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3D MRI Investigations of the Musculus Uvulae in Children with 22q11.2 Deletion Syndrome

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3D MRI INVESTIGATION OF THE MUSCULUS UVULAE IN CHILDREN WITH 22q11.2 DELETION SYNDROME

By

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ABSTRACT

22q11.2 Deletion Syndrome (22q) is the most common genetic cause for velopharyngeal dysfunction. Several velopharyngeal muscles in children with 22q have been shown to be hypoplastic, but no studies to date have examined the musculus uvulae in children with 22q. This study aimed to investigate the presence and characteristics of the musculus uvulae in children with 22q using3D modeling software MRI scans of 13 children (8 with 22q and 5 control participants) were used to measure the musculus uvulae using Amira 6 visualization modeling software. The muscle was segmented by selecting voxels displaying the musculus uvulae on successive oblique coronal slices and combining those voxels into a surface model. The muscle volume, length, diameter, vertical length, and horizontal width were measured from the surface model of the musculus uvulae. Mann-Whitney U tests were used to compare differences between the two groups. Results revealed the musculus uvulae to be significantly hypoplastic, shorter, and thinner in the group with 22q.The velum was also found to be thinner in the 22q group. Further investigations should be conducted using larger sample sizes to confirm these preliminary results.

ACKNOWLEDGEMENTS

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I am deeply grateful to my thesis chair, Dr. Lakshmi Kollara. This thesis could not have been completed without her dedication and enthusiasm for this project and my success. It cannot be understated the impact her guidance has had on my academic and professional career.

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INTRODUCTION

22q11.2 Deletion Syndrome

22q11.2 deletion syndrome (22q) is a genetic condition characterized by a microdeletion of about 50 genes that are associated with several anatomical and physiological abnormalities and medical conditions (Solot et al., 2019). It is estimated that 1 in 4000-6000 births present with 22q (Digilio et al., 2005; Solot et al., 2019). The conditions include congenital heart defect, palatal and facial abnormalities, T-cell immune deficiencies, among others (Digilio et al., 2005). It should be noted that there are other names for 22q, velo-cardio-facial syndrome and DiGeorge syndrome, and there is also 22q11.2 reduplication (Digilio et al., 2005; Solot et al., 2019). This study is focused on the velopharyngeal (VP) abnormalities found among individuals with 22q.

Velopharyngeal Dysfunction

VPD and other VP abnormalities can be coexisting conditions of broader diagnoses. 22q is the most common genetic cause of velopharyngeal dysfunction (VPD) (McDonald-McGinn et al., 1999, Digilio et al., 2005). VPD is characterized by an incomplete seal of the VP port due to abnormalities in anatomical structures requisite for VP closure (Ruotolo et al., 2006). Common abnormal VP manifestations in 22q include palatal abnormalities, hypoplastic velar and pharyngeal muscles, abnormal pharyngeal depths, platybasia, and adenoid hypoplasia (McDonald-McGinn et al., 1999; Hakvin et al., 2000; Ruotolo, 2006; Kollara et al., 2016). Hypernasality is a frequent co-occurrence in 22q as a result of velopharyngeal dysfunction (Digilio et al., 2005).

Due to vocal tract abnormalities, significant swallowing and speech problems are common in individuals with 22q. Solot et al. (2019) reported that 35-68% of the population with the syndrome display feeding or swallowing disorders. This study also reported 14% of the 22q population had laryngotracheal abnormalities that cause voice disorders (Solot et al., 2019). With respect to voice characteristics, hoarseness and breathiness are common and lead to vocal nodules and even unilateral vocal fold paralysis in this population (Solot et al., 2001; Solot et al., 2019).

Velopharyngeal Closure

VP closure describes the seal of the oral, nasal, and pharyngeal cavities which provides the pressure required for normal speech and swallowing (Witt et al., 2000; Inouye et al., 2015). VP closure requires the muscles of the pharynx and velum to come together to separate the nasal cavity from the oral cavity (Witt et al., 2000; Inouye et al., 2015; Kotlarek and Perry, 2018; Jordan et al., 2017). Under normal conditions, the lateral and posterior pharyngeal walls contract anteriorly while the velar muscles retract the velum posteriorly, meeting together to seal the VP port. The primary velar muscles involved in this action include the levator veli palatini (LVP) muscle and the musculus uvulae. The LVP is the primary elevator and retractor of the velum (Perry et al., 2014; Inouye et al., 2015; Kollara et al., 2016). Inadequate VP closure may result in hypernasal speech.

Previously Studied Velopharyngeal Muscles

Certain velopharyngeal muscles have been studied in this population, specifically the LVP, the superior pharyngeal constrictor (SPC), and the tensor veli palatini (TVP) (Zim et al.,

2003; Ruotolo, 2006; Perry et al., 2013; George et al., 2018; Kollara et al., 2019; Perry et al., 2020). In a non-sedated three-dimensional (3D) magnetic resonance imaging (MRI) study, the LVP muscle was analyzed in 30 children (ages 4-12; 15 with 22q and 15 control subjects). The LVP muscle was found to be 8% shorter and 20% thinner than the LVP of children in the control group (Kollara et al., 2019). The origin-to-origin distance of the LVP was found to be shorter for the 22q group than the control group by 16% in the same study (Kollara et al., 2019). Kollara et al. (2019) also found a significant difference in velar thickness for the 22q group. The velum was found to have a 23% reduction in thickness for the 22q group compared to the control group (Kollara et al., 2019). In two studies measuring the velar length for the population with 22q, no significant differences were found between the 22q group and the control (Ruotolo et al., 2006; Kollara et al., 2019).

