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Cleft Palate, Retrognathia and Congenital Heart Disease in Velo-Cardio-Facial Syndrome: A Phenotype Correlation Study

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Abstract

Objective—Velo-cardio-facial syndrome (VCFS) is caused by a microdeletion of approximately 40 genes from one copy of chromosome 22. Expression of the syndrome is a variable combination of over 190 phenotypic characteristics. As of yet, little is known about how these phenotypes correlate with one another or whether there are predictable patterns of expression. Two of the most common phenotypic categories, congenital heart disease and cleft palate, have been proposed to have a common genetic relationship to the deleted T-box 1 gene (*TBX1*). The purpose of this study

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is to determine if congenital heart disease and cleft palate are correlated in a large cohort of human subjects with VCFS.

Methods—This study is a retrospective chart review including 316 Caucasian non-Hispanic subjects with FISH or CGH microarray confirmed chromosome 22q11.2 deletions. All subjects were evaluated by the interdisciplinary team at the Velo-Cardio-Facial Syndrome International Center at Upstate Medical University, Syracuse, NY. Each combination of congenital heart disease, cleft palates, and retrognathia was analyzed by chi square or Fisher exact test.

Results—For all categories of congenital heart disease and cleft palate or retrognathia no significant associations were found, with the exception of submucous cleft palate and retrognathia (nominal p=0.0325) and occult submucous cleft palate and retrognathia (nominal p=0.000013).

Conclusions—Congenital heart disease and cleft palate do not appear to be correlated in human subjects with VCFS despite earlier suggestions from animal models. Possible explanations include modification of the effect of *TBX1* by genes outside of the 22q11.2 region that may further influence the formation of the palate or heart, or the presence of epigenetic factors that may effect genes within the deleted region, modifying genes elsewhere, or polymorphisms on the normal copy of chromosome 22. Lastly, it is possible that *TBX1* plays a role in palate formation in some species, but not in humans. In VCFS, retrognathia is caused by an obtuse angulation of the skull base. It is unknown if the correlation between retrognathia and cleft palate in VCFS indicates a developmental sequence related to skull morphology, or direct gene effects of both anomalies. Much work remains to be done to fully understand the complex relationships between phenotypic characteristics in VCFS.

Keywords

Velo-cardio-facial syndrome; 22q11 deletion; VCFS; cleft palate; submucous cleft palate; occult submucous cleft palate; retrognathia; TBX1; conotruncal heart anomalies; tetralogy of Fallot; aortic arch anomalies

Introduction

Velo-cardio-facial syndrome (VCFS; MIM #192430; [1]) is the most common microdeletion syndrome in humans with a reported population prevalence of approximately 1:2,000 [2,3]. VCFS is caused by a deletion from one copy of the q11.2 band of chromosome 22 [4,5], usually about 3 million base pairs containing approximately 40 genes. The deletion results from meiotic non-allelic homologous recombination events between flanking segmental duplications also known as low copy repeats [6–8]. VCFS is also known to be the most frequent syndrome associated with conotruncal heart anomalies and is the most common multiple anomaly syndrome associated with cleft palate [2,9]. There is a characteristic facial appearance that can include retrognathia, vertical maxillary excess, vertically long nose with a bulbous tip, suborbital congestion, hooded upper eyelids, overfolding of the helices and absent lobules, and occasionally mild hypertelorism. None of these findings occur in all cases [10]. Previous research using mouse models has suggested that craniofacial and cardiovascular anomalies may be related to haploinsufficiency of *TBX1*, a gene mapping to the deleted 22q11.2 region that encodes a member of the T-box family of transcription factors [11] and is known to influence the formation of structures associated with the neural crest [12–14].

To date, research has focused on looking for phenotype to genotype correlations in order to understand the mechanism for these variably expressed anomalies. One important question is whether there are genetic modifiers to explain the basis of altered expressivity in affected individuals. Such genes might be in the same genetic pathway as *TBX1* or independent

pathways. It has been shown for example, that modulating genes such as vascular endothelial growth factor (*VEGF)* and fibroblast growth factor (*FGF8*) can act on the same pathway as genes from the commonly deleted region at 22q11.2, thereby influencing the expression of primarily deleted genes such as *TBX1* [15,16]. The identification of genetic modifiers can be guided by the observed correlations between phenotypic expressions. The question could more specifically ask whether the same modifiers are responsible for varying phenotypes in different tissues, such as craniofacial and cardiovascular anomalies.

