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SHORT REPORT

# A highly penetrant form of childhood apraxia of speech due to deletion of 16p11.2

**This article has been corrected since Advance Online Publication and a corrigendum is also printed in this issue**

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**Individuals with heterozygous 16p11.2 deletions reportedly suffer from a variety of difficulties with speech and language. Indeed, recent copy-number variant screens of children with childhood apraxia of speech (CAS), a specific and rare motor speech disorder, have identified three unrelated individuals with 16p11.2 deletions. However, the nature and prevalence of speech and language disorders in general, and CAS in particular, is unknown for individuals with 16p11.2 deletions. Here we took a genotype-first approach, conducting detailed and systematic characterization of speech abilities in a group of 11 unrelated children ascertained on the basis of 16p11.2 deletions. To obtain the most precise and replicable phenotyping, we included tasks that are highly diagnostic for CAS, and we tested children under the age of 18 years, an age group where CAS has been best characterized. Two individuals were largely nonverbal, preventing detailed speech analysis, whereas the remaining nine met the standard accepted diagnostic criteria for CAS. These results link 16p11.2 deletions to a highly penetrant form of CAS. Our findings underline the need for further precise characterization of speech and language profiles in larger groups of affected individuals, which will also enhance our understanding of how genetic pathways contribute to human communication disorders.**
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## INTRODUCTION

Deletions of a ~600-kb region in human chromosomal band 16p11.2 occur in ~1/2000 individuals,[1,2] and have been associated with a number of neurodevelopmental disorders, including autism.[3] Individuals with heterozygous 16p11.2 deletions have increased head circumference,[1,4] high rates of obesity,[3,5] and seizures.[6] Cognitively, these individuals have a reduced intelligence quotient (IQ), approximately two SDs below that of familial noncarriers.[6] Furthermore, affected individuals have a variety of speech and/or language difficulties.[6,7] However, the precise nature of these difficulties remains unknown. Here, we focus on the speech domain, testing whether 16p11.2 deletions confer a risk of developing a specific speech disorder: Childhood Apraxia of Speech (CAS).[8]

CAS is a rare motor speech disorder that affects the production, sequencing and timing of sounds, syllables and words,[8] with a diagnosis distinct from other speech (e.g., stuttering) and language disorders (e.g., specific language impairment). Although CAS has been linked to a number of genes and genomic pathways, including *FOXP2* and *ELKS/ERC1*,[9–11] the confirmed genetic risk factors explain only a small proportion of cases.[12] Recently, however, three unrelated individuals with 16p11.2 deletions were identified in children diagnosed with CAS and screened for copy-number variations,[13,14] thus implicating 16p11.2 deletions as one of the genetic causes of CAS. However, a crucial question remains: how common is CAS among individuals with 16p11.2 deletions?

No standardized or systematic testing of speech abilities has been reported in a group of individuals with 16p11.2 deletions. Further, in existing studies, the terms 'speech' (the perception and production of speech sounds) and 'language' (understanding and use of syntax, morphology, semantics and pragmatics) are often used interchangeably. Yet these terms refer to broad multicomponent domains, and deficits within each encompass many distinct disorders. Precise phenotyping, with the use of correct terminology, diagnostic tools and criteria accessible and hence replicable to others, is thus critical to elucidate genotype–phenotype relations in this relatively common deletion syndrome. Here, we conduct the first systematic differential diagnostic assessment of speech disorders in a cohort of individuals with 16p11.2 deletions. In contrast to prior phenotype-driven studies of CAS,[13,14] we have taken a genotype-first approach[15] and recruited participants with 16p11.2 deletions to conduct speech and neuropsychological assessment.

## SUBJECTS AND METHODS

### Participants

Eleven children (8 males) ages 5.4–18.1 (mean 10.8) with 16p11.2 deletions from the Simons Variation In Individuals Project (Simons VIP) cohort

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(http://sfari.org/resources/simons-vip) participated (see Supplementary Table 1 for SFARIbase IDs). Participants were recruited from attendees of the 2013 Simons VIP Connect Family Meeting in Orlando, Florida. The study was advertised to attendees as a 'language study'. Inclusion criteria were: presence of a 16p11.2 deletion, an IQ of at least 80, based on previously performed phenotyping (cognitive testing is challenging for individuals with lower IQ), and age $\leq 18$ years. The presentation of CAS is known to change across the lifespan, with little or no information available on core features of the disorder to guide informed diagnosis in mid- to late adulthood. Hence, we excluded individuals older than 18 years. Thirteen individuals met these criteria, and 11 agreed to be tested. As described previously, all 11 children carry the canonical deletion (~600 kb, chr16.hg18:g.29557497_30107356del) at the locus, and do not have any other copy-number variant that is known to affect function, or another neurogenetic or neurological diagnosis unrelated to 16p11.2 (e.g., tuberous sclerosis).[16] Written consent and assent was provided, in accordance with the requirements of the Internal Review Board at MIT. A testing battery of ~1 h was conducted. Families were compensated with Amazon.com gift cards and other prizes.

