A META-ANALYSIS OF THE PREVALENCE OF COMMON CLINICAL CHARACTERISTICS IN VELOCARDIOFACIAL SYNDROME

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Background: Velocardiofacial syndrome (VCFS) is a congenital malformation syndrome with an estimated prevalence of 1:4,000 livebirths. Most cases are caused by a common 3 Mb deletion at 22q11.2. This syndrome exhibits wide inter- and intra-familial variability in phenotypic features including physical, developmental, neurological, and neuropsychiatric manifestations despite the general uniformity in deletion size. The purpose of this meta-analysis was to seek explanations for the differences in the reported prevalence rates of various findings; to more accurately estimate the prevalence of each of the nine traits examined; to provide insight into future research; and to improve the ability for genetic counselors and clinicians to provide more appropriate services and offer appropriate resources. *Methods:* A PubMed search was performed for keywords associated with VCFS. After an exhaustive search, twenty-nine articles were included. Nine traits of interest were chosen along with five predictor variables. From the articles, prevalence data was abstracted, overall prevalence data was calculated, and unweighted and weighted regression analyses were performed. *Results:* Ascertainment bias may be associated with the prevalence of ADHD; the prevalence of males does not appear to play a role in the discrepant data; the number of years ago a study was published is associated with prevalence of ADHD, cleft palate, palatal findings and VPI; age range is associated with the prevalence of congenital heart defects; having a *de novo* deletion is significantly associated with the prevalence of cleft palate and SMCP; and geographical location is significantly associated

with the prevalence of palatal anomalies. Overall prevalence rates are as follows: ADHD 17.2%, CHD 73.5%, cleft palate 11.6%, submucosal cleft palate 17.0%, velopharyngeal insufficiency 35.1%, any palatal anomaly 54.1%, any psychiatric disorder 34.4%, schizophrenia 12.6%, and hypotonia 64.5%. *Conclusions:* Due to small sample sizes, it is difficult to draw conclusions on the presented data; however, this analysis provides useful insight into future avenues of research especially with regards to behavioral and psychiatric illnesses. Many findings, especially psychiatric illnesses, associated with VCFS, pose a significant public health burden thus it would be of public health significance to find answers to some of the questions addressed in this study.

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PREFACE

I would like to acknowledge my committee members for their time, effort and support they have given me throughout this study. In particular, I would like to thank Dr. Mary Marazita for allowing me the freedom to pursue a study that would reflect my personal interests and supporting me over the past two years. Dr. Michael Barmada and Dr. Kevin Kip you were of great help to me and I thank you for your efforts in helping me create a quality analysis that I hope to have published. I would not have known where to begin (or end) without you! I would also like to thank you both for your continuous availability.

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1. INTRODUCTION

A meta-analysis of reported prevalence data can be a very useful tool to gain insight into the true prevalence of features associated with a genetic syndrome when variability in the literature exists. A meta-analysis serves not only to summarize data but also to seek explanations for the variability by analyzing the association between predictor variables and various outcomes. The current meta-analysis examines nine features that are commonly associated with Velocardiofacial syndrome (VCFS), a genetic syndrome that has numerous physical malformations and behavioral problems associated with it. Because of the wide variation in phenotype in conjunction with the wide variation in reported data regarding these phenotypic features, it is extremely difficult to provide genetic counseling and prognostic information to families. Furthermore, it is difficult for clinicians to determine who should have genetic testing for this syndrome. Many clinicians offer testing on a regular basis for any child with unexplained behavioral problems and/or speech problems. It would be more cost-effecient to be able to narrow down the population of who is most appropriate to be offered testing. It would also be extremely valuable for clinicians and genetic counselors to be able to tell families in the future which children are at-risk to develop the psychiatric disorders such as schizophrenia which we know can be associated with this syndrome. However, this information would not be useful for families until early intervention and preventive treatment is available to help these individuals.

In the current study, the following specific aims were pursued:

Specific Aim 1: To more accurately estimate the prevalence of nine traits commonly associated with Velocardiofacial syndrome

Specific Aim 2: To seek explanations for the extreme variability in prevalence rates reported for these nine traits

Specific Aim 3: To provide insight into future research that will be beneficial to clinicians, researchers and families dealing with VCFS

Specific Aim 4: To improve the ability of genetic counselors to provide a more accurate risk assessment and suggest appropriate resources

2. REVIEW OF LITERATURE

2.1. CLINICAL SPECTRUM OF VELOCARDIOFACIAL SYNDROME

Velocardiofacial syndrome or 22q11.2 deletion syndrome (MIM# 192430) is a relatively common congenital malformation syndrome with an estimated prevalence of 1:4,000 livebirths as shown in a large study of 325,000 Belgian children (Devriendt et al., 1998). This syndrome was first described by Shprintzen et al. (1978) as a congenital syndrome presenting with hypernasal speech associated with cleft palate or submucosal cleft, conotruncal cardiac defects, distinctive facies, learning disabilities, variable IQ, and disturbances in behavior; today, over 180 clinical features have been associated with VCFS. Other phenotypic syndromes commonly associated with the 22q11.2 deletion include DiGeorge (MIM# 188400) and CATCH22 (Cardiac anomalies, Abnormal facies, Thymic hypoplasia, Cleft palate, Hypocalcemia, and deletion of chromosome 22). Phenotypic features more often associated with DiGeorge syndrome include parathyroid hypoplasia, thymic hypoplasia, T cell deficits, and cardiac outflow tract defects. CATCH22 primarily acts as an acronym that encompasses all of the features associated with a deletion at 22q11.2. These syndromes were originally thought to be distinct disorders but are now thought to be part of a spectrum of clinical manifestations caused by a deletion at 22q.

