Clinical Genetic Studies of Speech and Language Disorders

Samantha Jane Turner
MSpPath, BA/BSc (Hons)

ORCID #0000-0002-7707-6228

Submitted in total fulfilment of the requirements of the degree

Doctor of Philosophy

The University of Melbourne

August 2017

Abstract

The neurobiology underlying childhood speech and language disorder is largely unknown. This thesis explores the genetic basis of speech dysfunction using a range of phenotyping approaches.

Reverse phenotyping was used to study the speech and language phenotype associated with ion channel gene diseases (Dravet syndrome, *GRIN2A* encephalopathy). Motor speech disorder was part of the characteristic phenotype of individuals with pathogenic variants in the glutamate receptor subunit gene *GRIN2A*, a newly discovered cause of epilepsy-aphasia spectrum syndromes that are inextricably linked to speech and language impairment (Chapter 4). Individuals with Dravet syndrome, a developmental and epileptic encephalopathy associated with mutations in the sodium channel subunit gene *SCN1A,* also had motor speech disorder in addition to cognitive and motor impairments (Chapter 5). This thesis is first to implicate ion channel genes in speech dysfunction.

The speech and language phenotype of *FOXP2* diseases was also studied (Chapter 3). The phenotype of the intragenic deletion case described here was comparable to that of other *FOXP2* mutation cases.

Detailed phenotyping of a multiplex family, suspected of harbouring a new and as yet unidentified speech and language gene, revealed multiple individuals with childhood apraxia of speech (Chapter 6). This crucial data will inform molecular studies to identify a new gene for motor speech disorder.

Declaration

This is to certify that:

1) The thesis comprises only my original work towards the degree of Doctor of Philosophy, except where indicated in the Preface.
2) Due acknowledgement has been made in the text to all other materials used.
3) The thesis is fewer than 100,000 words in length, exclusive of tables, figures, references and appendices.

Signed,

Samantha Jane Turner

Preface

The research projects presented in this thesis were done in collaboration with others. The nature of these contributions, as well as my own original work, is described below.

Chapter three includes a manuscript accepted for publication in the **American Journal of Medical Genetics Part A**. Chapters four and five include manuscripts accepted for publication in **Neurology**. I am the primary author of these three papers and contributed more than 50% of the experimental planning, execution and preparation of the research data for publication. This has been acknowledged by completion of 'co-author authorisation' and 'declaration of thesis with publication' forms that were submitted with this thesis, in line with The University of Melbourne requirements.

In the study described in chapter three, Ms Elena Aleksoska performed genomic DNA extractions and Dr Michael Hildebrand and Mr Anthony Damiano performed amplification reactions via polymerase chain reaction, Sanger sequencing of novel *FOXP2* variants, and interrogated genetic variant databases. Dr Michael Fahey referred and conducted a neurological examination in one proband. Dr Susan Block collected and rated conversational speech samples from one family.

In the study described in chapter four, Dr Angela Mayes completed some of the cognitive and receptive language testing and coordinated neuroimaging studies in two families. Dr Simone Mandelstam reviewed the brain magnetic resonance imaging scans from two families. Ms Andrea Verhoeven provided a second opinion assessment of velopharyngeal dysfunction.

In the study described in chapter five, Dr Amy Brown and Ms Marta Arpone conducted the neuropsychological experiments, under the supervision of Professor Vicki Anderson. Mrs Natalie Bryant and Ms Annie Roten assisted with videorecording the speech and language assessments.

Chapter six includes oral motor, speech and language assessment data that was collected by me as a research assistant, prior to the commencement of my PhD candidature. Dr Megan Spencer-Smith completed the neuropsychological assessments in this family. Dr Michael Hildebrand performed zygosity testing and Ms Amber Boys

performed single nucleotide polymorphism (SNP) microarray. Professor David Amor gave a second opinion regarding jaw malocclusion.

Professor Angela Morgan completed auditory-perceptual ratings of conversational speech samples and scored the oral motor assessments for all the studies, as well as providing overall supervision together with Professor Ingrid Scheffer.

Chapter one includes text on pages 33 and 34 that is quoted directly from the manuscript 'New genes for focal epilepsies with speech and language disorders,' which was published in **Current Neurology and Neuroscience Reports** in April 2015. I am the primary author of this paper. The published paper is included in Appendix 1.

This work was supported by a National Health and Medical Research Council Dora Lush Postgraduate Scholarship (GNT1017773), an Australian National University Gowrie Scholarship and a Speech Pathology Australia Nadia Verrall Memorial Research Grant.

Acknowledgements

Firstly, I would like to thank all the families that selflessly gave their time to participate in this research. I am hopeful that their contributions will one day translate into better therapies and outcomes for their families and others.

I am indebted to my PhD supervisors Professor Angela Morgan and Professor Ingrid Scheffer, two exceptional clinician-scientists who are incredibly caring women and role models. I am incredibly lucky to have such inspiring mentors – their passion for research and dedication to their respective fields is an example of excellence that I can only hope to emulate. My heartfelt thanks for the time they have invested in me, for their belief in my abilities and care in my professional and personal growth.

I have been fortunate to work with a wonderful team of researchers as part of the Genetics of Speech and Language Disorders project, including Dr Michael Hildebrand, Professor Alan Connelly, Professor Melanie Bahlo, Dr Frederique Liegeois, Professor David Amor, Dr Thomas Scerri, Dr Cristina Mei, Dr Angela Mayes, Dr Sarah Barton, Lauren Pigdon and Olivia Van Reyk. It has been exciting to watch our team grow, and to be involved from the beginning.

Thanks also must go to Professor Martin Delatycki and Professor Sarah Wilson for their support as part of my PhD advisory committee, and to Professor Eliane Roulet-Perez, Professor Vicki Anderson, Dr Amy Brown, Marta Arpone and A/Professor Adam Vogel for their fruitful collaborations. Thank you to the team at the Epilepsy Research Centre - especially Bronwyn, Rosie, Brigid, Amy and my dear Karen and Natalie - for their patience and help with my endless queries.

I am incredibly grateful to family and friends for their love and support over the years. Thanks to my friends who I have neglected terribly while working on this thesis, but have still been there with words of encouragement and wine. A big thank you to Shanna and Melody for their precious care of my children. Sincere thanks to my parents-in-law John and Selina for keeping the children entertained while I worked, and for their practical support and words of wisdom when very much needed. Thank you to my family - Sarah, Corey, Daniel, James, Elijah, Lauren, Jarrod, Ariane, Nathaniel,

Evelyn, Alannah, Natalie, Dean, Amelia, Charlotte and Benjamin - for all the laughter and tears, and for being such an important part of my life.

I owe a particular debt of gratitude to my parents Neil and Mary, who fostered my love of learning from a young age. They have always done everything in their power to support their children, including the unenviable task of caring for seven young grandchildren on Fridays. Thank you for being truly wonderful parents and for your boundless love.

Finally, three very special people have unwittingly shared this PhD journey with me, and have stayed steadfastly by my side through all the highs and lows. To my precious babies Imogen and Liam – life is brighter with your cheeky smiles, 'huggles' and unconditional love. To my husband Andrew – my better half in every sense – you have been a tower of strength over the past six years. We would not have reached this point without your tenacity and determination, and I am forever grateful for the sacrifices you have made for our family. I love you all dearly.

I can no other answer make but thanks, and thanks, and ever thanks…

- William Shakespeare

Publications

Original peer-reviewed articles arising from this research:

Turner SJ, Brown A, Arpone M, Anderson, V, Morgan, AT, Scheffer IE. Dysarthria and broader motor speech deficits in Dravet syndrome. Neurology 2017;88:743-749.

Turner SJ, Mayes AK, Verhoeven A, Mandelstam SA, Morgan AT, Scheffer IE. GRIN2A - an aptly named gene for speech dysfunction. Neurology 2015;84:1-8.

Turner SJ, Morgan AT, Roulet Perez E, Scheffer IE. New genes for focal epilepsies with speech and language disorders. Curr Neurol Neurosci Rep 2015; 15:35. IF3.6

Turner SJ, Hildebrand MS, Fahey M, Reilly S, Block S, Damiano, J, Dahl HM, Smith RJH, Bahlo M, Scheffer IE, Morgan AT. Small intragenic deletion in FOXP2 associated with childhood apraxia of speech. Am J Med Genet A 2013;161A:2321-6.

Original peer-reviewed articles arising from other works:

Corbett MA, **Turner SJ**, Gardner A, Silver J, Stankovich J, Leventer RJ, Derry CP, Carroll R, Ha T, Scheffer IE, Bahlo M, Jackson GD, Mackey DA, Berkovic SF, Gecz J. Familial epilepsy with anterior polymicrogyria as a presentation of COL18A1 mutations. Eur J Med Genet 2017;60:437-443.

Liegeois FJ, Hildebrand MS, Bonthrone A, **Turner SJ**, Scheffer IE, Bahlo M, Connelly A, Morgan AT. Early neuroimaging markers of FOXP2 intragenic deletion. Sci Rep 2016;6:35192.

Carvill GL, Regan BM, Yendle SC, O'Roak BJ, Lozovaya N, Bruneau N, Burnashev N, Khan A, Cook J, Geraghty E, Sadleir LG, Turner SJ, Tsai MH, Webster R, Ouvrier R, Damiano JA, Berkovic SF, Shendure J, Hildebrand MS, Szepetowski P, Scheffer IE, Mefford HC. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. Nat Genet 2013;45:1073-6.

Tsai MH, Vears DF, **Turner SJ**, Smith RL, Berkovic SF, Sadleir LG, Scheffer IE. Clinical genetic study of the epilepsy-aphasia spectrum. Epilepsia 2013;54:280-7.

Hynes K, Tarpey P, Dibbens LM, Bayly MA, Berkovic SF, Smith R, Al Raisi Z, **Turner SJ**, Brown NJ, Desai TD, Haan E, Hackett A, Turner G, Christodoulou J, Leonard H, Gill D, Stratton M, Gecz J, Scheffer IE. Epilepsy and mental retardation limited to females with PCDH19 mutations can present de novo or in single generation families. J Med Genet 2010;47:211-6.

Dibbens LM, Tarpey PS, Hynes K, Bayly M, Scheffer IE, Smith R, Bomar J, Sutton E, Vandeleur L, Shoubridge C, Edkins S, **Turner SJ**, Stevens C, O'Meara S, Tofts C, Barthorpe S, Buck G, Cole J, Halliday K, Jones D, Lee R, Madison M, Mironenko T, Varian J, West S, Widaa S, Wray P, Teague J, Dicks E, Butler A, Menzies A, Jenkinson A, Shepherd R, Gusella JF, Afawi Z, Mazarib A, Neufeld MY, Kivity S, Lev D, Lerman-Sagie T, Korczyn AD, Derry CP, Sutherland GR, Friend K, Shaw M, Corbett M, Kim H, Geschwind DH, Thomas P, Haan E, Ryan S, McKee S, Berkovic SF,

Futreal PA, Stratton MR, Mulley JC, Gécz J. X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment. Nat Genet 2008: 40: 776-81.

Scheffer IE, **Turner SJ**, Dibbens LM, Bayly MA, Friend K, Hodgson B, Burrows L, Shaw M, Wei C, Ullmann R, Ropers HH, Szepetowski P, Haan E, Mazarib A, Afawi Z, Neufeld MY, Andrews PI, Wallace G, Kivity S, Lev D, Lerman-Sagie T, Derry CP, Korczyn AD, Gecz J, Mulley JC, Berkovic SF. Epilepsy and mental retardation limited to females: an under-recognized disorder. Brain 2008: 131(Pt 4): 918-27.

Scheffer IE, Harkin LA, Grinton BE, Dibbens LM, **Turner SJ**, Xu R, Jackson G, Adams J, Connellan M, Petrou S, Wellard RM, Briellmann RS, Wallace RH, Mulley JC, Berkovic SF. Temporal lobe epilepsy and GEFS+ phenotypes associated with SCN1B mutations. Brain 2007;130:100–109.

Maxwell MJ, Dopheide SM, **Turner SJ**, Jackson SP. Shear induces a unique series of morphological changes in translocating platelets. Arterioscler Thromb Vasc Biol 2006:26:663-669.

Taylor I, Marini C, **Turner S**, Berkovic SF, Scheffer IE. Juvenile myoclonic epilepsy and idiopathic photosensitive occipital lobe epilepsy: is there overlap? Brain 2004;127:1878-86.

Marini C, Scheffer IE, Crossland KM, Grinton BE, Phillips FL, McMahon JM, **Turner SJ**, Dean JT, Kivity S, Mazarib A, Neufeld MY, Korczyn AD, Harkin LA, Dibbens LM, Mulley JC, Berkovic SF. Genetic architecture of idiopathic generalized epilepsy: Clinical genetic analysis of 55 multiplex families. Epilepsia 2004;45:467-78.

Awards

National Health and Medical Research Council Dora Lush Postgraduate Scholarship (2011-2016)

Gowrie Scholarship (2015-2016)

Speech Pathology Australia Nadia Verrall Memorial Grant

Table of Contents

List of Figures

List of Tables

Third party copyright material

Citation Information for third party copyright material	Location of item in thesis	Permission granted Y/N
Microarray data from the Allen Human Brain Atlas, Allen Institute for Brain Science. *FOXP2* gene in Donor H0351.1012. Available from: human.brain-map.org/microarray/gene/show/606634 *SCN1A* gene in Donor H0351.1012. Available from: human.brain-map.org/microarray/gene/show/6288 *GRIN2A* gene in Donor H0351.1012. Available from: human.brain-map.org/microarray/gene/show/2886 Allen Human Brain Atlas. Hawrylycz, M.J. et al. (2012) An anatomically comprehensive atlas of the adult human transcriptome, Nature 489: 391-399. doi: 10.1038/nature11405	page 99	Y
'Neural Correlates of the DIVA model' Figure 3.5 from Guenther FH. Neural Control of Speech. Cambridge, MA: MIT Press; 2016. Figure from page 103.	page 101	Y

List of Abbreviations

ASD	Autism spectrum disorder
ASHA	American Speech-Language-Hearing Association
CAS	Childhood apraxia of speech
CCS	Complexity of Communication Scale
CELF-4	Clinical Evaluation of Language Fundamentals, 4th Edition
CNRep	Children's Test of Nonword Repetition
CNV	Copy number variation
CSWS	Continuous spike wave in slow wave sleep
CTOPP	Comprehensive Test of Phonological Processing
DEAP	Diagnostic Evaluation of Articulation and Phonology
DCD	Developmental coordination disorder
DDK	Diadochokinesis
DIVA	Directions into Velocities of Articulators
DKEFS	Delis-Kaplan Executive Function System
DS	Dravet syndrome
DSM	Diagnostic and Statistical Manual of Mental Disorders
DZ	Dizygotic
EAS	Epilepsy aphasia spectrum
EDS-HT	Ehlers-Danlos syndrome, hypermobility type
EEG	Electroencephalogram
EMCS	Early Motor Control Scales
EVT-2	Expressive Vocabulary Test, 2nd Edition
FDA-2	Frenchay Dysarthria Assessment, 2nd Edition
fMRI	Functional magnetic resonance imaging
GFTA-2	Goldman-Fristoe Test of Articulation, 2nd Edition
HREC	Human Research Ethics Committees
ID	Intellectual disability
JHS	joint hypermobility syndrome
LDS	Loeys-Dietz syndrome
MZ	Monozygotic
NMDA	N-methyl-D-aspartate

pAC	posterior auditory cortex
PLS-5	Preschool Language Scales, 5th Edition
PPVT-4	Peabody Picture Vocabulary Test, 4th Edition
PVSP	Prosody-Voice Screening Profile
QTL	Quantitative trait locus
SLI	Specific language impairment
SNP	Single nucleotide polymorphism
SRT	Syllable Repetition Task
SSD	Speech sound disorder
TROG-2	Test For Reception of Grammar, 2nd Edition
vSC	ventral somatosensory cortex
WASI-2	Wechsler Abbreviated Scale of Intelligence, 2nd Edition
WRAT-4	Wide Range Achievement Test, 4th Edition
VMPAC	Verbal Motor Production Assessment for Children

CHAPTER 1
INTRODUCTION

Speech and language disorders are common in young children. In Australia, just over 20% of four year-old children have language impairment and 3.4% have speech disorder.[1, 2] Speech disorder is likewise prevalent in North American children, with estimated rates as high as 25% in five to seven year-olds.[3, 4] The impact of persistent speech and language disorder on academic performance, social and emotional development, mental health and later employment opportunities is well documented (reviewed in [2]). There is considerable interest in learning more about the underlying neurobiology of speech and language disorders in order to enhance early diagnosis of affected individuals, and provide targeted treatments to improve long term outcomes.

There is overwhelming evidence that speech and language disorders are genetic. Numerous studies report that individuals with language impairment, dyslexia and speech disorder have a positive family history. This finding has been replicated across studies, and using various methodological approaches (twin studies, case-control studies, familial aggregation studies, single pedigree reports, case history reports).[5-12] While most cases may follow complex multifactorial inheritance,[13] rare multiplex families with monogenic inheritance provided our first insight into the molecular underpinnings of speech disorder.

Terminology

"Speech and language disorder" is a broad term that encompasses a range of diagnoses, including speech disorder, language impairment and dyslexia.

Speech disorder

Speech disorder is any disruption to normal speech production. Errors may occur in the production of sounds, as well as the use sounds to convey meaning.[14] There are a variety of speech disorder subtypes, including articulation disorder, phonological impairment, stuttering, childhood apraxia of speech (CAS) and dysarthria (figure 1-1). Speech disorders can be broadly categorised as i) speech sound disorders or ii) motor speech disorders.

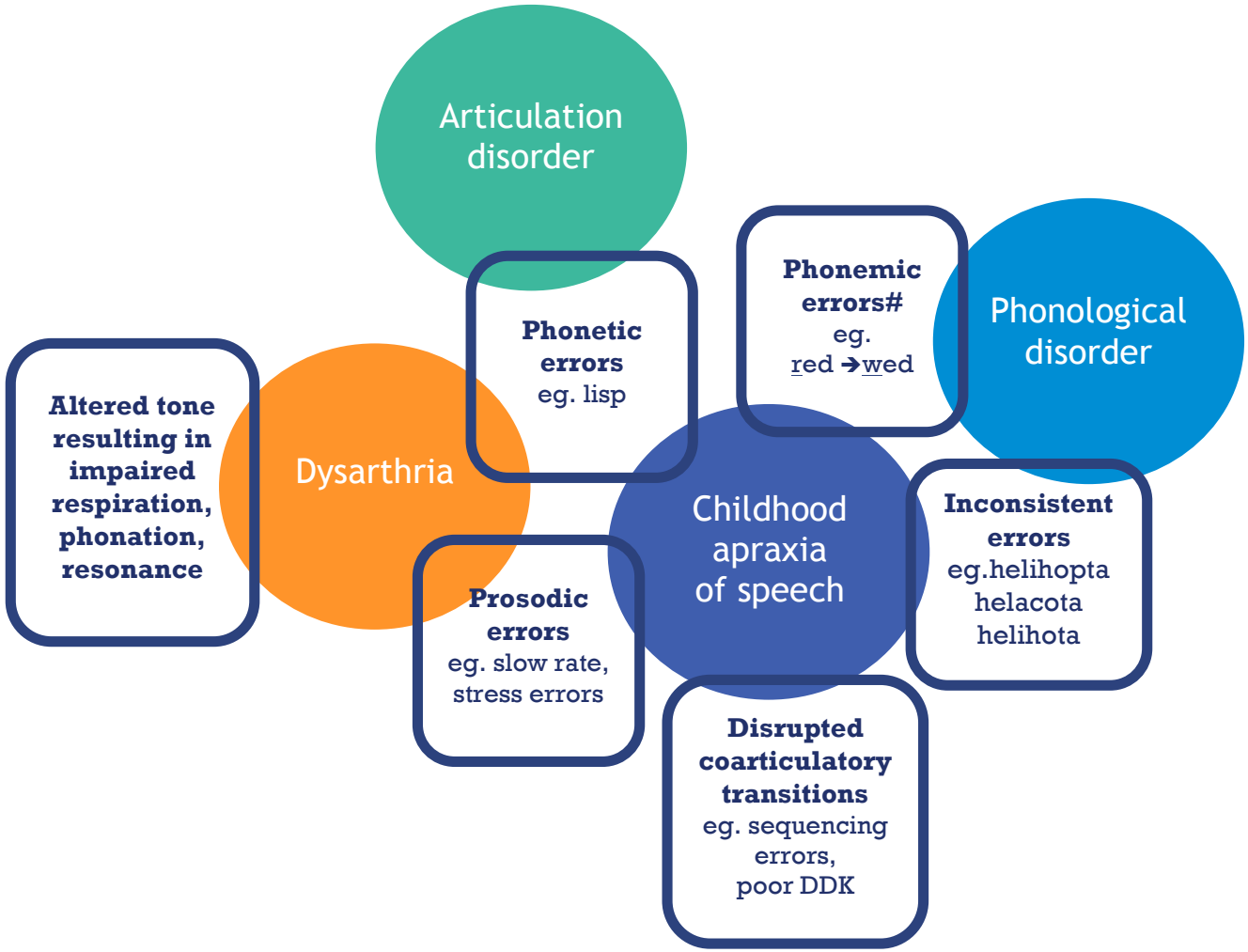


Figure 1-1 – Speech disorder subtypes are distinguished by speech error type

#Phonemic errors may be age appropriate (in normal development), delayed or atypical (in phonological disorder)[14]

Speech sound disorder

Speech sound disorder (SSD) is a term used to describe impairments in both articulation and phonology. Articulation disorder and phonological impairment are the most prevalent forms of speech disorder, and make up the majority of speech pathology patients.[15] An individual with an articulation disorder has difficulty with the production of speech sounds (phonetic level difficulty), whereas a phonological disorder is characterized by poor understanding of the use of sound patterns in a language (phonemic or cognitive-linguistic difficulty).[14] Phonological disorder is a higher-level disorder that involves the linguistic system, thus it is sometimes referred to as a language disorder.[14]

Motor speech disorder

CAS, dysarthria and stuttering are motor speech disorders that occur as a result of breakdown in one of the sensorimotor processes underlying speech production (sensorimotor planning, sensorimotor programming, and sensorimotor execution).[16]

CAS is described as 'a neurological paediatric SSD in which the precision and consistency of movements underlying speech are impaired'.[17] It is due to impairments in planning and programming speech movements, and occurs in the absence of neuromuscular deficits. Since it was first described in 1954, numerous terms have been used to identify CAS – developmental dyspraxia,[18] articulatory dyspraxia,[19] developmental verbal dyspraxia,[20] childhood verbal apraxia,[21] dilapidated speech,[22] developmental apraxia of speech,[23, 24] developmental verbal dyspraxia.[25, 26] The American Speech-Language-Hearing Association (ASHA) Ad Hoc Committee on Apraxia of Speech in Children recommend the term 'childhood apraxia of speech' which is used in this thesis. Disagreement amongst clinicians and researchers regarding diagnostic features of CAS lead the ASHA Ad Hoc Committee to propose three hallmark deficits; (a) inconsistent errors in repeated productions of syllables or words, (b) lengthened and disrupted coarticulatory transitions between sounds and syllables, and (c) inappropriate prosody.[17] Yet these features are not proposed as necessary or sufficient for diagnosis, and children with CAS may exhibit other features including groping, differences in performance on automatic versus volitional activities, and oral apraxia. Some researchers use a modified version of the Mayo Clinic classification

system for acquired apraxia of speech in adults, dubbed 'Strand's 10 point checklist'.[27, 28] A tri-level model of speech-output planning and programming is also proposed to diagnose CAS, and individuals must demonstrate deficits at all three levels (phonological plan, assembly of the phonetic programme, implementation of the motor-speech programme) for a diagnosis.[25] The diagnostic features in this model are compared to the ASHA consensus criteria and Strand's 10-point checklist in table 1.1 (over page).

Table 1-1. Comparison of three feature lists used to diagnose CAS

ASHA consensus criteria	Ozanne, 2005	Strand's 10 point checklist
	*must have symptoms at the motor level (2 & 3) for CAS diagnosis	*evidence of 4 of 10 behaviours for CAS diagnosis
Lengthened and disrupted coarticulatory transitions between sounds and syllables		Difficulty achieving initial articulatory configurations or transitory movement gestures
	DDK rate and sequence (2)	Slow DDK rate
	Groping (3)	Groping
	Increased errors in polysyllables, phrases, sentences (1)	Increased difficulty with multisyllabic words
	Inability to maintain phonotactic structure (1)	Syllable segregation
	Distorted substitutions	Non-English speech sounds/distortions
	Errors not accounted for by child's own phonological rules (1)	
	Metathesis, epenthesis	
		Intrusive schwa
	Prolongations or repetitions	
		Voicing errors
	Consonant deletion (3)	
	Sounds and words being produced spontaneously but not on imitation (3)	
	Use of phoneme in words that do not contain that phoneme, but errors on that phoneme in appropriate context (3)	
Inconsistent errors on consonants and vowels in repeated productions of syllables or words	Inconsistent articulation (1)	
Inappropriate prosody, especially in the realisation of lexical or phrasal stress	Prosodic disturbance (4)	Equal stress or lexical stress errors, slow rate
	Vowel errors (1)	Vowel distortions
	Oral apraxia (2)	
	No history of babbling (4)	

Dysarthria is a disorder of neuromuscular execution, affecting the range, rate, strength and co-ordination of muscles used for speech.[29] This may affect multiple speech subsystems including articulation, resonance, respiration and phonation. Dysarthria is typically characterised according to the Mayo Clinic classification system proposed by Darley, Aronson and Brown, which includes five subtypes (flaccid, spastic, ataxia, hypokinetic, hyperkinetic).[30, 31] The Mayo system was developed in adults with neurological disorders such as amyotrophic lateral sclerosis, and while commonly used in children with acquired dysarthria, the validity of using the classification system in this population is questionable.[32] With the exception of children with basal ganglia lesions, speech features in acquired childhood dysarthria do not resemble the adult type.[33] When children with similar brain lesions are compared, clusters of deviant speech features are not evident as they are for adult dysarthria.[33] Conditions associated with childhood dysarthria also differ from those in adults, and some such as cerebral palsy and Worster-Drought syndrome do not have an adult equivalent.[32] Childhood dysarthria is associated with lesions in the corticobulbar and corticospinal tracts, perisylvian and peri-rolandic cortices, basal ganglia, thalamus and cerebellum, which fits the adult model of motor speech execution.[29] Several studies have characterised speech features in childhood dysarthria associated with traumatic brain injury,[34] posterior fossa tumour,[35] hemispherectomy,[36], Kabuki syndrome,[37], cerebral palsy,[38] Down syndrome,[39] and epilepsy.[40]

Stuttering is another motor speech disorder that affects speech fluency, and is characterized by repetition of words or parts of words, prolongation of speech sounds or blocks in the progress of speech.[41]

Classification of speech disorders

Approaches to the classification of speech disorders are varied, and may be based on age of acquisition (congenital, developmental, acquired), severity (mild, moderate, severe), aetiology (organic vs. functional), surface speech patterns (linguistic) or underlying deficits in the speech processing chain (psycholinguistic) (discussed in [14]). The most widely recognized classification systems are those based on aetiology, linguistic and psycholinguistic approaches (reviewed in [42]). While distinct, these approaches share three broad subtypes in common: (1) a subtype with speech errors characterised by substitutions and omissions; (2) a subtype with articulation errors

characterized by distortions; and (3) a group with speech errors consistent with dysarthria, apraxia or both.[43]

The finalized version of the Speech Disorders Classification System consists of five speech classifications, five motor speech classifications and five dysarthria subtype classifications, with reference to perceptual and acoustic diagnostic markers to classify each subtype.[44] Yet the assessment tasks and data analysis software to identify these diagnostic makers are not widely available and have not been independently validated, thus the currently framework has limited clinical utility. The descriptive-linguistic approach to classification is based on speech output and patterns of sound errors. The system proposed by Dodd (2005) to classify 'functional' speech disorders where no organic aetiology is apparent, includes four speech disorder subtypes defined by speech error type (articulation disorder, delayed phonological development, deviant-consistent phonological disorder, deviant-inconsistent phonological disorder).[14] There is growing empirical support for the validity and clinical utility of Dodd's classification system.[42] The psycholinguistic approach utilises a theoretical model of speech processing, with speech disorder the result of breakdown at one or more levels of input (e.g. hearing loss, auditory processing difficulty), stored linguistic knowledge or output (e.g. motor programming).[45] The model was developed to analyse individual profiles, rather than broadly classify speech disorders.[45]

Language impairment

Language impairment affects comprehension and formulation of spoken language in the absence of hearing impairment, neurological impairment, or psychiatric disturbance.[46] The ability to understand and process spoken or written language is known as receptive language, while the ability to express oneself through speaking or writing is termed expressive language. Children with language impairment demonstrate deficits in language form (phonology, morphology, syntax), language content (semantics) or the function of language in communication (pragmatics).[47] There is debate over the terminology used for deficits in language, with numerous labels adopted over the last century (reviewed in [48]). 'Specific language impairment' (SLI) is a widely used term to describe language difficulties in a child with normal intelligence and adequate educational opportunity not attributable to other disorders e.g. hearing loss or autism spectrum disorder (ASD).[49] However SLI is a controversial label and no longer

included in the Diagnostic and Statistical Manual of Mental Disorders (DSM). Language impairment is currently proposed as an appropriate diagnostic label.[48]

Literacy impairment

Identified in 1895 as 'congenital word blindness', dyslexia is characterized by 'difficulties with accurate and/or fluent word recognition and by poor spelling and decoding abilities'.[50, 51] Difficulties occur in spite of adequate intelligence and opportunities at school, and are theorized to result from an underlying phonological deficit. Children with dyslexia have impaired phonological awareness (awareness that words can be broken down into smaller segments of sound or phonemes) persisting into adulthood (discussed in [52]). Phonological deficit theory does not fully explain the impairments in dyslexia, thus alternate theories are also proposed.[53]

Co-morbidity

Speech, language and literacy disorders are frequently co-morbid.[15, 54-56] Around half of children with SSD have co-morbid language impairment, with co-morbidity higher in preschool children.[3, 54, 55, 57] There is disagreement as to whether co-morbidity is higher for receptive or expressive language impairment.[15, 57] Children with SSD have weaker spelling skills relative to their cognition, language and reading ability.[55] They also have poor phonological awareness.[56]

Clinical genetic studies of speech disorders

As with many common medical conditions, clinical genetic studies in families and twins first established that speech and language disorders have a genetic basis. The majority of studies focused on probands with dyslexia, stuttering and specific language impairment.[58, 59] Far fewer studies have investigated probands with speech disorder, and will be discussed here in more detail.

Twin studies

Classical twin studies compare concordance of a disease or trait in identical (monozygotic or MZ) and non-identical (dizygotic or DZ) twin pairs.[60] Concordance refers to the presence of disease in both twins – a twin pair is concordant if the disease is present in both twins, and discordant if the disease is present in only one twin. MZ twins have an identical genotype, thus a heritable disease will show higher concordance

in MZ twins compared to DZ twins.[61] Studies of MZ and DZ twin pairs demonstrate that speech disorders are highly heritable.

Early twin studies established a genetic contribution to normal speech. In the Louisville Twin Study, articulation test scores were more highly correlated for three to eight year old MZ twins (within-pair correlation coefficient 0.9 compared with 0.56 for DZ twins).[62] MZ twins are also more likely to misarticulate the same sounds.[62, 63] Three to five year old MZ twins had 82% shared errors on an articulation test compared with 56% for DZ twins.[63] Similar types of errors were heard in both MZ and DZ twins.

SSDs also show higher concordance in MZ twins. In one study of six to 12 year old twins, around 80% had articulation disorder with 20/21 concordant MZ twins compared with 4/18 concordant DZ twins.[5] Concordant twins had a higher percentage of first-degree relatives with speech and language disorder (25% compared with 13.5% for DZ twins).[5] In the Western Research Reading project, probandwise concordance for six year old MZ twins with articulation difficulties was 86%, compared with 44% for DZ twins.[10] The presence of speech disorder was determined via parent report in both studies. Probandwise concordance in MZ twins was lower (70%; DZ twins 46%) when twins were directly assessed and strict DSM-III-R criteria for developmental articulation disorder were used.[9] Relaxing the diagnostic criteria saw an increase in probandwise concordance to 92% for MZ male and 100% for MZ female twins.[9] MZ twins with CAS are rare, with only one pair in the literature who had a 10q21.2-22.1 interstitial deletion inherited from their unaffected mother, suggesting this was at most a contributor to the genetic aetiology.[64]

A unique opportunity to study the relative contribution of genes vs. environment, the Colorado Adoption project provided evidence for the role of genetics in transmission of developmental speech disorders.[65] Children with a biological parent with speech disorder were up to 7.5 times more likely to have a speech disorder, compared to children raised by an adoptive parent with speech disorder.[65]

Family aggregation studies

By comparing the prevalence of a disorder in a specific group of relatives (e.g., siblings, second-degree relatives) to the prevalence in the general population, family aggregation studies also support the presence of a genetic contribution to disease.[60] Best estimates

from family studies are that 20-33% of first-degree relatives of children with speech and language disorders are also affected, compared with 3-7% in the general population.[66] Ingram's 1959 review of children diagnosed with 'Specific Developmental Disorders of Speech' was one of the first to document a family history of SSD.[67] The sample included children with impaired articulation due to oral structural anomalies and hearing loss, and half had co-morbid language impairment. Almost one quarter (18/80) had a parent with impaired speech, and around one third (24/80) had an affected sibling who had a similar speech phenotype.

Barbara Lewis and colleagues published a series of seminal family studies in SSD.[6, 7, 68-71] In these studies, SSD was not due to hearing loss, oral structural anomalies, neurological disorder or intellectual disability (ID), and probands had at least two phonological error types and often co-morbid language impairment. Parent questionnaire was used to collect family history data, and family members were classified as affected if they had ever been enrolled in speech/language therapy or met other study specific criteria. Probands with SSD had significantly more family members with speech and language disorders, dyslexia and learning disabilities.[7] The proportion was as high as 12.4% in one study (2.3% for controls).[68] Nuclear family members were more likely to be affected than more distant relatives, although studies differed as to whether this percentage was higher for male or female probands.[7, 70] Male probands had more affected fathers, whereas mothers and fathers of female probands were equally affected.[70] Significantly more male family members were affected, most often brothers.[7, 69, 70] Over half reported a family member with dyslexia.[68]

Speech sound production, phonological processing, language, reading, spelling and motor skills were directly assessed in four studies.[68-71] Compared to age-matched controls or family members without speech disorder, affected family members performed significantly worse on challenging speech tasks, including nonsense word repetition, multisyllabic real word repetition and tongue twisters.[68, 70, 71] Affected siblings also performed poorly on reading, spelling and language tasks and on a screening test for CAS.[68-70] While parents with SSD performed worse than unaffected parents, their scores were in the average range on all standardized measures.[69, 71] Parents with co-morbid language impairment had the worst performance on most measures.[71]

There are only a handful of family studies in CAS, with up to 70% of probands reporting a positive family history.[18, 22, 72-74] Family history data has been gathered from medical record review or parent report. One study directly assessed nuclear family members, and found probands with CAS had a higher percentage of affected first-degree relatives (direct testing 55%; interview 40%) compared to children with SSD with or without language impairment.[75] All three groups were above population estimates (6%).[3] Over half of CAS probands (59%) had at least one affected parent. Affected family members had speech disorder (ranging from mild articulation disorders to stuttering to more severe SSD), reading and language disorder, with males typically affected.[18, 22, 72-75]

Multiplex family studies

The first account of a multigenerational family with SSD was in 1957.[18] The proband had CAS, a paternal grandfather and uncle with stammering, and two paternal uncles with delayed speech development who were unintelligible as children, and did not pronounce words correctly as adults. Multiplex families are rare, and those with autosomal dominant inheritance indicate that some SSDs are monogenic. Other reported families show bilineal inheritance.[6, 11, 12]

The most famous CAS family in the literature is the KE family, a three-generation family from the United Kingdom with autosomal dominant inheritance of severe speech disorder and complete penetrance. The speech and language phenotype has been detailed across several studies.[76-81] Early reports conflicted as to whether the core deficit in the family was speech disorder[76] or 'dysphasia' characterized by severe phonological and grammatical problems.[77, 82] The core phenotype was subsequently established as oral and speech dyspraxia. Affected family members had moderate to severe speech disorder, with poor awareness of sound patterns and defective articulation.[76] Dysarthria was also described, including low and monotonous pitch, strained-strangled or hoarse-breathy voice, alternating loudness, prolonged phonemes and hypernasality.[83] They performed poorly on tests of oral praxis compared to age-matched controls and demonstrated impaired word and non-word repetition.[78, 80] Language and cognition were also poor relative to unaffected family members. Structural imaging in the KE family revealed bilateral abnormalities in speech-motor related areas (caudate nucleus, Broca's area, precentral gyrus, ventral cerebellum,

Wernicke's area, putamen) in affected family members.[84] Functional magnetic resonance imaging (fMRI) tasks also showed under-activation in speech-motor related regions (putamen, Broca's area, right hemisphere homologue of Broca's).[85] Gene discovery in the family identified the first gene for speech and language disorder, *FOXP2* (discussed below).

In another multiplex family study, one of four CAS pedigrees showed autosomal dominant inheritance.[6] The speech phenotype was variable, including CAS, articulation disorder, phonological disorder and stuttering. Dyslexia and learning disabilities were also reported, consistent with the findings of family aggregation studies. Familial subtypes have been examined in other multiplex families.[12, 86, 87] In CAS families tested on syllable repetition and finger tapping tasks, affected and unaffected individuals had slower alternating movements (repetition of disyllables /pata/, /taka/ and trisyllables /pataka/; two finger key presses) compared to repetitive movement (repetition of monosyllables /pa/, /ta/, /ka/; single finger key presses).[12] Motor sequencing ability (repetition of monosyllables vs. disyllables vs. trisyllables) was used as an endophenotype in a subsequent linkage study (discussed below). Sequential processing was another proposed CAS endophenotype, with affected individuals scoring lower on tasks involving high sequential processing loads (alternating syllable repetition, verbal processing, rapid alternating naming, non-word repetition, non-word reading and spelling).[86] This global sequential processing deficit was specific to CAS families, with residual deficits observed in adults with normal conversational speech who had a history of CAS.[86, 87]

Molecular genetic studies

Insight into molecular pathways underlying speech disorders came with the discovery of *FOXP2* as the first monogenic cause of motor speech disorder in 2001. There was little progress in the field subsequent to this finding, with only a handful of linkage studies and chromosomal rearrangements reported. The advent of next generation sequencing techniques saw a proliferation of studies identifying further genetic causes of speech disorder.

FOXP2

Genome-wide linkage in the KE family was successful in mapping the disorder to a 5.6-cM locus on 7q31 (SPCH1).[88] The linkage was consistent with autosomal dominant inheritance with full penetrance. An unrelated individual with a de novo 7q31 balanced translocation and similar phenotype was subsequently identified, and the translocation breakpoint found to disrupt *FOXP2*.[8, 89] *FOXP2* screening in the KE family revealed a missense mutation (R553H) that disrupted the forkhead DNA-binding domain of the encoded protein and segregated with the speech phenotype.[8] Point mutations, large deletions and chromosomal structural variations involving *FOXP2* have subsequently been reported (reviewed in [90, 91]). The core phenotype in all *FOXP2* related speech and language disorders is CAS.[92] The frequency of *FOXP2* mutations in CAS is unclear; one study of 49 unrelated individuals with CAS identified a nonsense mutation (R328X) in two affected siblings and their unaffected mother,[93] while a *FOXP2* variant of uncertain clinical significance was discovered in one of 24 individuals with CAS.[94]

FOXP2 encodes a transcription factor that acts either to repress or activate gene expression. Hundreds of neural targets have been identified, including genes associated with speech and language disorders, malformations, ASD, epilepsy and schizophrenia.[95-97] *FOXP2* plays an important role in neurodevelopment, directly or indirectly regulating genes involved in neurite outgrowth.[98] It is also implicated in vocal learning in songbirds and motor learning deficits in mice.[99, 100] There has been over a decade of research determining how *FOXP2* disruption leads to speech impairment, which has been comprehensively reviewed in [101-103].

Genes related to *FOXP2*

FOXP1

FOXP1 is another member of the forkhead-box family of transcription factors, and the closest paralogous gene to *FOXP2*. The *FOXP1* protein is co-expressed with *FOXP2* in several regions of the brain, and plays a critical role in neurodevelopment.[104] *FOXP1* acts as a transcriptional repressor, with exact neural targets yet to be determined.

Variants in *FOXP1* have been associated with an ID syndrome that may also feature ASD, behavioural problems such as aggression and hyperactivity, macrocephaly and dysmorphic features.[105-110] A likely pathogenic *FOXP1* variant was reported in one

individual with CAS.[111] Other individuals with *FOXP1* mutations may have CAS, and are described as having 'poor speech articulation', reduced intelligibility, difficulty producing word initial and final consonants and multisyllabic words, sound substitutions and inconsistent productions.[105-108] Expressive language skills are more impaired than receptive language, and in some cases oral motor dysfunction may be present including difficulty with lip protrusion, 'dyspraxia of the tongue', excessive drooling and mild oral dysphagia.[105, 106, 110]

CNTNAP2

CNTNAP2 encodes contactin-associated protein 2, a neuronal cell adhesion molecule that is distantly related to the family of neurexins. The gene is transcriptionally regulated by *FOXP2*. *CNTNAP2* has been implicated in numerous neurodevelopmental disorders, including SLI, ASD, ID, cortical malformations and epilepsy.[97, 112-117]

Variants in *CNTNAP2* are reported in individuals with CAS.[28, 94, 111] Only one missense variant in an individual with ID, ASD and CAS is likely pathogenic. The other reported variants are of unknown significance, or occur in non-coding parts of the gene and are associated with other copy number variants. A 7q33-35 deletion disrupting *CNTNAP2* is reported in one individual with stuttering,[118] however analysis of 602 cases with developmental stuttering found no increase in deleterious *CNTNAP2* variants compared to neurologically normal controls.[119] Two other studies report an association between SNPs in *CNTNAP2* and the endophenotype of nonsense-word repetition in cohorts with SLI and dyslexia.[112, 120]

SRPX2

SRPX2 encodes a secreted sushi-repeat protein expressed in neurons of the adult human brain. *SRPX2*, and plasminogen activator receptor uPAR that directly interacts with SRPX2, are both downregulated by *FOXP2*.[121] *SRPX2* has been implicated in the aetiology of speech disorder. A missense variant (N327S) in the *SRPX2* gene was identified in a French family with oral and speech dyspraxia, epilepsy and cognitive impairment.[122] This is a rare variant in the European population.[123] Family members with seizures were subsequently found to have mutations in the glutamate N-methyl-D-aspartate (NMDA) receptor subunit gene *GRIN2A* (see below).[124] Two family

members with CAS and no seizures did not have a *GRIN2A* mutation, yet did carry the *SRPX2* variant, which points to a possible role for this gene in speech function. A second *SRPX2* variant was reported in a proband with bilateral perisylvian polymicrogyria.[122] This cortical malformation is also seen in individuals with Worster-Drought or Foix-Chavany-Marie syndrome who have significant difficulties with speech, drooling and swallowing.[125, 126] Speech and oral motor function was not reported in the proband with the *SRPX2* variant.

GRIN2A

GRIN2A mutations were discovered as the first monogenic cause of Landau-Kleffner syndrome and related disorders within the epilepsy aphasia spectrum (EAS). [124, 127, 128] EAS syndromes share the electroencephalogram (EEG) signature of focal sharp waves in language regions, with associated speech and language impairment (discussed in [129] – see Appendix 1). *GRIN2A* mutations were identified in 9-20% of patients with EAS.[124, 127, 128, 130] "Individuals in five EAS families had speech dyspraxia without seizures, suggesting a role for *GRIN2A* in speech and language unrelated to seizures per se.

GRIN2A encodes the NR2A subunit of the glutamate NMDA receptor. NMDA receptors are ligand-gated ion channels involved in brain development, synaptic plasticity and memory. NMDA receptor functioning has also been linked to slow-wave activity during sleep.[131] The NMDA receptor is tetrameric, comprised of two NR1 subunits and two NR2 subunits (from NR2A-NR2D). The NR2A subunit is crucial to NMDA receptor functioning, controlling cell surface expression and localization, providing glutamate binding sites and modifying channel properties.[132-134]

GRIN2A mutations disrupt the ligand-binding domain of the NR2A subunit and alter NMDA receptor gating.[124, 128] Increased receptor activation due to impaired zinc mediated inhibition, failure to initiate protein translation or nonsense mediated decay of the mutant transcript may underlie other *GRIN2A* mutations.[127, 128] Mice expressing truncated NR2A show deficits in synaptic plasticity and reorganization and have impaired motor coordination.[135] *GRIN2A* mutations have been identified in familial and sporadic ID in concert with epilepsy or EEG abnormalities.[136]

In the human brain, NR2A is expressed in many cortical and subcortical structures including those relevant to speech and language.[137-143] Aberrant NMDA receptor

functioning in the basal ganglia may contribute to impaired motor speech planning/programming and execution, with these structures implicated in both childhood dysarthria and CAS.[29] NR2A expression has not been studied in Broca's area, a key expressive language region implicated in a range of speech and language disorders."[29, 84, 85] (quote from [129] - see Appendix 1).

BCL11A

A second transcription factor linked to motor speech disorder is the zinc-finger protein encoded by *BCL11A*, with a de novo microdeletion reported in a boy with CAS and dysarthria.[144] As well as motor speech disorder, he had oral and body dyspraxia, hypotonia, mild ID and expressive language impairment. An ID syndrome caused by de novo mutations in *BCL11A* is reported.[145] *BCL11A* is also implicated in ASD, and is one of three genes in the proposed critical region of the 2p15-p16.1 microdeletion syndrome characterized by moderate to severe ID, short stature, microcephaly, characteristic facial features, ASD and visual impairment.[145-147] The *BCL11A* protein downregulates axon branching and dendrite outgrowth, and is essential in postnatal development and lymphopoiesis.[148, 149] *BCL11A* is found in the dyslexia candidate region (DYX3) on chromosome 2, with other dyslexia candidate regions examined for linkage with speech disorder (discussed below).

GALT

Motor speech disorder is associated with mutations in the galactosemia gene *GALT*. Galactosemia is a disorder of galactose metabolism that can result in life-threatening complications in untreated infants.[150] Individuals with galactosemia are deficient in the enzyme galactose-1-phosphate-uridyl transferase enzyme encoded by the *GALT* gene. Individuals with classic galactosemia have CAS, dysarthria or an unspecified motor speech disorder.[27] Roughly 60% have the recurrent Q188R *GALT* mutation.

Copy number variants

"The critical role of copy number variations (CNVs) in human disease has become of increasing importance. The finding that CNVs, which include microdeletions and microduplications, exist in the normal population provided ground-breaking insights into genomic variation and, more recently, the pathogenic role of CNVs in disease[151]"(quote from [129] – see Appendix 1). Rare de novo and inherited CNVs have

been identified in CAS. Intriguingly, many of these CNVs have also been identified in individuals with EAS,[129] reinforcing shared molecular pathways between the two disorders.

7q11.23 duplication (Dup 7)

The majority of patients with Dup7 have SSD and in those who have been formally diagnosed, 75-94% have CAS or symptoms of CAS.[152-154] Most individuals have co-morbid dysarthria, phonological disorder or oral apraxia.[154] Cognitive skills range from severe ID to superior intellect, although most individuals have low average cognition.[152] Other common features include a characteristic facial phenotype, developmental coordination disorder (DCD), ASD, anxiety disorder, cerebellar dysfunction and hypotonia.[152, 153] Individuals with Dup7 have a 1.5-1.8 heterozygous duplication of the Williams-Beuren syndrome critical region, which contains 26 genes.[155] While most cases are de novo, 27% of probands have an affected parent.[153] Adults with Dup7 have signs of CAS, phonological disorder or dysarthria and oral apraxia, yet these features are not severe enough to warrant a diagnosis of speech disorder.[154] Learning difficulties and social phobia are also reported in adults with Dup7.[153]

Chromosome 16p

The short arm of chromosome 16 may prove to be a hotspot for speech function, with *GRIN2A* on 16p13 and several 16p CNVs identified in association with CAS.[94, 156-160]

Speech and language impairment is prevalent in individuals with 16p11.2 microdeletion syndrome, characterized as CAS in a handful of cases.[157-159] Impairments are not limited to speech, with lexical and syntactical processing and motor coordination difficulties also reported.[159] Verbal dyspraxia is reported in one individual with an EAS syndrome and a 16p11.2 duplication.[160] The 16p11.2 locus is approximately 600kb in size and contains 29 genes.[161] Other neurocognitive phenotypes commonly associated with 16p11.2 deletions and duplications include ID, ASD, and epilepsy.[161-166] While individuals with deletions are predisposed to obesity and macrocephaly, the opposite phenotype is associated with duplications (underweight/microcephaly).[162, 164, 167] Individuals with 16p11.2 CNVs who are phenotypically normal have also been reported.[162, 168]

Microdeletions of 16p13.2 that do not include the *GRIN2A* gene are reported in two unrelated individuals with CAS.[94]

12p13.33 microdeletion

Inherited and de novo 12p13.33 microdeletions have been reported in CAS.[169] Subtelomeric or interstitial deletions were found in nine individuals from six families with speech disorder, with five individuals diagnosed with CAS, oral motor impairment and language disorder. Deletions of the distal portion of the short arm of chromosome 12 are rare, with less than 20 cases reported in the literature.[170-177] Of these cases, speech function is described in three as 'slurred', 'slightly dysarthric' or difficult to understand. ID and attention-deficit hyperactivity disorder are commonly reported, with ASD, anxiety, behavioural problems and psychiatric disturbance in some. The smallest region of overlap across all reported 12p13.3 microdeletions contains the *ERC1* gene. A brain specific protein transcript encoded by *ERC1* is present in the presynaptic active zone in neurons, and binds RIM proteins to regulate neurotransmitter release.[178]

17p11.2 duplication

Approximately half of the patients with 17p11.2 duplication or Potocki-Lupski syndrome have CAS.[179] Other patients have articulation difficulties, speech delay or absent speech.[180] Core clinical features of Potocki-Lupski syndrome include ID, ASD, hypotonia, failure to thrive, sleep apnoea, and cardiovascular abnormalities.[180, 181] The reciprocal 17p11.2 microdeletion causes Smith-Magenis syndrome, and while CAS is not reported in these patients, almost all (96%) have delayed speech.[182] The *RAI1* (retinoic acid-inducible 1) gene is responsible for the major phenotypic features of both syndromes[179] yet its relationship to speech is unclear. CAS and oral apraxia are also reported in individuals with de novo supernumerary marker chromosomes derived from chromosome 17p.[183, 184]

Other CNVs

Other reported CNVs are not clearly pathogenic and further cases will help to determine whether they are causative variants for CAS.

The long arm of chromosome 15 is highly unstable with CNVs reported in ID, epilepsy, ASD and other neuropsychiatric disorders.[185-189] CAS, language impairment and limb apraxia are described in three of four siblings with a maternally inherited 15q11-q13.9 interstitial duplication.[190] The duplicated region was larger than in 15q duplication syndrome (15q11.2-q13.2), and covered the Prader Willi/Angelman syndrome critical region. Also overlapping this region is 15q13.3 microdeletion syndrome, with speech problems in around 16% of cases.[191] Speech dyspraxia, SSD and 'developmental or oro-motor dyspraxia with disarticulation' are reported in individuals with inherited and de novo deletions in this region.[188, 192, 193] A duplication near the end of 15q (15q26.3) in a proband with CAS and his affected and unaffected relatives was unlikely to be causative.[194]

An unbalanced 4q;16q translocation is reported in three siblings with CAS, language impairment, ID and dysmorphic features, inherited from their unaffected father with a balanced translocation.[195] The breakpoint at 4q35.2 may overlap a second chromosome 4 deletion in a child with CAS (4q35.1-35.2)[196] This child also carried a large chromosome 9 duplication (9p22.1-24.3)[196], thus the chromosome 4 locus was not clearly causative.