### Speech sampling and analysis

Speech samples for transcription were derived from audio (Sony Digital ICD-PX312, Sony, San Diego, CA, USA) and video (Sony HDR-CX260V) recordings of conversation with the examiner and/or parent/guardian during and between standardized tests, and also from three cognitive tasks (syllable repetition task (SRT), Comprehensive Test of Phonological Processing (CTOPP) and rapid automatized naming (RAN); see below). Conversations were elicited for a total of at least 10 min to seek a sample of at least 100 words.[17] Samples were transcribed by a speech language pathologist (EM) using broad phonetic transcription with supplemental diacritics to note distortion errors.

Samples were analyzed for speech features associated with articulation disorder, phonological disorder, dysarthria and CAS. A phonetic inventory was determined, including initial, medial and final consonant and vowel inventories. Percentage phoneme correct scores were calculated based on conversational speech. An overall impression of intelligibility was provided by EM and AM using a perceptual intelligibility rating scale.[18] Ability to repeat pronounceable nonsense words was additionally examined using nonword repetition and the SRT (see below). Phonological process analysis was conducted (Table 1). Traditional articulation errors were noted where present (e.g., interdental or lateral /s/ or /z/, distorted /r/). Motor speech features (encompassing symptoms of dysarthria and apraxia) were rated using the 'Mayo Clinic Motor Speech Characteristics Rating Scale'[19] (Table 1) and further diagnostic criteria for CAS were adapted from Murray *et al.*[20,21] based on the three American Speech and Hearing Association (ASHA)[8] consensus-based criteria (Table 2). Two SLPs (EM and AM) examined the resultant data to make a final diagnosis by consensus.

### Neuropsychological tests

Not every participant was able to perform all of the tasks because of general unwillingness to perform particular tasks, fatigue, insufficient time, and because two (male) participants were mostly nonverbal, as confirmed by parent report. Eight participants were tested on the SRT,[22] the nonword repetition subtest from the CTOPP,[23] and RAN tasks.[24] In the syllable and nonword repetition tasks, participants are presented with spoken syllable sequences or nonwords, respectively, of increasing length and are asked to repeat each one. The SRT[22] and nonword repetition tasks are particularly sensitive for detecting errors in children with speech sound disorder, including CAS. Poor performance on the SRT in particular, a tool that overcomes some of the methodological limitations of standard nonword repetition tasks (see the report by the American Speech-Language-hearing association[8] for a review), has been suggested to have diagnostic accuracy for identifying the signature transcoding deficits seen in CAS.[25] The RAN task requires participants to name in order, as quickly as possible, sequences of letters, digits, pictures of objects or colored dots. Seven participants were tested on the Peabody Picture Vocabulary Task (PPVT),[26] and four on the Test for Reception of Grammar (TROG).[27] In these tasks, participants are shown sets of four pictures accompanied by a word (PPVT) or sentence (TROG) and have to choose the picture that corresponds to the word/sentence. Non-verbal IQ was assessed in 10 of the 11 participants with the matrices subtest of the Kaufman Brief Intelligence Test.[28] Fine and gross motor skills were evaluated in 10 participants with the Bruininks–Oseretsky Test of motor proficiency (BOT),[29] which requires participants to string beads and copy and draw figures (fine motor skills), as well as catch a ball, walk on a straight line and jump on one leg (gross motor skills). Raw scores, computed for all tasks following the instructions in the manuals, were converted into standardized scores and percentiles. The parents/guardians of nine participants completed the Children's Communication Checklist (CCC).[30]

The phenotyping data have been uploaded to the Simons Foundation Autism Research Initiative (SFARI) database (available at http://sfari.org/resources/sfari-base).

