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Complete List of Authors:	Green, Claire; Sheffield Children's NHS Foundation Trust, Sheffield Clinical Genetics Willoughby, Joshua; Sheffield Children's NHS Foundation Trust, Sheffield Diagnostic Genetics Service Balasubramanian, Meena; Sheffield Children's NHS Foundation Trust, Sheffield Clinical Genetics Service;
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De Novo SETD5 Loss-of-Function Variant as a Cause for Intellectual Disability in a 10-year old boy with an Aberrant Blind Ending Bronchus

Short Title: *SETD5* variant and blind ending bronchus

Green C[1], Willoughby J[2], DDD Study[3], Balasubramanian M[1]

[1]Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, UK

[2]Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, UK

[3]DDD Study, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

Correspondence to:

Dr Meena Balasubramanian

Sheffield Clinical Genetics Service

Sheffield Children's NHS Foundation Trust

Sheffield, UK

Ph: +44 114 2717025

Fax: +44 114 2737467

E-mail: meena.balasubramanian@nhs.net

ABSTRACT

Although rare, 3p microdeletion cases have been well described in the clinical literature. The clinical phenotype includes; intellectual disability (ID), growth retardation, facial dysmorphism and cardiac malformations. Advances in chromosome microarray (CMA) testing narrowed the 3p25 critical region to a 124kb region, and recent Whole Exome Sequencing (WES) studies have suggested that the *SETD5* gene contributes significantly to the 3p25 phenotype. Loss-of-Function (LoF) variants in *SETD5* are now considered a likely cause of ID.

We report here a patient with a frameshift LoF variant in exon 12 of *SETD5*. This patient has features overlapping with other patients described with LoF *SETD5* variants to include; similar facial morphology, feeding difficulties, intellectual disability, behavioural abnormalities and leg length discrepancy. In addition, he presents with an aberrant blind ending bronchus.

This report adds to publications describing intragenic mutations in *SETD5* and supports the assertion that *de novo* LoF mutations in *SETD5* present with an overlapping but distinct phenotype in comparison with 3p25 microdeletion syndromes.

KEY WORDS

3p microdeletion, 3p25, *SETD5*, aberrant blind ending bronchus, intellectual disability, loss of function.

INTRODUCTION

Intellectual Disability (ID) has a worldwide prevalence of approximately 1%-3% and has become the most frequent reason for referral to paediatric genetic services [Maulik and Darmstadt 2007]. Due to its clinical and genetic heterogeneity, the underlying cause for ID remains unclear. However, advances in genetic testing have led to the elucidation of several novel genes linked to ID, some of the most successful studies have led to the identification of de novo LoF sequence variants in candidate genes [de Ligt and others 2012; Rauch and others; Vissers and others 2010].

Distal deletions of the short arm of chromosome 3 were first characterised by cytogenetic and FISH analysis [Aqua and others 1995; Verjaal and De Nef 1978]. This condition results in a well-described syndrome associated with a clinical phenotype that includes; intellectual disability, growth retardation, facial dysmorphism and cardiac malformations. The severity of the condition varies considerably, with the size of the deletion apparently correlating with the severity of the phenotype [Drumheller and others 1996].

A report by Kellogg and others [2013] described 4 patients with 3p25.3 deletions and ID, including 3 previously reported patients by Peltekova and others [2012], Riess and others [2012] and Gunnarsson and Foyn Bruun [2010]. These 3p25.3 deletion carriers had a narrow range of overlap comprising of 3 genes including *SETD5*. In addition to ID, patients had a common phenotype of depressed nasal bridge (3/4), low set ears (3/4) and philtrum differences (3/4). Other features were more variable including; cardiac malformations (2/4), ptosis (2/4), low birth weight/growth retardation (2/4), seizures (2/4) and microcephaly (2/4) [Kellogg and others 2013].

Following this, 7 patients with independent LoF variants in *SETD5* from a cohort of 996 patients with moderate/severe ID (0.7% of cohort) were reported. Features included; speech delay, behavioural problems and autism. Similar dysmorphology i.e. brachycephaly, prominent forehead, abnormal eyebrows, similar nose morphology (long, thin, tubular), eye morphology (long, narrow, upslanting palpebral fissures, mild ptosis, unilateral amblyopia, nystagmus, strabismus) and large, fleshy, low set ears were reported. Again, more variable features included; cardiac malformations (2/7), skeletal abnormalities (leg length discrepancy, scoliosis, kyphosis, lordosis) and genitourinary abnormalities (4/7) [Grozeva and others 2014].