Because many of the anomalous structures in VCFS are derived from the pharyngeal apparatus, a temporary embryological structure that forms gills in fish, it may be that much of the VCFS phenotype represents a series of structures of similar developmental origin, or "field defects." It is not clear, however, that this common embryological structure is sufficient to explain malformations of its derivative structures; field defects can be the result of any number of factors influencing development of more than one structure. Histological factors or dysplasias, vascular perfusion, and the effects of gene function at the end stages of development rather than the primordium all may play an important role in the final phenotypic presentation. Mechanical and developmental influences instigated by a single structural anomaly present during development, known as a sequence, can be another important factor influencing phenotypic expression [17]. For example, VCFS has been reported to be the second most common genetic cause of Robin sequence [18], a well known developmental sequence involving micrognathia or retrognathia, cleft palate, and upper airway obstruction. It has been reported that approximately 11% of Robin sequence cases are secondary to VCFS [18]. Both Potter sequence and holoprosencephaly sequence have also been reported in individuals with VCFS [19,20]. Therefore, it is already known that developmental sequences can be triggered by structural anomalies common to VCFS. However, a broader analysis of large populations of individuals with VCFS has not assessed relationships between phenotypes in a systematic manner.

VCFS occurs with considerable variability of expression for nearly all of its clinical features. This variability may be related to a combination of stochastic, environmental and genetic influences. It is possible that examining the distribution of phenotypes in VCFS may reveal patterns of co-occurring malformations that would suggest "field defects." Such patterns might help to guide the sorting of genome wide single nucleotide or copy number polymorphisms in relation to phenotypes. Cardiovascular and craniofacial anomalies are among the most common anomalies in humans and their association is seen in many multiple anomaly syndromes. It has been hypothesized that both may be causally related to hemizygosity of *TBX1*. The purpose of this paper is to determine if there are identifiable relationships between craniofacial and cardiovascular malformations in VCFS. We report on a retrospective chart review and statistical analysis of more than 300 subjects with VCFS who have had careful phenotypic analysis to determine the associations between congenital heart disease, palatal anomalies, and mandibular position.

Methods

Subjects

This retrospective chart review was reviewed and approved by the Institutional Review Board (IRB #3669) at Upstate Medical University. The study sample included 316 Caucasian non-Hispanic subjects with VCFS ascertained from a larger racially mixed sample. Only Caucasian non-Hispanic subjects were analyzed in this investigation to avoid potential variability in facial phenotypes that might be related to racial variability. All cases had deletions from 22q11.2 confirmed by fluorescence in situ hybridization or array comparative genome hybridization analysis. Subjects were first entered into the study with an age range of birth to sixty-five years. Nearly all subjects were seen at least three times by

the examiners typically over a period of several years, and sometimes for a period of more than thirty years. Clinical data were updated at the time of each examination. Data were entered into a database that categorized all known phenotypes associated with the syndrome.

Evaluation

All subjects were evaluated by the interdisciplinary team at the Velo-Cardio-Facial Syndrome International Center at Upstate Medical University in Syracuse, New York. Data obtained from the charts included direct physical examination, pediatric cardiology assessment, heart ultrasound studies, endoscopic and fluoroscopic studies of the palate and pharynx, laboratory results and radiographic imaging studies. Heart disease was classified according to type based on echocardiograms or angiograms. Structure of the soft palate was assessed by direct oral examination plus nasopharyngoscopy in all cases. Palatal anomalies were labeled as either normal, overt cleft palate, submucous cleft palate, or occult submucous cleft palate and asymmetry was also noted as previously described [9,21,22]. The category of submucous cleft palate was dependent on the presence of a bifid uvula in association with muscle separation on the nasal surface of the palate as seen endoscopically [9]. Occult submucous cleft palate was based on the presence of an intact uvula but muscle separation on the nasal surface of the soft palate or absence of the musculus uvulae as seen endoscopically [21]. Retrognathia was assessed by orthodontic evaluation based on cephalometric and clinical exam that indicated a morphologically normal mandible by measurement and comparison to age appropriate cephalometric norms with class II skeletal malocclusion based on consensus agreement between the examining dentist and the last author.

Data analysis

While each phenotypic feature was examined independently as a single category, some cardiac and craniofacial anomalies were additionally analyzed as grouped variables. All intra-cardiac and aortic arch defects were analyzed together. Intra-cardiac defects were combined into a group to include tetrology of Fallot, ventricular septal defects, atrial septal defects and truncus arteriosus. All variations of cleft palate were examined together as a group, and aberrant right and left subclavian arteries were also examined as a group. The associations between congenital heart disease and palatal anomalies were assessed individually for each subtype by Chi Square analysis, or by Fisher Exact Test when any cell value in the 2×2 table was less than eleven. Chi square analysis or Fisher Exact Test was also performed for the association of retrognathia and palatal anomalies and the association of heart anomalies with retrognathia.