Zim et al. (2003) compared the SPC muscle in individuals with and without 22q using MRI technology. 26 participants (18 male and 8 female) with 22q of ages ranging 3-29 years old were compared to an age and sex matched control group. The SPC muscle was found to be significantly thinner in individuals with 22q (mean difference = 0.82mm).

The TVP has been studied using 3D MRI technology in both adults and children. George et al. (2018) studied the TVP in 14 adult participants (6 with repaired cleft palate and 8 with no cleft history). The cleft group consisted of 3 females and 3 males with a mean age of 24 years while the control group consisted of 6 females and 2 males with a mean age of 22 years. The study concluded that the TVP muscle was a significantly hypoplastic (359mm^3 mean difference)

and shorter (3.14 mm mean difference) in the group with repaired cleft palate 358.97 mm^3 (George et al., 2018).

The TVP was also studied in 53 children with and without 22q using 3D MRI. 13 of the children were diagnosed with 22q and had a mean age of 8.3 years with a standard deviation of 2.8 years. 40 of the children had normal VP anatomy and a mean age of 8.4 years with a standard deviation of 0.59 years. None of the children with 22q did not display either an overt cleft palate or a submucous cleft palate. It should be noted that 4 of the children in the 22q group displayed LVP irregularity in the intravelar portion, and another 3 children from the clinical group showed LVP diastasis on the MRI data. The hearing severity index scores were created by audiologic and otologic data. A review of most recent audiologic records were obtained for audiologic data and any degree of hearing loss was recorded. Otoscopic examinations by a physician or nurse practitioner recorded otologic data. The TVP was found to be significantly shorter and less voluminous in those with 22q across all hearing severity ratings. As the hearing severity index score increased, the length and volume of the TVP decreased (Perry et al., 2020).

Musculus Uvulae

Although less discussed in the literature, the musculus uvulae (MU) significantly contributes to VP closure. The MU is made of two bundles originating in the palatal aponeurosis and forms into the uvula which hangs off the end of the soft palate in the oropharyngeal region It is considered a floating muscle because it has no formal insertion point. At rest, the musculus uvulae creates the convex shape of the velum (Azzam and Kuehn, 1977; Kuehn et al., 1988; Perry et al., 2019). Azzam and Kuehn (1977) emphasized the difference between the uvula and

the musculus uvulae so it should be noted that the uvula simply describes the dangling structure in the back of the oral cavity while the musculus uvulae refers to the muscle structure at the end of the velum. There is also a misconception that the muscle originates in the hard palate itself, but it in fact originates slightly posteriorly in the palatal aponeurosis (Boorman et al., 1985; Kuehn et al., 1988).

There are three identified functions of the MU. First, the MU provides bulky tissue to create a firmer seal of the velopharynx during VP closure (Azzam & Kuehn, 1977 Boorman et al., 1985). Additionally, it assists the LVP muscle in retracting and elevating the velum to the posterior pharyngeal wall by stiffening and extending the velum (Kuehn et al., 1988; Inouye et al., 2016). Kuehn et al. found that the MU and LVP coactivate during speech and swallowing (1988.) In a computational study of the musculus uvulae variability, Inouye et al. found that the musculus uvulae can create VP closure without the LVP involved in instances where the LVP muscle is not functioning at full capacity (2016). There is no known data about the musculus uvulae in the 22q population

Submucous Cleft Palate

Submucous cleft palate (SMCP) is a palatal abnormality associated with 22q (Ryan et al., 1997). A cleft that occurs at the level of the palatal muscles beneath the oral mucosa is considered a submucous cleft palate. SMCP can occur in classic and occult forms (Schenk et al., 2021). Classic SMCP is characterized by bifid uvula, presence of a zona pellucida, and notching in the posterior portion of the hard palate Occult SMCP, in contrast, is a cleft in the same location with diastasis in the intravelar portion of the LVP with at least one of the above of the

characteristics of classic SMCP. Secondarily, hypoplastic or absent MU and short palate are characteristics of SMCP (Schenk et al., 2021).

3D MRI

3D MRI is an attractive method for imaging the VP mechanism. This technology allows for observation of the VP complex in its entirety which gives it an advantage over the two-dimensional (2D) slices obtained in traditional MRI, X-Ray, or CT scan (Bae et al., 2011). 3D imaging is particularly beneficial for studying VP closure because the soft tissue muscles can be visualized *in vivo* and the movements of those muscles during swallowing can be observed simultaneously from the initiation of closure to its completion (Witt et al., 2000; Perry et al., 2015). It is important to note that the movement of muscles are only observable in MRI when the participant is not sedated which is a new feature of 3D MRI study of the 22q population (Kollara et al., 2017). MRI is also a less intrusive and easily repeatable means to study the VP anatomy which is clinically relevant when imaging pediatric subjects and those that might otherwise be averse to other imaging techniques like nasendoscopy which can be uncomfortable for pediatric participants (Kao et al., 2008; Perry et al., 2014).

PURPOSE

No studies to date have examined the musculus uvulae in individuals with 22q. The purpose of this study was to 1) determine if MRI can be used to visualize the musculus uvulae in individuals with 22q 2) provide quantitative and qualitative data on the musculus uvulae in individuals with 22q using advanced three-dimensional computer technology. It was hypothesized that the musculus uvulae in the 22q group would be hypoplastic and smaller in all dimensions than the musculus uvulae in the control group.