Kuechler and others [2015] went on to expand the phenotype further and described 4 unrelated patients with 4 different non-recurrent microdeletions on chromosome 3p25 narrowing down the smallest region of overlap to 94kb including only 2 coding genes, *SETD5* and parts of THUMPD3. Included in the cohort were 2 patients with intragenic *SETD5* variants. Patients were compared with those from Kellogg and others [2013], Peltekova and others [2012], Riess and others [2012] and Gunnarsson and Foyn Bruun [2010]. Both microdeletion carriers and intragenic *SETD5* variant carriers had a similar craniofacial phenotype of striking eyebrows (full, broad, straight, arched or with synophyrys), a tubular nose with broad nasal bridge, bulbous nasal tip, anteverted nares, a long philtrum and downturned corners of the mouth. Just like previous reports, features which showed incomplete penetrance were cardiac malformations and postaxial polydactyly.

An emerging behavioural phenotype was supported with almost all the LoF mutation carriers and 3 microdeletion carriers showing some behavioural problems. Patients with larger deletions had additional facial differences (blepharophimosis, abnormal slanting of palpebral fissures and ptosis). Microdeletion carriers were more

likely to be of short stature, microcephalic and hypotonic and microdeletion carriers also showed more severe speech impairment.

The only published cases of an inherited *SETD5* variant suggested a more variable phenotype. Two siblings with developmental delay and features consistent with previously reported *SETD5 de novo* cases were compared to their father who had only mild intellectual impairment with some features of *SETD5*. These patients did not have ritualised behaviour or autism, abnormalities in eye structure, gastrointestinal and/or abdominal wall defects or scoliosis/kyphosis [Szczałuba and others 2016].

Frequency data for *SETD5* mutations is difficult to obtain. However, the genetic database Decipher includes 34 patients with *SETD5* sequence variants (https://decipher.sanger.ac.uk/), the SFARI database of genes linked with autism has 47 *SETD5* mutations reported in its human gene module (https://sfari.org/) and a recent large study of 4,293 patients recruited from the Deciphering Developmental Disorders (DDD) study identified 17patients with *de novo SETD5* mutations [Deciphering Developmental Disorders 2017]. *SETD5* is, therefore, likely one of the most commonly mutated genes in developmental disorders [Deciphering Developmental Disorders 2017].

Adding to published reports, we present a patient with a *de novo* heterozygous c.1381_1388del, p.(Asn461fs) frameshift mutation in exon 12 of *SETD5*, who also has an aberrant blind ending bronchus, thus expanding the phenotype.

MATERIALS AND METHODS

This patient was recruited to the Deciphering Developmental Disorders (DDD) study. Trio-based exome sequencing was performed on the affected individual and their parents, as previously described [Wright and others 2014]. Each affected individual also had a high-resolution analysis for copy number abnormalities using array-based comparative genomic hybridization (aCGH). Putative *de novo* mutations were identified from exome data using DeNovoGear software [Ramu and others 2013] and were validated using targeted Sanger sequencing. Mutation nomenclature is according to HGVS guidelines with reference transcript NM_001080517.2.

CLINICAL REPORT

This patient is the second child of healthy non-consanguineous, White European parents with unremarkable family history. He was born at term with a birth weight of 2.976kg, following an uneventful pregnancy. Raised alpha fetoprotein (AFP) levels were noted but antenatal scans were normal. There were no postnatal complications. Failure to thrive and difficulty gaining weight were noted and he later required fundoplication. He was born with postaxial polydactyly in all extremities requiring surgical removal at the age of 4 months and tongue tie. Persistent cough was noted at age 2 days and recurrent infections have continued to affect him, with a later diagnosis of asthma. His chest infections were thought to be due to an aberrant blind ending bronchus identified on bronchoscopy. There were no concerns with his vision but conductive hearing loss was treated with grommet insertion.

Developmentally, at 10 months he could not sit unaided, he walked at 23 months. Leg asymmetry was detected subsequently. His first word was at 13 months, with a vocabulary of single words at 2 years. At a clinical psychologist

assessment at 5 ½ years old, a Verbal IQ of 83 (13th percentile) and Performance IQ of 98 (45th percentile) were recorded. Assessment also showed some weakness in social communication and a stutter with recurrent dribbling. However, he did not fulfil criteria for Autistic Spectrum Disorder. At age 7, he was attending mainstream school with additional support. He was reported at school to have a dyslexic profile with 'low average' level. In terms of behaviour, repetitive stereotyped behaviours were noted such as repeatedly touching his face. He was late to develop imaginary play and weakness in social interaction with peers was noted. Reassessment at age 9 highlighted his complex communication needs and problems acquiring language skills.

