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Severe speech impairment is a distinguishing feature of FOXP1-related disorder

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### **Severe speech impairment is a distinguishing feature of *FOXPI*-related disorder**

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#### PUBLICATION DATA

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#### ABBREVIATION

VABS Vineland Adaptive Behaviour Scales

**AIM** To delineate the speech and language phenotype of a cohort of individuals with *FOXP1*-related disorder.

**METHOD** We administered a standardized test battery to examine speech and oral motor function, receptive and expressive language, non-verbal cognition, and adaptive behaviour. Clinical history and cognitive assessments were analysed together with speech and language findings.

**RESULTS** Twenty-nine patients (17 females, 12 males; mean age 9y 6mo; median age 8y [range 2y 7mo–33y]; SD 6y 5mo) with pathogenic *FOXP1* variants (14 truncating, three

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missense, three splice site, one in-frame deletion, eight cytogenic deletions; 28 out of 29 were de novo variants) were studied. All had atypical speech, with 21 being verbal and eight minimally verbal. All verbal patients had dysarthric and apraxic features, with phonological deficits in most (14 out of 16). Language scores were low overall. In the 21 individuals who carried truncating or splice site variants and small deletions, expressive abilities were relatively preserved compared with comprehension.

**INTERPRETATION** *FOXP1*-related disorder is characterized by a complex speech and language phenotype with prominent dysarthria, broader motor planning and programming deficits, and linguistic-based phonological errors. Diagnosis of the speech phenotype associated with *FOXP1*-related dysfunction will inform early targeted therapy.

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Speech in Individuals with *FOXP1*-Related Disorder

Ruth O Braden et al.

### What this paper adds

- Individuals with *FOXP1*-related disorder have a complex speech and language phenotype.
- Dysarthria, which impairs intelligibility, is the dominant feature of the speech profile.
- No participants were receiving speech therapy for dysarthria, but were good candidates for therapy
- Features of speech apraxia occur alongside persistent phonological errors.
- Language abilities are low overall; however, expressive language is a relative strength.

[main text]

Heterozygous pathogenic variants disrupting *FOXP1* are associated with neurodevelopmental disorders. Intellectual disability, autism spectrum disorder, dysmorphic features, and behavioural problems have been the primary focus of phenotypic reports.<sup>1–8</sup> Speech and language features are of particular interest given that rare disruptive variants in *FOXP2*, the closest paralogue of *FOXP1*, were the first identified molecular cause of childhood apraxia of

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speech<sup>9</sup> and that *FOXP1* and *FOXP2* are coexpressed in brain regions known to be critical for such traits.<sup>1,10</sup> Childhood apraxia of speech refers to a breakdown in motor planning and programming that affects the production, sequencing, timing, and stress of sounds and words in a person's speech.<sup>11,12</sup> Often cooccurring with childhood apraxia of speech is dysarthria, a speech disorder affecting the strength, control, and coordination of the orofacial musculature used to produce clear speech.<sup>13,14</sup> Although such speech problems have been noted in some individuals with aetiological *FOXP1* variants, the nature of these impairments has been based on clinical records, without detailed assessment by a speech pathologist.<sup>1-7</sup> Several studies have described a generalized articulation or speech impairment, although the nature of the specific speech production disorder is impossible to discern due to a lack of detail. Generalized oromotor dysfunction and tongue apraxia have been reported in some cases.<sup>1,4</sup>

Multiple reports have described problems with language development in children with *FOXP1* disorder.<sup>3,4,6,7</sup> Delayed language milestones are usually seen, with most recorded cases acquiring first words later than the typical 12 months of age, and subsequent delays in the development of short sentences.<sup>1-8</sup> A proportion of children are minimally verbal, while those with verbal speech have ongoing language impairments.<sup>1-8</sup> Language impairments appear to affect both the receptive and expressive domains; relative strengths in receptive over expressive abilities have been noted.<sup>4,5</sup> Despite this, exploration of language subdomains (e.g. semantics, morphology) is limited to clinical observations and small cohort sizes; further investigation using standardized assessments is required to better delineate the linguistic profile.<sup>1-8</sup>

Speech production has not been systematically evaluated in children with *FOXP1*-related disorder and the relative involvement of subdomains of language are unexplored. With the evolving accessibility of genetic testing, especially whole-exome sequencing in the clinic, pathogenic *FOXP1* variants are being more frequently identified. In this study, we performed in-depth examination of the speech and language phenotypes of 29 unrelated individuals with pathogenic variants of this gene, the vast majority of whom (28 out of 29) are new individuals who have not been reported previously, to inform early diagnosis and management approaches.

## METHOD

### Patients

Patients aged 2 years and older with a confirmed pathogenic *FOXP1* variant were identified in the Victorian Clinical Genetics Services database or referred by their clinician from December 2017 to May 2020. Additional patients were referred by their families after

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advertisement of online recruitment flyers in parent support groups on Facebook and the Simons Foundation Network (<https://www.simonsfoundation.org/>).

Variants were identified through clinical testing or as part of other research studies using next-generation sequencing (whole-genome sequencing, exome sequencing, or panel testing) or chromosome microarray (single-nucleotide polymorphism array or comparative genomic hybridization array).