METHODS

Participant Information

Participants were recruited for this study upon approval of the study protocol from two insitutions (Kollara et al., 2019). 13 participants (8 with 22q and 5 as a control) were included for the study. The mean age for total participants was 9.22years (SD = 1.60). The mean age for the control group was 8.48 years (SD = 1.84) and the mean age for the 22q group was 9.68 years (SD = 1.57). Exclusion criteria for the group with 22q include evidence of an overt cleft palate, a medical history of cleft palate repair or VP surgery, additional genetic diagnoses, no less than six months post adenoidectomy or tonsillectomy. Exclusion criteria for the control group were a history of genetic syndromes, clefting, craniofacial anomalies, or abnormal velopharyngeal anatomy determined by an oral mechanism examination (Kollara et al., 2019). Resonance was rated by two experienced speech language pathologists on a four-point scale (0-3).

Table 1 Participant Demographics

PARTICIPANTS	AGE	SEX	RESONANCE RATING (0=NONE, 1=MILD)	NOTES
C1	10.05	male	None(0)	-
C2	9.10	female	None(0)	-
C3	8.05	female	None(0)	Asymmetric LVP sling
C4	6.08	male	None(0)	none
C5	9.11	female	None(0)	none
S1	10.11	female	None(0)	LVP is higher on the extravelar right than left
S2	9.04	male	None(0)	Disconnected intravelar portion of LVP sling
S3	6.01	male	Mild hypernasality(1)	Velum deviation to the left
S4	8.07	female	Mild hypernasality (1)	-
S5	12	female	None(0)	-
S6	8.09	female	Mild hypernasality(1)	LVP thicker on the right extravelar portion
S7	10.09	female	None(0)	-
S8	8	female	Mild hypernasality(1)	Zona pellucida and notching. Thin LVP with disconnected intravelar portion

MRI Protocol

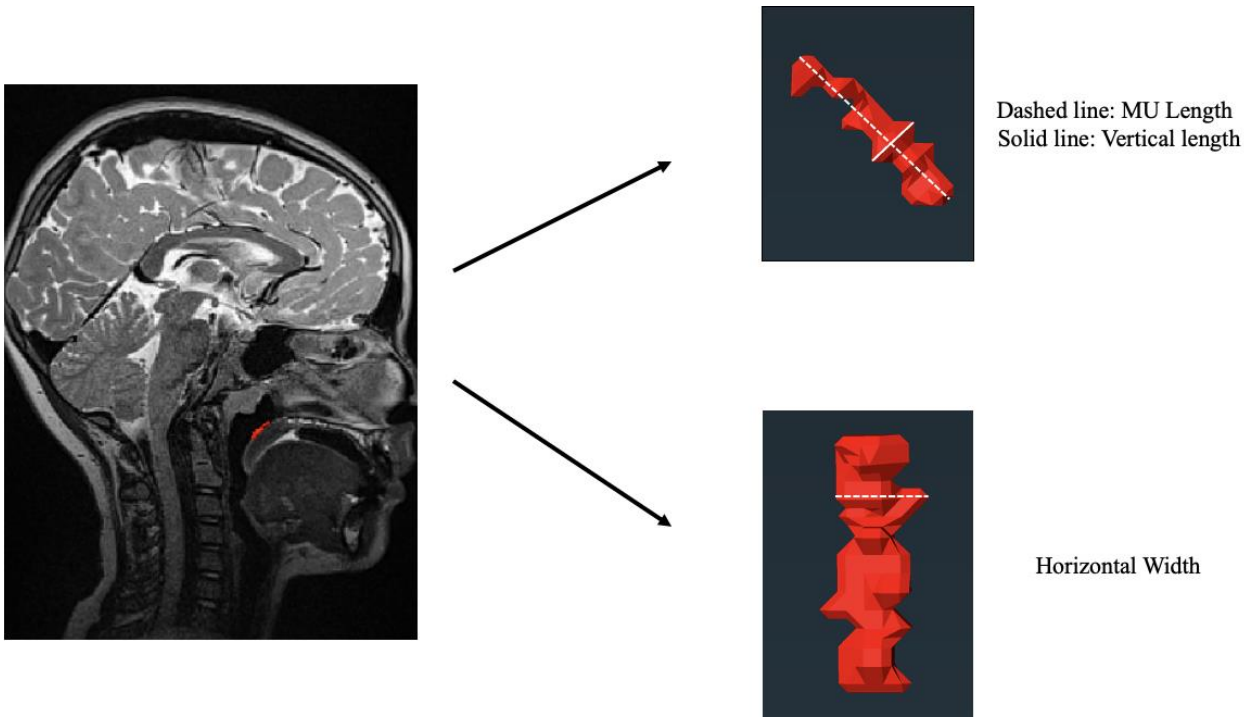
Participants were scanned non-sedated in the supine position, using a Siemens 3 Tesla Trio (Erlangen, Germany) MRI scanner with a 12-channel Siemens Trio head coil. Behavioral modifications were made to ensure the comfort of the participants using an MRI simulator. The MRI simulator presented the sounds and included a motion sensor so the researcher could guide the par to be still. This was an effective way to acclimate the participant and determine whether the participant was able to tolerate the machine protocol. The participants who were able to tolerate the MRI scanner were brought into the real MRI scanner room where they were able to explore the space for 3-5 minutes. A parent/guardian was able to stay in the room for the entire scanning protocol. An investigator communicated to the participant through headphones for the entire scan. In addition, each participant was allowed to listen to their choice in music or watch a pre-selected movie. These modifications are particularly important when scanning participant with 22q because there is increased risk for psychiatric conditions such as claustrophobia and schizophrenia associated with the syndrome (Philip & Bassett, 2011; Kollara et al., 2017). They were instructed to breathe through their nose with their mouth closed. This breathing style ensures the velum is relaxed in a lowered position (Perry, 2019). A velcro strap and cushions were placed around the head above the nasion to minimize motion during the scan. SPACE (Sampling Perfection with Application optimized Contrasts using different flip angle Evolution), which is a high- resolution, T2-weighted turbo-spin-echo three-dimensional anatomical scan, was used to obtain a large view of the oropharyngeal anatomy (25.6 x 19.2 x 15.5 cm) with .08 mm isotropic resolution. The acquisition time is less than five minutes (4 minutes 52 seconds), echo time is 268 milliseconds, and repetition time is 2.5 seconds (Perry et al., 2013).