## RESULTS

### Speech results and diagnosis

The limited verbal output for two participants precluded speech diagnosis (Tables 1 and 2). All nine remaining participants met the three ASHA-based consensus diagnostic criteria for CAS[8] (Table 2). All nine also had additional motor speech deficits that could be associated with either dysarthria or CAS, including phoneme imprecision (8/9), hypernasality (7/9), slow speech rate (6/9) and equal stress (9/9), characterized by the Mayo clinic rating scale.[19] Further, some features more commonly associated with dysarthria than CAS included mildly reduced overall loudness (7/9) and a breathy voice (6/9). The majority also demonstrated phonological errors (Table 1), with final consonant deletion, gliding, weak syllable deletion, cluster reduction and cluster simplification most common. Some of these error patterns could reflect motor features of CAS rather than being purely phonological in nature. Poorer performance with increasing stimulus length was noted on the SRT and nonword repetition tasks (Table 1), a hallmark characteristic of CAS. Participants scored in the 7th percentile (SEM = 1.22) on nonword repetition. Conversational speech intelligibility was reduced in all, with most rated as 'somewhat intelligible', with corroborating percent phoneme correct ratings (range 66–88%, Table 1) falling well below the expected intelligibility range for this age range.[31]

### Neuropsychological results

Here we include the two largely nonverbal participants excluded from the speech analyses (Table 3; Supplementary Table 2). Consistent with prior reports (e.g., see Zufferey *et al.*[6]), nonverbal IQ ($n = 10$) was about two SDs below the general population mean (average standard score = 78.8, SEM = 5.57; average percentile = 18.4, SEM = 4.25). Participants performed poorly on tasks assessing higher levels of language processing. In particular, on lexical knowledge (PPVT), participants ($n = 7$) scored on average in the 36th percentile (SEM = 10.2), and on grammatical comprehension (TROG), participants ($n = 4$) scored on average in the 21st percentile (SEM = 15.4). Participants ($n = 8$) scored relatively higher on the RAN task: in the 57th percentile (SEM = 12.7), with a large range. Finally, on the motor-skills assessment, participants ($n = 10$) scored on average in the 22nd percentile (SEM = 9.33), again with a large range. The summed CCC scores were in the 11th percentile on average, with the speech subscale revealing the lowest score (6th percentile on average, SEM = 3.59).

In terms of inter-task relationships (Supplementary Table 2), parental assessments of children's speech abilities (CCC speech subscale) predicted nonword repetition scores ($r = 0.52$), as did the overall CCC scores ($r = 0.46$). Interestingly, IQ did not show a strong relationship with nonword repetition scores ($r = 0.34$), although it did predict performance on PPVT ($r = 0.57$) and TROG ($r = 0.89$), consistent with prior reports revealing such correlations in both

**Table 1 Speech (phonetic, phonemic and intelligibility) features for the participants with 16p11.2 deletion**

| | | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Age (Sex) | | 8.0 (M) | 10.11 (M) | 8.9 (F) | 6.3 (F) | 5.4 (M) | 14.8 (M) | 10.10 (M) | 8.4 (F) | 18.1 (M) |
| No. words in transcription sample | | 200 | 150 | 418 | 147 | 292 | 145 | 219 | 208 | 180 |
| Intelligibility rating[a] | | 3 | 3 | 3 | 4 | 3 | 2 | 4 | 3 | 3 |
| PPC conversation | | NA | 88% | 83% | NA | 81% | NA | 66% | 85% | NA |
| CTOPP nonword repetition SS | | 3 | 2 | NA | 4 | 2 | 8 | 7 | 4 | 8 |
| SRT task 2 syllables RS | | 12 | 16 | NA | 9 | 13 | 16 | 15 | 15 | 16 |
| SRT task 3 syllables RS | | 4 | 12 | NA | 6 | 5 | 13 | 15 | 11 | 18 |
| SRT task 4 syllables RS | | NA | 8 | NA | NA | 5 | 3 | 9 | 1 | 7 |
| Delayed phonological errors[b] | Final consonant deletion | | | | | | | | | |
| | Context sensitive de/voicing | | | | | | | | | |
| | Fronting | | | | | | | | | |
| | Assimilation | | | | | | | | | |
| | Weak syllable deletion | | | | | | | | | |
| | Gliding of liquids | | | | | | | | | |
| | Cluster simplification | | | | | | | | | |
| | Cluster reduction | | | | | | | | | |
| | Stopping fricatives, affricates | | | | | | | | | |
| | Epenthesis | | | | | | | | | |
| | Metathesis | | | | | | | | | |
| Articulation errors | Interdental [s] | | | | | [c] | | | | |
| Motor Speech ratings[d] | Imprecise consonants | | 1 | 2 | 2 | 2 | 1 | 3 | 1 | 2 |
| | Prolonged phonemes | | | 2 | | 1 | | | 1 | |
| | Irregular articulatory breakdowns | 1 | | 2 | 1 | 1 | | 1 | 1 | 1 |
| | Distorted vowels | | 1 | 3 | 1 | 2 | 1 | | 1 | 3 |
| | Articulatory groping | | | 2 | 1 | | 1 | | | |
| | Increased errors with increased rate | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 2 |
| | High Pitch | 2 | | 1 | 2 | | | 1 | | |
| | Monopitch | | | | 1 | | | | 1 | 1 |
| | Overall Loudness (reduced) | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 |
| | Monoloudness | | 1 | | | | | 2 | 1 | |
| | Loudness decay | | | | | | | | 2 | 1 |
| | Breathy voice quality | 1 | 1 | 2 | 1 | 1 | | 2 | 3 | 2 |
| | Hypernasal resonance | 1 | 2 | 3 | 1 | 1 | | 2 | 3 | 1 |
| | Mixed nasality | | | | | | | | | 2 |
| | Slow speech rate | 1 | 1 | 1 | 1 | 2 | | | 1 | 2 |
| | Equal stress | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 1 |
| | Syllable segregation | | 1 | 2 | 1 | 1 | 2 | | | 2 |
| | Poorly sequenced SMRs | 1 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 |