Ethics approval was obtained from the Royal Children's Hospital Human Research Ethics Committee (no. HREC37353A). Written informed consent was obtained from the patient or their parents or legal guardian in the case of minors or adults with intellectual disability.

### **Measures**

Medical and developmental histories were obtained via parent interview and an online questionnaire.<sup>15</sup> Questionnaires were available in English, French, German, Portuguese, and Spanish. Medical reports (e.g. magnetic resonance imaging, electroencephalogram, speech pathology, and psychology assessments) were reviewed. Standardized assessments of speech, language, and adaptive behaviour were used to measure the communication abilities of all patients. Performance on each assessment was compared to normative data according to the respective test manual, as outlined in the next sections.

### **Speech**

Speech disorders refer to impairments in the perception and use of sounds for verbal communication. Speech was examined in patients with verbal language abilities for features of core paediatric speech conditions: articulation and phonological error patterns; dysarthria; and speech apraxia. Overall intelligibility and speech accuracy measures were used to describe functional speech production abilities. Speech was examined in the following ways.

#### ***Intelligibility and developmental speech sound production***

An overall measure of intelligibility (how easily a person is understood) was collected using the Intelligibility in Context Scale.<sup>16</sup> Articulation (motor production of sounds) and phonology (understanding the contrasts in sound that govern a language, e.g. 'cat' vs 'tat') were assessed in person or via telehealth using the Phonology subtest of the Diagnostic Evaluation of Articulation and Phonology.<sup>17</sup> Data were analysed for delayed and disordered articulation and phonological errors.<sup>17</sup> Overall speech accuracy was measured with percentage of consonants correct and classified by severity as: mild (>85% consonants correct); mild to moderate (65–84%); moderate to severe (50–64%); and severe (<50%).<sup>15,18</sup>

#### ***Dysarthria and speech apraxia***

Dysarthria was defined as a disorder of neuromuscular execution affecting one or more subdomains of respiration, phonation, articulation, resonance, or prosody. Speech samples

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were analysed for features of dysarthria, based on a 5-minute conversational speech sample, sustained vowel, single word, diadochokinetic, and reading task.<sup>14,15,19,20</sup> Features of apraxia were rated according to previous protocols.<sup>11,12,15</sup> Consistency of speech sound production was assessed using the Inconsistency subtest of the Diagnostic Evaluation of Articulation and Phonology,<sup>17</sup> where clinically indicated, since inconsistency of speech production is a core feature of apraxia but may also occur in inconsistent phonological disorders.<sup>17</sup>

### **Language**

Overall communication ability was measured using the Children's Communication Checklist-2,<sup>21</sup> a 70-item standardized parent questionnaire for verbal children aged from 4 to 16 years. In patients younger than 4 years of age with limited verbal abilities, the Communication and Symbolic Behaviour Scales Developmental Profile<sup>22</sup> was used to assess language. The Children's Communication Checklist-2 and Communication and Symbolic Behaviour Scales Developmental Profile scores were used to determine the presence and severity of language disorders. Age equivalence data for the Children's Communication Checklist-2 and Communication and Symbolic Behaviour Scales Developmental Profile were analysed to estimate the level of language delay in chronologically older children with linguistic abilities typical of younger age levels.

### **Cognition and adaptive behaviour**

Non-verbal intelligence was measured using the Wechsler Abbreviated Scale of Intelligence, Second Edition, Perceptual Reasoning Index if patients were able to attend the Murdoch Children's Research Institute in person.<sup>23</sup> Data from cognitive assessments completed within the previous year were obtained. The Vineland Adaptive Behaviour Scales (VABS), Third Edition, parent/caregiver form<sup>24</sup> provided standard scores for communication, socialization, activities of daily living abilities, and an overall composite. Scaled scores were calculated for the expressive, receptive, and written language subdomains and used as a measure of functional language ability.

Patients were grouped according to variant type: (1) truncating variants, splice site variants, and intragenic deletions predicted to cause loss of function ( $n=21$ ); (2) missense variants and in-frame deletions ( $n=4$ ); and (3) large deletions spanning multiple genes, including *FOXPI* ( $n=4$ ). A Wilcoxon signed-rank test was used to compare the severity of VABS, Third Edition receptive and expressive scaled scores in group A. Groups B and C were too small for robust statistical comparisons about receptive and expressive abilities to be drawn.

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## Oral motor structure and function

Oral motor structure and function were assessed using a systematic protocol.<sup>25</sup> Single movement and sequencing tasks were used to examine the precision and accuracy of speech- and non-speech-related movements of lingual, dental, mandibular, and facial structures.

## RESULTS

Our cohort comprised 32 patients; six were recruited from the Victorian Clinical Genetics Services database, two via clinician referral, and 24 from parent referral. Three males were excluded because pathogenicity could not be confirmed in one, one carried a variant of uncertain significance, and variant details were not available for the third male. Thus, 29 patients (17 females, 12 males) were included, with a mean age of 9 years 6 months (range 2y 7mo–33y, SD 6y 5mo). To our knowledge, only one patient (patient 24) in this study has been previously reported.<sup>5</sup> Patients were recruited internationally and spoke multiple languages including English, French, Portuguese, and Spanish. Health and medical surveys were translated to accommodate all linguistic backgrounds; published, standardized assessments were provided in each patient's preferred language, where available.