Amira Software Protocol

The images acquired from the scans were uploaded to Amira 6 Visualization Volume Modeling software (Thermo Fisher Scientific, Waltham, Massachusetts). Voxels on successive oblique coronal slices displaying the musculus uvulae were highlighted using the paintbrush tool. These voxels were combined to create a voxel set and segmented as the musculus uvulae surface model (Perry et al., 2019). Qualitative and quantitative measurements were made using the 3D reconstructions. The quantitative measures included total muscle volume, muscle length, vertical thickness, muscle diameter, and horizontal width. The measures were selected based on comparable studies in the literature. Qualitative variations of the muscle were noted on both sagittal and oblique coronal image planes.

Total muscle volume was measured on the muscle surface model using the volumetric tool. The muscle length was measured from the distance from the most anterior point to the most posterior point on the sagittal view. Vertical thickness of the muscle was measured as the most superior to most inferior point on the sagittal view. Horizontal width of the muscle was measured as the horizontal distance at the greatest width on the oblique coronal/axial view. The muscle diameter was measured as the thickness of the muscle on the transformed oblique coronal MRI scan. The velar thickness was measured on the midsagittal view as the distance from the velar knee to the oral surface of the velum.

Figure 1 Measures on the MU surface model



Statistical Analysis

Descriptive statistics are displayed in Table 2. Mann-Whitney U tests were used to assess the differences between the control and clinical groups due to non-normally distributed data. A p-value of $<.05$ was considered to be statistically significant.

RESULTS

All participants in the control group displayed a musculus uvulae. In 3 out of the 8 children in the 22q group, the musculus uvulae was found to be absent. As such, statistical analysis included only the five participants from the 22q group that had a musculus uvulae. Of the four participants who had mild hypernasality, two displayed a musculus uvulae and two did not.

Qualitative Findings

The musculus uvulae was observed on the oblique coronal view of the MR images to be a dark approximate circle surrounded by light band of fatty tissue above the center of the intravelar portion of the levator veli palatini sling. Preliminary findings show that the musculus uvulae is hypoplastic and smaller in vertical and horizontal dimensions in children with 22q. It is not consistently present across all participants with 22q. When viewing the musculus uvulae in the 22q group, the dark portion was viewed as less massive than in the control group and off the midline above the levator sling. The musculus uvulae in the control group contributed more bulky tissue to the velum than in the 22q group. Three participants in the 22q group had SMCP. Two of the three were occult SMCP with no other SMCP characteristics besides the absence of a musculus uvulae, and one of the three had classic SMCP.

Quantitative Findings

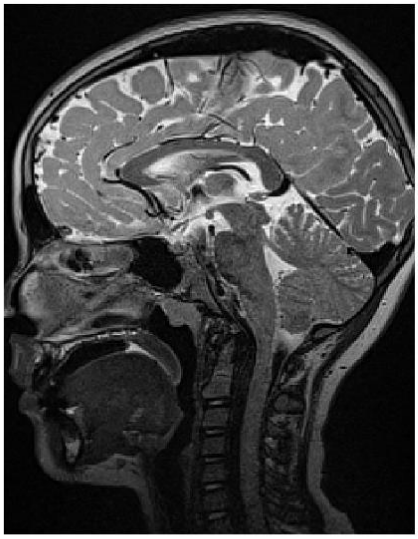
The findings from this study support the hypothesis that the musculus uvulae is visible on MR images. Significant quantitative variations were noted across the control and clinical groups. Significant differences ($p < 0.05$) were noted for volume, length, and thickness of the musculus

uvulae. Specifically, the musculus uvulae was hypoplastic (23.98mm^3 vs. 41.06mm^3), shorter (7.37mm vs. 10.09mm), and thinner (2.43mm vs. 3.02mm) in children with 22q. Velar thickness was also found to be significantly different between groups (6.54mm in 22q group vs. 7.71mm in control group). The decrease in thickness of the velum may in part be due to the hypoplasticity of the musculus uvulae. No statistically significant differences were found in the horizontal width or muscle diameter between the two groups.

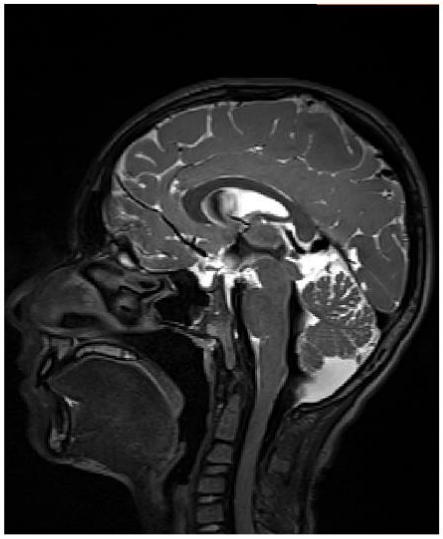
Table 2 Results

Variables	Mean (Standard Deviation)		Significance
	Control	22q	
MU Volume (mm^3)	41.07 (3.8)	23.98 (0.48)	.08 U= 25.0
MU Length (mm)	10.09 (1.3)	7.37 (1.6)	.032 U= 23.0
Vertical thickness (mm)	3.03 (0.28)	2.44 (0.02)	.008 U=25.0
Horizontal Width (mm)	3.25 (0.45)	3.27 (0.12)	1.0 U=12.5
MU diameter (mm)	2.45 (0.33)	2.53 (0.09)	.69 U=10.0
Velar thickness(mm)	7.71 (0.31)	6.55 (0.87)	.032 U=22.5

