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Original research

In-depth characterisation of a cohort of individuals with missense and loss-of-function variants disrupting *FOXP2*

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ABSTRACT

Background Heterozygous disruptions of *FOXP2* were the first identified molecular cause for severe speech disorder: childhood apraxia of speech (CAS), and yet few cases have been reported, limiting knowledge of the condition.

Methods Here we phenotyped 28 individuals from 17 families with pathogenic *FOXP2*-only variants (12 loss-of-function, five missense variants; 14 males; aged 2 to 62 years). Health and development (cognitive, motor, social domains) were examined, including speech and language outcomes with the first cross-linguistic analysis of English and German.

Results Speech disorders were prevalent (23/25, 92%) and CAS was most common (22/25, 88%), with similar speech presentations across English and German. Speech was still impaired in adulthood, and some speech sounds (eg, 'th', 'r', 'ch', 'j') were never acquired. Language impairments (21/25, 84%) ranged from mild to severe. Comorbidities included feeding difficulties in infancy (10/26, 38%), fine (13/26, 50%) and gross (13/26, 50%) motor impairment, anxiety (5/27, 19%), depression (6/27, 22%) and sleep disturbance (10/24, 42%). Physical features were common (22/27, 81%) but with no consistent pattern. Cognition ranged from average to mildly impaired and was incongruent with language ability; for example, seven participants with severe language disorder had average non-verbal cognition.

Conclusions Although we identify an increased prevalence of conditions like anxiety, depression and sleep disturbance, we confirm that the consequences of *FOXP2* dysfunction remain relatively specific to speech disorder, as compared with other recently identified monogenic conditions associated with CAS. Thus, our findings reinforce that *FOXP2* provides a valuable entry point for examining the neurobiological bases of speech disorder.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Heterozygous disruptions of *FOXP2* were the first identified molecular cause for severe speech disorder: childhood apraxia of speech (CAS), and yet few cases have been reported, limiting knowledge of the condition.

WHAT THIS STUDY ADDS

⇒ Here we provide the most comprehensive characterisation of individuals with pathogenic *FOXP2* variants, almost doubling the number of published families to date. We provide the first cross-linguistic analysis of speech and language across German and English. We show that the phenotype for pathogenic *FOXP2* variants remains relatively specific to speech disorder, compared with phenotypes associated with other monogenic conditions involving CAS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study guides the identification of cases with a *FOXP2*-related disorder for a clinical genetic diagnosis, improves prognostic counselling and leads to a better targeted clinical management.

INTRODUCTION

FOXP2 was the first gene implicated in a developmental speech and language disorder in the absence of intellectual disability.[1] A private heterozygous missense variant in *FOXP2* was found to cosegregate with childhood apraxia of speech (CAS) in 15 members of the multigenerational British 'KE family', while being absent from all unaffected relatives and healthy controls.[1] The study also characterised a balanced chromosomal translocation with a 7q31.2 breakpoint disrupting *FOXP2* in an unrelated proband with similar speech deficits.[1] CAS is a disorder of speech motor planning and programming that manifests in impaired sequencing of speech sounds into syllables and words with the correct prosody.[2] The condition is associated with delayed and protracted speech development.

Pathogenic single-nucleotide variants (SNVs) and intragenic indels that disrupt *FOXP2* are rare. To our knowledge, there have been a dozen of these SNVs/indels reported in the literature to date: the

original missense variant in the large KE family,[1] a non-sense (stop-gain) variant in two siblings and their mother,[3] a frame-shift in a sporadic patient[4] and eight variants across eight small families (intragenic deletions, non-sense, missense and frame-shift variants)[5] (online supplemental table 1), each occurring in a heterozygous state. The limited number of cases reported may in part be due to the relatively mild speech-focused phenotype associated with pathogenic SNVs/indels of *FOXP2*, compared with other neurogenetic childhood disorders. While debilitating for affected probands and families, a CAS phenotype does not often lead to clinical genetic testing or to ascertainment in gene discovery cohorts for other neurodevelopmental disorders such as autism spectrum disorder (ASD) or intellectual disability. All probands with pathogenic *FOXP2* variants reported to date share a severe speech disorder presentation, most commonly CAS. Yet there is preliminary evidence that, in some cases, SNVs or indels of *FOXP2* may cause a broader phenotype including subtle dysmorphology and co-occurring neurodevelopmental features such as ASD.[5]

There are other variants that affect *FOXP2* that are not SNVs or indels. A large deletion downstream of *FOXP2* was hypothesised to have a position effect on expression.[6] There have also been case series of large heterozygous 7q31 deletions or reciprocal-balanced translocations associated with more complex phenotypes involving disruptions of *FOXP2* in addition to neighbouring genes.[7–15] Such phenotypes are now clinically defined as having a *FOXP2*-plus-related disorder.[16]

A systematic prospective cohort study of the phenotype(s) associated with *FOXP2* variants is warranted to guide better identification of cases for clinical genetic diagnosis, improve prognostic counselling and lead to better targeted intervention. Here we examine speech, language, health and broader developmental phenotypes associated with pathogenic *FOXP2* SNVs/indels in a cohort of 28 probands from 17 unrelated families (7 previously published but not deeply characterised for speech and language[3–5] and 10 novel) expanding the genetic and clinical spectrum of the disorder. For the first time in any phenotypic study of *FOXP2*, the specificity of a linguistic phenotype, relative to broader neurodevelopmental skills (eg, communication ability compared with domains of social, motor and daily living skills), was examined using standardised tests. A novel cross-linguistic comparison of speech diagnoses in German-speaking versus English-speaking participants was also conducted to determine homogeneity of the speech phenotype across languages.

METHODS

Participants

Inclusion criterion was a molecular diagnosis of pathogenic variants (SNVs or intragenic deletions/duplications) in *FOXP2*, in individuals aged ≥6 months. Participants were recruited via clinical genetics colleagues or family self-referral in the Netherlands, France, Britain, Germany, the USA, the UK and Australia. Adult participants and caregivers of child participants provided informed consent to participate in the study. The assessment battery was tailored to cover a wide range of ages and languages.

Health and development

Health and medical information, including developmental milestones and existing diagnoses of neurodevelopmental conditions, were collected via an established direct (adult) or caregiver survey.[17–19] Health professional reports and consults confirmed caregiver survey responses. Feeding (Child Oral and Motor Proficiency Scale[20]) and drooling (Drooling Impact Scale[21]) measures were collected where age appropriate.

Speech

In English-speaking participants, phonology and articulation were assessed using standardised tools (Diagnostic Evaluation or Articulation and Phonology[22]) or Goldman Fristoe Test of Articulation—Second Edition.[23] Phonological delay versus disorder was delineated. Both for English-speaking (authors LDM, ATM) and German-speaking (author EM) participants, phonological and articulation errors were also analysed from a phonetic transcription of a 5 min conversational speech sample. Across both languages, CAS features were rated across three core diagnostic criteria[17 18 24]: inconsistent speech errors, lengthened and disrupted coarticulatory transitions and impaired prosody. These three criteria were further operationalised into rateable speech errors (see online supplemental table 2). Similarly, dysarthria was assessed using the Mayo Clinic Dysarthria Classification System rating scale[25] and evaluating oral motor structure and function.[26] The Intelligibility in Context Scale[27] documented how well an individual was understood by conversational partners, with a five-point scale ranging from 'never' to 'always' understood.

Language and literacy

Receptive vocabulary and expressive vocabulary were assessed using clinician-administered standardised tools dependent on an individual's age and language (see table 1 for assessments). Likewise, caregiver-administered standardised tools were used to measure speech and language skills. Assessment tools used were dependent on an individuals' communicative ability, age and language (table 1). Literacy abilities were documented by direct (adult) or caregiver reports, the Vineland Adaptive Behaviour Scales—Third Edition[28] written communication subdomain and academic records.

Adaptive behaviour and intellectual ability

The Vineland Adaptive Behaviour Scale, Third Edition (VABS-3) provided scores across language, socialisation, self-care, daily living, motor skills and a composite total adaptive behaviour score.[28] A non-parametric Kruskal-Wallis test examined the relative involvement of VABS-3 subdomain scores, and a Wilcoxon signed-rank test was performed between VABS-3 receptive and expressive language scores to highlight any differences between these domains.

General intellectual abilities were assessed with the age-appropriate Wechsler assessment (see table 1 for assessments). Where Full-Scale Intelligence Quotient (FSIQ) could not be obtained, Perceptual Reasoning Indexes and Matrix Reasoning subtest scores were calculated from the relevant Wechsler assessment. A further three assessment tools were used to assess intellectual abilities in four children (table 1). Diagnoses of neurodevelopmental disorders (eg, autism) were identified by caregiver report and confirmed with clinical records.

