
HP:0011342	Mild global developmental delay
HP:0011343	Moderate global developmental delay
HP:0011344	Severe global developmental delay
HP:0100543	Cognitive impairment
HP:0001249	Intellectual disability
HP:0001256	Intellectual disability,mild
HP:0002342	Intellectual disability, moderate
HP:0002187	Intellectual disability, profound
HP:0006887	Intellectual disability, progressive
HP:0010864	Intellectual disability, severe
HP:0002194	Delayed gross motor development
HP:0001270	Motor delay
HP:0002376	Developmental regression
HP:0000735	Impaired social interactions
HP:0001328	Specific learning disability
HP:0000750	Delayed speech and language development
HP:0001344	Absent speech
HP:0002371	Loss of speech
HP:0006863	Severe expressive language delay

Table 1. List of HPO codes and terms of neurodevelopmental phenotype entries.

Phenotype Categories	Cases with ND and no hypospadias N=645	Cases with ND and hypospadias, N=166	Chi squared test Fisher exact test, p value	2 proportion test p value
Abdominal wall	56	16	0.7594	
Pregnancy related	94	20	0.4539	
Structural brain abnormalities	92	40	**0.003**	**0.002**
Chest	56	11	0.4339	
Cardiovascular	110	47	**0.0019**	**0.001**
Ear	164	36	0.3637	
Endocrine	59	20	0.3033	
Epilepsy	110	24	0.4825	
Eye	394	102	1.00	
Face	221	51	0.4081	
Gastrointestinal	180	40	0.3784	
Growth	194	54	0.5711	
Haematological	17	5	0.7895	
Hand	221	62	0.4661	
Hearing	74	16	0.58	
Mouth	230	52	0.316	
Neck	15	3	1.00	
Neuromuscular	166	31	0.0675	
Nose	122	33	0.825	
Renal	73	14	0.327	
Respiratory	73	21	0.683	
Skeletal	292	67	0.293	
Skin	152	44	0.418	
Skull	222	56	0.927	
Voice	2	1	0.497	
Other	157	50	0.135	

Table 2. Number of cases with an abnormality in the disease categories in the two groups of neurodevelopmental delay with and without hypospadias and results of statistical anlayses showing p values derived from the Fisher exact and 2 proportion tests.

	Number of cases in group with hypospadias	Number of cases in group without hypospadias
Cardiovascular phenotypes		
Septal defects	15 (32%)	37 (34%)
Combination of large vessel and septal defects	7 (15%)	9 (8%)
PDA +/- septal defects	7 (15%)	12 (11%)
Complex combined heart disorders	7 (15%)	21 (19%)
Abnormalities of the large vessels	4 (8%)	8 (7%)
Valvular disorders	3 (6%)	7 (6%)
Other	4 (8%)	16 (14%)
Total	**47**	**110**
Structural brain abnormalities		
Complex structural brain defects	15 (37%)	27 (29%)
Abnormalities of the cerebral ventricles	6 (15%)	14 (15%)
Cerebral malformations	5 (12%)	18 (20%)
Cerebellar malformations	3 (7%)	5 (5%)
Abnormalities of the corpus callosum	3 (7%)	11 (12%)
Other	8 (20%)	17 (18%)
Total	**40**	**92**

Table 3. Description of cardiovascular and brain phenotypes encountered in cases of neurodevelopmental delay with and without hypospadias.

Figure legends

Figure 1. DDD participants selected for current study, from top down: DDD participants (n, 13,632), participants with 'Abnormality of the genital system' (n, 1,092), participants with hypospadias (n, 245) and participants without hypospadias (n, 847), participants with and without hypospadias who also have neurodevelopmental delay (n, 166 and 645, respectively).

Figure 2. The range of additional phenotype categories in the 166 DDD participants with neurodevelopmental delay and hypospadias.