(a) Title: Language development, hearing loss and intracranial hypertension in children with *TWIST1*-confirmed Saethre-Chotzen syndrome: a longitudinal retrospective review of patients in the Oxford Craniofacial Unit.

(b) Authors (and affiliations):
Kilcoyne, Sarah[1]
B.SpPath(Hons); B.Laws(Hons);Grad Dip Leg Prac; M.Laws (Health); MRCSLT

Luscombe, Carrie[1]
BSc; MRCSLT

Scully, Paula[2]
BSc (Hons); MSc; Clinical Scientist (Audiology)

Jayamohan, Jayaratnam[1]
MBBSm BSc, FRCS (SN)

Magdum, Shailendra[1]
FRCS

Wall, Steven[1]
FCS(SA) plast.

Johnson, David[1]
MA BM BCh DM FRCS (Plast)

Wilkie, Andrew O. M.[1,3]
DM FRCP

[1] Oxford Craniofacial Unit, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, OX3 9DU, UK
[2] Department of Audiology, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, OX3 9DU, UK
[3] MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK

(c) Corresponding author:
Sarah Kilcoyne,
Oxford Craniofacial Unit,
LG1 West Wing,
John Radcliffe Hospital,
Oxford, United Kingdom, OX39DU
01865234041
sarah.kilcoyne@ouh.nhs.uk

(d) Acknowledgements: Shahida Kiani, Sarah Overton, Dr Patrick Kennedy-Williams and Dr Ginette Phippen.

Abstract

Saethre-Chotzen syndrome (SCS) is an autosomal dominant condition defined by mutations affecting the *TWIST1* gene on chromosome 7p21.1. Previous research has identified an elevated prevalence of intracranial hypertension and hearing impairment associated with this syndrome. This study aimed to investigate the influence of hearing history and presence of intracranial hypertension on language development in children with SCS.

A retrospective case note analysis was performed for all patients with a confirmed *TWIST1* gene abnormality who attended the Oxford Craniofacial Unit and underwent a language assessment over a twenty-two-year period. Intracranial pressure monitoring, hearing status and language outcomes were examined in detail.

Thirty patients with genetically confirmed SCS and language assessment data were identified. Twenty-eight patients underwent surgical intervention; ten presented with intracranial hypertension (five prior to, and five after primary surgical intervention). Language data coinciding with the presentation of intracranial hypertension were available for eight children. 44% of children with intracranial hypertension presented with concurrent receptive and expressive language delay (n=4/8). For both children (n=2) with longitudinal language data available, the onset of intracranial hypertension reflected a concurrent decline in language skills. Audiometric data were available for 25 children, 80% (n=20/25) had a history of hearing loss. 50% of these had confirmed conductive hearing loss with middle ear effusion and the other 50% had presumed conductive hearing loss with middle ear effusion. 100% of the children with available hearing data in our study had evidence of middle ear effusion in at least one ear. Results also indicated that 43% (n=13/30) of the children presented with receptive and/or expressive language delay during childhood.

Given the importance of hearing for language development and the preliminary findings of a potential decline in language skills in children during periods of intracranial hypertension, regular follow up of hearing, language and intracranial hypertension is indicated in children with SCS.

Key Words: Saethre-Chotzen syndrome, *TWIST1*, hearing loss, language.

Introduction

Saethre-Chotzen syndrome (SCS) is an autosomal dominant condition defined by a genetic mutation or deletion affecting the *TWIST1* gene on chromosome 7p21.1.[1,2,3,4] The clinical presentation of individuals with SCS commonly includes unicoronal or bicoronal synostosis, a low set frontal hairline, hypertelorism, blocked naso-lacrimal ducts, ptosis and small ears with prominent helical crura.[5,6,7,8] Patients also often present with other variable facial and limb anomalies (including soft tissue syndactyly and broad great toes).[3,6,9,10] Patients may also present with a cleft palate.[11] SCS has an estimated prevalence of ~1:50,000 live births.[12]

Previous research has identified an increased rate of secondary calvarial surgery required for reasons of recurrent intracranial hypertension in children with SCS.[3] If untreated, intracranial hypertension may result in neurological impairment including visual loss, developmental delay or seizures,[3] all of which may have implications for a child's language development.[13,14]

Adequate hearing is also vital for the acquisition of oral language. Chotzen first identified the presence of hearing impairment in three individuals with SCS in 1932.[15] Subsequent investigations have also reported hearing loss in patients with SCS;[6,16,17,18,19,20,21] although many of these studies have small sample sizes. Paznekas et al. (1998) identified the presence of hearing loss in (n=4/32) patients, however did not distinguish the type of loss.[6] In contrast, Rosen et al. (2011) found that the majority of their patients with SCS experienced hearing loss (reporting conductive, mixed and sensorineural hearing loss) at some point during childhood (n=17/29). However, this study included children with both genetically-confirmed *TWIST1* mutation and those with clinical features of SCS.[8] Due to the similar presentation of children with Muenke Syndrome and SCS,[22] this may mean their sample inadvertently contained children with Muenke syndrome (FGFR3 Pro250Arg). This is important because children with Muenke syndrome are known to have high prevalence of low-frequency sensorineural hearing loss.[22,23] The phenotype associated with mutations in *TCF12* may also resemble SCS clinically.[24,25]