RESULTS

Participants

Twenty-eight participants with pathogenic *FOXP2* variants were recruited from 17 families, comprising 10 unreported families (families 1–3, 6, 12–17) and 7 that were previously reported but not deeply characterised for speech and language abilities

Table 1 Language skills and cognition in this cohort with pathogenic missense/loss-of-function variants disrupting *FOXP2*

Case	Age at assessment (year range)	Receptive language		Expressive language		Intellectual abilities	Literacy impairment	
		Vocabulary	Grammar	Vocabulary	Grammar		Spelling	Reading
1a	6–8	Mild*	Moderate†	Moderate‡	Severe††	Average¶ PRI 100	Y	Y
1b	36–38	Mild*	–	–	–	–	Y	Y
1c	30–32	Average*	Average†	–	–	Average¶ PRI 100	Y	Y
1d	60–62	Average*	Moderate†	–	–	Average¶ MR 40	Y	N
2	6–8	Severe*	Severe**	Severe**	Severe††	Borderline‡‡ FSIQ 78	Y	Y
3a	3–5	Severe§§		Severe§§		Borderline¶¶ FSIQ 73	Y	Y
3b	39–41	–	–	–	–	–	N	N
4a	18–20	Severe*	Moderate†	–	–	Mild*** FSIQ 67	Y	Y
4b	18–20	Moderate*	Severe†	–	–	Mild*** FSIQ 65	Y	Y
4c	15–17	Average*	Average†	–	–	Mild¶ FSIQ 62	Y	Y
5	6–8	Severe§§		Severe§§		Average¶ PRI 94 Borderline¶ FSIQ 73	Y	Y
6	12–14	Moderate†††	Moderate†	Severe‡	Severe††	Borderline¶ PRI 79	Y	Y
7a	15–17	Severe*	Moderate†	Mild	Below average‡‡‡	Average¶¶ MR 10	Y	Y
7b	15–17	Average*	Moderate†	Severe¶¶	Below average‡‡‡	Average¶¶ MR 8	Y	Y
8a	36–38	Average*	Severe †	Mild***	–	Moderate*** MR 4	N	N
8b	15–17	Moderate*	Severe†	Severe¶¶	Below average‡‡‡	Average¶¶ MR 10	N	N
9	18–20	Severe*	Severe†	–	Severe‡‡‡	Severe¶¶ MR 2 Mild§§§ FSIQ 67	Y	Y
10	9–11	Average§§§	Average†	Mild¶¶	Below average‡‡‡	Average¶¶ MR 17	N	N
11a	39–41	Average*	Average†	Average***	Average‡‡‡	Moderate*** MR 5	N	N
11b	6–8	Average§§§	Severe†	Average¶¶	Severe‡‡‡	Average¶¶¶ FSIQ 92	Y	Y
12a	3–5	Severe*	–	Severe****	Severe****	Average¶¶¶ FSIQ 85	NA	NA
12b	33–35	–	–	–	–	–	N	N
13	0–2	Average††††	–	Mild‡‡‡‡	–	–	NA	NA
14	3–5	Severe††††	–	Severe‡‡‡‡	–	–	NA	NA
15	3–5	Average§§§§		Severe‡	Severe††	–	Y	Y
16a	42–44	–	–	–	–	–	N	Y
16b	9–11	Mild§§	–	Mild§§	–	Borderline¶¶ FSIQ 78	Y	Y
17	0–2	Average††††	–	Average‡‡‡‡	–	–	NA	NA

Average=−1 < SD; mild=−1 to −1.5 SD; moderate= −1.5 to −2 SD; severe=< −2 SD. Below average=a qualitative descriptor based on the analysis of a transcribed conversation speech sample.
*Peabody Picture Vocabulary Test.[59]
†Test for Reception of Grammar, Second and German Editions.[60 61]
‡Children's Communication Checklist, Second Edition Semantics subdomain.[61]
§Wechsler Abbreviated Scale of Intelligence, Second Edition.[62]
¶Clinical Evaluation of Language Fundamentals Preschool, Second Edition.[63]
**Children's Communication Checklist, Second Edition syntax subdomain.
††Kaufman Assessment Battery for Children, Second Edition.[64]
‡‡Clinical Evaluation of Language Fundamentals, Fourth and Fifth Editions.[65 66]
§§Wechsler Intelligence Scale for Children, Fourth and Fifth Editions.[67 68]
¶¶Wechsler Adult Intelligence Scale, Second and Fourth Editions.[62 69]
***Receptive One-Word Picture Vocabulary Test, Fourth Edition,[70]
†††Analysis of transcribed conversation speech sample.
‡‡‡Kaufman Assessment Battery for Children, Second Edition.[64]
§§§Snijders-Oomen Non-verbal Intelligence Test.[71]
¶¶¶Schlichting Test for Language Production.
****Communication and Symbolic Behaviour Scales Development Profile Infant-Toddler Checklist Understanding subdomain.[72]
††††Communication and Symbolic Behaviour Scales Development Profile Infant-Toddler Checklist Words subdomain.
‡‡‡‡Vineland Adaptive Behaviour Scales, Third Edition, Expressive and Receptive subdomain scaled scores.[28]
§§§§Wechsler Preschool and Primary Scale of Intelligence, Fourth Edition.[73]
¶¶¶¶Universal Non-verbal Intelligence Test, Second Edition.[74]
-, not assessed; FSIQ, Full-Scale Intelligence Quotient; MR, matrix reasoning; N, no; PRI, perceptual reasoning index; Y, yes.

(families 4, 5, 7–11). Participants had a median age of 16 years 4 months, range 2 years 7 months to 62 years 7 months, and 14 (50%) were male (table 2). Most participants were referred for genetic testing due to the proband's striking speech and language impairment, except for family 3 (n=2) and participant 17. Family 3 underwent genetic testing as part of research testing for preterm children (ID 3a), and subsequently a variant was also identified in the father (ID 3b). Participant 17 was referred due to microcephaly and dysmorphic facial features.

Genetic results

The 17 families each had a unique *FOXP2* variant, comprising 12 loss-of function and 5 missense variants (table 2). The 12

Table 2 Genotypes and phenotypes of this cohort

Case	cDNA*	Protein	Variant type	ACMG criteria	ACMG classification	Inheritance	Country	Sex	Age, years†	Sleep	Motor	NDD
1a	c.1191dupA	p.Glu398Argfs‡31	Loss of function (frameshift)	PVS1, PM2, PP1	Pathogenic	Maternal	Aus	M	6–8	–	Fine and gross	–
1b						Maternal	Aus	F	36–38	–	–	–
1c						Maternal	Aus	F	30–32	+	Fine and gross	ASD, ADHD
1d						NA	Aus	F	60–62	+	–	–
2	(114 254 407–114 308 861)×3		Loss of function (intragenic duplication)			De novo	Aus	M	6–8	+	–	ASD, impulsivity, hyperactive, borderline IQ
3a	(114 036 269–114 347 379)×1		Loss of function (intragenic deletion)			Paternal	Aus	M	3–5	–	Fine	Borderline IQ, sensory and attention difficulties
3b						De novo	Aus	M	39–41	–	Fine	–
4a‡	c.982C>T	p.Arg328‡	Loss of function (non-sense)	PVS1, PS2, PM2, PP1	Pathogenic	Maternal	Aus	M	18–20	+	Fine	Tourette's syndrome, mild ID, ASD
4b‡						Maternal	Aus	F	18–20	+	Fine	Mild ID, ASD
4c						Maternal	Aus	F	15–17	+	Fine	Mild ID, ASD
5‡	c.1168_1169delCA	p.Gln415Valfs‡7	Loss of function (frameshift)	PVS1, PS2, PM2	Pathogenic	De novo	Aus	M	6–8	–	Mild tremor	Borderline IQ
6	c.1666C>T	p.Leu556Phe	Missense	PS2, PM1, PM2, PP3, PP4	Pathogenic	De novo	UK	F	12–14	+	Fine and gross	–
7a‡	(114 296 590–114 310 602)×1		Loss of function (intragenic deletion)			De novo	Ger	F – twin	15–17	–	Gross	–
7b‡						De novo	Ger	F – twin	15–17	–	Gross	–
8a‡	c.982C>T	p.Arg328‡	Loss of function (nonsense)	PVS1, PS2, PM2, PP1	Pathogenic	NA	Ger	F	36–38	–	–	NA
8b‡						Maternal	Ger	M	15–17	–	–	–
9‡	c.1607G>C	p.Arg536Pro	Missense	PS1, PS2, PM1, PM2, PP3, PP4	Pathogenic	Paternal (? mosaic Fa.)	Ger	F	18–20	NA	Gross	Autistic features, mild ID
10‡	c.1432C>T	p.Arg478‡	Loss of function (nonsense)	PVS1, PS2, PM2	Pathogenic	De novo	Ger	F	9–11	NA	Broad-based gait	–
11a‡	c.1514C>T	p.Pro505Leu	Missense	PS1, PS2, PM1, PM2, PP3, PP4	Pathogenic	NA	Ger	M	39–41	NA	NA	–
11b‡						Paternal	Ger	M	6–8	NA	NA	Borderline IQ
12a	c.1385C>G	p.Ser462‡	Loss of function (non-sense)	PVS1, PS2, PM2, PP1	Pathogenic	Maternal	NL	M	3–5	–	Fine and gross	SPD
12b						NA	NL	F	33–35	–	–	DCD, ASD
13	c.1513C>A	p.Pro505Thr	Missense	PS1, PS2, PM1, PM2, PP3, PP4	Pathogenic	De novo	NL	M	0–2	–	Fine and gross	–

Continued

Table 2 Continued

Case	cDNA*	Protein	Variant type	ACMG criteria	ACMG classification	Inheritance	Country	Sex	Age, years†	Sleep	Motor	NDD
14	c.1658 G>A	p.Arg553His	Missense	PVS1, PS2, PM2	Pathogenic	De novo	USA	M	3–5	+	Fine and gross	Hyperactive, attention deficit, restricted interests and behaviour
15	c.1428del	p.Lys417Asnfs‡7	Loss of function (frameshift)	PVS1, PS2, PM2	Pathogenic	De novo	USA	M	3–5	–	–	Sensory-seeking behaviours
16a	(113 844 506–114 106 056)×1		Loss of function (intragenic deletion)			De novo	USA	F	42–44	+	Gross	–
16b						Maternal	USA	F	9–11	+	Gross	Dyslexia, ASD, borderline IQ
17	c.1A>G	p.M1?	Translational start-site variant	PM1, PM2, PM4, PP3, PP4	Likely pathogenic	NA	USA	M	0–2	–	Fine and gross	–

*NM_014491, all deletions and the microduplication are **arr[hg19]7q31.1**.
†Ages in 2-year age ranges.
‡Previously published.
ADHD, attention-deficit hyperactive disorder; ASD, autism spectrum disorder; Aus, Australia; DCD, developmental coordination disorder; F, female; Ger, Germany; M, male; NA, not assessed; NDD, neurodevelopmental disorder; NL, Netherlands; OCD, obsessive–compulsive disorder; SPD, sensory processing disorder; UK, United Kingdom; USA, United States of America.