Abbreviations: CTOPP, comprehensive test of phonological processing; F, female; M, male; NA, not administered or not able to be scored; PPC, percent phonemes correct; RS, raw score; SRT, syllable repetition test.
[a]Intelligibility severity rating (rating scale 1–5, 1—completely intelligible; 5—completely unintelligible).
[b]Not age appropriate;[47] Shaded: feature present.
[c]Age appropriate.
[d]Motor speech disorder severity rating 1–4, 1 = mild deviation from typical speech, 4 = severely deviant.

healthy individuals (e.g., see Hodapp and Gerken[32] and Bell *et al.*[33]) and individuals with neuropsychiatric disorders (e.g., see Beck and Black[34]). PPVT and TROG were also highly correlated with each other ($r = 0.94$). BOT performance was weakly correlated with nonword repetition scores ($r = 0.23$) and not correlated with IQ ($r < 0.1$).

## DISCUSSION

Systematic differential diagnostic assessment of speech disorders in a cohort of children with 16p11.2 deletions revealed a high number of individuals with features of CAS: of eleven children, two were mostly nonverbal, and the remaining nine met the core consensus-based ASHA criteria for CAS.[8] This finding is striking, given that CAS is a specific speech disorder, distinct from—though often co-morbid with—other speech and language disorders, and exhibiting low prevalence in the general population (for e.g., one study estimates prevalence of ~0.01–0.02%[35]). Nonetheless, the cognitive profile associated with 16p11.2 deletion is not characterized by a selective deficit in speech production. Our participants were additionally intellectually impaired and had difficulties with lexical and syntactic processing and general motor coordination. The relatively low inter-task correlations, however, suggest that these language and general cognitive deficits are at least somewhat independent from the speech production difficulties.

Our findings are in line with a handful of previous reports of CAS[13] (2 cases);[14] (1 case); dysarthria[36] (2/3 cases);[37] (1/10 cases); and articulation difficulties[37] (2/10 cases);[38] (1/15 cases);[39] (1/18 cases) in individuals with 16p11.2 deletions (Supplementary Table 3). However, most studies of individuals with 16p11.2 deletions have used the non-specific term 'speech delay', making it impossible to identify the precise sub-type(s) of speech disorder. Our data go beyond these earlier reports by establishing, with detailed phenotyping, that many individuals with 16p11.2 deletions do not just have generalized speech and language disorders, but features of CAS in particular. Ours is the first genotype-driven study to apply the systematic analyses necessary