Results

No significant association was found for congenital heart disease and cleft palate or congenital heart disease and retrognathia (Table 1). In fact, for all categories of associations, no significant positive associations were found with the exception of occult submucous cleft palate and retrognathia (Chi square = 18.959, nominal p= 0.000013) and submucous cleft palate and retrognathia (Chi square = 4.57, nominal p= 0.0325). For overt cleft palate and retrognathia, the Chi square = 0.022 and the p = 0.8821 [23]. After adjusting for the number of comparisons assessed, only the association between occult submucous cleft palate and retrognathia remained significant. The frequencies of phenotypic findings in our population are detailed in Table 2.

Discussion

Heart anomalies, primarily conotruncal, have been reported to occur in approximately 70% of individuals with VCFS and include intracardiac anomalies such as tetralogy of Fallot (TOF), ventricular or atrial septal defects or persistent truncus arteriosus [24,25]. Major aortic arch anomalies include interrupted aortic arch type B, double aortic arch, coarctation, and right sided aortic arch. Pulmonic stenosis or atresia is also seen with frequency. VCFS is known to constitute more than 50% of all cases of interrupted aortic arch type B, more than 50% of all cases of truncus arteriosus, and at least 15% of all cases of tetralogy of Fallot. When right-sided aortic arch is present, the frequency of VCFS among individuals with TOF is much higher [24]. The most common heart anomaly in individuals with VCFS is probably ventricular septal defect, often of the malalignment type, with or without pulmonic stenosis or atresia [26].

The prevalence of congenital heart disease in our series diverges slightly from those reported in previous studies, although in general the distribution is comparable [27]. Some lower prevalence rates of cardiac findings such as TOF in our sample may be explained by a lower ascertainment bias towards acute cardiac problems than in previous studies where detections were made largely after referral for major heart anomalies. Subjects for this study were ascertained through the Velo-Cardio-Facial Syndrome International Center in Syracuse, NY where primary referrals for cardiac anomalies is not the primary reason for referral. Referrals for feeding difficulties, speech impairment, behavioral and psychiatric concerns, and developmental and educational problems are common in our sample. Many referrals are made during pre-school and school age years after diagnosis had been made elsewhere, and we have also seen many adults. It is likely that the referral base represents a broader spectrum of the syndrome's phenotypic expression and is more representative of the population of people with VCFS.

Structural palate anomalies are known to occur in approximately 70% of VCFS individuals. Overt palatal clefts occur in 20% or fewer of the VCFS cases, with submucous cleft or occult submucous cleft palate being far more common [10]. It has also been found that there is a high frequency of palatal asymmetry in the individuals with VCFS [22]. Reports of the association of congenital heart disease with cleft palate (without cleft lip) in the general population have shown that slightly more than 10% of individuals with palatal anomalies have congenital heart disease, but the number of VCFS cases among this 10% is unknown [28]. Furthermore, it is unlikely that cases of occult submucous cleft were recognized in the report of Geis et al. [28] because the concept and clinical findings of occult clefts were initially reported at about the same time [21] and were not well understood at the time. The association between a retruded mandible and palatal anomalies is well known in association with Robin sequence, but has not been studied specifically in association with milder variants of mandibular position, or milder variants of palatal anomalies, such as submucous or occult submucous cleft palate.

Among all infants with cleft palate, the co-occurrence of congenital heart disease is more frequent than would be expected by chance. Geis et al. [28] reported 10.24% frequency of heart anomalies in individuals with clefts of the secondary palate including submucous cleft and congenital palatal insufficiency (a term used to describe cases of occult submucous cleft at that time) in a sample of 151. Shprintzen et al. [29] reported a 7% frequency of major heart anomalies and 3% frequency of minor heart anomalies (such as patent ductus arteriosus or patent foramen ovale) in a sample of 580 cases that included overt, submucous and occult submucous clefts ascertained from a single craniofacial center. The association of congenital heart disease with cleft palate (without cleft lip) by chance would be 1/272 (the frequency of heart anomalies in the general population) [30] multiplied by 1/2000 live births

with cleft palate (without cleft lip) which would calculate to a frequency of 1/544,000 [28,31]. Therefore, the high co-occurrence of these two anomalies indicates that they are either somehow causally related or that they frequently have common causative factors. Because VCFS has been reported to account for at least 5.0% [29,32] of all children at cleft palate and craniofacial centers, it can be concluded that many of these occurrences are related to this single syndrome. It would therefore be important to understand the underlying causative factor in a syndrome with a known pathogenesis that may help to elucidate the common causes of each of these clinical features.