### *FOXPI* pathogenic variants

We describe 25 new *FOXPI* variants (Fig. 1). Previously reported variants included p.Arg514His<sup>2</sup> in patient 20, p.Arg514Cys<sup>1</sup> in patient 19, and arr[hg19]3p14.1(71 045 000–71 236 128)×1 in patient 24.<sup>5</sup> Patients 3 and 9 had the same new variant, p.Arg497\*. Pathogenic sequence variants included truncating variants (14 out of 29; 10 frameshift, four nonsense), missense variants (3 out of 29), splice site variants (3 out of 29), and an in-frame deletion (1 out of 29, encompassing 12 base pairs). All truncating variants were predicted to undergo nonsense-mediated decay. Of the eight patients with deletions detected on microarray, four had intragenic deletions encompassing multiple exons of *FOXPI* and four had larger deletions spanning additional neighbouring genes (range 4–20 loci). All but one variant occurred de novo (28 out of 29). Patient 22 had a paternally inherited variant; however, the father was unavailable for phenotypic analysis.

### Phenotypic features

Gross and fine motor delay affected all patients. Most (18 out of 21) had an intellectual disability, which was mild in 12, moderate in five, and severe in one. Overall adaptive behaviour scores were below the normal range for all (mean [SD] VABS Adaptive Behaviour Composite 65.6 [10.0], compared with average range 85–115) and were similar across communication domains (average 62.8 [16.4]), daily living skills (61.1 [13.7]), and socialization (65.8 [15.9]).

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Twelve out of 29 individuals (age range 4–19y) had an autism spectrum disorder diagnosis and a further eight had autistic features (e.g. restricted interests and/or repetitive behaviours; Fig. S1, online supporting information). Nine individuals had formal attention-deficit/hyperactivity disorder or attention deficit disorder diagnoses, with parents reporting attentional problems in a total of 15 patients (15 out of 29).

Vision problems were a common feature (23 out of 29); 18 out of 23 individuals wore glasses. Epilepsy occurred in 2 out of 29 patients; one patient had absence seizures (patient 7) and the other had epileptic encephalopathy with continuous spike–wave in sleep (patient 10).

A history of periodic conductive hearing loss secondary to ear infections was frequent (14 out of 29) and was resolved with antibiotics and tympanostomy tubes. Of note, 3 out of 29 participants had profound (>90dB hearing level) sensorineural hearing loss (patients 26, 27, and 28) but this only occurred in children with a large deletion encompassing *MITF*, which is known to cause sensorineural hearing loss.

Of the 25 individuals over the age of 3 years 6 months, all 25 had received support from occupational therapists or physiotherapists. Of the 18 school-age patients, five were attending a mainstream school with integration aide support, 12 a specialized educational setting, and one was home-schooled. Of the two adult patients, one lived semi-independently and worked part-time in a kitchen and one was living at home and not currently working. The *FOXP1* genotypes and phenotypes of the cohort in the present study are summarized in Table 1.

### Speech and language phenotype

All patients had received speech therapy. The first spoken words were delayed in almost all (27 out of 29) of the patients, with subsequent delays in putting two words together (Table 2). Of the 21 patients with verbal language abilities, five developed verbal communication before 3 years of age, 11 between 3 and 5 years, and five between 6 and 10 years. Of these patients, two used sign language or graphic communication systems to support their speech. The remaining eight patients had minimal verbal language. However, five of these were younger than 5 years of age and were receiving speech therapy, while the three older non-verbal individuals (aged 7–15y; patients 5, 7, and 29) used alternative communication systems (e.g. sign language and picture exchange systems).

### Speech

In the 21 verbal patients, speech was rated ‘sometimes to usually’ intelligible overall (Intelligibility in Context Scale average score 3.5 out of 5 [0.47]); while parents could usually understand their child’s speech (average 4.1 out of 5 [0.45]), intelligibility was low with unfamiliar listeners (average 3.0 out of 5 [0.69]). All 16 English-speaking patients with verbal language provided a speech sample for analysis (Table 3).

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### Speech disorder subtypes

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Features of dysarthria were present in all 16 assessed individuals. Resonance, articulation, and prosodic deficits were the most striking features (Table 3), with 12 out of 16 patients having moderate to marked deficits in at least two domains. Mild pitch, loudness, and voice deficits were also observed. Patients 10 and 22 had notable voice disorders that affected the intelligibility of their speech.

All verbal patients who provided a connected speech sample (14 out of 16) had features of speech apraxia (Table S1, online supporting information). Common features included inconsistent production of sounds (12 out of 14) or words (10 out of 14) across different trials, difficulty transitioning between sounds (13 out of 14), increased errors with increased word complexity (12 out of 14), frequent sound omissions (11 out of 14), distortions (11 out of 14), and prosodic errors (13 out of 14).