On examination age 2 ½, he was noted to have brachycephaly with prominence in forehead, metopic ridge, hypertelorism, clinodactyly and prominent left ear (Figure 1 which shows evolving facial dysmorphism with age). Left leg was 2 cms longer than right with a small café au lait mark (0.5cm) on left leg. Height was 89cm (25th centile), weight was 13.11kg (25th centile), and head circumference was 49cm (2nd-9th centile).

Investigations included metabolic tests; urine organic acids and amino acids, creatine kinase, alpha feta protein, thyroid function, mucopolysaccharides were normal. Echocardiogram and renal ultrasound were also reported normal. Cytogenetics showed a normal male karyotype (46,XY) and FISH testing for deletion or duplication of the TBX1 locus at 22q11.2 was negative. Testing for primary ciliary dyskinesia was also normal along with normal ophthalmology assessment which included electroretinogram. Panel genetic testing for Bardet Biedl Syndrome was normal. Patient was enrolled in the Deciphering Developmental Delay (DDD)

[DECIPHER ID: 259090] study which identified a de novo heterozygous c.1381_1388del, p.(Asn461fs) frameshift mutation in exon 12 of *SETD5*.

DISCUSSION

The 3p critical region was initially thought to be a 3-5Mb region [Aqua and others 1995]. Subsequent patients were described with a narrow region of overlapping regions [Gunnarsson and Foyn Bruun 2010; Kellogg and others 2013; Peltekova and others 2012; Riess and others 2012] and *SETD5* was considered to be the strongest candidate gene [Peltekova and others 2012; Shuib and others 2009]. Further evidence to support pathogenicity of *SETD5*, came from large cohorts of *de novo* mutation carriers [Iossifov and others 2014; Pinto and others 2016; Rauch and others 2012; Deciphering Developmental Disorders 2017].

The coding sequence of *SETD5* is 4329bp long and encodes 1442 amino acids. It is ubiquitously expressed and high levels have been seen in the cerebral cortex, the intestine and the eye [Kuechler and others 2015; Nagase and others 1997]. The gene is highly evolutionarily conserved suggesting that it is functionally important and is considered a member of the 'writers' group of epigenetic genes [Kleefstra and others 2006].

In silico domain analysis has showed that SETD5 is a multidomain protein containing a SET domain and a putative PHD domain [Kuechler and others 2015]. It thought have an important role in cell replication and gene expression through regulation of histone acetylation [Hu and others 2010; Jones and others 2008; Tanaka and others 2000; Yao and others 1998]. Genes encoding histone modifiers are increasingly recognised to have a contribution to ID [Berdasco and Esteller 2013].

Animal models have demonstrated that *SETD5* is important in mammalian development with *SETD5* deficient mouse embryos exhibiting severe developmental delay, vascular abnormalities, apoptosis, and reduced cellular proliferation; findings consistent with impairment of gene expression [Osipovich and others 2016].

Histone modifier genes are all dosage sensitive and haploinsufficiency is believed to be the disease mechanism. *In vitro* analysis has shown that variants in *SETD5* trigger nonsense mediated decay (NMD) pointing to LoF [Kuechler and others 2015]. Haploinsufficiency of a single gene has also proven to be casual for the specific phenotype in a number of microdeletion syndromes. There are several examples of this including; *EHMT1* in association with 9q34 deletion and *SATB2* in association with 2q33.1 deletion.

The phenotypic features of our patient with a *de novo SETD5* variant fit with previously described patients to include; ID, language delay, ritualised behaviour, feeding difficulties, abnormal ears, eyebrows, shape of nose and polydactyly (see Table I). Common features described in the literature that our patient did not have include; micrognathia (8/13), thin upper lip (8/13), gastrointestinal defects (5/13) and genitourinary defects (6/13).

The observation of brachycephaly was made only in our patient and 3 patients in the Grozeva and others [2014] cohort (3/7). Unsteady gait and hypertelorism are only described in our patient and 1 of the patients in the Szczałuba and others [2016] paper.