Cohen and MacLean (2000) reported that intelligence is usually in the normal range for children with SCS (in the absence of other malformations).[26] Gallagher et al. (2003) noted that patients with SCS may present with mild-to-moderate developmental delay and intellectual disability.[27] Da Costa et al. (2006) reviewed the development of five children with Saethre-Chotzen, and identified an average full scale IQ of 86 (range 49-104). Genetic changes in individuals with SCS are most commonly intragenic. Although learning differences may be noted in persons with intragenic pathogenic variants, severe delay or intellectual disability is not typical. Contrastingly, individuals with a microdeletion in 7p21.1 usually show significant learning deficits. Johnson et al. (1998) identified that significant learning difficulties were present in three patients with SCS who had megabase-sized deletions (>3 Mb) of 7p21.1, detailing that one patient did not speak until aged three years.[4] De Heer et al. (2005) identified that intellectual impairment is rare in patients with SCS, but found a much higher rate of intellectual impairment in individuals with *TWIST1* deletions than in those with a *TWIST1* intragenic

mutation.[7] Whilst some authors have alluded to the presence of speech and language delay in patients with SCS,[4,7] there is no comprehensive evaluation of language outcomes for this population in the literature.

Previous research into individuals with SCS has reinforced the importance of considering genetics, hearing and intracranial hypertension, all of which may have implications for a child's language development. However, to our knowledge this is the first study to focus specifically on how these factors combine to impact on language development in children with SCS.

Materials and Methods

A retrospective casenote review was undertaken of all patients with a diagnosis of Saethre-Chotzen syndrome and a confirmed *TWIST1* mutation attending the Oxford Craniofacial Unit during a 22-year period from 1995 to 2017.

Speech and Language data were collected as a part of the United Kingdom National Craniofacial Unit Speech and Language Assessment routine protocol. Assessments were conducted at ages 3-4 years, 6-7 years and 10 years. The Oxford Craniofacial Unit also conducts pre-operative assessments in addition to the National Protocol. Speech was assessed using a combination of the *Great Ormond Street Speech Assessment* (GOS.SP.ASS'98)[28] and informal clinical assessment. Receptive and expressive language were assessed using formal and standardized assessments appropriate for the child's age, including the *Receptive Expressive Emergent Language assessment (REEL-3),*[29] *Preschool Language Scales – third edition (PLS-3),*[30] *Clinical Evaluation of Language Fundamentals Preschool (UK) – second edition (CELF-P2),*[31] *Clinical Evaluation of Language Fundamentals (UK) – third edition (CELF-3),*[32] and the *Clinical Evaluation of Language Fundamentals (UK) – fourth edition (CELF-4).*[33]

Criteria for Language Difficulties:

The criteria for language difficulties were defined as a standard score of more than 1 SD below the mean score on one or more of the above standardized-measures, or a scaled score of 7 or less (equating to -1 SD+) on one or more subtests of one of the standardized measures.

Criteria for intracranial hypertension

The criteria for intracranial hypertension were in accordance with Wiegand & Richards' (2007) specification of a baseline average above 15 mm Hg or more than 3 B-waves in a 24-hour period.[34] Monitoring was conducted over a 24- to 48-hour period using an intraparenchymal Codman microsensor (Codman Microsensor, Johnson & Johnson Professional Inc.) placed in the right frontal lobe under a general anaesthetic. A recent review of 385 cases of intracranial pressure monitoring identified a low complication and morbidity rate relating to the use of this procedure in the craniofacial population.[35]

Criteria for Hearing Impairment

The hearing data were obtained from audiological assessments typically undertaken at the time of a child's pre-operative assessment. Where possible the average hearing loss (HL) was calculated from the hearing thresholds using Play or Pure Tone Audiometry and minimal response levels using Visual

Reinforcement Audiometry or Distraction Testing at 500, 1000, 2000 and 4000 Hz.

Abnormal test results were defined in accordance with the British Society of Audiology guidelines: 21-40 dB HL = mild; 41-70 dB HL = moderate; 71-95 dB HL = severe; >95 dB HL = profound[36]. For the majority of cases, minimum hearing levels obtained using Soundfield Audiometry used a screening level of 25 dB HL. Due to changes in departmental protocol over time, older cases were screened to a minimum level of 30 dB HL. For these cases, hearing levels of 30 dB HL were classified as satisfactory.