Figure 2 Midsagittal MRI comparison

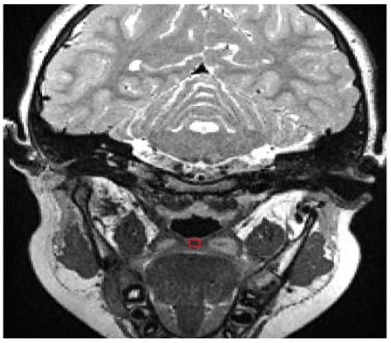


Midsagittal plane of control participant

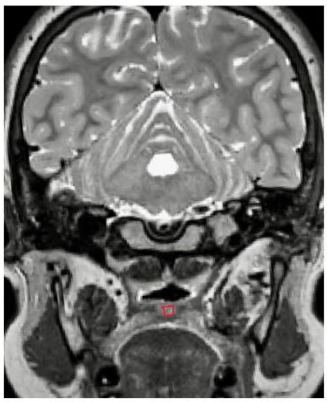


Midsagittal Plane of participant with 22q

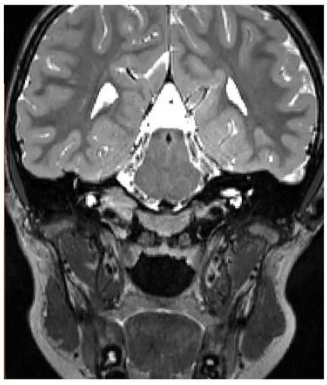
Figure 3 Oblique Coronal MRI Comparison



Oblique coronal view of control participant



Oblique coronal view of control participant with visible MU



Oblique coronal view of participant with 22q and absent MU

DISCUSSION

MRI Feasibility

3D MRI technology was successful in visualizing the musculus uvulae in this study. 3D MRI is the most effective method of visualizing and quantifying VP musculature to date (Perry & Kuehn, 2007; Bae et al., 2011, Perry et al., 2014; Kuehn, 2016; Kollara et al., 2017; Mason & Perry, 2017).

The first method of studying VP muscles was dissection and histology which allowed for thorough examination of the muscles and their make up, but it excluded in vivo study of the structures (Azzam & Kuehn, 1977; Kuehn, 2016). Simple radiologic imaging methods, such as X-Ray and CT scans have been used to study speech anatomy, but this technology primarily displays bony anatomy without delineating soft tissue structures, such as VP musculature (Bae et al., 2011). The most commonly utilized clinical tools for visualizing the VP anatomy are nasendoscopy and multi-view videofluoroscopy. They are, however, unable to visualize underlying muscle such as the musculus uvulae (Perry et al., 2014). MRI allows for the visualization of soft tissue anatomy and underlying muscle tissue as in the LVP and MU (Bae et al., 2011; Kuehn; 2016; Kollara et al., 2017). It is especially preferable for measuring those muscles because it can display the VP mechanism from the oblique coronal view (Bae et al., 2011; Perry et al., 2014; Kollara et al., 2017). MRI is also the only methodology for objectively identifying an absent musculus uvulae (Mason & Perry, 2017). Previous methods of identifying MU dysmorphology included the observation of a flat nasal velar surface on nasopharyngoscopy or a ‘V-shaped’ depression on the nasal surface of the velum on videofluoroscopy (Lewin et al.,

1980; Kuehn et al., 1988; Inouye et al., 2016). 3D MRI allows for definitive identification of musculus uvulae absence as well as objective MU measurements. Perry et al. developed the 3D MRI protocol for evaluating VP anatomy that is used in this study (2014).

Results Compared to the Literature

In this study, the musculus uvulae was analyzed in children with and without 22q on MRI and 3D visualization software. While there are no studies to compare measures taken from children with 22q, there was a study measuring the musculus uvulae in adults with and without cleft palate using the same technology (Perry et al., 2019). Measures from the control group of this study can be compared to the adults studied by Perry et al. (2019). The location and shape of the musculus uvulae on the oblique coronal view of the MRI scans were consistent with the description provided in the Perry et al. study published in 2019. The mean volume of the musculus uvulae in the control group (41.06mm^3) was approximately half of the mean volume of the musculus uvulae in typical adults (97.62mm^3) (Perry et al. 2019). The mean length of the muscle in the control group (10.09mm) was found to be approximately two thirds of the length of the muscle in adults (16.07mm) from that same study. Further investigation should be conducted to study whether there is a growth pattern associated with the musculus uvulae that can correlate to these findings.

Submucous Cleft Palate in Participants

Out of the eight participants in the 22q group, three presented with SMCP. One child had a classic SMCP. Two participants presented with occult SMCP. The child with the classic SMCP was observed to have a zona pellucida and bony notching along the midline of the hard palate

upon oral mechanism examination. On the oblique coronal slice of the MRI data, the child displayed a missing musculus uvulae. The first child with occult SMCP displayed a normal sling on the MRI and an absent MU. The second child with occult SMCP displayed asymmetrical thickness of the extravelar portions of the LVP and an absent MU. The absence of the musculus uvulae in three out of eight the children with 22q is consistent with the findings of Schenk et al. who found that four out the five children with submucous cleft palate lacked a musculus uvulae (2021).