Congenital heart defects appear to be more of a feature of the microdeletion syndrome [Gunnarsson and Foyn Bruun 2010; Peltekova and others 2012] with only 4/13 patients in the *SETD5* group affected. Knockout mice models have shown that *SETD5* may be important for embryonic stem cells to differentiate into

cardiomyocytes [Osipovich and others 2016] and given the nature of cardiac anomalies reported in the literature, it is reasonable to consider a cardiac assessment at the time of initial diagnosis.

Behavioural problems including obsessive compulsive disorder (OCD) and autism were common findings (6/13). Most of the patients with *SETD5* pathogenic variants have truncating variants (frameshift or nonsense variants) and whilst some *de novo* missense variants have been reported in large cohorts of autism patients as being causative, extended phenotypic descriptions are not available to assign causality (Table I). Based on data presented by [Li and others 2016; Neale and others 2012] there is insufficient evidence to link the reported *SETD5* missense variants with autism or susceptibility to autism. Our patient had been assessed for autism but did not meet criteria for a diagnosis. He did show signs of ritualised behaviour with some areas of weakness in social communication. This is increasingly being observed in children with underlying genetic conditions contributing to their behaviour profile i.e. their behavioural problems are not typical and hence, will not fulfil the diagnostic criteria for autism spectrum disorder. However, it is well–recognised that their learning needs and behavioural traits can be challenging to manage and needs appropriate assessment to tailor resources to their needs.

The notable difference in our patient is the presence of a blind-ending bronchus. Although recent studies have highlighted the integral role of *SETD5* in mammalian development, it remains unclear if the blind ending bronchus is related to the *SETD5* mutation or whether it has an independent cause.

CONCLUSION

We report a 10-year old boy with a *de novo* LoF variant in *SETD5* and provide a comprehensive review of published literature on this frequently reported ID gene. The emerging phenotype includes; ID, facial dysmorphology, skeletal anomalies, behavioural problems and speech and language difficulties. We report for the first time, aberrant blind ending bronchus as a possible association with this phenotype. Further case reports of this nature are required to expand the phenotype and understand variable expressivity of *SETD5* especially as genomic sequencing studies identify variants of interest in this gene.

FIGURE AND TABLE LEGENDS

Figure 1: Facial dysmorphism of this patient evolving with age demonstrating prominent forehead, upslanting palpebral fissures, bilateral low-set, posteriorly rotated ears, smooth philtrum.

Table I: Clinical features of patients with *de novo SETD5* variants in comparison to our patient.

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CONFLICTS OF INTERESTS

None to declare

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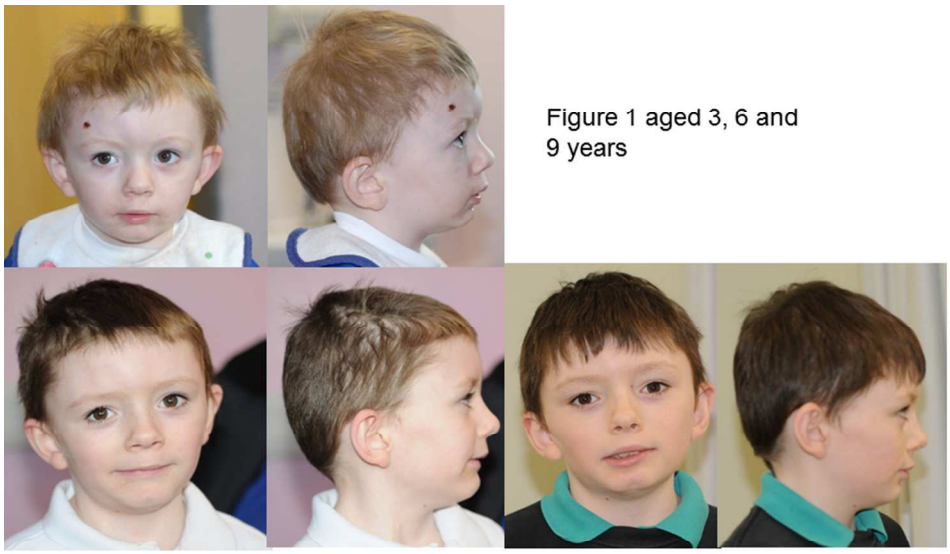

Figure 1: Facial dysmorphism of this patient evolving with age demonstrating prominent forehead, upslanting palpebral fissures, bilateral low-set, posteriorly rotated ears, smooth philtrum.