The characteristic facial findings in individuals with Velocardiofacial syndrome (VCFS) include narrow palpebral fissures, tubular nose often with hypoplastic nasal alae, broad nasal bridge, small “carp-shaped” mouth, malformed ears and a long face (see Figures 1 and 2). Other problems that have been noted include hypotonia in infancy, conductive hearing loss associated with cleft palate, poor fine motor skills, abundant scalp hair, slender hands and digits, small

stature and spinal anomalies such as scoliosis. Cardiac anomalies and cleft palate are commonly reported findings although the actual prevalence is difficult to determine due to many small studies, overlapping samples, and ascertainment bias. The concordance of clefting and heart anomalies is not common in the general population. Shprintzen et al. (1981) reported that of 275 patients at the Center for Craniofacial Disorders (CCFD) at Montefiore Hospital and Medical Center in Bronx, New York, only seven (2.5%) also had a congenital heart anomaly. This suggests that the concordance of clefting and heart anomalies should raise the suspicion of a VCFS diagnosis.

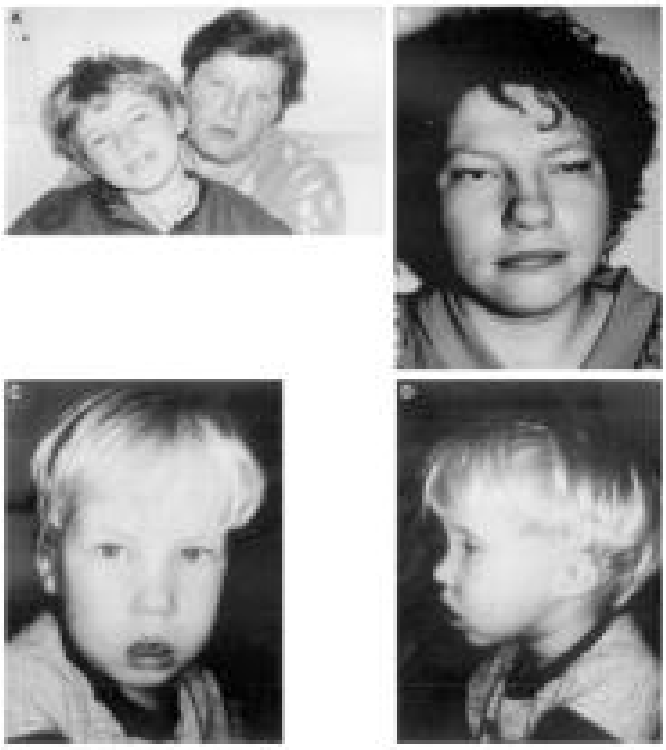

Figure 1 Phenotypic features of VCFS

Picture taken from Ryan et al. (1997); shows a phenotypic variability within families. Bottom left, child with long, myopathic face, small mouth, low set ears. Top right, mother with thick, fleshy nose with bulbous tip, small mouth, wide set eyes. Top left, shows mother and son with mild features of VCFS.

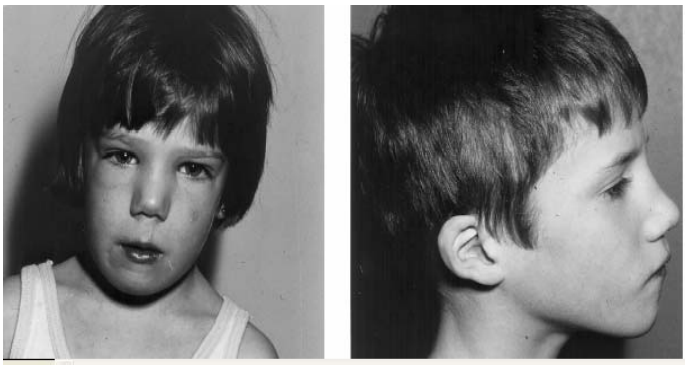

Figure 2 Children with VCFS

Picture from Vantrappen et al (1998); left: child with VCFS with long myopathic face, thick, tubular nose, wide set eyes and small mouth; right: child with VCFS with retruded mandible, low set ears, small mouth.

VCFS exhibits wide inter- and intra-familial variability in phenotypic features including physical, developmental, neurological, and neuropsychiatric manifestations despite the general uniformity in deletion size (see Figure 3). The variability in phenotype is so wide that many affected individuals are not identified until having an affected child with a more severe phenotype. It is likely that current prevalence data are underestimates due to mild cases that may never present to the medical community.