For the cases in whom bone conduction information was available hearing losses with bone conduction levels of <=20 dB HL indicate conductive hearing loss. Bone conduction levels of >20 dB HL with an air bone gap of >5 dB HL indicate mixed hearing loss and hearing losses with an air bone gap of ≤ 5 dB HL indicate sensorineural hearing losses.

Results of Tympanometry were analysed to determine the compliance of the tympanic membrane. Results were classified in accordance with Jerger's (1970) definitions of Type A, B or C tympanograms. Type A is normal. Type B is flat and correlates with either middle ear effusion, occlusion of the external auditory canal with wax, perforation of the tympanic membrane or presence of a grommet which can be differentiated by the ear canal volumes.[37,38] A Type C tympanogram usually indicates eustachian tube dysfunction or mastoid abnormality.

In the absence of bone conduction information, hearing losses with Type B tympanograms were classified as 'presumed conductive hearing losses associated with middle ear effusion' and hearing losses with Type A tympanograms were considered 'presumed sensorineural hearing losses'.

Results

Genetic characteristics

Forty-one patients with genetically-confirmed SCS were identified from a comprehensive search of the Oxford Craniofacial Unit database. Of these, 30 patients (15 male, 15 female) had language assessment data. The remaining patients received treatment prior to the formal speech and language assessment protocol being instituted, or missed their protocol assessment appointments. All patients had a confirmed heterozygous intragenic mutation (n=26) or complete deletion (n=4) in the *TWIST1* gene. Of these 11 arose *de novo* and the remainder were familial (Table 1).

Craniosynostosis

In keeping with the phenotype of SCS, the majority of patients had coronal synostosis. Our sample comprised five patients with left unicoronal synostosis, three with right unicoronal synostosis, 17 with bicoronal synostosis, one with sagittal synostosis, one with multi-suture synostosis and one with pansynostosis. Two patients had no synostosis (Table 1).

Primary and Secondary Surgical Interventions
In respect of surgical management, 14 patients underwent fronto-orbital advancement and remodeling (FOAR), six underwent posterior release then subsequent FOAR, two underwent subtotal calvarial remodeling, one underwent posterior distraction, one underwent posterior distraction and FOAR, one underwent posterior release followed by calvarial expansion and frontal release. The two patients with no synostosis did not require surgery. Five patients required calvarial expansion or remodeling following their primary procedure because of recurrent intracranial hypertension (Table 1).

Hearing
Twenty-five patients had both tympanometry and audiometry data, one patient had tympanometry only. Assessment information was drawn from Pure Tone Audiometry (n=2), Play Audiometry (n=2), Soundfield Visual Reinforcement Audiometry (n=16), Soundfield Performance Audiometry (n=1) and Distraction testing (n=4).Of the 25 patients who we had hearing assessment data for, 80% (n=20/25) had a degree of hearing loss in at least one ear. 40% (n=10/25) had a proven conductive hearing loss. The remaining 40% had (n=10/25) presumed conductive hearing loss (with a hearing loss in the presence of middle ear effusion, but with the absence of bone conduction information). 20% (n=5/25) had satisfactory hearing. None of the children were found to have a sensorineural or mixed hearing loss. However, as bone conduction testing was not completed in 50% of cases, sensorineural hearing loss cannot be ruled out.

Out of the 20 children with hearing loss, ten had a mild hearing loss and ten had a moderate hearing loss. 16 patients had a low frequency sloping hearing loss. Tympanometry data showed that 22 patients had a Type B pattern bilaterally and four had a unilateral Type B tympanogram. All the Type B Tympanograms had ear canal volumes suggesting presence of middle ear effusion. Review of patient notes indicated that 22 children (including 4 of whom we were unable to obtain formal hearing assessment data) had ongoing hearing concerns including the need for grommets (n=4), hearing aids (n=8), preferential seating in the classroom and/or support from specialist hearing impairment teachers (n=4), ongoing monitoring by audiologists/ENT (n=3) and long-term parental concern regarding hearing difficulties (n=8) (Table 2).

Language
Language assessment data were available for 30 children, with longitudinal data available for 11 children. Results indicated that 43% (n=13/30) of the children presented with receptive and/or expressive language difficulties at one assessment point in childhood (Figure1).

