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Short Communication

Whole genome investigation of an atypical autism case identifies a novel *ANOS1* mutation with subsequent diagnosis of Kallmann syndrome

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ABSTRACT

We report an actionable secondary finding from whole-genome sequencing (WGS) of a 10-year-old boy with autism. WGS identified non-synonymous variants in several genes, including a nonsense mutation in the *ANOS1* gene which is an X-linked cause of Kallmann syndrome. WGS can provide insights into complex genetic disorders such as autism, and actionable incidental findings can offer the potential for therapeutic interventions.

1. Introduction

The genetic aetiology of autism spectrum disorder (ASD) is complex, involving combinations of inherited and/or *de novo* gene variants. In recent years, the use of whole-exome sequencing (WES) and whole-genome sequencing (WGS) has identified thousands of DNA variants as potentially contributing to ASD [1]. The decline in cost of WGS has led to this technology being more widespread to help understand the genetics of complex disorders that involve both coding and non-coding DNA sequences such as ASD [2,3]. An important consideration for WGS is the incidental finding of actionable genetic variants that are unrelated to the medical condition being investigated. The estimated frequency of actionable variants in the population is approximately 2.5% [4], which provides an opportunity for preventing adverse health outcomes in a significant number of individuals. In recent years, recommendations for reporting actionable secondary findings, as well as a list of reportable genes, have been developed by the American College of Medical Genetics and Genomics, based on the potential medical, legal, social and economic impacts [5]. Further reports of WGS outcomes will help inform the impact of secondary findings on the treating physician, individuals, families and society. In this study, we report an actionable secondary finding from WGS of an individual with autism.

2. Methods

2.1. Study participants

This study investigated a 10-year-old proband and his typically developing 12-year-old brother who did not meet ASD criteria. The proband was first seen at the age of 2 years when he presented with features of ASD and some dysregulation of behaviour. His general examination at that time was normal apart from a mildly reduced penile length which was initially treated with testosterone. He was then lost to follow-up for a while, re-presenting later at 5 years of age with clear autistic features and ongoing micropenis. It was impossible to assess for anosmia given his autistic features. This together with some sensory seeking behaviours made it difficult to interpret his ability to describe smells and taste. His examination continues to be otherwise normal with growth trending along the 5th centile with testosterone therapy and weight along the 25th centile. He has normal blood pressure and cardiac examination. An early MRI had found a small hypo-enhancing focus in the anterior pituitary and normal optic chiasm but was not demonstrated on a repeat MRI (post-diagnosis of Kallmann syndrome) at 13 years of age. On this recent MRI, the Left Olfactory nerve is not able to be visualised and what is suggestive of a hypoplastic Right olfactory nerve is seen where the Right nerve would normally be located. The rest of the MRI is normal. He has mildly delayed bone age of 2 SD below the mean for age by Greulich and Pyle standards. Other medical

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issues in the proband have included severe multifocal pyelonephritis with multiple small abscesses on imaging. Despite an extensive workup, the aetiology of his ASD features remained unknown. Both children were seen at the Mater Children's Hospital and Queensland Children's Hospital for diagnostic purposes and ongoing clinical assessments. The research protocol was approved by the Mater Human Research Ethics Committee (HREC/14/MHS/211). Written informed consent was obtained from both children and their parents.

2.2. DNA sequencing and analyses

Genomic DNA was isolated from peripheral blood mononuclear cells of the proband and his sibling, and then sequenced on an Illumina X10 system (Macrogen Inc). WGS data were aligned to the human reference genome GRCh37 using bwa mem version 0.7.13 [6]. SNP and Indel variants were called jointly *via* GATK 3.7 HaplotypeCaller [7] and analysed using GEMINI version 0.18 [8]. Structural variants were called using DELLY and selected variants were examined manually in IGV [9]. The *ANOS1* variant (c.1340G > A) was confirmed by Sanger sequencing of DNA from an independent blood sample from the proband.

3. Results and discussion

In this study, WGS was used to investigate the potential genetic aetiology of ASD in a 10-year-old boy. To eliminate genetic variants that are unlikely to be contributing to ASD, we included WGS data obtained from his typically developing brother. Through this approach, we identified putative loss-of-function variants in nine genes, as well as short intronic deletions in four neuronal-expressed genes implicated in autism and other neurological disorders, none of which were present in the unaffected sibling (Table 1). Three of these (*GRIK4*, *DLG2*, *GRIN3A*) are heterozygous mutations present as segregating variation based on the 1000 Genomes populations [10] although the *DLG2* [11] and *GRIN3A* [12] deletions appear to be rare with a minor allele frequency (MAF) < 0.01. The presumably inherited deletion in *GABBR2* (MAF = 0.41) is homozygous and removes a number of annotated transcription factor binding sites, which is interesting given the gene's active expression profile in the brain and published links to ASD [13]. The deletion in *GRIK4* (MAF = 0.08) is particularly interesting as it occurs in a putative regulatory region and has been reported as an ASD candidate gene. A previous study identified a possibly damaging *de novo* missense variant in *GRIK4* of an individual with ASD [14]. In addition, overexpression of *Grik4* in the forebrain of mice led to altered synaptic transmission with abnormal behaviours of social impairment, enhanced anxiety and depressive states [15]. Whilst disruption of *GRIK4*, possibly in combination with other mutations highlighted in this report, may potentially contribute to the ASD features of the proband, further cohort studies and molecular studies would be required to implicate some combination of these variants in ASD. We await the outcome of future population studies to determine whether those genes shown in Table 1 have any involvement in ASD.