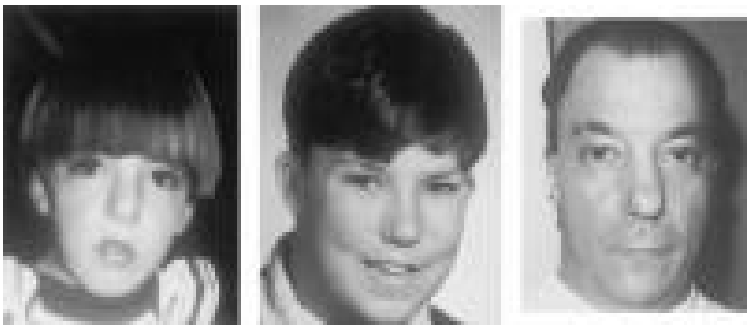

Figure 3 Phenotypic variability within families with VCFS

Photo from McDonald-McGinn et al. (2001); shows father on right with his two children, all affected. Child on left has autism, small stature, long myopathic face, epicanthal folds, prominent nasal root, bulbous nasal tip; middle picture shows child with learning disabilities and auricular abnormalities; father on right has no history of medical or educational difficulties, minor dysmorphic features.

2.1.1. TWIN STUDIES

Twin studies can be used to identify genetic and environmental contributions to the variation seen in a particular disorder. Monozygotic (MZ) twins in particular can provide information and raise interesting questions regarding genotype-phenotype correlations. Several studies have reported MZ twin pairs with 22q11 deletions that are discordant in phenotype. Discordance in MZ twins shows that phenotypic variability cannot be explained by differences in genotype alone. Goodship et al. (1995), described MZ twin males concordant for a 22q11 deletion sharing typical facial dysmorphism and nasal speech, yet only one twin had a heart defect, tetralogy of Fallot. In this case, the twins were born to clinically normal, unrelated parents who had no family history of congenital heart defects or disease. The twins were said to have a single placenta and the probability of dizygosity of only 0.000156. Zygosity was determined by Southern blot analysis and probing for four hypervariable DNA polymorphisms as well as red cell antigens. High resolution cytogenetic analysis showed 46, XY, del(22)(q11.21q11.23) in each twin which was confirmed by FISH analysis. One hundred metaphases were counted in

each twin and all one hundred showed the deletion. This, in addition to the dysmorphic facial features being so similar, makes it unlikely that mosaicism is an explanation for the discordance. It is important to consider however that heart malformations in general are more common in MZ twins and when present usually only affect one twin in the pair; the incidence of tetralogy of Fallot though is much higher in children with del22q11 than MZ twins (Goodship et al., 1995). This study concluded that this twin pair was predisposed to a heart defect due to the chromosomal abnormality and that other factors perhaps relating to the twinning process itself must have invoked the heart defect in one twin and not the other.

Singh et al. (2002) describe four additional MZ twin pairs with apparent discordance. The first pair, MZ females, again shared the typical facial features of VCFS but twin 2 had an aortic defect requiring surgery during childhood. The next pair, MZ males born to clinically normal parents, had abnormal facies but very little else in common. Twin 1 had a cardiac defect, thymic hypoplasia, velopharyngeal insufficiency, metal retardation (MR) and short stature while the other twin had no such complications. In the next pair, twin 1 had a normal cardiovascular system with other abnormalities while twin 2 had similar abnormalities in addition to a cardiac defect from which she died at day 5 of life. The final pair of MZ twins in this report was female in which both twins had tetralogy of Fallot with pulmonary atresia. Twin 1 had facial dysmorphism, abnormal outflow septation and misalignment of the great vessels. This twin died at age 11 months of sepsis. Twin 2 had in addition to tetralogy of Fallot, facial dysmorphism and low-normal thymic function. These studies in conjunction with the fact that over 85% of patients with VCFS have a comparable 3Mb deletion raise interesting questions regarding the variable nature of the phenotype as well as the susceptibility of this region to become deleted.

2.2. INHERITANCE OF THE 22q11 DELETION

The recurrence risk for VCFS is low in cases of *de novo* deletions though there is a small risk of germline mosaicism. In cases of inherited or familial deletions, an individual with the deletion has a 50% (1:2) chance of passing it on to each of their offspring apparently due to autosomal dominant inheritance. Genetic counseling in regards to this risk remains difficult due to the variability in phenotype. It has been shown in several studies that parents often have a milder phenotype than their affected offspring. This can be at least partially attributed to ascertainment bias since adults with VCFS who go on to reproduce would be more likely to have a milder phenotype (e.g. no major cardiac malformation).