A 1q21.1 microdeletion is reported in one individual with CAS,[196] with 'articulation abnormalities' and poor non-word repetition a common finding in a cohort with 1q21.1 CNVs.[197] Eleven pathogenic 1q21.1 deletions are reported on the DECIPHER database in individuals with various phenotypes, including speech and language delay, ID and seizures.[198]

An 10q21.2-22.1 interstitial deletion was reported in identical twin boys with CAS and their unaffected mother.[64] Nine individuals with delayed speech and language development, global developmental delay or ID and likely pathogenic deletions within the 10q21.2-22.1 region are reported in the DECIPHER database.[198] Pathogenic 10q21.3 deletions, including the cell adhesion protein *CTNNA3* gene, are also implicated in the EAS syndromes.[199]

Linkage studies

Linkage studies have identified other chromosomal regions of interest. In one multiplex family described above,[12] motor sequencing ability (repetition of monosyllables vs.

disyllables vs. trisyllables) was used as an endophenotype in a subsequent linkage study.[200] Linkage mapping revealed four regions of interest (6p21, 7q32, 7q36, 8q23) however only the two chromosome 7 regions achieved non-parametric LOD scores above 3.[200] Presence of speech disorder was used to determine affected status in second linkage study in two families.[194] Linkage to 5p15.1-p14.1 and 17p13.1-q11.1 was reported in one family and *CDH18* identified as the primary gene of interest.[194] *ZGRF1* on 4q25-q28.2 was a gene of interest in the second family.[194]

Quantitative trait locus (QTL) mapping has been used to examine dyslexia candidate gene regions (DYX5 on 3p12-q13; DYX8 on 1p36; DYX2 on 6p22; DYX1 on 15q21).[201-204] Quantitative traits included speech skills, language skills, phonological short-term memory, phonological processing, and oromotor skills. QTL mapping determines any significant correlation between phenotypic similarity and identity-by-descent sharing (whether related individuals have inherited identical alleles from a common ancestor) in chromosome regions of interest.[205] Two studies found significant linkage for measures of phonological memory to chromosome 3 and DYX1 on 15q21.[201, 204] Suggestive linkage to chromosome 15, but not the DYX1 locus was reported in a separate study.[203] Suggestive linkage to chromosomes 3 and 6 for speech assessment measures (Goldman-Fristoe Test of Articulation, Percentage of Consonants Correct),[201, 204] and linkage to chromosome 1 has also been reported.[202, 204] These linkage studies used relatively small data sets (77 families[201]; 65[204]; 126[203];126[202]), and data from several hundred nuclear families are likely to be needed for sufficient power.[205]

Summary

Clinical and molecular genetic studies to date provide overwhelming evidence for a genetic aetiology for CAS and other SSDs. MZ twins with SSD show higher concordance than DZ twins, and probands with SSD and CAS have significantly more affected family members than those with unimpaired speech. Affected relatives have mild articulation disorders to more severe SSDs, language and literacy impairments. While most SSDs may be caused by a combination of multiple genetic variants, multiplex family studies demonstrate that some SSDs are monogenic. Recent gene technology advances have seen a surge in new genes and chromosome regions identified for SSD.

Aims of the present study

This thesis aims to use detailed clinical phenotyping to gain further insight into the underlying biology of speech and language disorders. 'Genotype-first' and 'phenotype-first' approaches will be adopted, which have proven successful in identifying new genetic causes of SSD, but their potential to further our understanding in this field has not been fully explored. The clinical phenotype associated with a novel *FOXP2* variant will be examined, as well as mutations in two developmental and epileptic encephalopathy genes, where speech and language impairments are noted but have not been studied in detail ('genotype-first'). The phenotype of affected and unaffected individuals from a multiplex family with speech disorder will also be analysed, and detailed phenotypic data will inform subsequent genotyping studies not part of this thesis ('phenotype-first'). For both approaches, a comprehensive assessment battery will be designed to characterize the clinical features presenting in individuals, with speech, language, oral motor function, cognition and literacy skills evaluated in depth. This detailed phenotypic data will inform differential diagnosis of each individual's speech and language disorder, with reference made to specific diagnostic criteria. The speech and language phenotype will then be compared across individuals, to identify and refine the speech and language features associated with particular genetic conditions. In the multiplex family, segregation of phenotypes will also be examined to determine mode of inheritance.

CHAPTER 2
METHODS

The thesis comprised four related projects examining the aetiology of speech and language disorders. The first three projects describe the speech and language phenotype associated with mutations in known disorders and genes, while study four focused on a familial speech disorder of unknown cause. Study one examined the speech and language phenotype of an individual with a novel mutation in *FOXP2*, the first gene identified for severe motor speech disorder in the absence of intellectual impairment. Study two analysed the speech phenotype associated with mutations in *GRIN2A*, a newly discovered cause of EAS syndromes that are inextricably linked to speech and language impairment. Study three examined the speech and language phenotype of one of the best known developmental and epileptic encephalopathies, Dravet syndrome. The focus of study four was a large multigenerational family with speech sound disorder, suspected of harbouring a new and as yet unidentified speech and language gene. The familial phenotype was comprehensively studied, which informed subsequent neuroimaging and gene finding studies that do not form part of this thesis.

Recruitment

FOXP2 study

Two probands with novel variants in *FOXP2* were recruited to the study. They were part of a cohort of eight probands with motor speech disorder identified from Professor Angela Morgan and Professor Ingrid Scheffer's 'Genetics of Speech Disorders' study. All eight probands were screened for mutations in *FOXP2*.[90] The two proband families agreed to participate, and four individuals with *FOXP2* variants and two unaffected relatives were studied.

GRIN2A study

Four probands with EAS syndromes and *GRIN2A* mutations were ascertained from a large cohort of 519 probands with a range of developmental and epileptic encephalopathies of unknown cause. The cohort was recruited from the epilepsy clinic at Austin Health and other neurology clinics in Australia and overseas, or were identified through Professor Scheffer's 'Genetics of Epilepsy' study. The cohort was tested for mutations in *GRIN2A*.[128] Four of the 519 probands had *GRIN2A* mutations; all had EAS syndromes and the *GRIN2A* variant segregated with the

disorder in their families. Three families agreed to participate, and eleven individuals with *GRIN2A* mutations were studied.

Dravet syndrome study

Patients with electroclinical features of Dravet syndrome (DS) and a confirmed *SCN1A* mutation participated. Patients were identified through Professor Scheffer's Dravet clinic at Austin Health. Twenty-six patients were invited to participate - three families refused and three were unavailable during the study. The final cohort comprised twenty patients with DS.

Multiplex family study

Large multiplex families with six or more affected individuals across several generations, suggestive of Mendelian inheritance of a speech and/or language disorder, were studied. The study was not restricted to one type of speech or language disorder, as suitable large families are relatively rare. Gene discovery has previously been successful in one family with CAS and dysarthria (KE family), thus families with motor speech disorder were of particular interest. Various sources were used to identify suitable large families (figure 2-1). A recruitment advertisement (Appendix 2) was sent to speech pathologists working in community health, early intervention and private practice to advertise the study in their clinic. It was also forwarded to the administrator of the Facebook group 'Families living with verbal dyspraxia' to advertise on their Facebook page. A short piece about the study was published in "Speak Out" magazine to advertise the study to members of Speech Pathology Australia (Appendix 3). Patients with a familial motor speech disorder were identified through a CAS clinic at The Royal Children's Hospital. Potential participants were asked to contact the research team directly if they were interested in the study. Alternatively, they consented for their contact details to be passed on by their treating speech pathologist or doctor. Probands were also identified through Professor Morgan and Professor Scheffer's other research studies (Genetic Basis of Epilepsy, Genetic Basis of Speech and Language Disorders, Genetics of Speech Disorders). All potential participants were contacted via mail or telephone and invited to participate in the study. Once recruited, participants were asked to inform other members of their family about the study and ask whether they would consent to participate.

Eighteen probands were identified and invited to participate (figure 2-1); nine consented to participate in the study, six did not respond to the initial recruitment letter and were unable to be contact via phone, and three did not return consent forms after speaking to the researcher. Clinical assessments were initiated in six families; three were unable to be seen during the study due to logistical constraints. One multigenerational family with CAS that looked likely to be segregating a gene of major dominant effect was targeted for detailed phenotyping, neuroimaging and molecular genetic analysis. Twenty-eight affected and unaffected family members from four branches of the family underwent phenotypic analysis and/or gave DNA samples. Individuals from three other branches of the family were contacted but did not return consent forms, and eight family members refused to participate in the study.

The study had approval from the Human Research Ethics Committees (HREC) at The Royal Children's Hospital (HREC 27053) and Austin Health (HREC H2011/04390). Written informed consent was obtained for all participants or from their parents in the case of minors or those with ID. All participants signed a Participant Information and Consent Form (Appendix 4).

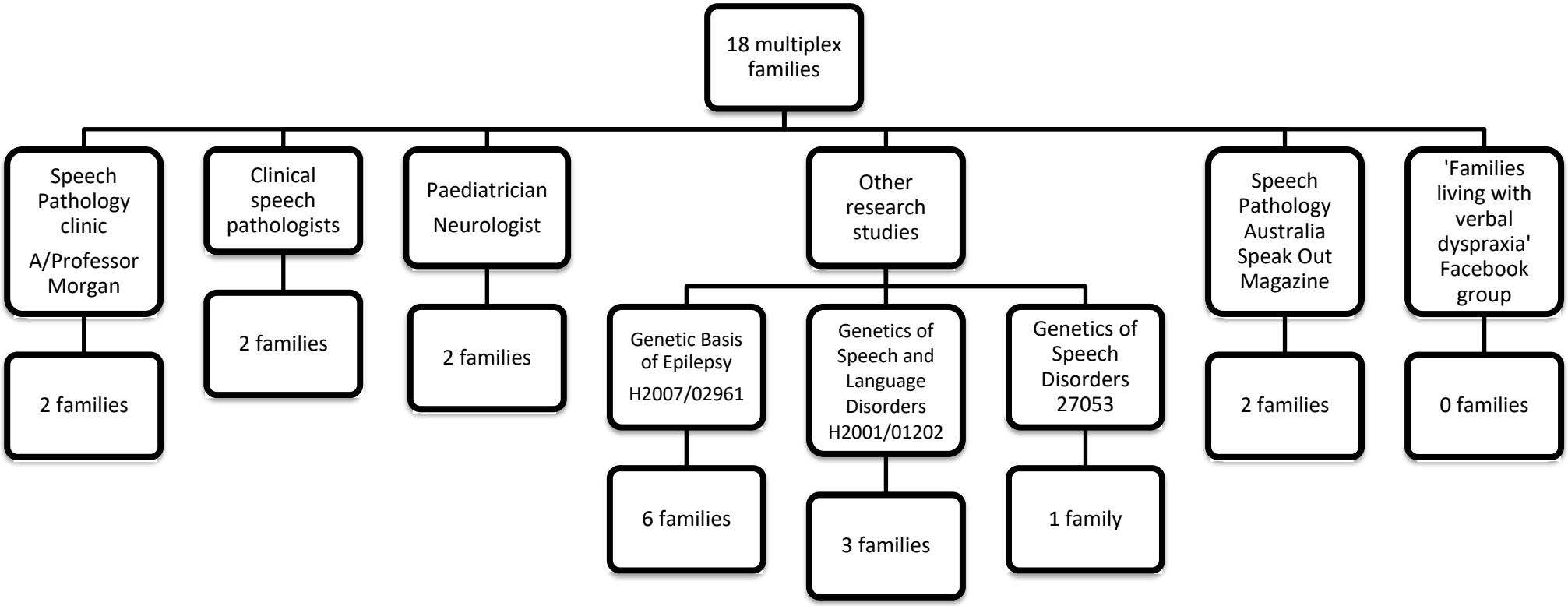


Figure 2-1. Sources used to identify suitable multiplex families

Assessment battery

A comprehensive assessment battery was used to focus on the speech and/or language phenotype presenting in each individual. Testing on related areas of literacy, cognition and oral motor function was also conducted, to support differential diagnosis and better interpretation of the speech and language phenotype. The battery was designed following an extensive review of the literature and comprised the latest measures to precisely characterise these behavioural traits. The battery was tailored to the age and ability of the participants. Different standardised assessments were used for children and adults to allow age-based standardised scores to be calculated. The battery was shortened for individuals with ID who were unable to complete all the measures, and an alternative battery was designed for non-verbal individuals with DS. This resulted in slight variation across the four studies (see table 2-1).

The assessment was typically 1½ to 2 hours per individual. Participants attended one or more appointments at the Melbourne Brain Centre or The Royal Children's Hospital for the assessment. For those participants living in regional Victoria, or living interstate or overseas, the assessment was conducted in the family home. Data collection involved the following Victorian, interstate and international field trips – Sydney (November 2011), New Zealand (July 2012), Bunyip (November 2012 and February 2015), Seymour and Avenel (January and March 2013), Brisbane (March and June 2013), Sydney (April 2013), Albury & Benalla (May 2013), Wallan and Kangaroo Flat (June 2013) and Healesville (July 2013).

Responses to the tasks were noted and clinical observations were made during the assessment. Audiovisual recordings were made for later review and analysis using a Marantz PMD671 digital recorder, Countryman Isomax headset microphone and a Sony DCR-SR85 digital camera. Speech tasks were transcribed using broad phonetic transcription, with diacritic marks used for misarticulations. A case history questionnaire was used to gather detailed information regarding birth, development and medical history, and history of communication or speech problems (Appendix 5). Assessment reports and therapy notes were obtained from the participant's treating speech pathologist where possible. Information regarding any relevant medical conditions and the results of past investigations (i.e. pure tone audiometry, EEG,

neuroimaging, genetic testing) were obtained from the hospital unit record or the participant's treating doctor.

To ensure the assessment data was robust, a second experienced clinician (ATM, speech pathologist, 15 years clinical experience) completed auditory-perceptual ratings of conversational speech samples and scored the oral motor assessments. Percentage agreement ratings were calculated to determine inter-rater agreement. In the case of discrepant ratings, the two clinicians discussed the scoring criteria and reached consensus on scoring.

Results from the assessment battery, clinical observations, case history information and previous assessment findings were analysed together to determine the speech and language phenotype presenting in each individual. The pattern of behaviours was compared with existing disorders, to determine how the speech or language disorder presenting in the individual fits with current classification.

Table 2-1 – Battery of standardised and informal assessments included in each study

	***FOXP2* study**	***GRIN2A* study**	**Dravet syndrome study**	**Multiplex family study**
Oral motor skills	8 year old proband Verbal Motor Production Assessment for Children (VMPAC) Adults - Frenchay Dysarthria Assessment, 2nd Edition (FDA-2)	FDA-2	Under 3 years or Minimally Verbal - Early Motor Control Scales 3 to 12 years - VMPAC 12 years and older - FDA-2	VMPAC OR FDA-2
Speech	Conversational speech sample Diagnostic Evaluation of Articulation and Phonology (DEAP) (proband only) Syllable Repetition task (SRT) (proband only) Nonword Memory Test OR Comprehensive Test of Phonological Processing (CTOPP) (adults only)	Conversational speech sample Nonword Memory Test Multisyllabic Word Repetition task Apraxia Battery for Adults, 2nd Edition Maximum vowel prolongation Monosyllable repetition rate (pa, ta, ka) Trisyllable repetition rate (pataka)	Minimally Verbal – Behavioural sample - Complexity of Communication Scale Verbal - Conversational speech sample DEAP	Conversational speech sample Goldman Fristoe Test of Articulation Nonword Memory Test OR CTOPP Nonword Repetition subtest (adults only) Children's Test of Non-Word Repetition OR SRT (children only) Multisyllabic Word Repetition task Tongue Twister task DEAP Inconsistency assessment Maximum vowel prolongation Monosyllable repetition rate (pa, ta, ka) Trisyllable repetition rate (pataka)

Table 2-1 (continued)

	FOXP2 study	*GRIN2A* study	Dravet syndrome study	Multiplex family study
Language	8 year old proband – Clinical Evaluation of Language Fundamentals, 4th Edition (CELF-4) Adults – Peabody Picture Vocabulary Test, 4th Edition (PPVT-4) Expressive Vocabulary Test, 2nd Edition (EVT-2) Test For Reception of Grammar, 2nd Edition (TROG-2)	Up to 21 years of age– CELF-4 Over 21 years of age – PPVT-4 EVT-2 TROG-2	Verbal patients (up to 7 years) Preschool Language Scales, 5th Edition (PLS-5) Verbal patients (5-21 years) CELF-4 OR PPVT-4/EVT-2/TROG-2 AND Children's Communication Checklist, 2nd Edition[206]	Up to 5 years of age - PLS-5 5 to 21 years – CELF-4 Over 21 years of age – PPVT-4 EVT-2 TROG-2
Cognition	8 year old proband – External clinical assessment with the Wechsler Intelligence Scale for Children, 4th Edition, Australian Standardized Edition (WISC-IV)[207] Adults – Wechsler Abbreviated Scale of Intelligence, 2nd Edition (WASI-II)	WASI-II	Bayley Scales of Infant and Toddler Development, 3rd Edition[208] OR WISC-IV OR WASI-II AND Vineland Adaptive Behaviour Scales, 2nd Edition[209] for estimation of Intellectual Disability range	Kaufman Brief Intelligence Test, 2nd Edition[210] Digit Span subtest from the WISC-IV OR Wechsler Adult Intelligence Scale, 4th Edition[211] Colour-Word Interference Task of the Delis-Kaplan Executive Function System[212] Behaviour Rating Inventory of Executive Function[213]
Phonological processing				CTOPP
Literacy	Wide Range Achievement Test, 4th Edition (WRAT-4)			WRAT-4

Phenotypic analysis

Oral motor structure and function

An oral motor assessment was conducted to examine the structural and functional integrity of the articulators, to support the differential diagnosis of speech disorder. The presence of craniofacial anomalies e.g. cleft lip/palate, indicated a structurally based speech disorder. Reduced strength, tone, accuracy, range of motion, or coordination of the muscles for speech suggested a motor speech disorder (dysarthria). Difficulty planning non-speech oral motor movement suggested oral motor apraxia.

The Verbal Motor Production Assessment for Children (VMPAC)[214] was used to assess speech and non-speech oral motor function in children aged 3 to 12 years. A review of standardised paediatric oral motor assessments found the VMPAC to be the most robust.[215] Administration time was approximately 30 minutes. Four subtests of the VMPAC were administered - Global Oromotor Control, Focal Oromotor Control, Sequencing and Connected Speech and Language. The Global Oromotor Control subtest evaluated parameters such as postural control and tone and adequate breath support for speech. The Focal Oromotor Control subtest examined quality of neuromuscular execution (e.g. symmetry and smoothness of movement) with individual and sequenced movements of the lips, tongue, jaw, and face assessed in non-speech and speech tasks. The Sequencing subtest examined the child's proficiency in producing sounds, syllables and words in sequence. Visual and tactile prompts were given if the individual failed to perform the task accurately following verbal instructions, with scoring across the three modalities (Verbal, Visual, Tactile). Some children with DS and ID had difficulty comprehending the verbal instructions and were confused when given a tactile prompt, thus their visual modality score was felt to be most representative of their performance. The Connected Speech and Language subtest examined lip, tongue and jaw movement and their interaction in connected speech. It also evaluated the child's ability to formulate a story based on four sequenced pictures. For each subtest, a percentage correct score was calculated and converted into a severity score with reference to normative data. Mild, moderate or severe severity scores on one or more subtests of the VMPAC indicated impaired oral motor functioning.

The Frenchay Dysarthria Assessment, 2nd Edition (FDA-2)[216] was used to examine non-speech oral motor skills in individuals older than 12 years of age. Administration time was approximately 20 minutes. The FDA-2 assesses general oral motor control (i.e. ability to move the lips and tongue etc.) and features of speech including pitch, volume and resonance. It is divided into seven sections (Reflexes, Respiration, Lips, Palate, Laryngeal, Tongue, Intelligibility). Each task was measured on a nine-point scale, and compared to normative data for adults without dysarthria, with scores of 7 or below indicative of oral motor impairment.

Some individuals who presented with abnormal nasal resonance were examined for features of submucous cleft palate and velopharyngeal insufficiency (see protocol – Appendix 6). Two individuals with *GRIN2A* mutations and abnormal nasal resonance were referred to an otolaryngeal surgeon, and underwent nasendoscopy to evaluate palatal structure and function.

The pre-publication version of the Early Motor Control Scales (EMCS)[217] was used with minimally verbal individuals with DS and ID unable to cooperate with standardised testing. The EMCS evaluates motor control of the voice, jaw, face, lips and tongue for accurate production of emerging speech. The 'Abnormal Structure and Function' and 'Predominant Combined Control – Motor Speech Control' subscales were rated using the behavioural sample collected for the Complexity of Communication Scale (see below). The 'Abnormal Structure and Function' subscale evaluates any abnormal structure/function, including jaw sliding, abnormal posture, excessive drooling, or asymmetrical facial structure. The 'Motor Speech Control' subscale evaluates movement of the jaw, face, lips, and tongue, with movement rated as *holistic*, *beginning differentiated* or *beginning independent*. A *beginning differentiated* movement pattern is noted in typically developing children aged 12-20 months, while independent control of voicing, jaw, face, lips and tongue is generally completed by 6-7 years.[217] Individuals were diagnosed with oral motor impairment if movement was rated as *holistic* or *beginning differentiated* across one or more speech subsystems (voicing, jaw, face, lips, tongue) in individuals aged 6 years and older.

Differential diagnosis of speech disorder

SSD

Speech production in single words and connected speech, and consistency of production were used to differentially diagnose the different SSD subtypes. Speech sound disorders were classified according to Dodd's Differential Diagnosis system[14]:

Articulation disorder: impaired ability to pronounce specific speech sounds (usually /s/ or /r/) with consistent substitution or distortion of the target sound in words or in isolation.

Phonological delay: all the error patterns derived to describe a child's speech that occur during normal development but are typical of younger children.

Consistent phonological disorder: consistent use of one or more unusual, non-developmental error patterns such as backing or initial consonant deletion.

Inconsistent phonological disorder: phonological system shows at least 40% variability of production, in the absence of oral motor difficulties.

Single word speech tasks included the Sounds-in-Words subtest of the Goldman-Fristoe Test of Articulation, Second Edition (GFTA-2)[218] or the Articulation and Phonology assessments of the Diagnostic Evaluation of Articulation and Phonology (DEAP).[219] These are standardised assessments that use a series of coloured pictures of objects and actions to elicit target sounds. The GFTA-2 has normative data up to 21 years, 11 months and the DEAP up to 8 years, 11 months. Administration time was approximately 15-20 minutes.

Connected speech tasks included reading a short passage (The Rainbow Passage), a picture description task (The Cookie Theft) and a ten-minute conversation with the researcher. These tasks are regarded as more indicative of an individual's functional communication.[220, 221] Administration time was approximately 12 minutes. For young children and children with ID who found it difficult to engage in conversation, a connected speech sample was collected during administration of the connected speech task of the VMPAC,[214] or the free play portion of the Preschool Language Scales, 5th Edition (PLS-5).[222]

The speech tasks were analysed to determine the individual's phonetic inventory and presence of articulation or phonological error patterns. Articulation errors were noted where a perceptually acceptable version of a particular phoneme was not produced in isolation or in any phonetic context.[14] Phonological errors affected syllable structure or involved substitution of one sound for another, and were classified with reference to the normative study by Barbara Dodd and colleagues.[223] Phonological errors were classified as age-appropriate (used by at least 10% of same-aged children in the normative sample), delayed (used by more than 10% of younger children but not same-aged children in the normative sample) or unusual (not used by more than 10% of children of any age in the normative sample).

Consistency of speech production was examined using the DEAP Inconsistency assessment, which measures token-to-token variability (variable production of a sound in multiple repetitions of the same word). Individuals were asked to name 25 pictures three times in one session, with other tasks administered in between. Administration time was approximately 20 minutes. Children up to seven years of age were regarded as having inconsistent speech production when at least 40% of words were produced inconsistently on this task. Inconsistent errors were regarded as atypical for individuals older than seven years of age (B. Dodd, personal communication). Inconsistent errors in repeated productions of syllables or words is also one of the key diagnostic criteria for CAS (see below).[17]

Where additional impairments were noted and the speech disorder could not be classified according to the above criteria, perceptual speech characteristics in conversational speech and maximum performance tasks were used to examine whether the individual had CAS or dysarthria.

CAS

CAS was diagnosed based on the presence of consensus diagnostic criteria identified by ASHA[17] with reference to the list of primary features summarised by Elizabeth Murray and colleagues:[224]

Inconsistent errors on consonants and vowels in repeated productions of syllables or words: determined as described above.

Lengthened and disrupted coarticulatory transitions between sounds and syllables: evidenced by slow repetition of trisyllables (pataka) or difficulty repeating the sequence correctly, sound errors such as intrusive schwa, epenthesis (insertion of sounds in a word), metathesis (rearranging sounds in a word), omissions and voicing errors, increasing errors with word length and phonological complexity, difficulty maintaining syllable integrity and/or groping.

Inappropriate prosody: evidenced by lexical stress errors, misplaced or equal stress in conversational speech or scores below 90% on the Prosody-Voice Screening Profile (PVSP).[225]

Dysarthria

Dysarthria was diagnosed based on the presence of perceptual speech characteristics that denoted deficits in respiration, articulation, pitch, loudness, voice quality, resonance and prosody, specified in the Mayo dysarthria classification system.[31, 226] Examples of deviant speech characteristics include audible inspiration and inhalatory stridor (respiration); imprecise consonants and prolonged phonemes (articulation); monopitch and pitch breaks (pitch); monoloudness (loudness); hoarse, harsh, breathy or strained strangled voice (voice quality); hypernasality or hyponasality (resonance); slow rate and excess and equal stress (prosody).

Audiovisual recordings of conversational speech were rated for perceptual speech features using the pen-and-paper version of PVSP (Study one) or a Dysarthria Rating Scale[227] (Studies two and three). For the PVSP, utterances in the conversational speech sample were coded for 31 inappropriate prosody-voice codes (15 prosody codes; 16 voice codes). The percentage of utterances with appropriate phrasing, rate, stress, loudness, pitch and quality was determined. Scores below 90% were indicative of inappropriate prosody-voice, one of the consensus diagnostic criteria for CAS. The Dysarthria Rating Scale comprised 32 items rated on a 4-point, 5-point or 7-point scale, that examined the different speech subsystems - prosody (including features of pitch, loudness, phrasing, rate, stress), respiration, phonation, resonance and articulation – as well as overall intelligibility. Scores of two or above on the 4-point and 5-point scale, and any score other than four on the 7-point scale were indicative of impairment, with

deviant speech characteristics across the different speech subsystems diagnostic for dysarthria.

Maximum performance tasks were used to determine the upper limits of subsystems required for accurate speech production (respiration, phonation, articulation) and to distinguish the different motor speech disorders.[226] Tasks included maximum prolongation of vowel /a/ and fricative /s/ and maximum repetition rate of monosyllables (pa, ta, ka) and trisyllables (pataka). Difficulty repeating a trisyllabic sequence or slow repetition rate are commonly reported features of CAS.[17] Reduced vowel prolongation and slow repetition of monosyllables are reported in children with spastic dysarthria.[228] Tasks were administered as per a published protocol.[228] Administration time was approximately 10 minutes. Three trials of each task were recorded and only responses judged to be phonetically correct were selected for further analysis. Maximum sound prolongation (/a/, /s/) was the duration in seconds of the best trial. For maximum repetition rate, ten monosyllables and 12 trisyllables were analysed, and the number of syllables repeated per second calculated. Maximum repetition rate of monosyllables was defined as the fastest correctly produced monosyllabic sequence. Maximum repetition rate of trisyllables was the fastest repeated sequence of pataka. Best performance on all tasks was compared with published data.[226, 229, 230]

Two subtests of the Apraxia Battery for Adults, 2nd Edition[231] were administered to adults with *GRIN2A* mutations to support differential diagnosis of CAS. Administration time was approximately 15 minutes. In the Repeated Trials subtest, individuals were asked to repeat a word three times, and their responses were examined to determine whether word production had improved, deteriorated or remained unchanged across the three trials. Trials 1 and 3 were compared to determine the amount of change between trials. In the Increasing Word Length subtest, individuals were asked to repeat words of increasing length (eg. thick, thicken, thickening) and their responses were scored on a scale from 0-2 (2 = correct response; 0 = no response or failed attempts to produce a word). Raw scores were used to determine the level of impairment (none, mild, moderate or severe) for each subtest.

Speech endophenotypes

Endophenotypes are measurable traits of a complex disease hypothesised to be less genetically complex than the disorder itself.[232, 233] They may clarify classification and diagnosis, and result in more straightforward genetic analysis.[232] Oral motor skills, phonological memory and phonological awareness are proposed endophenotypes of speech disorder that have been linked to various chromosomal regions (reviewed in [233]) Challenging speech tasks (repetition of multisyllabic real words, non-words and tongue twisters) were included in this study as biomarkers of speech dysfunction to simplify genetic analysis in speech disorder families. Non-word repetition tasks tap phonological encoding skills and phonological memory,[45, 234] and individuals with speech disorder and their immediate family members perform significantly worse on this and other challenging speech tasks compared to controls.[68, 235] For all tasks, individuals listened to a recording of an Australian speaker presenting the stimuli, and were instructed to repeat exactly what they had heard. Administration time was approximately 20 minutes.

The Children's Test of Nonword Repetition (CNRep) [236] was used with children aged 4-8 years. The CNRep is a standardised assessment consisting of 40 non-words, including ten 2-syllable, ten 3-syllable, ten 4-syllable, ten 5-syllable words. Standard scores and percentile ranks were determined with reference to normative data. The Nonword Memory Test[237] or the Nonword Repetition subtest from the Comprehensive Test of Phonological Processing (CTOPP)[238] were used for individuals older than 8 years. The Nonword Memory Test consists of 28 non-words, with an equal number of 2-syllable, 3-syllable, 4-syllable and 5-syllable words. The total number of words correctly repeated was compared to mean scores (+/- standard deviation) for the normative sample. The CTOPP Nonword Repetition task has 18 items of increasing length, including one 7-syllable word. Scaled scores were calculated with reference to normative data, with norms available up to 24 years, 11 months.

The Multisyllabic Word Repetition task[234] and the Tongue Twister task[239] were administered to individuals 8 years and older. The Multisyllabic Word Repetition task consists of 52 words that are generally familiar to school age children. Words are three to six syllables in length and contained complex phonetic sequences (consonant clusters and/or limited phonetic differentiation across syllables). The Tongue Twister task includes ten tongue twisters around eight syllables in length, and ten control sentences

matched for syntactic complexity, syllable count and sentential stress pattern. Raw scores were calculated as the number of items correctly repeated. Scores on both tasks were compared to the mean scores (+/- standard deviation) of individuals with a history of moderate-severe speech disorder and aged-matched individuals with typical speech.[235]

For children with a reduced phonetic inventory or severe speech disorder, the Syllable Repetition Task (SRT)[240] was used. The 18 stimulus items are presented by an American speaker, and include eight 2-syllable (CVCV), six 3-syllable (CVCVCV) and four 4-syllable (CVCVCVCV) items incorporating four early occurring consonants (/b/, /d/, /m/, /n/) and one early occurring vowel. SRT score was calculated as the percentage of correct consonants (Number of correct consonants/Total number of consonants x 100) with percentage scores compared to the normative sample.[241]

In the Multiplex family study, incorrect responses on the non-word and multisyllabic word repetition tasks were analysed for EMA (epenthesis, metathesis, assimilation) errors. There is consensus in the literature that these errors are diagnostic for CAS.[242] Epenthetic errors were defined as additions of across-manner sounds (eg. /ɔrkrɪstrə/ for orchestra), metathetic errors were rearrangement of target sounds within words (eg. /sɪmənɪm/ for cinnamon) and atypical assimilation errors were where a target sound was changed to resemble another sound in the word (eg. /pɛrərɛl/ for parallel). EMA errors were counted if they occurred at least twice in two different words.[243]

Early expressive communication

The Complexity of Communication Scale (CCS)[244] was used to measure early expressive communication in minimally verbal individuals with DS. The CCS is an ordinal scale with eleven levels encompassing pre-intentional, intentional and symbolic forms of communication. The CCS was rated using a behavioural sample collected based on a scripted interaction, which took into account the individual's preferred objects and activities. During the 20-30 minute interaction, the individual was given opportunities to communicate when a desired activity was stopped or they required help to access a desired object. The individual's communication behaviour for each opportunity was rated using detailed scoring guidelines (N Brady, personal

communication). The highest CCS score corresponded to the individual's best communicative performance.

Language disorder

Standardised language assessments were used to examine receptive and expressive language functioning in children and adults. Administration time was up to one hour. Raw scores were converted to standard scores with reference to normative data.

The PLS-5[222] was used with children up to 4 years, 11 months of age, or up to 7 years, 11 months for children with ID. The Auditory Comprehension subscale includes tasks assessing comprehension of basic vocabulary, concepts and grammatical markers. The Expressive Communication subscale requires individuals to name common objects, use concepts that describe objects and express quantity, use specific prepositions, grammatical markers and sentence structure.

The Clinical Evaluation of Language Fundamentals, 4th Edition (CELF-4)[245] was used with individuals aged 5 to 21 years of age. The Receptive Language Index score measures performance on tasks assessing comprehension of grammatical rules, relationships between words, and the ability to follow oral commands containing functional language. The Expressive Language index score measures performance on tasks evaluating the ability to recall and reproduce sentences of varying length and complexity, to formulate complex sentences and to complete sentences using grammatical rules.

For adults, the Peabody Picture Vocabulary Test, 4th Edition (PPVT-4)[246] was used to examine receptive vocabulary skills. The Expressive Vocabulary Test, 2nd Edition (EVT-2)[247] and the Test For Reception of Grammar, 2nd Edition (TROG-2)[248] were used to evaluate expressive vocabulary and comprehension of grammatical contrasts respectively. The PPVT-4 and EVT-2 were also used for children older than 7 years, 11 months with ID who were unable to be examined using the CELF-4.

Receptive language disorder was diagnosed when an individual received a standard score of 80 or below on the PLS-5 Auditory Comprehension subscale, the CELF-4 Receptive Language Index, or on the PPVT-4 or the TROG-2. Expressive language disorder was diagnosed when the individual received a standard score of 80 or below

on the PLS-5 Expressive Communication subscale, CELF-4 Expressive Language Index or the EVT-2. Receptive and/or expressive language disorder was also diagnosed if the individual previously scored below the average range on a standardized language assessment, as documented in their speech pathology assessment report.

Phonological processing

The CTOPP was used to assess phonological processing skills in individuals aged 5 to 24 years.[238] Standardised assessment tasks examined phonological awareness (the ability to attend to, identify and manipulate sounds in spoken words), phonological memory (use of the speech sound system to store information in short term memory) and rapid naming (retrieval of sound-based information from long-term memory). Children with speech disorder perform poorly on phonological awareness tasks compared to peers, with phonological awareness a strong predictor of reading ability, while phonological memory and speeded naming are proposed endophenotypes of speech and language disorder (reviewed in [249]). Administration time was approximately 30 minutes. Composite standard scores were calculated with reference to normative data.

Reading and spelling impairment

Literacy difficulties are often comorbid with speech sound disorder and language impairment.[55] The Wide Range Achievement Test, 4th Edition (WRAT-4)[250] was used to assess literacy skills in individuals aged five to 94 years. Three subtests of the WRAT-4 were administered (Word Reading, Sentence Comprehension, Spelling). Administration time was approximately 15 minutes. The Word Reading subtest examined letter identification and recognition of single words, while the Sentence Comprehension subtest measured the ability to comprehend ideas and information contained in sentences. Scores from these subtests were combined into a Reading Composite score. The Spelling subtest examined the ability to encode sounds into written form through a dictated spelling format. Standard scores for each subtest were calculated with reference to normative data.

A past history of reading or spelling difficulty was also gleaned from speech pathology reports or the case history questionnaire.

A diagnosis of reading or spelling impairment was made if an individual scored 84 or below on at least one of the WRAT-4 subtests (Word Reading, Sentence Comprehension, Spelling) OR they had previously performed below the average range on a standardised literacy assessment as documented in their speech pathology assessment report OR they reported having extra support with reading or spelling in the classroom i.e. a modified spelling program or integration aide support.

Cognitive impairment

In the *FOXP2* and *GRIN2A* studies, verbal and non-verbal cognitive skills were examined using the Wechsler Abbreviated Scale of Intelligence, 2nd Edition (WASI-2).[251] The Full Scale IQ score gives an estimate of general cognitive ability. The four subtest form (Block Design, Matrix Reasoning, Vocabulary, Similarities) was administered, or the two subtest form (Vocabulary, Matrix Reasoning) when there were time constraints during testing. Perceptual Reasoning and Verbal Comprehension Index scores were calculated on the four-subtest form. The Perceptual Reasoning Index measures non-verbal abilities and visuomotor/coordination skills, while the Verbal Comprehension Index measures verbal reasoning ability. Scores were calculated with reference to normative data.

In the Multiplex family and Dravet syndrome studies, a neuropsychologist completed the cognitive assessment. For the multiplex family study, this was part of a broad neuropsychological battery that examined intellectual functioning, memory and learning and executive functioning. Standardised cognitive assessments were chosen based on the age and intellectual ability of the individual (table 2-1).

A diagnosis of ID was made by a neuropsychologist, and was based on an intelligence quotient of 70 or below, and in the Dravet syndrome study, deficits in at least two areas of adaptive behaviour (communication, self care, home living, social skills, self direction, leisure and work, learning).

Pedigree analysis

In the Multiplex family study, the pattern of phenotypes across family members was scrutinized to identify unique phenotypic clustering, and to determine whether the mode of inheritance was consistent with autosomal dominant, autosomal recessive, X-

linked, mitochondrial or polygenic inheritance. SNP genotyping and whole exome sequencing were subsequently conducted by molecular collaborators, with the aim of finding the causative gene/s (data not part of this work).

CHAPTER 3

SMALL INTRAGENIC DELETION OF *FOXP2* ASSOCIATED WITH CAS AND DYSARTHRIA

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Small Intragenic Deletion in *FOXP2* Associated With Childhood Apraxia of Speech and Dysarthria

Samantha J Turner,[1] Michael S. Hildebrand,[2] Susan Block,[3] John Damiano,[2] Michael Fahey,[4] Sheena Reilly,[1,5] Melanie Bahlo,[6,7] Ingrid E. Scheffer,[1,2,8]* and Angela T. Morgan[1,5]

[1]Department of Paediatrics, The Royal Children's Hospital, The University of Melbourne, Parkville, Victoria, Australia

[2]Department of Medicine, The University of Melbourne, Austin Health, Heidelberg, Victoria, Australia

[3]Department of Human Communication Sciences, La Trobe University, Bundoora, Victoria, Australia

[4]Department of Paediatrics, Monash University, Clayton, Victoria, Australia

[5]Population Health, Genes and Environment Theme, Murdoch Childrens Research Institute, Parkville, Victoria, Australia

[6]Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

[7]Department of Mathematics and Statistics, The University of Melbourne, Parkville, Victoria, Australia

[8]Florey Institute, Melbourne, Victoria, Australia

Manuscript Received: 21 November 2012; Manuscript Accepted: 25 April 2013

Relatively little is known about the neurobiological basis of speech disorders although genetic determinants are increasingly recognized. The first gene for primary speech disorder was *FOXP2*, identified in a large, informative family with verbal and oral dyspraxia. Subsequently, many de novo and familial cases with a severe speech disorder associated with *FOXP2* mutations have been reported. These mutations include sequencing alterations, translocations, uniparental disomy, and genomic copy number variants. We studied eight probands with speech disorder and their families. Family members were phenotyped using a comprehensive assessment of speech, oral motor function, language, literacy skills, and cognition. Coding regions of *FOXP2* were screened to identify novel variants. Segregation of the variant was determined in the probands' families. Variants were identified in two probands. One child with severe motor speech disorder had a small de novo intragenic *FOXP2* deletion. His phenotype included features of childhood apraxia of speech and dysarthria, oral motor dyspraxia, receptive and expressive language disorder, and literacy difficulties. The other variant was found in a family in two of three family members with stuttering, and also in the mother with oral motor impairment. This variant was considered a benign polymorphism as it was predicted to be non-pathogenic with in silico tools and found in database controls. This is the first report of a small intragenic deletion of *FOXP2* that is likely to be the cause of severe motor speech disorder associated with language and literacy problems.

Key words: childhood apraxia of speech; forkhead box P2 gene; genotype–phenotype correlations; intragenic deletion; speech and language disorders

How to Cite this Article:
Turner SJ, Hildebrand MS, Block S, Damiano J, Fahey M, Reilly S, Bahlo M, Scheffer IE, Morgan AT. 2013. Small intragenic deletion in *FOXP2* associated with childhood apraxia of speech and dysarthria.
Am J Med Genet Part A 161A:2321–2326.

S.J. Turner, M.S. Hildebrand, I.E. Scheffer, and A.T. Morgan contributed equally to this work.

Conflict of interest: none.
Grant sponsor: Australian Research Council (ARC) Discovery Project; Grant number: DP120100285; Grant sponsor: National Health & Medical Research Council (NHMRC) CJ Martin Overseas Biomedical Postdoctoral Training Fellowship; Grant sponsor: NHMRC Dora Lush Postgraduate Scholarship; Grant sponsor: NHMRC Career Development Award; Grant number: 607315; Grant sponsor: Victorian Government's Operational Infrastructure Support Program ARC Future Fellowship.

Abbreviations: *CNTNAP2*, contactin-association protein-like 2; CAS, childhood apraxia of speech; *FOXP2*, forkhead box P2 gene; *SRXP2*, sushi-repeat containing protein X-linked 2.

*Correspondence to:
Professor Ingrid Scheffer, Epilepsy Research Centre, The University of Melbourne, Melbourne Brain Centre at Austin Health, 245 Burgundy Street, Heidelberg, Victoria 3084, Australia.
E-mail: scheffer@unimelb.edu.au

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 5 August 2013
DOI 10.1002/ajmg.a.36055

INTRODUCTION

Genetic determinants are increasingly recognized as underpinning the neurobiology of speech disorder. *FOXP2* was the first gene associated with severe speech disorder, identified in the large "KE family" [Lai et al., 2001]. Point mutations, large deletions (including contiguous gene deletions), and chromosomal structural variations including translocations and uniparental disomy have been reported [MacDermot et al., 2005; Feuk et al., 2006; Shriberg et al., 2006; Zeesman et al., 2006; Lennon et al., 2007; Palka et al., 2012; Rice et al., 2012; Zilina et al., 2012]. In a cohort with severe speech disorder, the prevalence of etiological variants is 2% [MacDermot et al., 2005], with *FOXP2* haploinsufficiency felt to underlie the speech phenotype [Lai et al., 2001].

Delineating the phenotype(s) associated with different *FOXP2* mutations is challenging. In 26 cases with mutations and reported speech and language data (Supplementary Fig. in supporting information online) the core phenotype is a severe motor speech disorder, with most cases having verbal dyspraxia, also known as childhood apraxia of speech (CAS). CAS is a disorder of speech motor programming in which there is impaired precision and consistency of speech movements [ASHA, 2007]. Oral motor dyspraxia may also occur [Alcock et al., 2000; Vargha-Khadem et al., 1998]. Speech is often "unintelligible," with omission, substitution, and distortion of consonants and vowels, inconsistent errors across multiple repetitions and prosodic impairments [Hurst et al., 1990; Feuk et al., 2006; Shriberg et al., 2006; Zeesman et al., 2006; Rice et al., 2012]. Spastic dysarthria can also occur with hypernasality, impaired laryngeal quality, and difficulties modulating pitch and loudness [Morgan et al., 2010; Shriberg et al., 2006]. Severe receptive and expressive language disorder usually occurs [Tomblin et al., 2009; Vargha-Khadem et al., 1995; Zeesman et al., 2006]. In terms of cognition, verbal skills are generally poorer than non-verbal skills with average non-verbal skills documented in some cases [Rice et al., 2012; Tomblin et al., 2009; Vargha-Khadem et al., 1995]. Reading and spelling impairments are also reported [Rice et al., 2012; Vargha-Khadem et al., 2005].

FOXP2 encodes a transcription factor that acts to either repress or activate gene expression, with hundreds of neural targets identified [Spiteri et al., 2007; Vernes et al., 2007]. These include *CNTNAP2* and *SRPX2* that are associated with speech and language disorders, brain malformations, autism, epilepsy, and schizophrenia [Gregor et al., 2011]. Interestingly, a novel *FOXP2* missense mutation has also been associated with polymicrogyria and epilepsy [Roll et al., 2010].

We recruited eight Australian families in which the proband had a speech disorder. We phenotyped the patients and their families and sequenced the coding regions of *FOXP2*. We identified novel variants in two probands. We present the genotype–phenotype correlation of these cases.

CLINICAL REPORT

Family A—A-II-1

A-II-1 is an 8-year-old boy with severe motor speech disorder.

History. A-II-1 was born at term by emergency caesarian for fetal distress. He was born in good condition with Apgar scores of 6 at 1 min and 9 at 5 min. In the neonatal period, he commenced bottle-feeding as he had difficulty attaching to the breast despite an adequate suck. He has a history of mild oral dysphagia, and at 8 years had difficulty chewing with poor bolus formation and clearance. His speech, oral-motor, and motor milestones were delayed (crawling 11 months, walking 19 months, excessive drooling up to 18 months, two words together 3 years). He had an early history of conductive hearing loss. Ventilation tubes were inserted at 1, 2, and 4 years, and adenoidectomy was performed at 4 years. Hearing was normal at 4.5 years. Moderate to severe receptive and expressive language impairment was diagnosed at 2 years and at 6 years 2 months he scored below the 1st centile (Clinical Evaluation of Language Fundamentals 4th edition, CELF-4; Semel et al., 2006). Motor apraxia was diagnosed at 5.5 years, impacting on fine motor skills. He had fortnightly speech and occupational therapy from 2 to 5 years, then ongoing weekly therapy, with little change in presentation over time. Cognitive assessment at 7 years 3 months (Wechsler intelligence scale for children, 4th edition; Wechsler, 2004) revealed borderline verbal comprehension (IQ 71) with average perceptual reasoning (IQ 94). Working memory (IQ 77) and processing speed (IQ 70) were borderline.

Neurological examination at 7 years 5 months showed mild tremor and cogwheel rigidity. He had four beats of clonus bilaterally in the lower limbs with brisk reflexes without spread and downgoing plantar responses. He had a decreased ability to copy hand shapes and ocular motor apraxia. His strabismus was surgically corrected. MRI brain scan and electroencephalogram were normal.

A-II-1's speech and oral motor skills, language and literacy skills were evaluated at 8 years (see Table I).

Speech and oral motor skills. The Diagnostic Evaluation of Articulation and Phonology (DEAP) [Dodd et al., 2002] and conversational speech sample were rated for phonological and articulation errors. He demonstrated a reduced consonant inventory, with /v, z, θ, ð, ʃʒ, ʒ, ʤ/ absent. He was unable to imitate consonant sounds in isolation. Articulatory production was imprecise with prolongation of vowels and reduced strength of articulatory contacts. Quantitative analysis showed only 55 Percent Phonemes Correct (PPC), with 39% consonants and 72% vowels produced correctly. Delayed phonological processes were prominent including fronting, backing, cluster reduction, weak syllable deletion, final consonant deletion, gliding, and deaffrication. He had an atypical error pattern, replacing plosives, approximants and fricatives for the glottal fricative /h/ (e.g., "tiger" to "haiher").

Typical features of CAS were noted. He showed inconsistent errors in repeated productions of words (DEAP Inconsistency subtest score 72%). He performed poorly on a nonsense syllable repetition task (52% consonants correct; Syllable Repetition task; Shriberg et al., 2009). On a perceptual rating tool (Prosody-Voice Screening Profile; Shriberg et al., 1990), <90% utterances in spontaneous speech were scored as appropriate (considered a fail) on suprasegmentals of prosody (rate 67%, stress 25%) and voice (pitch 83%, quality 0%). Speech was slow and sounded "singsong" due to misplaced stress. He had abnormal mixed nasality, nasal emission and at times had a harsh vocal quality. Taken together, these features made his speech sound markedly abnormal and greatly impacted on his intelligibility.

TABLE I. Standardized Assessment Results for Family A and B

		A-II-1	A-I-1	A-I-2	B-I-1	B-I-2	B-II-1	B-II-2
	Age at assessment	**8 y**	**41 y**	**37 y**	**55 y**	**54 y**	**31 y**	**29 y**
Nonword Repetition	Syllable repetition task[a]	52%	—	—				
	Nonword Memory Test[b]	—	20	21				
	Nonword Repetition[c]	—	—	—	10/18	11/18	11/18	11/18
Oral motor skills	VMPAC/FDA	Oral-motor apraxia	WNL	WNL	Marked oromotor impairment	Imprecise tongue and lip movement in speech	Reduced lip rounding	Imprecise tongue movement in speech
Language								
Receptive	CELF-4[d]	55	—	—				
	PPVT-4[d]	—	100	92	90	114	104	88
	TROG-2[d]	—	95	90				
Expressive	CELF-4[d]	45	—	—				
	EVT-2[d]	—	102	105				
Cognition (WISC/WASI)	Full scale IQ[d]	73	97	111	88	92	114	88
	Verbal[d]	71	80	104				
	Performance[d]	94	116	117				
	Working memory[d]	77	—	—				
	Processing speed[d]	70	—	—				
	Digit span[e]	4	13	9				
Literacy	WRAT-4 word reading[d]	70	97	106	—	—	—	—
	WRAT-4 spelling[d]	72	89	106				
	WRAT-4 sentence comprehension[d]	—	99	99				

WNL: within normal limits.
Syllable repetition task [Shriberg et al., 2009]; Nonword Memory Test [Gathercole and Baddeley, 1996]; Nonword Repetition [Comprehensive Test of Phonological Processing] [Wagner et al., 1999]; VMPAC: Verbal Motor Production Assessment for Children [Hayden and Square, 1999]; FDA: Frenchay Dysarthria Assessment [Enderby, 2003]; CELF-4: Clinical Evaluation of Language Fundamentals 4th Edition, Australian standardised edition [Semel et al., 2006]; PPVT-4: Peabody Picture Vocabulary Test [Dunn and Dunn, 2007]; TROG-2: Test for Reception of Grammar, 2nd edition [Bishop, 2003]; EVT-2: Expressive Vocabulary Test, 2nd edition [Williams, 2007]; WISC-4: The Wechsler Intelligence Scale for Children, 4th edition [Wechsler, 2004]; WASI: The Wechsler Abbreviated Scale of Intelligence [Wechsler, 1999]; WRAT-4: The Wide Range Achievement Test, 4th edition [Wilkinson and Robertson, 2006].
[a]Mean = 90%, SD = 5.7 (males 6–8 yrs).
[b]Mean = 22.05, SD = 4.39.
[c]Mean = 10/18.
[d]Mean = 100, SD = 15.
[e]Mean = 10, SD = 3.

He had a severe speech and non-speech oral motor impairment. He scored below the 5th centile on Global Oromotor Control, Focal Oromotor Control, and Sequencing subtests of the Verbal Motor Production Assessment for Children (VMPAC) [Hayden and Square, 1999]. Precision and sequencing of non-speech and speech movement were severely affected. Movements involving the lips (e.g. blow) and tongue (e.g. stick out tongue) were poorly executed. He was unable to imitate strings of consonants and vowels (e.g. kata, pataka–pataka–pataka–pataka). He also had reduced tongue strength and contraction of orofacial muscles. Thus features of both dysarthria and CAS were seen.

Language. Assessment on the CELF-4, Australian Standardized Edition [Semel et al., 2006] revealed severely impaired receptive (0.1 centile) and expressive (<0.1 centile) language skills.

Literacy. The Wide Range Achievement Test, 4th edition (WRAT-4) [Wilkinson and Robertson, 2006] revealed severely impaired word reading (2nd centile) and spelling skills (3rd centile). His phonological awareness was severely impaired (3rd centile) on the Comprehensive Test of Phonological Processing [Wagner et al., 1999].