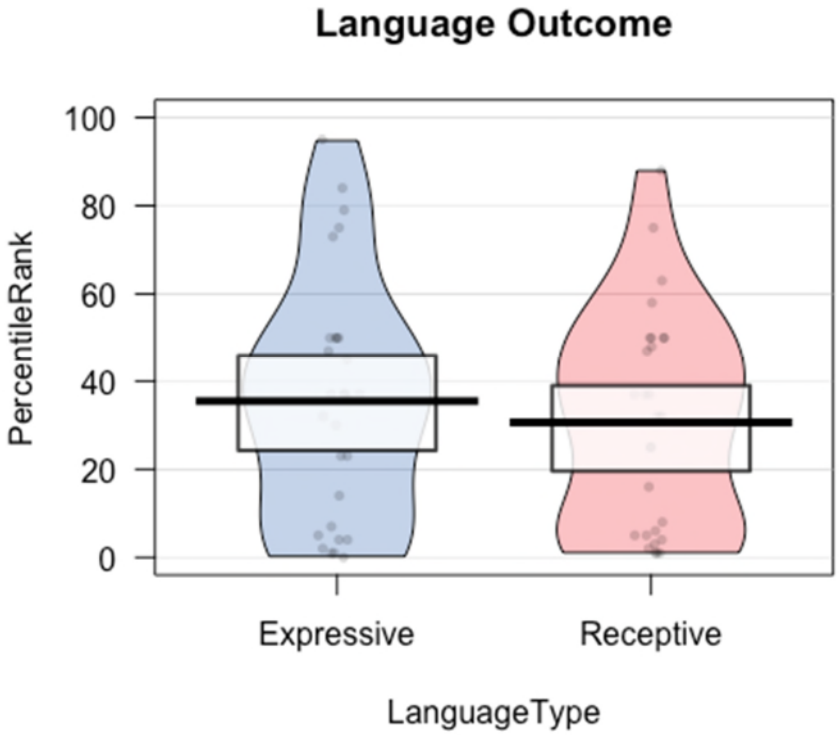

Figure 1. Receptive and Language Outcomes.

When language results were considered according to genotype, we found that 75% (n=3/4) patients with whole gene deletions presented with language difficulties on formal, standardized assessment. The remaining patient (Patient 28) with a whole gene deletion presented with appropriate language development when assessed at five months of age; although she is yet to reach the age of her next assessment according to the national assessment protocol, parental report indicates she has been receiving speech and language therapy support in the community. When language results were analysed for patients without whole gene deletions, 34% (n=10/26) of the children presented with receptive and/or expressive language delay at one assessment point in childhood. Hence the majority of children had age-appropriate language development (Table 2).

Statistical analysis of the relationship between hearing impairment, language, synostosis type and gender was not possible because there were too few patients who had normal hearing.

Cleft Palate

Patient 1 (who presented with a whole gene deletion) has a history of a submucous cleft palate, repaired prior to formal language assessment. This patient did present with significant language difficulties, however formal speech assessment conducted by a Cleft Palate Specialist Speech and Language Therapist identified appropriate speech sound production, therefore Patient one's expressive language difficulties could not be explained by the presence of cleft-type speech characteristics. No other SCS patients in our series had a cleft palate.

Intracranial Hypertension and Language Development

Ten children presented with intracranial hypertension on intracranial pressure monitoring. Five children presented with intracranial hypertension prior to primary surgical intervention. Five children presented with intracranial hypertension after their primary surgical procedure (Table 4).

Language data coinciding with the presentation of intracranial hypertension were available for 8/10 children; four children presented with normal receptive and expressive language and four presented with receptive and expressive language difficulties, which persisted in subsequent language assessments. For the remaining child (Patient 1), language data were available for the year prior to their pressure monitoring procedure, which indicated receptive and expressive language difficulties. This child had a large whole gene deletion (3.42-5.49 Mb).

Baseline language data (pre-dating the presentation of intracranial hypertension) were available for two of the children who presented with language difficulties. When the results of their language assessment were examined longitudinally, a decline in language skills that corresponded with the onset of intracranial hypertension was observed in each case. Both patients had previously presented with appropriate language development, until the onset of intracranial hypertension. Both patients then presented with expressive and receptive language difficulties on formal assessment, which coincided with the onset of intracranial hypertension (Figure 2). Both children also had a history of conductive hearing loss which had remained stable on audiological examination.