The frequency of inherited VCFS deletions varies and has been reported to be between 6% and 28%; therefore the vast majority of cases occur sporadically (McDonald-McGinn et al, 2001; Ryan et al, 1997). A study by McDonald-McGinn et al. (2001), which ascertained 30 familial cases of VCFS through a population of three hundred and seventy affected individuals, suggests that the true incidence of inherited deletions is most likely around 10%. A preponderance of maternal transmission is reported in the majority of studies of inherited cases (Driscoll et al., 1992, Kelly et al., 1993). For example, Digilio et al. (2003) reviewed the prevalence of parental transmission as well as the clinical phenotype of the affected parent. In a series of eighty-seven patients, an affected parent was identified in fifteen (15 or 17.2%) and of these, ten were mothers, five were fathers; all were shown to have the typically deleted 3 Mb deletion. After careful examination of the parents with the deletion, one or more clinical feature was identified in all except one mother who displayed a very mild clinical phenotype. Although this was a small number of familial cases, this preferential maternal transmission has been demonstrated in

other studies as well such as Swillen et al. (1998) and Ryan et al. (1996). In the latter study, eighty-one patients had an inherited deletion, sixty-one were maternal in origin and eighteen were paternal in origin. With regards to cases of *de novo* deletions in the same study, initial estimates showed a paternal excess, however when the data was combined with previous reports by Demczuk et al. (1995), no parent of origin preference was noted. Hypothesized explanations for an excess of maternally inherited deletions include decreased fertility in male carriers, ascertainment bias, and imprinting. Imprinting can most likely be ruled out since most individuals with reported maternal uniparental disomy 22 have a normal phenotype (Balmer et al., 1999). Paternal uniparental disomy however, has not been reported to date.

In contrast to studies supporting a maternal excess of inherited deletions, there does not appear to be a parent-of-origin preference in *de novo* cases. Saitta et al. (2004) examined sixty-five cases of *de novo* deletions and used multiple microsatellite markers to determine the parent of origin; thirty-five cases were maternal in origin and thirty were paternal in origin. A smaller study by Ravnan et al. (1996) identified thirty-one deletions in a group of one hundred individuals with suspicious features of VCFS. Of these thirty-one cases, four were familial and three of these four were maternally inherited. There appears to be no difference in phenotype when the deletion is familial in nature versus when it occurs *de novo*.

2.3. CYTOGENETIC ANALYSIS OF 22q11 DELETION

Due to advances in cytogenetic technology and the use of fluorescent in situ hybridization (FISH), detection of VCFS is more accurate and the results are easier to interpret as compared to traditional high-resolution Giemsa banding. It is well established that the cytogenetic

abnormality in VCFS is a microdeletion at chromosomal location 22q11.2; most cases (87%) involve a 3 Mb region of the genome. Approximately 7-8% have a smaller 1.5 Mb deletion that lies within the 3 Mb region; thus this 1.5 Mb region is involved in over 95% of cases. An additional 4% have one of two other nested deletions and the remainder have unique deletions (Shaikh 2000, Ivanov 2003, Saitta, 2004) (Fig .1). Despite the vast majority of cases having a common 1.5 Mb deletion, there is great variability in the disorder as discussed earlier.

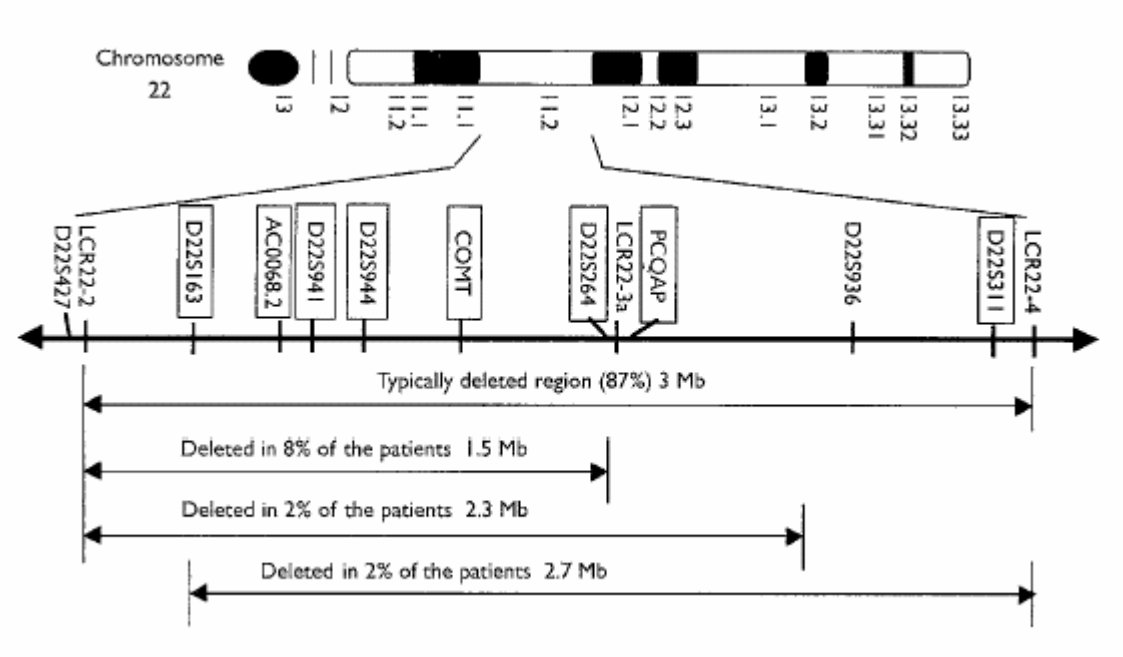

Figure 4: Chromosome 22: common deletions associated with VCFS

Shows the distribution of the genotyped markers in the typically deleted region of Velocardiofacial syndrome as well as the low copy repeats on chromosome 22q11.2. Ivanov, D. et al., 2002