Articulation errors were found in 13 out of 16 patients who completed the Diagnostic Evaluation of Articulation and Phonology (e.g. lateral and interdental lisps, difficulty pronouncing ‘r’ and ‘th’ sounds). Fourteen out of 16 had phonological delays that included cluster reduction (11 out of 14), sound omissions (11 out of 14), gliding (9 out of 14), stopping (8 out of 14), voicing errors (8 out of 14), weak syllable deletion (7 out of 14), and fronting (6 out of 14). Eight out of 16 had disordered phonological errors (errors that are not part of typical speech development at any age), namely vowel errors (8 out of 8), with other unusual error patterns observed in smaller numbers (e.g. substituting the sound ‘l’ with ‘n’, or ‘k’ with ‘f’). The percentage of consonants correct was calculated for all 16 patients and scores ranged from severe impairment (3 out of 16) to moderate to severe (2 out of 16), mild to moderate (7 out of 16), and mild (4 out of 16).

### Language

In the 26 out of 29 patients who completed the VABS, overall language abilities were poor, with average receptive and expressive scaled scores in the low range (Table 2). When analysed by *FOXP1* variant type, patients in group A (truncating or splice site variants and intragenic deletions) had overall lower receptive and expressive language scores on the VABS (average receptive scaled score 8.2, expressive score 9.3) compared to those in group B (missense or in-frame deletion variants; average receptive score 12.00, expressive score 12.7). In group A, stronger expressive compared to receptive language abilities were seen ( $p=0.006$ ). For those in group B, receptive and expressive abilities were qualitatively more similar; however, since there were only four such patients, a robust statistical comparison was not possible. VABS scores were unavailable for three patients because the assessment was not published in their native language; however, language impairment was confirmed in these

patients based on the results from clinical speech pathology assessment. Similar language profiles to those seen on the VABS, Third Edition, were found in the 16 verbal patients who completed the Children's Communication Checklist-2. Average General Communication Composite scores were low for all individuals (average General Communication Composite 23.46 compared with the average range 85–115), with speech, syntax, coherence, and use of context scores relatively poor compared to other subdomains.

Broader social skills measured on the VABS were commensurate with receptive and expressive language abilities (Table 2). Qualitatively, most children were socially motivated and interested in playing with same-aged peers but had difficulty understanding social rules, such as recognizing when others may not be interested in their chosen game or maintaining and changing conversation topics.

Seven young patients aged 2 to 4 years completed the Communication and Symbolic Behaviour Scales Developmental Profile. Use of basic gestures, joint attention, and play was developing in all seven. All were beginning to produce early sounds (e.g. 'm' for 'mum' or 'b' for 'baba') but most had restricted sound inventories for their age. Most spoke a handful of words although these were often approximations and difficult for unfamiliar adults to understand.

### **Oral motor structure and function**

Nineteen out of 29 patients completed an oral assessment (denominators vary because some individuals completed only a brief battery). Oral motor assessments were unavailable for 10 patients due to language or cognitive barriers preventing assessment by the speech pathologist where many oral motor tasks require two- or three-step commands (e.g. 'Show me how you bite and blow'). Irregular dentition was the most frequent oral structural impairment (9 out of 16), mostly characterized by large gaps between the front teeth (6 out of 16). Four patients had malocclusion of the jaw, although it was challenging to assess severity on video assessments (<https://www.geneticsofspeech.org.au/example-of-the-speech-profile-of-a-child-with-foxp1-disorder/>); 10 out of 29 had frequent dental carries.

In terms of oral motor movement and function, the following abnormalities were seen: limited movement of cheeks and upper lip during speech (9 out of 19); lip (2 out of 19) and jaw asymmetry (3 out of 19); jaw slide during speech tasks (8 out of 19); and open mouth posture at rest (3 out of 19). Tongue protrusion and lateral movements were limited in 10 out of 19, although accuracy improved with prompting and modelling. Excessive drooling (9 out of 29) persisted throughout the early school years and resolved by later childhood. Both speech and oral motor sequencing tasks (e.g. speech task 'oo ee', oral motor task 'Blow then smile') were effortful and uncoordinated in 6 out of 8 patients who completed these.

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## DISCUSSION

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We delineated the speech, language, and oral motor phenotype associated with heterozygous pathogenic disruptions of *FOXP1*. Most patients had a complex speech production disorder characterized by dysarthric, apraxic, phonological, and articulation errors. In addition, language was impaired and cognitive abilities varied from average abilities to profound intellectual disability. We showed that the speech and language phenotype is a core distinguishing feature of *FOXP1*-related disorder, extending our understanding of the accepted neurodevelopmental phenotype comprising intellectual disability, behavioural problems, and features of autism spectrum disorder.