Because palatal anomalies and congenital heart disease occur together at such a high frequency in VCFS, the causation must in some way be linked. It is possible that the expression of the heart and craniofacial anomalies in VCFS are both caused by the underexpression of a single gene because of hemizygosity. Using mouse and zebrafish models, previous publications have demonstrated a probable causal relationship between the deletion of *TBX1* and the presence of congenital heart disease in humans with VCFS [12,33,34]. Homozygous inactivation of *TBX1* in mice has also yielded cleft palate, mandibular hypoplasia, ear malformations, and low birth weight and length [12,33]. Cleft palate and other craniofacial anomalies were not found in mice hemizygous for *TBX1* deletions although outflow tract anomalies of the cardiovascular system were. In our sample, the data demonstrate a lack of significant concordance between the presence of congenital heart disease and cleft palate in 348 individuals with VCFS. Specifically, although both anomalies occur frequently in the syndrome, in many instances palatal anomalies occur in the absence of congenital heart disease, and conversely, congenital heart disease occurs frequently in the absence of cleft palate. Therefore, the lack of significant association between the effects of a hemizygous deletion of *TBX1* on the human heart and on palatal anomalies raises several important questions regarding the mechanism of expression for the deletion. One possible explanation is that the effect of *TBX1* is modified by genes outside of the 22q11.2 region that may modulate the formation of either the palate or the heart [15,16]. Another possible explanation is that epigenetic factors may have an effect on genes within the deleted region or modifying genes elsewhere. A third possible explanation is that *TBX1* is not directly related to palate formation in humans although it may play a role in other species. It is also possible that polymorphisms in the genes present on the normal copy of choromosome 22 may provide some protection against certain malformations. The lack of association, however, would seem to suggest that the presence of congenital heart disease does not lead to the presence of palatal anomalies in a causative manner by developmental sequence. In other words, it is unlikely that events related to perfusion and vascular supply to the craniofacial complex cause structural anomalies of the palate.

An analysis of the co-occurrence of retrognathia and congenital heart disease also revealed no significant association although the homozygous mouse deleted for *TBX1* showed significant changes in mandibular structure. It has been reported that mandibular morphology in VCFS is normal [35] and that only its position is different secondary to platybasia and posterior positioning of the glenoid fossa and temporomandibular joint. While the cause and possible genomic correlates of the abnormal skull base flexion in VCFS have not been identified as yet, mandibular position could potentially relate to the association of retrognathia with palate anomalies. For example, VCFS has been reported to be the second most common genetic cause of Robin sequence [18], a developmental sequence involving micrognathia, cleft palate, and upper airway obstruction. It has been reported that approximately 11% of Robin sequence cases are secondary to VCFS [18]. Therefore, it is already known that structural anomalies can be causally linked in VCFS. However, a broader analysis of large populations of individuals with VCFS has not assessed relationships between phenotypes in a systematic manner and it is possible that a subset of

genetic risk factors for craniofacial features combined with the deletion might also contribute to the etiology and expression of Robin sequence.

The only positive association between the phenotypes assessed in this study was that of retrognathia and occult submucous cleft palate or submucous cleft palate. This finding would seem to be contrary to the model of Robin sequence that postulates that posterior positioning of the mandible results in the tongue being positioned high in the oral cavity against the skull base at approximately 9 weeks post fertilization thereby preventing fusion of the palatal shelves that are trying to migrate medially to form the hard and soft palate [36–38]. There are several possible hypotheses that might be applicable, none of them being mutually exclusive. First, since retrognathia in VCFS is related to an obtuse angulation of the skull base, it is possible that cleft palate and retrognathia in VCFS have in common certain develomental abrnomalities of skull morphology. Previous studies have demonstrated that platybasia is a common finding in VCFS based on mid sagittal radiographs, such as cephalometric images that have measured the skull base in 2 dimensions (the mid-sagittal plane) [35,39]. It is possible that the flattening of the skull base may be part of or an initiating factor in other skull shape abnormalities, such as increased width in addition to increased angulation. Such changes could alter the relationship of the palate, maxilla, mandible, and the structures attached such as the pharynx and soft palate as previously demonstrated by Arvystas and Shprintzen [35]. If the skull base were wider than normal, this might make palatal fusion and migration of muscle tissue into the ectodermal envelope of the palate more difficult. Although these alterations may not be sufficient to cause overt clefting in all cases, it may be sufficient to prevent complete migration of muscle tissue into the velar envelope. It has also been established that the muscle tissue of the palate and pharynx is histologically abnormal; muscle fibers were found to be fewer in number and thinner in diameter when compared to control samples [40]. It is therefore possible that muscle migration is abnormal in terms of the amount of muscle mass available to infiltrate the palatal envelope during embryogenesis. It is not known if this type of primary muscle anomaly is related to *TBX1*, another gene deleted from the 22q11.2 locus, or perhaps a downstream regulation of a gene elsewhere in the genome. Any or all of these factors could be contributory.