The region that is typically deleted in VCFS is associated with other syndromes and recurrent rearrangements as well, including duplication 22q, Cat-Eye syndrome, a translocation (t11;22) that gives rise to derivative (22) syndrome, and other translocations and deletions that are associated with DiGeorge syndrome and Conotruncal Face Anomaly syndrome (CFAS).

Recurrent constitutional rearrangements such as these suggest that genomic instability exists in this region or that this is a site of preferential recombination that leads to a greater frequency of rearrangements. The high prevalence of VCFS in addition to the fact that most cases are sporadic in nature provide evidence that there is likely a common molecular basis for rearrangements at 22q11. Many studies concur that four large (200-500 kilobases), highly homologous low copy repeats (LCR's A, B, C, D) presumably confer instability and therefore lead to aberrant recombination in this region (Saitta, 2004; Edelmann et al., 1999; Shaikh., 2000; Shaikh., 2001). These low copy repeats are found within and flanking the 3Mb typically deleted region associated with VCFS (Fig 1). Additional LCR's exist on chromosome 22 but do not appear to have significant involvement in recurrent rearrangements.

There had been much discussion in past literature with regards to whether deletion 22q syndrome is a contiguous gene deletion syndrome or whether there exists a single gene responsible for the phenotype. The absence of an obvious genotype-phenotype correlation in conjunction with numerous reports of patients with the classic VCFS/DiGeorge phenotype and an atypical deletion excludes this disorder from being a contiguous gene deletion syndrome. In terms of the single gene theory, several genes have been proposed as candidate genes responsible for the phenotype while the other deleted genes in the region may act as modifiers of the phenotype thereby explaining the variability. It has been hypothesized that VCFS is a developmental field defect and the phenotypic differences observed reflect the events that affect the differentiation of structures during neural crest cell migration. Neural crest cells are critical in the development of the jaw, thymus, outflow tract of the heart (conotruncal region) and disruption of the migration of these cells can cause abnormalities in these structures as well as facial dysmorphism, all

structures which have been reported to be associated with having a deletion of 22q. Several genes within the deleted region have been shown to be involved in neural crest cell migration. One gene in particular is the *UFD1L* gene (DeDecker et al., 2001). *UFD1L* was shown to be deleted in all one hundred-eighty two patients in a study performed by Yamagishi et al. (1999). Furthermore expression of this gene was shown in various structures that are frequently affected in individuals with the deletion such as the conotruncal region, palatal precursors, and the hippocampus which may have a role in learning disabilities. Other genes thought to be critical in neural crest cell migration include the *TUPLE1* gene, a transcription factor expressed at critical point in development of the outflow tract of the heart, and *TBX1* another transcription factor expressed in early embryogenesis of the pharyngeal pouches.

2.4. SCHIZOPHRENIA IN VCFS

Schizophrenia is a severe and disabling mental illness with unknown etiology characterized by delusions, hallucinations and disorganized speech and behavior. It has an estimated prevalence of 1% in the general population and 25-30% in individuals with VCFS (Murphy et al, 1999; Pulver et al, 1994; and Bassett et al, 2000). Schizophrenia like many other psychiatric disorders appears to have both genetic and non-genetic factors influencing its development. Research shows that children with one parent meeting criteria for schizophrenia are 10-15 times more likely to develop schizophrenia as adults; those with two affected parents are 40 times more likely to develop schizophrenia when compared to children with normal parents (Cornblatt and Erlenmeyer-Kimling, 1985). Moreover, twin studies have shown that the genetically identical twin of an individual with schizophrenia has a 40-50% chance of also developing schizophrenia. Determining specific genetic and environmental factors would greatly increase the ability to

develop appropriate treatments and/or preventive measures and initiate early intervention strategies.