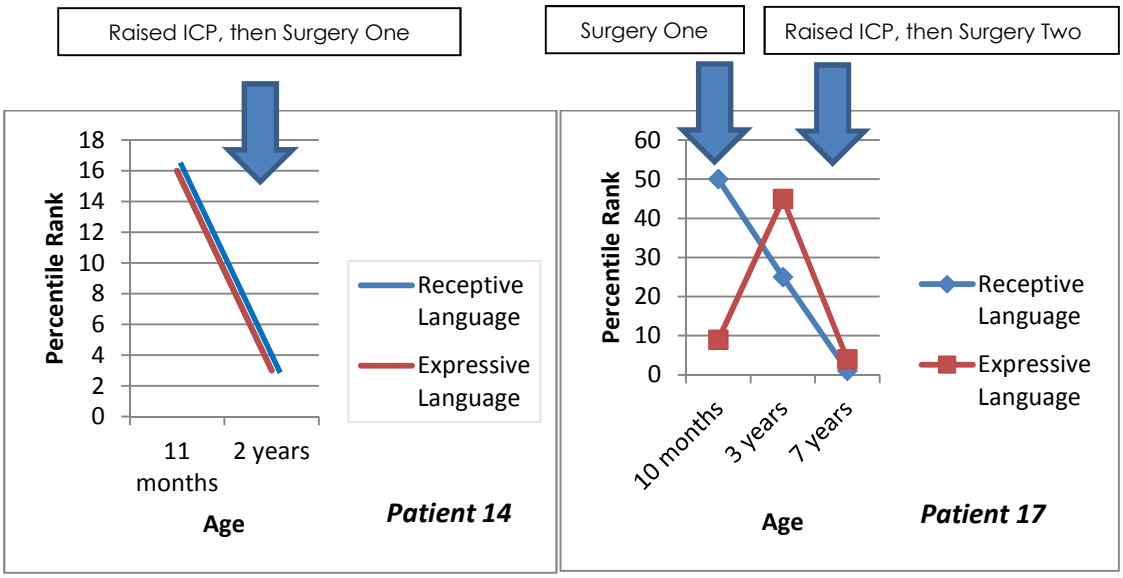

Figure 2. Longitudinal Language, Intracranial Pressure Results and Surgery – Patient 14 and Patient 17

Genetics and Language Development

Johnson et al. (1998) highlighted that children with megabase-sized deletions presented with a developmental delay.[4] Here, we provide additional detail about

the language profiles of children with whole gene deletions. In accordance with Johnson's (1998) findings, all children presented with language delays on formal, standardized assessment, with the exception of Patient 28 (who is yet to have her three year old assessment in accordance with the protocol)[4] (Table 3).

Patient Number	Age of Assessment	Language		Size of 7p Deletion (kb)
		Receptive	Expressive	
1	7 years 11 months	Severe delay	Severe delay	3.42-5.49Mb
12	2 years	Moderate delay	Moderate Delay	1.29Mb
	6 years 9 months	Moderate delay	Moderate delay	
27	5 years 9 months	Severe delay	Severe delay	2.91 Mb
28	5 months	Age-appropriate Skills *	Age-appropriate Skills*	0.41 Mb

**Evidence of delay at 3 years, awaiting formal assessment*

Table 3. Language assessment results of children with whole gene deletions.

Discussion

This is the first study to investigate language and hearing outcomes in children with genetically-confirmed SCS in relation to the presence of intracranial hypertension.

Our finding that 80% of children had a hearing loss in the presence of middle ear effusion (Table 2) corresponds with elevated prevalence of abnormal hearing in children with SCS reported elsewhere.[8] 100% of the children with available hearing data in our study had evidence of middle ear effusion in at least one ear whereas the prevalence of middle ear effusion in children in the general population is reported to be 20% at around 2 years old and this reduces to 8% by the age of 7-8 years.[39, 40, 41, 42] A point of difference in the present study, compared with that by Rosen et al. (2014)[8] is that only children with genetically-confirmed SCS were included, avoiding the possibility that children with Muenke syndrome or *TCF12*-related craniosynostosis were incorporated into our sample.

Conductive hearing loss caused by middle ear effusion impacts the low frequencies most commonly. This is consistent with 16 patients in our sample having a low frequency sloping conductive hearing loss. Due to the fluctuating nature of conductive hearing loss and the indicators of middle ear effusion in all patients in our cohort, it is likely that the children, who presented with normal hearing on the day of assessment, also had a history of temporary conductive hearing loss. As none of the children had a sensorineural or mixed hearing loss

our study supports the theory that children with SCS are most likely to have a temporary conductive hearing loss associated with middle ear effusion.

It is important to consider the rate of language impairment in our sample (46%) (Table 3) in the context of the prevalence of language impairment in the normal population, which is 6.5%.[43] Whilst most children presented with appropriate language development, results indicate a higher rate of language difficulties in children with SCS when compared to the general population. In addition, in the Oxford Craniofacial Unit, language assessments are undertaken in a quiet environment with the child sat in close proximity to the Speech and Language Therapist. Classrooms are typically noisy environments and therefore these language assessments may not be representative of the child's functional skills in the classroom environment.

Stoler et al (2009) investigated the frequency of palatal anomalies in 51 patients with SCS and identified that a cleft palate was observed in 6% of their sample.[11] Only one patient in our sample had a cleft palate, which was associated with a whole gene deletion.