**Table 2 Speech features categorized under the three consensus diagnostic criteria for CAS diagnosis (ASHA 2007)**

| CAS Consensus diagnostic criteria | Speech features associated with CAS diagnostic criteria | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 |
|---|---|---|---|---|---|---|---|---|---|---|
| CAS (1) Inconsistent errors | Same word/syllable different on repetitions | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| | Same C/V different across different words | ■ | | | ■ | | | | ■ | |
| CAS (2) Lengthened & disrupted coarticulatory transitions | Speech motor behaviors, 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 noted) | ■ | | | ■ | ■ | | | | |
| | Prolongation errors | | | | ■ | ■ | | | | |
| | Nonphonemic productions/distorted substitutions | ■ | ■ | ■ | ■ | | | | | ■ |
| | Hypernasality/nasal emissions | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | ■ |
| | Slowed & disrupted DDK sequence | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ |
| CAS (3) Inappropriate prosody | Equal stress or lexical stress errors | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | |
| | Altered suprasegmental features | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ |
| | Prolongation errors | | | | | ■ | | | | |
| CAS criteria met | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Abbreviations: ASHA, American Speech and Hearing Association; CAS, childhood apraxia of speech.
Shaded: feature present.

**Table 3 Neuropsychological test results**

| | *P1* | *P2* | *P3* | *P4* | *P5* | *P6* | *P7* | *P8* | *P9* | *P10* | *P11* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (sex) | 8.0 (M) | 10.11 (M) | 8.9 (F) | 6.3 (F) | 5.4 (M) | 14.8 (M) | 10.10 (M) | 8.4 (F) | 18.1 (M) | 9.11 (M) | 16.3 (M) |
| KBIT matrices percentile | 6 | 27 | 37 | 32 | 16 | 19 | NA | 1 | 27 | 1 | <0.1 |
| PPVT percentile | NA | 77 | 16 | NA | NA | 45 | 58 | NA | 50 | 6 | 0.5 |
| TROG percentile | NA | 66 | NA | NA | NA | 16 | NA | NA | NA | 1 | 1 |
| RAN percentile | 40 | 85.3 | 92 | 16 | NA | 97.8 | 76 | 48.5 | 2 | NA | NA |
| BOT percentile | 14 | 7 | 31 | 1 | 1 | 99 | 27 | 31 | 12 | NA | 1 |

Abbreviations: BOT, Bruininks–Oseretsky Test of motor proficiency; F, female; KBIT, Kaufmann Brief Intelligence Test; M, male; NA, not administered; PPVT, Peabody Picture Vocabulary Task; RAN, rapid automatized naming; TROG, Test for Reception of Grammar. NA: Not administered.

to distinguish features of CAS from other speech and language disorders in individuals with 16p11.2 deletions.

One limitation in our speech phenotyping was the absence of an oral peripheral exam. An oral exam can determine the presence of altered tone (spasticity, flaccidity), reduced range and rate of movement of the articulators, or structural deficits (high arched palate, submucous cleft, bifid uvula). These data can assist in more precisely differentiating between dysarthria, apraxia and structural speech deficits.

As in many other clinical reports, our sample may not be fully free of ascertainment bias; genetic anomalies in these children were discovered after they presented cognitive and/or behavioral problems and were evaluated by medical professionals. Yet, any such bias is unlikely to be specific to CAS and is instead a general bias toward neurodevelopmental problems. Although we found that 9/9 of the 16p11.2 individuals we tested met criteria for CAS (two additional participants were nonverbal and thus not tested for CAS), we do not conclude that features of CAS will be found in every individual with a 16p11.2 deletion. Nevertheless, given the rarity of CAS in the general population, and the high proportion of features of CAS cases in our sample, it seems likely that CAS is a reasonably frequent component of the phenotype of the 16p11.2 deletion syndrome.

One important aim for future research will be to test a larger number of individuals with 16p11.2 deletions, to precisely quantify the penetrance of this genomic rearrangement with respect to features of CAS. Another important direction would be to attempt to link CAS-related deficits to some aspects of brain structure and function. Some recent studies have reported neurological markers associated with the 16p11.2 deletion syndrome (e.g., increased brain volume, including increased gray and white matter, subcortical and cerebellar volumes,[40,41] some regional differences,[41] and aberrant white matter microstructure[42]). However, the neurobiological mechanisms underlying these effects are not yet well understood.[43,44] Also, there have been no consistent reports of relationships between speech/language problems in 16p11.2 deletion cases and neuroanatomical abnormalities[4,36–39] and no investigations into potential dysfunction of language networks[45] or brain regions related to speech articulation.[46] Finally, it will be of considerable interest to eventually determine which of the 25 or so genes that are contained within the

deleted region contribute to the different neurodevelopmental aspects of this major chromosomal rearrangement.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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