His parents, A-I-1 and A-I-2, demonstrated average performance on non-word repetition, cognition, language, and literacy tests (Table I). A-I-1, his 41-year-old father, reported mild articulation impairment as a child, with substitution of /θ/ for /f/ (e.g. "**th**ing" to "**f**ing") persisting as an adult. Oral motor skills were normal. He had a positive family history of speech/language difficulties.

Family B

History. Gestational maternal toxemia was reported for B-I-2. She has bipolar affective disorder, and voice and swallowing difficulties (choking, nasopharyngeal reflux) presenting in adulthood. B-II-2 had neonatal feeding difficulties and delayed milestones (walking 18 months, first words 18–24 months). He has a history of migraines and a suspected tonic–clonic seizure at 18 years. MRI brain scan and electroencephalogram were normal in B-I-2 and B-II-2. All family members received tuition for reading and comprehension difficulties. There is a history of stuttering in B-I-1's extended family.

Speech and oromotor skills. B-I-1, B-II-1, and B-II-2 all presented with moderate-severe stuttering characterized by repeti-

tions, prolongations, blocks, fillers, circumlocution, and word avoidance. Speech rate was normal. Structural abnormalities, oromotor dyspraxia, or dysarthria were not evident. B-I-2 presented with a perceptibly high-pitched, rough, strained, and breathy voice and some nasal resonance. She has no history of speech difficulties. All family members demonstrated average performance on nonword repetition, cognition, and receptive vocabulary tests (Table I).

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committees of The Royal Children's Hospital and Austin Health.

PCR and Sanger Sequencing

FOXP2 was amplified using gene-specific primers designed to the reference human transcript (Ensembl ID: ENSG00000128573; http://www.ensembl.org). Oligonucleotide sequences are available on request. Amplification reactions were cycled using a standard protocol on a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA). Bidirectional sequencing of all exons and flanking regions was completed with a BigDye v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. Sequencing products were resolved using a 3730xl DNA Analyzer (Applied Biosystems).

RESULTS

Eight probands were sequenced. Their phenotypes included CAS in seven and stuttering in one. Direct sequencing of the 17 coding exons and splice site boundaries of *FOXP2* revealed novel variants in families A and B (Fig. 1). The variant in family A was de novo whilst the variant in family B was observed in three individuals, including two affected with stuttering. The de novo variant was a novel two base-pair deletion (c.1243_1244delCA) in individual A-II-1 (Fig. 1A,B), predicted to lead to a glutamine to valine substitution at position 415 followed by a frameshift and then a premature stop codon (p.Gln415Val*5). This deletion was not present in 192 ethnically matched Australian Caucasian blood bank controls (384 chromosomes), dbSNP132 (NCBI), 1000 Genomes (Wellcome Trust Sanger Institute and Harvard Medical School), or Exome Variant Server (EVS; University of Washington, 6,503 individuals) databases. We identified a novel missense variant (c.1321C>A) in three individuals (Fig. 1C), predicted to lead to a proline to threonine substitution at position 441 (p.P441T; Fig. 1D). Although the variant was not present in the matched Australian controls, the dbSNP132 or 1000 Genomes databases, it was present in the EVS database (1/6,503 individuals). The Grantham score for P441T was 38, while PolyPhen-2 and SIFT predicted it to be "probably damaging" and "tolerated", respectively.

DISCUSSION

Five point mutations, one insertion, 16 large deletions, and four chromosomal rearrangements of *FOXP2* have previously been reported [Zeesman et al., 2006; Lennon et al., 2007; Palka et al., 2012; Rice et al., 2012; Zilina et al., 2012]. The de novo deletion in A-II-1 is the first small intragenic deletion reported in *FOXP2*. It is likely to be pathogenic as it occurs before the forkhead DNA-binding domain and is predicted to lead to haploinsufficiency for this critical functional domain via truncation of the protein or nonsense-mediated decay of the transcript. An RNA sample could not be obtained from the patient to confirm the effect of the deletion.

A-II-1's clinical presentation is consistent with other *FOXP2* mutation cases. He has a severe motor speech disorder, with features of CAS and dysarthria. The features of CAS included inconsistent errors, lengthened and disrupted coarticulatory transitions (prolonged vowels, sound/syllable deletions, difficulty sequencing strings of sounds), and inappropriate prosody characterized by slow rate and stress errors [ASHA 2007]. Other commonly proposed characteristics of CAS [ASHA, 2007] present in A-II-1 included oral motor dyspraxia, impaired diadochokinesis, vowel errors, sounds produced spontaneously but not on imitation and atypical phonological errors. He also showed impaired nonword repetition reflecting similar findings in the KE family [Vargha-Khadem et al., 1998]. Dysarthric features in A-II-1 included imprecise production of consonants, abnormal nasal resonance, nasal emission, and harsh vocal quality. Co-morbid speech dyspraxia and dysarthria have only been reported rarely with *FOXP2* mutations: in the KE family who had a missense mutation, and two parent-child pairs, one with a chromosome deletion and another with a translocation [Shriberg et al., 2006; Morgan et al., 2010; Rice et al., 2012]. Taken together, this suggests that the FOXP2 speech phenotype is not restricted to impaired motor speech planning/programming, as in CAS, but is also associated with impairments in speech execution [Morgan et al., 2010].

In addition, A-II-1 has concomitant receptive and expressive language disorder and impaired literacy. These impairments are not due to a global cognitive deficit as he has average non-verbal cognitive skills, also reported in other *FOXP2* mutation cases [Rice et al., 2012]. While language impairments are common, literacy impairments associated with *FOXP2* mutations have rarely been examined and may be more prevalent than recognized to date. Impaired word reading, spelling and phonological awareness skills are seen in A-II-1, as noted in the KE family and a mother with 7q31 deletion [Rice et al., 2012; Vargha-Khadem et al., 1995]. Our findings support earlier studies that *FOXP2* is associated with severe motor speech disorder, oral-motor and motor dyspraxia, language and literacy impairments.

The novel missense variant in family B is of unknown significance. It was identified in two of the three individuals with stuttering who had no features of CAS or dysarthria. It was also found in a third family member with marked oral motor impairments, a history of swallowing difficulties and features of dysarthria. Stuttering has not previously been associated with *FOXP2* mutations. However, sound/syllable/word repetitions, articulatory blocks, and prolongations were reported in a mother and daughter with a t(7;13)(q31.1:q13.2) translocation, with the breakpoint disrupting *FOXP2* [Shriberg et al., 2006]. From a molecular viewpoint, the P441 residue is highly conserved and not present in controls, however it is present on the EVS database (1/6503 individuals) in an individual in whom the stuttering phenotype may not have been studied. The variant is not predicted to be pathogenic by pathogenicity prediction tools PolyPhen-2 [Adzhu-

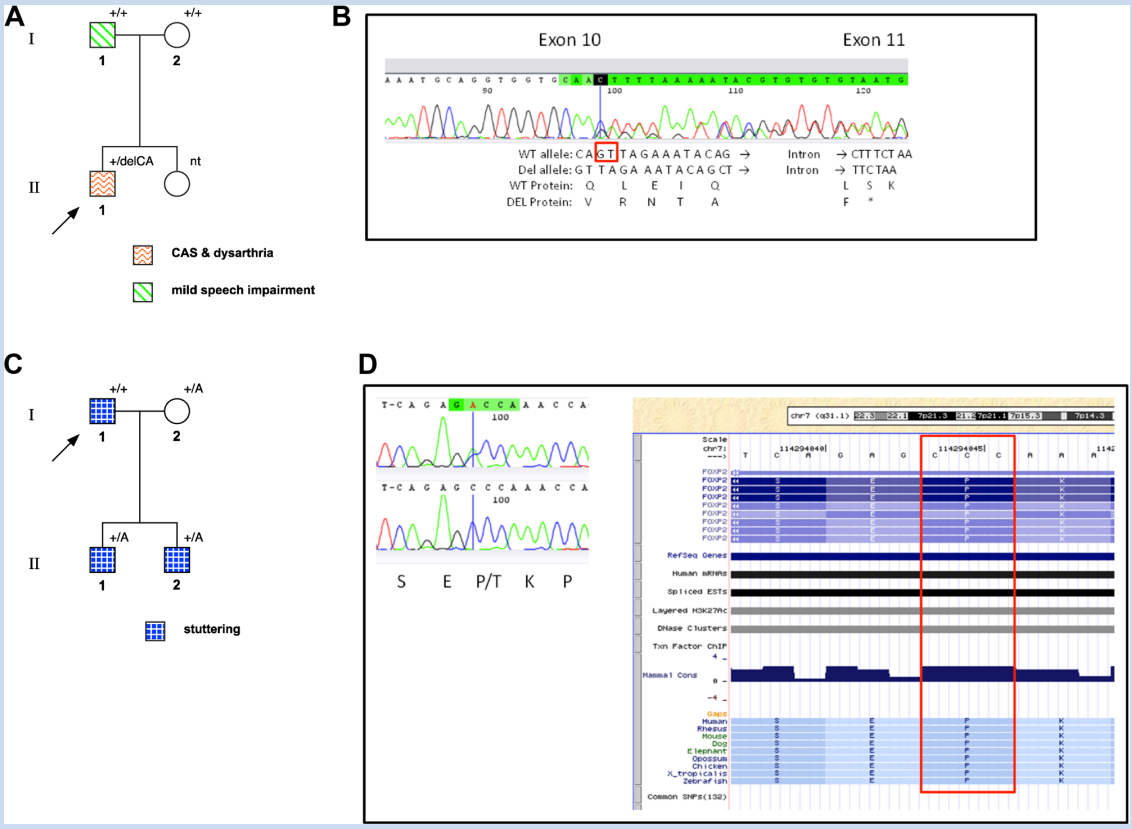


FIG. 1. Pedigree and genotypic data for families A and B. A: Pedigree of family A. Genotypes for the c.1243_1244 nucleotides are shown. B: De novo deletion (c.1243_1244delCA) in individual A-II-1. C: Pedigree of family B. Genotypes for the c.1321 nucleotide are shown. D: Missense variant (c.1321C>A; p.P441T) in individuals B-I-2, B-II-1 and B-II-2 (top left) that was not identified in controls (bottom left). Conservation of the p.P441 residue in the FOXP2 protein as depicted on the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) is shown on the right.

bei et al., 2010] and SIFT [Kumar et al., 2009]. Given the lack of segregation with the stuttering phenotype and weak evidence to support molecular pathogenicity, we believe the p.P441T variant is unlikely to be pathogenic.

We identified a novel *FOXP2* mutation in one family. The small de novo intragenic deletion is likely to be pathogenic and correlates with the phenotype of severe motor speech disorder characterized by CAS and dysarthria. *FOXP2* plays an important role in motor control but may have more diverse implications for literacy and language development.

ACKNOWLEDGMENTS

The authors sincerely thank the families for their participation in this study. We thank Elena Aleksoska (Melbourne Brain Centre, The University of Melbourne) for performing genomic DNA extractions. We thank Professor Larry Shriberg for use of the Prosody-Voice Screening Profile, and Professor Susan Gathercole for use of the Nonword Memory Test.

REFERENCES

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. 2010. A method and server for predicting damaging missense mutations. Nat Methods 7:248–249.

Alcock KJ, Passingham RE, Watkins KE, Vargha-Khadem F. 2000. Oral dyspraxia in inherited speech and language impairment and acquired dysphasia. Brain Lang 75:17–33.

American Speech-Language-Hearing Association (ASHA). 2007. Childhood apraxia of speech [technical report]. Available from. http://www.asha.org/policy.

Bishop DJ. 2003. Test for Reception of Grammar. 2nd edition. London: Pearson Assessment.

Dodd B, Hua Z, Crosbie S, Holm A, Ozanne A. 2002. Diagnostic evaluation of articulation and phonology. London: Pearson Assessment.

Dunn LM, Dunn DM. 2007. The Peabody Picture Vocabulary Test. 4th edition. Minneapolis: NCS Pearson Inc.

Enderby P. 2003. Frenchay Dysarthria Assessment. San Diego: College-Hill Press.

Feuk L, Kalervo A, Lipsanen-Nyman M, Skaug J, Nakabayashi K, Finucane B, Hartung D, Innes M, Kerem B, Nowaczyk MJ, Rivlin J, Roberts W, Senman L, Summers A, Szatmari P, Wong V, Vincent JB, Zeesman S, Osborne LR, Cardy JO, Kere J, Scherer SW, Hannula-Jouppi K. 2006. Absence of a paternally inherited FOXP2 gene in developmental verbal dyspraxia. Am J Hum Genet 79:965–972.

Gathercole SE, Baddeley AD. 1996. Nonword Memory Test. University of Bristol.

Gregor A, Albrecht B, Bader I, Bijlsma EK, Ekici AB, Engels H, Hackmann K, Horn D, Hoyer J, Klapecki J, Kohlhase J, Maystadt I, Nagl S, Prott E, Tinschert S, Ullmann R, Wohlleber E, Woods G, Reis A, Rauch A, Zweier C. 2011. Expanding the clinical spectrum associated with defects in CNTNAP2 and NRXN1. BMC Med Genet 12:106.

Hayden D, Square P. 1999. The Verbal Motor Production Assessment for Children. Texas: The Psychological Corporation.

Hurst JA, Baraitser M, Auger E, Graham F, Norell S. 1990. An extended family with a dominantly inherited speech disorder. Dev Med Child Neurol 32:352–355.

Kumar P, Henikoff S, Ng PC. 2009. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4:1073–1081.

Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. 2001. A forkhead-domain gene is mutated in a severe speech and language disorder. Nature 413:519–523.

Lennon PA, Cooper ML, Peiffer DA, Gunderson KL, Patel A, Peters S, Cheung SW, Bacino CA. 2007. Deletion of 7q31.1 supports involvement of FOXP2 in language impairment: Clinical report and review. Am J Med Genet Part A 143A:791–798.

MacDermot KD, Bonora E, Sykes N, Coupe AM, Lai CS, Vernes SC, Vargha-Khadem F, McKenzie F, Smith RL, Monaco AP, Fisher SE. 2005. Identification of FOXP2 truncation as a novel cause of developmental speech and language deficits. Am J Hum Genet 76:1074–1080.

Morgan AT, Liegeois F, Vargha-Khadem F. 2010. Motor speech outcome as a function of the site of brain pathology: A developmental perspective. In: Maassen B, van Lieshout P, editors. Speech motor control: New developments in basic and applied research. Oxford: Oxford University Press. pp 95–115.

Palka C, Alfonsi M, Mohn A, Cerbo R, Guanciali Franchi P, Fantasia D, Morizio E, Stuppia L, Calabrese G, Zori R, Chiarelli F, Palka G. 2012. Mosaic 7q31 deletion involving FOXP2 gene associated with language impairment. Pediatrics 129:e183–e188.

Rice GM, Raca G, Jakielski KJ, Laffin JJ, Iyama-Kurtycz CM, Hartley SL, Sprague RE, Heintzelman AT, Shriberg LD. 2012. Phenotype of FOXP2 haploinsufficiency in a mother and son. Am J Med Genet Part A 158A:174–181.

Roll P, Vernes SC, Bruneau N, Cillario J, Ponsole-Lenfant M, Massacrier A, Rudolf G, Khalife M, Hirsch E, Fisher SE, Szepetowski P. 2010. Molecular networks implicated in speech-related disorders: FOXP2 regulates the SRPX2/uPAR complex. Hum Mol Genet 19:4848–4860.

Semel E, Wiig E, Secord W. 2006. Clinical evaluation of language fundamentals, Australian standardised edition. 4th edition. Marrickville: Harcourt Assessment.

Shriberg L, Kwiatkowski J, Rasmussen C. 1990. The prosody-voice screening profile. Tuscson, AZ: Communication Skill Builders.

Shriberg LD, Ballard KJ, Tomblin JB, Duffy JR, Odell KH, Williams CA. 2006. Speech, prosody, and voice characteristics of a mother and daughter with a 7;13 translocation affecting FOXP2. J Speech Lang Hear Res 49:500–525.

Shriberg LD, Lohmeier HL, Campbell TF, Dollaghan CA, Green JR, Moore CA. 2009. A nonword repetition task for speakers with misarticulations: The Syllable Repetition Task (SRT). J Speech Lang Hear Res 52:1189–1212.

Spiteri E, Konopka G, Coppola G, Bomar J, Oldham M, Ou J, Vernes SC, Fisher SE, Ren B, Geschwind DH. 2007. Identification of the transcriptional targets of FOXP2, a gene linked to speech and language, in developing human brain. Am J Hum Genet 81:1144–1157.

Tomblin JB, O'Brien M, Shriberg LD, Williams C, Murray J, Patil S, Bjork J, Anderson S, Ballard K. 2009. Language features in a mother and daughter of a chromosome 7;13 translocation involving FOXP2. J Speech Lang Hear Res 52:1157–1174.

Vargha-Khadem F, Gadian DG, Copp A, Mishkin M. 2005. FOXP2 and the neuroanatomy of speech and language. Nat Rev Neurosci 6: 131–138.

Vargha-Khadem F, Watkins K, Alcock K, Fletcher P, Passingham R. 1995. Praxic and nonverbal cognitive deficits in a large family with a genetically transmitted speech and language disorder. Proc Natl Acad Sci U S A 92:930–933.

Vargha-Khadem F, Watkins KE, Price CJ, Ashburner J, Alcock KJ, Connelly A, Frackowiak RS, Friston KJ, Pembrey ME, Mishkin M, Gadian DG, Passingham RE. 1998. Neural basis of an inherited speech and language disorder. Proc Natl Acad Sci U S A 95:12695–12700.

Vernes SC, Spiteri E, Nicod J, Groszer M, Taylor JM, Davies KE, Geschwind DH, Fisher SE. 2007. High-throughput analysis of promoter occupancy reveals direct neural targets of FOXP2, a gene mutated in speech and language disorders. Am J Hum Genet 81:1232–1250.

Wagner RK, Torgesen JK, Rashotte CA. 1999. Comprehensive Test of Phonological Processing. Austin: PRO-ED.

Wechsler D. 1999. The Wechsler Abbreviated Scale of Intelligence. London: Pearson Assessment.

Wechsler D. 2004. The Wechsler Intelligence Scale for Children. 4th edition. London: Pearson Assessment.

Wilkinson GS, Robertson GJ. 2006. Wide Range Achievement Test. 4th edition. Lutz: Psychological Assessment Resources.

Williams KT. 2007. Expressive Vocabulary Test. 2nd edition. London: Pearson Assessment.

Zeesman S, Nowaczyk MJ, Teshima I, Roberts W, Cardy JO, Brian J, Senman L, Feuk L, Osborne LR, Scherer SW. 2006. Speech and language impairment and oromotor dyspraxia due to deletion of 7q31 that involves FOXP2. Am J Med Genet Part A 140A:509–514.

Zilina O, Reimand T, Zjablovskaja P, Mannik K, Mannamaa M, Traat A, Puusepp-Benazzouz H, Kurg A, Ounap K. 2012. Maternally and paternally inherited deletion of 7q31 involving the FOXP2 gene in two families. Am J Med Genet Part A 158A:254–256.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

CHAPTER 4

GRIN2A – AN APTLY NAMED GENE FOR SPEECH DYSFUNCTION

GRIN2A

An aptly named gene for speech dysfunction

Samantha J. Turner, MSpPath
Angela K. Mayes, PhD
Andrea Verhoeven, BAppSc (Sp Path)
Simone A. Mandelstam, MBChB
Angela T. Morgan, PhD*
Ingrid E. Scheffer, MBBS, PhD*

Correspondence to Prof. Scheffer: scheffer@unimelb.edu.au

ABSTRACT

Objective: To delineate the specific speech deficits in individuals with epilepsy-aphasia syndromes associated with mutations in the glutamate receptor subunit gene *GRIN2A*.

Methods: We analyzed the speech phenotype associated with *GRIN2A* mutations in 11 individuals, aged 16 to 64 years, from 3 families. Standardized clinical speech assessments and perceptual analyses of conversational samples were conducted.

Results: Individuals showed a characteristic phenotype of dysarthria and dyspraxia with lifelong impact on speech intelligibility in some. Speech was typified by imprecise articulation (11/11, 100%), impaired pitch (monopitch 10/11, 91%) and prosody (stress errors 7/11, 64%), and hypernasality (7/11, 64%). Oral motor impairments and poor performance on maximum vowel duration (8/11, 73%) and repetition of monosyllables (10/11, 91%) and trisyllables (7/11, 64%) supported conversational speech findings. The speech phenotype was present in one individual who did not have seizures.

Conclusions: Distinctive features of dysarthria and dyspraxia are found in individuals with *GRIN2A* mutations, often in the setting of epilepsy-aphasia syndromes; dysarthria has not been previously recognized in these disorders. Of note, the speech phenotype may occur in the absence of a seizure disorder, reinforcing an important role for *GRIN2A* in motor speech function. Our findings highlight the need for precise clinical speech assessment and intervention in this group. By understanding the mechanisms involved in *GRIN2A* disorders, targeted therapy may be designed to improve chronic lifelong deficits in intelligibility. ***Neurology*® 2015;84:586–593**

GLOSSARY

ADRESD = autosomal dominant rolandic epilepsy with speech dyspraxia; **EAS** = epilepsy-aphasia syndromes; **ECSWS** = epileptic encephalopathy with continuous spike and wave during sleep; ***GRIN2A*** = glutamate receptor, ionotropic, *N*-methyl D-aspartate 2A; **IEAD** = intermediate epilepsy-aphasia disorder.

Language and speech impairment are integral to the epilepsy-aphasia syndromes (EAS). At the severe end of the epilepsy-aphasia spectrum lie two disorders associated with regression and continuous spike and wave during sleep, defined by bilaterally synchronous discharges occupying >85% of slow-wave sleep. Language regression, typically with verbal auditory agnosia, is characteristic of Landau-Kleffner syndrome, often associated with treatable focal seizures. Global regression is usual in epileptic encephalopathy with continuous spike and wave during sleep (ECSWS) associated with multiple seizure types. Next in the continuum is intermediate epilepsy-aphasia disorder (IEAD) with abnormal cognitive development or regression, with or without seizures, with epileptiform activity occupying <85% sleep.[1] At the mild end, impaired language and literacy skills are described in benign childhood epilepsy with centrotemporal spikes.[2] Ictal oromotor and speech impairment as well as interictal speech sound disorder have also been reported.[3,4] Speech dyspraxia occurs in rare families with rolandic epilepsy and cognitive impairment.[5–7] Impairment in language (understanding and use of words) is central to the

Supplemental data at Neurology.org

*These authors contributed equally to this work.

From the Department of Paediatrics (S.J.T., S.A.M., A.T.M., I.E.S.), The University of Melbourne, The Royal Children's Hospital, Parkville; Language and Literacy Group (A.K.M., A.T.M.), Population Health Theme, Murdoch Childrens Research Institute, Parkville; Speech Pathology Department (A.V.), The Royal Children's Hospital, Parkville; Department of Radiology (S.A.M.), The University of Melbourne, Parkville; Epilepsy Research Centre (I.E.S.), Department of Medicine, The University of Melbourne, Austin Health, Melbourne; and Florey Institute of Neuroscience and Mental Health (S.A.M., I.E.S.), Melbourne, Australia.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

EAS, yet speech (how speech sounds are produced or articulated) has not been carefully investigated.

Inherited and de novo mutations in *GRIN2A*, encoding the NR2A subunit of the glutamate NMDA receptor, are found in 9% to 20% of probands with EAS.[8–10] We identified mutations in *GRIN2A* in 4 of 519 patients with epileptic encephalopathies of unknown cause and found all 4 patients had EAS disorders.[8] This finding was replicated in French and German studies.[9,10] Here, we studied the speech phenotype of 3 families with EAS associated with *GRIN2A* mutations.

METHODS We studied 11 individuals from 3 families with EAS and *GRIN2A* mutations.[1,5,8] A range of tasks was performed to assess speech, oral motor skills, cognition, and language (table 1). Audiovisual recordings of assessments were made using a Marantz PMD671 digital recorder, Countryman Isomax headset microphone, and a Sony DCR-SR85 digital camera. Two speech pathologists (S.J.T., A.T.M.) independently rated the perceptual speech characteristics of conversational samples using a dysarthria rating scale,[11] then reached consensus on discrepant ratings. Word and nonword repetition tasks (Nonword Memory Test, multisyllabic word repetition task) and subtests of the Apraxia Battery for Adults, Second Edition (ABA-2), were used to assess motor speech planning and programming. Word and nonword repetition raw scores were calculated as the number of words correctly produced and compared with adult normative data.[12,13] Raw scores on the ABA-2 were compared with normative data.[14] Maximum performance tasks (maximum vowel prolongation, maximum repetition rate of monosyllables and trisyllables) were used to independently assess subsystems required for accurate speech production: respiration, phonation, and articulation.[15] Three trials of each task were performed, and the best performance was compared with adult normative data.[16] The Frenchay Dysarthria Assessment, Second Edition, was used to examine nonspeech oral motor skills.[17] Performance was measured on a 9-point scale, with scores 7 and below indicative of impairment.

Perceptual speech characteristics and performance on word and nonword repetition and maximum performance tasks were used to distinguish the different motor speech disorders. Dysarthria was diagnosed based on the presence of speech deficits at any level of the speech subsystem (respiration, phonation, articulation, resonance, prosody) due to abnormalities in the strength, speed, range, steadiness, tone, or accuracy of movements, specified in the Mayo dysarthria classification system.[16] Diagnosis of speech dyspraxia was based on features identified in the American Speech and Hearing Association Childhood Apraxia of Speech Technical Report,[18] including inconsistent errors, disrupted coarticulatory transitions, and inappropriate prosody.

Receptive and expressive language skills were measured using the Clinical Evaluation of Language Fundamentals, Fourth Edition, with normative data available up to 21 years.[19] For adults older than 21 years, receptive vocabulary skills were examined using the Peabody Picture Vocabulary Test, Fourth Edition, expressive vocabulary using the Expressive Vocabulary Test, Second Edition, and comprehension of grammatical contrasts using the Test for Reception of Grammar, Second Edition.[20–22] Standard scores were computed using normative data provided for each test. Information regarding early language skills, as well as electroclinical and imaging data, was obtained from the families and confirmed from their medical records. Cognitive function was measured using the 4-subtest form of the Wechsler Abbreviated Scale of Intelligence, Second Edition,[23] or the 2-subtest form when there were time constraints during testing.

Standard protocol approvals, registrations, and patient consents. This study was approved by the Human Research Ethics Committees of The Royal Children's Hospital and Austin Health (RCH HREC 27053, Austin HREC H2011/04390). Written informed consent was obtained from all participants, including from parents in the case of minors or those with intellectual disability. Consent covered use of video footage for publication.

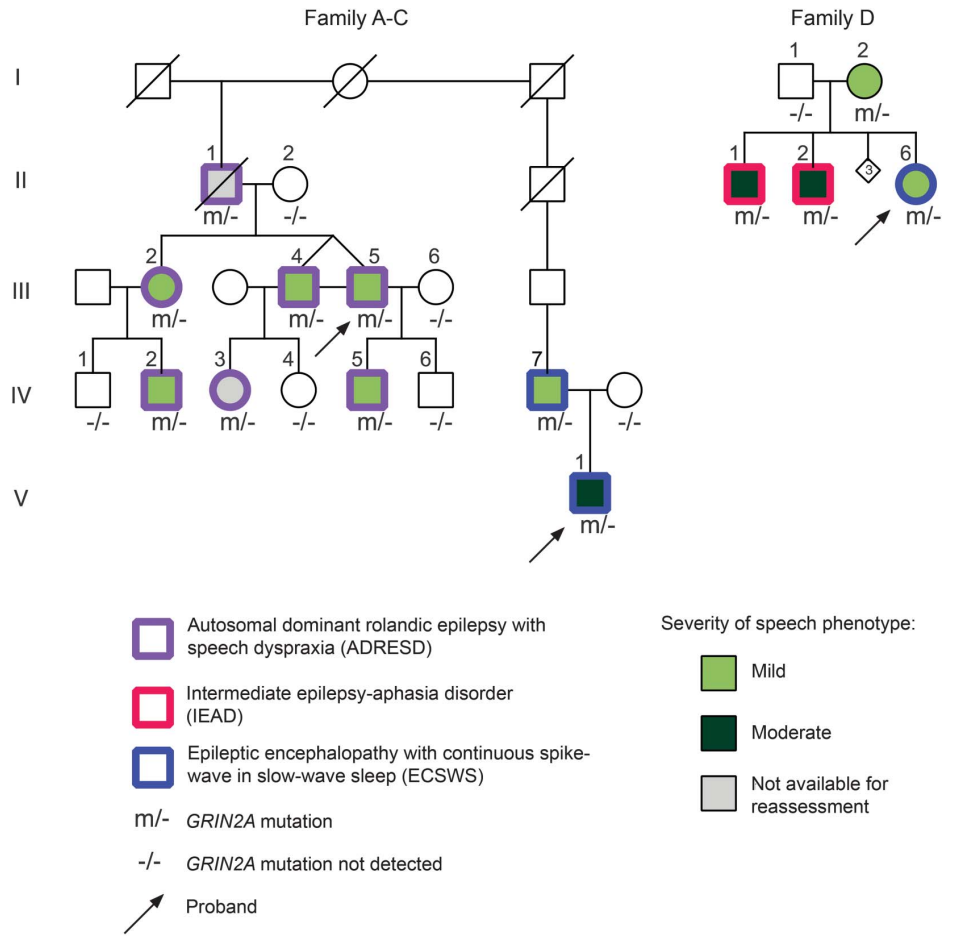
Table 1 Tasks used to assess speech, oral motor skills, cognition, and language

Task	Reference
Speech	
Conversational speech sample: speech errors and dysarthria rating scale	11
Nonword Memory Test	13
Multisyllabic word repetition task	12
Apraxia Battery for Adults, Second Edition: Repeated Trials and Increasing Word Length subtests	14
Maximum vowel prolongation	16
Monosyllable repetition rate (pa, ta, ka); trisyllable repetition rate (pataka)	15, 16
Oral motor skills	
Frenchay Dysarthria Assessment, Second Edition	17
Language	
Up to 21 y	
Clinical Evaluation of Language Fundamentals, Fourth Edition	19
Older than 21 y	
Peabody Picture Vocabulary Test, Fourth Edition	20
Expressive Vocabulary Test, Second Edition	21
Test for Reception of Grammar, Second Edition	22
Cognition	
Wechsler Abbreviated Scale of Intelligence, Second Edition	23

RESULTS The cohort comprised 11 individuals from 3 families named according to the codes (A, C, D) used in the report identifying *GRIN2A* as the causative gene for ease of reference.[1,5,8] Family A included 5 members with autosomal dominant rolandic epilepsy with speech dyspraxia (ADRESD).[5] Family C was a father–son pair with ECSWS who had the same *GRIN2A* mutation and shared an identical haplotype with family A, but the families were not known to be related. Recent genealogic work has determined the relationship between the 2 families (figure, family A-C). Family D comprised 2 brothers with IEAD, their sister with ECSWS, and their mother who did not have a history of seizures and had not received antiepileptic medication (figure).

The median age of the affected individuals studied was 48 years (mean 38 years, range 16–64 years). At

Figure Pedigrees of families A–C and D



Family A was originally reported in Scheffer et al.,[5] 1995; family D is family I in Tsai et al.,[1] 2013.

the time of this study, none of the 10 individuals with a seizure disorder had ongoing seizures. Only 3 individuals were on antiepileptic medication (AC-V-1, AC-IV-5, D-II-1); in one individual, the antiepileptic medication was for behavioral management rather than seizures (D-II-1). No epileptiform abnormalities were present on the last EEG in 7 individuals (table 3). Three (AC-IV-2, D-II-2, D-II-6) had epileptiform abnormalities on their studies at ages 8 to 12 years but have not had subsequent studies. Six individuals underwent brain MRI at the time of the study, which was normal in 4 (AC-III-2, AC-III-5, AC-IV-2, AC-V-1). A Chiari I malformation was found in AC-IV-5, and left hippocampal sclerosis in AC-IV-7. Brain MRI was reported as normal in 3 (AC-III-4, D-II-1, and D-II-6). D-I-2 did not undergo EEG or MRI studies and her son D-II-2 did not have an MRI study.

The more severe epilepsy phenotypes may be associated with more severe speech phenotypes, but larger numbers of cases are required to show whether this is a true correlation.

Individuals with *GRIN2A* mutations showed abnormalities in both motor speech planning/programming (i.e., speech dyspraxia) and execution (i.e., dysarthria).

Speech features. Conversational speech intelligibility was moderately impaired in 3 individuals (AC-V-1, D-II-1, D-II-2) and mildly reduced in 7 (AC-III-2, AC-III-4, AC-III-5, AC-IV-2, AC-IV-5, AC-IV-7, D-I-2) (table 2; see video on the *Neurology*® Web site at Neurology.org). Impairments occurred across the domains of articulation, phonation, resonance, and prosody. All individuals demonstrated impaired articulation, characterized by imprecise production of consonants (11/11, 100%) and vowels (8/11, 73%). Phonological-level speech production errors included substitution of consonants and vowels, reduction of consonant clusters, and omission of sounds and/or

Table 2 Conversational speech abnormalities: Frequency (n = number of individuals/11) and severity ratings

	Frequency, n (%)	Severity, %	
		Mild	Moderate
Prosodic features			
Pitch level	6 (55)	100	
Variation of pitch	10 (91)	70	30
Steadiness of pitch	0 (0)		
Loudness level	1 (9)	100	
Variation of loudness	1 (9)	100	
Maintenance of loudness	1 (9)	100	
Phrase length	6 (55)	83	17
General rate	8 (73)	75	25
Maintenance of rate	8 (73)	100	
General stress pattern	7 (64)	86	14
Respiratory features			
Breath support for speech	4 (36)	100	
Resonance			
Hypernasality	6 (55)	50	50
Hyponasality	0 (0)		
Mixed nasality	1 (9)	100	
Phonation			
Harshness	7 (64)	86	14
Strain-strangled	0 (0)		
Intermittent breathiness	2 (18)	100	
Hoarseness	3 (27)	100	
Glottal fry	4 (36)	100	
Wetness	1 (9)	100	
Articulation			
Precision of consonants	11 (100)	64	36
Length of phonemes	8 (73)	75	25
Precision of vowels	8 (73)	100	
Perceptual vocal abnormalities			
Pitch breaks	2 (18)	100	
Excessive fluctuation of pitch	5 (45)	100	
Excessive loudness variation	0 (0)		
Rate fluctuations	0 (0)		
Prolonged intervals	5 (45)	60	40
Short rushes of speech	5 (45)	100	
Forced inspiration/expiration	0 (0)		
Audible inspiration	6 (55)	50	50
Grunt at end of expiration	0 (0)		

syllables. A number of phonological errors were heard, for example, /f/ for /θ/ (e.g., "fing" for "thing") and /d/ or /v/ for /ð/ (e.g., "dat" for "that"). Prosodic impairments were common, and speech was typically slow, with stress errors (7/11, 64%), shortening of phrases (6/11, 55%), and prolonged intervals between syllables and words (5/11, 45%). Breath support for speech was generally adequate. Laryngeal impairments manifested as difficulty modulating pitch (monopitch 10/11, 91%; pitch fluctuations 5/11, 45%) as well as hoarse, harsh, or breathy vocal quality (11/11, 100%; harsh and breathy voice in AC-IV-5). Altered resonance was characterized by hypernasality (7/11, 64%; mixed nasality in AC-IV-7), with nasal flare and nasal air escape at times. No speech features were rated as severely impaired.

Two individuals (AC-IV-7, AC-V-1) underwent nasendoscopy by an otolaryngeal surgeon. This revealed normal structures and excluded obvious velopharyngeal weakness. Difficulty with motor control of velopharyngeal closure was noted. AC-V-1 had minimal air escape on non-nasal sustained sounds, which increased in connected speech. AC-IV-7 had a small granuloma on the left vocal fold process of the arytenoid, but laryngeal function was normal.

Speech tasks. Individuals had dysarthria with motor execution difficulties on maximum performance tasks (table 3). Maximum vowel duration was reduced in 8 of 11 individuals, with the task not completed by one (D-II-6). Maximum repetition rate of monosyllables was also slow in 10 of 11 individuals, with repetition of /ta/ impaired in 9 of 11, followed by /ka/ (8/11) and /pa/ (7/11). Two individuals (AC-IV-2, AC-V-1) ran out of breath during these tasks, and 2 (AC-IV-2, AC-IV-7) were unable to maintain loudness during vowel prolongation.

Speech dyspraxia with difficulties in motor planning and programing was observed (table 3). Most individuals (7/11) had difficulty repeating a trisyllabic sequence (pataka). Four (AC-IV-5, AC-III-5, AC-V-1, D-II-6) were unable to repeat the sequence correctly, with sequencing errors noted for most individuals (8/11) across multiple repetitions. All individuals also had difficulty repeating nonwords and multisyllabic words compared with control data, with more errors as word length increased (2 syllables 14%–71% correct compared with 5 syllables 0%–43% correct on Nonword Memory Test; mild-severe impairment on ABA-2 Increasing Word Length subtest).

Oral motor assessment. AC-III-5 and AC-IV-2 had involuntary movements of the tongue at rest. Dystonic posturing of the tongue at rest and deviation to the left was noted in D-II-1. Slow or poorly coordinated tongue movement was evident in all individuals but one (D-II-2). Tongue

Table 3 Results of speech and language tasks

	Individual											Normative data
	AC-IV-5	AC-IV-2	AC-III-5	AC-III-4	AC-III-2	AC-V-1	AC-IV-7	D-II-6	D-II-2	D-II-1	D-I-2	
Sex	M	M	M	M	F	M	M	F	M	M	F	
Diagnosis	ADRESD	ADRESD	ADRESD	ADRESD	ADRESD	ECSWS	ECSWS	ECSWS	IEAD	IEAD	—	
Age at assessment, y	24	27	58	58	64	19	49	16	19	20	49	
EEG												
Age	21 y	8 y	39 y	39 y	45 y	16 y	14 y	12 y	12 y	12 y	—	
Type	Awake	Sleep	Sleep	Sleep	Awake	Awake	Awake	Sleep	Sleep	Sleep		
Result	Normal	Bilateral CTS	Normal	Normal	Normal	Mild diffuse and L posterior temporal slowing	Rare nonepileptiform, temporal sharp waves	Frequent multifocal sharps	Bilateral CTS	Normal		
Cognition												
Nonverbal	65	96	63	Full scale: 89	78	59	Full scale: mild ID[a]	Full scale: 67[b]	86	Full scale: 65[b]	87	
Verbal	79	78	92	—	86	51	—	—	—	—	—	
Vowel prolongation	20.3	4.9	18.8	26.4	18.0	6.6	10.3	—	7.2	6.4	6.9	M 22.6–34.6; F 15.2–26.5
Maximum repetition rate, syllables/s												
/pa/	5.9	4.0	4.3	4.4	3.8	2.9	5.0	5.2	6.0	4.9	4.8	5.0–7.1; med 6.3
/ta/	4.5	3.3	3.8	3.6	3.6	2.7	4.4	4.9	4.9	4.7	3.9	4.8–7.1; med 6.2
/ka/	4.5	3.3	3.9	3.6	3.0	2.9	3.7	3.6	4.4	4.6	4.2	4.4–6.4; med 5.8
/pataka/	Incorrect	1.3	Incorrect	2.8	3.0	Incorrect	4.6	Incorrect	5.6	6.5	5.7	3.6–7.5; med 5.0
Word repetition, no. correct												
Nonwords (n = 28)	7	3	8	14	10	2	11	15	7	9	16	m 22.05; SD 4.38
Multisyllabic (n = 52)	27	19	36	29	32	3	37	34	32	26	45	m 51.65; SD 0.6
Receptive language												
CELF-4	—	—	—	—	—	45	—	69	53	50	—	m 100; SD 15
PPVT-4	95	80	99	94	90	—	—	—	—	—	93	m 100; SD 15
TROG-2	90	90	95	62	—	—	—	—	—	—	85	m 100; SD 15
Expressive language												
CELF-4	—	—	—	—	—	45	—	76	45	45	—	m 100; SD 15
EVT-2	74	83	85	84	87	—	—	—	—	—	97	m 100; SD 15

Abbreviations: ADRESD = autosomal dominant rolandic epilepsy with speech dyspraxia; CELF-4 = Clinical Evaluation of Language Fundamentals, Fourth Edition; CTS = centrotemporal spikes; ECSWS = epileptic encephalopathy with continuous spike and wave during sleep; EVT-2 = Expressive Vocabulary Test, Second Edition; ID = intellectual disability; IEAD = intermediate epilepsy-aphasia disorder; m = mean; med = median; PPVT-4 = Peabody Picture Vocabulary Test, Fourth Edition; SD = standard deviation; TROG-2 = Test for Reception of Grammar, Second Edition.

[a] Previously reported.[5,8]

[b] Two-subtest form of the Wechsler Abbreviated Scale of Intelligence, Second Edition, completed because of time constraints.

movement was generally poorer on nonspeech tasks (protrusion, elevation) compared with speech.

In speech, lip movements were reduced and/or poorly coordinated, with the posture of the top lip suggestive of increased tone (AC-IV-5, AC-III-5, AC-IV-7, D-II-6). Subtle asymmetry in lip retraction was noted in 2 individuals (AC-III-5, AC-V-1), and as the lips came to rest in one (AC-IV-7). Lip seal was adequate. Mild oral dysphagia was reported (AC-IV-5, AC-III-5, AC-V-1) including difficulty chewing or food "getting stuck," together with instances of expectoration (AC-V-1) or aspiration (e.g., peanut inhalation in AC-III-5). Early saliva control difficulties were noted in AC-IV-5 and AC-V-1, and required medication (AC-V-1).

Language. Moderate to severe language impairment was present in individuals younger than 21 years with ECSWS and IEAD, with receptive and expressive language skills below the first percentile in AC-V-1, D-II-1, and D-II-2 (table 3). No specific pattern of impairment was evident across the domains of language (e.g., semantics, syntax, morphology; see table 4). The degree of impairment in the brothers with IEAD was greater than anticipated given their cognitive skills. Language was congruent with cognitive skills in the individuals with ECSWS (AC-V-1, D-II-6). Adults with ADRESD performed comparatively better, and apart from a few (AC-IV-5, AC-IV-2, AC-III-4), scores fell within 1 SD of the mean on receptive and expressive language tasks. Delayed language development or impaired language skills were documented before onset of seizures in 7 individuals. Early language skills were also delayed in AC-III-5 and normal in AC-III-2 and AC-III-4 based on parental report.

D-I-2, who had no history of seizures or regression, had average cognitive and language skills; however, it is impossible to exclude that she had difficulties as a child. The language assessment was not completed in AC-III-4 and AC-IV-7.

DISCUSSION *GRIN2A* has recently been identified as the first gene associated with EAS and therefore is likely to play a critical role in speech and cognitive-linguistic function. This present cohort is the largest studied to date with comprehensive speech and language data. The *GRIN2A* speech phenotype consists of a combination of speech dyspraxia with impaired motor planning and programing, and dysarthria with impairments in speech execution. Although variations among affected individuals were noted, their speech was typified by imprecise articulation of consonants and vowels and hypernasality, with prosodic disturbance. Poorly coordinated lip and tongue movements were seen, with abnormal tone and reduced and asymmetrical lip movement. These abnormalities, distinguished by listening to their speech, are supported by findings on quantifiable assessments of dysarthria. Performance on maximum vowel prolongation and diadochokinesis (alternating rapid movements) tasks examining maximum repetition rate of monosyllabic and trisyllabic sequences was poor. Impaired trisyllabic repetition is characteristic of speech dyspraxia.[15] Reduced vowel prolongation and slow monosyllabic repetition are also observed in dysarthria associated with spastic quadriplegia; our patients did not have cerebral palsy.[15] Although speech dyspraxia has been previously recognized in EAS disorders,[5] dysarthria has not been a key feature.

Significant language impairment was also seen in adolescents and young adults with ECSWS and IEAD. The language of adults with ADRESD seemed comparatively better. This may have been due to continual improvement in language performance through life. Alternatively, different language assessments were used with individuals older than 21 years, so we cannot rule out that the contrast in language phenotype from adolescence to late adulthood was attributable to methodologic differences. Cognitive impairment was present in most cases. Earlier assessment in the original family with ADRESD revealed impaired comprehension of linguistic-semantic concepts and deficits in expressive vocabulary.[5]

Deficits in language skills were evident before seizure onset in 8 of 10 individuals with seizures, and 7

Table 4 CELF-4 subtest scores

Individual	Sex	Diagnosis	Age at assessment, y	Recalling sentences	Formulated sentences	Word classes	Word definitions	Understanding spoken paragraphs	Semantic relationships
AC-V-1	M	ECSWS	19	1	1	1	1	1	1
D-II-6	F	ECSWS	16	7	7	4	5	8	1
D-II-2	M	IEAD	19	1	1	1	5	3	1
D-II-1	M	IEAD	20	1	1	1	1	1	3

Abbreviations: CELF-4 = Clinical Evaluation of Language Fundamentals, Fourth Edition; ECSWS = epileptic encephalopathy with continuous spike and wave during sleep; IEAD = intermediate epilepsy-aphasia disorder.

of 10 had a nonepileptiform EEG prior to speech and language assessment. This suggests that epileptiform abnormalities were not the cause of the speech and language impairments. Of note, *GRIN2A* mutations have been identified in individuals with speech disorder in the absence of seizures, in 2 members of the families studied here (AC-II-1 deceased,[5] D-I-2) and 3 unrelated families with atypical rolandic epilepsy and speech dyspraxia.[9] As we have studied a relatively small sample of 11 affected individuals, it is possible that the deficits observed are attributable to other familial genetic or environmental determinants. It is, however, noteworthy that we found similar impairments across the families with different mutations of *GRIN2A*. Larger numbers of cases with *GRIN2A* mutations will further refine the phenotypic spectrum of this disease.

The speech deficits described suggest an important role for *GRIN2A* and NMDA receptors in normal speech production. The NR2A subunit of the glutamate NMDA receptor, encoded by *GRIN2A*, is expressed in regions involved in speech production[24] including the anterior cingulate, thalamus, putamen, cerebellum, anterior and dorsolateral prefrontal cortex, and caudate.[25,26] NR2 subunits are crucial to NMDA receptor functioning, controlling cell surface expression and localization,[27] providing glutamate binding sites,[28] and modifying channel properties.[25] Patients with anti-NMDA-receptor encephalitis, with antibodies against NR1-NR2 subunits, have absent or unintelligible speech and echolalia.[29] Mice expressing truncated NR2A show impaired motor coordination, as well as deficits in synaptic plasticity and reorganization.[30] Speech motor planning and execution deficits were observed in our cohort. Discovery of *GRIN2A* mutations in cohorts with speech disorder without epilepsy will add further support to the importance of this gene in normal speech production.

AUTHOR CONTRIBUTIONS

S.J.T., A.T.M., and I.E.S. designed the study and wrote the manuscript. A.T.M. and I.E.S. supervised the study. S.A.M. analyzed the MRI brain scans. S.J.T., A.K.M., A.T.M., and A.V. performed phenotypic analysis.

ACKNOWLEDGMENT

The authors sincerely thank the families for their participation in this study. The authors thank Associate Professor Lynette Sadleir for referring a family and Professor Susan Gathercole for use of the Nonword Memory Test. The authors are grateful to Professor Eliane Roulet-Perez for helpful advice regarding the manuscript.

STUDY FUNDING

S.J.T. is supported by a National Health and Medical Research Council (NHMRC) Postgraduate Scholarship (101777) and Speech Pathology Australia Nadia Verrall Memorial Research Grant. A.T.M. is supported by an NHMRC Career Development Award (607315). I.E.S. is supported by an NHMRC Program Grant (628952) and Practitioner Fellowship (1006110). This project was also supported by an Australian Research Council (ARC) Discovery Project (DP120100285) to A.T.M. and I.E.S.

DISCLOSURE

S. Turner, A. Mayes, A. Verhoeven, S. Mandelstam, and A. Morgan report no disclosures relevant to the manuscript. I. Scheffer serves on the editorial boards of the *Annals of Neurology*, *Neurology®*, and *Epileptic Disorders*; may accrue future revenue on a pending patent re: therapeutic compound; has received speaker honoraria from Athena Diagnostics, UCB, GSK, and Transgenomic; has received funding for travel from Athena Diagnostics, UCB, and GSK; and receives/has received research support from the NHMRC, ARC, Health Research Council of New Zealand, The University of Melbourne, American Epilepsy Society, the Jack Brockhoff Foundation, the Weizmann Institute, CURE, US Department of Defense, and the Perpetual Charitable Trustees. Go to Neurology.org for full disclosures.

Received May 5, 2014. Accepted in final form October 10, 2014.

REFERENCES

1. Tsai MH, Vears DF, Turner SJ, et al. Clinical genetic study of the epilepsy-aphasia spectrum. Epilepsia 2013;54:280–287.
2. Overvliet GM, Besseling RM, Vles JS, et al. Nocturnal epileptiform EEG discharges, nocturnal epileptic seizures, and language impairments in children: review of the literature. Epilepsy Behav 2010;19:550–558.
3. Clarke T, Strug LJ, Murphy PL, et al. High risk of reading disability and speech sound disorder in rolandic epilepsy families: case-control study. Epilepsia 2007;48:2258–2265.
4. Lundberg S, Frylmark A, Eeg-Olofsson O. Children with rolandic epilepsy have abnormalities of oromotor and dichotic listening performance. Dev Med Child Neurol 2005;47:603–608.
5. Scheffer IE, Jones L, Pozzebon M, Howell RA, Saling MM, Berkovic SF. Autosomal dominant rolandic epilepsy and speech dyspraxia: a new syndrome with anticipation. Ann Neurol 1995;38:633–642.
6. Roll P, Rudolf G, Pereira S, et al. SRPX2 mutations in disorders of language cortex and cognition. Hum Mol Genet 2006;15:1195–1207.
7. Kugler SL, Bali B, Lieberman P, et al. An autosomal dominant genetically heterogeneous variant of rolandic epilepsy and speech disorder. Epilepsia 2008;49:1086–1090.
8. Carvill GL, Regan BM, Yendle SC, et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. Nat Genet 2013;45:1073–1076.
9. Lesca G, Rudolf G, Bruneau N, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat Genet 2013;45:1061–1066.
10. Lemke JR, Lal D, Reinthaler EM, et al. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. Nat Genet 2013;45:1067–1072.
11. Murdoch BE. Dysarthria: A Physiological Approach to Assessment and Treatment. Cheltenham, UK: Stanley Thornes; 1998.
12. Lewis BA, Freebairn L. Residual effects of preschool phonology disorders in grade school, adolescence, and adulthood. J Speech Hear Res 1992;35:819–831.
13. Gathercole SE, Baddeley AD. Nonword Memory Test. Bristol, UK: University of Bristol; 1996.
14. Dabul BL. Apraxia Battery for Adults, 2nd ed. Austin: PRO-ED; 2000.
15. Thoonen G, Maassen B, Wit J, Gabreels F, Schreuder R. The integrated use of maximum performance tasks in differential diagnostic evaluations among children with motor speech disorders. Clin Linguist Phon 1996;10:311–336.

16. Duffy JR. Motor Speech Disorders: Substrates, Differential Diagnosis and Management, 3rd ed. St. Louis: Mosby; 2013.
17. Enderby P, Palmer R. Frenchay Dysarthria Assessment, 2nd ed. Austin: PRO-ED; 2008.
18. ASHA. Technical Report: Childhood Apraxia of Speech. Available at: www.asha.org/policy/tr2007-00278.htm#sec1.1. Accessed March 12, 2010.
19. Semel E, Wiig E, Secord W. Clinical Evaluation of Language Fundamentals, Australian Standardised Edition, 4th ed. Marrickville, Australia: Harcourt Assessment; 2006.
20. Dunn LM, Dunn DM. The Peabody Picture Vocabulary Test, 4th ed. Minneapolis: NCS Pearson Inc.; 2007.
21. Williams KT. Expressive Vocabulary Test, 2nd ed. London: Pearson Assessment; 2007.
22. Bishop DJ. Test for Reception of Grammar, 2nd ed. London: Pearson Assessment; 2003.
23. Wechsler D. The Wechsler Abbreviated Scale of Intelligence, 2nd ed. London: Pearson Assessment; 2011.
24. Liegeois FJ, Morgan AT. Neural bases of childhood speech disorders: lateralization and plasticity for speech functions during development. Neurosci Biobehav Rev 2012;36:439–458.
25. Monyer H, Sprengel R, Schoepfer R, et al. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 1992;256:1217–1221.
26. Conti F, Barbaresi P, Melone M, Ducati A. Neuronal and glial localization of NR1 and NR2A/B subunits of the NMDA receptor in the human cerebral cortex. Cereb Cortex 1999;9:110–120.
27. Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science 1995;269:1737–1740.
28. Kendrick SJ, Lynch DR, Pritchett DB. Characterization of glutamate binding sites in receptors assembled from transfected NMDA receptor subunits. J Neurochem 1996;67:608–616.
29. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 2008;7:1091–1098.
30. Sprengel R, Suchanek B, Amico C, et al. Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. Cell 1998;92:279–289.