254x190mm (96 x 96 DPI)

Table I: Clinical features of patients with *de novo SETD5* variants in comparison to our patient

	Our patient	Grozeva et al.2013 Patient 1	Grozeva et al.2013 Patient 2	Grozeva et al.2013 Patient 3	Grozeva et al.2013 Patient 4	Grozeva et al.2013 Patient 5	Grozeva et al.2013 Patient 6	Grozeva et al.2013 Patient 7	Kuechler et al.2015 Patient 1	Kuechler et al.2015 Patient 2	Szczaluba et al.2016 Proband	Szczaluba et al.2016 Brother	Szczaluba et al.2016 Father
Mutation	Chr3:9486924-9486932 c.1381_1388 del p.Asn461Profs*15	Chr3:9486739 c.1195A>T p.Lys399**	Chr3:9486877 c.1333C>T p.Arg445*	Chr3:9489453 c.1866C>G p.Tyr622**	Chr3:9490142 c.2177_2178del p.Thr726Asnfs*39	Chr3:9512419 c.3001C>T p.Arg1001*	Chr3:9517216 c.3771dup p.Ser1258Glufs*65	Chr3:9517302 c.3846del p.Ser1286Leufs*84	Chr3:9477570_9477650del c.547_567+60del p.Pro183_Lys189del	Chr3:9490270 C>T c.2302C>T p.Arg768*	Chr3:009512336C>G;_0010805517.2. Ser973*	Chr3:009512336C>G;_0010805517.2. Ser973*	Chr3:009512336C>G;_0010805517.2. Ser973*
Gender	M	M	M	M	M	M	M	M	F	F	M	M	M
Descent	Caucasian	NR	NR	NR	NR	NR	NR	NR	Caucasian	Caucasian	NR	NR	NR
Age (years) at last examination	6 5/12	NR	NR	NR	NR	NR	NR	NR	20	9 10/12	4 2/12	12 2/12	31
Height	109.7cm 2nd centile	NR	50-75th centile	2nd centile	NR	25-50th centile	NR	NR	178cm 2.26 SD	134cm -1.18 SD	96cm -2.1SD Below 3rd	78cm 0SD 50th	170cm -0.8SD 10th-25th
Weight	18.05kg 9th centile	NR	NR	9th centile	NR	25-50th centile	NR	NR	67kg	28.5kg	14kg 1.5SD 3rd centile	5kg -5.4SD Below 3rd	64kg -0.4SD 25th-50th
OFC	51.5cm 9th centile	25th centile	75-98th centile	50-75th centile	10-25th centile	75-91st centile	75th centile	10th centile	58cm 2.46 (SD)	50.5cm -1.26 (SD)	51cm -0.3SD 25th-50th	47.5cm -0.2SD 25th-50th	56cm -0.5SD 25th
Uneventful Pregnancy	Raised AFP Normal scans	NR	NR	NR	NR	NR	NR	NR	+	+	NR	NR	NR
Gestation	term	34/40	38/40	term	term	35.5/40	term	term	40/40	40/40	41/40	36/40	NR
Birth Weight (kg)	2.92 9th centile	2.47	2.69	2.99	3.66	2.41	2.95	small	3.20	3.07	2.97 -1SD 15th	2.35 2.1SD 1st-3rd	NR
Feeding Difficulties	Y - reflux	Y	Y – swallowing difficulties	N	N	Y – chewing difficulties	Y- chewing and swallowing difficulties	N	NR	NR	Y	Y	NR
Dribbling	Y	N	N	N	N	Y	Y	Y	NR	NR	NR	NR	NR
Ears	Prominent left ear Posteriorly rotated bilateral low set	Large ears Periauricular pit	Fleshy ear lobes	Large ears Long,narrow low set ears	NR	Fleshy ear lobes	NR	Long,narrow low set ears	NR	Low set/malformed ears	N	Bilateral ear creases	Y
Eyes	Hypertelorism	Upslanting palpebral fissures	Left eye amblyopia	Long narrow fissures Upslanting palpebral fissures	Nystagmus and strabismus Upslanting palpebral fissures	Long narrow fissures Mild ptosis Upslanting palpebral fissures	Upslanting palpebral fissures	Down slanting palpebral fissures	Myopia/astigmatism	Strabismus Mildly down slanting palpebral fissures	N	Hypertelorism	N
Eye brows	Pencilled fine eyebrows	Synophrys Straight eyebrows	Full eyebrows	Synophrys	NR	Cyst in eyebrows	NR	Synophrys Broad eyebrows	NR	NR	N	N	N
Nose	Tubular nose Prominent nasal root	Tubular nose	Broad bridge, Bulbous tip Anteverted nares Depressed nasal	Prominent high nasal root Tubular nose Prominent nares	Prominent high nasal root Tubular nose	Broad bridge Bulbous tip Anteverted nares Depressed nasal bridge Prominent high	Depressed nasal bridge	Prominent high nasal root	Broad bridge, Bulbous tip Anteverted nares	Broad bridge, bulbous tip Anteverted nares Long philtrum	Abnormally shaped	Depressed nasal bridge Abnormally shaped Short nose	Abnormally shaped