Of great interest was our finding of a novel nonsense mutation (W447X) in the *ANOS1* gene of the proband. Mutations in *ANOS1* are the most common genetic cause of Kallmann syndrome (OMIM #308700) that is an X-linked cause of anosmia and gonadotropin deficiency with delayed puberty and infertility [16]. The role of *ANOS1* in ASD is currently unclear. One study reported a R423X mutation in *ANOS1* of a 14-year-old male with atypical ASD and Kallmann syndrome [17]. However, another study reported the same mutation in an individual with Kallmann syndrome but that case did not present with ASD [18]. Furthermore, disruption of the chromosomal region Xp22.31 which contains *ANOS1*, has been linked to ASD, however, this region also contains several genes that may be responsible for ASD [19,20].

To date, more than 60 mutations have been reported in *ANOS1* of individuals with Kallmann syndrome [16,21]. *ANOS1* encodes a 680 amino acid protein that is important for axonal guidance and migration of GnRH and olfactory neurons [22]. A hallmark feature of this syndrome is the delayed or arrested pubertal maturation, which can be prevented with testosterone replacement therapy in males at the onset of puberty, followed by gonadotrophin or pulsatile gonadotropin-releasing hormone (GnRH) therapy for induction of spermatogenesis [22]. More recent clinical examinations of the proband at 13 years of age show his testosterone level remains low with normal pituitary hormone level and delayed puberty, which is consistent with the endocrine features of Kallmann syndrome. Other studies have reported several pathogenic nonsense mutations that truncate the ANOS1 protein between amino acids 421 and 631 [16], suggesting that the W447X variant detected in the present study is the cause of Kallmann syndrome

Table 1
Sequence variants detected in the proband.

Gene	Gene ID	*ASD candidate	Location	Variant type	Nucleotide and protein change
Heterozygous loss-of-function variants					
DCHS2	54798	No	4q31.3	Frameshift	c.4095_4098delCAAA, p.Asn1365fs
GPRC6A	222545	No	6q22.1	[a]Frameshift	c.2323dupT, p.Tyr775fs
				[a]Frameshift	c.2110dupT, p.Tyr704fs
				[a]Frameshift	c.1798dupT, p.Tyr600fs
BARHL1	56751	No	9q34.13	Frameshift	c.946dupC, p.Leu316fs
CCDC7	79741	No	10p11.22	[a]Frameshift	c.699_702delAAAT, p.Asn234fs
				[a]Frameshift	c.675_678delAAAT, p.Asn226fs
				[a]Frameshift	c.603_606delAAAT, p.Asn202fs
PGM2L1	283209	No	11q13.4	Frameshift	c.1493delC, p.Pro498fs
NEU3	10825	No	11q13.4	[a]Stop lost	c.1287A > T, p.Ter429Tyrext*?
				[a]Stop lost	c.1059A > T, p.Ter353Tyrext*?
				[a]Stop lost	c.1386A > T, p.Ter462Tyrext*?
KRTAP1-5	83895	No	17q21.2	Frameshift	c.396delC, p.Cys133fs
USF2	7392	No	19q13.12	Frameshift	c.447delG, p.Arg150fs
Hemizygous loss-of-function variant					
ANOS1	3730	No	Xp22.31	Stop gained	c.1340G > A, p.Trp447*
Deletions in potential regulatory regions of genes					
GRIK4	2900	Yes	11q23.3	Deletion	11:120387644–120388371
DLG2	1740	No	11q14.1	Deletion	11:85263028–85274777
GRIN3A	116443	Yes	9q31.1	Deletion	9:104496701–104498219
GABBR2	9568	Yes	9q22.23	Deletion	9:101309048–101311670

* ASD candidate gene reported on the SFARI gene database (https://gene.sfari.org/database/human-gene/).
[a] Variant sequence in mRNA transcripts and protein isoforms [a]1, [b]2 and [c]3.

in the proband. There is no family history of Kallmann syndrome on the maternal or paternal side of the family, suggesting that the W447X variant in the proband may be a *de novo* mutation.

In summary, we report an actionable secondary finding of Kallmann syndrome from WGS analysis of a child with ASD. This outcome demonstrates the medical benefit of reporting secondary findings from WGS, and highlights that individuals and their parents, as well as the treating physician can be unaware of Kallmann syndrome prior to puberty. This information has enabled timely endocrine management of the proband and highlights the potential for secondary findings when undertaking WGS.

Declaration of Competing Interest

None declared.

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