Musculus Uvulae Contribution to VP Closure

Under normal conditions, the MU has three functions including occupying space during VP closure, modifying velar stiffness, and extending the velum during speech and swallowing (Azzam & Kuehn, 1977; Kuehn et al., 1988; Inouye et al., 2016). With abnormal MU anatomy, increased difficulty in VP closure is observed (Inouye et al., 2016). This can be especially exasperated in individuals whose closure pattern is dependent on longitudinal contraction, such as coronal, circular, and circular with a Passavant's ridge (Kummer et al., 2012; Inouye et al., 2016).

In a study of individuals with classic and occult SMCP, Lewin et al. identified that people whose only displayed a hypoplastic or missing musculus uvulae have a 4mm or lessgap in the VP port during phonation which yielded hypernasal speech (1980). Croft et al. (1978) also observed hypernasal speech in subjects with only MU dysmorphology, identified by a midline depression in the velum. VP dysfunction and hypernasal speech was related to missing or hypoplastic MU in the 3D computational model study from Inouye et al. (2016). In this study, there was not a clear correlation between the absence of the MU and increase in hypernasal

speech. This is likely to due to the small sample size of the study. Additional participants with more severe hypernasality scores will be included in following studies.

Velopharyngeal Surgery

Several studies concerning the MU suggest the surgical practice of adding either fat, muscle, or artificial implant for patients with absent or hypoplastic MU to compensate for the lack of bulk in the velar midline which has been found to contribute significantly to VP closure. Kuehn et al. (1988) recommended muscle tissue transfer as opposed to fat or implant because of the important stiffening and extending functions of the MU, not just its space occupying function (1988). Inouye et al. (2016) highlighted that several VP surgeries do not allow for MU reconstruction or the general preservation of longitudinal intrinsic velar muscle which limits the velum's extension such as the Z-plasty procedure. The present study has confirmed the hypoplasia and absence of the MU in the 22q population so surgical considerations should be made in terms of preserving longitudinal muscle and bulk in the velum.

Limitations

The small sample size is a limitation of this study. Given the paucity in the literature concerning musculus uvulae in children and the confirmation from this study that the musculus uvulae in children is visible on this technology, further investigation should be conducted in larger sample sizes to confirm these preliminary results.

CONCLUSION

The MU plays an important role in enhancing the efficacy of VP closure (Sumida et al., 2014). This study provides preliminary data on the musculus uvulae in children with and without 22q. Assessment of the MU and LVP muscle using MRI and 3D technology sheds additional insight on the pathophysiology of VPD in 22q. It is evident that children with 22q exhibit significant variations in their speech muscles, which negatively impacts speech production, Therefore, it is important that we examine the musculus uvulae and characterize its contributions to speech resonance in this clinically challenging population.

APPENDIX A
IRB LETTER OF APPROVAL

UNIVERSITY OF CENTRAL FLORIDA

Institutional Review Board
FWA00000351
IRB00001138, IRB00012110
Office of Research
12201 Research Parkway
Orlando, FL 32826-3246

APPROVAL

April 27, 2021

Dear Lakshmi Kollara Sunil:

On 4/27/2021, the IRB reviewed the following submission:

Type of Review:	Initial Study
Title:	Magnetic Resonance Imaging of the Velopharynx in Children and Adults
Investigator:	Lakshmi Kollara Sunil
IRB ID:	STUDY00002748
Funding:	Name: UCF/College of Health and Public Affairs, Grant Office ID: FP00002913
Grant ID:	FP00002913;
IND, IDE, or HDE:	None
Documents Reviewed:	• HIPAA authorization form_4.5.21.pdf, Category: HIPAA; • Coding document.xlsx, Category: Other; • consent form_adult, Category: Consent Form; • consent form_parent for child, Category: Consent Form; • flyer, Category: Recruitment Materials; • Johnson_support letter_NCH.pdf, Category: Letters of Support; • Kellog_support letter_NCH.pdf, Category: Letters of Support; • MRI screening form.pdf, Category: Other; • questionnaire- Objectives 1 and 2.docx, Category: Survey / Questionnaire; • research protocol , Category: IRB Protocol; • script-phone_email.docx, Category: Other;

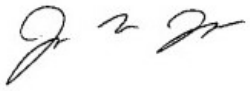
The IRB approved the protocol on 4/27/2021.

In conducting this protocol, you are required to follow the requirements listed in the Investigator Manual (HRP-103), which can be found by navigating to the IRB Library within the IRB system. Guidance on submitting Modifications and a Continuing Review or Administrative Check-in are detailed in the manual. When

you have completed your research, please submit a Study Closure request so that IRB records will be accurate.

If you have any questions, please contact the UCF IRB at 407-823-2901 or irb@ucf.edu. Please include your project title and IRB number in all correspondence with this office.

Sincerely,



Racine Jacques, Ph.D.
Designated Reviewer

REFERENCES

Azzam, N., & Kuehn, D.P. (1977). The Morphology of Musculus Uvulae. *Cleft Palate Journal*,*14*(1), 78–87. https://pubmed.ncbi.nlm.nih.gov/264280/

Bae, Y., Kuehn, D.P., Sutton, B.P., Conway, C.A., & Perry, J.L. (2011). Three-Dimensional Magnetic Resonance Imaging of Velopharyngeal Structures. *Journal of Speech Language and Hear Research*,*54*, 1538– 1545. https://pubs.asha.org

Boorman, J.G., & Sommerlad, B.C. (1985). Musculus uvulae and levator palati: their anatomical and functional relationship in velopharyngeal closure. *British Journal of Plastic Surgery*, *38*(3), 333– 338. https://doi.org/10.1016/0007-1226(85)90237-1

Cohen, J.L., Crowley, T.B., McGinn, D.E., McDougall, C., Unolt, M., Lambert, M.P., Emanuel, B.S., Zackai, E.H., & McDonald-McGinn, D.M. (2018). 22q and two: 22q11.2 deletion syndrome and coexisting conditions. *American Journal of Medical Genetics Part A*, *176*(10), 2203–2214. https://doi.org/10.1002/ajmg.a.40494

Croft C.B., Shpritzen R.J., Daniller, A.I., & Lewin, M.L. (1978). The occult submucous cleft palate and the musculus uvulae. *The Cleft Palate Journal*, *150*(15), 150-154.