In respect of analysis of language results according to genotype, 3/4 patients with whole gene deletions presented with language difficulties. Importantly, the risk for developmental delay in individuals with deletions involving *TWIST1* is approximately 90%, or eightfold, greater than in individuals with intragenic pathogenic variants.[44] Individuals with a *TWIST1* deletion and normal development have been reported;[7,45] however, developmental attainment likely depends on the size of the deletion. Johnson et al. (1998) noted that clinical examination of patients with SCS indicated similar dysmorphic presentation of patients with a deletion to those with an intragenic mutation.[4] However, the patients with large deletions had significant learning difficulties, because the deletion of neighbouring genes that is responsible for developmental delay. This indicates the importance of understanding the genotype of patients to facilitate appropriate management.

Woods et al. (2009) noted the increased risk of intracranial hypertension in patients with SCS.[3] We considered language development alongside intracranial hypertension and identified a decline in language skills corresponding with the onset of raised pressure. Whilst no firm conclusions can be drawn from this small number of participants, research has identified that it is unusual for a child's language skills to decline as they develop without the presence of neurobiological comorbidity (e.g. autism) or neurological morbidity (e.g. epilepsy). Ukommune et al. (2011) conducted a population study of 1113 children in Victoria, Australia, and identified that for the majority of children, their language development category is fairly well-defined and remains stable (entropy statistic 0.84).[46] An investigation of the clinical characteristics of language regression in children identified that the mean age of language regression was 21.2 months,[47] suggesting that the decline in language skills of Patient 13 at 7 years and 11 months of age was unusual.

To date no research has demonstrated a convincing relationship between the presence and severity of intracranial hypertension and delayed

development.[48,49,50,51] The finding here of language regression in the context of intracranial hypertension, is not conclusive and is constrained by the small sample and limited longitudinal data. Further investigation into the longitudinal impact of intracranial hypertension on language development is required. Importantly, such a heterogenous and rare group will almost never allow a statistically robust analysis. It will be likely that inference and 'clinical common sense' will always need to be involved in interpreting evidence in this area.

Study numbers were limited by the inclusion only of children who had received a comprehensive language assessment; further restricting a sample size already constrained by the relative rarity of the SCS diagnosis. Limited longitudinal information about children's hearing also meant we were unable to determine whether the conductive hearing loss resolved with age and management. However, parental report, need for support from Teachers of the Deaf, and the use of hearing aids into early adolescence indicates the long-term functional impact of hearing loss for children with SCS. Further investigation into longitudinal outcomes of hearing is warranted. Additionally, limited baseline language data precluded meaningful analysis of the longitudinal implications of intracranial hypertension for language development. However, strengths of this study are that only children with genetically-confirmed SCS were included; despite this strict criterion, it is the largest study of intracranial hypertension, language and hearing in SCS of which we are aware.

Finally, the mild clinical phenotype of some patients with SCS may mean investigation of hearing, speech and language is not routinely undertaken. It is recommended that all patients with SCS have regular hearing assessments to rule out any impact this is having on oral language development and permit timely referral to appropriate early intervention services. Children would also benefit from early speech and language therapy to remediate the presence of any language difficulties. Intracranial hypertension and its possible risk factors for language development should also be routinely considered.

Conclusion

Patients with SCS are at a high risk of conductive hearing loss. Additional analysis suggests increased levels of receptive and expressive language difficulties. Whilst previous research has highlighted the need for regular audiological assessment of children with SCS, the need for regular speech and language assessments in this patient population have been largely underappreciated. Close monitoring for intracranial pressure is also indicated. Further investigation into the impact of the onset of intracranial hypertension on language development is recommended.

Table 1 – Genetic Analysis and Origin, Raised ICP and Surgical Intervention for Patients with *TWIST1*-confirmed SCS

Patient Number	Synostosis				Nucleotide Substitution	Amino_Acid_Substitution/ Size of whole gene deletion	Reference
1	Unicoronal: left	Abnormal (secondary)	FOAR Calvarial expansion by lateral release	*de novo*	Whole gene deletion	3.42-5.49 Mb	Woods et al.[3], case 1
2	Bicoronal		FOAR	*de novo*	c.465C>A	p.Y155*	Woods et al.[3], case 2
3	Bicoronal		FOAR Posterior release Calvarial expansion Frontal release	Familial	c.485_488del [a]	p.V162Afs*68	Woods et al.[3], case 27
4	Bicoronal		FOAR	Familial	c.376G>T	p.E126*	Woods et al.[3], case 6
5	Bicoronal		Occipital release by distraction osteogenesis	*de novo*	c.415C>A	p.P139T	Woods et al.[3], case 7
6	Unicoronal: right		FOAR	Familial	c.452T>G	p.L151R	Novel mutation