Figure 1 Schematic representation of 17 pathogenic *FOXP2* variants in this cohort from 28 individuals in 17 families (NM_014491).

loss-of-function variants included 3 frameshifts, 4 stop-gain/nonsense variants, 1 variant abolishing the translational start site, 3 intragenic deletions and 1 intragenic duplication. All missense variants were located in the forkhead-box DNA-binding domain of the encoded protein (figure 1). Eleven of the 28 participants had confirmed de novo variants, 12 inherited their variant from a parent, and for 5 participants the inheritance status was unknown (table 2; online supplemental figure 1). Participant 9 was previously reported to have inherited the *FOXP2* variant from their father who had the same variant in a mosaic state, and who was unavailable to take part in the present study. Deletions and sequence variants were submitted to Decipher (https://decipher.sanger.ac.uk/).

Health and development

Over a third of the assessed participants had feeding difficulties in infancy (10/26), and some had excessive drooling (5/21) in the early years of life (online supplemental table 3; table 2). Gross motor impairments during early development (13/26) and fine motor impairment (13/26) were also present, with a subset of participants having both fine and gross motor impairment (7/26). Of those with fine motor impairment, participant 12b had a formal diagnosis of developmental coordination disorder. In a small number of individuals, hypotonia (IDs 6, 12a) or microcephaly (ID 17) was also noted. Two participants had hearing impairment: mild, conductive hearing loss (25-39dBHL, ID 15) and moderate mixed hearing loss (40-69dBHL, ID 1d). Sleep disturbances were relatively common (10/24), mostly characterised as difficulty falling asleep (5/24) or frequent waking (4/24). Visual impairments (8/26) were present (online supplemental table 3). Physical features were reported in most participants (22/27, four previously reported (online supplemental table 3)), but with no distinct morphological profile across families. Recorded physical features involved the nose (upturned nose: ID 1a; prominent nose: IDs 1b, 1c; hypoplastic alae nasae: ID 4b, 4c; high nasal root: ID 8b; rounded, fleshy or prominent nasal tip: IDs 1b, 1c, 3a, 5, 8b), philtrum (short/flat philtrum: IDs 2, 17), ears (prominent/protruding ears: IDs 1a, 1b, 12b; anteverted ears: ID 14), eyes (periorbital fullness: IDs 3a, 5, 13; prominent eyes: ID 16b), jaw (retrognathia: IDs 1a, 1b, 3a, 13) and lips (full lips IDs: 1a, 1b, 1c, 4a, 4b, 13; thin upper lip: 3a). In individual cases, mild finger pads (ID 10), tapering fingers (ID 8b), single palmar crease (ID 17) and clinodactyly (ID 12a) were also noted.

Co-occurring diagnoses

A quarter of participants had a diagnosis of ASD (7/27; 2 diagnosed in adulthood) and one had autistic traits but did not meet the criteria. Participant 1c had a diagnosis of attention-deficit

hyperactivity disorder (ADHD). Hyperactivity (2/27), attention difficulties (2/27) and restricted interests and behaviour (2/27) were also noted in further participants without formal ASD or ADHD diagnoses. Mental health conditions such as anxiety (5/27), depression (6/27) and obsessive–compulsive disorder (2/27) were reported in five adults and one adolescent.

Most participants (school aged or older) with pathogenic *FOXP2* variants attended mainstream schools (14/23); seven attended special education schools and two attended a mix of special education and mainstream schools. Learning support (eg, teaching aide, individualised learning plan) was common (15/23) across all settings. All five preschool participants attended specialist preschool settings for children with additional learning needs. All caregivers of school-aged children and adolescents reported that their child's academic progress had been most impacted by their speech and language impairments.

Communication development

Speech development was characterised by limited babbling and a reduced phonetic (sound) inventory relative to peers across the first 7 years of life when a full inventory is typically acquired. Some developed first words around the typical age of development (12–15 months, 9/25), whereas others were slightly (15–18 months, 1/26) or more significantly (>18 months, 14/26) delayed (table 3). One participant (ID 14) had not said their first words yet in early childhood (3–5 years old). Eight participants had not yet mastered combining words (IDs 1a, 6, 9, 12a, 13, 14, 15, 17). Only three participants combined words in line with the typical development milestone of 2–3 years of age. The remaining participants combined words between 4 to 5 years (4/22), 6 to 7 years (2/22) and 8 years or older (5/22), representing protracted development relative to the typical developmental milestone of 2–3 years.

Speech

CAS was the most common speech diagnosis (88%, 22/25) (figure 2, table 3), with features including frequent sound omissions, the same consonant or vowel being produced differently across different words, impaired sequencing of phonemes and syllables, voicing errors, syllable segregation, difficulty achieving initial articulatory configurations, equal stress, altered suprasegmental features and slow rate (online supplemental table 3).

Table 3 Speech features and educational placement of individuals with pathogenic SNVs/indels disrupting *FOXP2*

Case	Age first words	Age first sentences	CAS	Phonological errors	Dysarthria	Oral motor impairment	Schooling	Support*
1a	>18 mo	NYA	+	Disorder	–	+	MS PS	+
1b	12–15 mo	2–3 years	+	Delay	–	+	MS SC and diploma	–
1c	2–3 years	>8 years	+	Delay	–	+	MS SC and diploma	+
1d	<12 mo	2–3 years	+	–	–	+	MS PS and diploma	–
2	>18 mo	4–5 years	+	Disorder	–	+	Mixed MS and specialist	+
3a	>15 mo	2–3 years	–	Disorder	–	+	MS PS	+
3b	NA	NA	–	–	–	–	MS SC and diploma	–
4a	>18 mo	>8 years	+	Disorder	+	+	MS SC	+
4b	>18 mo	>8 years	+	Disorder	–	+	MS SC	+
4c	>18 mo	>5 years	+	Disorder	–	+	MS SC	+
5	12–15 mo	6–7 years	+	Disorder	+	+	MS SC	+
6	>18 mo	NYA	+	Disorder	–	+	Specialist SC	+
7a	4–5 years	7–8 years	+	–	–	NA	Specialist	–
7b	4–5 years	7–8 years	+	–	–	NA	Specialist	–
8a	NA	NA	+	–	–	NA	MS SC	–
8b	4–5 years	NA	+	–	–	NA	Specialist	–
9	4–5 years	NYA	+	NA	NA	+	Specialist	–
10	12–15 mo	NA	+	–	–	NA	MS school	+
11a	NR	NA	–	–	–	NA	School for speech and language disorders, advanced technical college	–
11b	12–15 mo	NA	+	Disorder	–	NA	Pre for speech and language disorders	–
12a	>18 mo	NYA	+	Disorder	–	NA	Specialist	+
12b	>18 mo	6–7 years	NA	NA	NA	NA	MS and specialist, higher vocational education	–
13	NYA	NYA	+	Disorder	–	NA	Specialist pre	+
14	NYA	NYA	NA	NA	NA	NA	Specialist pre	+
15	12–15 mo	NYA	+	Disorder	–	NA	Specialist pre	+
16a	12–15 mo	4–5 years	NA	NA	NA	NA	MS SC and degree	–
16b	12–15 mo	4–5 years	+	Disorder	–	NA	MS PS	+
17	12–15 mo	NYA	+	Disorder	–	+	Specialist pre	–

+=feature present, –=feature absent
*Support in the form of support staff in the classroom and/or individualised education plans.
CAS, childhood apraxia of speech; mo, months; MS, mainstream; NA, not assessed; NYA, not yet achieved; OCD, obsessive–compulsive disorder; Pre, preschool; PS, primary/elementary school; SC, secondary school; SNVs, single-nucleotide variants; wks, weeks.

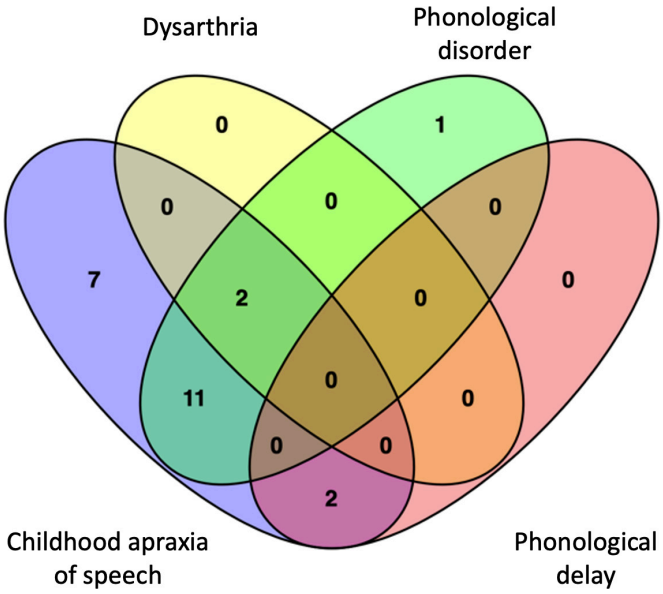

Figure 2 Speech disorders of participants with pathogenic missense/loss-of-function variants disrupting *FOXP2* (n=25, two participants had no speech disorder).

CAS was present in most English (86.7%, 13/15) and German (87.5%, 7/8) speakers.