WriteClick® rapid online correspondence

The editors encourage comments about recent articles through WriteClick:

Go to *Neurology.org* and click on the "WriteClick" tab at the top of the page. Responses will be posted within 72 hours of submission.

Before using WriteClick, remember the following:

- WriteClick is restricted to comments about studies published in *Neurology* within the last eight weeks
- Read previously posted comments; redundant comments will not be posted
- Your submission must be 200 words or less and have a maximum of five references; reference one must be the article on which you are commenting
- You can include a maximum of five authors (including yourself)

CHAPTER 5

DYSARTHRIA AND BROADER MOTOR SPEECH DEFICITS IN DRAVET SYNDROME

Dysarthria and broader motor speech deficits in Dravet syndrome

Samantha J. Turner, MSpPath
Amy Brown, PhD
Marta Arpone, MSc
Vicki Anderson, PhD
Angela T. Morgan, PhD*
Ingrid E. Scheffer, MBBS, PhD*

Correspondence to Dr. Scheffer: scheffer@unimelb.edu.au

ABSTRACT

Objective: To analyze the oral motor, speech, and language phenotype in 20 children and adults with Dravet syndrome (DS) associated with mutations in *SCN1A*.

Methods: Fifteen verbal and 5 minimally verbal DS patients with *SCN1A* mutations (aged 15 months-28 years) underwent a tailored assessment battery.

Results: Speech was characterized by imprecise articulation, abnormal nasal resonance, voice, and pitch, and prosody errors. Half of verbal patients had moderate to severely impaired conversational speech intelligibility. Oral motor impairment, motor planning/programming difficulties, and poor postural control were typical. Nonverbal individuals had intentional communication. Cognitive skills varied markedly, with intellectual functioning ranging from the low average range to severe intellectual disability. Language impairment was congruent with cognition.

Conclusions: We describe a distinctive speech, language, and oral motor phenotype in children and adults with DS associated with mutations in *SCN1A*. Recognizing this phenotype will guide therapeutic intervention in patients with DS. ***Neurology*® 2017;88:1–7**

GLOSSARY

CCS = Complexity of Communication Scale; **DS** = Dravet syndrome; **GEFS+** = genetic epilepsy with febrile seizures plus; **ID** = intellectual disability; **MV** = minimally verbal; **V** = verbal; **VNS** = vagal nerve stimulator.

Dravet syndrome (DS) is an infantile-onset developmental epileptic encephalopathy with poor outcome. Typically, a 6-month-old infant presents with febrile hemiclonic status epilepticus in the setting of reputedly normal development, and then develops multiple seizure types over the next 4 years, with developmental slowing from 1–2 years of age.[1] More than 80% of cases have mutations of the sodium channel gene *SCN1A*. Intellectual disability (ID) is usual, with almost all patients having severe ID.

Speech and language function in adults and children with DS has not been specifically characterized. Three pediatric studies have examined language (understanding and use of words) in the context of a broader neuropsychological battery and include *SCN1A* positive and negative cases.[2–4] The results are varied, ranging from cohorts with severe ID and severe language impairment to others with mild to moderate ID and borderline to average naming and comprehension.

In terms of speech (how speech sounds are produced or articulated), dysarthria and speech planning difficulties have been reported anecdotally.[2,4–6] Oral motor skills have not been investigated.

We aimed to determine whether there was a characteristic developmental speech, language, and oral motor phenotype in children and adults with DS associated with mutations in *SCN1A*. Recognition of progressive patterns of dysfunction will inform diagnosis and guide therapeutic intervention.

Supplemental data at Neurology.org

*These authors contributed equally to this work.

From the Department of Paediatrics, The University of Melbourne (S.J.T., M.A., V.A., A.T.M., I.E.S.), and Department of Psychology (V.A.), The Royal Children's Hospital; Neuroscience of Speech Group, Clinical Sciences Theme (S.J.T., A.T.M.), Australian Centre for Child Neuropsychological Studies (A.B., M.A., V.A.), Murdoch Childrens Research Institute, Melbourne; Epilepsy Research Centre, Department of Medicine (I.E.S.), The University of Melbourne, Austin Health; and Florey Institute of Neuroscience and Mental Health (I.E.S.), Melbourne, Australia.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

METHODS Patients attending a DS clinic were invited to participate; the entire cohort comprised 26 patients with the electroclinical features of DS and an *SCN1A* mutation. Three families refused and 3 were unavailable during the study. Diagnosis was confirmed by a pediatric neurologist with expertise in DS (I.E.S.). All *SCN1A* mutations were located in highly conserved regions or reported to alter protein expression or function.

Standard protocol approvals, registrations, and patient consents. The study was approved by Austin Health Human Research Ethics Committee (Austin HREC H2011/04390). Written informed consent was obtained from all participants or their parents (for minors or those with ID). Consent covered use of video footage for publication.

Speech and language assessment. Two batteries were administered depending on the patient's ability (table 1). Standardized assessments were used where possible, for comparison with typically developing children.

The minimally verbal patients (MV) had little or no speech and were unable to cooperate with standardized assessment, while the verbal group (V) had conversational speech. Testing focused on oral motor, speech, and language skills.

Oral motor tasks and perceptual speech characteristics of conversational samples were independently rated by 2 speech pathologists (S.J.T., A.T.M.). The *SCN1A* mutation, psychological assessment results, medications, and seizure history were reviewed.

RESULTS The cohort comprised 20 patients with DS (11 female), with 15 in the V and 5 in the MV group (table 2). Median age was 11½ years (mean 13 years, range 15 months–28 years). Fifteen had de novo *SCN1A* mutations and 3 inherited mutations. Inherited mutations were from an unaffected mother (patient 10), a father with genetic epilepsy with febrile seizures plus (GEFS+; patient 15), and a mother (patient 20 here) with DS and a de novo *SCN1A* mutation (patient 7). Inheritance for 2 individuals (6, 18) could not be confirmed as their fathers were not available for testing.

In children under 2 years of age, development was normal. In older patients, cognitive skills varied markedly, with intellectual functioning ranging from low average (1) to borderline (1) to mild (5), moderate (2), and severe (9) ID. No pattern of performance was seen on verbal vs perceptual reasoning tasks. All patients were on antiepileptic drugs, with 17/20 taking 3 or more. Three individuals had a vagal nerve stimulator (VNS) (table 2).

Oral motor skills. See table 2 and video 1 (at Neurology.org). All 5 MV individuals showed impaired oral motor control apart from individual 2 who had independent jaw and tongue movement.[7]

Lip and tongue movement was reduced, asymmetrical, or poorly coordinated in 12/15 V individuals. Notably, 2/3 without impairment were the youngest patients in the cohort aged under 2 years. Lip retraction (say "ee") was generally within normal limits, while lip rounding (say "oo") was weak or asymmetrical. Two individuals (17 and 18) could not overcome an open mouth posture at rest to round the lips. Tongue protrusion, elevation, and lateral movement were also impaired, with 6 (8, 9, 11, 15, 16, 18) unable to elevate the tongue and 3 (16, 17, 18) showing involuntary tongue movement.

Overriding impaired motor programming and planning issues affected performance of speech motor and nonspeech oral motor tasks (table 2). Poor postural control of the trunk and head also affected lip and tongue movement.

Saliva control issues were prominent in 8/20 individuals, likely compounded by benzodiazepine therapy in 7 cases. Saliva control management included medication in 3 (3, 5, 18) and salivary duct surgery in 1 that was not beneficial (18). Mild dysphagia was reported in 5/20 individuals, including 1 with a VNS (17). Three had percutaneous endoscopic gastrostomy for nutrition (3, 4, 11).

Speech. See table 3 and video 2. All MV individuals had intentional communication. Three had potentially

Table 1 Comprehensive assessment battery

	Minimally verbal	Verbal
Oral motor skills	Early Motor Control Scales[7] (abnormal structure and function; predominant combined control–motor speech control subscales)	Early Motor Control Scales[7]—under 3 years or Verbal Motor Production Assessment for Children[15] (global motor control, focal oromotor control, sequencing subtests)—3 to 12 years or Frenchay Dysarthria Assessment, 2nd edition[16]—12 years and older
Speech	Behavioral sample: Complexity of Communication Scale[17]	Conversational speech sample Speech errors Dysarthria rating scale[18] Diagnostic Evaluation of Articulation and Phonology[19]
Language		Preschool Language Scales, 5th edition[20]—up to 7 years or Clinical Evaluation of Language Fundamentals, 4th edition[21]—5 to 21 years or Peabody Picture Vocabulary Test, 4th edition,[22] Expressive Vocabulary Test, second edition,[23] Test For Reception of Grammar, second edition[24]
Cognition	Clinical observation and attempt of formal cognitive assessment with Wechsler Abbreviated Scale of Intelligence, 2nd edition,[25] or Wechsler Intelligence Scale for Children, 4th edition, Australian Standardized Edition,[26] and Vineland Adaptive Behaviour Scales, 2nd edition,[27] for estimation of intellectual disability range	Bayley Scales of Infant and Toddler Development, 3rd edition,[28] or Wechsler Abbreviated Scale of Intelligence, 2nd edition,[25] and Vineland Adaptive Behaviour Scales, 2nd edition,[27] for estimation of intellectual disability range

Table 2 Patient details and results of oral motor and language assessment (n = 20)

	Minimally verbal, individual (sex)					Verbal, individual (sex)				
	1 (F)	2 (F)	3 (M)	4 (M)	5 (M)	6 (F)	7 (F)	8 (F)	9 (F)	10 (F)
SCN1A mutation[a]	R946C DN	S128X DN	E1008X DN	F14SfsX78 DN	K38NfsX54 DN	c.602+1 G>A	R222X I, Ma	M960R DN	R613X DN	F256TfsX3 I, Ma
Age at onset	6 wk	3 mo	5.5 mo	4.5 mo	2.5 mo	5.5 mo	6 wk	2.5 mo	6 mo	4.5 mo
ASD	X	√	√	X	X	X	X	X	X	X
AEDs at assessment	VPA, CZP, STP	VPA, TPM, CZP	VPA, TPM, AZM, CZP	VPA, CLB, TPM, STP	STP, CLB, TPM, VPA, LEV, VNS	VPA, CLB, TPM	VPA, TPM	VPA, CZP, LEV, TPM	VPA, TPM, CLB	TPM
Age at assessment	6 y 2mo	9 y 3 mo	12 y	12 y 8 mo	24 y	15 mo	15 mo	4 y 6 mo	5 y 2 mo	6 y
Cognition[b]	Severe ID	Severe ID[c]	Severe ID	Severe ID	Severe ID	95[c]	90	60[c]	75	67
Language										
Receptive[d]	NC	NC	NC	NC	NC	100	100	74	69	48[c]
Expressive[d]	NC	NC	NC	NC	NC	85	105	68	68	50[c]
Dysphagia	X	X	PEG—nutrition	PEG—nutrition	X	X	X	X	X	X
Oral motor and speech motor										
Drooling	X	X	√	√	√	X	X	X	X	X
Lips: rounding, retraction	Can say "ee"	Can smile	Can smile	Can smile, say "ee"	Can smile	Can smile	Can say "ee"	Rounding Abn	N[e]	N
Tongue: elevation, protrusion, lateral	NS	Can elevate /s/	NS	Can elevate /t/,/d/	NS	Can elevate /t/,/d/	Can elevate /d/,/l/	Elevation, lateral Abn[e]	Elevation Abn	N
Multiple movement: blow + smile, a-m-u, ka-la	NC	NC	NC	NC	NC	NC	NC	Abn	Abn	Abn
Motor planning/programming	NC	NC	NC	NC	NC	NC	NC	VSeq[f]	VSeq[f]	VSeq[f]
VMPAC	—	—	—	—	—	—	—	VGlobal[f]	—	VGlobal[f]
	—	—	—	—	—	—	—	VFocal[f]	VFocal[f]	VFocal[f]
Other publications	—	29	30,31	30,31	31,32	—	—	—	—	—

	Verbal, individual (sex)									
	11 (M)	12 (F)	13 (M)	14 (F)	15 (M)	16 (M)	17 (F)	18 (M)	19 (M)	20 (F)
SCN1A mutation[a]	D1416H DN	R101Q DN	c.602+1G>A DN	I1545V DN	A239T I, P	A1326P DN	F575SfsX48 DN	F1707V	V944E DN	R222X DN
Age at onset	6 mo	5 mo	5 mo	8.5 mo	6 mo	6 mo	7 mo	3 mo	7 mo	6 mo
ASD	√	X	X	X	√	X	X	X	X	X
AEDs at assessment	CLB, VPA, TPM, STP	VPA, CLB, TPM, LEV	STP, CLB, TPM, LEV, CZP	STP, VPA, TPM	VPA, TPM, CLB	CZP, VPA, TPM, VNS	VPA, STP, CLB, VNS	CLB, VPA, TPM, LTG	STP, VPA, CLB	LEV
Age at assessment	6 y 8 mo	9 y 5 mo	10 y 9 mo	11 y 7 mo	14 y 6 mo	17 y 4 mo	23 y 10 mo	27 y 2 mo	27 y 11 mo	28 y 5 mo

Continued

Table 2 Continued

	Verbal, individual (sex)									
	11 (M)	**12 (F)**	**13 (M)**	**14 (F)**	**15 (M)**	**16 (M)**	**17 (F)**	**18 (M)**	**19 (M)**	**20 (F)**
Cognition[b]	46	46[c]	Severe ID	68[c]	48[c]	Severe ID[c]	56	40	Severe ID	87
Language										
Receptive[d]	50	58	33	58	Unable to finish	55	45	20	NC	PPVT 76 TROG 71
Expressive[d]	50	53	26	61		48	59	20	NC	84
Dysphagia	√PEG	X	√	X	√	X	√	√	X	X
Oral motor and speech motor										
Drooling	X	X	X	X	√	√	√	√	√	X
Lips: rounding, retraction	Abn[e]	N	Abn[e]	N	FDA-2: retraction 7, alternate 6	FDA-2: retraction 9, alternate 6	FDA-2: retraction 5, alternate 3	FDA-2: retraction 7, alternate 3[e]	NC	FDA-2: retraction 8, alternate 8
Tongue: elevation, protrusion, lateral	Elevation, lateral Abn[e]	N	Abn[e]	Lateral Abn[e]	Trunk control inhibited movement	FDA-2: protrusion 3, elevation 1, lateral 2	FDA-2: protrusion 4, unable to finish	FDA-2: protrusion 6,[e] elevation 1, lateral 5[e]	NC	FDA-2: protrusion, elevation, lateral 9
Multiple movement: blow + smile, a-m-u, ka-la	Abn	Abn	Abn	Decreased lip movement	Abn	Abn	Abn	Abn	NC	N
Motor planning/ programming	Abn	VSeq[f]	VSeq[f]	VSeq 95th percentile	Abn, groping	Abn	Abn	Abn	NC	N
VMPAC	VGlobal[f]	VGlobal[f]	VGlobal[f]	VGlobal[f]	—	—	—	—	—	—
	VFocal[f]	VFocal[f]	VFocal[f]	VFocal[f]	—	—	—	—	—	—
Other publications	—	—	31	30,31	30	31	30,31	30,31,33	30,31,33	30,31,33,34

Abbreviations: Abn = abnormal; AED = antiepileptic drug; ASD = autism spectrum disorder; AZM = acetazolamide; CLB = clobazam; CZP = clonazepam; DN = de novo; FDA-2 = Frenchay Dysarthria Assessment, 2nd ed (scores 7 or below correspond to less than 1% of the normative sample); I = inherited; ID = intellectual disability; LEV = levetiracetam; LTG = lamotrigine; Ma = maternal; N = normal; NC = not able to cooperate with testing; NS = not shown; P = paternal; PEG = percutaneous endoscopic gastrostomy; PPVT = Peabody Picture Vocabulary Test; STP = stiripentol; TPM = topiramate; TROG = Test for Reception of Grammar; VFocal = VMPAC Focal Oromotor Control; VGlobal = VMPAC Global Motor Control; VMPAC = Verbal Motor Production Assessment for Children; VNS = vagal nerve stimulator; VPA = valproic acid; VSeq = VMPAC Sequencing.

Severe ID = standardized assessment attempted but valid score could not be obtained; IQ estimated to be less than 40. Three individuals (6, 7, 20) showed no oral motor impairment; 2 were unable to finish the assessment due to fatigue (17) and a seizure during testing (11). Individual 19 did not cooperate with the assessment.

[a] Nomenclature according to Claes et al. (Hum Mutat 2009).[35]

[b] Scores indicate full-scale IQ (Wechsler Intelligence Scale for Children–IV, Wechsler Abbreviated Scale of Intelligence–II; mean 100, SD 15) or ID ranges based on Vineland Adaptive Behaviour Scales scores and full-scale IQ results if available.

[c] External clinical assessment.

[d] Normative data—mean 100, SD 15; scores 70 and below: <2 SD below the mean.

[e] Motor planning difficulty.

[f] Below fifth percentile.

Table 3 Perceptual speech assessment in verbal patients with conversational speech (n = 13[a])

	Frequency		Severity, n		
	n	%	Mild	Moderate	Severe
Respiration					
Breath support for speech	13	100	4	7	2
Audible inspiration	6	46	2	4	
Forced inspiration/expiration	4	31	2	2	
Grunt at end of expiration	0	0			
Voice					
Intermittent breathiness	10	77	10		
Wetness	5	38	4	1	
Strain-strangled	4	31	2	2	
Hoarseness	3	23	3		
Glottal fry	3	23	3		
Harshness	0	0			
Pitch					
Variation of pitch (monopitch)	10	77	7	3	
Steadiness of pitch (tremor)	10	77	8	2	
Pitch level	8	62	7	1	
Excessive fluctuation of pitch	6	46	2	3	1
Pitch breaks	5	38	3	2	
Loudness					
Maintenance of loudness	8	62	7	1	
Loudness level (overall loudness)	6	46	6		
Variation of loudness (monoloud)	6	46	6		
Excessive loudness variation	5	38	4	1	
Articulation					
Precision of consonants	13	100	6	5	2
Length of phonemes	13	100	7	6	
Precision of vowels	13	100	7	6	
Resonance					
Hyponasality	8	62	5	3	
Mixed nasality	2	15	1	1	
Hypernasality	1	8	1		
Prosody					
General stress pattern	13	100	4	9	
Phrase length	11	85	2	9	
General rate	10	77	6	4	
Maintenance of rate	8	62	8		
Prolonged intervals	8	62	6	2	
Rate fluctuations	3	23	2	1	
Short rushes of speech	3	23	2	1	
Intelligibility					
Overall intelligibility	12	92	5	4	3

[a] The 2 youngest individuals in the verbal group (patients 6 and 7) aged 15 months did not have conversational speech.

communicative behavior (Complexity of Communication Scale [CCS] score 7b) such as eye contact, gesture, vocalization (1, 5), or using an adult's hand as a tool (3) regarded as nonsymbolic communication. Two (2, 4) had symbolic communication (CCS score 10), using single words recognized by an unfamiliar observer.

In the V group, conversational speech intelligibility was severely impaired in 3 (16, 18, 19), moderately impaired in 4 (11, 13, 15, 17), mildly reduced in 5 (8, 9, 10, 12, 14), and normal in 1 (20). All had inadequate breath support for speech. Speech was typified by imprecise articulation of consonants and vowels, abnormal nasal resonance, breathy or strain-strangled voice, low pitch, and prosodic errors (e.g., excess stress on unstressed parts of speech, slow rate, short phrases). Sound errors included voicing errors, distortion of fricatives /s, z, ʃ/, affricates /tʃ, dʒ/ and /l/, delayed phonological processes (final consonant deletion, gliding, fronting, stopping, cluster reduction, /f/ for /θ/, /d/ for /ð/) and atypical phonological processes (backing, replacing sounds with /j/ or /h/, insertion of schwa vowel). The vocal quality of individuals 16 and 17 may be attributed to VNS functioning; however, their voice was similar to patients without a VNS.

Language. Thirteen patients cooperated with language testing. Severely impaired receptive and expressive language (>2 SD below the mean) was seen in 9/13, a severe expressive deficit in patient 8 (receptive moderate), and moderately impaired receptive language in patient 20. The 2 youngest patients scored in the average range at age 15 months (table 2).

DISCUSSION A distinctive speech and language phenotype was found in 20 patients with DS associated with *SCN1A* mutations. Oral motor impairment was common, compounded by poor postural control of the trunk, neck, and head. Motor planning and programming difficulties were striking. Speech was characterized by imprecise articulation of consonants and vowels, abnormal nasal resonance, breathy or strain-strangled voice, and errors in pitch and prosody. Language impairment involving receptive and expressive language was seen in all bar the 2 youngest children. Nonverbal individuals had intentional communication.

Our language findings were more severe than previously reported in 2 earlier studies, which found borderline to average comprehension (9/12 and 9/9 children) and naming (8/12 and 4/9 children).[3,4] This disparity is likely due to past studies including children of a younger age range (up to 13 years of age) and with better cognitive profile. Further, around half of previously reported patients had *SCN1A* mutations. Our findings are comparable to a cohort aged up to 16 years, in which 3/20 children had preserved language.[2]

We found a trend towards a more severe speech phenotype in adults than children, with 3/4 of verbal adults being moderately to severely unintelligible. Current therapeutic regimens for DS are more targeted than in the past, which may lead to amelioration of speech impairment. The oldest adult had very mild impairments in respiration, articulation, phonation, and prosody; however, her phenotype is distinct from the rest of the cohort, as she had normal speech intelligibility, no oral motor impairment, and normal intellect, which is rare in DS. The youngest girls, aged 15 months, presented with age-appropriate language and oral motor skills, which likely reflects the typical developmental trajectory of DS, with normal development slowing in the second year of life.

Interestingly, 5 patients (aged 6–27 years) reported mild dysphagia, similar to the frequency reported in older adults from their fourth decade (5/22 patients).[5] Larger numbers of patients are needed to determine whether there is a correlation between the severity of the speech phenotype and features such as age, type and inheritance of *SCN1A* mutation, seizure types, and medication. Looking at cognitive outcome more broadly, previous studies have shown no correlation of *SCN1A* mutation class, age at seizure onset, type, and number, and MRI abnormalities.[8,9]

The voltage-gated sodium channel $Na_V1.1$, encoded by *SCN1A*, is found in brain regions important for speech and language function including the cerebellum, sensory motor cortex, basal ganglia, hippocampus, middle temporal gyrus, and middle frontal gyrus.[10] Hyperexcitability due to loss of function of GABAergic inhibitory interneurons expressing $Na_V1.1$ underlies seizures in DS.[11] Abnormal inhibition may also be important for speech and language function in DS. Interestingly, abnormal excitation due to mutations in the excitatory glutamate receptor subunit gene *GRIN2A* is associated with motor speech impairment in epilepsy-aphasia syndromes.[12] Studies in milder *SCN1A* phenotypes such as GEFS+ may clarify the role of sodium channels in speech and language impairment.

Moreover, structural changes in speech and language brain regions have been reported and include precentral gyrus, cerebellum, brainstem, corpus callosum, corticospinal tracts, and association fibers (left inferior fronto-occipital fasciculus, left uncinate fasciculus),[13] and influence phenotypic heterogeneity.

Understanding the speech and language phenotype in DS is crucial to planning early intervention. Targeted dysarthria therapy has been successful in other pediatric populations with mild to severe dysarthria[14] and could potentially also improve speech intelligibility of verbal patients with DS.

AUTHOR CONTRIBUTIONS

S.J.T., A.T.M., and I.E.S. designed the study and wrote the manuscript. A.T.M., I.E.S., and V.A. supervised the study. S.J.T. and A.T.M. performed phenotypic analysis. A.B. and M.A. performed most standardized developmental/intellectual functioning assessments.

ACKNOWLEDGMENT

The authors thank the families for their participation in this study; Associate Professor Nancy Brady for use of the Complexity of Communication Scale; Deborah Hayden and Brookes Publishing for permission to use the prepublication version of the Early Motor Control Scales; and Natalie Bryant and Annie Roten for assistance with filming assessments.

STUDY FUNDING

S.J.T. is supported by National Health and Medical Research Council (NHMRC) Postgraduate Scholarship (101777), Australian National University Gowrie Scholarship, and Speech Pathology Australia Nadia Verrall Memorial Research Grant. V.A. is supported by NHMRC Senior Practitioner Fellowship (2010–2019). A.T.M. is supported by NHMRC Career Development Award (607315, 2010–2015) and Practitioner Fellowship (1105008, 2016–2020). I.E.S. is supported by NHMRC Program Grant (628952, 2011–2015; 1091593, 2016–2020) and Senior Practitioner Fellowship (1006110, 2011–2015; 1104831, 2016–2020). The project is also supported by Australian Research Council Discovery Project (DP120100285) to A.T.M. and I.E.S.

DISCLOSURE

S. Turner, A. Brown, M. Arpone, V. Anderson, and A. Morgan report no disclosures relevant to the manuscript. I. Scheffer serves on the editorial boards of *Neurology®* and *Epileptic Disorders*; may accrue future revenue on a pending patent re: Therapeutic compound; has received speaker honoraria from Athena Diagnostics, UCB, GSK, Eisai, and Transgenomics; has received funding for travel from Athena Diagnostics, UCB, and GSK; and receives/has received research support from the NHMRC, ARC, NIH, Health Research Council of New Zealand, March of Dimes, the Weizmann Institute, CURE, US Department of Defense, and the Perpetual Charitable Trustees. Go to Neurology.org for full disclosures.

Received June 9, 2016. Accepted in final form November 23, 2016.

REFERENCES

1. Dravet C, Bureau M, Oguni H, Cokar O, Guerrini R. Dravet syndrome (severe myoclonic epilepsy in infancy). In: Bureau M, Genton P, Dravet C, et al, eds. Epileptic Syndromes in Infancy, Childhood and Adolescence, 5th ed. Montrouge, France: John Libbey Eurotext Ltd.; 2012: 125–156.
2. Casse-Perrot C, Wolff M, Dravet C. Neuropsychological aspects of severe myoclonic epilepsy in infancy. In: Jambaque I, Lassonde M, Dulac O, eds. The Neuropsychology of Childhood Epilepsy. New York: Plenum Press/Kluwer Academic; 2001:131–140.
3. Battaglia D, Chieffo D, Siracusano R, et al. Cognitive decline in Dravet syndrome: is there a cerebellar role? Epilepsy Res 2013;106:211–221.
4. Chieffo D, Battaglia D, Lettori D, et al. Neuropsychological development in children with Dravet syndrome. Epilepsy Res 2011;95:86–93.
5. Catarino CB, Liu JY, Liagkouras I, et al. Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology. Brain 2011;134:2982–3010.
6. Genton P, Velizarova R, Dravet C. Dravet syndrome: the long-term outcome. Epilepsia 2011;52(suppl 2):44–49.
7. Hayden D, Wetherby AM, Cleary JE, Prizant BM. Early Motor Control Scales: Prepublication Version. Baltimore: Paul H. Brookes Publishing Co.; 2011.

8. Brunklaus A, Ellis R, Reavey E, Forbes GH, Zuberi SM. Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome. Brain 2012;135: 2329–2336.
9. Villeneuve N, Laguitton V, Viellard M, et al. Cognitive and adaptive evaluation of 21 consecutive patients with Dravet syndrome. Epilepsy Behav 2014;31:143–148.
10. Whitaker WR, Faull RL, Waldvogel HJ, Plumpton CJ, Emson PC, Clare JJ. Comparative distribution of voltage-gated sodium channel proteins in human brain. Brain Res Mol Brain Res 2001;88:37–53.
11. Yu FH, Mantegazza M, Westenbroek RE, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci 2006;9:1142–1149.
12. Turner SJ, Mayes AK, Verhoeven A, Mandelstam SA, Morgan AT, Scheffer IE. GRIN2A: an aptly named gene for speech dysfunction. Neurology 2015;84:586–593.
13. Perez A, Garcia-Penton L, Canales-Rodriguez EJ, et al. Brain morphometry of Dravet syndrome. Epilepsy Res 2014;108:1326–1334.
14. Pennington L, Parker NK, Kelly H, Miller N. Speech therapy for children with dysarthria acquired before three years of age. Cochrane Database Syst Rev 2016;7:CD006937.
15. Hayden D, Square P. The Verbal Motor Production Assessment for Children. San Antonio, TX: The Psychological Corporation; 1999.
16. Enderby P, Palmer R. Frenchay Dysarthria Assessment, 2nd ed. Austin, TX: PRO-ED; 2008.
17. Brady NC, Fleming K, Thiemann-Bourque K, et al. Development of the communication complexity scale. Am J Speech-Language Pathol 2012;21:16–28.
18. Murdoch BE. Dysarthria: A Physiological Approach to Assessment and Treatment. Cheltenham, UK: Stanley Thornes; 1998.
19. Dodd B, Hua Z, Crosbie S, Holm A, Ozanne A. Diagnostic Evaluation of Articulation and Phonology. London: Pearson Assessment; 2002.
20. Zimmerman I, Steiner V, Pond R. Preschool Language Scales, Australian and New Zealand Language Adapted Edition, 5th ed. Sydney: Pearson; 2011.
21. Semel E, Wiig E, Secord W. Clinical evaluation of language fundamentals. In: Australian Standardised Edition, 4th ed. Marrickville, Australia: Harcourt Assessment; 2006.
22. Dunn LM, Dunn DM. The Peabody Picture Vocabulary Test, 4th ed. Minneapolis, MN: NCS Pearson Inc.; 2007.
23. Williams KT. Expressive Vocabulary Test, 2nd ed. London: Pearson Assessment; 2007.
24. Bishop DJ. Test for Reception of Grammar, 2nd ed. London: Pearson Assessment; 2003.
25. Wechsler D. The Wechsler Abbreviated Scale of Intelligence, 2nd ed. London: Pearson; 2011.
26. Wechsler D. Wechsler Intelligence Scale for Children, Australian Standardised Edition, 4th ed. Sydney: Pearson Clinical and Talent Assessment; 2005.
27. Sparrow SS, Cicchetti DV, Balla DA. Vineland Adaptive Behavior Scales, 2nd ed. London: Pearson; 2005.
28. Bayley N. Bayley Scales of Infant and Toddler Development, 3rd ed. London: Pearson; 2005.
29. Carvill GL, Weckhuysen S, McMahon JM, et al. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. Neurology 2014;82:1245–1253.
30. Harkin LA, McMahon JM, Iona X, et al. The spectrum of SCN1A-related infantile epileptic encephalopathies. Brain 2007;130:843–852.
31. Rodda JM, Scheffer IE, McMahon JM, Berkovic SF, Graham HK. Progressive gait deterioration in adolescents with Dravet syndrome. Arch Neurol 2012;69:873–878.
32. Singh R, Andermann E, Whitehouse WP, et al. Severe myoclonic epilepsy of infancy: extended spectrum of GEFS+? Epilepsia 2001;42:837–844.
33. Jansen FE, Sadleir LG, Harkin LA, et al. Severe myoclonic epilepsy of infancy (Dravet syndrome): recognition and diagnosis in adults. Neurology 2006;67:2224–2226.
34. Vadlamudi L, Dibbens LM, Lawrence KM, et al. Timing of de novo mutagenesis: a twin study of sodium-channel mutations. New Engl J Med 2010;363:1335–1340.
35. Claes LRF, Deprez L, Suls A, et al. The *SCN1A* variant database: a novel research and diagnostic tool. Hum Mutat 2009;30:E904–20.

CHAPTER 6
MULTIPLEX FAMILY STUDY

Family G is a multigenerational Australian family of European ancestry suspected of harbouring a new and as yet unidentified speech and language gene. The family was initially referred as one child (III-13) was diagnosed with CAS, and had five of eight siblings who required speech pathology intervention. Both of the proband's parents had therapy for speech disorders as children, and there is a strong family history of speech and language disorder on the maternal side. The three youngest children later presented with delayed or disordered speech; III-16 was an infant when the family were initially referred and III-17 and III-18 were born subsequently (pedigree in figure 6-1).

The family were chosen for detailed phenotypic analysis, as the pedigree looked likely to be segregating a gene of major effect. Thirty-nine individuals from seven branches of the family were invited to participate in the study (figure 6-2). Two unaffected branches of the family were unable to be contacted. Twenty-eight affected and unaffected family members consented to participate in the study. Information regarding each individual's birth, development, medical history and history of speech and language impairment is detailed (see Appendix 8). Phenotypic assessment was completed and analysed as described in the Methods chapter. Assessments were conducted at The Royal Children's Hospital, Melbourne Brain Centre or in the family home, including seven field trips to regional Victoria. Key diagnostic findings of the 21 affected individuals are summarised in Table 6-1.

Clinical phenotyping data has informed molecular genetic studies. SNP genotyping by molecular collaborators has been completed in this family, with whole exome sequencing underway with the aim of finding the causative gene.

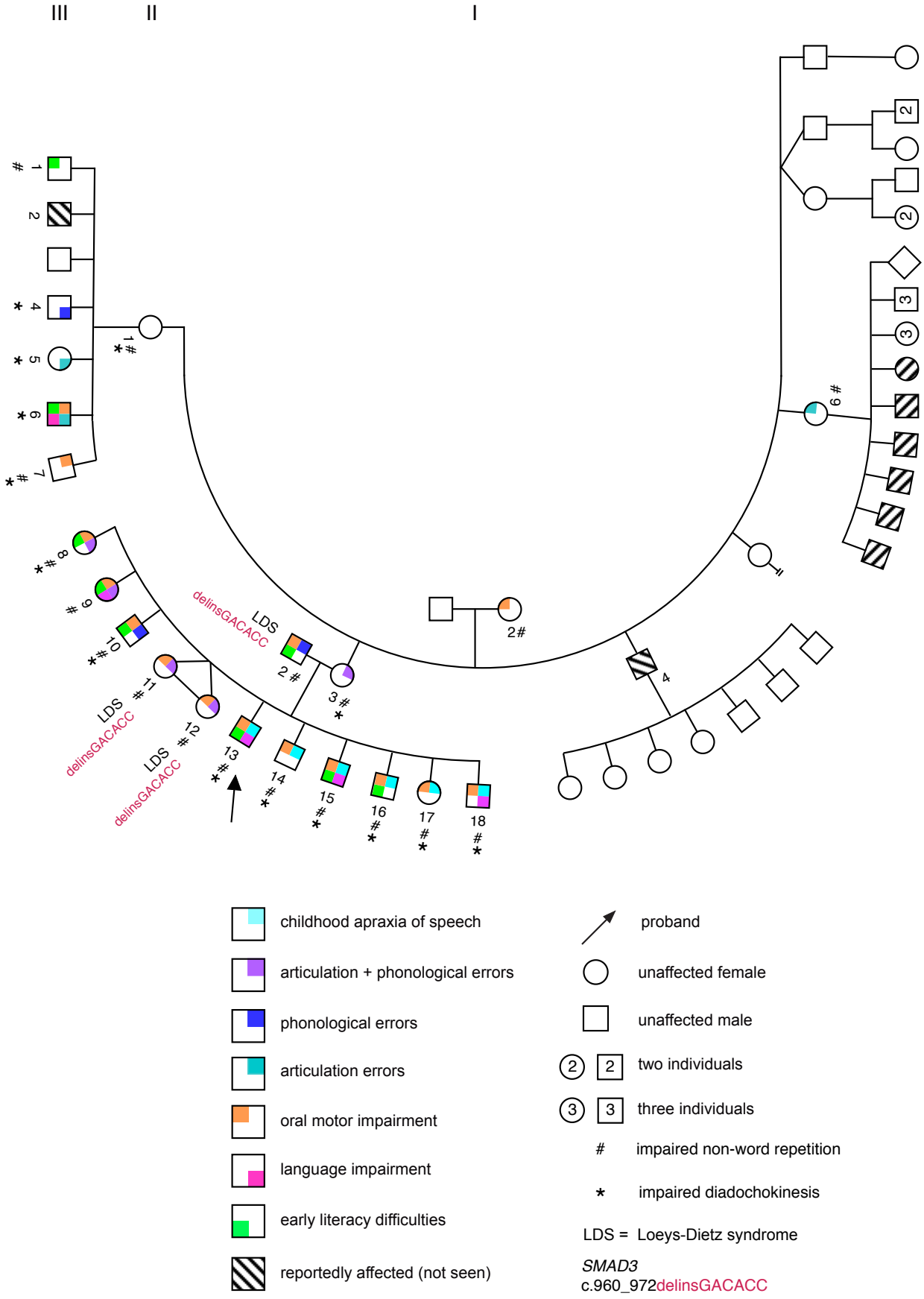


Figure 6-1. Pedigree of family G

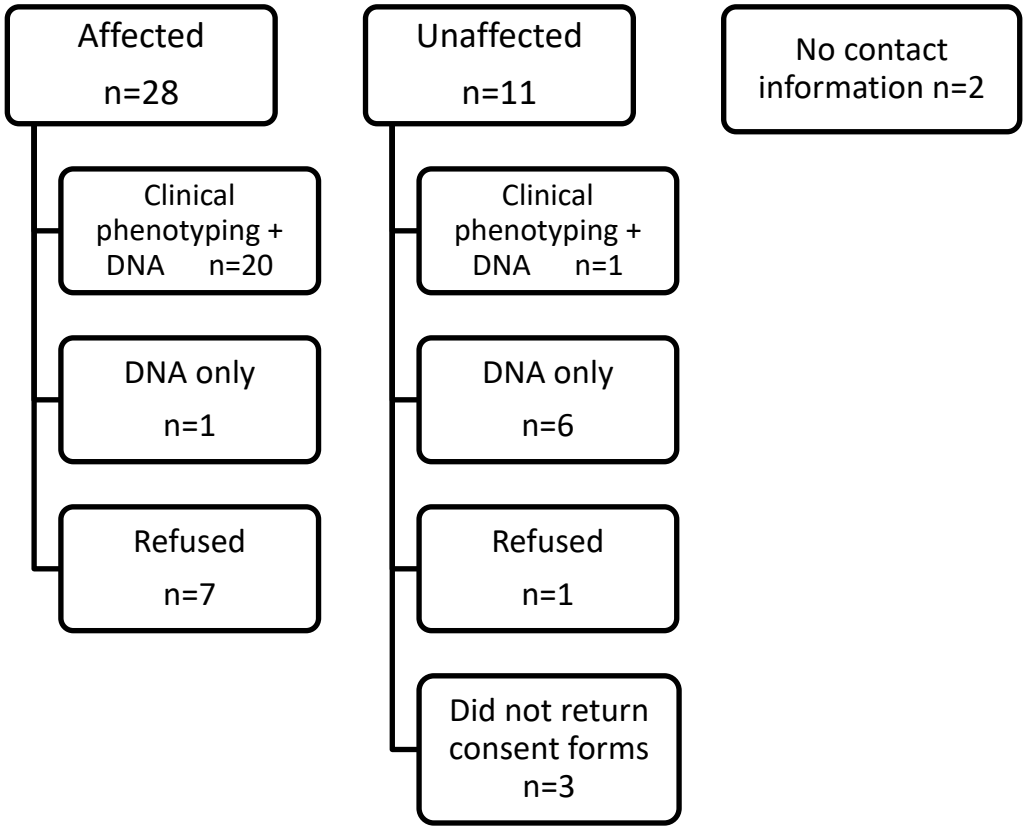


Figure 6-2. Participation of 39 family members in the study

Table 6-1. Key diagnostic features of 21 individuals from family G who underwent phenotypic analysis.

Individual	I-2	II-1	II-2	II-3	II-6	III-1	III-4	III-5	III-6	III-7	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15	III-16	III-17	III-18
Relationship to proband	GMo	Aunt	Fa	Mo	Aunt	Cousin	Cousin	Cousin	Cousin	Cousin	Sib	Sib	Sib	Sib	Sib	P	Sib	Sib	Sib	Sib	Sib
Sex	F	F	M	F	F	M	M	F	M	M	F	F	M	F	F	M	M	M	M	F	M
Oral motor impairment	+	-	+	-	NT	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
CAS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Articulation errors	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	+	+	-	+	+	-
Phonological errors	-	-	+	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Language impairment	-	-	-	-	-	-	-	-	+	NT	-	+	-	-	-	+	-	+	-	-	+
Early literacy difficulties	-	-	+	-	-	+	-	-	+	-	+	+	+	-	-	+	-	+	+	n/a	n/a

GMo: grandmother; Fa: father; Mo: mother; Sib: sibling; P: proband; F: female; M: male; + feature present; - feature absent; NT: not tested; n/a: not applicable

Clinical phenotypic assessment

Oral motor structure and function (figure 6-3; table 6-2)

All family members except one underwent an oral motor assessment (VMPAC in 13 cases, partial assessments for III-11 and III-16; FDA-2 in 7 cases). II-6 refused to complete the assessment.

Two individuals wore orthodontic appliances as teenagers (II-3, III-9 - fitted after the initial speech assessment) and two required orthodontic treatment (twins III-11 and III-12). Eight had oral structural anomalies (anterior open bite in III-12; mandibular retrognathia in III-11, III-12, III-13, III-17; high arched palate in II-3, III-8, III-9, III-17; bifid uvula III-11, III-12, III-18). Forward resting tongue position and mild tongue thrust was reported in III-9 and III-11.

Poor postural control was evident in four individuals (III-10, III-11, III-12, III-14). Six had reduced tongue strength against mild resistance (III-6, III-7, III-10, III-13, III-14, III-15) and eight had involuntary tongue movements, which were manifested as fasciculations in II-1, III-4, III-5 and III-6. I-2 and II-2 had some lip asymmetry at rest, and II-2 also during retraction. I-2 and III-9 reported mild dysphagia, including a sensation of food getting stuck and occasional choking. I-2 had altered nasality in speech. Five of 16 individuals tested had reduced vowel prolongation (II-2, III-4, III-9, III-14, III-17).

Ten of 11 children were impaired on VMPAC Sequencing, with scores ranging from borderline to severe. III-11 and III-16 also demonstrated difficulties, but did not complete all the subtest items for scoring. All children had difficulty sequencing non-speech movement (eg. bite and blow), with only single movements performed or movements repeated, added or performed out of sequence. Sequences of consonant and vowels (e.g. m-o-i, m-o-i, m-o-i, m-o-i) were also problematic. One child (III-9) demonstrated some mild difficulty (i.e. smile and bite instead of bite and blow, a-u-m instead of a-m-u) but scored within normal limits on the Sequencing subtest.

Twelve children were impaired on the VMPAC Focal Oromotor Control subtest, with all but one scoring in the severe range. III-16 also had difficulties but did not complete all the subtest items for scoring. When non-speech movements were examined, all

except III-9 had reduced lip rounding when puckering to kiss, and five had reduced retraction (III-7, III-13, III-15, III-16, III-18) with asymmetrical lip movement in III-6, III-10 and III-18. Reduced jaw excursion, asymmetrical movement or jaw sliding were noted in five (III-10, III-11, III-14, III-17, III-18). Most had tongue imprecision, with asymmetrical movement in three (III-11, III-14, III-16) and groping in eight (III-10, III-11, III-13, III-14, III-15, III-16, III-17, III-18). Lip, tongue and jaw movement were also imprecise in speech tasks (i.e. say i-u-a).

Adults assessed on the FDA-2 had poor oromotor control of the tongue. Three (I-2, II-2, III-1) had reduced tongue elevation, with slow and uneven tongue protrusion also seen in I-2 and imprecise lateral movement in II-2. Imprecise tongue movement in conversational speech was noted in II-1, II-3, III-4 and III-5.

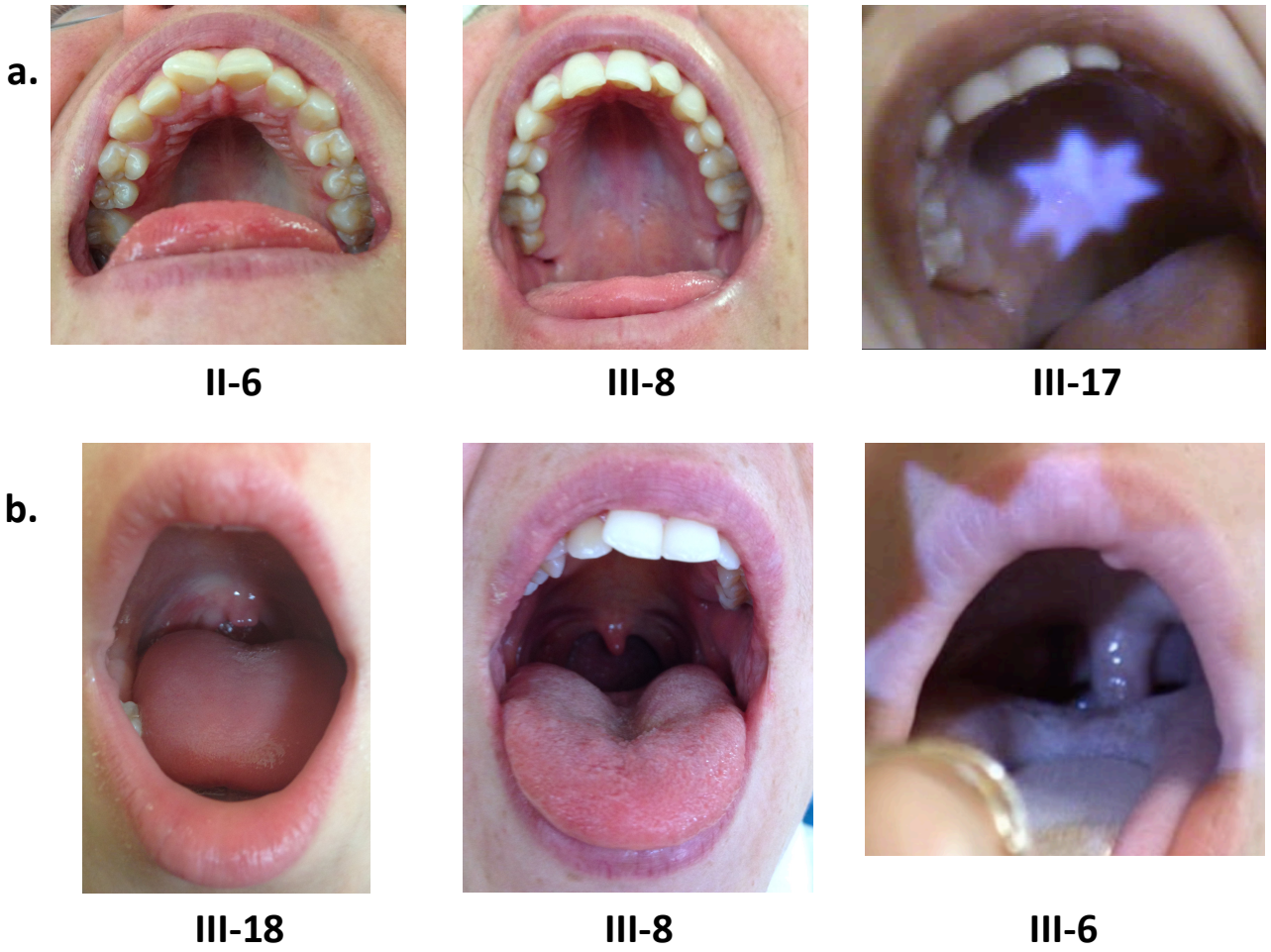


Figure 6-3. Structural findings of palate and uvula in individuals with speech disorder. a. High arched palate in three individuals with speech disorder. b. Bifid uvula in one child with CAS (III-18) and normal uvula in two other individuals with speech disorder (III-8; III-6).

Table 6-2. Results on three subtests of the Verbal Motor Production Assessment for Children for 13 individuals

Individual		III-6	III-7	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15	III-16	III-17	III-18
Age at assessment		9y5m	8y	14y5m	12y9m	10y7m	9y4m	9y4m	7y5m	5y3m	5y5m	3y5m	4y8m	3y8m
Global motor control	**%**	85	85	100	100	80	95	95	80	65	90	95	95	90
	severity	**severe**	**severe**	WNL	WNL	**severe**	**mod**	**mod**	**severe**	**severe**	**severe**	WNL	**mild**	**mild**
Focal oromotor	**%**	83	72	85	87	73	74	88	67	65	48	nc	66	52
	severity	**severe**	**severe**	**severe**	**severe**	**severe**	**severe**	**severe**	**severe**	**severe**	**severe**	nc	**severe**	**mod**
Sequencing	**%**	80	43	85	97	83	nc	86	52	61	41	nc	58	36
	severity	**severe**	**severe**	**mild**	WNL	**mod**	**nc**	**border**	**severe**	**mod**	**severe**	nc	**border**	**mild**

y: years; m: months; WNL: within normal limits; mod: moderate; nc: did not complete all tasks for scoring; border: borderline

Results in the clinically impaired range are highlighted in bold.

Speech disorder (table 6-3; appendix 7)

A connected speech sample (conversational speech, Rainbow Passage and picture description task) was collected from all 21 family members, and the GFTA-2 was administered to all individuals aged up to 21 years. Seventeen individuals had speech disorder, and ten met consensus criteria for CAS.

Speech sound errors

Nine individuals had both articulation and phonological speech errors (II-3, III-8, III-9, III-11, III-12, III-13, III-14, III-16, III-17), five had only phonological speech errors (II-2, III-4, III-10, III-15, III-18) and three only articulation errors (II-6, III-5, III-6). II-2 also had motor execution difficulties in conversational speech, with slightly unclear articulation and rushes of speech.

Consistent articulation errors included dentalised production of alveolar fricatives /s/ and /z/, plosive /d/ and lateral approximant /l/, dentalised or lateral production of postalveolar fricative /ʃ/ and dentalised production of affricates /tʃ/ and /dʒ/. The proband III-13 had derhotacized /r/. Five had labiodental production of bilabials (III-11, III-12, III-13, III-16, III-17). Four children aged 9 years or younger (III-11, III-14, III-16, III-17) had inconsistent sound distortions that may have been resolving. In typically developing children, accurate sound production is achieved by approximately 9 years of age.[252]

Delayed phonological processes including final consonant deletion, stopping of fricatives, cluster reduction and gliding were heard in III-14. Older family members had fronting of fricative (/θ/ replaced with [f] eg. "thing" to 'fing') and /ð/ replaced with [v]. II-1 inconsistently replaced /ð/ with [v] in conversational speech. In addition to phonological processes that were age appropriate or delayed, seven children also had atypical processes (reported in III-8 at 4 years, 7 months; III-13 at 4 years; III-14 at 5 years, 3 months) such as backing, replacing consonant clusters with [fw], replacing plosives /p, t, k/ with glottal fricative [h], deletion of medial sounds and gliding of fricatives and affricates.