Most patients developed verbal skills, despite delays in early speech milestones. However, intelligibility was poor even in older patients, although most improved with age and intensive speech therapy. This poor intelligibility was predominantly due to dysarthria combined with some speech praxis deficits in verbal patients. Dysarthric and apraxic features denote perturbed motor speech control across programming, planning, and execution domains. While apraxic features were evident, the speech phenotype associated with *FOXP1*-related disorder is different and multifaceted compared to the dominant profile of severe speech apraxia that is characteristically seen in *FOXP2*-related disorder. Moreover, individuals with pathogenic *FOXP2* variants have language and social impairments that are less severe and occur in the context of more preserved non-verbal IQ.<sup>26,27</sup>

In addition to impairments in motor programming, planning, and execution, the high frequency of phonological errors seen signals linguistic, not just motor, involvement and further highlights the phenotypic complexity of *FOXP1*-related disorder. It is important that speech problems are not dismissed simply as a concomitant feature to broader neurodevelopmental deficits and that patients are instead referred for targeted speech therapy, which improves outcomes for each of these diagnoses.<sup>28-30</sup>

Language disorder was almost universal in our cohort and correlated with the type of pathogenic variant to some extent. In particular, patients with loss-of-function variants had more severely affected language profiles compared to those with missense/in-frame variants. Within the group of patients with loss-of-function variants, expressive language was considerably stronger than receptive language, in contrast to patients with missense variants who had similar receptive and expressive abilities. Our findings contradict previous work that reported a relative strength in receptive compared to expressive language in individuals with pathogenic *FOXP1* variants.<sup>4,5</sup> One explanation for this is that we used a different prospective standardized assessment to the tools used in past studies, which allowed us to make direct

comparisons between expressive and receptive abilities. Other studies had small sample sizes or were based on clinical observation rather than standardized assessment. Given the severe speech impairments we observed, these studies may have underestimated expressive language abilities. Due to our cohort size, we also had sufficient power to compare language ability within the loss-of-function group, which has not been previously reported. However, it is important to note that our group sizes were relatively small; further studies of larger cohorts would allow greater understanding and higher-powered statistical comparisons to be drawn between speech and language abilities in individuals with other variant types (e.g. missense or in-frame deletions). Natural history studies would enable greater understanding of speech and language trajectories. Regarding specific linguistic subdomains, vocabulary and semantic abilities were a relative strength over syntax in our cohort. Many patients had difficulty providing context and linking ideas together in conversation, which likely contributed to their social difficulties.

Of the eight minimally verbal patients, five were children aged under 5 years who were developing early speech sounds, words, and gestures, while the three older patients used graphic communication systems and sign language. Patient 29 had the largest deletion in our cohort, which likely explained her more severe phenotype, compared with the other two older minimally verbal individuals, who had truncating variants. Exploration of the gestural and graphic communication abilities of minimally verbal patients with *FOXP1*-related disorder would be a helpful direction for future research, to better understand underlying communication abilities and whether these reflect the disordered speech characteristics observed in the verbal patients in this study.

In addition to comprehensive speech and language phenotyping, our results confirmed the frequency of neurodevelopmental features in patients with *FOXP1*-related disorder, including intellectual disability, autistic features, and behavioural and attention problems. A limitation of this study was that we were unable to obtain cognitive assessment scores for all patients using the same assessment tool. This was unavoidable in most instances due to geographical constraints; however, this is an important consideration for future research. While it was not possible to make statistical comparisons between language and cognitive profiles in this study, all but one of the patients with moderate or severe intellectual disability had a relatively concordant language profile, with receptive and expressive scores in the low range. This contrasted with patients with mild intellectual disability, who had less severe language difficulties, including some with receptive and expressive scores in the average range (e.g. patients 17 and 25). Patient 22 is of particular interest because they had cognitive abilities in the average range despite severe speech, language, and behavioural difficulties. This patient

has an intragenic deletion (arr[hg19] 3p13(71,145,830-71,523,110)×1) that is predicted to remove exons 4 to 7 of *FOXP1*, yet this deletion may still allow the production of a shorter *FOXP1* isoform (NP\_001336271.1) that retains key functional domains, including the zinc finger, leucine zipper, and forkhead box DNA binding domains. In vitro studies of this shorter isoform in human B cells showed it to be functionally equivalent to the wild-type allele in terms of transcriptional regulation, despite lacking the N-terminal 100 amino acids of the full-length FOXP1 protein.<sup>31</sup> We postulate that the presence of the shorter isoform explains the milder phenotype in patient 22, whose deletion was inherited from a father who was not available for phenotypic assessment.

In conclusion, we have shown that individuals with heterozygous pathogenic variants of *FOXP1* have a complex set of speech deficits that occur in the context of a broader neurodevelopmental disorder. Dysarthria is the dominant feature of the profile, although motor speech deficits occur alongside persistent phonological errors, which have a severe, long-lasting impact on intelligibility. Language is more severely impaired in patients with truncating variants who have a relative strength in expressive over receptive abilities. Overall, our findings demonstrate that speech and language impairments represent a core and distinctive feature of the phenotype associated with the *FOXP1*-related disorder.