Another possible explanation for the association of retrognathia and cleft palate is that these are primary anomalies associated with gene effect. Although it is not known if occult submucous cleft palate represents a milder form of overt cleft palate, it is likely that this is the case. It is therefore curious that retrognathia in VCFS is associated only with the mildest form of structural palatal anomaly. The implication is that the overwhelming majority of clefts are not associated with a developmental sequence (Robin sequence) but are rather primary anomalies associated with gene action. Although Robin sequence occurs in a percentage of VCFS cases, presumably the cases with the most severe retrognathia, and VCFS constitutes a sizable percentage of Robin sequence cases, the total percentage of Robin type clefts is small compared to the total sample of structural palatal anomalies. Because retrognathia in VCFS is related to platybasia resulting in a posterior displacement of the temporomandibular joint, it is possible that there is a major gene effect related to cranial base formation and palatal formation that affects both. It is interesting to note that clinically we find that relatively few of the overt clefts in VCFS involve the hard palate and relatively few submucous clefts have notching of the posterior border of the hard palate. The majority of the palatal anomalies in the syndrome are isolated to the velum. In future phenotypic studies, it would be worthwhile to measure the width of the skull base in relation to the presence of palatal anomalies to determine if there is a causal relationship.

As a result of careful phenotypic observation and analysis of a large sample of VCFS subjects, we have shown that cardiac anomalies, palatal malformations, and mandibular

anomalies do not seem to segregate together, but seem to vary in frequency independent of each other. We also found that certain types of palatal clefts do appear to be associated with the posterior displacement of the mandible in VCFS. Phenotypic correlation studies such as this may serve as a guide for ongoing genetic studies and further studies to understand the basis of these correlations. Additionally, this study points out the importance of keeping the larger picture in mind when presented with a patient with VCFS, where a large number of concerns may present that as of yet cannot be anticipated based on the presenting clinical picture. Relatively little is currently known about the relationships between the other 190+ phenotypic presentations in VCFS, though using our clinical data we hope to continue working to unmask the complex relationships between all of the various phenotypic presentations in VCFS.

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Table 1

Correlations between cardiac, cleft and mandibular phenotypes. [Note: Reported p-values are not adjusted to account for the number of statistical tests run.]