			bridge			nasal root Tubular nose							
Mouth/ Lower face	Small mouth Short Philtrum	Long philtrum Micrognathia Thin upper lip High palate	Long philtrum Thin upper lip	Long philtrum Thin upper lip	Small mouth Long philtrum Micrognathia	Long philtrum Thin upper lip	NR	Short philtrum Small mouth Micrognathia Thin upper lip High palate	Long philtrum Downturned corners of the mouth	Long philtrum Downturned corners of the mouth	Long philtrum Open mouth with an everted full lower lip Micrognathia Cleft palate	Long philtrum Thin upper lip Micrognathia	Long philtrum Micrognathia
Teeth	NR	NR	Crowded teeth	NR	NR	NR	Crowded teeth	Crowded teeth	NR	NR	NR	NR	NR
Digits	Post axial polydactyly Clinodactyly	NR	NR	Clinodactyly	post axial polydactyly	NR	NR	NR	Long and thin fingers	Prominent finger joints Broad distal phalanges Sandal gaps	Post axial polydactyly	Post axial polydactyly Single transverse palmar creases	N
Skeletal	Leg length discrepancy Unusual gait	Leg length discrepancy Scoliosis	Leg length discrepancy Scoliosis Sacral dimple	Lordosis	NR	Sacral dimple Stiff legged gait	Lordosis Stiff legged gait	NR	NR	NR	N	N	Pectus Excavatum
Cranio-facial features	Brachycephaly Prominent forehead Metopic ridge	Brachycephaly	Brachycephaly	NR	NR	Prominent forehead	NR	Brachycephaly	NR	NR	Triangular face	Triangular face	Triangular face
Neurology	NR	NR	NR	NR	NR	NR	NR	NR	1 febrile seizure at 8 years	NR	Hypotonic Unsteady gait	Hypotonic	N
Abnormal organ development	Blind ended bronchus	Hypospadias	Inguinal hernia Undescended testes	Hypospadias	NR	Paraumbilical hernia Undescended testes	NR	Inguinal hernia Nocturnal enuresis	NR	NR	bilateral vesico-ureteral reflux with posterior urethral valve	Bilateral cryptoorchism bilateral vesico-ureteral reflux	N
Heart	NR	VSD,PDA	MVP	NR	NR	NR	NR	NR	NR	NR	ASD,PDA, persistent left superior vena cava	ASD	N
Developmental Delay	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Severe	Y - Mild ID
Walking (age)	19-23 months	24 months	36 months	18 months	NR	24 months	38 months	20 months	24-36 months	20 months	18 months	NR	NR
Speaking (age at first words)	13 months	4 years	4 years	12 months	Late	18 months	2 years	NR	4 years	4 years	4 years	NA	NR
Speech problems	Stammer Motor dyspraxia Speech dysfluency Expressive language delay	NR	Stammer	Stammer	NR	Expressive language delay	Expressive language delay	Expressive language delay	NR	NR	Severe delay-only a few words at 4	NR	N
Behaviour	Repetitive stereotyped activities	Repetitive stereotyped activities Autistic OCD	Repetitive stereotyped activities	NR	NR	Repetitive stereotyped activities	Autistic OCD	OCD	Dominant in know, anxious in unknown situations	Mild ADD	N	N	N
Other	Recurrent infections	NR	NR	NR	NR	NR	NR	NR	NR	Recurrent infections	NR	Exophthalmos	NR

Mutation nomenclature according to HGVS guidelines (http://varnomen.hgvs.org /), using NCBI reference transcript NM_001080517.2.
Abbreviations: NR=Not Recorded, M=Male, F=Female, Y=Yes, N=No, SD=Standard Deviations, AFP=Alpha FetoProtein, ASD= Artrioventricular Septal Defect VSD=Ventricular Septal Defect, PDA=Patent Ductus Arteriosus, MVP=Mitral valve prolapse, OCD=Obsessive Compulsive Disorder, ADD=Attention Deficit Disorder, ID = Intellectual Disability