Some participants had multiple co-occurring speech sound disorders (figure 2). Dysarthria (8.3%, 2/24) was infrequent and mild in severity, characterised as mixed nasality and harsh vocal quality (IDs 4a, 5). Phonological disorder was common (58.3%, 14/24), especially in children (<18 years); although for some (IDs 4a, 4b) this persisted into adulthood (table 3). A further two adults had a severe phonological delay, that is, typical phonological error patterns that appear in the speech of younger individuals but that should have resolved by 7 years of age (online supplemental table 4a; IDs 1b, 1c). Disordered oral motor movements were present both on speech (eg, say 'pataka') and non-speech tasks (eg, bite then blow) (92.9%, 13/14).

Two adults and one child did not have signs of CAS at the time of testing (IDs 3a, 3b, 11a). Participant 11a had a speech and language disorder in childhood for which they received therapy, but could not recall having CAS. The other adult participant (ID 3b) reported being a 'quiet' child but did not have speech therapy, and his son (ID 3a) had a phonological disorder without CAS.

Phonetic inventories were analysed for 12 English-speaking participants (online supplemental table 4a,b). Strikingly, 66.7% (8/12) of English-speaking participants did not have the affricate /dʒ/ (eg, 'j' in 'jump') and 58.3% (7/12) did not have its voiceless counterpart, /tʃ/ (eg, 'ch' in 'chair'). Many were also missing the later developing sounds of /ɹ/ (eg, 'r' in 'rabbit', 66.7%, 8/12), /θ/ (eg, voiceless 'th' in 'thin', 58.3%, 7/12) and /ð/ (eg, voiced 'th' in 'this', 58.3%, 7/12) (online supplemental table 4a,b). Other phonemes absent in some English speakers' inventories were /ʃ/ ('sh', 41.7%, 5/12), /ŋ/ ('ng', 41.7%, 5/12), /l/ (27.3%, 3/11) and /s/ (27.3%, 3/11). The phonemes /dʒ/ and /tʃ/ were not present in most German participants (4/6).

Average intelligibility for children, assessed via the ICS, ranged from 'never' understood (20%, 2/10), to 'rarely' (33.3%, 3/10) to 'sometimes' understood (50%, 5/10). For adults, average intelligibility ranged from 'sometimes' (16.7%, 1/6) to 'usually' understood (66.7%, 4/6). Only one participant was 'always' understood (ID 3b).

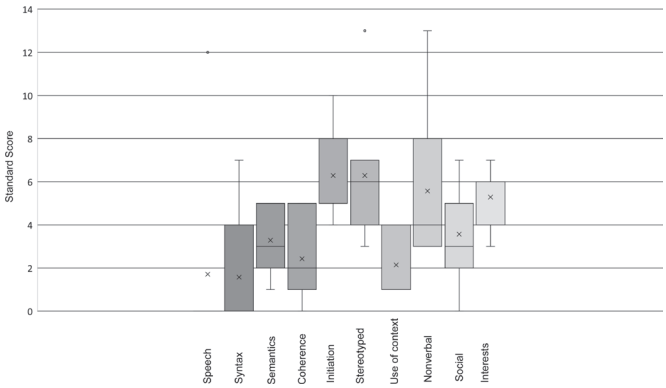

Figure 3 Children's Communication Checklist subdomains[61] in participants with pathogenic *FOXP2* variants (n=7, average=10, SD=3). Scores ≤6 are within normal limits, and scores <5 are low. Line=median, x=average, •=outlier.

Language and literacy

More than half of the cohort (56%, 14/25) had mild to severe receptive vocabulary impairment (table 3; online supplemental figure 2). Receptive grammar was commonly affected (72.2%, 13/18) ranging from moderate to severely impaired. Expressive vocabulary (83.3%, 15/18) and expressive grammar impairments were also common (86.7%, 13/15).

Speech was the most severely affected communication domain (mean score=1.7) for the Children's Communication Checklist, Second Edition (CCC-2) (completed for IDs 1a, 2, 3a, 5, 6, 15, 16b; figure 3, normative mean=10, SD=3).

Five participants were minimally verbal (<30 words; IDs 9, 12a, 13, 14, 15; between 2 and 18 years old). Three of the five used high-tech Augmentative and Alternative Communication (AAC) devices (eg, a speech-generating application on a tablet), while the remaining two used some sign language.

Spelling (17/24) and reading (17/24) impairments were common (table 3). This was reflected in the results from the VABS-3 (written subdomain mean=9.25, normative mean=15, SD=2). Four participants were at the preliteracy stage (<5 years of age).

Adaptive behaviour and cognition

Receptive and expressive language performance was low in those assessed (n=11, mean=9.5 in both domains; online supplemental figure 3) and not significantly different between the two domains (p=0.79). Language performance (mean=70.73) was significantly different from (p=0.01) and higher than socialisation (mean=61.18) and daily living (mean=57.45). Motor skills were an area of relative strength, although impaired compared with norms (mean=77.18), although the sample size was small and results should be interpreted with caution. Further, normative data for motor skills are only available up to 9 years 11 months; however, none of the older participants reached the ceiling on motor skills.

Language skills were incongruent with intellectual skills for many, as seven participants with severe language impairment scored within the average range on non-verbal subtests (table 3). IQ was formally assessed in 20 participants (FSIQ n=10, non-verbal IQ n=10). In the non-verbal testing, seven performed in the average range, one was borderline and two were moderately impaired. For an FSIQ, two performed within the average range, four in the borderline range (70–85 IQ) and four had a mild intellectual disability (50–69 IQ).

DISCUSSION

We systematically delineated the speech and cognitive phenotype in 28 probands from 17 unrelated families (10 of which are novel) with heterozygous pathogenic missense/loss-of-function variants disrupting *FOXP2*, and completed the first cross-linguistic analysis of this disorder. Our data confirm aberrant speech and language development as a central feature. While speech presentation improves over time, with a reduction in CAS severity and improvement in phonological production, the disorder is characterised by impaired speech intelligibility that persists into adulthood, with most adults in our cohort being understood only 'sometimes' or 'usually' (rather than 'always') understood.

In terms of intellectual ability, scores ranged from below average to average in our cohort. For individuals with FSIQ data available, most (8/10) were scored as having borderline or mild intellectual disability, while for those who had only non-verbal IQ data available, most (7/10) were average. This range and profile are in line with previous findings for individuals with pathogenic SNVs/indels in *FOXP2*, with most individuals falling in the low average range and below for FSIQ and non-verbal IQ. The critical point of note here is that FSIQ takes into account the language metric of vocabulary knowledge, hence why FSIQ is generally more impaired than non-verbal IQ skills for individuals with this speech and language phenotype. At the same time, we also confirm observations from prior literature that the profile of this disorder differs from classic intellectual disability syndromes, in that severe speech and/or language impairments can occur against a background of non-verbal cognition within the normal range[29] as observed for seven of our probands with available data.

ASD has previously been reported in only a small number of individuals carrying pathogenic SNVs/indels of *FOXP2* (n=2/46; online supplemental table 1). The findings in our cohort indicate that there may be a higher prevalence of ASD in this disorder (25.9% of our cohort) than in the general population (~1%–2%),[30] although further research is needed to account for the discrepancy between our current findings and the prior literature. Of note, pathogenic variants in the closely related orthologue *FOXP1* are known to substantially increase the risk for ASD.[31] Common non-coding polymorphisms in introns of *FOXP2* have shown associations with ADHD in large-scale genome-wide association studies, in the context of a multifactorial framework.[32] The current study clearly shows that, by contrast, high-penetrance SNVs/indels disrupting this same locus do not yield elevated susceptibility to ADHD, with a prevalence in our cohort (1/27=4%) that is similar to that in the general population.[33]

Sleep disturbances were common in our cohort and have been previously associated with idiopathic CAS[34] and other neurodevelopmental disorders, such as ASD, intellectual disability and ADHD.[35] The aetiology of sleep problems in such disorders is currently unknown, but they are posited to have biological and psychopathological causes. Although ASD, intellectual disability and ADHD were present here to varying degrees (as discussed above), within our cohort, sleep disturbance is also noted in children without those diagnoses.

We provide novel insights into other clinical diagnoses of mental health conditions that might be associated with pathogenic *FOXP2* variants. In particular, anxiety (19%) and depression (22%) had a higher prevalence than in the general population (between 2% and 4%)[33] and than in other neurodevelopmental disorders which were also present in our cohort, such as mild intellectual disability (~3%–4%).[36] Anxiety has previously been associated with idiopathic CAS,[37 38] and speech and language disorders are known to have possible negative impacts on mental health.[37–40] It is difficult to ascertain whether mental health disorders are part of the phenotypic spectrum due to pathogenic *FOXP2* variants, or occur as a secondary consequence of the communication deficits experienced by affected individuals, as is seen in other speech disorders, such as stuttering.[41] All participants with anxiety and depression were older than 16 years old, perhaps indicating that these mental health conditions arise later in life due to the impact of the communication impairment.

Gross motor impairment is thought to be relatively uncommon in individuals with pathogenic SNVs/indels disrupting *FOXP2*.[42] However, two-thirds of the assessed participants in this study indicated having difficulty with gross motor skills during development. *FOXP2* disruption therefore appears to impact brain circuits involved in fine as well as gross motor development. Gross motor skill learning deficits have been identified in knock-out animal models.[43]

We did not find convincing evidence of a dysmorphism phenotype in individuals with pathogenic *FOXP2* variants. Although physical features were noted for 81% of participants, most were minor and only shared among individuals from the same family. There was no consistent pattern of morphology seen across multiple unrelated probands in the cohort.