Numerous independent loci have been linked to schizophrenia; some such as the linkage on 22q11.2 seem to be robust in that a large proportion of deleted individuals develop schizophrenia (Murphy et al. 1999, Shprintzen, 1992, and Pulver, 1994). A study conducted by Murphy et al. (1999) evaluated 50 adults with VCFS in an attempt to characterize the psychiatric phenotype. Once categorized, individuals with schizophrenia and VCFS (SZ/VCFS, n=12) were compared to a control group of individuals with schizophrenia but without a deletion on chromosome 22q (SZ). This study revealed that 42% (21 individuals) had a history of a major psychiatric disorder, and 30% (15 individuals) had a history of psychosis. Twenty-four percent (24% or 12 individuals) met DSM-IV criteria for schizophrenia. Six individuals with VCFS and psychosis were referred by psychiatric services thus presenting an ascertainment bias, but excluding these six subjects, the rate of psychosis remains much higher (18%) than would be expected. It was suggested that the high prevalence of schizophrenia in particular in this study might reflect a non-specific association with mental retardation since 33% of those with VCFS had mental retardation (predominantly mild). However, the rate of schizophrenia in the mentally retarded population outside of VCFS is estimated to be around 3% (Murphy et al, 1999), higher than that of the general population yet still much lower than the observed 24% in this study. Therefore, this explanation does not likely account for the increased prevalence rate observed.

Additionally, this study found a significantly later age of onset of schizophrenia in the SZ/VCFS group when compared to the SZ group. Mean age of onset in the SZ/VCFS group was 26 years whereas in the SZ group it was 19 years. Conflicting data regarding age of onset of

schizophrenia in individuals with VCFS exists as other studies demonstrate an association with early-onset schizophrenia. The incidence of 22q11 deletion was examined in a series of patients with childhood-onset schizophrenia (COS). Usiskin et al. (1999) screened forty-seven patients with COS and found that three (6.4%) had a deletion of chromosome 22q11. All three individuals were said to have premorbid impairments of language, motor and social development. These results provide evidence that these deletions are in fact associated with schizophrenia and may be associated with an earlier age of onset.

There is also conflicting reports of the rates of schizophrenia in those with VCFS. In a study by Papolos et al. (1996; n=25), 64% met DSM-III-R criteria for a spectrum of bipolar affective disorders but there were no individuals meeting similar criteria for schizophrenia. Some studies have examined the rate of the 22q deletion in populations of patients with schizophrenia in an attempt to better characterize the incidence. One study by Lindsay et al. (1995) identified the deletion in two out of one hundred (2/100) individuals with schizophrenia, these individuals were not prospectively evaluated for features of VCFS. A second study (Sugama et al., 1999) screened three hundred twenty six patients admitted to a Japanese psychiatric hospital for features suggestive of VCFS, twelve were identified. Of these twelve patients, six were underwent further evaluation by FISH and one was found to have the deletion on chromosome 22. Another study by Bassett et al. (1998) assessed seventeen subjects with schizophrenia or schizoaffective disorder with two or more features suggestive of VCFS. Of these seventeen subjects, ten were found to have a deletion by FISH. Discrepancies in the published data are likely due to small sample sizes and differences in the modes of ascertainment. Most of these studies support a link between schizophrenia and a deletion on chromosome 22q11.2.

2.4.1. IMPAIRED ATTENTION AND SCHIZOPHRENIA

Schizophrenia is characterized by psychosis, negative symptoms such as flat affect or decreased pleasure, and cognitive impairment especially in terms of attentional deficits. Cognitive domains that have been studied most extensively in schizophrenia are attention, memory and executive function. Impaired sustained attention is a strong premorbid indicator of risk for schizophrenia as noted in numerous studies (Cornblatt and Keilp, 1994; Liu, et al, 2002). A study by Liu et al, 2002, suggested that impairment in sustained attention may differentiate schizophrenia from other psychotic disorders. When schizophrenics were compared to bipolar patients with and without psychosis and individuals with major depression without psychosis, those with schizophrenia had the highest level of impairment followed those with bipolar disorder. The Continuous Performance Test, which measures sustained attention, showed that the deficits observed in patients with bipolar disorder improved from the time of inpatient admission to discharge whereas those in schizophrenics remained unchanged. They concluded that the deficits measured by the CPT are stable indicators of the vulnerability to schizophrenia. Other studies concur that there is no significant difference in the performance of acute schizophrenics versus those in remission. Additional studies involving the CPT test have also shown that it was possible to distinguish normal controls from patients with schizophrenia who are in remission (are not acute). Others have shown that attention performance is worse in first-degree relatives with schizophrenia than those who have no family history (Suwa et al., 2004; D'Amato et al., 1998). Thus it has been proposed that poor performance on attentional tasks may suggest a vulnerability to the development of schizophrenia.

2.4.2. ABNORMALITIES IN DOPAMINE NEUROTRANSMISSION

It has long been postulated that disturbances in dopamine neurotransmission may play an active role in the development of schizophrenia. Dopamine has many roles within the brain; in the prefrontal cortical region in particular, it is linked to functions such as memory, attention and problem solving. Deficits involving these cognitive domains are cardinal features of schizophrenia as discussed earlier. The mesolimibic pathway of the brain is thought to contribute to the positive symptoms (hallucinations, delusions) seen in schizophrenia while the mesocortical pathway is thought to be associated with the negative symptoms (flat affect, lack of desire or emotion) of schizophrenia. The mesolimibic and mesocortical pathways are two of the four major pathways where dopamine is found. Evidence for the dopamine hypothesis of schizophrenia or psychosis came from the effects of drugs such as cocaine or other amphetamines which greatly increase the levels of dopamine causing psychotic-like symptoms. In addition, the discovery of a group of drugs called phenothiazines which include commonly used anti-psychotic drugs, block the uptake of dopamine at receptors and reduce psychotic symptoms. Although dopamine is not likely to be the only neurotransmitter involved in psychosis, there is much evidence to support its importance. Interestingly, *COMT* is a gene in the 22q deleted region which has been implicated in the pathogenesis of schizophrenia due to its effect on dopamine catabolism and further evidenced by the frequency of schizophrenia in those with a deletion in this region.