Digilio, M.C., Marino, B., Capolino, R., & Dallapiccola, B. (2005). Clinical manifestations of Deletion 22q11.2 syndrome (DiGeorge/Velo- Cardio-Facial syndrome). *Images Pediatric Cardiology*, *7*(2), 23–34. https://pubmed.ncbi.nlm.nih.gov/22368650/

George, T.N., Kotlarek, K.J., Kuehn, D.P., Sutton, B.P., & Perry, J.L. (2018). Differences in the Tensor Veli Palatini Between Adults With and Without Cleft Palate Using High-Resolution 3- Dimensional Magnetic Resonance Imaging. *Cleft Palate Craniofacial* Journal, *55*(5), 697– 705. https://doi.org/10.1177/1055665617752802

Havkin, N., Tatum, S.A., & Shprintzen, R.J. (2000). Velopharyngeal insufficiency and articulation impairment in velo-cardio-facial syndrome: the influence of adenoids on phonemic development. *International Journal Pediatric Otorhinolaryngolgy*, *54*(2–3), 103–110. https://doi.org/10.1016/s0165- 5876(00)00350-5

Inouye, J.M, Perry, J.L., Lin, K.Y., & Blemker, S.S. (2015). A Computational Model Quantifies the Effect of Anatomical Variability on Velopharyngeal Function. *Journal Speech Language Hearing Research*, *58*(4), 1119–1133. https://doi.org/10.1044/2015_jslhr-s-15-0013

Inouye, J.M., Lin, K.Y., Perry, J.L., & Blemker S.S. (2016). Contributions of the Musculus Uvulae to Velopharyngeal Closure Quantified With a 3-Dimensional Multimuscle Computational Model. *Annals Plastic Surgery*., *77*: S70–S75. https://doi.org/10.1097/sap.0000000000000777

Jordan, H.N., Schenck, G.C., Ellis, C., Rangarathnam, B., Fang, X., & Perry, J.L. (2017). Examining Velopharyngeal Closure Patterns Based on Anatomic Variables. *Journal of Craniofacial Surgery*, *28*(1), 270–274. https://doi.org/10.1097/scs.0000000000003284

Kao, D.S., Soltysik, D.A., Hyde, J.S., & Gosain, A.K. (2008). Magnetic Resonance Imaging as an Aid in the Dynamic Assessment of the Velopharyngeal Mechanism in Children. *Plastic Reconstruction Surgery*, *122*(2), 572–577. https://doi.org/10.1097/prs.0b013e31817d54d5

Kollara, L., Perry, J.L., & Hudson, S. (2016). Racial Variations in Velopharyngeal and Craniometric Morphology in Children: An Imaging Study. *Journal of Speech Language & Hearing Research, 59*(1), 27– 38. https://doi.org/10.1044/2015_jslhr-s-14-0236

Kollara, L., Schenck, G., Jaskolka, M., & Perry, J.L. (2017). Examining a New Method to Studying Velopharyngeal Structures in a Child With 22q11.2 Deletion Syndrome. *Journal of Speech Language & Hearing Research, 60*(4), 892–896. https://doi.org/10.1044/2016_jslhr-s-16-0084

Kollara, L., Baylis, A.L., Kirschner, R.E., Bates, D.G., Smith, M., Fang X., & Perry J.L. (2019). Velopharyngeal Structural and Muscle Variations in Children With 22q11.2 Deletion Syndrome: An Unsedated MRI Study. *Cleft Palate Craniofacial Journal*, *56*(9), 1139–1148. https://doi.org/10.1177/1055665619851660

Kotlarek, K.J., & Perry, J.L. (2018). Velopharyngeal Anatomy and Physiology. *Perspective ASHA Special Interest Groups*, *3*(5), 13–23. https://doi.org/10.1044/persp3.sig5.13

Kuehn, D.P. (2016). Five Decades of Research Experience in Speech Anatomy and Physiology. *Perspective ASHA Spec Interest Groups*. *1*(5), 4–12. https://doi.org/10.1044/persp1.sig5.4

Kuehn ,D.P., Folkins, J.W., & Linville, R.N. (1988). An electromyographic study of the musculus uvulae. *Cleft Palate Journal*, *25*(4), 348–355. https://pubmed.ncbi.nlm.nih.gov/3203466/

Kummer, A.W., Clark, S.L., Redle, E.E., Thomsen, L.L., & Billmire, D.A. (2012). Current practice in assessing and reporting speech outcomes of cleft palate and velopharyngeal surgery: A survey of cleft palate/craniofacial professionals. *Cleft Palate-Craniofacial Journal*, *49*(2), 146-152.