	Any Cleft Palate	Overt Cleft Palate	Submucous Cleft Palate	Occult Submucous Cleft Palate	Retrognathia
Any Heart Defect (intracardiac or aortic arch)	sample size = 314 n=141 x2= 0.049 p= 0.8248	sample size= 317 n= 19 p= 0.3059	sample size= 316 n=54 x2= 0.713 p=0.3984	sample size= 313 n=70 x2= 0.022 p=0.8820	sample size= 274 n=86 x2= 2.275 p=0.1315
Pulmonary Atresia/Stenosis	sample size= 305 n=23 p=1.0000	sample size= 308 n=3 p=0.7519	sample size= 307 n=10 p=0.8404	sample size= 304 n=10 p=0.8415	sample size= 267 n=15 x2= 0.319 p=0.5722
Vascular Ring	sample size= 294 n=6 p=0.1333	sample size= 297 n=1 p=1.0000	sample size= 296 n=1 p=0.1126	sample size= 293 n=4 p= 1.0000	sample size= 257 n=6 p= 0.7534
Aberrant LSC	sample size= 286 n=8 p= 1.0000	sample size= 289 n=1 p= 1.0000	sample size= 288 n=3 p= 1.0000	sample size= 285 n=4 p= 1.0000	sample size= 250 n=6 p= 1.0000
Aberrant RSC	sample size= 287 n=16 p= 1.0000	sample size= 290 n=2 p= 0.6969	sample size= 289 n=4 p= 0.3232	sample size= 286 n=10 p= 0.3396	sample size= 251 n=11 p=0.6266
Any Aberrant SC	sample size= 287 n=20 p= 0.8059	sample size= 290 n=2 p= 1.0000	sample size= 289 n=7 p= 0.6643	sample size= 286 n=11 x2= 0.251 p= 0.6164	sample size= 251 n=14 x2= 0.055 p= 0.8146
Right Sided AA	sample size= 310 n=29 p= 0.2838	sample size= 313 n=3 p= 1.0000	sample size= 312 n=11 x2= 0.002 p= 0.9643	sample size= 309 n=15 x2= 0.856 p= 0.3548	sample size= 271 n=17 x2= 0.111 p= 0.7390
IAA Type B	sample size= 309 n=17 p= 0.3195	sample size= 312 n=3 p=0.4798	sample size= 311 n=8 p= 1.0000	sample size= 308 n=6 p= 0.1973	sample size= 271 n=14 p= 0.1796
Truncus	sample size= 309 n=8 p= 0.1901	sample size= 312 n=0 p= 0.6163	sample size= 311 n=4 p= 1.0000	sample size= 308 n=4 p= 0.7727	sample size= 273 n=7 p= 0.3387
PDA	sample size= 309 n=21 p= 0.8090	sample size= 312 n=4 p= 0.2529	sample size= 311 n=9 p= 0.6593	sample size= 308 n=8 p= 0.5295	sample size= 273 n=10 p= 1.0000
TOF	sample size= 310 n=33 p= 0.8465	sample size= 313 n=6 p= 0.2413	sample size= 312 n=10 p= 0.2895	sample size= 309 n=18 x2= 0.586 p= 0.4439	sample size= 274 n=16 x2= 0.6 p= 0.4386
VSD	sample size= 307	sample size= 310	sample size= 309	sample size= 306	sample size= 271

	Any Cleft Palate	Overt Cleft Palate	Submucous Cleft Palate	Occult Submucous Cleft Palate	Retrognathia
	n=71 x2= 0.667 p= 0.4141	n=10 p= 0.5075	n=26 x2= 1.031 p= 0.3099	n=36 x2= 0 p= 1.0000	n=52 x2= 5.042 p= 0.0247
ASD	sample size= 309 n=25 x2= 2.886 p= 0.0894	sample size= 312 n=3 p= 1.0000	sample size= 311 n=13 x2= 0.204 p= 0.6515	sample size= 308 n=9 p= 0.0731	sample size= 272 n=15 x2= 0.003 p= 0.9563
Intracardiac (Truncus, TOF, VSD, or ASD)	sample size= 312 n=118 x2= 0.554 p= 0.4567	sample size= 315 n=17 x2= 1.21 p= 0.2713	sample size= 314 n=46 x2= 0.511 p= 0.4747	sample size= 311 n=57 x2= 0.134 p= 0.7143	sample size= 274 n=74 x2= 1.506 p= 0.2198
Retrognathia	**sample size= 276** **n=121** **x2= 6.644** **p= 0.00995**	sample size= 276 n=12 x2= 0.022 p= 0.8821	**sample size= 276** **n=37** **x2= 4.574** **p= 0.0325**	**sample size= 275** **n=73** **x2= 18.959** **p= 0.000013**	

Table 2

Frequency of Phenotypes

Phenotype	Sample size	% with phenotype
Any Heart Defect (Intracardiac or Aortic Arch)	327	59.33%
Intracardiac Defect (Truncus, TOF, ASD, VSD)	324	50.31%
Truncus	321	4.36%
PDA	321	8.10%
TOF	322	14.29%
ASD	321	12.15%
VSD	319	30.72%
IAA Type B	320	7.81%
Right Sided Aortic Arch	321	11.21%
Coarctation of the Aorta	320	1.56%
Double Aortic Arch	318	0.94%
Any Aberrant Subclavian	297	9.43%
Aberrant Right Subclavian	297	7.07%
Aberrant Left Subclavian	296	3.72%
Pulmonary Atresia/Stenosis	316	10.44%
Vascular Ring	304	3.95%
Any Cleft Palate	327	77.37%
Overt Cleft Palate	330	9.09%
Submucous Cleft Palate	329	30.70%
Occult Submucous Cleft Palate	326	37.42%
Retrognathia	278	51.44%