Regardless of the associated developmental features noted here, CAS was the most striking and consistent phenotypic characteristic in the present cohort. Dysarthria was far less common than CAS, clarifying the role of *FOXP2* in the planning and programming of movement sequences, as supported by animal models.[43] Adult participants typically had more intact, although incomplete, phonetic inventories than younger participants. In our study, more than half of all English-speaking participants with pathogenic missense/loss-of-function variants in *FOXP2* were missing one or more of the phonemes: /tʃ, dʒ, ɹ, θ, ð/ from their inventory. Three of these phonemes (/ɹ, θ, ð/) are in the 'late eight' sounds of English speech development, while affricates /tʃ, dʒ/ sit within the 'middle eight', referring to whether they are acquired earlier or later during typical phoneme acquisition.[44]

There may be a window for plasticity and acquisition of new phonemes. Children with speech sound disorders are more likely to have persistent speech error patterns if these are not resolved by 8½ years old.[44] We might speculate that *FOXP2* dysfunction has the greatest impact between 2 and 7 years old when most phonemes are acquired.[45 46] Intriguingly, neural expression of orthologues of *FOXP2* in model organisms has been shown to vary during different periods of vocal development,[47–49] for example being upregulated in parts of the brain of the male Zebra finch during a developmental window that is important for vocal learning.[50] Reduced expression of this gene in mice alters the development and continuing plasticity of neuronal networks,[51] impairs synaptic plasticity in striatal and cerebellar circuits and affects the learning of motor skills.[52 53] Perhaps, the lack of acquisition of 'late eight' sounds and affricates in children with *FOXP2* disruptions may relate to the closing of the relevant developmental window. Other theories to explain the lack of acquisition of these phonemes include reduced functional load and ambient frequency for these phonemes[54] or the motoric complexity of these sounds[55] which rely heavily on tongue coordination and movement, known to be impaired in children with CAS.[55–57] Further research is required to disentangle these relationships.

Speech impairment in the three participants without CAS was minimal or even absent, contrasting with unaffected, previously reported cases of *FOXP2* disruption.[13] The speech of participant 17 was also less impaired than that of other children, although he had a diagnosis of CAS. Participant 11a was not referred for genetic testing for his speech, but rather to determine whether the variant in his child was de novo or inherited. Of note, family 3 and participant 17 were the only participants who had not been referred for testing primarily on the basis of speech and learning impairments. Thus, it is possible that there is a broader range of speech phenotypes associated with pathogenic *FOXP2* variants, and that individuals with milder presentations are unlikely to be referred for genetic testing.

Although there was no statistically significant difference between receptive and expressive language as reported by caregivers, other standardised tests indicated receptive language was more intact than expressive language. Literacy skills were also low across the cohort, in line with the high rate of literacy challenges for individuals with idiopathic CAS.[58]

The speech domain on the CCC-2 confirmed that speech was the most severely impaired form of communication in children. AAC systems should be considered for children with pathogenic *FOXP2* variants due to the protracted speech development and severe speech impairment which persists for many throughout their lifetime.

We were unable to identify any clear phenotype–genotype correlation in the present cohort as we did not have sufficient power due to too few cases of missense and loss-of-function variants. The severity of speech and language disorder differed even among individuals with the same *FOXP2* variant in the same family. Family 3 had a relatively mild presentation compared with the other individuals in the study, despite having an intragenic deletion encompassing all *FOXP2* exons. Participant 17 had a translational start-site variant and a mild phenotype, with a larger phonemic repertoire and expressive vocabulary than other participants of similar ages in the cohort. This variant may not cause a clear-cut loss of function since there are alternative transcription start sites, potentially leading to a shorter protein.

In conclusion, CAS and language impairments are the most discernable features associated with heterozygous pathogenic missense/loss-of-function variants disrupting *FOXP2*. We also provide the evidence of additional neurodevelopmental features in subsets of our cohort, such as mild intellectual disability, ASD, anxiety, depression and sleep disturbances. There appear to be no distinctive physical features consistently associated with *FOXP2* disruptions. The phenotype associated with pathogenic variants that directly disrupt *FOXP2* remains relatively specific to speech disorder, compared with phenotypes associated with other monogenic conditions involving CAS.[58] Thus, our findings demonstrate that *FOXP2* provides an especially valuable entry point for examining the neurobiological bases of speech disorder.

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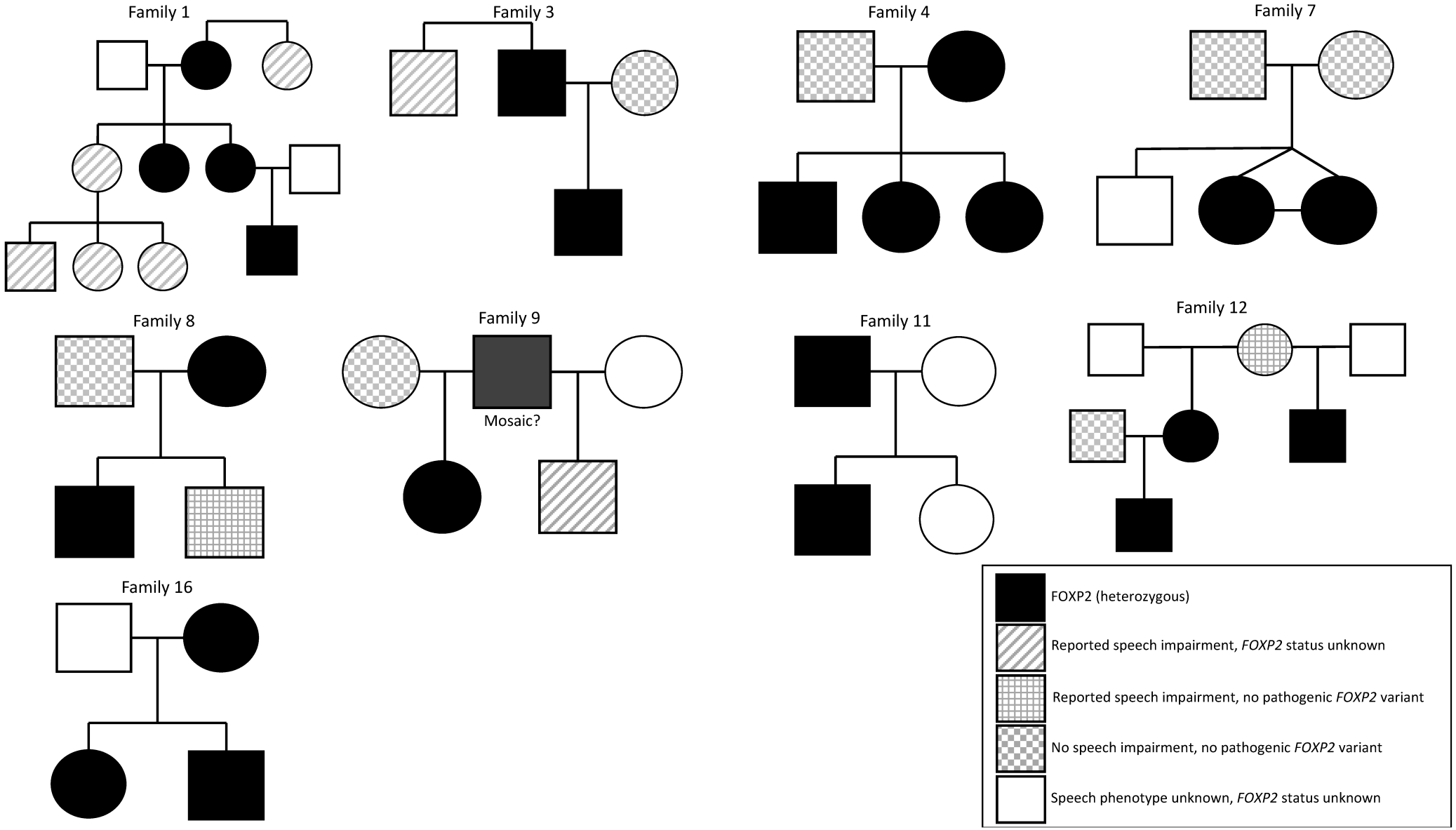
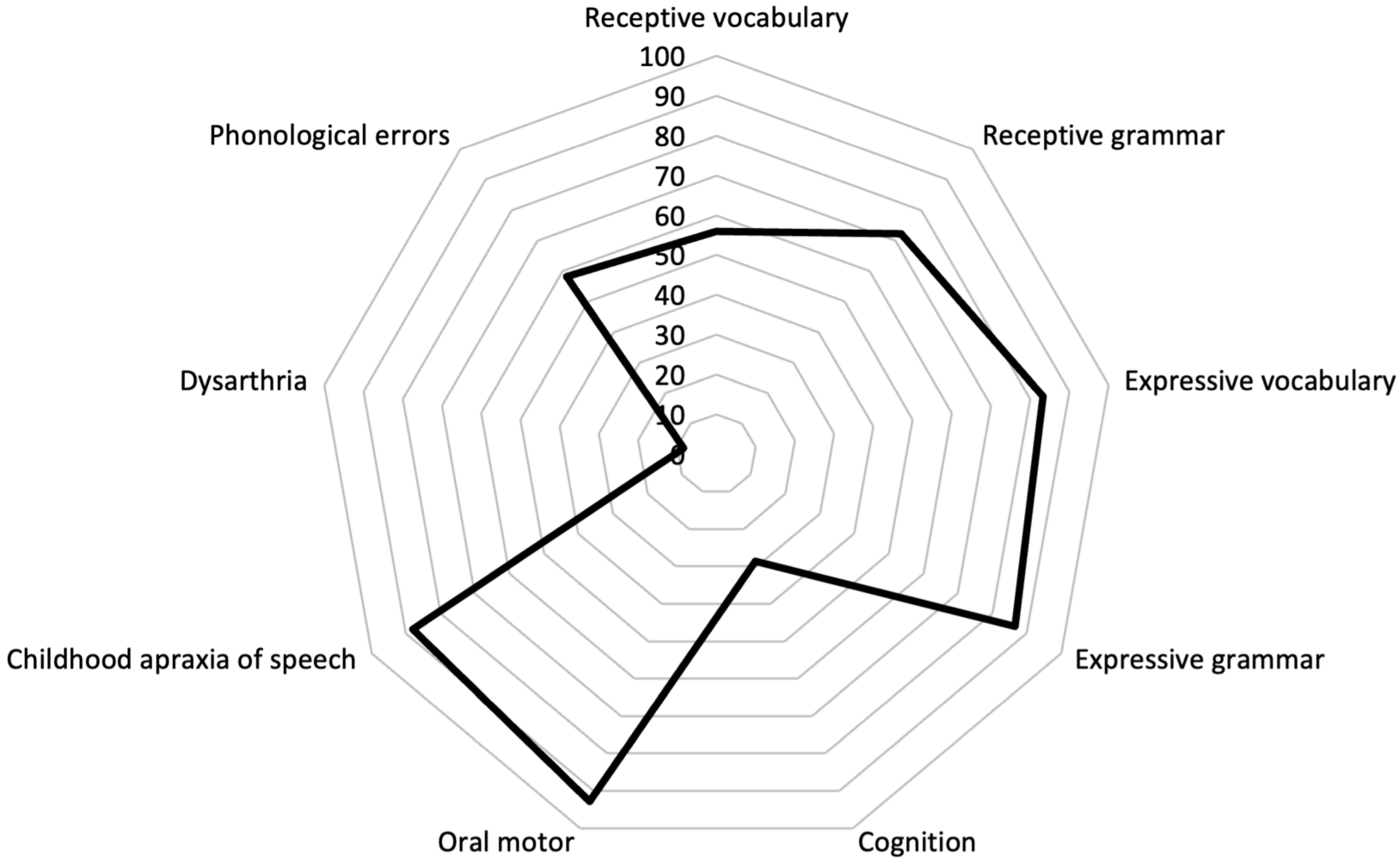
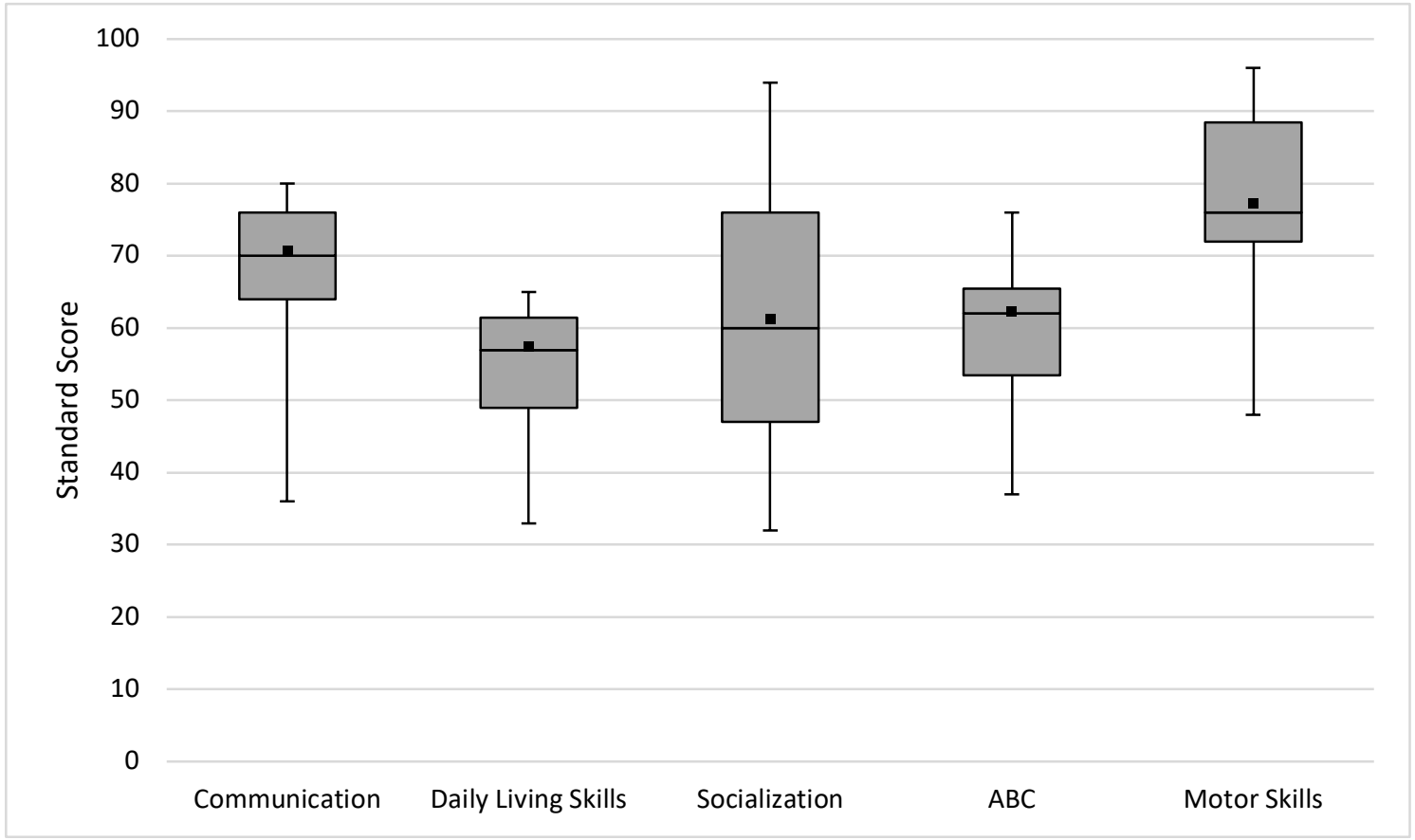