7	No synostosis		No surgery	Familial (sibling of case 7)	c.452T>G	p.L151R	Novel mutation
8	Bicoronal	Abnormal (primary)	FOAR	Familial	c.397_417dup21	p.K133_P139dup	El Ghouzzi et al.[1], independent occurence
9	Bicoronal		Posterior release FOAR	Familial	c.405C>G	p.I135M	Woods et al.[3], case 8
10	Bicoronal		FOAR	*de novo*	c.340A>G	p.N114D	Woods et al.[3], Case 30
11	Bicoronal		Posterior calvarial distraction FOAR	*de novo*	c.479_480insG	p.Y160*	Novel mutation
12	Bicoronal	Abnormal (primary)	Posterior release FOAR	*de novo*	Whole gene deletion	1.29 Mb	
13	Unicoronal: right	 Abnormal (secondary)	Posterior release FOAR Partial calvarial remodelling	Familial	c.472T>C	p.F158L	Woods et al.[3], Case 17
14	Bicoronal	 Abnormal (secondary)	FOAR Calvarial expansion	Familial (sibling of case 14)	c.472T>C	p.F158L	Woods et al.[3], Case 18

			FOAR partial calvarial remodelling				
15	Bicoronal	Abnormal (primary)	FOAR	Familial	c.406C>T	p.P136S	Woods et al.[3], Case 19.
16	Bicoronal		FOAR	Familial (relative of case 15)	c.406C>T	p.P136S	
17	Unicoronal: left	Abnormal (secondary)	FOAR Calvarial expansion	Familial (relative of case 15)	c.406C>T [a]	p.P136S	
18	Bicoronal	Abnormal (primary)	FOAR	Familial	c.331delG	p.V111Sfs*14	Woods et al.[3], Case 20
19	Sagittal	Abnormal (primary)	FOAR	*de novo*	c.362C>T	p.T121I	Woods et al.[3], Case 23
20	Unicoronal: left		FOAR	Familial	c.397_417dup21	p.K133_P139dup	El Ghouzzi et al.[1], independent occurrence
21	No synostosis		No surgery	Familial (sibling of case 20)	c.397_417dup21	p.K133_P139dup	
22	Multiple suture		FOAR	*de novo*	c.396_416dup21	p.K133_P139dup	Woods et al.[3], Case 28

23	Unicoronal: left		FOAR	Familial	c.397_417dup21	p.K133_P139dup	
24	Bicoronal		Posterior release FOAR	Familial	c.329_333del	p.R110Hfs*126	Woods et al.[3], Case 29
25	Unicoronal: left		FOAR	Familial	c.397_417dup21	p.K133_P139dup	Woods et al.[3], Case 32
26	Unicoronal: right		FOAR	Familial	c.397_417dup21	p.K133_P139dup	El Ghouzzi et al.[1], independent occurrence
27	Pansynostosis		Calvarial expansion	*de novo*	Whole gene deletion	2.91 Mb	
28	Bicoronal		Posterior distraction	Familial	Whole gene deletion	0.41 Mb	
29	Bicoronal	 Abnormal (secondary)	Posterior distraction FOAR	*de novo*	c.81_82delinsCT	p.Q27_Q28delinsH*	Novel mutation
30	Bicoronal		Posterior distraction FOAR and lateral expansion	*de novo*	c.385_405dup21	p.A129_l135dup	Howard et al.[2], independent occurrence

[a] Mutation identified in affected relative
primary = intracranial hypertension prior to primary surgical procedure; secondary = intracranial hypertension that developed after primary surgical procedure.

Table 2 – Gender, Hearing, Speech and Language Outcomes*

Patient Number	Gender	Age of Language Ax *(years; months)*	Synostosis	Receptive Language	Expressive Language	Hearing severity	Tympanometry Results	Functional/Ongoing Impact of Hearing Loss **
1	M	7;11	Unicoronal: left	Severe delay	Severe delay (not related to submucous cleft palate)	Data not available	Data not available	Age 8: Bilateral hearing aids, hearing support at school.
2	F	6;8	Bicoronal	Moderate delay	Age-appropriate	Mild conductive	Bilateral Type B	Age 8: Grommets had fallen out, considering hearing aids. Parental concern regarding hearing.
3	M	0;5	Bicoronal	Age-appropriate	Age-appropriate	Mild presumed conductive	Right Type C Left Type B	
		1;5		Age-appropriate	Age-appropriate			
4	M	6;5	Bicoronal	Age-appropriate	Age-appropriate	Satisfactory	Bilateral Type B	
5	F	0;7	Bicoronal	Age-appropriate	Age-appropriate	Satisfactory at 30 dB HL	Type B left Type A right	Age 8: Persistent glue ear. Under regular hearing review.
		3;8		Age-appropriate	Age-appropriate	Mild	Bilateral Type B	Age 3: Fluctuating hearing reported.
6	F	3;9	Unicoronal: right	Age-appropriate	Age-appropriate	Satisfactory at 30 dB HL	Bilateral Type B	