Table 6-3. Results of speech and maximum performance tasks for 19 individuals with and without speech disorder

Individual		I-2	II-1	II-2	II-3	II-6	III-1	III-4	III-5	III-6	III-7	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15	III-16	III-17	III-18
GFTA	**Age**	n/a	n/a	n/a	n/a	n/a	nt	15y9m	14y	9y7m	6y7m	14y5m	12y9m	10y7m	9y4m	9y4m	7y5m	5y3m	5y5m	3y5m	4y8m	3y8m
	SS							**59**	**82**	**61**	108	**<40**	**40**	93	87	87	**55**	**70**	**44**	**81**	**63**	**59**
Age for other tasks		67y	43y	46y	44y	36y	19y4m	15y9m	14y	11y7m	8y	17y6m	16y2m	13y11m	12y8m	12y8m	10y5m	8y7m	6y4m	4y5m	4y8m	3y8m
NWR (n=28)		**CNR 6/18**	**16**	**13**	**7**	**CNR 5/18**	**CNR SS 6**	17	22	16	**SS 66***	**16**	**15**	**14**	**13**	**14**	**6**	**SS <50***	**SS 72***	**SS 77***	**SS 74***	**SRT 42%**
MSW (n=52)		nt	**39**	**47**	**41**	nt	nt	43	49	44	27	46	44	36	38	45	20	27	n/a	n/a	n/a	n/a
Inconsistency (%)		nt	nt	8	4	nt	nt	nt	nt	20	nt	0	12	12	16	4	32	24	**52**	36	**48**	**64**
Maximum repetition rate (syllables/ second)	**/pa/**	nt	3.8	6.8	6.2	nt	nt	**4.4**	**5.3**	6.3	4.5	**4.2**	5.4	5.4	4.8	6.2	I	I	I	I	n/a	n/a
	/ta/	nt	3.8	**4.4**	I	nt	nt	**3.7**	**4.5**	6.5	4.9	**4.5**	**3.7**	5.6	**4.4**	I	**4.3**	**3.2**	inc	inc	n/a	n/a
	/ka/	nt	4.0	**4.1**	5.0	nt	nt	**3.4**	**4.4**	5.4	**3.8**	**3.2**	4.9	5.4	5.7	I	**4.0**	**3.0**	inc	inc	n/a	n/a
	/pataka/	nt	**4.6**	5.8	**5.3**	nt	5.8	**4.5**	**4.2**	inc	**3.1**	**3.6**	6.2	**3.9**	6.0	5.7	inc	Inc	Inc	Inc	Inc	inc

GFTA: Goldman-Fristoe Test of Articulation, 2nd Edition; n/a: no norms for age/not age-appropriate; nt: not tested; y: years; m: months; SS: standard score; NWR: non-word repetition; n: number; CNR: CTOPP nonword repetition; SRT: Syllable Repetition task; MSW: multisyllabic word repetition; sec: seconds; I: response inconsistent; inc: sequence incorrect *Children's Test of Non-Word Repetition; #difficulty articulating /s/

Results in the clinically impaired range are highlighted in bold

Word and non-word repetition

Family members with and without speech disorder had difficulty with tasks designed to stress the phonological system. All individuals completed a non-word repetition task, including the CNRep in five cases, Nonword Memory Test in 12, and CTOPP non-word repetition in three. The youngest child (III-18) completed the SRT, as his speech errors made responses to the CNRep difficult to transcribe and score. Eighteen had poor non-word repetition, including four (I-2, II-1, III-1, III-7) who did not have speech disorder. On the multisyllabic word repetition task, most (11/14) had scores equivalent to individuals with a history of speech disorder, however II-1 and II-3 performed far below speech disordered peers. One individual with speech disorder (III-5) had average performance on both non-word and multisyllabic word repetition tasks.

Diagnosis of CAS (table 6-4)

The proband was diagnosed with CAS at 4 years of age. Other family members were examined for features of CAS in the present study. Diagnosis was based on the presence of all three consensus-based diagnostic criteria proposed by ASHA.[17] Clinical features used to define each of these three criteria were based on those reported in Murray et al, 2014.[224]

The proband and five of his siblings met the three consensus diagnostic criteria for CAS. Other family members demonstrated some features of CAS.

Criterion 1 – Inconsistent errors in repeated productions of syllables and words

Token-to-token variability was examined in 14 individuals using the Inconsistency assessment from the DEAP.[219] Thirteen had inconsistent errors, with lower inconsistency scores seen in older individuals. Both phonetic and phonemic errors were heard. Four children younger than seven years scored close to or above the 40% threshold for inconsistency (III-15, III-16, III-17, III-18). Inconsistent errors are atypical for individuals older than seven years of age (B. Dodd, personal communication) with all older children and adults but one (III-8) showing inconsistent errors. The proband and his younger brother and cousin had high inconsistency scores (scores 20% and above in III-6, III-13, III-14) while his parents and older siblings had inconsistency scores below 20% (II-2, II-3, III-9, III-10, III-11, III-12).

Table 6-4. CAS speech features (categorised under the three ASHA consensus diagnostic criteria) present in 13 individuals

Criteria	Speech features associated with each criteria	II-2	II-3	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15	III-16	III-17	III-18
Inconsistent errors	Same word/syllable different on repetitions	■	■		■	■	■	■	■	■	■	■	■	■
	Same C/V different across different words				■		■	■	■	■	■	■	■	■
Lengthened and disrupted coarticulatory transitions between sounds and/or syllables	*Any one of these*: Speech motor behaviours, including groping													
	Any one of these: Slowed diadochokinetic (DDK) rates/disrupted DDK sequence		■	■		■			■	■	■	■	■	■
	Any one of these: Difficulty sequencing phonemes and syllables		■		■	■	■	■	■	■	■	■	■	
	Any one of these: Difficulty achieving initial articulatory configurations/transitory movement gestures													
	Any two or more of these: Syllable segregation								■					■
	Any two or more of these: Intrusive schwa			■					■		■	■	■	■
	Any two or more of these: Epenthesis		■		■		■	■	■		■	■		
	Any two or more of these: Frequent omission errors		■	■	■	■	■	■	■	■	■	■	■	■
	Any two or more of these: Addition errors	■	■	■	■	■	■	■	■	■	■			
	Any two or more of these: Prolongation errors								■					
	Any two or more of these: Repetitions of sounds and syllables									■				
	Any two or more of these: Voicing errors			■				■	■		■	■	■	■
	Any two or more of these: Non-phonemic productions or distorted substitutions			■	■		■		■		■	■	■	
	Any two or more of these: Nasality and/or nasal emissions			■							■		■	■
	Any two or more of these: Errors increase with word length and phonological complexity					■	■		■	■	■	■	■	■
	Any two or more of these: Metathesis		■		■	■	■	■	■	■		■	■	
	Any two or more of these: Difficulty maintaining syllable integrity								■	■	■	■	■	■
Inappropriate prosody	Equal stress or lexical stress errors	■							■	■	■	■	■	■
	Prolongation errors								■					
	Vowel errors	■	■	■	■	■	■	■	■	■	■	■	■	■
	Vowel distortion													
	Altered suprasegmental characteristics	■							■	■	■		■	

Criterion 2 - Lengthened and disrupted coarticulatory transitions between sounds and/or syllables

Nineteen family members performed a DDK task (not including I-2, II-6) to assess the ability to program sequences of speech movements. Seven were unable to repeat a trisyllabic sequence (pataka) correctly, and seven had slow repetition of the sequence compared to controls. Sixteen performed a monosyllable repetition task (not III-1; normative data unavailable for III-17 and III-18) which assessed ability to coordinate repetitive movements of the lips, jaw and tongue, and is considered indicative of motor planning or execution difficulties. Fourteen (all except III-6 and III-10) were impaired on the task. Six had difficulty repeating the correct monosyllable, with inconsistent productions across repetitions. Ten had slow repetition of one or more monosyllables (pa, ta, ka) (II-1, II-2, III-4, III-5, III-7, III-8, III-9, III-11, III-13, III-14).

Responses to the speech tasks (GFTA-2; DEAP Inconsistency assessment; Nonword Memory Test/CTOPP Nonword repetition/CNRep; Multisyllabic Word Repetition task) were analysed for errors that indicated lengthened and disrupted coarticulatory transitions. Two or more error features were evident in all the children with CAS, as well as their older siblings, mother II-3 and aunt II-1. Sequencing errors (epenthesis/metathesis), addition and omission errors were most frequently heard across individuals. Four (II-1, III-7, III-11, III-14) made more errors on the Nonword Memory Test as the task became more complex and word length increased.

Criterion 3 - Inappropriate prosody

Perceptual judgements were made regarding prosodic disturbance. The proband had marked impairments in prosody, with lexical stress errors, sound prolongations and fast rate. Lexical stress errors were also heard in III-16, III-17 and III-18. II-2, III-14 and III-15 had misplaced or equal stress in conversational speech.

Language disorder (table 6-5)

All family members apart from III-7 completed the language assessment, with partial assessments for I-2, II-1, II-6 and III-1 (CELF-4 in 14 individuals 21 years and under; PPVT-4/TROG-2/EVT-2 in 2; PPVT-4 only in 3). III-16 had an external language assessment at the time of the study.

Fifteen individuals had average language skills. Four (III-6, III-9, III-15, III-18) had receptive language impairment with scores at the 5th percentile or below. III-13 and III-15 had expressive language impairment, scoring at the 1st percentile or below.

External testing subsequent to the study revealed impaired receptive and average expressive language in the proband III-13, and average receptive language in III-15 (see Case History - Appendix 8).

Reading and spelling impairment (tables 6-6 and 6-7)

Seven individuals (II-2, III-1, III-6, III-8, III-9, III-10, III-13) reported reading or spelling difficulties in primary school that required intervention (see Case History). III-15 and III-16 had intervention for literacy difficulties subsequent to the study.

Reading and spelling skills were assessed in all but the four youngest family members (III-15, III-16, III-17, III-18) on the WRAT-4. Phonological awareness was examined in nine individuals using the CTOPP. Phonological awareness testing was attempted in III-15, however was discontinued as he was unable to complete the practice items correctly.

All individuals tested had average phonological awareness scores, and only III-1 had below average reading and spelling. Six had reading or spelling skills in the high average to superior range (standard scores 110 and above) including II-2 who had extra support for literacy difficulties as a child. III-10 had reportedly performed below age expectations on criterion-referenced assessments of literacy, but scored within the average range on standardised testing.

Table 6-5. Language assessment results for 20 individuals

Individual	I-2	II-1	II-2	II-3	II-6	III-1	III-4	III-5	III-6	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15	III-16	III-17	III-18
Age at assessment	67y	43y	43y	41y	36y	19y4m	15y9m	14y	9y7m	14y2m	12y9m	10y7m	9y3m	9y3m	7y4m	5y3m	5y8m	4y5m	4y8m	2y2m
Receptive Language	118	113	107 (PPVT) 104 (TROG)	106 (PPVT) 104 (TROG)	111	97	nt	nt	**70**	95	**76**	94	94	91	88^	100	**72^**	101	98	**76**
Expressive Language	nt	nt	102	89	nt	nt	93	108	89	104	100	97	95	106	**63^**	99	**49^**	90	83	82

Table 6-6. Results of the Wide Range Achievement Test for 17 individuals

Individual	I-2	II-1	II-2	II-3	II-6	III-1	III-4	III-5	III-6	III-7	III-8	III-9	III-10	III-11	III-12	III-13	III-14
Age at assessment	67y	43y	45y	42y	36y	19y4m	15y9m	14y	9y7m	8y	15y7m	16y2m	12y	11y6m	11y6m	9y4m	7y5m
Word Reading	105	97	98	92	94	**78**	98	112	89	89	98	100	90	107	111	89	97
Sentence Comprehension	115	104	103	95	90	90	nt	nt	90	nt	106	101	102	104	101	92	101
Reading Composite	110	100	100	92	90	**82**	nt	nt	88	nt	101	100	95	105	106	89	99
Spelling	113	95	121	101	103	**82**	99	124	92	101	107	117	87	113	115	96	98

y: years; m: months; PPVT: Peabody Picture Vocabulary Test, 4th Edition; TROG: Test for Reception of Grammar, 2nd edition; nt: not tested
^external clinical assessment

Table 6-7. Results of the Comprehensive Test of Phonological Processing for 9 individuals

Individual	III-1	III-6	III-8	III-9	III-10	III-11	III-12	III-13	III-14
Age at assessment	19y4m	9y7m	14y2m	12y9m	10y7m	9y3m	9y3m	7y5m	5y3m
Phonological awareness	100	106	91	106	91	100	115	97	89
Phonological memory	**76**	**79**	100	**79**	**79**	97	97	**70**	82
Rapid Naming	88	103	100	130	109	133	130	ns	ns

y: years; m: months; ns: unable to score

Results in the clinically impaired range are highlighted in bold.

Cognition (table 6-8)

Thirteen family members underwent neuropsychological assessment, with a partial assessment completed for III-14 due to his age. External cognitive assessment results were available for III-15.

All family members apart from III-1 and III-6 had average non-verbal cognitive skills. Four had impaired verbal skills (III-11, III-12, III-14, III-15) and five (III-1, III-6, III-8, III-10, III-13) had verbal working memory impairment. All family members tested were able to retrieve sound-based information from long-term memory (CTOPP Rapid Naming), with scores unable to be calculated for the proband or his younger brother III-14. All had appropriate verbal memory and learning, apart from III-1 who had variable skills and III-13 who had mild attention and working memory difficulties. III-8 and III-10 had difficulty monitoring their performance on a test of executive function (III-8: Delis-Kaplan Executive Function System (DKEFS) Word naming subtest, total errors 1st percentile; III-10: DKEFS Inhibition subtest, uncorrected errors 5th percentile), with III-10 reported to have problems with planning and organising, and regulating his own behaviour in daily life.

Table 6-8. Neuropsychology assessment results for 14 individuals

Individual			I-2	II-1	II-2	II-3	III-1	III-6	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15
Age at assessment			67y	43y	43y	41y	19y4m	9y7m	14y2m	12y9m	10y7m	9y3m	9y3m	7y1m	5y3m	5y8m
Cognition	**KBIT-2**	**Non-verbal**	111	109	109	100	**67**	**74**	116	90	95	106	111	84	107	95^
		Verbal	98	104	98	106	82	89	87	84	87	**77**	**77**	94	**80**	**74^**
Memory	**Digit Span**	**Forward**	10	11	9	9	**4**	**6**	13	11	8	10	10	6	n/a	n/a
		Backward	11	11	8	10	7	7	**5**	12	**5**	11	14	**5**	n/a	n/a
Executive function	**BRIEF**	**Behavioural regulation**	n/a	n/a	n/a	n/a	n/a	nt	43	44	**78**	46	40	46	44	nt
		Metacognition	n/a	n/a	n/a	n/a	n/a	nt	58	51	**70**	54	38	56	42	nt
	DKEFS	**Inhibition**	13	12	11	12	11	11	8	13	11	14	13	n/a	n/a	n/a
		Inhibition/ Switching	13	9	11	13	12	13	9	15	12	12	15	n/a	n/a	n/a

y: years; m: months; KBIT-2: Kaufman Brief Intelligence Test, 2nd Edition; n/a: no norms for age/not age-appropriate; BRIEF: Behavior Rating Inventory of Executive Function; nt: not tested; DKEFS: Delis-Kaplan Executive Function System
^external clinical assessment

Results in the clinically impaired range are highlighted in bold.

Summary of the phenotype

The family comprised 56 individuals with 20 confirmed affected individuals over three generations. The dominant profile in the family was SSD. Six individuals presented with CAS, three with articulation errors, three with delayed phonological errors, and five with both articulation and phonological errors (four with delayed errors, one with delayed and disordered errors). Oral motor impairment evidenced by reduced, imprecise or poorly coordinated lip, tongue or jaw movement in non-speech and speech tasks was seen in 15 individuals. Individuals with and without speech disorder had difficulty on tasks examining sequencing of non-speech and speech movements. Family members with and without speech disorder also performed poorly on tasks designed to stress the phonological system, including repetition of multisyllabic real words and non-words.

Four individuals had receptive language impairment, with expressive language impaired in two children with CAS. Early literacy difficulties were reported in nine individuals, yet most demonstrated above average reading and spelling skills in their teenage years and into adulthood. Cognitive problems were not present for the majority of family members. All individuals tested had average non-verbal cognitive skills apart from one with borderline intellect and one with mild ID; this individual had a distinct phenotype, with autism spectrum disorder and below average reading and spelling skills, and no speech or oral motor disorder. Five individuals had verbal working memory impairment.

The proband's mother (II-3), aunt (II-1) and older siblings had some features of CAS (inconsistent errors, slow DDK rate, difficulty sequencing phonemes, omission and addition errors, intrusive schwa) but did not meet the three core criteria for diagnosis. Presentation of CAS is known to change across the lifespan with regard to severity and speech features.[26] Speech-sound errors in individuals with CAS may normalise over time.[243] Teenagers with CAS show improved articulation of target phonemes, but continue to have difficulty with novel and complex words.[253] Adults with some symptoms of CAS in the setting of a 7q11.23 duplication have difficulty with challenging speech tasks (multisyllabic word repetition, tongue twisters) but their speech is not severe enough to be diagnosed with speech disorder.[154] Thus these individuals may have had CAS when younger.

Examining patterns of inheritance

Affected individuals in this family demonstrated impairments in speech, oral motor skills, language and literacy, with the type and number of domains affected varying across individuals (figure 6-1). When the four domains are considered together as a broad phenotype, the inheritance pattern in this family is bilineal; individuals on both the paternal and maternal sides have speech disorder, impaired oral motor skills, language disorder and impaired reading and spelling. When examined individually, it is possible that language disorder may be inherited through the maternal side as the father of the proband was unaffected; one paternal cousin was reported to have language and literacy difficulties yet this was unconfirmed.

Endophenotypes were scrutinized to determine whether one or more traits showed monogenic inheritance. DDK may be a useful biomarker in this family; fourteen individuals on the maternal side performed the DDK task poorly, including two without speech disorder (II-1, III-7). Both II-1 and III-7 made more errors on the non-word repetition task as word length increased; III-7 was also severely impaired on the Sequencing subtest of the VMPAC and II-1 had sequencing errors on the challenging speech tasks. Thus an underlying motor planning/programming deficit may be present in this family, which is inherited on the maternal side and may be under the control of a single gene.

Other traits also appeared to be maternally inherited. Verbal working memory impairment was inherited on the maternal side, with three children in the proband family and two maternal cousins affected. Examination of speech error subtypes also suggested that articulation errors were inherited on the maternal side. However the proband's father may have presented with articulation errors when younger, and early speech pathology reports were not available to confirm this. Some individuals with articulation errors also had a history of jaw malocclusion and tongue thrust. Impaired multisyllabic word and non-word repetition, which was a core deficit in affected members of the KE family,[78] did not prove to be a useful endophenotype in the G family as it did not segregate with speech disorder status, and both parents of the proband were affected. Of note, the proband's mother and maternal aunt were the only two family members whose scores were below those of speech-disordered controls on the multisyllabic word test.

Comorbid connective tissue disorder may be influencing the phenotype in this family. The proband's father and twin sister have Loeys-Dietz syndrome (LDS) and a confirmed *SMAD3* mutation. LDS is an autosomal dominant aortic aneurysm syndrome involving multiple organ systems (craniofacial, musculoskeletal, integumental, ocular). It is characterised by a triad including hypertelorism, bifid uvula or cleft palate and aortic aneurysm. While speech has not been examined in LDS, speech impairments are reported in related connective tissue disorders including Marfan syndrome and joint hypermobility syndrome or Ehlers-Danlos syndrome, hypermobility type (JHS/EDS-HT).[254-256] In these disorder, hypermobility of the temporomandibular joint, hypotonia of the oral and laryngeal structures, hypoplastic lingual frenulum and reduced proprioception are hypothesised to underlie speech deficits (reviewed in [257]). Interestingly, several studies report clinical overlap between generalised joint hypermobility, JHS/EDS-HT and DCD.[257, 258] DCD is in turn associated with CAS.

Other family members have features associated with connective tissue disorder including joint hypermobility (III-9, III-10, III-13, III-16), scoliosis (III-9), pes planus (III-8, III-9, III-10), hip dislocation (III-13), high arched palate (II-3, III-8, III-9, III-17) and bifid uvula (III-18). Some of these features are also reported in the extended family on the maternal side, including I-2, raising the possibility that a separate connective tissue disorder may be present in the family.

The speech disorder phenotype in this family may follow monogenic, oligogenic or polygenic forms of inheritance. Autosomal dominant inheritance is possible as there is more than one affected generation, and males and females are equally likely to have speech disorder. CAS is present in a single generation of the proband family, and may be due to inheritance of recessive alleles from each parent. Given the bilineal history of speech and language disorder, a number of susceptibility genes from both sides of the family may also have given rise to the speech disorder phenotype. A few major genes may have combined to result in expression of speech disorder through oligogenic inheritance. Alternatively, common functional polymorphisms in a number of genes may have been inherited together via polygenic inheritance. For both oligogenic and polygenic inheritance, each susceptibility gene modifies the risk but is not sufficient alone to cause the disease. It can be difficult to distinguish oligogenic from polygenic disorders, and in large pedigrees, polygenic inheritance may also be mistaken for autosomal dominant inheritance.

The involvement of environmental factors in this family should not be overlooked, as all the offspring have lived in a similar environment for their growth and development. Indeed most cases of speech disorder are postulated to follow complex multifactorial inheritance,[13] where multiple genes and environmental factors determine an individual's affected status. Yet studies examining the relative contribution of genes and environment to speech disorders highlight the important role of genetic factors in transmission of speech disorders in families.[65]

Chapter 7
Discussion

The past five years has seen a surge in new genetic discoveries for motor speech disorder, with dysfunction linked to mutations in genes encoding transcription factors (*FOXP2*, *FOXP1*, *BCL11A*), cell migration and adhesion proteins (*CNTNAP2*, *SRPX2*) and an enzyme for galactose metabolism (*GALT*). This thesis is the first to implicate ion channel genes in speech dysfunction. Motor speech disorder is part of the characteristic phenotype of individuals with pathogenic variants in the glutamate receptor subunit gene *GRIN2A* (Chapter 4) and sodium channel subunit gene *SCN1A* (Chapter 5). A genotype-first approach was successful in identifying these speech phenotypes. However, the reverse approach may bear further fruit. Detailed phenotyping of a multiplex family revealed multiple individuals with CAS (Chapter 6), and this crucial data will inform linkage analysis in the family. This phenotype-first approach in the KE family was key to discovery of the first speech disorder gene *FOXP2*. The phenotype of the intragenic deletion case described here was comparable to that of the KE family and other *FOXP2* mutation cases (Chapter 3).

Comparing *FOXP2*, *GRIN2A* and *SCN1A* phenotypes

The three genes studied in this thesis are all associated with motor speech disorder, yet each gene presents a distinct speech phenotype. Phenotypic differences are not restricted to speech (figure 7-1). Most notably, *GRIN2A* and *SCN1A* are both genes for epilepsy, with *GRIN2A* mutations in up to 20% of patients with EAS syndromes and *SCN1A* mutations in more than 80% of DS cases.[124, 127, 128, 259, 260] Seizures and epilepsy are not reported with *FOXP2* mutations.

Understanding speech features associated with disruption of *FOXP2*, *GRIN2A* and *SCN1A* will not only aid differential diagnosis, but is also important to identifying symptoms that require treatment. In *SCN1A*-DS for example, alternative and augmentative forms of communication should be introduced from a young age to support minimally verbal children or those with severely unintelligible speech. For verbal DS children, early intervention may include strategies focusing on voice and speech production to improve speech intelligibility. Understanding the phenotype is crucial to early intervention planning, and early identification and intervention will optimise developmental outcomes for patients.

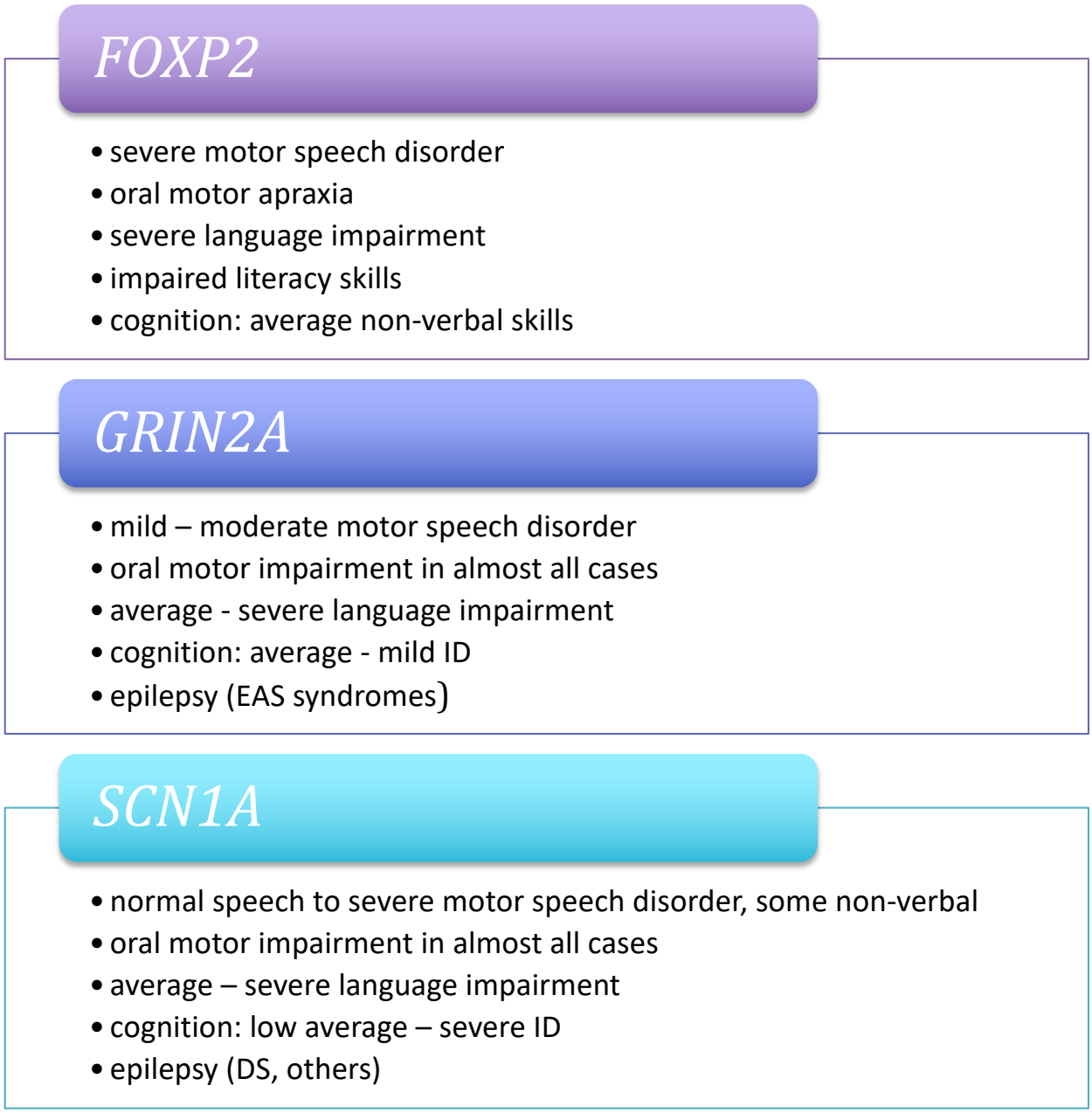


Figure 7-1. Phenotypic features associated with mutations in *FOXP2*, *GRIN2A* and *SCN1A*

Speech intelligibility

Mutations in *FOXP2* result in highly unintelligible speech, while only one quarter of patients with *SCN1A* mutations were severely unintelligible. In contrast, most cases (70%) with *GRIN2A* mutations were only mildly unintelligible and none were severe. There was a trend toward a more severe speech phenotype in older patients with *SCN1A* mutations, and in *GRIN2A* patients with a more severe epilepsy phenotype.

CAS

CAS is the core speech phenotype associated with *FOXP2* mutations.[92] Non-word repetition has been proposed as an endophenotype of *FOXP2* disruption, as most individuals with mutations find this task particularly challenging.[261] Performance on a DDK task is significantly impaired in individuals with *FOXP2* mutations. CAS is also part of the *GRIN2A* speech phenotype. Similarly to *FOXP2*, individuals with *GRIN2A* mutations had difficulty repeating nonwords and multisyllabic real words, and repeating a trisyllabic sequence (pataka).

Dysarthria (Table 7-1)

Dysarthria is the core speech phenotype of *SCN1A*-positive DS. A distinguishing feature in the DS cohort was vocal tremor, which has been reported in only one KE family member with a *FOXP2* missense mutation[83] and no *GRIN2A* cases. Vocal tremor is characteristic of hyperkinetic dysarthria, and may also be present in up to half of patients with ataxic dysarthria.[226] Most *SCN1A*-DS patients had excess and equal stress (100%) and hyponasality (62%) which are also associated with ataxic dysarthria. Other distinctive features of hyperkinetic dysarthria present in *SCN1A*-DS patients were intermittent breathiness (77%) and forced inspiration/expiration (31%). Other speech features (excess loudness variation, prolonged phonemes, distorted vowels, prolonged intervals) are heard in both dysarthria types. Hyperkinetic dysarthria is associated with diseases of the basal ganglia control circuit, while ataxic dysarthria is associated with damage to the cerebellum.[226] Extrapyramidal (dyskinetic movement of the tongue, rigidity, dystonic gait), cerebellar (ataxia, tremor) and pyramidal signs are noted in DS patients.[262] [262, 263]

Dysarthria associated with *FOXP2* mutations is typically classified as spastic. Spastic dysarthria results from bilateral damage to upper motor neurons of pyramidal or extra-pyramidal tracts.[226] Distinctive speech characteristics included low and monotonous pitch, strained-strangled or hoarse-breathy voice and hypernasality. Other features in individuals with *FOXP2* mutations not associated with spastic dysarthria included alternating loudness and prolonged phonemes.

Table 7-1. Perceptual speech features associated with *FOXP2*, *GRIN2A* and *SCN1A*

	FOXP2	*GRIN2A*	*SCN1A*
Respiration		Inadequate breath support for speech in 36%	Inadequate breath support for speech
Voice	Strained-strangled, hoarse-breathy or rough	Harsh	Breathy or strained-strangled
			Wet voice
Pitch	Low pitch	Low pitch	Low pitch
	Monopitch	Monopitch	Monopitch
			Tremor
Loudness	Soft		Soft
	Monoloud		Monoloud
	Alternating loudness		Alternating loudness
	Difficulty maintaining sufficient volume throughout phrase		Difficulty maintaining sufficient volume throughout phrase
Resonance	Hypernasal	Hypernasal	Hyponasal
Articulation	Imprecise consonants and vowels	Imprecise consonants and vowel	Imprecise consonants and vowel
Prosody	Slow rate	Slow rate	Slow rate
	Equal and excess stress	Equal and excess stress	Equal and excess stress
	Short phrases	Short phrases	Short phrases

Dysarthria associated with *GRIN2A* mutations is more challenging to classify, as the speech features in *GRIN2A*-positive EAS patients are characteristic of multiple dysarthria types. These individuals most likely have a mixed dysarthria. Most patients had a low and monotonous pitch also seen in *FOXP2* mutation cases, and a slow rate, however they did not have the strained-strangled voice distinctive of spastic dysarthria. They had hypernasal speech, which is a distinguishing feature of both spastic and flaccid dysarthria. Lower motor neuron signs, including nasal emission and fasciculations, seen in some *GRIN2A* patients also suggested flaccid dysarthria. Half of the patients had audible inspiration, prolonged intervals and short rushes of speech typical of hyperkinetic dysarthria.

Oral motor

All three genes are associated with oral motor impairment, including oral dyspraxia. Differences in muscle tone may distinguish the two ion channel genes, with spasticity of the top lip and dystonic posturing of the tongue noted with *GRIN2A* mutations, while open mouth posture, weak lip rounding and inability to elevate the tongue were noted in some *SCN1A*-DS cases. Lip and tongue movement was slow, asymmetrical and poorly coordinated for both disorders. In DS patients, motor programming and planning issues impacted performance of oral motor tasks. Many patients had difficulty imitating or following commands to perform volitional movements, and some evidenced groping or were unable to inhibit previously performed movements. It was often difficult to distinguish whether poor performance of oral motor tasks was due to impaired neuromuscular execution, overriding motor programming and planning issues or both.

Mild oral dysphagia affected a similar proportion of individuals with *GRIN2A* and *SCN1A* mutations, and was also observed in the *FOXP2* small deletion case. Saliva control difficulties were most prominent in *SCN1A* individuals, but were also seen in individuals with mutations of *GRIN2A* and *FOXP2*.

Cognition and language

FOXP2 mutations present a very different picture from the other two genes with regard to cognition and language. Individuals with *FOXP2* disruption typically have moderate to severe language disorder, and intact non-verbal cognitive skills. A similar pattern was seen in only two *GRIN2A* cases and one *SCN1A* case. Other individuals with intact cognition had average language skills or only mild impairments. The majority (almost 90%) of individuals

with *SCN1A* mutations had ID and congruent language skills. In contrast, language skills were variable in *GRIN2A* ID cases. Literacy impairments are likely under-recognised in individuals with mutations of all three genes.

A multiplex family with a distinct phenotype

Several features distinguished the G family phenotype from those associated with disruption of *FOXP2*, *GRIN2A* and *SCN1A*. Most notably, the majority of G family members had average non-verbal cognitive skills, language and literacy. The family also did not have a history of epilepsy. Language disorder was common in individuals with mutations in *FOXP2*, and the developmental and epileptic encephalopathy genes *GRIN2A* and *SCN1A*. Similarly to *FOXP2*, the core speech phenotype in the G family was CAS, and affected family members had difficulty with DDK, multisyllabic real word and non-word repetition tasks. Yet they did not have co-morbid dysarthria, and severity of the speech phenotype appeared to improve with age. While oral motor impairment was seen in the G family members, they did not have oral apraxia as described in individuals with *FOXP2* mutations, or altered tone as seen in individuals with *GRIN2A* and *SCN1A* mutations. These differences suggest that different gene/s are responsible for the G family phenotype. Molecular genetic studies confirm that the family do not have mutations in *FOXP2* or *GRIN2A*.

Challenges in CAS diagnosis

A major limitation in CAS research is the lack of validated differential diagnostic markers of the disease.[17] Indeed there is no universally accepted classification system for paediatric speech and language disorders generally. The three consensus criteria proposed by ASHA ten years ago are not necessary nor sufficient for diagnosis.[17] The criteria were also equivocal until recently operationalized,[224] thus studies vary widely in how they have applied the criteria to diagnose CAS. Moreover, the consensus criteria have not been universally adopted across molecular genetic studies of CAS. Many studies use a modified form of the Mayo Clinic system for acquired apraxia of speech, adapted for paediatric motor speech disorders.[27, 28, 111, 157] Others use alternative CAS feature lists,[144, 154, 169] or have a poorly defined speech phenotype.[105-108, 122, 158] Diagnosis of individuals who have limited verbal output or whose speech features have largely resolved poses a challenge. The diagnostic criteria currently in use have limited utility in young children or individuals with cognitive impairment who do not have single words. The criteria may also not be sensitive enough to pick up the residual

deficits often reported in adults with a history of CAS, whose conversational speech has normalised.[75, 86, 154] Severity and features of CAS are well reported to change across the lifespan.[26, 243] Adding to the complexity is that some speech features in CAS overlap those of dysarthria – prosodic impairment is one example - and it can be a challenge to tease the two speech disorders apart. Co-morbid CAS and dysarthria are reported in a number of different genetic speech disorders (*FOXP2*, *GRIN2A*, *BCL11A*, *GALT,* dup7) thus it may be an arbitrary separation in some cases.

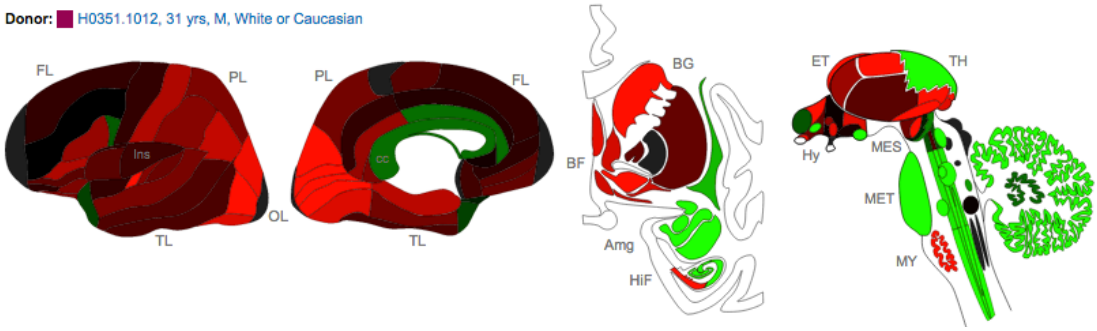
There have been attempts to identify one or a set of standardized behavioural diagnostic markers that may discriminate all cases of CAS.[44, 264-266] The proposed markers are largely restricted to verbal children thus have limited utility in the diagnosis of non-verbal individuals, or those with resolved CAS. Such an approach does not account for the phenotypic variation seen across individuals with CAS.[253] It is also unlikely to provide further insight into the biology underlying CAS, as this approach groups cases with differing aetiologies under the one diagnostic label.

A neurobiological approach

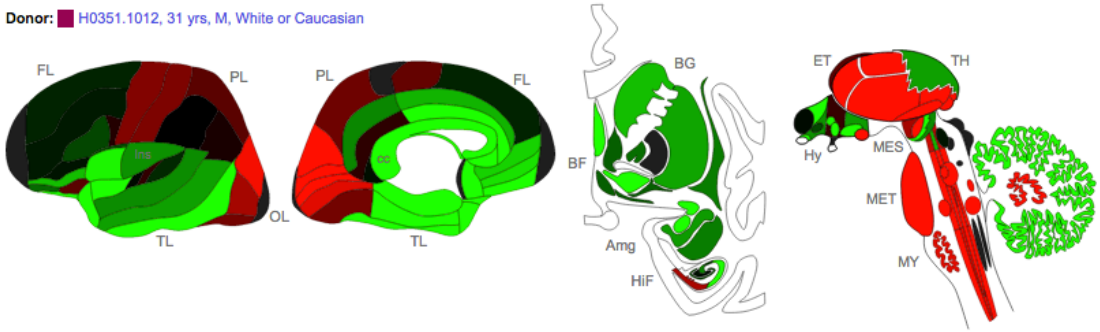
An alternative approach has been taken in this thesis. In the four studies, precise clinical phenotyping was used to identify a set of speech features shared by individuals who had the same genetic aetiology. The next step is to determine the impact of gene disruption on brain structure and function. This neurobiological approach is the way forward in motor speech disorder research.[32] Characterisation of speech features and brain structure and function will pinpoint clusters of features shared by individuals with identical aetiologies, and give rise to identifiable speech syndromes. *FOXP2*-related speech and language disorders are a prime example.[92, 261, 267]

As *FOXP2*, *GRIN2A* and *SCN1A* show distinct patterns of expression in the brain (figure 7-2), breakdown in different parts of the speech network are likely to underlie speech dysfunction for each gene. It is well established that *FOXP2* haploinsufficiency leads to structural and functional changes in speech-critical brain regions (discussed below). How mutations in *GRIN2A* and *SCN1A* impact on brain structure and function is still unclear, although there is some evidence that disruption of both genes cause abnormal neural development.

FOXP2



SCN1A



GRIN2A

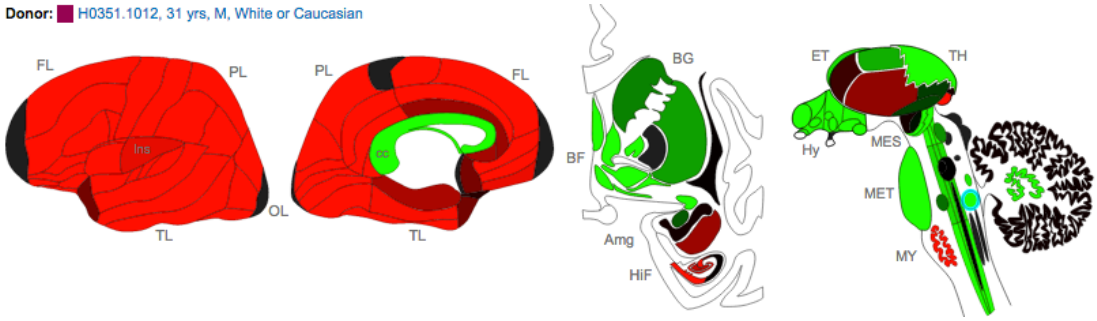


Figure 7-2. Distinct brain expression patterns are evident for *FOXP2*, *SCN1A* and *GRIN2A*. Microarray data from Allen Brain Atlas (normalized expression values, red: relatively high expression; green = relatively low expression).

Amg: amygdala; BF: basal forebrain; BG: basal ganglia; CC: corpus callosum; ET: epithalamus; FL: frontal lobe; HiF: hippocampal formation; Hy: hypothalamus; ins: insula; MES: mesencephalon; MET: metencephalon; MY: myelencephalon; OL: occipital lobe; PL: parietal lobe; TL: temporal lobe; TH: thalamus

Neural networks for speech and *FOXP2*

Neuroimaging studies in the original KE family and our case with an intragenic deletion (A-II) suggest that *FOXP2* disruption is likely to cause atypical development of the basal ganglia.[267] Our case A-II had bilateral grey matter anomalies in the caudate nucleus and globus pallidus, with structural and functional changes in basal ganglia also reported in the KE family.[84, 85, 261, 267, 268] In a neural network model of speech production (Directions into Velocities of Articulators or DIVA, figure 7-3) the basal ganglia is hypothesised to be involved in the timing and release of a speech motor program.[269] Malfunction in the inferior frontal gyrus (pars opercularis and triangularis) may be another marker of *FOXP2* disruption.[267] Reduced grey matter density and functional under-activation during a non-word repetition task is reported in the KE family, with functional deactivation in pars opercularis in A-II.[84, 261, 267] In the DIVA model, the left ventral premotor cortex (vPMC; posterior inferior frontal gyrus, ventral precentral gyrus, anterior insula) forms the **speech sound map** that when activated forwards a motor program to the primary motor cortex (precentral gyrus) via a cerebellar loop.[269] Other neural structures that have structural or functional anomalies in affected KE family members include the precentral gyrus, sensorimotor cortex, right homologue of Broca's area, supramarginal gyrus, planum temporale, posterior temporal gyrus, supplementary motor area,[84, 85, 261, 268] and all are represented in the DIVA model. The **speech sound map** forwards the expected auditory, tactile and proprioceptive targets for the speech sound being produced to the **auditory target map** in the posterior auditory cortex (pAC; planum temporale, posterior superior temporal gyrus and sulcus) and the **somatosensory target map** in the ventral somatosensory cortex (vSC; ventral postcentral gyrus, supramarginal gyrus) via a cortico-cerebellar loop. State and error maps in the pAC and vSC receive auditory and somatosensory feedback from the auditory system and speech articulators, and transform sensory feedback into motor commands to correct speech sound errors via the **feedback control map** (right vPMC). The **initiation map** in the supplementary motor area is responsible for launching the motor program (sequence of gestures to produce a speech sound). Thus almost all components of feedforward and feedback control in the DIVA model are affected by *FOXP2* disruption.

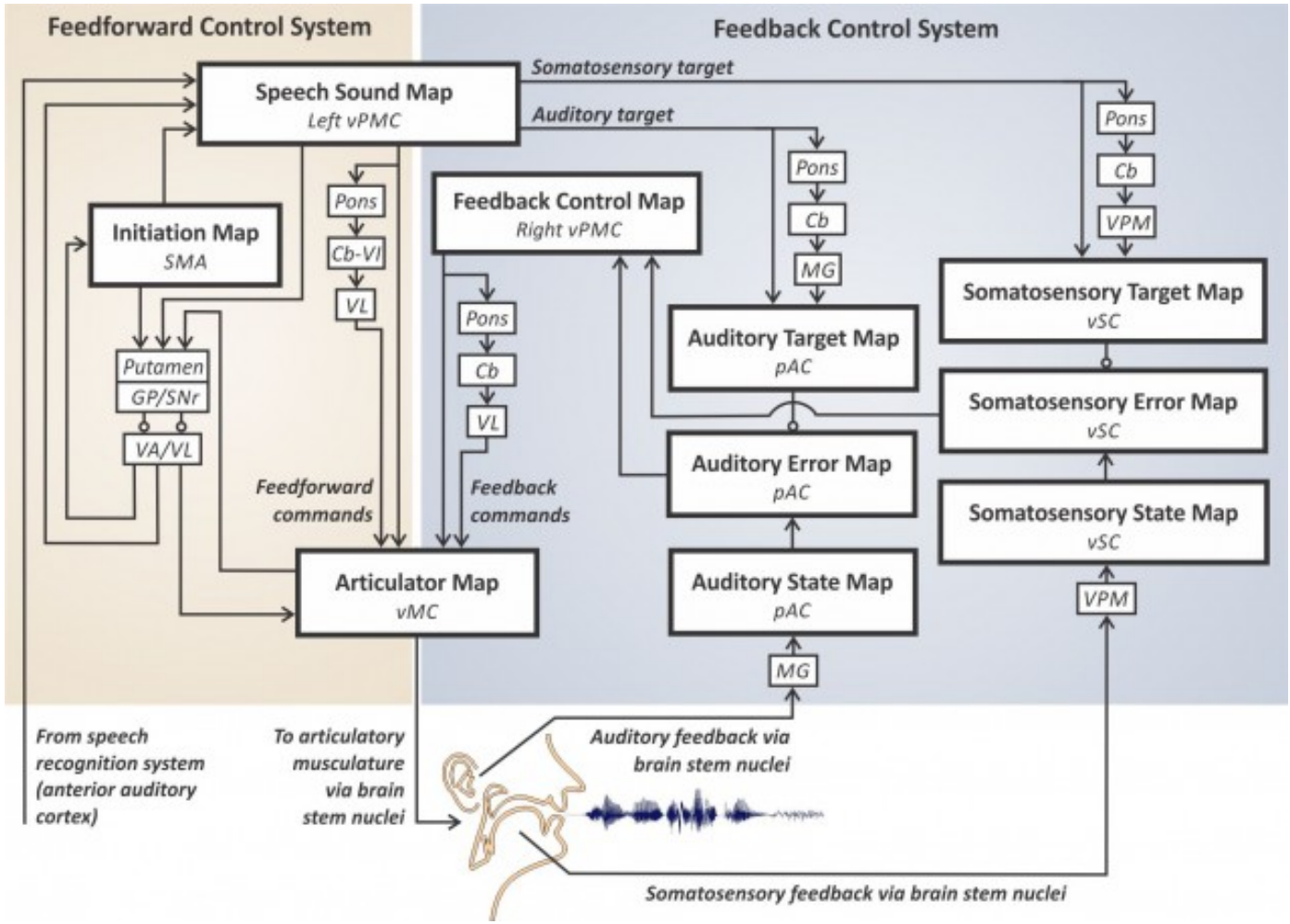


Figure 7-3. Directions into Velocities of Articulators (DIVA) neural network model of speech production. Reproduced with permission from Frank H. Guenther, Neural Control of Speech, published by The MIT Press.

Cb, cerebellum; Cb-VI, cerebellum lobule VI; GP, globus pallidus; MG, medial geniculate nucleus of the thalamus; pAC, posterior auditory cortex; SMA, supplementary motor area; SNr, substantia nigra pars reticula; VA, ventral anterior nucleus of the thalamus; VL, ventral lateral nucleus of the thalamus; vMC, ventral motor cortex; VPM, ventral posterior medial nucleus of the thalamus; vPMC, ventral premotor cortex; vSC, ventral somatosensory cortex

SCN1A disruption and the brain

Neuroimaging studies in patients with DS provide evidence that *SCN1A* disruption causes structural changes in the brain.[270-273] Global grey and white matter reductions are reported, with regional white matter reductions in corticospinal tracts, association fibres (left inferior fronto-occipital fasciculus, left uncinate fasciculus), corpus callosum, cerebellum and brainstem.[273] Reduced cortical folding is also reported in the right precentral gyrus.[273]

Neural networks related to interictal epileptiform discharges have also been examined via EEG-fMRI studies of *SCN1A*-positive DS patients.[274] Most showed activation in the prefrontal cortex and deactivation in the parietal cortex (postcentral gyrus, angular gyrus), with changes in the thalamus, basal ganglia, cerebellum and posterior cingulate cortex in a small number of patients. Taken together, these studies point to a possible role of the prefrontal cortex including the inferior frontal gyrus, insula, pre- and postcentral gyri, cerebellum, thalamus and basal ganglia in *SCN1A*-related motor speech dysfunction. *SCN1A* is strongly expressed in the pre and postcentral gyri, but only weakly expressed in the other structures (figure 7-2).

Interestingly, age related changes are reported in the brains of healthy adults in association with an *SCN1A* polymorphism.[275] Carriers of the allele show increased activity in the right inferior frontal cortex and posterior cingulate cortex bilaterally with age, and reduced grey matter in frontal and insula regions.[275] There was a trend toward a more severe speech phenotype in older DS patients in my study, which may be associated with age-related changes in these parts of the brain.

GRIN2A disruption and the brain

Continuous spike wave in slow wave sleep (CSWS) is the archetypal EEG pattern of the EAS syndromes, including *GRIN2A*-related disorders.[276] The location of epileptiform discharges makes it likely that several key speech regions are impacted, and this is supported by EEG-fMRI studies in EAS patients.[277] The inferior frontal gyrus and postcentral gyrus bilaterally, and left precentral gyrus, supramarginal gyrus, angular gyrus and caudate nucleus are positively correlated with rolandic regions before, during and after epileptiform discharges in childhood epilepsy with centrotemporal spikes.[278] *GRIN2A* is strongly expressed in all of these neural structures, apart from the thalamus

and caudate nucleus (figure 7-2). Positron emission tomography in one patient with a mutation implicates the superior temporal gyrus in *GRIN2A* dysfunction.[279] These studies suggest that the neural networks disrupted in *GRIN2A*-related motor speech disorders are comparable to those of *FOXP2*, including the primary motor cortex, vPMC, vSC and part of the pAC.

Channelopathies as a mechanism for motor speech dysfunction

Ion channels are critical to initiation of action potentials in neurons, yet their dysfunction has not previously been explored as a cause of motor speech impairment. This thesis points to a role for two different types of ion channels - glutamate receptors and sodium channels - in motor speech dysfunction. Understanding the functional consequences of disruption to the genes that encode these ion channels may provide crucial insights into the underlying neurobiology of speech disorder.

Sodium channels encoded by *SCN1A* are expressed on GABAergic inhibitory interneurons and play a critical role in regulating spike output.[280] Mutations in *SCN1A* result in loss of function of the mutant channel.[259, 281] *SCN1A* knockout mice also show reduced sodium currents from GABAergic inhibitory interneurons.[280, 282] Impaired function of the inhibitory circuit is hypothesized to cause hyperexcitability that underlies seizures in patients with DS.[280, 282] Thus increased neuronal excitability may be an important mechanism for *SCN1A*-related motor speech disorder.

Mutations in *GRIN2A* have also been shown to alter expression and function of the glutamate receptor it encodes. Loss of function is predicted for *GRIN2A* deletions, truncations and frameshift mutations.[199, 283] Variable effects are reported for *GRIN2A* missense mutations.[284-287] Some missense mutations result in loss of function, with reduced protein expression, decreased NMDA receptor trafficking to the cell surface and altered agonist binding.[287] Other missense mutations cause overactivation of the mutant receptor.[284, 285] Mutant glutamate receptors show gradual loss of zinc inhibition,[127] a mechanism that protects neurons from overexcitation. Interestingly, two missense mutations reported in individuals with verbal dyspraxia and no seizures have been studied functionally. One mutation (D731N) reported in a mother with verbal dyspraxia and her daughter with verbal dyspraxia and rolandic epilepsy showed complete loss of function, and was unable to bind the agonists glutamate or glycine.[124,

[287] The missense variant (T531M) in Family D, whose speech has been phenotyped as part of this thesis (Chapter 4), affects NMDA receptor gating by increasing the mean duration of the open state.[128] The NMDA receptor blocker memantine reduces hyperactivity of mutant receptors in vitro, and targeted memantine therapy in a patient with epileptic encephalopathy associated with a *GRIN2A* missense variant was effective in reducing seizures.[284]

Limitations of the current study

Small samples were included in this thesis, therefore the study findings may not generalize to larger groups of patients with mutations in the same genes. For example, the *GRIN2A* cohort did not include all EAS syndromes where mutations have been reported. Different syndromes that weren't studied, including LKS and atypical benign partial epilepsy, may present a different speech phenotype. The youngest *GRIN2A*-EAS individual was 16 years of age, thus how the speech phenotype manifests in childhood is also unknown. While only 11 individuals were included in the *GRIN2A* study, they represented all available patients with mutations in Australia and New Zealand that had consented to participate.