Supplementary Figure 1. Family pedigrees of inherited cases of pathogenic missense/loss-of-function variants disrupting *FOXP2*



Supplementary Figure 2. Phenotypic distribution of speech, language, and intellect in assessed participants with pathogenic *FOXP2* variants.
Cognition refers to general cognitive abilities measured by Full Scale Intelligence Quotient, and perceptual reasoning indices and matrix reasoning sub-tests. Data retrieved from Table 3.



Supplementary Figure 3 . Vineland Adaptive Behaviour Scales, Third Edition (42) domains for participants with pathogenic *FOXP2* variants (n=10). ABC = adaptive behaviour composite (overall score). Scores <70 are low, 71-85 moderately low and 86-114 adequate. Line = median, ■ = average.

Supplementary Table 1 Cases of *FOXP2*-related disorder previously published in the literature ordered reverse chronologically

Author	Variant	N	**Age** (year range)	Sex	Speech	Oromotor	Language	Cognition	Physical features	Motor	Other	FOXP2-only or plus
Nagy *et al* 2021	7.87 Mb interstitial deletion 7q31.1q31.31	4	15-17	F	CAS, delayed speech	NR	Receptive language below average, social difficulties	Intellectual disability	Scoliosis, dysmorphic features, strabismus	Early motor development normal, walked 15 months, uncoordinated, walked with bent knees	MRI NAD	Plus
			27-29	F	CAS	NR	Some social difficulties	NR	Dysmorphic features	NR	NR	Plus
			24-26	F	Dyspraxia	NR	No social difficulties	Borderline IQ (81)	No dysmorphic features	NR	NR	Plus
			48-50	F	CAS	NR	No social difficulties	NR	Dysmorphic features	NR	NR	Plus
Rieger *et al* 2020	4.7 Mb deletion arr[GRCh37] 7q31.2q31.31 (downstream of *FOXP2*)	3	9-11	M	Spoke in full sentences with impaired articulation, delayed speech development	NR	Below average language, delayed language development	Hyperactivity, attention difficulties	Mild dysmorphic features	Motor milestones typical	MRI NAD	Plus
			NR *de novo*	F	Dysarthria	NR					Attended special school	Plus

			3-5	F	Far below average for speech production, speech delay, imprecise articulation	NR	Normal comprehension of sentences, only 25-word vocabulary, age-appropriate development on first words		Mild dysmorphic features	NR		Plus
Argyropoulos *et al* 2019	missense (p.Arg553His) **KE FAMILY**	10			Poor performance in non-word repetition	Poor performance non-speech oromotor tasks					fMRI: pronounced volume reduction right hemispheric & total cerebellar Crus I	Only
Akahoshi & Yamaoto 2018	*de novo* intersitial deletion 7q31.1q31.3	1	27-29	F	NR	Hypotonia & poor sucking in infancy	NR	Binet–tanaka test: 53 (mild ID)	Horizontal line in her palm, high-arched palate, mandibular protrusion	Gross motor delayed	Sleep difficulties, schizophrenia	Plus
Reuter *et al*, 2017	*de novo* 14kb deletion 7q31.1	2	9-11	F	Short sentences with slurred articulation	NR	Reading & spelling below average	FSIQ 78 VCI 77, PRI 86, WMI 82, PSI 88	Mild dysmorphic features	NR	Delayed, EEG NAD	Only