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served on scientific advisory boards for UCB, Eisai, GlaxoSmithKline, BioMarin, Nutricia, RogCon, Xenon Pharmaceuticals, Chiesi, and Encoded Therapeutics. She has received speaker honoraria from GlaxoSmithKline, UCB, BioMarin, Biocodex, and Eisai. She has received funding for travel from UCB, Biocodex, GlaxoSmithKline, BioMarin, and Eisai. She has served as an investigator for Zogenix, Zynerva, Ultragenyx, GW Pharmaceuticals, UCB, Eisai, Epygenyx, Anavex Life Sciences, Ovid Therapeutics, Encoded Therapeutics, and Marinus. She has consulted for Zynerva, Atheneum Partners, Ovid Therapeutics, Epilepsy Consortium, Care Beyond Diagnosis, and UCB. She receives/has received research support from the NHMRC of Australia, Health Research Council of New Zealand, CURE, Australian Epilepsy Research Fund, Medical Research Future Fund, and National Institutes of Health/ National Institute of Neurological Disorders and Stroke.

#### Supporting information

Commented [SC13]: Typesetter: B head

The following additional material may be found online:

**Figure S1:** Comparison of cohort characteristics

**Table S1:** Features of speech apraxia in our cohort

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**Table 1:** *FOXP1* genotypes and phenotypes in the cohort of the present study

Sex	Age	Visual impairment	Cognitive impairment	FSIQ or PIQ score	Behavioural difficulties	Neurodevelopmental and medical diagnoses	Variant (NM_001244814.3 transcript)	
<i>Patients with truncating (nonsense or frameshift) variants</i>								
1	F	3y 6mo	-	Mild	FSIQ=66	+	SPD	c.532C>T p.(Gln178*)
2	M	4y	+	Too young, GDD		-	-	c.1354dup p.(Ile452Asnfs*9)
3	M	5y	+	Too young, GDD		+	ASD	c.1489C>T p.(Arg497*)
4	F	7y	-	Moderate <sup>a</sup>	NC	+	ASD, ADHD, SPD	c.1241delT p.(Leu414Argfs*60)
5	M	7y	+	Moderate <sup>a</sup>		+	ASD, SPD	c.1420_1427del p.(Ile474Glyfs*14)
6	F	8y	+	Moderate <sup>a</sup>		+	ASD, SPD	c.1458_1461dupAACA p.(Leu488Asnfs*4)
7	F	9y	+	Moderate		+	ASD, Absence seizures	c.1103dup p.(His368Glnfs*93)
8	F	10y	+	Mild <sup>a</sup>		+	ASD, ADHD, SPD, DCD	c.987_990del p.(Glu330Metfs*21)
9	F	10y	+	Mild <sup>a</sup>		-	-	c.1489C>T p.(Arg497*)
10	M	10y	+	Mild	PIQ=67	+	ADHD, EECSWS	c.1240_1241delCT p.(Leu414Aspfs*46)
11	F	13y	+	Mild <sup>a</sup>		+	SPD	c.1333_1335delinsAA p.(Val445Asnfs*29)
12	F	14y	+	Borderline	FSIQ=78	-	-	c.1141C>T p.(Gln381*)
13	M	16y	+	Moderate <sup>a</sup>		+	ASD, ADHD	c.945_946insT p.(Val316Cysfs*15)
14	M	19y	+	Mild	PIQ=54	-	ASD, ADHD	c.606del p.(Gly203Alafs*3)
<i>Patients with splice site variants</i>								
15	M	3y 3mo	+	Too young, GDD		+	-	c.664G>T p.(Gly222Cys) (loss of donor splice site)
16	M	7y	-	- <sup>a</sup>		+	ASD, ADHD, pectus carinatum	c.1146+1G>A
17	M	33y	+	Mild <sup>a</sup>		+	-	c.1889+5G>T
<i>Patients with missense variants or in-frame deletions</i>								
18	F	2y 7mo	-	Too young, GDD		-	-	c.1490G>C p.(Arg497Pro)
19	F	3y 9mo	+	Too young, GDD		-	-	c.1540C>T p.(Arg514Cys)
20	F	4y	+	Too young, GDD		+	ASD	c.1541G>A p.(Arg514His)
21	F	12y	+	Mild		+	ADHD, SPD	c.1590_1601del p.(Gly531_Trp534del)
<i>Patients with intragenic deletions</i>								

22 <sup>b</sup>	M	5y	-	Average	PIQ=108	+	ASD, SPD, pectus carinatum	arr[hg19] 3p13(71,145,830-71,523,110)×1 pat
23	F	8y	-	Mild	PIQ=64	+	-	arr[hg19] 3p13(71,019,900-71,096,114)×1
24 <sup>c</sup>	M	11y	-	Mild	PIQ=66	+	ASD, ADD, SPD	arr[hg19] 3p14.1(71,045,000-71,236,128)×1
25	F	15y	+	Mild	PIQ=53	-	ADHD	arr[hg19] 3p14.1(70,435,706_71,256,962)×1
<i>Patients with large deletions</i>								
25	M	2y 0mo	+	Too young, GDD		-	DCD, SNHL	arr[hg19] 3p14.1(69413875_71194154)×1
27	F	3y 3mo	+	Too young, GDD		-	SNHL	arr[hg19] 3p14.1p13(67,662,260-73,418,054)x1
28	F	6y	+	Mild	PIQ=67	NR	SNHL	arr[hg19] 3p14.1(64,391,455-71,527,617)×1
29	F	15y	+	Severe	NC	-	-	arr[hg19] 3p14.1-p11.2(71,045,000-87,604,635)x1