Lewin, M.L., Croft, C.B., & Shprintzen, R.J. (1980). Velopharyngeal Insufficiency due to hypoplasia of the musculus uvulae and occult submucous cleft palate. *Plastic Reconstruction Surgery*, *65*(5), 585–591. https://doi.org/10.1097/00006534-198005000-00008

Mason, K., & Perry, J. (2017). The Use of Magnetic Resonance Imaging (MRI) for the Study of the Velopharynx. *Perspective ASHA Spec Interest Groups, 2*(5), 35–52. https://doi.org/10.1044/persp2.sig5.35

Perry, J.L., Kuehn, D.P., & Sutton, B.P. (2013). Morphology of the Levator Veli Palatini Muscle Using Magnetic Resonance Imaging. *The Cleft Palate Craniofacial Journal*, *50*(1), 64–75. https://doi.org/10.1597/11-125

Perry, J.L, Sutton, B.P., Kuehn, D.P., & Gamage, J.K. (2014). Using MRI for Assessing Velopharyngeal Structures and Function. *Cleft Palate Craniofacial Journal*, *51*(4), 476–485. https://doi.org/10.1597/12-083

Perry, J.L., Chen, J.Y., Kotlarek, K.J., Haenssler, A., Sutton, B.P., Kuehn, D.P., Sitzman, T.J., & Fang, X. (2019). Morphology of the Musculus Uvulae In Vivo Using MRI and 3D Modeling Among Adults With Normal Anatomy and Preliminary Comparisons to Cleft Palate Anatomy. *Cleft Palate Craniofacial Journal, 56*(8), 993–1000. https://doi.org/10.1177/1055665619828226

Perry, J.L., Kollara, L., Sutton, B.P., Kuehn, D.P., & Fang, X. (2019). Growth Effects on Velopharyngeal Anatomy From Childhood to Adulthood. *Journal of Speech Language & Hearing Research*, *62*(3), 682– 692. https://doi.org/10.1044/2018_jslhr-s-18-0016

Perry, J.L., Kotlarek, K.J., Spoloric, K., Baylis, A., Kollara, L., Grischkan, J.M., Kirschner, R.E., Bates, D.G., Smith, M., & Findlen, U. (2020). Differences in the Tensor Veli Palatini Muscle and Hearing Status in Children With and Without 22q11.2 Deletion Syndrome. *Cleft Palate Craniofacial Journal, 57*(3), 302–309. https://doi.org/10.1177/1055665619869142

Philip, N., & Bassett, A. (2011). Cognitive, Behavioural and Psychiatric Phenotype in 22q11.2 Deletion Syndrome. *Behavioral Genetics, 41*(3), 403–412. https://doi.org/10.1007/s10519-011- 9468-z

Ruotolo, R.A., Veitia, N.A., Corbin, A., McDonough, J., Solot, C.B., McDonald-McGinn, D., Zackai, E.H., Emanuel, B.S., Cnaan, A., LaRossa, D., Arens, R., & Kirschner, R.E. (2006). Velopharyngeal Anatomy in 22q11.2 Deletion Syndrome: A Three-Dimensional Cephalometric Analysis. *Cleft Palate Craniofacial Journal*, *43*(4), 446. https://doi.org/10.1597/04-193r.1

Ryan, A.K., Goodship, J.A., Wilson, D.I., Philip, N., Levy, A., Seidel, H., Schuffenhauer, S., Oechsler, H., Belohradsky, B., Prieur, M., Aurias, A., Raymond, F.L., Clayton-Smith, J., Hatchwell, E., McKeown, C., Beemer, F.A., Dallapiccola, B., Novelli, G., Hurst, J.A., & Scambler, P.J. (1997). Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *Journal of Medical Genetics*, *34*(10), 798–804. https://doi.org/10.1136/jmg.34.10.798

Schenk, G.C., Perry, J.L, O'Gara, M. M., Linde, A.M., Grasseschi, M.F., Wood, R.J, Lacey, M.S., Fang, X. (2021). Velopharyngeal Muscle Morphology in Children with Unrepaired Submucous Cleft Palate: An Imaging Study. *The Cleft Palate-Craniofacial Journal, 58*(3), 313-323. DOI: 10.1177/1055665620954749

Solot, C.B., Gerdes, M., Kirschner, R.E., McDonald-McGinn, D.M., Moss, E., Woodin, M., Aleman, D., Zackai, E.H., Wang, P.P. (2001). Communication issues in 22q11.2 deletion syndrome: Children at risk. *Genetics in Medicine,* 3(1), 67-71. DOI: 10.1097/00125817-200101000-00015

Solot, C.B., Sell, D., Mayne, A., Baylis, A.L., Persson, C., Jackson, O., & McDonald-McGinn, D.M. (2019). Speech-language disorders in 22q11.2 deletion syndrome: Best practices for diagnosis and management. *American Journal of Speech Language Pathology*, 28(3), 984–999. https://doi.org/10.1044/2019_ajslp-16-0147

Sumida, K., Kashiwaya, G., Shinichiro, S., Masui, T., Ando, Y., Yamashita, K., Fujimura, A., & Kitamura, S. (2014). Anatomical status of the human musculus uvulae and its functional implications. *Clinical Anatomy*, 27(7), 1009-1015. DOI: 10.1002/ca.22431

Witt, P.D., Marsh, J.L., McFarland, E.G., & Riski, J.E. (2000). The evolution of velopharyngeal imaging. *Annals of Plastic Surgical,* 45(6),665–673. https://doi.org/10.1097/00000637-200045060-00019

Zimm, S., Schelper, R., Kellman, R., Tatum, S., Ploutz-Snyder, R., Shprintzen, R. (2003). Thickness and histologic and histochemical properties of the superior pharyngeal constrictor muscle in velocardiofacial syndrome. *Archives of Facial Plastic Surgery*, 5(6): 503-510. doi: 10.1001/archfaci.5.6.503.