			9-11	F	Short sentences with slurred articulation	NR	Reading & spelling below average	FSIQ 79 VCI 67, PRI 88, WMI 82, PSI 103	Mild dysmorphic features	NR	Delayed, EEG NAD	Only
Reuter *et al*, 2017	nonsense (p.Arg353*)	2	12-14	M	Dyspraxia, slurred articulation	NR	Severely impaired expressive & receptive	FSIQ 72 VCI 57, PRI, WMI, PSI 77-96	Mild dysmorphic features	NR	Delayed, EEG NAD	Only
			33-35	F	Dyspraxia, slurred articulation, stuttering	NR	NR	Learning difficulties (self-reported)	NR	NR		Only
Reuter *et al*, 2017	missense (p.Arg561Pro)	2	9-11	F	Single words only	NR	Severely impaired	FSIQ 70	Mild hypotonia, no dysmorphic features	Delayed	Autistic features, pneumonia	Only
			NR	M	Delayed speech development	NR	NR	NR	NR	NR	NR	Only
Reuter et al, 2017	nonsense (p.Arg503*)	1	6-8	F	Persistent speech impairment, delayed speech development	Choking in infancy	NR	Average IQ, poor auditory memory	Mild dysmorphic features	NR	Strabismus, hyperopia, astigmatism possible seizure at 10m	Only
Reuter et al, 2017	*de novo* nonsense (p.Arg589*)	1	9-11	M	Vocabulary of 2 words	NR	Impaired expressive & receptive	NR	Mild dysmorphic features	Impaired fine motor skills	Delayed, ASD, left exotropia	Only
Reuter *et al*, 2017	*de novo* frameshift (p.Phe563Leufs*28)	1	18-20	M	Stuttering, slurred articulation, stilted intonation, delayed	NR	NR	Good verbal comprehension (not formally tested)	Mild dysmorphic features, 6cm café au lait macule	NR	ASD	Only

					speech development							
Reuter *et al*, 2017	nonsense (p.Arg503*)	3	6-8	M	Vocabulary of 5 words, delayed speech development	NR	NR	NR	Mild dysmorphic features	NR	Hyperactivity, tantrums	Only
			3-5	F	Three-word sentences	NR	NR	NR	Mild dysmorphic features	Walking at 18m due to hip dysplasia		Only
			36-38	M	Simple sentences with slurred pronunciation	NR	NR	NR	Mild dysmorphic features	Delayed		Only
Reuter *et al*, 2017	missense (p.Pro530Leu)	2	6-8	M	Slurred articulation, unintelligible, delayed speech development	NR	Average vocabular, below average receptive grammar	FSIQ 72	NAD	NR		Only
			39-41	M	Articulation disorder, delayed speech development	NR	NR	Average	NR	NR	Strabismus, right exotropia MRI NAD,	
Turner *et al*, 2013	*de novo* frameshift p.Gln415Val*5	1	6-8	M	CAS & dysarthria	Oral motor dyspraxia, mild oral dysphagia	Severely impaired expressive & receptive severe reading & spelling impairment	FSIQ 73 VCI 71, PRI 94, WMI 77, PSI 70	Submucous cleft palate (repaired)	Motor apraxia	Delayed, strabismus, MRI & EEG NAD	Only

Zilina *et al*, 2012	8.3Mb deletion 7q31.1-q31.31	2	3-5	F	CAS	Difficulty chewing, swallowing, drooling	Poor vocabulary	Moderate developmental delay	Dysmorphic	NR	Delayed, autistic features, kidney & eye abnormalities	Plus
			27-29	F	CAS	Difficulty chewing, swallowing, drooling	NR	Low average FSIQ 88	Dysmorphic	NR	Delayed, poor social skills, eye abnormalities	Plus
Zilina *et al*, 2012	6.5Mb deletion 7q31.1-q31.2	2	6-8	F	CAS	NAD	Poor vocabulary	Moderate ID	Dysmorphic, mild ataxia	Motor delayed	Aggressive	Plus
			NR	F	CAS	NAD	NR	Apparent ID	NR	Motor delayed	Aggressive	Plus
Palka *et al*, 2012	*de novo* 14.8Mb mosaic deletion 7q31.1-q31.3	1	9-11	F	CAS	NR	Impaired expressive & receptive	Borderline FSIQ 71, PIQ, 88, VIQ 57	High arched palate, lordosis	Fine motor praxis & balance problems	Delayed	Plus
Rice *et al*, 2012	1.57Mb deletion 7q31.1-q31.2	2	3-5	M	Severe CAS, delayed speech development	Messy bottle feeder, gagging & drooling	Severely impaired expressive, average receptive	Borderline FSIQ 71, PIQ 75, VIQ 77	NAD	Fine & gross motor planning difficulty	NR	Plus
			24-26	F	CAS, dysarthria, delayed speech development	Gagging & drooling as infant, delayed swallow	Severely impaired	Low average FSIQ 89, PIQ 92, VIQ 87	Surgery for L esotropia	Motor NAD	PPD-NOS	Plus
Roll *et al*, 2010	missense (p.Met406Thr)	4	NR	2F 2M	NR	NR	Impaired (proband)	Cognitive impairment (proband)	NR	NR	Polymicrogyria (proband)	Only

Tomblin *et al*, 2009 Shriberg *et al*, 2006	balanced translocation t(7;13)(q31.1;q13.2) disrupting *FOXP2*	2	50-52	F	CAS & spastic dysarthria	No orofacial apraxia	Impaired expressive & receptive	Low average FSIQ 88, PIQ 95 VIQ 81	NAD	NR		Plus
			18-20	F	CAS & spastic dysarthria	No orofacial apraxia	Impaired expressive & receptive	Low average FSIQ 81, PIQ 8D, VIQ 81	NR	NR		Plus
Lennon *et al*, 2007	9.1Mb deletion 7q31.1–7q31.31	1	6-8	M	CAS	Drooling, low oral-motor tone	Severely impaired expressive & receptive	Moderate ID	Dysmorphic		Global delay	Plus
Zeesman *et al*, 2006	*de novo* 16Mb deletion 7q31.2-q32.2	1	3-5	F	Verbal dyspraxia	Oromotor dyspraxia	Severely impaired expressive & receptive	Below average-average	Dysmorphic		Delayed	Plus
Feuk *et al*, 2006	*de novo* deletion 7q31.1-q31.3 (15Mb Patient 1; 11Mb Patient 3); 7q31.2-q32 (13Mb Patient 2; 15Mb Patient 4); 7q22-q31.3 (15Mb Patient 5)	5		NR	CAS	Oromotor difficulties	Impaired expressive & receptive	Cognitive impairment	NR	NR	Delayed, patient 3: ASD	Plus
Feuk *et al*, 2006	translocation t(3;7)(q23;q31.2)	1	NR	NR	CAS	Oromotor difficulties	Impaired expressive & receptive	Cognitive impairment	NR	NR	Delayed	Plus

	disrupting *FOXP2*											
Feuk *et al*, 2006	*de novo* deletion 7q31.2-q32 (26Mb Patient 18; 14Mb Patient 20); 7q22-q31.33 (22Mb Patient 19); q31.1-q33 (30Mb Patient 21 & 22)	5	NR	NR	Severe dyspraxia	NR	Language delay	NR	NR	NR	Patient 18: ASD, global delay (patients 18, 21 & 22)	Plus
Feuk *et al*, 2006	maternal uniparental disomy of chromosome 7 that reduces *FOXP2* expression	7	NR	NR	CAS, delayed speech development	Oromotor dyspraxia	Impaired expressive, average receptive	NR	NR	NR	Silver-Russell Syndrome Patient 13: ASD	Plus
MacDermot *et al*, 2005	nonsense (p.Arg328*)	3	3-5	M	CAS	NR	Impaired expressive & receptive	NR	NR	NR	Delayed	Only
			0-2	F	Minimally verbal	Oropharyngeal dyspraxia	Impaired expressive & receptive	NR	NR	Motor dyspraxia	Delayed	Only
			NR	F	Poor clarity, delayed	NR	Simple grammar	Comprehension difficulties	NR	NR		Only

					speech development							
MacDermot *et al*, 2005	Heterozygous insertion of CAGCAGCAACAA into exon 5	1	NR	NR	CAS	NR	NR	NR	NR	NR		Only
Vargha-Khadem *et al*, 1995; Lai *et al*, 2001	missense (p.Arg553His) **KE FAMILY**	15	mean 24		CAS, delayed speech development	Orofacial dyspraxia	Impaired expressive & receptive, reading/spelling impairments	Borderline VIQ (mean 75), average PIQ (mean 86)	NAD	Motor NAD	Impaired performance on phonological loop working memory assessment (Schulze *et al* 2017)	Only
Lai *et al*, 2000	*de novo* balanced translocation t(5;7)(q22;q31.2) disrupting *FOXP2*	1	3-5	M	Verbal dyspraxia	Severe orofacial dyspraxia	Impaired expressive & receptive	Normal non-verbal skills	NAD	Delayed		Plus

ASD = autism spectrum disorder, CAS = childhood apraxia of speech, EEG = electroencephalogram, FSIQ = full scale intelligence quotient, ID = intellectual disability, m = months, MRI = magnetic resonance imaging, NAD = no abnormalities detected, NR = not reported, PIQ = performance intelligence quotient, VIQ = verbal intelligence quotient, y = years

Note: A further 15 cases with pure interstitial deletions of chromosome 7 are reviewed in Palka *et al* 2012, however speech and language data were not reported. Zhao *et al* 2016 published one case of a female child with a *de novo* 3.2 Mb sub microscopic deletion 7q31.2–7q31.31 (downstream of *FOXP2*) and Sangu *et al* 2017 reported one case of a male child with

a *de novo* de novo 1.9-Mb microdeletion in 7q31.33q32.1 (*FOXP2* is not within deletion region). Adgebola *et al* 2014 also report an individual with a deletion 3Mb away from *FOXP2*.