<sup>a</sup>Standardized cognitive assessment data were not available; severity of intellectual disability was based on medical reports, school placement information, and parent and clinician impression. <sup>b</sup>All variants occurred de novo except for patient 22, who had a paternally inherited variant. <sup>c</sup>This patient was previously reported in Le Fevre et al.<sup>5</sup> -, feature not observed. FSIQ, full-scale IQ; PIQ, performance IQ; SPD, sensory processing disorder; GDD, global developmental delay; ASD, autism spectrum disorder; NC, assessment score could not be calculated; ADHD, attention-deficit/hyperactivity disorder; DCD, developmental coordination disorder; EECSSWS, epileptic encephalopathy with continuous spike-wave in sleep; NR, feature not reported; SNHL, sensorineural hearing loss.

**Table 2:** Language, literacy, and social skill abilities of our cohort

	Communication milestones (age when achieved)		Primary mode communication	Language ability <sup>a</sup>			Social skills <sup>a</sup> (average=64.56)
	Spoken words	Short sentences		Expressive (average=8.89)	Receptive (average=7.89)	Written (average=7.24)	
<i>Patients with truncating (nonsense or frameshift) variants</i>							
1	NYA	Minimally verbal <sup>b</sup>	Gestural/sign	Low	Low	Moderate low	Low
2	NYA	Minimally verbal <sup>b</sup>	Gestural/sign	Low	Low	Low	Low
3	>18mo	2-3y	Verbal	Low	Low	Moderate low	Low
4	12-15mo	4-5y	Verbal	Low	Low	Low	Low
5	NYA	Minimally verbal <sup>b</sup>	Graphic	c	c	c	c
6	>18mo	4-5y	Verbal	Low	Low	Low	Low
7	NYA	Minimally verbal <sup>b</sup>	Gestural/sign, graphic	Low	Low	Low	Low
8	<12mo	2-3y	Verbal	c	c	c	c
9	>18mo	4-5y	Verbal	Moderate low	Low	Low	Low

10	<12mo	≥8y	Verbal	Low	Low	Low	Low
11	12–15mo	2–3y	Verbal	Moderate low	Moderate low	Low	Moderate low
12	>18mo	4–5y	Verbal	Moderate low	Moderate low	Moderate low	Low
13	12–15mo	≥8y	Verbal	Moderate low	Low	Low	Low
14	12–15mo	4–5y	Verbal	Moderate low	Low	Low	Low
<i>Patients with splice site variants</i>							
15	>18mo	Minimally verbal <sup>b</sup>	Gestural/sign	Low	Low	Adequate	Moderate low
16	15–18mo	4–5y	Verbal	Moderate low	Adequate	Low	Moderate low
17	>18mo	4–5y	Verbal	Adequate	Adequate	Moderate low	Adequate
<i>Patients with missense variants or in-frame deletion</i>							
18	12–15mo	2–3y	Verbal	Moderate low	Adequate	NA	Moderate low
19	12–15mo	2–3y	Verbal	Adequate	Adequate	Adequate	Moderate low
20	15–18mo	4–5y	Verbal, graphic	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>
21	>18mo	4–5y	Verbal	Moderate low	Low	Low	Low
<i>Patients with intragenic deletions</i>							
22	15–18mo	4–5y	Verbal	Moderate low	Low	Adequate	Moderate low
23	>18mo	≥8y	Verbal	Moderate low	Low	Low	Moderate low
24	>18mo	6–7y	Verbal	Moderate low	Low	Low	Low
25	12–15mo	6–7y	Verbal	Adequate	Adequate	Low	Moderate low
<i>Patients with large deletions</i>							
26	NYA	Minimally verbal <sup>b</sup>	Gestural/sign	Low	Low	NA	Low
27	NYA	Minimally verbal <sup>b</sup>	Verbal, gestural/sign	Low	Low	Moderate low	Low
28	12–15mo	4–5y	Verbal, gestural/sign	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>
29	NYA	Minimally verbal <sup>b</sup>	Graphic	Low	Low	Low	Low

<sup>a</sup>Language ability according to scores on the Vineland Adaptive Behaviour Scales, Third Edition,<sup>24</sup> where scores <9 are in the low range, 10–12 moderately low, and 13–17 adequate. <sup>b</sup>This patient was yet to achieve this milestone and was classified as having minimal verbal language. <sup>c</sup>Vineland Adaptive Behaviour Scales scores were not available; however, impairment was confirmed based on the results from the clinical speech pathology assessment. NYA, not yet achieved; NA, not applicable.