Catechol-O-methyltransferase (COMT) is an enzyme involved in the breakdown of the catecholamine neurotransmitters including dopamine, epinephrine and norepinephrine. COMT is responsible for more than 60% of the degradation of dopamine in the frontal cortex region of

the brain (Malhotra et al., 2002). The gene that encodes this enzyme, also known as *COMT*, has been investigated as a candidate gene for schizophrenia. The functional polymorphism, G → A, at codon 158 results in a substitution of methionine (Met) for valine (Val) and has been identified in association with schizophrenia. This substitution alters the activity of the *COMT* gene with the Val allele resulting in high activity and the Met allele resulting in low activity. Homozygosity for the Met allele leads to a 3-4 fold reduction in enzyme activity when compared to homozygosity of the Val allele (Ohmori et al., 1998, Egan et al., 2001). Heterozygotes for the two alleles (Val/Met) have activity levels midway between the homozygote levels. Thus homozygotes for the Val allele have increased dopamine catabolism and are hypothesized to be associated with deficits involved in the functions of the prefrontal region of the brain, where dopamine plays an active role. In studies on laboratory animals, low levels of dopamine have demonstrated cognitive impairments whereas in human studies, the enhancement of dopaminergic activity has improved cognitive functioning. These provided evidence for the role of the *COMT* gene in schizophrenia.

Conflicting data has been published in the literature regarding the effects of the Val and Met alleles on cognition and also their prevalence in the schizophrenic population. Some studies have shown an association of the high activity Val allele and schizophrenia (Wonodi et al, 2003; Egan et al, 2001) or similarly, that individuals with the Met allele performed better on tests of prefrontal cognitive functions (Malhotra et al., 2002 and Bilder et al., 2002). Nevertheless, other studies have found no association between the Val and Met alleles and performance on tasks involving similar cognitive functions. A study by Shifman et al. (2002) did not reveal an association with the Val/Met alleles and schizophrenia but did however demonstrate a highly

significant association between the *COMT* gene and schizophrenia by haplotype analysis. It is possible that some of this conflict may be explained by ascertainment bias or by differences in testing techniques.

2.5. ATTENTION DEFICT/HYPERACTIVITY DISORDER AND VCFS

It has been well established that hemizygosity for the 22q11 region is associated with several behavioral and psychiatric illnesses in both childhood and adulthood. The behavioral phenotype in VCFS has been characterized more specifically by developmental delays, cognitive and language deficits as well as the presence of psychiatric disorders, and childhood abnormalities of attention, mood and anxiety. Children with VCFS often display bland affect, social difficulties, and extremes of behavior, from shy and withdrawn to disinhibited and impulsive (Murphy, 2004; Gerdes et al., 1999). Studies of cognition and behavior profiles of children with a deletion of 22q reveal a range in IQ levels from normal to moderate mental retardation. Verbal IQ is often significantly higher than performance IQ regardless of IQ status (Moss et al., 1999; Swillen et al., 1997). They report that although many children with VCFS have delayed language skills, which often represent later problems in reading and spelling, verbal IQ scores are consistently higher than performance. Attention deficit-hyperactivity disorder (ADHD) has also been associated with 22q deletion syndrome and has been hypothesized to play a role in the aforementioned neurocognitive profile (verbal IQ > performance IQ). The IQ profile in children with ADHD without VCFS is often the opposite in that they typically have higher performance IQ's than verbal (Moss et al., 1999).

Attention deficit-hyperactivity disorder typically becomes apparent in the preschool or early school-age years. ADHD is not easy to diagnose as all children may exhibit some of the symptoms at a low level. Typical symptoms or behaviors are inattention, hyperactivity and impulsivity. Symptoms may appear at different times and may be more or less pronounced in certain settings such as school, home or social relationships. According to the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV), there are three subtypes of ADHD: predominantly hyperactive-impulsive, predominantly inattentive, or combined type. Diagnostic guidelines state that behaviors consistent with ADHD must be demonstrated to be inappropriate for the person's age, appear before age seven years, continue for at least six months and create a handicap in two areas of the person's life including social situations, home-life, or the classroom setting.