Small sample sizes also meant that trends noted in the different studies, such as potential deterioration in speech with increasing age in patients with *SCN1A*-DS, could not be examined further.

Another limitation may be the speech and language assessments included in the phenotypic battery. Subjective judgements are made when scoring these tests, which may lead to discrepant findings. However, there was good inter-rater agreement between the two experienced clinicians in the current studies. Different assessments were also used for children and adults so that age-based standardised scores could be calculated. For example, the VMPAC was used to assess oral motor skills in children under 12, and the tasks and procedure for scoring are distinct from the FDA-2, which was used in individuals 13 years and older. This made it impossible to directly compare scores across younger and older individuals in the same study, or in the same family. Yet, these assessments represent the 'gold standard' for examining oral motor, speech, language and literacy skills, and are routinely used in clinical practice.

The challenge inherent in diagnosing CAS has been discussed in detail above. Other factors that may influence the speech and language phenotype, such as speech pathology intervention, seizures and medication, were not examined in this thesis and may be another potential limitation.

Future directions

Studying further cohorts of patients is crucial to understanding genotype-phenotype relationships. Relatively small groups of patients have been studied here, and larger numbers will help to clarify correlations between the speech phenotype and other features such as age or seizure type. Examining a larger cohort of patients with *GRIN2A* mutations will confirm whether speech is more impaired in individuals with a more severe epilepsy phenotype, as observed in our cohort. Longitudinal studies in *SCN1A* patients will help to determine whether speech deteriorates with age, a trend noted in this thesis. If this observation holds true, examining a larger cohort of patients over an extended time frame will allow us to determine at what age the deterioration occurs and how it manifests. Examining mildly affected individuals with mutations, for example Genetic Epilepsy with Febrile Seizures Plus and mutations in *SCN1A*, may expand the phenotypic spectrum associated with different genes. Phenotyping cohorts with other ion channel gene mutations will clarify the role of other ion channels in motor speech dysfunction.

Structural and functional neuroimaging studies will establish which neural networks are implicated in *GRIN2A* and *SCN1A*-related speech dysfunction.

Chromosomal regions that harbour several genes or CNVs implicated in speech dysfunction, including 7q (*FOXP2*, *CNTNAP2*, 7q11.23 duplication, linkage to 7q32 and 7q36), 16p (*GRIN2A*, 16p11.2 microdeletion, 16p13.2 microdeletion, 4q35.2/16p translocation) and 15q (15q11q13.9 duplication, 15q13.3 microdeletion, 15q26.3 duplication, DYX1), may prove to be 'speech hotspots' that may harbour other as yet unidentified pathogenic variants. Chromosomal hotpots for ID are already well established (reviewed in [288]). These potential 'speech hotspots' may be one focus of future molecular genetic studies.

Most importantly, further research is needed to develop novel therapies that specifically target the impairments identified in these groups of patients. Randomized placebo-

controlled trials of memantine in patients with EAS secondary to *GRIN2A* mutations is one such example, which may improve speech and language outcome, as well as reduce seizure burden. Identification of new speech disorder genes may offer further opportunities for a precision medicine approach targeting the underlying mutation.

REFERENCES

1. Eadie P, Morgan A, Ukoumunne OC, Ttofari Eecen K, Wake M, Reilly S. Speech sound disorder at 4 years: prevalence, comorbidities, and predictors in a community cohort of children. Dev Med Child Neurol. 2015 Jun;57(6):578-84.

2. Reilly S, Wake M, Ukoumunne OC, et al. Predicting language outcomes at 4 years of age: findings from Early Language in Victoria Study. Pediatrics. 2010 Dec;126(6):e1530-7.

3. Shriberg LD, Tomblin JB, McSweeny JL. Prevalence of speech delay in 6-year-old children and comorbidity with language impairment. J Speech Lang Hear Res. 1999 Dec;42(6):1461-81.

4. Law J, Boyle J, Harris F, Harkness A, Nye C. Prevalence and natural history of primary speech and language delay: findings from a systematic review of the literature. Int J Lang Commun Disord. 2000 Apr-Jun;35(2):165-88.

5. Lewis BA, Thompson LA. A study of developmental speech and language disorders in twins. J Speech Hear Res. 1992 Oct;35(5):1086-94.

6. Lewis BA. Familial phonological disorders: four pedigrees. J Speech Hear Disord. 1990 Feb;55(1):160-70.

7. Lewis BA. Pedigree analysis of children with phonology disorders. J Learn Disabil. 1992 Nov;25(9):586-97.

8. Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. A forkhead-domain gene is mutated in a severe speech and language disorder. Nature. 2001 Oct;413(6855):519-23.

9. Bishop DV, North T, Donlan C. Genetic basis of specific language impairment: evidence from a twin study. Dev Med Child Neurol. 1995 Jan;37(1):56-71.

10. DeThorne LS, Hart SA, Petrill SA, et al. Children's history of speech-language difficulties: genetic influences and associations with reading-related measures. J Speech Lang Hear Res. 2006 Dec;49(6):1280-93.

11. Carrigg B, Parry L, Baker E, Shriberg LD, Ballard KJ. Cognitive, Linguistic, and Motor Abilities in a Multigenerational Family with Childhood Apraxia of Speech. Arch Clin Neuropsychol. 2016 Oct;31:1006-1025.

12. Peter B, Raskind WH. A multigenerational family study of oral and hand motor sequencing ability provides evidence for a familial speech sound disorder subtype. Top Lang Disord. 2011 Apr;31(2):145-67.

13. Fisher SE, Lai CS, Monaco AP. Deciphering the genetic basis of speech and language disorders. Annu Rev Neurosci. 2003;26:57-80.

14. Dodd B. Differential diagnosis and treatment of children with speech disorder. Dodd B, editor. West Sussex: Whurr; 2005.

15. Broomfield J, Dodd B. Children with speech and language disability: caseload characteristics. Int J Lang Commun Disord. 2004 Jul-Sep;39(3):303-24.

16. Caruso AJ, Strand E. Clinical Management of Motor Speech Disorders. New York: Thieme Medical Publishers, Inc; 1999.

17. ASHA. Childhood Apraxia of Speech [Technical Report]. Available from www.asha.org/policy2007.

18. Morley ME. Developmental Articulatory Apraxia. The Development and Disorders of Speech in Childhood. London: Livingstone; 1957. p. 217-31.

19. Eisenson J. Aphasia and language disorders in children. New York: Harper and Row; 1972.

20. Edwards M. Developmental verbal dyspraxia. Br J Disord Commun. 1973 Apr;8(1):64-70.

21. Chappell GE. Childhood verbal apraxia and its treatment. J Speech Hear Disord. 1973 Aug;38(3):362-8.

22. Ferry PC, Hall SM, Hicks JL. Dilapidated speech: developmental verbal dyspraxia. Dev Med Child Neurol. 1975 Dec;17(6):749-56.

23. Rosenbek J, Hansen R, Baughman CH, Lemme M. Treatment of developmental apraxia of speech: A case study. Language, Speech and Hearing Services in Schools. 1974;5:13-22.

24. Yoss KA, Darley FL. Developmental apraxia of speech in children with defective articulation. J Speech Hear Res. 1974 Sep;17(3):399-416.

25. Ozanne A. Childhood apraxia of speech. In: Dodd B, editor. Differential diagnosis and treatment of children with speech disorder. 2nd ed. London: Whurr; 2005.

26. RCSLT. Developmental Verbal Dyspraxia Policy Statement 2011. Available from www.rcslt.org/speech_and_language_therapy/rcslt_position_papers

27. Shriberg LD, Potter NL, Strand EA. Prevalence and phenotype of childhood apraxia of speech in youth with galactosemia. J Speech Lang Hear Res. 2011 Apr;54(2):487-519.

28. Centanni TM, Sanmann JN, Green JR, et al. The role of candidate-gene CNTNAP2 in childhood apraxia of speech and specific language impairment. Am J Med Genet B Neuropsychiatr Genet. 2015 Oct;168(7):536-43.

29. Liegeois FJ, Morgan AT. Neural bases of childhood speech disorders: lateralization and plasticity for speech functions during development. Neurosci Biobehav Rev. 2012 Jan;36(1):439-58.

30. Darley FL, Aronson AE, Brown JR. Clusters of deviant speech dimensions in the dysarthrias. J Speech Hear Res. 1969 Sep;12(3):462-96.

31. Darley FL, Aronson AE, Brown JR. Differential diagnostic patterns of dysarthria. J Speech Hear Res. 1969 Jun;12(2):246-69.

32. Morgan AT, Liegeois F. Re-thinking diagnostic classification of the dysarthrias: a developmental perspective. Folia Phoniatr Logop. 2010;62(3):120-6.

33. van Mourik M, Catsman-Berrevoets CE, Paquier PF, Yousef-Bak E, van Dongen HR. Acquired childhood dysarthria: review of its clinical presentation. Pediatr Neurol. 1997 Nov;17(4):299-307.

34. Morgan AT, Mageandran SD, Mei C. Incidence and clinical presentation of dysarthria and dysphagia in the acute setting following paediatric traumatic brain injury. Child Care Health Dev. 2010 Jan;36(1):44-53.

35. Morgan AT, Liegeois F, Liederkerke C, et al. Role of cerebellum in fine speech control in childhood: persistent dysarthria after surgical treatment for posterior fossa tumour. Brain Lang. 2011 May;117(2):69-76.

36. Liegeois F, Morgan AT, Stewart LH, Helen Cross J, Vogel AP, Vargha-Khadem F. Speech and oral motor profile after childhood hemispherectomy. Brain Lang. 2010 Aug;114(2):126-34.

37. Morgan AT, Mei C, Da Costa A, et al. Speech and language in a genotyped cohort of individuals with Kabuki syndrome. Am J Med Genet. 2015 Jul;167(7):1483-92.

38. Scholderle T, Staiger A, Lampe R, Strecker K, Ziegler W. Dysarthria in Adults With Cerebral Palsy: Clinical Presentation and Impacts on Communication. J Speech Lang Hear Res. 2016 Apr 01;59(2):216-29.

39. Rupela V, Velleman SL, Andrianopoulos MV. Motor speech skills in children with Down syndrome: A descriptive study. Int J Speech Lang Pathol. 2016 Oct;18(5):483-92.

40. Turner SJ, Brown A, Arpone M, Anderson V, Morgan AT, Scheffer IE. Dysarthria and broader motor speech deficits in Dravet syndrome. Neurology. 2017 Feb 21;88(8):743-9.

41. Onslow M. Behavioural management of stuttering. 1st ed. Sydney: Livingston Press; 1993.

42. Waring R, Knight R. How should children with speech sound disorders be classified? A review and critical evaluation of current classification systems. Int J Lang Commun Disord. 2013 Jan;48(1):25-40.

43. Tyler AA. Speech sound disorders in children: exploring subgroups. Top Lang Disord. 2011;31:93-5.

44. Shriberg LD, Strand EA, Fourakis M, et al. A Diagnostic Marker to Discriminate Childhood Apraxia of Speech From Speech Delay: I. Development and Description of the Pause Marker. J Speech Lang Hear Res. 2017 Apr 14;60(4):S1096-S117.

45. Stackhouse J, Wells B. Children's speech and literacy difficulties : a psycholinguistic framework. San Diego, California: Wiley; 1997.

46. Tomblin JB, Records NL, Buckwalter P, Zhang X, Smith E, O'Brien M. Prevalence of specific language impairment in kindergarten children. J Speech Lang Hear Res. 1997 Dec;40(6):1245-60.

47. ASHA. Definitions of communication disorders and variations [Relevant Paper]. 1993. Available from www.asha.org/policy.

48. Reilly S, Tomblin B, Law J, et al. Specific language impairment: a convenient label for whom? Int J Lang Commun Disord. 2014 Jul;49(4):416-51.

49. Consortium SLI. Highly significant linkage to the SLI1 locus in an expanded sample of individuals affected by specific language impairment. Am J Hum Genet. 2004 Jun;74(6):1225-38.

50. Hinshelwood J. Word-blindness and visual memory. Lancet. 1895;146:1564-70.

51. Lyon GR, Shaywitz SE, Shaywitz BA. A definition of dyslexia. Annals of Dyslexia. 2003;53:1-14.

52. Shaywitz SE. Dyslexia. N Engl J Med. 1998 Jan 29;338(5):307-12.

53. Ramus F, Rosen S, Dakin SC, et al. Theories of developmental dyslexia: insights from a multiple case study of dyslexic adults. Brain. 2003 Apr;126(Pt 4):841-65.

54. Sices L, Taylor HG, Freebairn L, Hansen A, Lewis B. Relationship between speech-sound disorders and early literacy skills in preschool-age children: impact of comorbid language impairment. J Dev Behav Pediatr. 2007 Dec;28(6):438-47.

55. Lewis B, Freebairn L, Taylor G. Correlates of spelling abilities in children with early speech sound disorders. Reading Writing Interdisciplinary. 2002;15:389-407.

56. Bird J, Bishop DV, Freeman NH. Phonological awareness and literacy development in children with expressive phonological impairments. J Speech Hear Res. 1995 Apr;38(2):446-62.

57. Shriberg L, Austin D. Comorbidity of speech-language disorder: Implications for a phenotype marker for speech delay. In: Paul R, editor. The speech-language connection. Baltimore, MD: Paul H. Brookes; 1998.

58. Stromswold K. Genetics of spoken language disorders. Hum Biol. 1998 Apr;70(2):297-324.

59. Kang C, Drayna D. Genetics of speech and language disorders. Annu Rev Genomics Hum Genet. 2011;12:145-64.

60. Helbig I, Scheffer IE, Mulley JC, Berkovic SF. Navigating the channels and beyond: unravelling the genetics of the epilepsies. Lancet Neurol. 2008 Mar;7(3):231-45.

61. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. Nat Rev Genet. 2002 Nov;3(11):872-82.

62. Matheny AP, Jr., Bruggemann CE. Children's speech: hereditary components and sex differences. Folia Phoniatr. 1973 Dec;25(6):442-9.

63. Locke JL, Mather PL. Genetic factors in the ontogeny of spoken language: evidence from monozygotic and dizygotic twins. J Child Lang. 1989 Oct;16(3):553-9.

64. McNeill BC, Gillon GT, Dodd B. A longitudinal case study of the effects of an integrated phonological awareness program for identical twin boys with childhood apraxia of speech (CAS). Int J Speech Lang Pathol. 2009;11(6):482-95.

65. Felsenfeld S, Plomin R. Epidemiological and offspring analyses of developmental speech disorders using data from the Colorado Adoption Project. J Speech Lang Hear Res. 1997 Aug;40(4):778-91.

66. Felsenfeld S, McGue M, Broen PA. Familial aggregation of phonological disorders: results from a 28-year follow-up. J Speech Hear Res. 1995 Oct;38(5):1091-107.

67. Ingram TT. Specific developmental disorders of speech in childhood. Brain. 1959 Sep;82:450-67.

68. Lewis BA, Ekelman BL, Aram DM. A familial study of severe phonological disorders. J Speech Hear Res. 1989 Dec;32(4):713-24.

69. Lewis BA, Freebairn L. Subgrouping children with familial phonologic disorders. J Comm Disord. 1997 Sep-Oct;30(5):385-401; quiz -2.

70. Lewis BA, Freebairn L. Speech production skills of nuclear family members of children with phonology disorders. Lang Speech. 1998 Jan-Mar;41 (Pt 1):45-61.

71. Lewis BA, Freebairn LA, Hansen AJ, Miscimarra L, Iyengar SK, Taylor HG. Speech and language skills of parents of children with speech sound disorders. Am J Speech Lang Pathol. 2007 May;16(2):108-18.

72. Thoonen G, Maassen B, Gabreels F, Schreuder R, de Swart B. Towards a standardised assessment procedure for developmental apraxia of speech. Eur J Disord Commun. 1997;32(1):37-60.

73. Riley GD. Developmental verbal dyspraxia: A clinical perspective. Aust J Hum Commun Disord. 1984;12:83-91.

74. Horwitz SJ. Neurological findings in developmental verbal apraxia. Sem Speech Lang. 1984;5(2):111-8.

75. Lewis BA, Freebairn LA, Hansen A, Gerry Taylor H, Iyengar S, Shriberg LD. Family pedigrees of children with suspected childhood apraxia of speech. J Commun Disord. 2004 Mar-Apr;37(2):157-75.

76. Hurst JA, Baraitser M, Auger E, Graham F, Norell S. An extended family with a dominantly inherited speech disorder. Dev Med Child Neurol. 1990 Apr;32(4):352-5.

77. Gopnik M, Crago MB. Familial aggregation of a developmental language disorder. Cognition. 1991 Apr;39(1):1-50.

78. Vargha-Khadem F, Watkins KE, Price CJ, et al. Neural basis of an inherited speech and language disorder. Proc Natl Acad Sci U S A. 1998 Oct 13;95(21):12695-700.

79. Vargha-Khadem F, Watkins K, Alcock K, Fletcher P, Passingham R. Praxic and nonverbal cognitive deficits in a large family with a genetically transmitted speech and language disorder. Proc Natl Acad Sci U S A. 1995 Jan 31;92(3):930-3.

80. Alcock KJ, Passingham RE, Watkins KE, Vargha-Khadem F. Oral dyspraxia in inherited speech and language impairment and acquired dysphasia. Brain Lang. 2000 Oct 15;75(1):17-33.

81. Alcock KJ, Passingham RE, Watkins K, Vargha-Khadem F. Pitch and timing abilities in inherited speech and language impairment. Brain Lang. 2000 Oct 15;75(1):34-46.

82. Gopnik M. Feature-blind grammar and dysphagia. Nature. 1990 Apr 19;344(6268):715.

83. Morgan AT, Liegeois F, Vargha-Khadem F. Motor speech outcome as a function of the site of brain pathology: A developmental perspective. In: Maassen B, van Lieshout P, editors. Speech Motor Control: New developments in basic and applied research. Oxford: Oxford University Press; 2010. p. 95-115.

84. Belton E, Salmond CH, Watkins KE, Vargha-Khadem F, Gadian DG. Bilateral brain abnormalities associated with dominantly inherited verbal and orofacial dyspraxia. Hum Brain Mapp. 2003 Mar;18(3):194-200.

85. Liegeois F, Baldeweg T, Connelly A, Gadian DG, Mishkin M, Vargha-Khadem F. Language fMRI abnormalities associated with FOXP2 gene mutation. Nat Neurosci. 2003 Nov;6(11):1230-7.

86. Peter B, Button L, Stoel-Gammon C, Chapman K, Raskind WH. Deficits in sequential processing manifest in motor and linguistic tasks in a multigenerational family with childhood apraxia of speech. Clin Linguist Phon. 2013 Mar;27(3):163-91.

87. Button L, Peter B, Stoel-Gammon C, Raskind WH. Associations among measures of sequential processing in motor and linguistics tasks in adults with and without a family history of childhood apraxia of speech: a replication study. Clin Linguist Phon. 2013 Mar;27(3):192-212.

88. Fisher SE, Vargha-Khadem F, Watkins KE, Monaco AP, Pembrey ME. Localisation of a gene implicated in a severe speech and language disorder. Nat Genet. 1998 Feb;18(2):168-70.

89. Lai CS, Fisher SE, Hurst JA, et al. The SPCH1 region on human 7q31: genomic characterization of the critical interval and localization of translocations associated with speech and language disorder. Am J Hum Genet. 2000 Aug;67(2):357-68.

90. Turner SJ, Hildebrand MS, Block S, et al. Small intragenic deletion in FOXP2 associated with childhood apraxia of speech and dysarthria. Am J Med Genet. 2013 Sep;161A(9):2321-6.

91. Reuter MS, Riess A, Moog U, et al. FOXP2 variants in 14 individuals with developmental speech and language disorders broaden the mutational and clinical spectrum. J Med Genet. 2017 Jan;54(1):64-72.

92. Morgan A, Fisher SE, Scheffer I, Hildebrand M. FOXP2-Related Speech and Language Disorders. 2016 Jun 23 [Updated 2017 Feb 2]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews [Internet]. Seattle: University of Washington; 1993-2018.

93. MacDermot KD, Bonora E, Sykes N, et al. Identification of FOXP2 truncation as a novel cause of developmental speech and language deficits. Am J Hum Genet. 2005 Jun;76(6):1074-80.

94. Laffin JJ, Raca G, Jackson CA, Strand EA, Jakielski KJ, Shriberg LD. Novel candidate genes and regions for childhood apraxia of speech identified by array comparative genomic hybridization. Genet Med. 2012 Nov;14(11):928-36.

95. Spiteri E, Konopka G, Coppola G, et al. Identification of the transcriptional targets of FOXP2, a gene linked to speech and language, in developing human brain. Am J Hum Genet. 2007 Dec;81(6):1144-57.

96. Vernes SC, Spiteri E, Nicod J, et al. High-throughput analysis of promoter occupancy reveals direct neural targets of FOXP2, a gene mutated in speech and language disorders. Am J Hum Genet. 2007 Dec;81(6):1232-50.

97. Gregor A, Albrecht B, Bader I, et al. Expanding the clinical spectrum associated with defects in CNTNAP2 and NRXN1. BMC Med Genet. 2011;12:106.

98. Vernes SC, Oliver PL, Spiteri E, et al. Foxp2 regulates gene networks implicated in neurite outgrowth in the developing brain. PLoS Genet. 2011 Jul;7(7):e1002145.

99. Wohlgemuth S, Adam I, Scharff C. FoxP2 in songbirds. Curr Opin Neurobiol. 2014 Oct;28:86-93.

100. Groszer M, Keays DA, Deacon RM, et al. Impaired synaptic plasticity and motor learning in mice with a point mutation implicated in human speech deficits. Curr Biol. 2008 Mar 11;18(5):354-62.

101. Fisher SE, Scharff C. FOXP2 as a molecular window into speech and language. Trends Genet. 2009 Apr;25(4):166-77.

102. Graham SA, Fisher SE. Decoding the genetics of speech and language. Curr Opin Neurobiol. 2013 Feb;23(1):43-51.

103. Graham SA, Deriziotis P, Fisher SE. Insights into the genetic foundations of human communication. Neuropsychol Rev. 2015 Mar;25(1):3-26.

104. Li S, Weidenfeld J, Morrisey EE. Transcriptional and DNA binding activity of the Foxp1/2/4 family is modulated by heterotypic and homotypic protein interactions. Mol Cell Biol. 2004 Jan;24(2):809-22.

105. Le Fevre AK, Taylor S, Malek NH, et al. FOXP1 mutations cause intellectual disability and a recognizable phenotype. Am J Med Genet. 2013 Dec;161A(12):3166-75.

106. Horn D, Kapeller J, Rivera-Brugues N, et al. Identification of FOXP1 deletions in three unrelated patients with mental retardation and significant speech and language deficits. Hum Mutat. 2010 Nov;31(11):E1851-60.

107. Carr CW, Moreno-De-Luca D, Parker C, et al. Chiari I malformation, delayed gross motor skills, severe speech delay, and epileptiform discharges in a child with FOXP1 haploinsufficiency. Eur J Hum Genet. 2010 Nov;18(11):1216-20.

108. Lozano R, Vino A, Lozano C, Fisher SE, Deriziotis P. A de novo FOXP1 variant in a patient with autism, intellectual disability and severe speech and language impairment. Eur J Hum Genet. 2015 Dec;23(12):1702-7.

109. Hamdan FF, Daoud H, Rochefort D, et al. De novo mutations in FOXP1 in cases with intellectual disability, autism, and language impairment. Am J Hum Genet. 2010 Nov 12;87(5):671-8.

110. Sollis E, Graham SA, Vino A, et al. Identification and functional characterization of de novo FOXP1 variants provides novel insights into the etiology of neurodevelopmental disorder. Hum Mol Genet. 2016 Feb 1;25(3):546-57.

111. Worthey EA, Raca G, Laffin JJ, et al. Whole-exome sequencing supports genetic heterogeneity in childhood apraxia of speech. J Neurodev Disord. 2013;5(1):29.

112. Vernes SC, Newbury DF, Abrahams BS, et al. A functional genetic link between distinct developmental language disorders. N Engl J Med. 2008 Nov;359(22):2337-45.

113. Bakkaloglu B, O'Roak BJ, Louvi A, et al. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. Am J Hum Genet. 2008 Jan;82(1):165-73.

114. Arking DE, Cutler DJ, Brune CW, et al. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. Am J Hum Genet. 2008 Jan;82(1):160-4.

115. Smogavec M, Cleall A, Hoyer J, et al. Eight further individuals with intellectual disability and epilepsy carrying bi-allelic CNTNAP2 aberrations allow delineation of the mutational and phenotypic spectrum. J Med Genet. 2016 Dec;53(12):820-7.

116. Alarcon M, Abrahams BS, Stone JL, et al. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. Am J Hum Genet. 2008 Jan;82(1):150-9.

117. Penagarikano O, Abrahams BS, Herman EI, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell. 2011 Sep;147(1):235-46.

118. Petrin AL, Giacheti CM, Maximino LP, et al. Identification of a microdeletion at the 7q33-q35 disrupting the CNTNAP2 gene in a Brazilian stuttering case. Am J Med Genet. 2010 Dec;152A(12):3164-72.

119. Han TU, Park J, Domingues CF, et al. A study of the role of the FOXP2 and CNTNAP2 genes in persistent developmental stuttering. Neurobiol Dis. 2014 Sep;69:23-31.

120. Peter B, Raskind WH, Matsushita M, et al. Replication of CNTNAP2 association with nonword repetition and support for FOXP2 association with timed reading and motor activities in a dyslexia family sample. J Neurodev Disord. 2011 Mar;3(1):39-49.

121. Roll P, Vernes SC, Bruneau N, et al. Molecular networks implicated in speech-related disorders: FOXP2 regulates the SRPX2/uPAR complex. Hum Mol Genet. 2010 Dec;19(24):4848-60.

122. Roll P, Rudolf G, Pereira S, et al. SRPX2 mutations in disorders of language cortex and cognition. Hum Mol Genet. 2006 Apr;15(7):1195-207.

123. Piton A, Redin C, Mandel JL. XLID-causing mutations and associated genes challenged in light of data from large-scale human exome sequencing. Am J Hum Genet. 2013 Aug;93(2):368-83.

124. Lesca G, Rudolf G, Bruneau N, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat Genet. 2013 Aug;45(9):1061-6.

125. Clark M, Carr L, Reilly S, Neville BG. Worster-Drought syndrome, a mild tetraplegic perisylvian cerebral palsy. Review of 47 cases. Brain. 2000 Oct;123(Pt 10):2160-70.

126. Kuzniecky R, Andermann F, Guerrini R. Congenital bilateral perisylvian syndrome: study of 31 patients. The CBPS Multicenter Collaborative Study. Lancet. 1993 Mar;341(8845):608-12.

127. Lemke JR, Lal D, Reinthaler EM, et al. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. Nat Genet. 2013 Aug;45(9):1067-72.

128. Carvill GL, Regan BM, Yendle SC, et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. Nat Genet. 2013 Aug;45(9):1073-6.

129. Turner SJ, Morgan AT, Perez ER, Scheffer IE. New genes for focal epilepsies with speech and language disorders. Curr Neurol Neurosci Rep. 2015 Jun;15(6):35.

130. Conroy J, McGettigan PA, McCreary D, et al. Towards the identification of a genetic basis for Landau-Kleffner syndrome. Epilepsia. 2014 Jun;55(6):858-65.

131. Miyamoto H, Katagiri H, Hensch T. Experience-dependent slow-wave sleep development. Nat Neurosci. 2003 Jun;6(6):553-4.

132. Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science. 1995 Sep;269(5231):1737-40.

133. Laube B, Hirai H, Sturgess M, Betz H, Kuhse J. Molecular determinants of agonist discrimination by NMDA receptor subunits: analysis of the glutamate binding site on the NR2B subunit. Neuron. 1997 Mar;18(3):493-503.

134. Monyer H, Sprengel R, Schoepfer R, et al. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science. 1992 May;256(5060):1217-21.

135. Sprengel R, Suchanek B, Amico C, et al. Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. Cell. 1998 Jan;92(2):279-89.

136. Endele S, Rosenberger G, Geider K, et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet. 2010 Nov;42(11):1021-6.

137. Akbarian S, Sucher NJ, Bradley D, et al. Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. J Neurosci. 1996 Jan;16(1):19-30.

138. Kosinski CM, Standaert DG, Counihan TJ, et al. Expression of N-methyl-D-aspartate receptor subunit mRNAs in the human brain: striatum and globus pallidus. J Comp Neurol. 1998 Jan;390(1):63-74.

139. Scherzer CR, Landwehrmeyer GB, Kerner JA, et al. Expression of N-methyl-D-aspartate receptor subunit mRNAs in the human brain: hippocampus and cortex. J Comp Neurol. 1998 Jan;390(1):75-90.

140. Conti F, Barbaresi P, Melone M, Ducati A. Neuronal and glial localization of NR1 and NR2A/B subunits of the NMDA receptor in the human cerebral cortex. Cereb Cortex. 1999 Mar;9(2):110-20.

141. Bi H, Sze CI. N-methyl-D-aspartate receptor subunit NR2A and NR2B messenger RNA levels are altered in the hippocampus and entorhinal cortex in Alzheimer's disease. J Neurol Sci. 2002 Aug 15;200(1-2):11-8.

142. Hynd MR, Scott HL, Dodd PR. Differential expression of N-methyl-D-aspartate receptor NR2 isoforms in Alzheimer's disease. J Neurochem. 2004 Aug;90(4):913-9.

143. Clinton SM, Meador-Woodruff JH. Abnormalities of the NMDA Receptor and Associated Intracellular Molecules in the Thalamus in Schizophrenia and Bipolar Disorder. Neuropsychopharmacology. 2004 Jul;29(7):1353-62.

144. Peter B, Matsushita M, Oda K, Raskind W. De novo microdeletion of BCL11A is associated with severe speech sound disorder. Am J Med Genet. 2014 Aug;164A(8):2091-6.

145. Dias C, Estruch SB, Graham SA, et al. BCL11A Haploinsufficiency Causes an Intellectual Disability Syndrome and Dysregulates Transcription. Am J Hum Genet. 2016 Aug 4;99(2):253-74.

146. Hancarova M, Simandlova M, Drabova J, Mannik K, Kurg A, Sedlacek Z. A patient with de novo 0.45 Mb deletion of 2p16.1: the role of BCL11A, PAPOLG, REL, and FLJ16341 in the 2p15-p16.1 microdeletion syndrome. Am J Med Genet. 2013 Apr;161A(4):865-70.

147. De Rubeis S, He X, Goldberg AP, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature. 2014 Nov;515(7526):209-15.

148. Liu P, Keller JR, Ortiz M, et al. Bcl11a is essential for normal lymphoid development. Nat Immunol. 2003 Jun;4(6):525-32.

149. Kuo TY, Hong CJ, Hsueh YP. Bcl11A/CTIP1 regulates expression of DCC and MAP1b in control of axon branching and dendrite outgrowth. Mol Cell Neurosci. 2009 Nov;42(3):195-207.

150. Berry GT. Classic Galactosemia and Clinical Variant Galactosemia. 2000 Feb 4 [Updated 2017 Mar 9]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews [Internet]. Seattle: University of Washington; 1993-2018.

151. Sebat J, Lakshmi B, Troge J, et al. Large-scale copy number polymorphism in the human genome. Science. 2004 Jul;305(5683):525-8.

152. Mervis CB, Klein-Tasman BP, Huffman MJ, et al. Children with 7q11.23 duplication syndrome: psychological characteristics. Am J Med Genet. 2015 Jul;167(7):1436-50.

153. Morris CA, Mervis CB, Paciorkowski AP, et al. 7q11.23 Duplication syndrome: Physical characteristics and natural history. Am J Med Genet. 2015 Dec;167A(12):2916-35.

154. Velleman SL, Mervis CB. Children with 7q11.23 Duplication Syndrome: Speech, Language, Cognitive, and Behavioral Characteristics and their Implications for Intervention. Perspect Lang Learn Educ. 2011 Oct;18(3):108-16.

155. Mervis CB, Morris CA, Klein-Tasman BP, Velleman SL, Osborne LR. 7q11.23 Duplication Syndrome. 2015 Nov 25. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews [Internet]. Seattle: University of Washington; 1993-2018.

156. Turner SJ, Mayes AK, Verhoeven A, Mandelstam SA, Morgan AT, Scheffer IE. GRIN2A: An aptly named gene for speech dysfunction. Neurology. 2015 Jan 16;84(6):586-93.

157. Raca G, Baas BS, Kirmani S, et al. Childhood Apraxia of Speech (CAS) in two patients with 16p11.2 microdeletion syndrome. Eur J Hum Genet. 2013 Apr;21(4):455-9.

158. Newbury DF, Mari F, Sadighi Akha E, et al. Dual copy number variants involving 16p11 and 6q22 in a case of childhood apraxia of speech and pervasive developmental disorder. Eur J Hum Genet. 2013 Apr;21(4):361-5.

159. Fedorenko E, Morgan A, Murray E, et al. A highly penetrant form of childhood apraxia of speech due to deletion of 16p11.2. Eur J Hum Genet. 2016 Feb;24(2):302-6.

160. Dimassi S, Labalme A, Lesca G, et al. A subset of genomic alterations detected in rolandic epilepsies contains candidate or known epilepsy genes including GRIN2A and PRRT2. Epilepsia. 2014 Feb;55(2):370-8.

161. Zufferey F, Sherr EH, Beckmann ND, et al. A 600 kb deletion syndrome at 16p11.2 leads to energy imbalance and neuropsychiatric disorders. J Med Genet. 2012 Oct;49(10):660-8.

162. Shinawi M, Liu P, Kang SH, et al. Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. J Med Genet. 2010 May;47(5):332-41.

163. Rosenfeld JA, Coppinger J, Bejjani BA, et al. Speech delays and behavioral problems are the predominant features in individuals with developmental delays and 16p11.2 microdeletions and microduplications. J Neurodev Disord. 2010 Mar;2(1):26-38.

164. Walters RG, Jacquemont S, Valsesia A, et al. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. Nature. 2010 Feb;463(7281):671-5.

165. Weiss LA, Shen Y, Korn JM, et al. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med. 2008 Feb;358(7):667-75.

166. Reinthaler EM, Lal D, Lebon S, et al. 16p11.2 600 kb Duplications confer risk for typical and atypical Rolandic epilepsy. Hum Mol Genet. 2014 Nov;23(22):6069-80.

167. Jacquemont S, Reymond A, Zufferey F, et al. Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. Nature. 2011 Aug;478(7367):97-102.

168. Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, et al. Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. Eur J Med Genet. 2009 Mar-Jun;52(2-3):77-87.

169. Thevenon J, Callier P, Andrieux J, et al. 12p13.33 microdeletion including ELKS/ERC1, a new locus associated with childhood apraxia of speech. Eur J Hum Genet. 2013 Jan;21(1):82-8.

170. Baker E, Hinton L, Callen DF, Haan EA, Dobbie A, Sutherland GR. A familial cryptic subtelomeric deletion 12p with variable phenotypic effect. Clin Genet. 2002 Mar;61(3):198-201.

171. Rooryck C, Stef M, Burgelin I, et al. 2.3 Mb terminal deletion in 12p13.33 associated with oculoauriculovertebral spectrum and evaluation of WNT5B as a candidate gene. Eur J Med Genet. 2009 Nov-Dec;52(6):446-9.

172. Macdonald AH, Rodriguez L, Acena I, et al. Subtelomeric deletion of 12p: Description of a third case and review. Am J Med Genet. 2010 Jun;152A(6):1561-6.

173. Abdelmoity AT, Hall JJ, Bittel DC, Yu S. 1.39 Mb inherited interstitial deletion in 12p13.33 associated with developmental delay. Eur J Med Genet. 2011 Mar-Apr;54(2):198-203.

174. Velinov M, Beldia G, Gu H, Tsiouris JA, Jenkins EC, Brown WT. Psychotic manifestations in a patient with mental retardation and a 6.2 megabase deletion at the distal short arm of chromosome 12. CNS Spectr. 2008 Jun;13(6):515-9.

175. Vargas H, Beldia G, Korosh W, et al. A 4.5 Mb terminal deletion of chromosome 12p helps further define a psychosis-associated locus. Eur J Med Genet. 2012 Oct;55(10):573-6.

176. Silva IM, Rosenfeld J, Antoniuk SA, Raskin S, Sotomaior VS. A 1.5Mb terminal deletion of 12p associated with autism spectrum disorder. Gene. 2014 May;542(1):83-6.

177. Faria RS, de Oliveira CP, da Costa MM, et al. Concurrent Loss of Heterozygosity and Mosaic Deletion of 12p13.32pter. Cytogenet Genome Res. 2016;148(2-3):174-8.

178. Wang Y, Liu X, Biederer T, Sudhof TC. A family of RIM-binding proteins regulated by alternative splicing: Implications for the genesis of synaptic active zones. Proc Natl Acad Sci U S A. 2002 Oct;99(22):14464-9.

179. Neira-Fresneda J, Potocki L. Neurodevelopmental Disorders Associated with Abnormal Gene Dosage: Smith-Magenis and Potocki-Lupski Syndromes. J Pediatr Genet. 2015 Sep;4(3):159-67.

180. Potocki L, Bi W, Treadwell-Deering D, et al. Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype. Am J Hum Genet. 2007 Apr;80(4):633-49.

181. Treadwell-Deering DE, Powell MP, Potocki L. Cognitive and behavioral characterization of the Potocki-Lupski syndrome (duplication 17p11.2). J Dev Behav Pediatr. 2010 Feb-Mar;31(2):137-43.

182. Elsea SH, Girirajan S. Smith-Magenis syndrome. Eur J Hum Genet. 2008 Apr;16(4):412-21.

183. Shaw CJ, Stankiewicz P, Bien-Willner G, et al. Small marker chromosomes in two patients with segmental aneusomy for proximal 17p. Hum Genet. 2004 Jun;115(1):1-7.

184. Kogan JM, Miller E, Ware SM. High resolution SNP based microarray mapping of mosaic supernumerary marker chromosomes 13 and 17: delineating novel loci for apraxia. Am J Med Genet. 2009 May;149A(5):887-93.

185. Kevelam SH, Jansen FE, Binsbergen E, et al. Copy number variations in patients with electrical status epilepticus in sleep. J Child Neurol. 2012 Feb;27(2):178-82.

186. Dibbens LM, Mullen S, Helbig I, et al. Familial and sporadic 15q13.3 microdeletions in idiopathic generalized epilepsy: precedent for disorders with complex inheritance. Hum Mol Genet. 2009 Oct;18(19):3626-31.

187. Sharp AJ, Mefford HC, Li K, et al. A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. Nat Genet. 2008 Mar;40(3):322-8.

188. Miller DT, Shen Y, Weiss LA, et al. Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders. J Med Genet. 2009 Apr;46(4):242-8.

189. Shinawi M, Schaaf CP, Bhatt SS, et al. A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes. Nat Genet. 2009 Dec;41(12):1269-71.

190. Boyar FZ, Whitney MM, Lossie AC, et al. A family with a grand-maternally derived interstitial duplication of proximal 15q. Clin Genet. 2001 Dec;60(6):421-30.

191. Lowther C, Costain G, Stavropoulos DJ, et al. Delineating the 15q13.3 microdeletion phenotype: a case series and comprehensive review of the literature. Genet Med. 2015 Feb;17(2):149-57.

192. van Bon BW, Mefford HC, Menten B, et al. Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome. J Med Genet. 2009 Aug;46(8):511-23.

193. Pettigrew KA, Reeves E, Leavett R, et al. Copy Number Variation Screen Identifies a Rare De Novo Deletion at Chromosome 15q13.1-13.3 in a Child with Language Impairment. PloS One. 2015;10(8):e0134997.

194. Peter B, Wijsman EM, Nato AQ, Jr., et al. Genetic Candidate Variants in Two Multigenerational Families with Childhood Apraxia of Speech. PloS One. 2016;11(4):e0153864.

195. Shriberg LD, Jakielski KJ, El-Shanti H. Breakpoint localization using array-CGH in three siblings with an unbalanced 4q;16q translocation and childhood apraxia of speech (CAS). Am J Med Genet. 2008 Sep;146A(17):2227-33.

196. Chilosi AM, Lorenzini I, Fiori S, et al. Behavioral and neurobiological correlates of childhood apraxia of speech in Italian children. Brain Lang. 2015 Nov;150:177-85.

197. Bernier R, Steinman KJ, Reilly B, et al. Clinical phenotype of the recurrent 1q21.1 copy-number variant. Genet Med. 2016 Apr;18(4):341-9.

198. Firth HV, Richards SM, Bevan AP, et al. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet. 2009 Apr;84(4):524-33.

199. Lesca G, Rudolf G, Labalme A, et al. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. Epilepsia. 2012 Sep;53(9):1526-38.

200. Peter B, Matsushita M, Raskind WH. Motor sequencing deficit as an endophenotype of speech sound disorder: a genome-wide linkage analysis in a multigenerational family. Psychiatr Genet. 2012 Oct;22(5):226-34.

201. Stein CM, Schick JH, Gerry Taylor H, et al. Pleiotropic effects of a chromosome 3 locus on speech-sound disorder and reading. Am J Hum Genet. 2004 Feb;74(2):283-97.

202. Miscimarra L, Stein C, Millard C, et al. Further evidence of pleiotropy influencing speech and language: analysis of the DYX8 region. Human Hered. 2007;63(1):47-58.

203. Stein CM, Millard C, Kluge A, et al. Speech sound disorder influenced by a locus in 15q14 region. Behav Genet. 2006 Nov;36(6):858-68.

204. Smith SD, Pennington BF, Boada R, Shriberg LD. Linkage of speech sound disorder to reading disability loci. J Child Psychol Psychiatry. 2005 Oct;46(10):1057-66.

205. Fisher SE, DeFries JC. Developmental dyslexia: genetic dissection of a complex cognitive trait. Nat Rev Neurosci. 2002 Oct;3(10):767-80.

206. Bishop DVM. Children's Communication Checklist. 2nd ed: Pearson; 2003.

207. Wechsler D. The Wechsler Intelligence Scale for Children. 4th ed. London: Pearson Assessment; 2004.

208. Bayley N. Bayley Scales of Infant and Toddler Development. 3rd ed. London: Pearson; 2005.

209. Sparrow SS, Cicchetti DV, Balla DA. Vineland Adaptive Behavior Scales. 2nd ed. London: Pearson; 2005.

210. Kaufman AS, Kaufman NL. Kaufman Brief Intelligence Test. 2nd ed. Bloomington, MN: Pearson; 2004.

211. Wechsler D. Wechsler Adult Intelligence Scale. 4th ed. London: Pearson Assessment; 2008.

212. Delis DC, Kaplan E, Kramer JH. Delis-Kaplan Executive Function System. San Antonio, TX: The Psychological Corporation; 2001.

213. Gioia GA, Isquith PK, Guy SC, Kenworthy L. Behavior Rating Inventory of Executive Function. Lutz, FL: PAR; 2000.

214. Hayden D, Square P. The Verbal Motor Production Assessment for Children. Texas: The Psychological Corporation; 1999.

215. McCauley RJ, Strand EA. A review of standardized tests of nonverbal oral and speech motor performance in children. Am J Speech Lang Pathol. 2008 Feb;17(1):81-91.

216. Enderby P, Palmer R. Frenchay dysarthria assessment. 2nd ed. Austin: PRO-ED; 2008.

217. Hayden D, Wetherby AM, Cleary JE, Prizant BM. Early Motor Control Scales - prepublication version. Paul H. Brookes Publishing Co.; 2011.

218. Goldman R, Fristoe M. Goldman-Fristoe Test of Articulation. 2nd ed. Circle Pines, MN: American Guidance Service Inc; 2000.

219. Dodd B, Hua Z, Crosbie S, Holm A, Ozanne A. Diagnostic Evaluation of Articulation and Phonology. London: Pearson Assessment; 2002.

220. Bunton K, Kent RD, Duffy JR, Rosenbek JC, Kent JF. Listener agreement for auditory-perceptual ratings of dysarthria. J Speech Lang Hear Res. 2007 Dec;50(6):1481-95.

221. Weismer G, Jeng JY, Laures JS, Kent RD, Kent JF. Acoustic and intelligibility characteristics of sentence production in neurogenic speech disorders. Folia Phoniatr Logop. 2001 Jan-Feb;53(1):1-18.

222. Zimmerman I, Steiner V, Pond R. Preschool Language Scales, Australian and New Zealand Language Adapted Edition. 5th ed. Pearson; 2011.

223. Dodd B, Holm A, Hua Z, Crosbie S. Phonological development: a normative study of British English-speaking children. Clin Linguist Phon. 2003 Dec;17(8):617-43.

224. Murray E, McCabe P, Ballard KJ. A systematic review of treatment outcomes for children with childhood apraxia of speech. Am J Speech Lang Pathol. 2014 Aug;23(3):486-504.

225. Shriberg L, Kwiatkowski J, Rasmussen C. The Prosody-Voice Screening Profile. Tuscson, AZ: Communication Skill Builders; 1990.

226. Duffy JR. Motor Speech Disorders; Substrates, Differential Diagnosis and Management. 3rd ed. St Louis, Mo: Mosby; 2013.

227. Murdoch BE. Dysarthria: A physiological approach to assessment and treatment. Cheltenham, UK: Stanley Thornes; 1998.

228. Thoonen G, Maassen B, Wit J, Gabreels F, Schreuder R. The integrated use of maximum performance tasks in differential diagnostic evaluations among children with motor speech disorders. Clin Linguist Phon. 1996;10(4):311-36.

229. Kent RD, Kent JF, Rosenbek JC. Maximum performance tests of speech production. J Speech Hear Disord. 1987 Nov;52(4):367-87.

230. Fletcher SG. Time-by-count measurement of diadochokinetic syllable rate. J Speech Hear Res. 1972 Dec;15(4):763-70.

231. Dabul BL. Apraxia Battery for Adults. 2nd ed. Austin: PRO-ED; 2000.

232. Gottesman, II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry. 2003 Apr;160(4):636-45.

233. Lewis B. Genetic Influences on Speech Sound Disorders. In: Paul R, Flipsen P, editors. Speech Sound Disorders in Children. San Diego: Plural Publishing; 2010. p. 51-70.

234. Catts HW. Speech production/phonological deficits in reading-disordered children. J Learn Disabil. 1986 Oct;19(8):504-8.

235. Lewis BA, Freebairn L. Residual effects of preschool phonology disorders in grade school, adolescence, and adulthood. J Speech Hear Res. 1992 Aug;35(4):819-31.

236. Gathercole SE, Baddeley AD. The Children's Test of Nonword Repetition. London: The Psychological Corporation; 1996.

237. Gathercole SE, Baddeley AD. Nonword Memory Test. University of Bristol; 1996.

238. Wagner RK, Torgesen JK, Rashotte CA. Comprehensive Test of Phonological Processing. Austin: PRO-ED; 1999.

239. Haber LR, Haber RN. Does silent reading involve articulation? Evidence from tongue twisters. Am J Psychol. 1982 Fall;95(3):409-19.

240. Shriberg LD, Lohmeier HL, Campbell TF, Dollaghan CA, Green JR, Moore CA. A nonword repetition task for speakers with misarticulations: the Syllable Repetition Task (SRT). J Speech Lang Hear Res. 2009 Oct;52(5):1189-212.

241. Shriberg L, Lohmeier HL. The Syllable Repetition Task (SRT) [Technical Report 14]. 2008. Available from www.waisman.wisc.edu/phonology/techreports 2008.

242. Shriberg LD, Ballard KJ, Tomblin JB, Duffy JR, Odell KH, Williams CA. Speech, prosody, and voice characteristics of a mother and daughter with a 7;13 translocation affecting FOXP2. J Speech Lang Hear Res. 2006 Jun;49(3):500-25.

243. Shriberg LD, Austin D, Lewis BA, McSweeny JL, Wilson DL. The speech disorders classification system (SDCS): extensions and lifespan reference data. J Speech Lang Hear Res. 1997 Aug;40(4):723-40.

244. Brady NC, Fleming K, Thiemann-Bourque K, et al. Development of the communication complexity scale. Am J Speech Lang Path. 2012 Feb;21(1):16-28.

245. Semel E, Wiig E, Secord W. Clinical Evaluation of Language Fundamentals, Australian Standardised Edition. 4th ed. Marrickville: Harcourt Assessment; 2006.

246. Dunn LM, Dunn DM. The Peabody Picture Vocabulary Test. 4th ed. Minneapolis: NCS Pearson Inc; 2007.

247. Williams KT. Expressive Vocabulary Test. 2nd ed. London: Pearson Assessment; 2007.

248. Bishop DJ. Test for Reception of Grammar. 2nd ed. London: Pearson Assessment; 2003.

249. Lewis BA, Avrich AA, Freebairn LA, Taylor HG, Iyengar SK, Stein CM. Subtyping Children With Speech Sound Disorders by Endophenotypes. Top Lang Disord. 2011;31(2):112-27.

250. Wilkinson GS, Robertson GJ. Wide Range Achievement Test. 4th ed. Lutz: Psychological Assessment Resources; 2006.

251. Wechsler D. The Wechsler Abbreviated Scale of Intelligence. 2nd ed. London: Pearson; 2011.

252. Smit AB, Hand L, Freilinger JJ, Bernthal JE, Bird A. The Iowa Articulation Norms Project and its Nebraska replication. J Speech Hear Disord. 1990 Nov;55(4):779-98.

253. Stackhouse J, Snowling M. Developmental verbal dyspraxia. II: A developmental perspective on two case studies. Eur J Disord Commun. 1992;27(1):35-54.

254. Hunter A, Morgan AW, Bird HA. A survey of Ehlers-Danlos syndrome: hearing, voice, speech and swallowing difficulties. Is there an underlying relationship? Br J Rheumatol. 1998 Jul;37(7):803-4.

255. Faivre L, Khau Van Kien P, Callier P, et al. De novo 15q21.1q21.2 deletion identified through FBN1 MLPA and refined by 244K array-CGH in a female teenager with incomplete Marfan syndrome. Eur J Med Genet. 2010 Jul-Aug;53(4):208-12.

256. Arverdson JC, Heintskill B. Ehlers-Danlos syndrome. In: McNeil MR, editor. Clinical management of sensorimotor speech disorders. 2nd ed. New York: Thieme; 2009. p. 314-16.

257. Ghibellini G, Brancati F, Castori M. Neurodevelopmental attributes of joint hypermobility syndrome/Ehlers-Danlos syndrome, hypermobility type: Update and perspectives. Am J Med Genet C Semin Med Genet. 2015 Mar;169C(1):107-16.

258. Celletti C, Mari G, Ghibellini G, Celli M, Castori M, Camerota F. Phenotypic variability in developmental coordination disorder: Clustering of generalized joint hypermobility with attention deficit/hyperactivity disorder, atypical swallowing and narrative difficulties. Am J Med Genet C Semin Med Genet. 2015 Mar;169C(1):117-22.

259. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am J Hum Genet. 2001 Jun;68(6):1327-32.

260. Depienne C, Trouillard O, Saint-Martin C, et al. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. J Med Genet. 2009 Mar;46(3):183-91.

261. Liegeois F, Morgan AT, Connelly A, Vargha-Khadem F. Endophenotypes of FOXP2: dysfunction within the human articulatory network. Eur J Paediatr Neurol. 2011 Jul;15(4):283-8.

262. Jansen FE, Sadleir LG, Harkin LA, et al. Severe myoclonic epilepsy of infancy (Dravet syndrome): recognition and diagnosis in adults. Neurology. 2006 Dec 26;67(12):2224-6.

263. Genton P, Velizarova R, Dravet C. Dravet syndrome: the long-term outcome. Epilepsia. 2011 Apr;52 Suppl 2:44-9.

264. Shriberg LD, Aram DM, Kwiatkowski J. Developmental apraxia of speech: II. Toward a diagnostic marker. J Speech Lang Hear Res. 1997 Apr;40(2):286-312.

265. Shriberg LD, Campbell TF, Karlsson HB, Brown RL, McSweeny JL, Nadler CJ. A diagnostic marker for childhood apraxia of speech: the lexical stress ratio. Clin Linguist Phon. 2003 Oct-Nov;17(7):549-74.