**Table 3:** Speech characteristics of the verbal patients in our study cohort<sup>a</sup>

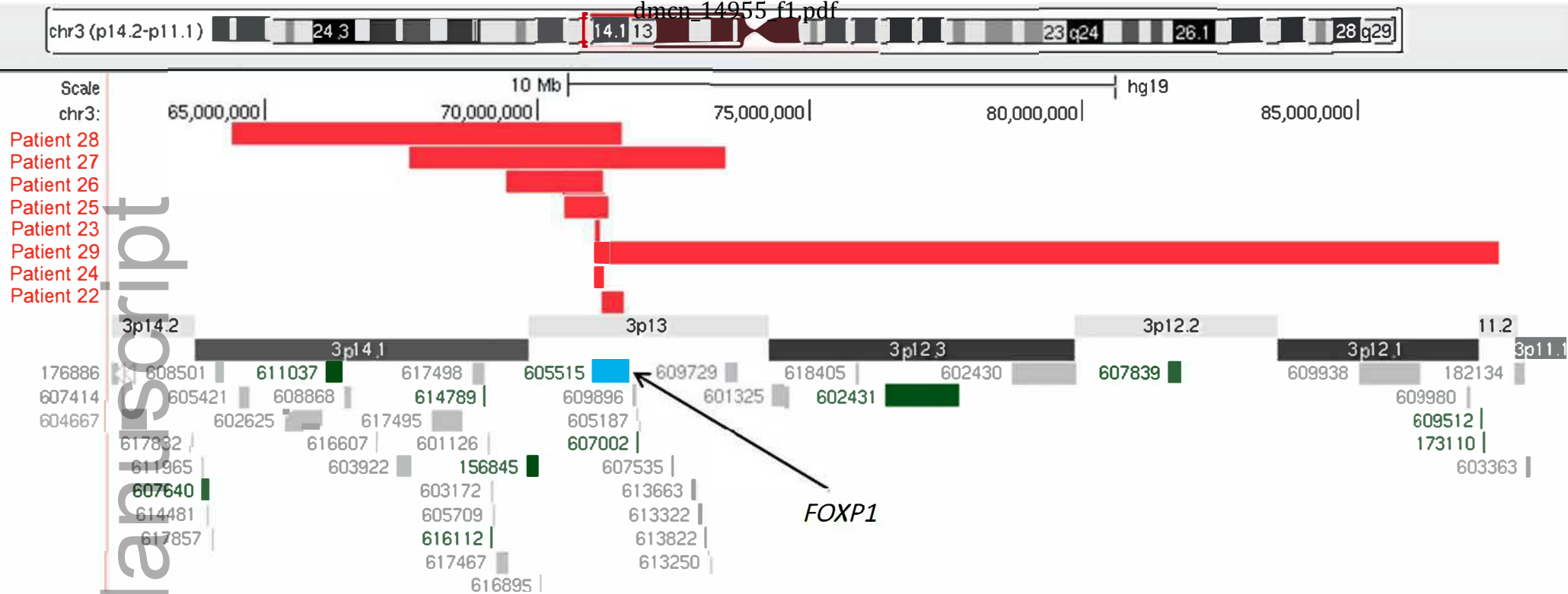
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	Articulation errors	Phonological errors	Percentage consonants correct severity	Dysarthric features <sup>b</sup>				Speech apraxia features
				Articulation	Prosody	Resonance	Voice	
<i>Patients with truncating (nonsense or frameshift) variants</i>								
4	+	+	Mild to moderate	Marked	Moderate	Mild	Mild	+
6	+	+	Moderate to severe	Marked	Mild	Mild	Moderate	Data not available
9	+	+	Mild to moderate	Moderate	Mild	Mild	Mild	+
10	-	+	Severe	Marked	Marked	Moderate	Marked	+
11	-	+	Mild	Moderate	Moderate	Mild	Mild	+
13	+	+	Mild to moderate	Moderate	Moderate	Moderate	Mild	+
14	+	+	Mild to moderate	Marked	Moderate	Moderate	Mild	+
<i>Patients with splice site variants</i>								
17	+	+	Mild	Moderate	Moderate	Moderate	Mild	+
<i>Patients with missense variants or in-frame deletions</i>								
18	+	+	Mild to moderate	Moderate	Mild	Mild	Moderate	Data not available
19	+	+	Mild to moderate	Moderate	Mild	Normal	Normal	+
21	+	-	Mild	Marked	Mild	Marked	Mild	+
<i>Patients with intragenic deletions</i>								
22	+	+	Severe	Moderate	Moderate	Mild	Marked	+
23	+	+	Moderate to severe	Marked	Moderate	Moderate	Mild	+
24	+	+	Mild to moderate	Marked	Moderate	Moderate	Mild	+
25	-	+	Mild	Moderate	Moderate	Moderate	Mild	+
<i>Patients with large deletions</i>								
28	+	+	Severe	Marked	Marked	Moderate	Mild	+

<sup>a</sup>Data displayed are for the 16 English-speaking patients who provided a speech sample for analysis. <sup>b</sup>Severity rated according to Duffy.<sup>19</sup> -, feature absent.

**Figure legend**

**Figure 1:** *FOXP1* variants in our cohort. (a) University of California Santa Cruz browser view (GRCh37/hg19) showing the locations of *FOXP1* heterozygous deletions detected by microarray in relation to cytogenetic location and OMIM (Online Mendelian Inheritance in Man) genes including *FOXP1*. (b) Decipher browser view showing locations of truncating (nonsense and frameshift), missense, splice site, and in-frame deletion variants in relation to the FOXP1 protein.

**(a)****(b)**