Supplementary Table 2 Speech apraxia features in this cohort with pathogenic missense/loss-of-function variants disrupting *FOXP2*

Speech apraxia features^	1a	1b	1c	2	3a	3b	4a	4b	4c	5	6	7a	7b	8b	10	11a	11b	12a[a]	13[a]	15[a]	17
(1) Inconsistent errors																					
Same C/V different across different words			+	+				+		+	+	+	+				+	NA	NA	+	+
Same word/syllable different on repetitions (percent)	+									+	+						+	NA	NA		+
Inconsistency of production*	68%	NA	NA	NA	NA	NA	NA	NA	NA	72%	44%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
(2) Lengthened & disrupted coarticulatory transitions																					
Speech motor behaviours, including groping during sound production		+	+				+	+	+	+										+	+
Difficulty sequencing phonemes & syllables	+	+	+					+	+	+	+							+	+	+	+
Voicing errors					+		+	+	+		+	+		+			+			+	+
Errors increase with word length & phonological complexity		+	+				+	+	+		+				+						+
Syllable segregation	+			+			+	+		+	+	+	+	+							+
Difficulty achieving initial articulatory configurations or transitory movement gestures		+	+				+		+	+		+		+	+				+		
Difficulty maintaining syllable integrity	+			+				+	+									+	+	+	+
Repetitions of sounds & syllables			+									+		+	+	+					
Epenthesis/intrusive schwa											+										+
Metathesis											+	+									
Addition errors																					
Frequent omissions (>10)	+						+	+	+		+	+	+	+			+	+	+	+	+
Prolongation errors	+			+			+		+												
Nonphonemic productions/distorted substitutions	+			+			+				+	+	+	+			+			+	+
Hypernasality/nasal emissions		+								+			+	+	+			NA	+	+	
Slowed & disrupted DDK sequence	+	+		+	+					+	+	+			+		+	NA	NA	NA	+
(3) Inappropriate prosody																					

Equal stress or lexical stress errors	+			+		+	+	+	+	+						+			+	+
Altered suprasegmentals	+			+		+	+	+	+	+	+	+		+						+
Prolongation errors	+			+		+		+												
Slow rate		+	+				+	+	+	+	+	+			+	NA	NA	NA		+

^ Rated perceptually using the criteria for rating Childhood Apraxia of Speech from the ASHA CAS Technical Report (2007), *Percent of single words said differently over 3 trials, using the DEAP inconsistency subtest (Dodd et al., 2002), + = feature present, NA = not assessed, [a] = Limited assessment of CAS features possible due to being minimally verbal.

Supplementary Table 3 Additional health and medical phenotypic features in individuals with pathogenic missense/loss-of-function variants disrupting *FOXP2*

Case	Vision impairment	Physical features	Mental health	MRI/CT	Feeding	Drooling	Other medical
1a	-	Upturned nose, mild retrognathia, mildly prominent ears, full lips	-	Incidental L anterior temporal arachnoid cyst	-	+	Tongue tie, Hx ear infections
1b	-	Prominent nose with fleshy nasal tip, mild retrognathia, mildly prominent ears, full lips	-	Hypoplasia of vermis	-	-	Tonsillectomy
1c	Glasses, myopia	Prominent nose with fleshy nasal tip, full lips	Anxiety, depression	Atrophic cerebellar vermis, mega cisterna magna, mild ventriculomegaly	-	-	-
1d	Glasses, hypermetropia	NA	Anxious behaviour	NA	-	-	Moderate, mixed hearing impairment
2	-	Mildly wide mouth, mildly short philtrum	Anxious behaviour	NA	+	+	-
3a	Glasses, squint	Mild periorbital fullness, rounded nasal tip, mild retrognathia, mildly thin upper lip	-	NA	-	-	Stage 2 chronic kidney disease, milk allergy
3b	Glasses, myopia	-	-	NA	-	-	-
4a	-	Synophyrus, mildly full lips, toe/toe nail abnormalities, large feet	OCD, anxiety, depression	NA	+ Hx	-	-
4b	-	Mildly full lips, mildly hypoplastic alar nasae, toe/toe nail abnormalities, large feet	OCD, anxiety, depression	NA	+ Hx	-	-
4c	-	Mildly hypoplastic alar nasae, toe/toe nail abnormalities	Anxiety, depression	NA	-	-	-

5	Strabismus	Mild periorbital fullness, rounded nasal tip	-	NA	+	-	Submucous cleft palate
6	-	Round face	-	MRI NAD	-	-	Eczema, hypotonia
7a	-	Epicanthic folds, mildly up slanting palpebral fissures, prominent incisors, sandal gaps	-	NA	-	NA	-
7b	-	Epicanthic folds, mildly up slanting palpebral fissures, prominent incisors, sandal gaps	-	NA	-	NA	-
8a	-	-	Depression	NA	-	NA	-
8b	-	High nasal root, a pointed nasal tip, prominent incisors, overbite, tapering fingers	-	NA	-	NA	Recurrent middle ear infections in infancy
9	-	-	-	NA	-	NA	-
10	NA	Narrow palpebral fissures, high arched palate, mild finger pads.	-	NA	+	NA	Possible seizure at 10mo
11a	Extropia, strabismus	-	NA	NA	NA	NA	NA
11b	NA	-	NA	NA	NA	NA	NA
12a	-	Flat face, upturned nose, small mouth, clinodactyly	-	NA	-	-	Hypotonia
12b	Glasses, myopia	Overbite, flat affect, protruding ears	-	NA	-	-	-
13	-	Full lips, mild periorbital fullness, slightly short palpebral fissures, mild retrognathia	-	NA	+	+	Laryngomalacia, tracheomalacia, laryngeal cleft
14	-	Mildly anteverted ears	-	CT NDA	+	-	-

15	-	-	-	CT NDA	+	-	Mild, bilateral, conductive hearing impairment, eczema
16a	Glasses, myopia	Overbite	Anxiety, depression	NA	-	-	Petite mal seizure unspecified x1 at 10yrs
16b	-	Round face, slightly prominent eyes	-	NA	+	+	Eczema, allergies, chronic constipation, seasonal asthma
17	-	Epicanthic folds, flat philtrum, single palmar crease right hand, microcephaly	-	MRI periventricular gliosis & right frontal migration abnormality	+	+	Eczema

+ = feature present, - = feature absent, CT = computed tomography, Hx = history (previous), mo = months, MRI = magnetic resonance imaging, NA = not applicable, NA = not assessed, NAD = no abnormalities detected, yrs = years

Supplementary Table 4a Phonemic inventory of English-speaking participants with pathogenic missense/loss-of-function variants disrupting *FOXP2*

	Case	**1a**	**1b**	**1c**	**2**	**3a**	**3b**	**4a**	**4b**	**4c**	**5**	**6**	**17**
	Age at assessment (years; months)	7;3	38;8	30;2	5;3	5;3	41;8	20;10	18;6	16;05	8	13;4	2;10
Expected age of acquisition	**Phonetic feature**												
3;0-3;5	Plosives (bilabial)	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g
	Nasals	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ
	Fricatives (Labiodental, Alveolar, Glottal, Velar, Uvular)	f, v, s, z, h	f, v, s, z, h	f, v, s, z, h	f, v, s, z, h	f, v, s, z, h	f, v, s, z, h	f, v, s#, z#, h	f, v, s, z, h	f, v, s, z, h	f, v, s, z, h	f, v, s, z, h	f, v, s, z, h
	Glides (bilabial, palatal) and liquid (alveolar)	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l	w, j, l
3;6-3;11	Affricate (postalveolar, voiceless)	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ	t͡ʃ
4;0-4;5	Fricative (palatal, voiced) Affricate (alveolar, voiced)	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ	ʒ d͡ʒ
5;0-5;5	Fricative (postalveolar, voiceless)	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ
6;0-6;5	Glide (alveolar)	ɹ	ɹ	ɹ	ɹ	ɹ	ɹ	ɹ	ɹ	ɹ	ɹ	ɹ	ɹ
7;0 and above	Fricatives (dental, voiceless and voiced)	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð	θ, ð

interdental lisp present. ● Sounds acquired, ● Sounds not yet acquired. Table normative data based on Dodd et al. (62), age at which 90% of n=684 British children acquired speech sounds.

Supplementary Table 4b Phonemic inventory of German-speaking participants with pathogenic missense/loss-of-function variants disrupting *FOXP2*

	Case Age at assessment (years; months)	**7a** 15;3	**7b** 15;3	**8b** 16;8	**10** 9;9	**11a** 41	**11b** 8;5
Expected age of acquisition	**Phonetic feature**						
3;0-3;5	Plosives (bilabial)	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g	p, b, t, d, k, g
	Nasals	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ	m, n, ŋ
	Fricatives (Labiodental, Alveolar, Glottal, Velar, Uvular)	f, v, s*, z*, h, x, ʁ	f, v, s*, z*, h, x, ʁ	f, v, s*#, z*, h, x, ʁ	f, v, s*, z*, h, x, ʁ	f, v, s*, z*, h, x, ʁ	f, v, s*, z*, h, x, ʁ
	Affricate	pf	pf	pf	pf	pf	pf
	Approximants (alveolar, palatal)	j, l	j, l	j, l	j, l	j, l	j, l
3;6-3;11	Affricate (alveolar, voiceless)	t͡s*	t͡s*	t͡s*	t͡s*	t͡s*	t͡s*
4;0-4;5	Fricative (palatal, voiceless)	ç	ç	ç	ç	ç	ç
4;6-4;11	Fricative (postalveolar, voiceless)	ʃ	ʃ	ʃ	ʃ	ʃ	ʃ
Other phonemes in German	Affricate (post-alveolar, voiceless) Fricative (post-alveolar, voiced) Affricate (post-alveolar, voiced)	t͡ʃ ʒ d͡ʒ	t͡ʃ ʒ d͡ʒ	t͡ʃ ʒ d͡ʒ	t͡ʃ ʒ d͡ʒ	t͡ʃ ʒ d͡ʒ	t͡ʃ ʒ d͡ʒ

[z] is not present in Southern German dialects (always substituted by [s]), therefore in parenthesis. # interdental lisp present. ● Sounds acquired, ●Sounds not yet acquired, ●Sounds absent in the speech sample. Table normative data based on Fox & Dodd (63), age at which sounds were acquired by 90% of n=177 German children. *[s], [z], [ts]: in Fox & Dodd (1999), 35% of children were not able to produce these sounds phonetically correct; however, as these sounds are mostly substituted by the interdental sounds [θ] and [ð], these substitutes are taken as allophones. Thus, phonemically, these sounds are rated as phonemically correct.