266. Murray E, McCabe P, Heard R, Ballard KJ. Differential diagnosis of children with suspected childhood apraxia of speech. J Speech Lang Hear Res. 2015 Feb;58(1):43-60.

267. Liegeois FJ, Hildebrand MS, Bonthrone A, et al. Early neuroimaging markers of FOXP2 intragenic deletion. Sci Rep. 2016 Oct;6:35192.

268. Watkins KE, Vargha-Khadem F, Ashburner J, et al. MRI analysis of an inherited speech and language disorder: structural brain abnormalities. Brain. 2002 Mar;125(Pt 3):465-78.

269. Guenther FH. Neural Control of Speech. Cambridge, MA: MIT Press; 2016.

270. Guerrini R, Striano P, Catarino C, Sisodiya SM. Neuroimaging and neuropathology of Dravet syndrome. Epilepsia. 2011 Apr;52 Suppl 2:30-4.

271. Striano P, Mancardi MM, Biancheri R, et al. Brain MRI findings in severe myoclonic epilepsy in infancy and genotype-phenotype correlations. Epilepsia. 2007 Jun;48(6):1092-6.

272. Barba C, Parrini E, Coras R, et al. Co-occurring malformations of cortical development and SCN1A gene mutations. Epilepsia. 2014 Jul;55(7):1009-19.

273. Perez A, Garcia-Penton L, Canales-Rodriguez EJ, et al. Brain morphometry of Dravet syndrome. Epilepsy Res. 2014 Oct;108(8):1326-34.

274. Moehring J, von Spiczak S, Moeller F, et al. Variability of EEG-fMRI findings in patients with SCN1A-positive Dravet syndrome. Epilepsia. 2013 May;54(5):918-26.

275. Meier S, Demirakca T, Brusniak W, et al. SCN1A affects brain structure and the neural activity of the aging brain. Biol Psychiatry. 2012 Oct;72(8):677-83.

276. Myers KA, Scheffer IE. GRIN2A-Related Speech Disorders and Epilepsy. 2016 Sep 29. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews [Internet]. Seattle: University of Washington; 1993-2018.

277. Siniatchkin M, Groening K, Moehring J, et al. Neuronal networks in children with continuous spikes and waves during slow sleep. Brain. 2010 Sep;133(9):2798-813.

278. Xiao F, An D, Lei D, et al. Real-time effects of centrotemporal spikes on cognition in rolandic epilepsy: An EEG-fMRI study. Neurology. 2016 Feb;86(6):544-51.

279. DeVries SP, Patel AD. Two patients with a GRIN2A mutation and childhood-onset epilepsy. Pediatr Neurol. 2013 Dec;49(6):482-5.

280. Ogiwara I, Miyamoto H, Morita N, et al. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. J Neurosci. 2007 May 30;27(22):5903-14.

281. Mulley JC, Scheffer IE, Petrou S, Dibbens LM, Berkovic SF, Harkin LA. SCN1A mutations and epilepsy. Hum Mutat. 2005 Jun;25(6):535-42.

282. Yu FH, Mantegazza M, Westenbroek RE, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci. 2006 Sep;9(9):1142-9.

283. Reutlinger C, Helbig I, Gawelczyk B, et al. Deletions in 16p13 including GRIN2A in patients with intellectual disability, various dysmorphic features, and seizure disorders of the rolandic region. Epilepsia. 2010 Sep;51(9):1870-3.

284. Pierson TM, Yuan H, Marsh ED, et al. GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine. Ann Clin Transl Neurol. 2014 Mar;1(3):190-8.

285. Yuan H, Hansen KB, Zhang J, et al. Functional analysis of a de novo GRIN2A missense mutation associated with early-onset epileptic encephalopathy. Nat Commun. 2014;5:3251.

286. Serraz B, Grand T, Paoletti P. Altered zinc sensitivity of NMDA receptors harboring clinically-relevant mutations. Neuropharmacology. 2016 Oct;109:196-204.

287. Addis L, Virdee JK, Vidler LR, Collier DA, Pal DK, Ursu D. Epilepsy-associated GRIN2A mutations reduce NMDA receptor trafficking and agonist potency - molecular profiling and functional rescue. Sci Rep. 2017 Dec;7(1):66.

288. Mefford HC. Genotype to phenotype-discovery and characterization of novel genomic disorders in a "genotype-first" era. Genet Med. 2009 Dec;11(12):836-42.

APPENDICES

Appendix 1 – Current Neurology and Neuroscience Reports review

Curr Neurol Neurosci Rep (2015) 15:35
DOI 10.1007/s11910-015-0554-0

GENETICS (V BONIFATI, SECTION EDITOR)

New Genes for Focal Epilepsies with Speech and Language Disorders

Samantha J. Turner[1,2,6] · **Angela T. Morgan**[1,2,3] · **Eliane Roulet Perez**[4] · **Ingrid E. Scheffer**[1,5,6]

Abstract The last 2 years have seen exciting advances in the genetics of Landau-Kleffner syndrome and related disorders, encompassed within the epilepsy-aphasia spectrum (EAS). The striking finding of mutations in the *N*-methyl-D-aspartate (NMDA) receptor subunit gene *GRIN2A* as the first monogenic cause in up to 20 % of patients with EAS suggests that excitatory glutamate receptors play a key role in these disorders. Patients with *GRIN2A* mutations have a recognizable speech and language phenotype that may assist with diagnosis. Other molecules involved in RNA binding and cell adhesion have been implicated in EAS; copy number variations are also found. The emerging picture highlights the overlap between the genetic determinants of EAS with speech and language disorders, intellectual disability, autism spectrum disorders and more complex developmental phenotypes.

Keywords Epilepsy-aphasia spectrum · Speech · Language · Gene · Landau-Kleffner syndrome · Epileptic encephalopathy with continuous spike-wave during sleep · Benign childhood epilepsy with centro-temporal spikes · *GRIN2A* · *RBFOX* genes · Copy number variants · Dysarthria · Speech dyspraxia · Oromotor dyspraxia · Continuous spike-wave in slow sleep · Rolandic · Atypical benign partial epilepsy · Autosomal dominant rolandic epilepsy with speech dyspraxia

Introduction

In their landmark paper describing six children with a syndrome of acquired aphasia with convulsive disorder, Landau and Kleffner first made the connection between epileptiform abnormalities and speech and language impairment [1].

This article is part of the Topical Collection on *Genetics*

✉ Ingrid E. Scheffer
scheffer@unimelb.edu.au

Samantha J. Turner
s.turner4@student.unimelb.edu.au

Angela T. Morgan
angela.morgan@mcri.edu.au

Eliane Roulet Perez
Eliane.Roulet-Perez@chuv.ch

[1] Department of Paediatrics, The University of Melbourne, The Royal Children's Hospital, Parkville, Australia

[2] Language and Literacy Group, Murdoch Childrens Research Institute, Parkville, Australia

[3] Speech Pathology Department, The Royal Children's Hospital, Parkville, Australia

[4] Paediatric Neurology and Neurorehabilitation Unit, Hospitalier Universitaire Vaudois, Lausanne, Switzerland

[5] Florey Institute of Neuroscience and Mental Health, Melbourne, Australia

[6] Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Melbourne, Australia

Published online: 29 April 2015

Almost 60 years later, the nature of this relationship is still being disentangled. While the aetiology of these disorders has been controversial, our first insights into causation have recently emerged with genes discovered for Landau-Kleffner syndrome and related disorders. In this review, we discuss recent gene findings, including the exciting discovery of *GRIN2A* mutations as the first monogenic cause of EAS disorders.

Epilepsy-Aphasia Spectrum

While the link between epilepsy and aphasia was first made with the identification of Landau-Kleffner syndrome (LKS) [1], the concept is now much broader and denotes an association between speech and language disorders and the EEG signature of focal sharp waves in language regions. The epilepsy-aphasia spectrum (EAS) has grown from our understanding of the inter-relationship between ranges of epilepsy syndromes sharing these features. At the mild end is the commonest focal epilepsy of childhood, benign childhood epilepsy with centro-temporal spikes (BECTS), and at the severe end lie the epileptic encephalopathies of LKS and epileptic encephalopathy with continuous spike-wave during sleep (ECSWS) (Fig. 1) [2–4].

Despite the use of *aphasia* in the term EAS, many patients do not experience complete loss of language and this word is used to denote a range of speech and language disorders. While the terms *speech* and *language* are often used interchangeably, they have different meanings (see Table 1).

LKS is the archetypal epilepsy syndrome in which children have catastrophic loss of both receptive and expressive language [1, 5]. It typically begins with a verbal auditory agnosia where children are unable to recognize speech and environmental sounds such as the telephone ringing. This deterioration occurs in the setting of normal hearing and non-verbal intelligence. Loss of language occurs around the time that epileptiform abnormalities appear, although pre-existing language delay may be present. The EEG of LKS typically shows sleep-activated bilaterally synchronous or asynchronous temporal spikes evolving into continuous spike-wave in slow sleep (CSWS) at some time during the illness, although this may take time to emerge and may fluctuate. CSWS is defined as bilaterally synchronous discharges occupying >85 % of non-REM sleep. Epilepsy occurs in 70 % of cases typically with rolandic seizures that may evolve to bilateral convulsive seizures. It is usually easily controlled with anti-epileptic drugs (AEDs).

In contrast, ECSWS is characterized by global regression affecting behaviour, learning, memory, attention, motor and social skills [6, 7]. Children have more severe epilepsy with

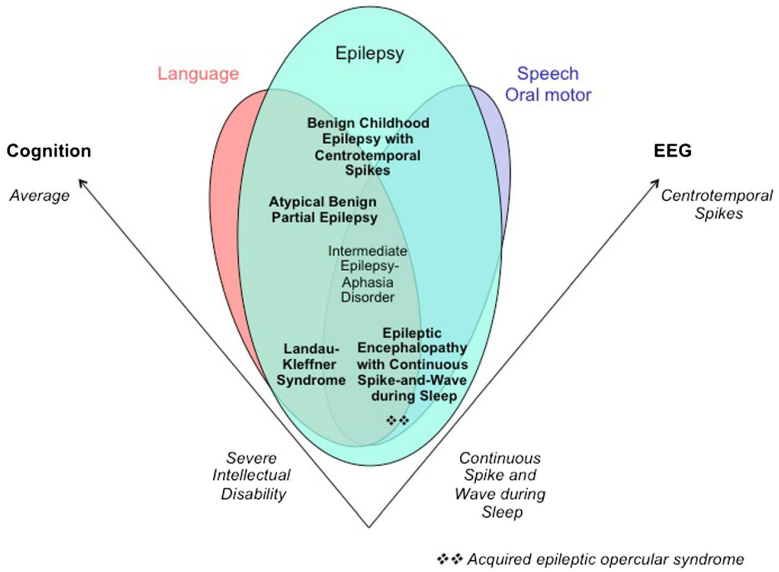


Fig. 1 The epilepsy-aphasia spectrum (EAS). The EAS is a broad concept denoting an association between epilepsy, speech and language disorders and the EEG signature of centro-temporal spikes. At the mild end of the spectrum is benign childhood epilepsy with centro-temporal spikes, with language impairments as well as speech and oromotor dyspraxia. At the severe end lie the epileptic encephalopathies of Landau-Kleffner Syndrome (LKS) and epileptic encephalopathy with continuous spike-wave during sleep (ECSWS), with severe loss of receptive and expressive language. Other cognitive and motor difficulties can occur with ECSWS without speech and language problems. Deterioration in oral motor function (oromotor dyspraxia, dysarthria, drooling) with CSWS is seen in the acquired epileptic opercular syndrome

Table 1 Definition of speech and language disorder subtypes

Speech and language disorders	Glossary of terms
Speech disorder	Any disruption to normal speech production, including the use of sounds to convey meaning [107]
Articulation disorder	Consistent fine motor problem of speech sound production [108], e.g. 'lisp' or lateral production of /s/
Phonological disorder	A higher-level disorder characterized by poor knowledge and use of sound patterns of their language [107], e.g. 'tat' for 'cat' with /t/ substituted for /k/
Childhood apraxia of speech (synonyms: speech dyspraxia, developmental verbal dyspraxia)	Impaired planning and programming of speech movements in the absence of weakness or altered tone [109]. Difficulty putting sounds and syllables together to form words; variability and more errors with increasing word length are important features. Prosodic errors are also seen
Dysarthria	Execution of fine motor movements involved in speech production is impaired, affecting the range, rate, strength or control of speech motor function [61•]. Deficits occur at any level of speech system (i.e. pitch, vocal quality, loudness, articulation, rate, resonance, respiration or prosody) impacting upon intelligibility or fluency of speech [110, 111].
Stuttering	Speech fluency disorder characterized by repetition of words or parts of words, prolongation of speech sounds or blocks, usually at the beginning of a word or a sentence [112]
Language impairment	Affects comprehension or formulation of spoken language
Receptive language	Ability to understand spoken language
Expressive language	Ability to express oneself through speaking
	Deficits include language form (phonology, syntax, morphosyntax), language content (semantics) or the function of language in communication (pragmatics) [113]
Specific language impairment	Language difficulties in a child with normal intelligence and adequate educational opportunity, not attributable to other disorders (e.g. hearing loss or autism); this label is currently under debate [114, 115]
Verbal auditory agnosia	Inability to recognize words and may include speech and environmental sounds

multiple seizure types including focal, convulsive and atypical absence seizures, which are often refractory to medication. ECSWS constitutes a broader syndrome and may not always have a predominant speech and language component [6]. In some instances, CSWS may be maximal in the prefrontal regions. In contrast, focal discharges in the rolandic region may be associated with oromotor dyspraxia [8, 9]. A more complex picture may be seen with deterioration in oral motor function (oromotor dyspraxia, dysarthria, drooling) in CSWS associated with the acquired epileptiform opercular syndrome [10].

BECTS is a self-limited childhood focal epilepsy associated with subtle cognitive impairment in some cases [11•, 12, 13]. Cognitive deficits include attention, executive function and memory dysfunction [14–17]. Speech and oromotor deficits are increasingly recognized [8, 9]. Poor performance on a range of tasks may be seen including impairment of receptive and expressive vocabulary, the ability to define words, apply word structure rules (morphology) and semantic and phonological verbal fluency [14, 18–20]. Literacy difficulties are common, including reading and spelling of single words, reading speed and comprehension, and impaired phonological awareness [12, 19–22]. Rolandic seizures are characterized by an aura of perioral paresthesia, oromotor features, guttural sounds and drooling. Hemiconvulsive or bilateral convulsive seizures also occur. In 80 % of children, seizures arise exclusively in sleep. Seizures are easily controlled by AEDs if they are sufficiently frequent to require treatment. The EEG hallmark of centro-temporal spikes (CTSs), occurring in unilateral or bilateral-independent doublets and triplets, is activated by sleep.

A rare entity, atypical benign partial (focal) epilepsy (ABPE), resembles BECTS but is associated with more severe seizures including drop attacks due to negative myoclonus [23, 24]. Regression occurs and primarily impacts on motor skills, speech, executive function and attention. The EEG shows active CTS both awake and in sleep when it may evolve to CSWS.

Autosomal dominant rolandic epilepsy with speech dyspraxia (ADRESD) is a rare type of autosomal dominant epilepsy with rolandic epilepsy and speech dyspraxia initially identified in an Australian family and later in other countries [25–28]. These families show phenotypic heterogeneity with variable severity in different family members.

For some individuals with cognitive regression, delay or developmental plateauing, their EEG features do not fulfil the strict criteria for CSWS. This intermediate phenotype was recently called intermediate epilepsy-aphasia disorder (IEAD) to denote those individuals where the bilaterally synchronous discharges occupy less than 85 % of slow-wave sleep [2].

It is unclear whether the epileptiform activity of the EAS causes the speech and language disorders or is simply a marker of the underlying disease. Focal discharges may disrupt speech and language networks at critical ages of language

development. In LKS, the severity of language impairment correlates with the presence and duration of CSWS [29–32]. Conversely, abnormal functioning of underlying brain regions may contribute to both seizures and speech and language impairment. This concept is supported by the presence of pre-existing language delay in some cases prior to the appearance of epileptiform activity [33]. Also, the resolution of epileptiform abnormalities may not correlate with improvement in speech and language function [29, 32, 33]. It is quite likely that both mechanisms are at play in the neurobiology of the EAS.

Aetiology of EAS

Until recently, the aetiology of EAS disorders remained largely elusive. Indeed there was debate whether the cause was congenital or acquired. In LKS, an autoimmune basis has been implicated because of the marked steroid responsiveness of the disorder [34, 35]. ECSWS occurs in patients with a range of cortical and subcortical (thalamic) lesions, but in many cases, the brain MRI shows no abnormalities.

BECTS was originally classified as the archetypal form of *idiopathic partial epilepsy* for which an hereditary predisposition was postulated [36]. Until recently, evidence for genetic factors has been relatively weak. A study of 18 monozygotic twin pairs found none to be concordant for BECTS [37, 38]. A single discordant monozygotic twin pair with LKS has been reported [39]. It has been suggested that the age-dependent EEG trait of CTS, rather than the seizure disorder, is genetically determined. Mechanisms such as environmental or epigenetic factors have been invoked to account for seizure expression.

In contrast, clinical genetic studies of the whole EAS support complex inheritance with phenotypic heterogeneity. While a handful of small families include members with BECTS and ECSWS [2, 40, 41•, 42], the most frequent phenotypes in affected relatives of EAS probands are febrile seizures and focal seizures [2, 40]. In addition, there are rare monogenic multiplex families with rolandic epilepsy and speech dyspraxia, such as ADRESD [25–28]. Thus, the discovery of the first genes for EAS (see below) provides a starting point to unravel the interaction between genetic and environmental factors.

Genes for EAS

GRIN2A

Three groups simultaneously discovered *GRIN2A* as a gene for EAS in 2013 [43••, 44••, 45••]. *GRIN2A* was the sole gene in the overlapping critical region of 16p13 microdeletions observed in three patients with moderate-to-severe intellectual disability, dysmorphic features and rolandic seizures [46]. A specific epilepsy syndrome was not described.

GRIN2A mutations were identified in all syndromes within the EAS accounting for 9–20 % of cohorts with LKS, ECSWS and ABPE [43••, 44••, 45••, 47] (Table 1). Both familial and de novo mutations were reported, although familial mutations predominated, perhaps reflecting ascertainment bias [43••]. *GRIN2A* mutations were also found in ADRESD [25, 45••], including in a French family previously reported to have a mutation of *SRPX2* [26]. In some families with EAS, unaffected carriers were observed [43••]. *GRIN2A* mutations were rare in BECTS as only 13/358 (3.6 %) cases harboured a mutation [43••, 44••, 45••]. Thus, the overall mutation rate was much higher in the complex, more severe EAS phenotypes than in BECTS at the mild end of the spectrum.

GRIN2A encodes the NR2A subunit of the glutamate *N*-methyl-D-aspartate (NMDA) receptor. NMDA receptors are ligand-gated ion channels involved in brain development, synaptic plasticity and memory. NMDA receptor functioning has also been linked to slow-wave activity during sleep [48]. The NMDA receptor is tetrameric, comprised of two NR1 subunits and two NR2 subunits (from NR2A to NR2D). The NR2A subunit is crucial to NMDA receptor functioning, controlling cell surface expression and localization [49], providing glutamate binding sites [50] and modifying channel properties [51].

GRIN2A mutations disrupt the ligand binding domain of the NR2A subunit and alter the NMDA receptor gating [43••, 44••, 45••]. Increased receptor activation due to impaired zinc-mediated inhibition, failure to initiate protein translation or nonsense-mediated decay of the mutant transcript may underlie other *GRIN2A* mutations [44••, 45••]. Mice expressing truncated NR2A show deficits in synaptic plasticity and reorganization and have impaired motor coordination [52]. *GRIN2A* mutations have been identified in familial and sporadic intellectual disability in concert with epilepsy or EEG abnormalities [53].

In the human brain, NR2A is expressed in many cortical and subcortical structures including those relevant to speech and language [54–60]. Aberrant NMDA receptor functioning in the basal ganglia may contribute to impaired motor speech planning/programming and execution, with these structures implicated in both childhood dysarthria and childhood apraxia of speech [61•]. NR2A expression has not been studied in Broca's area, a key expressive language region implicated in a range of speech and language disorders [61•, 62, 63].

Individuals in five EAS families had speech dyspraxia without seizures (Table 1), suggesting a role for *GRIN2A* in speech and language unrelated to seizures per se [64••]. The characteristic speech and language phenotype of individuals with *GRIN2A* mutations includes speech dyspraxia and dysarthria [25, 64••]. Oral motor, language and cognitive tasks are also impaired. Although variation between affected

Table 2 Mutations in *GRIN2A* and associated EAS phenotypes

	Mutation	Epilepsy phenotype								Other
		LKS	ECSWS	IEAD	Atypical RE	Typical RE	ADRESD	ABPE	No of seizures	
Carvill	c.1007+1G>A		2				7			
	c.2T>C	2							1[a]	
	c.1592C>T		2	1					1[a]	
Lesca	c.1123–2A>G substitution	1	3, 1[b]		3, 1[b]				1	
	≤75 kb microdeletion of 16p13.2		3[a]						1	
	≤15 kb microdeletion of 16p13.3	3 including 2[a]								Benign childhood epilepsy
	c.4161C>A		1							Benign childhood epilepsy
	c.1510C>T		2						1	
	c.1447G>A		1[b]		1[b]				1	
	c.1553G>A		1[a]		1[a]				1[a]	
	c.2191G>A				1[a]				1[a]	
	c.3751G>A				1					Absence of epilepsy
	c.2146G>A				7[a]				1[a]	SRPX2
	c.2797G>A	1							1	
	c.551T>G		1						1	
	c.2081T>C, de novo	1								
	c.1954T>G, de novo		1							
	c.1642G>A, de novo	1								
	c.2007G>T		1							
	c.883G>A					1				
Lemke	c.728C>T					1				Learning difficulties
	c.2041C>T	1							1	Learning disability
	c.1007+1G>A	1								
	c.1108C>T					1				
	c.2140G>A		1							
	c.2927A>G		1							
	c.594G>A							1		
	c.1001T>A		1							Focal epilepsy, panayiotopoulos syndrome
	c.2334_2338delCTTGC		1						1	
	c.2829C>G		1			1				FS/CTS
	c.2007+1G>A		1							Epilepsy
	c.236C>G		1			3			1	
	c.547T>A					1			1	
	c.692G>A	1							1	2×CTS
	c.869C>T					1				
	c.1306T>C							1		
	c.2095C>T					1				
	c.2113A>G					3			2	
	c.2179G>A					1				
	c.2200G>C					2			1	
	c.2314A>G							1		
	c.2441T>C					1			1	

Table 2 (continued)

Mutation	Epilepsy phenotype								Other
	LKS	ECSWS	IEAD	Atypical RE	Typical RE	ADRESD	ABPE	No of seizures	
c.2710A>T					2				FS/CTS
c.2927A>G							1		
c.90delTins(T)2					2			2	
c.1585delG					3				
c.1637_1639delCTT		1							CTS
c.1007+1G>A							1	1	Epilepsy
c.1007+1G>A	1				1			1	
c.1007+1G>A		1							CTS
Microdeletion of 16p13.3					2		1		
Duplication of 16p13.4		1							

LKS Landau-Kleffner syndrome, *ECSWS* epileptic encephalopathy with continuous spike-wave in slow-wave sleep, *IEAD* intermediate epilepsy-aphasia disorder, *RE* rolandic epilepsy/benign epilepsy of childhood with centro-temporal spikes, *ADRESD* autosomal dominant rolandic epilepsy with speech dyspraxia, *ABPE* atypical benign partial epilepsy, *BCE* benign childhood epilepsy, *CTS* centro-temporal spikes

[a] Speech dyspraxia

[b] Dysphasia

individuals is seen, their speech is typified by imprecise articulation of consonants and vowels and hypernasality, with prosodic disturbance. Phonological speech errors were also present, including sound substitutions, reduction of consonant clusters and omission of sounds or syllables. Moderate-to-severe receptive and expressive language impairment may occur with language deficits evident prior to the onset of seizures.

The *GRIN2A* speech phenotype is similar to that seen in individuals with mutations in *FOXP2*, the first gene associated with severe speech disorder; however, *FOXP2* mutations are associated with a more severe speech phenotype without epilepsy [65]. FOXP2 encodes a transcription factor that regulates the expression of hundreds of neural targets [66, 67]. Impaired speech motor planning/programming and execution occur with mutations in *FOXP2* [68, 69]. The speech of individuals is often *unintelligible* [70–74] compared with the mildly reduced intelligibility observed in individuals with *GRIN2A* mutations [64••]. There is also a disparity in the severity of language impairment with most *FOXP2* mutation cases showing severe deficits in comparison with the moderate-to-average language skills found in *GRIN2A* mutation carriers [75]. Poor non-word repetition is a common finding in both *FOXP2* and *GRIN2A* mutation cases [64••, 76].

RBFOX1 and *RBFOX3*

EAS disorders have recently been associated with variants in *RBFOX1* and *RBFOX3* [77•]. *RBFOX1* has been previously implicated in epilepsy and neurodevelopmental disorders including intellectual disability, autism spectrum disorders and ADHD [78–83]. Microdeletions and truncating variants in *RBFOX1* or *RBFOX3* have been reported in a range of small families with EAS including individuals with CTS, BECTS and ECSWS. Unaffected carriers were also observed, suggesting that these changes were disease risk factors rather than causative mutations [77•]. *RBFOX* genes encode RNA-binding proteins that control alternative splicing of exons. *RBFOX1* is a neuronal splicing regulator of an extensive network of genes involved in neuronal development and maturation [84], and also controls neuronal hyperexcitation [85]. *RBFOX1* disruption alters the expression of downstream genes important for neuronal development, maintenance and proliferation [84]. *RBFOX3* regulates splicing and nonsense-mediated decay of another *RBFOX* gene (*RBFOX2*) [86]. Interestingly, *RBFOX1* is also a direct target of the transcription factor *FOXP2* [87]. Future studies will help to elucidate the pathogenic role of *RBFOX* genes in EAS disorders.

Copy Number Variants

The critical role of copy number variations (CNVs) in human disease has become of increasing importance. The finding that CNVs, which include microdeletions and microduplications, exist in the normal population provided ground-breaking insights into genomic variation and, more recently, the pathogenic role of CNVs in disease [88]. Rare de novo and inherited CNVs have been identified in patients with EAS syndromes [41•, 89–91, 92•, 93•] (Table 2) (Table 3).

The best example of the power of CNVs in EAS was the 16p13 deletion that led to the discovery of *GRIN2A*. An overlapping deletion in three patients with rolandic seizures and

Table 3 De novo and inherited copy number variations reported in EAS syndromes

Copy number variation	EAS phenotype	Reference
Chromosome 1		
del1q21.1	BECTS	Mefford et al., 2010
del1q24.3/del5q22.1*	Typical RE	Dimassi et al., 2014
dup1q25.3	ECSWS	Lesca et al., 2012
delXq27/1q27.2*	Typical RE, ADHD	Dimassi et al., 2014
dup1q32	EAS	Mefford et al., 2011
dup1q44	LKS	Lesca et al., 2012
dup1p21.2-21.1	ECSWS	Lesca et al., 2012
Chromosome 2		
dup2p21	LKS woESES	Lesca et al., 2012
del19q13.33q13.41/dup2p21*	Typical RE	Dimassi et al., 2014
Chromosome 3		
del3q22.1/dup5q32*	Typical RE, migraine	Dimassi et al., 2014
dup3q24q25.1/dup6q26*	Typical RE, verbal dyspraxia	Dimassi et al., 2014
del3q25	ECSWS	Lesca et al., 2012
dup3q26.32-33	ECSWS	Lesca et al., 2012
dup3q28-q29	ECSWS	Lesca et al., 2012
dup3q29	ECSWS	Lesca et al., 2012
dup3p11.2	ECSWS	Lesca et al., 2012
dup3p26.3	ECSWS	Lesca et al., 2012
Chromosome 4		
dup4q13.1/del7q21.13*	Typical RE, dysphasia, behaviour	Dimassi et al., 2014
dup4q31.1	Atypical RE, ADHD, learning difficulties	Dimassi et al., 2014
del4q32.2	Typical RE, dysphasia	Dimassi et al., 2014
dup4q35.1	BECTS	Mefford et al., 2010
del4p16	EAS	Mefford et al., 2011
Chromosome 5		
dup5p12/dup5q31.3/dup16q23.1*	ECSWS	Kevelam et al., 2012
del5p14.1	ECSWS	Lesca et al., 2012
del5q11.2	ECSWS	Lesca et al., 2012
del1q24.3/del5q22.1*	Typical RE	Dimassi et al., 2014
del3q22.1/dup5q32*	Typical RE, migraine	Dimassi et al., 2014
Chromosome 6		
dup3q24q25.1/dup6q26*	Typical RE, verbal dyspraxia	Dimassi et al., 2014
del6q27	LKS with ESES	Lesca et al., 2012
Chromosome 7		
dup4q13.1/del7q21.13*	Typical RE, dysphasia, behaviour	Dimassi et al., 2014
del7q22	ECSWS	Lesca et al., 2012
dup7q35	LKS woESES	Lesca et al., 2012
Chromosome 8		
del8p22	ECSWS	Lesca et al., 2012
del8p23.1	BECTS	Mefford et al., 2010
del8p23.1	ECSWS	Lesca et al., 2012
del8p23.2	LKS woESES	Lesca et al., 2012
dup8q11.23	LKS with ESES	Lesca et al., 2012
del8q21	ECSWS	Lesca et al., 2012

Table 3 (continued)

Copy number variation	EAS phenotype	Reference
del8q22.3	ECSWS	Lesca et al., 2012
Chromosome 9		
dup9p13.2	LKS woESES	Lesca et al., 2012
dupXp22.11/dup9q34.3*	ECSWS	Kevelam et al., 2012
Chromosome 10		
del10q21.1	ECSWS	Lesca et al., 2012
dup10q21.1	ECSWS	Lesca et al., 2012
del10q21.3	ECSWS LKS with ESES	Lesca et al., 2012
Chromosome 11		
dup11p13	LKS woESES	Lesca et al., 2012
dup11p15.5	ECSWS	Lesca et al., 2012
del11q14	LKS with ESES	Lesca et al., 2012
Chromosome 13		
dup13q33.3	Typical RE, ADHD	Dimassi et al., 2014
del13q21.2	ECSWS	Lesca et al., 2012
Chromosome 14		
del14q21.3	ECSWS	Lesca et al., 2012
dup14q21.3	ECSWS	Lesca et al., 2012
del14q22.1/dup20p12.2*	Atypical RE, dysphasia, verbal dyspraxia	Dimassi et al., 2014
Chromosome 15		
del15q13.3	LKS	Kevelam et al., 2012
del15q21.3/dup16p13.11*	Typical RE	Dimassi et al., 2014
Chromosome 16		
dup16p11.2/dup17p13.3*	Atypical RE, ADHD	Dimassi et al., 2014
dup16p11.2	Atypical RE, verbal dyspraxia	Dimassi et al., 2014
del16p12.1	BECTS	Mefford et al., 2010
del16p13.11	BECTS	Mefford et al., 2010
del15q21.3/dup16p13.11*	Typical RE	Dimassi et al., 2014
del16p13.2	LKS woESES	Lesca et al., 2012
del16p13.2	Atypical RE, attention difficulties	Dimassi et al., 2014
dup16p11.2	Atypical RE, verbal dyspraxia	Dimassi et al., 2014
dup5p12/dup5q31.3/dup16q23.1*	ECSWS	Kevelam et al., 2012
del16q23.3	ECSWS	Lesca et al., 2012
Chromosome 17		
del17p12	Typical RE, attention difficulties	Dimassi et al., 2014
dup16p11.2/dup17p13.3*	Atypical RE, ADHD	Dimassi et al., 2014
Chromosome 19		
dup19p13.3	Typical RE, migraine	Dimassi et al., 2014
del19q13.33q13.41/dup2p21*	Typical RE	Dimassi et al., 2014
Chromosome 20		
dup20q13	ECSWS	Mefford et al., 2011
del20q13.3	LKS woESES	Lesca et al., 2012
del20p12.1	ECSWS	Lesca et al., 2012
dup20p12.1	LKS woESES	Lesca et al., 2012
del14q22.1/dup20p12.2*	Atypical RE, dysphasia, verbal dyspraxia	Dimassi et al., 2014

Table 3 (continued)

Copy number variation	EAS phenotype	Reference
Chromosome 22		
dup22q11.21/dup22q11.21	Atypical RE, moderate ID	Dimassi et al., 2014
del22q11.21	Atypical RE, migraine, ADHD, parasomnia	Dimassi et al., 2014
del22q13.32-33	ECSWS	Lesca et al., 2012
X chromosome		
dupXq21.1	Atypical RE, ADHD	Dimassi et al., 2014
delXq27/1q27.2*	Typical RE, ADHD	Dimassi et al., 2014
delXq28	Atypical RE, ADHD, severe ID	Dimassi et al., 2014
delXq28	ECSWS	Lesca et al., 2012
dupXp11.4	Atypical RE, ADHD, nocturnal enuresis	Dimassi et al., 2014
dupXp21.1	ECSWS	Lesca et al., 2012
dupXp22.11/dup9q34.3*	ECSWS	Kevelam et al., 2012
delXp22.12	ECSWS	Lesca et al., 2012
delXp22.31	ECSWS	Lesca et al., 2012
dupXp22.33	Atypical benign focal epilepsy of childhood	Kevelam et al., 2012

ADHD attention-deficit/hyperactivity disorder, *BECTS* benign epilepsy of childhood with centro-temporal spikes, *EAS* epilepsy-aphasia syndrome, *ECSWS* epileptic encephalopathy with continuous spike-wave in slow-wave sleep, *ID* intellectual disability, *LKS* Landau-Kleffner syndrome, *LKS woESES* LKS without electrical status epilepticus during slow-wave sleep, *LKS with ESES* LKS with electrical status epilepticus during slow-wave sleep, *RE* rolandic epilepsy

complex developmental disorders focused attention on this gene [46]. This was followed by the detection of a de novo partial deletion of *GRIN2A* in a child with LKS in a cohort of 61 patients with EAS [41•]. Interestingly, this patient inherited another rare intronic deletion of *CDH4* from his father who had transient language regression in childhood.

While most reported CNVs associated with EAS are unique, some include genomic regions or genes implicated in other developmental disorders [41•, 89, 91, 92•, 93•]. Disrupted genes include *CNTNAP2* on chromosome 7q35, associated with specific language impairment, autism spectrum disorders, intellectual disability, epilepsy and schizophrenia [94, 95]. CNVs found in EAS also implicate *CDH13*, *FGF12* and *ATP13A4*, which are candidate genes for language impairment [96, 97]. Rare inherited and de novo CNVs (39 gains, 43 losses) reported in 61 EAS patients included genes implicated in autism spectrum disorder: *MDGA2*, *SHANK3*, *DIAPH3* and *MACROD2* [41•]. Interestingly, not all the EAS patients carrying these CNVs had autistic traits. A significant number of patients carried CNVs that included genes encoding cell adhesion and closely related proteins (cadherin, protocadherin, contactin, catenin), suggesting that cell adhesion molecules play an important role in the pathophysiology of EAS.

CNVs on chromosomes 15q13 and 16p11 are in regions related to recurrent microdeletions (16p11.2 microdeletion syndrome, 15q13.3 microdeletion syndrome) where phenotypes include developmental delay, epilepsy, dysmorphic features and autism spectrum disorders [98–100]. Microduplications of 16p11.2 have recently been identified as a risk factor for rolandic epilepsy (1.3 % compared with 0.5 % in general population) [93•]. A 16p11.2 microdeletion syndrome is also linked to childhood apraxia of speech [101–103]. A 16p11.2 microduplication was identified in a child with verbal dyspraxia and EAS [92•]. A study of 13 patients with EAS found that four had a total of seven CNVs with six novel gains (duplications) and one recurrent loss of 15q13.3 in a patient with LKS [91]. The 15q region has also been linked to the EEG trait of centro-temporal spikes in families with BECTS [104]. The microdeletion encompassed the alpha 7 cholinergic neuronal nicotinic receptor gene, *CHRNA7*, implicated as the gene responsible for the clinical features underlying 15q13.3 microdeletion syndrome [105].

Taken together, these findings highlight that specific genomic regions are important for a range of developmental disorders and can direct the search for new EAS genes.

Conclusion

The fascinating interaction between focal epilepsy and speech and language disorders has long been known. It was only recently that molecular determinants have been discovered that inform the underlying neurobiology [43••, 44••, 45••]. Their discovery cements the association of an epilepsy-

aphasia spectrum ranging from self-limited rolandic epilepsies to severe epileptic encephalopathies [2]. The direct contribution of seizures and sleep-activated epileptiform discharges per se to speech, language and cognitive function in the context of a genetic aetiology remains to be unraveled.

The finding of NMDA receptor subunit mutations in a significant proportion of cases offers the opportunity for a personalized medicine approach to target the underlying loss of function mutations [43••, 44••, 45••]. Properly designed randomized placebo-controlled trials of memantine, a NMDA antagonist, in patients with EAS secondary to *GRIN2A* mutations may enable focused therapy that could improve long-term outcome of speech and language impairment in addition to epilepsy [106].

Compliance with Ethics Guidelines

Conflict of Interest Eliane Roulet Perez declares that she has no conflict of interest.

Samantha J. Turner has received grants from the National Health and Medical Research Council (postgraduate scholarship) and the Speech Pathology Australia (Nadia Verrall Memorial Research Grant).

Angela T. Morgan has received grants from the National Health and Medical Research Council (Career Development Award) and the Australian Research Council (Discovery Project).

Ingrid E. Scheffer has received grants from the NHMRC (CI, Program Grant 2011–2015), the NIH (PI, Centres without Walls funding "Epi4K" 2011–2015) and the ARC (Discovery Grant 2012–2014).

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Landau WM, Kleffner FR. Syndrome of acquired aphasia with convulsive disorder in children. Neurology. 1957;7:523–30.
2. Tsai MH, Vears DF, Turner SJ, et al. Clinical genetic study of the epilepsy-aphasia spectrum. Epilepsia. 2013;54:280–7.
3. Deonna T, Roulet-Perez E. Early-onset acquired epileptic aphasia (Landau-Kleffner syndrome, LKS) and regressive autistic disorders with epileptic EEG abnormalities: the continuing debate. Brain Dev. 2010;32:746–52.
4. Rudolf G, Valenti MP, Hirsch E, Szepetowski P. From rolandic epilepsy to continuous spike-and-waves during sleep and Landau-Kleffner syndromes: insights into possible genetic factors. Epilepsia. 2009;50 Suppl 7:25–8.
5. Tassinari CA, Cantalupo G, Dalla Bernardina B, et al. Encephalopathy related to status epilepticus during slow sleep (ESES) including Landau-Kleffner syndrome. In: Bureau M, Genton P, Dravet C, Delgado-Escueta A, Tassinari CA, Thomas P, et al., editors. Epileptic syndromes in infancy, childhood and adolescence. 5th ed. London: John Libbey Eurotext; 2012. p. 255–75.
6. Roulet Perez E, Davidoff V, Despland PA, Deonna T. Mental and behavioural deterioration of children with epilepsy and CSWS: acquired epileptic frontal syndrome. Dev Med Child Neurol. 1993;35:661–74.
7. Tassinari CA, Bureau M, Dravet C, Roger J, Daniele-Natale O. Electrical status epilepticus during sleep in children (ESES). In: Sterman MB, Shouse MN, Passouant P, editors. Sleep and epilepsy. New York: Academic; 1982. p. 465–79.
8. Deonna TW, Roulet E, Fontan D, Marcoz JP. Speech and oromotor deficits of epileptic origin in benign partial epilepsy of childhood with rolandic spikes (BPERS). Relationship to the acquired aphasia-epilepsy syndrome. Neuropediatrics. 1993;24:83–7.
9. Roulet E, Deonna T, Despland PA. Prolonged intermittent drooling and oromotor dyspraxia in benign childhood epilepsy with centrotemporal spikes. Epilepsia. 1989;30:564–8.
10. Shafrir Y, Prensky AL. Acquired epileptiform opercular syndrome: a second case report, review of the literature, and comparison to the Landau-Kleffner syndrome. Epilepsia. 1995;36:1050–7.
11. • Guerrini R, Pellacani S. Benign childhood focal epilepsies. Epilepsia. 2012;53 Suppl 4:9–18. **Comprehensive review of the BECTS literature.**
12. Northcott E, Connolly AM, Berroya A, et al. The neuropsychological and language profile of children with benign rolandic epilepsy. Epilepsia. 2005;46:924–30.
13. Hommet C, Billard C, Motte J, et al. Cognitive function in adolescents and young adults in complete remission from benign childhood epilepsy with centro-temporal spikes. Epileptic Disord. 2001;3:207–16.
14. Baglietto MG, Battaglia FM, Nobili L, et al. Neuropsychological disorders related to interictal epileptic discharges during sleep in benign epilepsy of childhood with centrotemporal or rolandic spikes. Dev Med Child Neurol. 2001;43:407–12.
15. Croona C, Kihlgren M, Lundberg S, Eeg-Olofsson O, Eeg-Olofsson KE. Neuropsychological findings in children with benign childhood epilepsy with centrotemporal spikes. Dev Med Child Neurol. 1999;41:813–8.
16. Weglage J, Demsky A, Pietsch M, Kurlemann G. Neuropsychological, intellectual, and behavioral findings in patients with centrotemporal spikes with and without seizures. Dev Med Child Neurol. 1997;39:646–51.
17. Massa R, de Saint Martin ARC. EEG criteria predictive of complicated evolution in idiopathic rolandic epilepsy. Neurology. 2001;57:1071–9.
18. Riva D, Vago C, Franceschetti S, et al. Intellectual and language findings and their relationship to EEG characteristics in benign childhood epilepsy with centrotemporal spikes. Epilepsy Behav. 2007;10:278–85.
19. Monjauze C, Tuller L, Hommet C, Barthez MA, Khomsi A. Language in benign childhood epilepsy with centro-temporal spikes abbreviated form: rolandic epilepsy and language. Brain Lang. 2005;92:300–8.
20. Staden U, Isaacs E, Boyd SG, Brandl U, Neville BG. Language dysfunction in children with rolandic epilepsy. Neuropediatrics. 1998;29:242–8.
21. Clarke T, Strug LJ, Murphy PL, et al. High risk of reading disability and speech sound disorder in rolandic epilepsy families: case-control study. Epilepsia. 2007;48:2258–65.
22. Papavasiliou A, Mattheou D, Bazigou H, Kotsalis C, Paraskevoulakos E. Written language skills in children with benign childhood epilepsy with centrotemporal spikes. Epilepsy Behav. 2005;6:50–8.

23. Aicardi J, Chevrie JJ. Atypical benign partial epilepsy of childhood. Dev Med Child Neurol. 1982;24:281–92.
24. Doose H, Brigger-Heuer B, Neubauer B. Children with focal sharp waves: clinical and genetic aspects. Epilepsia. 1997;38: 788–96.
25. Scheffer IE, Jones L, Pozzebon M, Howell RA, Saling MM, Berkovic SF. Autosomal dominant rolandic epilepsy and speech dyspraxia: a new syndrome with anticipation. Ann Neurol. 1995;38:633–42.
26. Roll P, Rudolf G, Pereira S, et al. SRPX2 mutations in disorders of language cortex and cognition. Hum Mol Genet. 2006;15:1195–207.
27. Kugler SL, Bali B, Lieberman P, et al. An autosomal dominant genetically heterogeneous variant of rolandic epilepsy and speech disorder. Epilepsia. 2008;49:1086–90.
28. Michelucci R, Scudellaro E, Testoni S, et al. Familial epilepsy and developmental dysphasia: description of an Italian pedigree with autosomal dominant inheritance and screening of candidate loci. Epilepsy Res. 2008;80:9–17.
29. Robinson RO, Baird G, Robinson G, Simonoff E. Landau-Kleffner syndrome: course and correlates with outcome. Dev Med Child Neurol. 2001;43:243–7.
30. Lanzi G, Veggiotti P, Conte S, Partesana E, Resi C. A correlated fluctuation of language and EEG abnormalities in a case of the Landau-Kleffner syndrome. Brain Dev. 1994;16:329–34.
31. Cole AJ, Andermann F, Taylor L, et al. The Landau-Kleffner syndrome of acquired epileptic aphasia: unusual clinical outcome, surgical experience, and absence of encephalitis. Neurology. 1988;38:31–8.
32. Rossi PG, Parmeggiani A, Posar A, Scaduto MC, Chiodo S, Vatti G. Landau-Kleffner syndrome (LKS): long-term follow-up and links with electrical status epilepticus during sleep (ESES). Brain Dev. 1999;21:90–8.
33. Soprano AM, Garcia EF, Caraballo R, Fejerman N. Acquired epileptic aphasia: neuropsychologic follow-up of 12 patients. Pediatr Neurol. 1994;11:230–5.
34. Nevsimalova S, Tauberova A, Doutlik S, Kucera V, Dlouha O. A role of autoimmunity in the etiopathogenesis of Landau-Kleffner syndrome? Brain Dev. 1992;14:342–5.
35. Connolly AM, Chez MG, Pestronk A, Arnold ST, Mehta S, Deuel RK. Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders. J Pediatr. 1999;134:607–13.
36. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 1989;30:389–399.
37. Vadlamudi L, Harvey AS, Connellan MM, et al. Is benign rolandic epilepsy genetically determined? Ann Neurol. 2004;56:129–32.
38. Vadlamudi L, Kjeldsen MJ, Corey LA, et al. Analyzing the etiology of benign rolandic epilepsy: a multicenter twin collaboration. Epilepsia. 2006;47:550–5.
39. Feekery CJ, Parry-Fielder B, Hopkins IJ. Landau-Kleffner syndrome: six patients including discordant monozygotic twins. Pediatr Neurol. 1993;9:49–53.
40. Vears DF, Tsai MH, Sadleir LG, et al. Clinical genetic studies in benign childhood epilepsy with centrotemporal spikes. Epilepsia. 2012;53:319–24.
41.• Lesca G, Rudolf G, Labalme A, et al. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. Epilepsia. 2012;53:1526–38. **This paper identifies copy number variants in LKS and ECSWS, many which highlight genomic regions or genes associated with ASD or speech and language disorders.**
42. De Tiege X, Goldman S, Verheulpen D, Aeby A, Poznanski N, Van Bogaert P. Coexistence of idiopathic rolandic epilepsy and CSWS in two families. Epilepsia. 2006;47:1723–7.
43.•• Lesca G, Rudolf G, Bruneau N, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat Genet. 2013;45:1061–6. **One of the three seminal papers published together (Carvill et al. 2013; Lesca et al. 2013; Lemke et al. 2013) identifying GRIN2A as the first monogenic cause of EAS disorders. Until this discovery, the pathophysiological basis of these disorders was unknown and controversial. They showed that 20 % of unrelated probands with EAS had GRIN2A mutations.**
44.•• Lemke JR, Lal D, Reinthaler EM. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. Nat Genet. 2013;45: 1067–72. **One of the three seminal papers published together (Carvill et al. 2013; Lesca et al. 2013; Lemke et al. 2013) identifying GRIN2A as the first monogenic cause of EAS disorders. Until this discovery, the pathophysiological basis of these disorders was unknown and controversial.**
45.•• Carvill GL, Regan BM, Yendle SC, et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. Nat Genet. 2013;45: 1073–6. **One of the three seminal papers published together (Carvill et al. 2013; Lesca et al. 2013; Lemke et al. 2013) identifying GRIN2A as the first monogenic cause of EAS disorders. Until this discovery, the pathophysiological basis of these disorders was unknown and controversial. This paper showed that 9 % of EAS probands had GRIN2A mutations.**
46. Reutlinger C, Helbig I, Gawelczyk B, et al. Deletions in 16p13 including GRIN2A in patients with intellectual disability, various dysmorphic features, and seizure disorders of the rolandic region. Epilepsia. 2010;51:1870–3.
47. Conroy J, McGettigan PA, McCreary D, et al. Towards the identification of a genetic basis for Landau-Kleffner syndrome. Epilepsia. 2014;55:858–65.
48. Miyamoto H, Katagiri H, Hensch T. Experience-dependent slow-wave sleep development. Nat Neurosci. 2003;6:553–4.
49. Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science. 1995;269:1737–40.
50. Laube B, Hirai H, Sturgess M, Betz H, Kuhse J. Molecular determinants of agonist discrimination by NMDA receptor subunits: analysis of the glutamate binding site on the NR2B subunit. Neuron. 1997;18:493–503.
51. Monyer H, Sprengel R, Schoepfer R, et al. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science. 1992;256:1217–21.
52. Sprengel R, Suchanek B, Amico C, et al. Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. Cell. 1998;92:279–89.
53. Endele S, Rosenberger G, Geider K, et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet. 2010;42:1021–6.
54. Akbarian S, Sucher NJ, Bradley D, et al. Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. J Neurosci. 1996;16:19–30.
55. Kosinski CM, Standaert DG, Counihan TJ, et al. Expression of N-methyl-D-aspartate receptor subunit mRNAs in the human brain: striatum and globus pallidus. J Comp Neurol. 1998;390:63–74.
56. Scherzer CR, Landwehrmeyer GB, Kerner JA, et al. Expression of N-methyl-D-aspartate receptor subunit mRNAs in the human brain: hippocampus and cortex. J Comp Neurol. 1998;390:75–90.
57. Conti F, Barbaresi P, Melone M, Ducati A. Neuronal and glial localization of NR1 and NR2A/B subunits of the NMDA receptor in the human cerebral cortex. Cereb Cortex. 1999;9:110–20.