7	F	3;10	No synostosis	Mild delay	Age-appropriate	Data not available	Data not available	
8	F	0;11	Bicoronal	Age-appropriate	Age-appropriate	Mild presumed conductive	Bilateral Type B	
		3;11		Age-appropriate	Age-appropriate	-	-	
9	M	6;7	Bicoronal	Significant delay	Age-appropriate	Moderate presumed conductive	Bilateral Type B	Age 7: Considering repeat grommets
		3;1		Significant delay	Moderate delay			
10	M	1;2	Bicoronal	Age-appropriate	Age-appropriate	Mild presumed conductive	Bilateral Type B	Age 2: Monitored by ENT
		3;9		Age-appropriate	Not assessed			
11	M	3;5	Bicoronal	Age-appropriate	Age-appropriate	Moderate presumed conductive	Bilateral Type B	Age 6: Second set of grommets. Age 7: ongoing hearing difficulties reported.
12	F	2;0	Bicoronal	Moderate delay	Moderate delay	Moderate presumed conductive	Bilateral Type B	Age 5: Hearing difficulties resolved.
		6;9		Moderate delay	Age-appropriate	-	-	
13	M	5;9	Unicoronal: right	Age-appropriate	Age-appropriate	Satisfactory right. Mild presumed	Type B left, type C right	

						conductive left		
14	F	0;11	Bicoronal	Age-appropriate	Age-appropriate	Moderate conductive	Bilateral Type B	
		2;1		Delay	Delay	Mild	Bilateral Type B	
15	M	7;0	Bicoronal	Moderate Delay	Severe Delay	Moderate right, Mild left conductive	Bilateral Type B	Age 15: Bilateral hearing aids
		8;10		Not assessed	Severe Delay			
16	M	1;0	Bicoronal	Age-appropriate	Age-appropriate	Mild conductive	Bilateral Type B	Age 6: Hearing aids. Sitting at the front of the class at school.
		3;1		Age-appropriate	Age-appropriate	-	-	
17	F	0;10	Unicoronal: left	Age-appropriate	Moderate Delay	Moderate conductive	Bilateral Type B	Age 8: Bilateral hearing aids. Support from Teacher of the Deaf.
		3;5		Age-appropriate	Age-appropriate	-	-	
		7;2		Moderate-severe delay	Mild Delay	Mild-moderate	Bilateral Type B	
18	F	10;3	Bicoronal	Age-appropriate	Age-appropriate	Data not available	Data not available	Age 12: ENT support for enlarged adenoids

19	M	3;3	Sagittal	Age-appropriate	Age-appropriate	Moderate conductive	Bilateral Type B	Age 13: Bilateral hearing aids. Audiologist noted: very narrow ear canals.
20	F	3;7	Unicoronal: left	Age-appropriate	Age-appropriate	Mild presumed conductive	Bilateral Type B	Age 3: Bilateral grommets
21	M	3;3	No synostosis	Age-appropriate	Age-appropriate	Data not available	Data not available	Age 4: Ongoing glue ear.
22	M	3;5	Multiple suture	Age-appropriate	Age-appropriate	Moderate presumed conductive	Bilateral Type B	Age 14: Hearing reduced when has a cold.
23	M	1;6	Unicoronal: left	Significant delay	Mild delay	Moderate presumed conductive	Bilateral Type B	Age 4: Right-sided hearing aid. Audiologist noted: tiny ears and ECV.
24	F	2;3	Bicoronal	Age-appropriate	Age-appropriate	Moderate conductive	Bilateral Type B	
25	M	3;3	Unicoronal: left	Moderate delay	Mild delay	Mild conductive	Bilateral Type B	Age 9: Hearing aid. Support from school hearing services.
		6;1		Age-appropriate	Not assessed	-	-	
26	F	3;9	Unicoronal: right	Age-appropriate	Age-appropriate	Satisfactory at 30 dB HL	Bilateral Type B	Age 6: Ongoing glue ear.
27	F	5;9	Pansynostosis	Severe delay	Severe Delay	Mild conductive	Bilateral Type B	

28	F	0;5	Bicoronal	Age-appropriate	Age-appropriate	Data not available	Bilateral Type B	Age 3: Grommets had fallen out. Referred for hearing aids.
29	F	1;0	Bicoronal	Age-appropriate	Age-appropriate	Satisfactory	Left Type B. Right Type A	Age 2: Parental concern regarding hearing.
30	M	0;7	Bicoronal	Age-appropriate	Age-appropriate	Mild conductive	Bilateral Type B	Age 3: Reported hyperacusis

**Hearing assessment not always contemporaneous with language assessment.*
*** Based on parental report of assessments conducted elsewhere, or current hearing status/support.*

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