58. Bi H, Sze CI. N-methyl-D-aspartate receptor subunit NR2A and NR2B messenger RNA levels are altered in the hippocampus and entorhinal cortex in Alzheimer's disease. J Neurol Sci. 2002;200: 11–8.
59. Hynd MR, Scott HL, Dodd PR. Differential expression of N-methyl-D-aspartate receptor NR2 isoforms in Alzheimer's disease. J Neurochem. 2004;90:913–9.
60. Clinton SM, Meador-Woodruff JH. Abnormalities of the NMDA receptor and associated intracellular molecules in the thalamus in schizophrenia and bipolar disorder. Neuropsychopharmacology. 2004;29:1353–62.
61.• Liegeois FJ, Morgan AT. Neural bases of childhood speech disorders: lateralization and plasticity for speech functions during development. Neurosci Biobehav Rev. 2012;36:439–58. **Systematic review examining the evidence linking motor speech disorders (apraxia of speech and dysarthria) and brain abnormalities in children and adolescents with developmental, progressive or childhood-acquired conditions.**
62. Belton E, Salmond CH, Watkins KE, Vargha-Khadem F, Gadian DG. Bilateral brain abnormalities associated with dominantly inherited verbal and orofacial dyspraxia. Hum Brain Mapp. 2003;18:194–200.
63. Liegeois F, Baldeweg T, Connelly A, Gadian DG, Mishkin M, Vargha-Khadem F. Language fMRI abnormalities associated with FOXP2 gene mutation. Nat Neurosci. 2003;6:1230–7.
64.•• Turner SJ, Mayes AK, Verhoeven A, Mandelstam SA, Morgan AT, Scheffer IE. GRIN2A: an aptly named gene for speech dysfunction. Neurology. 2015;84:586–93. **Study delineating the distinctive speech phenotype associated with GRIN2A mutations, a finding that will readily aid in diagnosis.**
65. Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. A forkhead-domain gene is mutated in a severe speech and language disorder. Nature. 2001;413:519–23.
66. Spiteri E, Konopka G, Coppola G, et al. Identification of the transcriptional targets of FOXP2, a gene linked to speech and language, in developing human brain. Am J Hum Genet. 2007;81: 1144–57.
67. Vernes SC, Spiteri E, Nicod J, et al. High-throughput analysis of promoter occupancy reveals direct neural targets of FOXP2, a gene mutated in speech and language disorders. Am J Hum Genet. 2007;81:1232–50.
68. Morgan AT, Liegeois F, Vargha-Khadem F. Motor speech outcome as a function of the site of brain pathology: a developmental perspective. In: Maassen B, van Lieshout P, editors. Speech motor control: new developments in basic and applied research. Oxford: Oxford University Press; 2010. p. 95–115.
69. Turner SJ, Hildebrand MS, Block S, et al. Small intragenic deletion in FOXP2 associated with childhood apraxia of speech and dysarthria. Am J Med Genet A. 2013;161A:2321–6.
70. Hurst JA, Baraitser M, Auger E, Graham F, Norell S. An extended family with a dominantly inherited speech disorder. Dev Med Child Neurol. 1990;32:352–5.
71. Feuk L, Kalervo A, Lipsanen-Nyman M, et al. Absence of a paternally inherited FOXP2 gene in developmental verbal dyspraxia. Am J Hum Genet. 2006;79:965–72.
72. Zeesman S, Nowaczyk MJ, Teshima I, et al. Speech and language impairment and oromotor dyspraxia due to deletion of 7q31 that involves FOXP2. Am J Med Genet A. 2006;140:509–14.
73. Shriberg LD, Ballard KJ, Tomblin JB, Duffy JR, Odell KH, Williams CA. Speech, prosody, and voice characteristics of a mother and daughter with a 7;13 translocation affecting FOXP2. J Speech Lang Hear Res. 2006;49:500–25.
74. Rice GM, Raca G, Jakielski KJ, et al. Phenotype of FOXP2 haploinsufficiency in a mother and son. Am J Med Genet A. 2012;158A:174–81.
75. Watkins KE, Dronkers NF, Vargha-Khadem F. Behavioural analysis of an inherited speech and language disorder: comparison with acquired aphasia. Brain. 2002;125:452–64.
76. Vargha-Khadem F, Watkins KE, Price CJ, et al. Neural basis of an inherited speech and language disorder. Proc Natl Acad Sci U S A. 1998;95:12695–700.
77.• Lal D, Reinthaler EM, Altmuller J, et al. RBFOX1 and RBFOX3 mutations in rolandic epilepsy. PLoS One. 2013;8:e73323. **Identifies deletions and truncating mutations of RBFOX1 and RBFOX3 in some individuals with rolandic epilepsy in complex pedigrees.**
78. Lal D, Trucks H, Moller RS, et al. Rare exonic deletions of the RBFOX1 gene increase risk of idiopathic generalized epilepsy. Epilepsia. 2013;54:265–71.
79. Zhao WW. Intragenic deletion of RBFOX1 associated with neurodevelopmental/neuropsychiatric disorders and possibly other clinical presentations. Mol Cytogenet. 2013;6:26.
80. Elia J, Glessner JT, Wang K, et al. Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. Nat Genet. 2012;44:78–84.
81. Davis LK, Maltman N, Mosconi MW, et al. Rare inherited A2BP1 deletion in a proband with autism and developmental hemiparesis. Am J Med Genet A. 2012;158A:1654–61.
82. Martin CL, Duvall JA, Ilkin Y, et al. Cytogenetic and molecular characterization of A2BP1/FOX1 as a candidate gene for autism. Am J Med Genet B Neuropsychiatr Genet. 2007;144B:869–76.
83. Bhalla K, Phillips HA, Crawford J, et al. The de novo chromosome 16 translocations of two patients with abnormal phenotypes (mental retardation and epilepsy) disrupt the A2BP1 gene. J Hum Genet. 2004;49:308–11.
84. Fogel BL, Wexler E, Wahnich A, et al. RBFOX1 regulates both splicing and transcriptional networks in human neuronal development. Hum Mol Genet. 2012;21:4171–86.
85. Gehman LT, Stoilov P, Maguire J, et al. The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain. Nat Genet. 2011;43:706–11.
86. Dredge BK, Jensen KB. NeuN/Rbfox3 nuclear and cytoplasmic isoforms differentially regulate alternative splicing and nonsense-mediated decay of Rbfox2. PLoS One. 2011;6: e21585.
87. Ayub Q, Yngvadottir B, Chen Y, et al. FOXP2 targets show evidence of positive selection in European populations. Am J Hum Genet. 2013;92:696–706.
88. Sebat J, Lakshmi B, Troge J, et al. Large-scale copy number polymorphism in the human genome. Science. 2004;305:525–8.
89. Mefford HC, Muhle H, Ostertag P, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. PLoS Genet. 2010;6:e1000962.
90. Mefford HC, Yendle SC, Hsu C, et al. Rare copy number variants are an important cause of epileptic encephalopathies. Ann Neurol. 2011;70:974–85.
91. Kevelam SH, Jansen FE, Binsbergen E, et al. Copy number variations in patients with electrical status epilepticus in sleep. J Child Neurol. 2012;27:178–82.
92.• Dimassi S, Labalme A, Lesca G, et al. A subset of genomic alterations detected in rolandic epilepsies contains candidate or known epilepsy genes including GRIN2A and PRRT2. Epilepsia. 2014;55:370–8. **This paper identifies 30 rare microduplication and microdeletions in patients with rolandic epilepsy.**
93.• Reinthaler EM, Lal D, Lebon S, et al. 16p11.2 600 kb duplications confer risk for typical and atypical rolandic epilepsy. Hum Mol Genet. 2014;23:6069–80. **This recent study demonstrates that duplications of 16p11.2 represent a significant genetic risk factor for typical and atypical rolandic epilepsy.**

94. Rodenas-Cuadrado P, Ho J, Vernes SC. Shining a light on CNTNAP2: complex functions to complex disorders. Eur J Hum Genet. 2014;22:171–8.
95. Strauss KA, Puffenberger EG, Huentelman MJ, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. N Engl J Med. 2006;354:1370–7.
96. Consortium SLI. Highly significant linkage to the SLI1 locus in an expanded sample of individuals affected by specific language impairment. Am J Hum Genet. 2004;74:1225–38.
97. Kwasnicka-Crawford DA, Carson AR, Roberts W, et al. Characterization of a novel cation transporter ATPase gene (ATP13A4) interrupted by 3q25-q29 inversion in an individual with language delay. Genomics. 2005;86:182–94.
98. Sharp AJ, Mefford HC, Li K, et al. A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. Nat Genet. 2008;40:322–8.
99. Ballif BC, Hornor SA, Jenkins E, et al. Discovery of a previously unrecognized microdeletion syndrome of 16p11.2-p12.2. Nat Genet. 2007;39:1071–3.
100. Malhotra D, Sebat J. CNVs: harbingers of a rare variant revolution in psychiatric genetics. Cell. 2012;148:1223–41.
101. Laffin JJ, Raca G, Jackson CA, Strand EA, Jakielski KJ, Shriberg LD. Novel candidate genes and regions for childhood apraxia of speech identified by array comparative genomic hybridization. Genet Med. 2012;14:928–36.
102. Raca G, Baas BS, Kirmani S, et al. Childhood apraxia of speech (CAS) in two patients with 16p11.2 microdeletion syndrome. Eur J Hum Genet. 2013;21:455–9.
103. Newbury DF, Mari F, Sadighi Akha E, et al. Dual copy number variants involving 16p11 and 6q22 in a case of childhood apraxia of speech and pervasive developmental disorder. Eur J Hum Genet. 2013;21:361–5.
104. Neubauer BA, Fiedler B, Himmelein B, et al. Centrotemporal spikes in families with rolandic epilepsy: linkage to chromosome 15q14. Neurology. 1998;51:1608–12.
105. Hoppman-Chaney N, Wain K, Seger PR, Superneau DW, Hodge JC. Identification of single gene deletions at 15q13.3: further evidence that CHRNA7 causes the 15q13.3 microdeletion syndrome phenotype. Clin Genet. 2013;83:345–51.
106. Pierson TM, Yuan H, Marsh ED, et al. GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine. Ann Clin Transl Neurol. 2014;1:190–8.
107. Dodd B. Differential diagnosis and treatment of children with speech disorder. 2nd ed. London: Whurr; 2005.
108. Broomfield J, Dodd B. Children with speech and language disability: caseload characteristics. Int J Lang Commun Disord. 2004;39:303–24.
109. ASHA. Childhood apraxia of speech [technical report]. www.asha.org/policy/tr2007-00278.htm#sec1.1.
110. Darley FL, Aronson AE, Brown JR. Clusters of deviant speech dimensions in the dysarthrias. J Speech Hear Res. 1969;12:462–96.
111. Darley FL, Aronson AE, Brown JR. Differential diagnostic patterns of dysarthria. J Speech Hear Res. 1969;12:246–69.
112. Onslow M. Behavioural management of stuttering. 1st ed. Sydney: Livingston; 1993.
113. ASHA. Definitions of communication disorders and variations [relevant paper]. 1993.
114. Bishop DV. Ten questions about terminology for children with unexplained language problems. Int J Lang Commun Disord. 2014;49:381–415.
115. Reilly S, Tomblin B, Law J, et al. Specific language impairment: a convenient label for whom? Int J Lang Commun Disord. 2014;49:416–51.

Appendix 2: Recruitment flyer for Multiplex family study

Research into the genetics of speech disorder

Would you be willing to take part in our project?

Austin Health and The Royal Children's Hospital are running a project looking at the causes of speech disorder. This is part of our ongoing commitment to world class research aimed at improving health.

We are studying how genes may be involved in speech disorders in adults and children.

We are looking for child and adult participants who have any type of speech disorder and have a strong family history of speech disorders to take part in our project. We are also keen for other family members who have a speech disorder to participate.

What is involved?
We would like you/your child to come to an appointment at the Melbourne Brain Centre (Austin Health) or at The Royal Children's Hospital. We will reimburse you for travel costs.
We will ask you/your child to:

- Allow us to obtain medical history and speech disorder information
- Allow us to tape a sample of speech
- Perform speech assessments and tasks of learning/understanding
- Have a hearing test, if required
- Give a blood sample (or saliva sample if preferred) for genetic testing
- We may ask you/your child to have an MRI brain scan (not everyone will be asked to have a scan). Children under 7 years of age will not be asked to have an MRI brain scan.

If you are interested in helping us with this research, or if you would like further information about the project, please contact:

Ms Samantha Turner
PhD Scholar
Department of Paediatrics
The University of Melbourne/The Royal Children's Hospital

Dr Angela Morgan
Senior Research Fellow
Hearing, Language & Literacy
Murdoch Childrens Research Institute

245 Burgundy Street
Heidelberg VIC 3084
Telephone (03) 9035 7281
Facsimile (03) 9035 7307
Email s.turner4@student.unimelb.edu.au

Appendix 3: Advertisement in Speech Pathology Australia Speak Out magazine

Research Study: Call for Child & Adult participants

Speech disorders in families.

It is of no surprise to clinicians working with children that speech disorders are highly heritable. Many of our clients with speech disorder also have a sibling or parent with speech difficulties. Sets of identical twins are more often affected with speech sound disorders than sets of non-identical twins (86% vs 45% probandwise concordance).[1-3]

Children with speech sound disorders also have more affected parents and siblings compared to unaffected children (21% compared to 2%).[4-7] The percentage of affected first degree relatives is even higher (55%) for children affected with a more severe speech phenotype such as childhood apraxia of speech.[8] Despite overwhelming evidence that speech disorders are genetic, only a small number of genes have been identified that account for rare affected individuals.

Our research study aims to discover further genes associated with childhood speech disorders. We are studying large multigenerational families with speech disorders. In such families, molecular genetic studies can be performed to identify a disease-causing gene. We will characterise the clinical features of multiple family members across several generations, and use this information to direct the search for genes associated with speech disorder. The study is being conducted by Dr Angela Morgan (Speech Pathologist), Professor Ingrid Scheffer (Paediatric Neurologist) and Ms Samantha Turner (Speech Pathologist) at The Royal Children's Hospital, the Murdoch Children's Research Institute and the University of Melbourne.

We are looking for child and adult participants who have any type of speech disorder and have a strong family history of speech disorders to take part in our project. We are also recruiting children with rare and unusual speech disorders in which a genetic cause might be suspected.

If you are interested in helping us with this research or if you would like further information, we would love to hear from you.

Please contact:
Samantha Turner, PH: (03) 9035 7281 or email: s.turner4@student.unimelb.edu.au

1. Lewis B.A. & Thompson L.A. (1992). J Speech Hear Res 35, 1086-1094.
2. Bishop D.V.M. et al. (1995). Dev Med Child Neurol 37, 56-71.
3. DeThorne L.S. et al. (2006). J Speech Lang Hear Res 49, 1280-1293.
4. Lewis B.A. et al. (1989). J Speech Hear Res 32, 713-24.
5. Lewis B.A. (1992). J Learn Disabil 25, 586.
6. Lewis B.A. & Freebairn L. (1998). Lang Speech 41, 45.
7. Lewis B.A. et al. (2007). Am J Speech Lang Path 16, 108-118
8. Lewis B.A. et al. (2004). J Comm Disord 37, 157-175.

Speak Out April 2012

Appendix 4: Participant Information Statement and Consent form

PARTICIPANT

INFORMATION STATEMENT AND CONSENT FORM

HREC Project Number: 27053

Research Project Title: Genetics of Speech Disorders

Principal Researcher: Dr Angela Morgan, Senior Research Fellow

Thank you for taking the time to read this Information Statement. This Information Statement and Consent Form is 6 pages long. Please make sure you have all the pages.

You are invited to participate in a research project that is explained below.

What is an Information Statement?
These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether or not you would like to take part in the research.

Please read this Information Statement carefully. You can ask us questions about anything in it. You may want to talk about the project with your family, friends or health care worker.

Participation in this research project is voluntary. If you don't want to take part, you don't have to. You can withdraw from the project at any time without explanation and this will not affect your access to the best available treatment options and care from The Royal Children's Hospital.

Once you have understood what the project is about, if you would like to take part please sign the consent form at the end of this information statement. You will be given a copy of this information and consent form to keep.

---

1. What is the research project about?
This project is looking at the genetic causes of speech disorder. Speech disorder disrupts the natural flow of communication. This can have an impact on self-esteem, school, work and general well-being. It is a common problem, with 5% of the population experiencing a speech disorder at some time in their lives.

Childhood speech disorder typically begins between two and five years of age. Many children will grow out of it, but others will go on to have a persistent speech disorder. Previous research into families and twins has shown that speech disorder often runs in families. This suggests that some families may inherit one or more genes, which make it more likely that they will have a speech disorder. In addition, other genes may be inherited which make it more likely that a person will recover, or a speech disorder will persists.

Genes are the instructions inside you that tell your body what to look like and how to work. Genes are arranged on chromosomes, and these chromosomes are inside almost every cell of your body. Each cell will have about 30,000 genes located on 46 chromosomes inside them. Genes get passed down in families from parents to children. Because speech disorder can run in families, it is possible that speech disorder is caused by a change in a gene.

To date, only a small number of genes for childhood speech disorder without other neurological causes have been identified. We hope to discover other genes that are associated with speech disorder. We aim to use new genetic techniques to search for genes related to speech difficulty in families where there are a number of members with speech disorder, as well as in individuals where we think that the speech disorder may have a genetic basis (e.g., in severe and persistent speech disorder).

We hope that by working with families with a history of speech disorder, or individual children/adults who may have a genetic basis to their speech disorder, that the genetics of speech impairment will become clearer. By trying to identify gene changes associated with speech disorders, we will learn more about what causes someone to have speech problems, be able to identify people who are at high risk of speech disorder, and develop better treatments.

1. Who is funding this research project?

This project is funded by small philanthropic grants from the Shepherd Foundation, ANZ Trustees, Perpetual Charitable Trustees and the Austin Hospital Medical Research Fund.

2. Why am I being asked to be in this research project?

We are asking you to take part in this project because you have a family history of speech disorder, or because you have a speech disorder.

3. What do I need to do to be in this research project?

We would like you to come to an appointment in the Speech Pathology Department at The Royal Children's Hospital. If you have any difficulties coming to the hospital for this appointment, we can organise to come to your home. The appointment may take up to 3 hours.

We will ask you to:

- Perform a series of assessments that determine your ability to understand and produce words, sounds and sentences as well as making different movements with your mouth, eg. poking out your tongue or pursing your lips.
- Perform a number of tasks that will test your cognitive ability.
- Give a blood or a saliva sample so that we can extract your DNA.
  - A blood sample is the best way for us to do this. We will take around 18mls of blood (about 4 teaspoons) from an elbow vein.
  - If you feel uncomfortable about giving us a blood sample, we will ask you for a saliva sample. We will give you a "spit kit" to give us around 5ml (1 teaspoon) of saliva. You can do this in a private room at the hospital if you wish.
- Complete a questionnaire about your birth and development history, medical history and history of communication or speech problems
- Have a hearing test if we think you have a hearing problem. We will organize a referral for you to have the hearing test at a community clinic at a separate appointment.

The assessments will be audio and video-recorded. We would also like to obtain information from your speech therapy or medical records. The information we need includes if/when you were diagnosed with speech disorder, the type of speech disorder you have, details of any treatments you have had, and whether you have any other medical disorders. We may also get this information from your speech therapist if you are currently in therapy.

We may ask you to have a Magnetic Resonance Imaging scan (MRI) in order to help us fully understand the nature of your speech disorder, or the speech disorder in your family. We will explain the test to you in detail, and you can decide whether or not you agree to participate in this part of the study. The MRI scan will be conducted at the Brain Research Institute at the Melbourne Brain Centre in Heidelberg. The appointment may take up to 1½ hours in total.

MRI stands for magnetic resonance imaging. A MRI scanner is a machine that uses electromagnetic radiation (radio waves) in a strong magnetic field to take clear pictures of the inside of the body. Electromagnetic radiation is not the same as ionising radiation used, for example, in X-rays. The pictures taken by the machine are called MRI scans.

We will ask you to lie on a table inside the MRI scanner. The scanner will record information about your brain. It is very important that you keep very still during the scanning. When you lie on the table, we will make sure you are in a comfortable position so you can keep still. The scanner is very noisy and we can give you some earphones to reduce the noise. During the scan you will be asked to complete some simple tasks, including looking at a computer screen, listening to single words (e.g., 'born') through headphones and repeating words.

The scan takes approximately 40 minutes in total. The rest of the time will be spent explaining the procedure and providing you with time to ask questions about the MRI scan.

If requested, we can send you a DVD so that you can learn about fMRI and decide whether you would like to take part in the study.

1. What are my alternatives to taking part in this project?
You do not have to take part in this project if you do not want to.

If you take part and change your mind, you can stop at any time without telling us why. If you withdraw from the project we will use any information collected from you unless you tell us not to.

Your decision will not affect any treatment or care you get, or your relationship with The Royal Children's Hospital.

2. What are the possible benefits for me?
We cannot promise that you will get any benefits from this project.

3. What are the benefits to other people in the future?
The real benefit from this project will come in the future. We hope we will be able to provide better information to parents about whether their child who has speech disorder is likely to have ongoing difficulties, or whether the problems are likely to be short-lived. This is important so that we can begin to find out some of the reasons that speech disorders occur and it may help us to work out who will benefit most from early intervention and treatment.

4. What are the possible risks, side-effects and/or discomforts?
Blood tests
There are no major risks associated with a blood test. It is possible you may feel some discomfort during the blood test. You may feel a sting when the needle is put in your arm to take the blood. We can use a cream to numb the skin before the blood is taken. It is possible there may be some bruising, swelling or bleeding where the needle enters the skin. Some people can feel a little light-headed when blood is taken.

Saliva sample
There are no risks or side-effects from a saliva sample. Some people may be embarrassed about spitting into a jar. We will offer you a private space for collection of the sample.

Genetic tests
The genetic tests we perform may tell us something about you or your wider family. This may have an impact on how family members relate to one another. We are only searching for genes that are related to speech disorders, but it is possible that we may find genes responsible for other genetic conditions that you do not know about. If we find that you have any genetic condition that you do not know about, we will contact you to discuss the findings and refer you to a genetic counsellor. Molecular karyotype analysis may provide important diagnostic information regarding your speech disorder.

You may be required to inform insurance companies or employers in the future of any genetic information that you learn about yourself through this project.

By chance, we may discover that parents and children or siblings may not be biologically related. Information regarding paternity or maternity will not be available through this project.

If any new information about possible risks becomes known during the project, we will tell you immediately. If you have any questions with regards to any of this information then please contact us.

MRI risks
There are no proven long-term risks related with MRI scans, as used at The Royal Children's Hospital in this project. MRI is considered a safe procedure when performed at a centre with appropriate guidelines. However, the magnetic attraction for some metal objects can pose a safety risk, so it is important that metal objects are not taken into the scanner room.

We will thoroughly examine you to make sure there is no reason for you not to have the scan. You must tell us if you have metal implanted in your body, such as a pacemaker, or metal pins after being involved in an accident.

The MRI scan could be inconvenient because you must remain very still while in the scanner. There is also a lot of machine noise during scanning.

What happens if something unusual is found in my scans?
The scans we are taking are for research purposes. They are not intended to be used like scans taken for a

full clinical examination. The scans will not be used to help diagnose, treat or manage a particular condition.

A specialist will look at your MRI scans for features relevant to the research project. On rare occasions, the specialist may find an unusual feature that could have a significant risk to your health. If this happens, we will contact you to talk about the findings.

In the unlikely event that we find an unusual feature, it could have consequences for you. It might affect your ability to work in certain professions, or get life or health insurance.

However, if we do find an unusual feature and tell you about it, you may be able to get treatment that might be of benefit.

We cannot guarantee that we will find any/all unusual features.

Please take time to consider the advantages and disadvantages of discovery of a health risk before deciding to take part in this research project.

There may be unforeseen or unknown risks. In the unlikely event that you suffer an injury because of participating in this project, the public health service will provide hospital care and treatment at no cost to you.

1. What are the possible inconveniences?

The only inconvenience is the time to come to the appointment.

2. What will be done to make sure my information is confidential?

Any information we collect for this research project that can identify you will be treated as confidential. We can disclose the information only with your permission, except as required by law.

All information will be stored securely in locked filing cabinets in the Speech Pathology Department at The Royal Children's Hospital and the Murdoch Childrens Research Institute. Your information will also be stored on a password-protected computer database. As part of their normal protocol, a backup copy of your MRI scan will be kept at the Brain Research Institute for five years.

The following people may access information collected as part of this research project:

- the research team involved with this project
- The Royal Children's Hospital Human Research Ethics Committee

The information will be identifiable. This means that your name and/or other personal details will stay on the information. Your DNA sample will be re-identifiable. This means that we will remove your name and give the DNA sample a special code number. Only the research team can match your name to your code number, if it is necessary to do so.

In accordance with relevant Australian and/or Victorian privacy and other relevant laws, you have the right to access and correct the information we collect and store about you. Please contact us if you would like to access your information.

DNA samples collected as part of this study will be stored in a laboratory in the Department of Medicine (Austin Health) located at the Melbourne Brain Centre. We would like to keep your information and DNA sample indefinitely for use in future projects related to this one. If you do not want your information and sample used in future projects, it will be kept for seven years after the research study ends and after this time it will be destroyed.

Your DNA sample may be sent to another research group for genetic analysis. Your sample will be labelled with an identifier code to protect your confidentiality. We may also provide data to the researchers including your year of birth, gender and affected status, but will not send personal details such as your name. After testing is completed, any remaining DNA will be stored by the other research group and may be stored indefinitely. Your sample may be sent overseas, and may be sent to a research group within the National Institutes of Health (NIH) in the United States of America. Samples sent overseas are not covered by Australian laws or regulations. Samples sent to an NIH group may be used by other researchers at hospitals and universities for research in any type of disease – this is a policy of the NIH and any requests for additional clinical or genetic information will need to be approved by a Human Research Ethics Committee.

We are considering the creation of a nation-wide database of patients with speech disorder for genetic research in the future. This future research will also adhere to the strict ethical standards of The Royal Children's Hospital.

When we write or talk about the results of this project, information will be provided in such a way that you cannot be identified. In some cases, we may ask to use a photograph or video-recording of you in research presentations or scientific research articles. The results of this research will be used by the student

researcher Samantha Turner to obtain a PhD degree

1. Will I be informed of the results when the research project is finished?
We will send you a summary of the overall project results. The summary will be of the whole group of participants, not your individual results. We will send a summary report of your assessment results if requested.

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Dr Angela Morgan

Contact telephone: (03) 8341 6458

If you have any concerns about the project or the way it is being conducted, and would like to speak to someone independent of the project, please contact:

Director, Ethics & Research, The Royal Children's Hospital on telephone: (03) 9345 5044.

CONSENT FORM

HREC Project Number: 27053

Research Project Title: Genetics of Speech Disorders

Version Number: 8 **Version Date:** 17/10/2011

- I voluntarily consent to take part in this research project.
- I believe I understand the purpose, extent and possible risks of my involvement in this project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).
- I understand I will receive a copy of this Participant Information Statement and Consent Form.

OPTIONAL CONSENT

☐ I do	☐ I do not	consent to the storage of my blood/tissue sample for use in **future ethically approved** research projects (related to this project/disease)

☐ I do	☐ I do not	consent to my photograph or video recording being used in scientific presentations or research articles

Participant Name	Participant Signature	Date
Name of Witness to Participant's Signature	Witness Signature	Date

I have explained the project to the participant who has signed above, and believe that they understand the purpose, extent and possible risks of their involvement in this project.

Research Team Member Name	Research Team Member Signature	Date

Note: All parties signing the Consent Form must date their own signature.

Appendix 5: Case History Questionnaire

Proband Family Name:

FULL NAME
MAIDEN NAME

ADDRESS

TELEPHONE **Home**
Business
Mobile
Email

Speech Pathologist
Address

Telephone

GP/Paediatrician/Neurologist
Address

Phone

Interviewee	**Relationship**	**Telephone**	**Pen colour**

DIAGNOSIS

DATE OF INTERVIEW:

DOB

AGE

UR number(s)

UR(s) at

SEX **M** **F**

Monoligual
Bilingual

Languages spoken

Handedness: **Right** **Left:**
Ambidexterous

Interviewed by:

Angela Morgan
Samantha Turner
Other

PREGNANCY & PERINATAL PERIOD **Information obtained from:** .....

The Pregnancy

Were there any problems during the pregnancy? | Y | N |

.....

.....

.....

.....

				Gestation				Gestation
During:	**Viral illness/infections**	**Y**	**N**	**weeks**	**Oedema**	**Y**	**N**	**weeks**
	Bleeding	**Y**	**N**	**weeks**	**Hypertension**	**Y**	**N**	**weeks**
	Drugs	**Y**	**N**	**weeks**	**Proteinuria**	**Y**	**N**	**weeks**
	Smoking	**Y**	**N**	**weeks**	**Polyhydramnios**	**Y**	**N**	**weeks**
	Alcohol	**Y**	**N**	**weeks**	**Oligohydramnios**	**Y**	**N**	**weeks**
	Diabetes	**Y**	**N**	**weeks**				

The Birth

Gestation ..... **weeks** **Mother's age at birth** ..... **years**

Place of birth .....

Onset of labour: **Spontaneous** **Medical induction** **Surgical induction**

Presentation **Cephalic** **Breech** **Footling** **Shoulder** **Transverse** **Oblique** **Other:** .....

Type of delivery: **Normal vaginal**
Forceps: **Type** .....
Vacuum extraction
Caesarian section **Elective** **for** .....
Emergency **for** .....

Condition at birth: **Good** **Poor**

Maternal complications after the birth .....

.....

Neonatal period

Birthweight ..... **kg** ..... **lb/oz**

Taken to special care nursery?
Duration ..... **days**

Taken to NICU?
Duration ..... **days**

Problems in first month of life: .....

.....

.....

DEVELOPMENT

Were there any concerns about your development?? Y | N

Were your height and weight normal while you were growing up?

Height	**Y**	**N**
Weight	**Y**	**N**

Motor milestones

Did you-			**If no, what age?**
Hold your head up by 4 months?	**Y**	**N**	
First sit alone by 12 months?	**Y**	**N**	
Crawl by 12 months?	**Y**	**N**	
Eat solid food by 12 months?	**Y**	**N**	
First walk alone by 16 months?	**Y**	**N**	
Fed yourself by 2 years?	**Y**	**N**	
Toilet train by 3 years?	**Y**	**N**	
First use scissors by 3 years?	**Y**	**N**	

Speech milestones:

Did you-			**If no, what age?**
Coo/babble by 4 months?	**Y**	**N**	
Respond to name by 8 months?	**Y**	**N**	
Use jargon by 12 months?	**Y**	**N**	
Imitate sounds by 12 months?	**Y**	**N**	
Say your first word by 15 months?	**Y**	**N**	
Say two words together by 24 months?	**Y**	**N**	
Use short sentences by 36 months?	**Y**	**N**	

Have you ever lost skills or gone backwards in your learning? Y | N

Age? Skills lost?

Have you ever been diagnosed with:

			Age identified
Learning difficulties	**Y**	**N**	
Intellectual disability	**Y**	**N**	
Behavioural problems	**Y**	**N**	
Autism	**Y**	**N**	
Speech/language problems	**Y**	**N**	

Have you ever seen a speech pathologist or had early childhood intervention? Y | N

Who did you see?

How old were you?

What were the concerns?

EDUCATION AND WORK

What level did you reach at school?

Never attended school	**Technical School**
Special School	**University**
Primary School	**Higher graduate Degree or Diploma**
Secondary School	

What level did your primary care giver reach at school? **<Year 11** **Yr 11-12** **tertiary**

Did you need extra help in the classroom eg. Integration aide? Special classes? **Y** **N**

Have you ever been kept back a grade at school? **Y** **N**

What has been your main type of work?

skilled/professional	**full time**
semi-skilled	**part time**
unskilled	**unemployment/pension/money from government**

Are you the primary income earner in your family? **Y** **N**

What work does the primary income earner in your family do?

skilled/professional	**full time**
semi-skilled	**part time**
unskilled	**unemployment/pension/money from government**

PAST MEDICAL HISTORY

Are you on any medications?

.....

.....

Have you ever had -

Otitis media or ear infections?	**Y**	**N**	**Allergies or asthma?**	**Y**	**N**
Frequent colds/sinus infections?	**Y**	**N**	**Respiratory or breathing problems?**	**Y**	**N**
Grommets?	**Y**	**N**	**Seizures/Convulsions?**	**Y**	**N**
Tonsils/adenoids removed?	**Y**	**N**	**Head Injury?**	**Y**	**N**
Hearing or auditory processing problems?	**Y**	**N**			

Investigations

Have you ever had:

EEG	**Y**	**N**
CT/MRI scan	**Y**	**N**
Videofluroscopy	**Y**	**N**
Laryngoscopy	**Y**	**N**

.....

.....

.....

.....

COMMUNICATION AND SWALLOWING

Physical

Do you have a tongue tie, cleft lip/palate or other craniofacial abnormalities?	**Y**	**N**
Do you have difficulty moving your lips, tongue or jaw?	**Y**	**N**
Have you ever had difficulties with gross motor movements ie. kicking a ball, running?	**Y**	**N**
Have you ever had difficulties with fine motor movements ie. writing, using scissors?	**Y**	**N**

Details

Dysphagia

Did you have any feeding problems as a baby?		**Y**	**N**
Have you ever had difficulties with chewing or swallowing food?		**Y**	**N**
Have you ever had any difficulties drinking liquids?		**Y**	**N**
Do you cough, choke or gag during mealtimes?		**Y**	**N**
Have you ever had problems with drooling or controlling saliva?		**Y**	**N**
Do you have a history of -	**Gastro-oesophogeal reflux?**	**Y**	**N**
	Aspiration?	**Y**	**N**
	Tube feeding?	**Y**	**N**
	Poor weight gain?	**Y**	**N**

Details

Speech

Have you ever stuttered?	**Y**	**N**
Have you ever had difficulties making speech sounds?	**Y**	**N**
Have you ever left sounds out of words?	**Y**	**N**
Have you ever substituted sounds in words?	**Y**	**N**
Do you find it hard to say words with several syllables eg. psgetti for spaghetti?	**Y**	**N**
Have people ever had trouble understanding your speech?	**Y**	**N**

Details

Language

Have you ever had trouble understanding instructions or directions?	**Y**	**N**
Have you ever had trouble finding the exact word?	**Y**	**N**
Do you have a poor memory for names of people or objects?	**Y**	**N**
Have you ever had difficulties with reading?	**Y**	**N**
Have you ever had difficulties with writing	**Y**	**N**

Details

Pragmatics

Have you ever had difficulties:-

looking at people when you are having a conversation?	**Y**	**N**
listening to what other people have to say?	**Y**	**N**
starting a conversation?	**Y**	**N**
taking turns in a conversation?	**Y**	**N**
asking or answering questions?	**Y**	**N**
maintaining an appropriate distance between yourself and others when talking?	**Y**	**N**
talking at the right speed (not too fast, not too slow)?	**Y**	**N**
understanding jokes or riddles?	**Y**	**N**
understanding facial expressions or body language?	**Y**	**N**

Details .....

.....

.....

Voice/Pitch

Has your voice ever been described as breathy, rough, strained, nasal, high/low pitched?	**Y**	**N**
Do you speak too loudly or too softly?	**Y**	**N**

Details .....

.....

.....

FAMILY

Marital Status

Single **Defacto** **Divorced**

Married **Widow** **Separated**

Partner

Are you related to your partner in any way other than marriage?

If YES, describe: .....

Children **Name of other parent** **Consanguinity?**

.....	.....	**Y**	**N**
.....	.....	**Y**	**N**
.....	.....	**Y**	**N**

Family structure: **two caregivers** **separated/ dual custody, or cared for by other family** **single caregiver**

Stillbirths/miscarriages?	**Y**	**N**

Number and weeks gestation: .....

FAMILY PEDIGREE

Family history:

Feeding/swallowing problems?	**Y**	**N**	**Speech problems?**	**Y**	**N**
Autism spectrum disorder?	**Y**	**N**	**Language Disorder?**	**Y**	**N**
Learning difficulties/Intellectual disability?	**Y**	**N**	**Reading difficulties?**	**Y**	**N**
Cleft palate or other craniofacial abnormalities?	**Y**	**N**	**Hearing problems?**	**Y**	**N**
Neurological problems/seizures?	**Y**	**N**			

Checklist:
Names
Maiden names
DOB or YOB
Birthplace of ancestors
Consanguinity

Appendix 6: Protocol to examine velopharyngeal insufficiency

Classical triad of signs of submucous cleft palate (reviewed in Moss et al, 1988):

- bifid uvula
- translucent vertical line down the soft palate (zona pellucida)
- v-shaped notch at the junction of the hard and soft palate

Other signs:
Feeding difficulties under 12 months (slowness, nasal regurgitation)
Speech problems with VPI (nasal escape, hypernasal resonance), articulatory errors, history of otitis media

1. **Say 'ahhhh' (as in bat) and protrude the tongue**
    - **look at hard palate, soft palate and velum**
2. **Palpate palate**

Hypernasality, nasal emission and patterns of articulatory errors are indicators of adequacy of velopharyngeal function (Yorkston, 2010). Velopharyngeal insufficiency is often characterized by hypernasality, nasal emission, phonation difficulty, and compensatory misarticulations.

Connected speech, sentences with oral sounds, sentences with nasal phonemes, low pressure sentences, and high pressure phonemic contexts should all be used to assess articulation, resonance and presence of nasal emission (LeDuc, 2008).

3. **Sentence repetition**

- **p/b: Popeye plays baseball.**
- **t/d: Take Teddy to town. Do it for Daddy.**
- **k/g: Give Kate the cake. Go get the wagon.**
- **f/v: Fred has five fish. Drive the van.**
- **s/z: I see the sun in the sky.**
- **sh: She went shopping.**
- **ch: I ride a choo choo train.**
- **j: John told a joke to Jim.**
- **l: Look at the lady.**
- **r: Run down the road. I have a red fire truck.**
- **th: Thank you for the toothbrush.**
- **Blends: splash, sprinkle, street**

Syllable repetition is a fast and easy way to assess articulation, resonance and presence of nasal emission (Kummer, 2011).

1. **/pa pa/**
2. **/pi pi/**
3. **/ka ka/**
4. **/ki ki/**

Tests of hypernasality and hyponasality (Kummer, 2011)

5. **Count 60-70 or repeat the numbers 60 or 66 over and over**
    - **assess hypernasality and nasal air emission**
6. **Count 90-99**
    - **assess production of /n/ in connected speech**

Check for –

- preference for nasal sounds
- lack of high-pressure consonants (plosives, fricatives and affricates) or reduced oral air pressure (speech soft or muffled)?
- sibilants, particularly /s/ - nasal air emission?
- audible nasal emission
- any unusual phonological processes, e.g., backing plosives or fricatives to pharyngeal sounds, backing to glottal stop.
- compensatory articulation productions i.e. pharyngeal or glottal sounds

Conflicting views in the literature regarding use of low-tech assessment of resonance (i.e. mirror test, listening tubes, straws, feeling sides of nose, nasal pinching) (Kummer, 2011; Lass & Pannbacker, 2015).

References

Lass, NJ, Pannbacker, M. (2015). Low-Tech assessment of resonance disorders. Evidence-Based Communication Assessment and Intervention, 9, 43-50.

LeDuc (2008). Cleft palate and/or velopharyngeal dysfunction: assessment and treatment. Perspectives on school-based issues (ASHA), 9, 155-161.

Kummer, AW (2011). Perceptual assessment or resonance and velopharyngeal function. Semin Speech Lang, 32, 159.

Moss ALH, Piggott RW, Jones KJ (1988). Submucous cleft palate. BMJ, 297, 85.

Yorkston, KM (2010). Management of velopharyngeal impairment. In: Management of motor speech disorders in children and adults. ProEd ch.7

Appendix 7: Speech sound errors in G family members with speech disorder

Individual	II-2	II-3	II-6	III-4	III-5	III-6	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15	III-16	III-17	III-18
Age	Adult	Adult	Adult	15y9m	14y	9y7m	17y6m	16y2m	13y11m	12y8m	12y8m	10y5m	8y7m	6y4m	4y5m	4y8m	3y8m
Articulation errors		dental /s/	dental /s/		dental /s, z/	dental /s, z, l/	dental /s, z, l/	dental /s, d, l/		dental /s/, /l/# (at 9y)		derhotacized /r/				lateral /s/#	
		dental /ʃ/					/ʃ/	dental /ʃ/			lateral /ʃ/	/ʃ/			interdental /ʃ/# (at 3y)		
							dental /tʃ, dʒ/	dental /tʃ, dʒ/				/tʃ/	lateral /tʃ/#				
Typical phonological processes -																	
Assimilation																	delayed
FCD													delayed				extensive - atypical
De/voicing										delayed /f/ for /v/# /s/ for /z/#		delayed /s/ for /z/		delayed /k/ for /g/ /ds/ for /dʒ/	delayed /s/ for /z/ /d/ for /t/	delayed /s/ for /z/	delayed /d/ for /t/
Stopping of fricatives													delayed /b/ for /v/	delayed /b/ for /v/ /p/ for /θ/	delayed /b/ for /v/	delayed /b/ for /v/ /p/ for /f/	delayed /b/ for /v/ /p, b/ for /f/
																age app. /d, b/ for /ð/ /p/ for /θ/	age app. /b, t/ for /ʃ/ /d/ for /ð/ /b/ for /θ/
Weak syllable deletion																delayed	age app.

y: years; m: months; # inconsistent error; FCD: final consonant deletion; age app: age appropriate process

table continued over page

Individual	II-2	II-3	II-6	III-4	III-5	III-6	III-8	III-9	III-10	III-11	III-12	III-13	III-14	III-15	III-16	III-17	III-18
Age	Adult	Adult	Adult	15y9m	14y	9y7m	17y6m	16y2m	13y11m	12y8m	12y8m	10y5m	8y7m	6y4m	4y5m	4y8m	3y8m
Fronting	delayed /f/ for /θ/	delayed /f/ for /θ/ /v/ for /ð/					delayed /f/ for /θ/ /v/ for /ð/	delayed /f/ for /θ/	delayed (at 10y) /f/ for /θ/ /v/ for /ð/	delayed /f/ for /θ/	delayed /f/ for /θ/	delayed /m/ for /n, ŋ/ /f/ for /θ/	age app. /f/ for /θ/	delayed /s/ for /ʃ/ Age app. /f/ for /θ/	delayed /d/ for /g/ Age app. /f/ for /θ/	delayed /d/ for /g/ /n/ for /ŋ/ /s/ for /ʃ/ /tz/ for /tʃ/	age app. /d/ for /g, k/ /n/ for /ŋ/
CR													delayed	delayed	age app.		age app.
Deaffrication														delayed /ʃ/ for /tʃ/		age app. /ʃ/ for /tʃ/	
Gliding				delayed								delayed /w/ for /l/ /l/ for /r/	delayed	delayed /w/ for /l, r/	age app. /w/ for /l, r/	age app. /w/ for /r/	age app. /w/ for /l, r/
Atypical phonological processes							/fw/ for consonant clusters (at 4y)							/fw/ for consonant clusters	/fw/ for consonant clusters	/fw/ for consonant clusters	
							/h/ for /f/ (at 4y)							/h/ for /p, k, t, θ/	/h/ for /k, p, t/	/h/ for / k, t/	
												backing (at 7y)	backing (at 5y)	backing		backing	backing
							affrication in blends (at 4y)								/tʃ/ for /ʃ, dʒ/	/s/ for /dʒ/	
																	gliding of fricatives & affricates
															MCD		MCD

y: years; m: months; age app: age appropriate process; CR: cluster reduction; MCD: medial consonant deletion

Appendix 8 – G family case histories

Case history information was taken from a variety of sources. Birth, development and medical history, including the results of any hearing assessments, were taken from hospital or other medical records or reported by the mother. The results of formal speech, language, literacy or cognitive assessments were taken from past speech pathology or psychology reports.

Proband (III-13)

The proband was a 10 year, 5 month old boy with CAS.

He was induced at 41 weeks' gestation after an uneventful pregnancy. He weighed 4175g at birth and was well in the neonatal period. There was no history of feeding difficulties, and he breastfed until 12 months of age. Motor milestones were normal, although he was described as clumsy with fine and gross motor skills into the school years (writing, riding a bike). Speech development was delayed, with no babbling, single words around 3 years and two words together at 3 years, 9 months. There was no history of regression. Hearing was normal at 3½ years. Motor speech planning difficulties were noted at 3 years, 10 months. His speech was frequently unintelligible, he had a reduced consonant inventory (no fricatives apart from glottal fricative /h/; no affricates), reduced syllable shapes, sound substitutions and omissions (17% consonants correct), distorted vowels, atypical phonological processes (eg. initial consonant deletion) and inconsistent word attempts. He had difficulty sequencing two or more sounds and groping was evident on oral motor assessment. He was diagnosed with CAS at 4 years, 4 months by his local speech pathologist. He commenced fortnightly speech therapy around 3 years, which increased to weekly from 4 years of age. He also had integration aide support in the classroom and a modified spelling program, and reportedly made steady gains. Cognitive assessment at 5 years (Wechsler Preschool and Primary Scale of Intelligence, 3rd Edition) revealed borderline verbal intelligence (IQ 74), with average non-verbal intelligence (IQ 100) and processing speed (IQ 86). He was severely delayed in the Communication domain on the Vineland Adaptive Behaviour Scales (standard score 46). Expressive language impairment was first diagnosed at 3 years, 10 months, with scores on the 5th percentile and below on the CELF at 3 years, 5 years, 7 years and 8 years, 11 months. Gains were noted at 11 years and 13 years, with average expressive language scores (CELF-4 Expressive Language

Score 85 at 11;10 years and 80 at 13;4 years). Receptive language impairment was noted at 13 years, 4 months (CELF-4 Receptive Language Score 74), with average receptive language scores (80 or above) on previous assessments.

III-8

III-8 was born at 38 weeks' gestation following a pregnancy complicated by first trimester haemorrhage and cholecystitis. Pain relief medication was given too close to delivery, and she spent 24 hours in the Special Care Nursery with breathing difficulties. She had normal early development (sitting 6 months; first words 8 months; crawling 9 months; walking 12 months). Hearing was reportedly normal. Speech disorder was diagnosed at 4 years, 7 months, with phonetic and phonemic errors identified. She had average receptive and expressive language skills (CELF-Preschool Receptive Language Index score 104, Expressive Language Index score 106). Auditory processing difficulties were reported by her mother II-3, but not formally assessed. She had regular speech therapy for two years. Educational assessment at 6 years, 8 months revealed impaired spelling (South Australian Spelling Test: spelling age 6-6.3 years) and average reading (Neale Analysis of Reading Ability: reading age 6 years, 7 months; reading comprehension age 7 years, 1 month). She had integration aide support in the classroom in years 7 and 8.

III-9

III-9's birth and development were normal. She had a history of recurrent otitis media from birth to 2 years, however hearing assessment at 3 years showed hearing in the normal range bilaterally. Speech disorder was diagnosed around 4 years, and she had weekly to fortnightly speech therapy for one and a half years. Reading difficulties were noted around 8 years of age, but she had no extra support in the classroom. Speech pathology assessment at 10 years, 1 month revealed tongue thrust with distortion of alveolar, postalveolar and affricate phonemes. Orthognathic surgery was recommended for malocclusion of the jaw, and she wore an orthodontic appliance as a teenager.

III-10

III-10's birth and early development were normal. Hearing was normal. He had a history of impaired literacy skills. Phonological awareness difficulties and below grade level reading and spelling were identified at 6 years, 8 months (Brigance Inventory of Basic Skills: Word Recognition 40% Grade 1 level; Spelling 80% Grade 1 level). He participated in a speech pathology literacy program at 8 years, 9 months, and had integration aide support in the classroom for English and maths. Reading and spelling were below age expectations at 11 years, 5 months (Schonell Graded Word Reading and Spelling Test - Reading age 9 years; Spelling age 8.9 years). He had persisting literacy difficulties in his teenage years. His language skills were average at 8 years, 10 months and 11 years, 5 months (CELF-4 Receptive Language score 94 at 8;10 and 94 at 11;5, Expressive Language score 104 at 8;10 and 102 at 11;5). Cognitive assessment at 8 years, 7 months revealed average intelligence (Wechsler Intelligence Scale for Children, 4th Edition Full Scale score 90; Verbal Comprehension 81; Perceptual Reasoning 88). Auditory processing skills were variable, with auditory memory below average at 8 years, 10 months but average at 13 years, 10 months (Test of Auditory Processing Skills (TAPS-3) Auditory Memory standard score 83 at 8;10, 90 at 13;10). Conversely, auditory comprehension and reasoning were average at 8 years, 10 months but below average at 13 years, 10 months (TAPS-3 Auditory Cohesion standard score 100 at 8;10, 73 at 13;10). He had no further assessment for auditory processing disorder.

III-11 and III-12

III-11 and III-12 are identical twins, confirmed by zygosity testing. They were induced at 36 weeks following a pregnancy complicated by severe cholecystitis and twin-to-twin transfusion syndrome. They spent five days in the special care nursery, and III-12 had phototherapy for neonatal jaundice. Speech and motor milestones were normal. Hearing was normal. III-12 was diagnosed with speech disorder at 6 years, 7 months, and had integration aide support in the classroom for one year. III-11 reportedly had no speech, language or literacy difficulties.

Both twins had clinical features of connective tissue disorder Loeys-Dietz syndrome (LDS) type III, including scoliosis, moderate restrictive lung disease, bifid uvula, wide arm span and arachnodactyly with hypermobility of the joints, and were shown to carry

the paternal mutation in the *SMAD3* gene (c.[960_972delinsGACACC]; p[Cys320Trpfs*19]). Orthodontic assessment revealed Class II division 1 dentition with mandibular retrognathia in both twins, with forward resting position and mild tongue thrust in II-18, and anterior open bite in III-12. Neither twin had temporomandibular joint dysfunction.

III-14

III-14's birth and early development were normal. He had mild jaundice in the neonatal period. Hearing was normal. He had not had speech pathology assessment prior to his enrolment in the study at 5 years, 3 months.

III-15

III-15 was induced at 41 weeks following a pregnancy complicated by cholecystitis. He had delayed early development and did not acquire single words until 3 years; gross motor developmental was age appropriate. He had otitis media with effusion and moderate conductive hearing loss in the left ear at 4 years, 5 months. He had weekly to fortnightly speech therapy from 4.5 years. Global developmental delay was diagnosed at 4 years, 9 months. General examination was normal with normal heard circumference and no neurological abnormalities. He had some autistic traits but did not meet diagnostic criteria for ASD. Receptive and expressive language impairment was diagnosed at 4 years, 7 months of age (CELF-P2 Receptive Language Score 60, Expressive Language Score 66). Receptive language improved with performance in the average range at 7 years, 2 months (CELF-4 Receptive Language score 103), however expressive language remained at the 1st percentile (CELF-4 Receptive Language score 65). Cognitive assessment at 5 years, 8 months revealed average non-verbal intelligence (Wechsler Primary and Pre-School Scale of Intelligence, 3rd Edition Performance Index score 95; Verbal Index score 74). He had integration aide support in the classroom from prep and was in the Reading Recovery program at 10 years of age.

III-16

III-16 was induced at 41 weeks following a pregnancy complicated by cholecystitis. Hearing was normal. He had not had speech pathology assessment prior to his

enrolment in the study at 3 years, 5 months. He had speech therapy at 8 years for literacy difficulties.

III-17

III-17 was induced at 41 weeks following a pregnancy complicated by cholecystitis and high blood pressure. Early development and hearing were normal. Speech pathology assessment at 5 years, 8 months revealed severe speech disorder and age appropriate language skills. She had regular speech therapy and integration aide support in the classroom.

III-18

III-18 was born at 41 weeks following a normal pregnancy and augmented normal delivery. An ejection systolic murmur was noted post-natally. He had delayed early development with 10 single words at 2 years, and 2 word combinations around 3 years of age. Speech pathology assessment at 3 years revealed severely delayed speech and language.

II-2

For II-2, the father of the main kindred, details regarding his birth and early development were not available. He was hospitalised with bacterial meningitis at 2 years. He reported a history of speech disorder, and had two years of speech therapy at 9 years. Target sounds included plosives (bilabial /b/; alveolar /t/), fricatives (postalveolar /ʃ/, alveolar /s/, labiodental /f, v/, dental /θ/) and an affricate /tʃ/. He was 'slow' in school and had difficulty with reading and spelling up to 11 years. He was university educated (Bachelor of Economics, Graduate Diploma in Finance) and worked in management.

II-2 had LDS type III and a confirmed frameshift mutation in *SMAD3* (c.[960_972delinsGACACC]; p[Cys320Trpfs*19]). There is a family history of LDS and schizophrenia. II-2's father and a sibling were described as 'slow' in school. He had one nephew who had speech therapy at 5 years for language and literacy difficulties.

II-3

II-3 had mildly delayed early development, with walking at 18 months (single words at 12 months). It was unclear whether she had hearing difficulties as a child, as a perforated eardrum was not identified until late. She had a history of mild speech disorder. She had 6-12 months of elocution lessons at 8 years, targeting velar plosive /g/, alveolar and lateral approximants /r/ and /l/), alveolar and dental fricatives /s/, /z/ and /θ/ and affricate /ʧ/. She wore an orthodontic appliance as a teenager. As an adult, she avoided large or unfamiliar words she found difficult to say.

II-1

II-1 had normal early development. She attended elocution lessons with her sister II-3, but had no history of speech difficulties.

Her children III-1, III-2 and III-6 had cognitive impairment and literacy difficulties. III-1 also had a diagnosis of Asperger syndrome, now known as ASD. III-4, III-5 and III-7 were reportedly unaffected.

II-4

II-4 had delayed early speech development. His mother reported that he had reading and spelling difficulties around 8-9 years. He had difficulty writing words and required 1:1 help with reading.

II-4 reported that he and his children had no speech, language or reading difficulties.

II-6

II-6 had normal early development and no history of speech disorder. She reportedly had night terrors as a toddler, which persisted until approximately 10 years of age.

II-6 reported that six of her 13 children had speech, language or literacy difficulties. III-7 and III-34 had impaired literacy skills, whilst III-33, III-35, III-36 and III-37 had impaired speech and language. Only III-36 had seen a speech pathologist. III-7 also had late speech development and a history of febrile seizures.

I-2

I-2 had elocution lessons for a mild speech disorder at around 8-9 years. Target sounds were labiodental fricatives /v/ and /f/. She reported having difficulty pronouncing some words as an adult. She did not have early literacy difficulties, and English was her strongest subject. She studied nursing following her secondary schooling

There was a history of epilepsy, learning difficulties and autism spectrum disorder in her extended family.