Several studies have evaluated children with VCFS to determine the rate of ADHD in this population. Papolos et al., (1996) performed psychiatric evaluations on twenty-five individuals over the age of five years with VCFS and found that five of them (20%) met DSM-III-R criteria for ADHD and four others (16%) met criteria for Attention Deficit Disorder without hyperactivity (formerly known as ADD but now considered to be part of the ADHD spectrum). A more recent study by Gothelf et al., (2004) evaluated fifty-one patients with VCFS, ADHD was diagnosed in twenty-one individuals (41.2%). Furthermore, this study examined the first degree relatives of patients with ADHD and found a significantly higher incidence of ADHD in these family members as compared to the first degree relatives of the patients without ADHD (OR = 5.9, p=0.006). In this study there was no significant difference in IQ of the two groups of patients (those with and without ADHD). The rate of ADHD in the general population is

estimated to be 3-7% of children, much lower than that shown by Papolos et al and Gothelf et al. The increased frequency of ADHD in children with VCFS suggests that the deleted region of genes on chromosome 22q may contribute to the etiology of ADHD. Interestingly, another group demonstrated that the *COMT* gene, which is also located in the typically deleted region, was associated with ADHD in a non-VCFS population of males (Gothelf et al., 2004).

2.6. CARDIAC ANOMALIES AND VCFS

Congenital heart defects (CHD) may occur as either an isolated defect or as part of a genetic syndrome. Certain types of cardiac defects are often over-represented in syndromes when compared with the general population. Typical cardiac defects associated with deletion 22q syndrome are conotruncal, meaning that they derive from the embryonic aortic arches and the ventricular septum. Malalignment and incomplete septation of these structures gives rise to the most commonly observed defects in VCFS: ventricular septal defects (VSD), tetralogy of Fallot (TOF), and right aortic arch (RAA). Truncus arteriosus, interrupted aortic arch and TOF are the most common congenital heart defects observed in DiGeorge syndrome; thus because the deletions in DGS and VCFS are indistinguishable, any one of these defects may be important in the diagnosis. Abnormalities of the cardiac valves or myocardium are rarely seen in deletion 22 syndrome. Moreover, it has been observed that patients with a CHD may be diagnosed as non-syndromic in infancy but are later found to have a deletion. This is because some features associated with the deletion are not detectable until later in childhood such as delayed speech, hypernasal speech or VPI, and subtle facial dysmorphism. The frequency of identifying a deletion in these individuals is dependent upon the particular cardiac defect that is present.

Studies have been done on populations of individuals with presumably isolated cardiac defects to determine the frequency of deletions. It has been suggested that a 22q deletion may account for up to 15% of individuals with an isolated conotruncal cardiac defect (Cuneo, 2001; DeDecker, 2001). An early study which examined seventeen individuals with a conotruncal defect not previously diagnosed with a deletion, showed that five patients (5/17) had a deletion at 22q (Goldmuntz, 1993). In 1997, Goldmuntz et al demonstrated that in a population of 50 seemingly non-syndromic individuals with cardiac defects (TA, IAA or TOF), nine had a deletion. Both of these studies have small sample sizes however, a subsequent study also by Goldmuntz et al (1998), ascertained a group of two hundred fifty-one patients diagnosed with one of seven different conotruncal defects; these individuals were then screened for a 22q11 deletion. Of these two hundred fifty-one patients, forty-five patients (17.9%) had a detectable deletion by FISH. The deletion was most frequently associated with IAA (50% or 12/24), TA (34.5% or 10/29), TOF (15.9% or 20/126), and posterior malalignment type ventricular septal defect without interruption of the aortic arch (PMVSD) (33.3% or 2/6). These findings demonstrate that IAA, TA and TOF may be important clues to the early diagnosis of deletion 22q syndrome.

2.7. PALATAL DEFECTS AND VCFS

2.7.1. CLEFT PALATE

Non-syndromic inheritance of facial clefting (cleft lip, cleft palate or cleft lip and palate) is multifactorial in nature and occurs with varying frequency depending upon family history. For isolated cleft palate in particular, the risk to future offspring is up to 7% with one affected parent, 2-5% when one sibling is affected, and up to 20% when one sibling and one parent are affected.

In general, isolated clefts of the palate are twice as common in females as in males and the rate is constant among different ethnic groups. Isolated cleft palate accounts for about 30% of facial clefts.

Certain chromosome abnormalities are associated with an increased occurrence of clefting; facial clefts are associated with a genetic syndrome 15-60% of the time. Common syndromes that may include cleft palate are Apert syndrome, Stickler syndrome, Treacher Collins and deletion 22q syndrome. The incidence of cleft palate in the VCFS population has been reported to be as low as 8% (Swillen, 1997) and as high as 85-98% (Thomas et al., 1997) depending upon the mode of ascertainment; clearly those ascertaining subjects through craniofacial centers will have much higher incidences. The majority of studies however report ranges closer to 13-28% when using mixed ascertainment methods. Nevertheless, there is considerable discrepancy in these reports.

Patients with cleft palate often have additional medical problems relating to their cleft. Eustachian tube dysfunction is commonly seen due to abnormal insertion of muscles into the hard palate and/or hypoplasia of the Eustachian tube. This can potentially result in conductive hearing loss. Eustachian tube dysfunction typically decreases as the child ages and in many cases normalizes by mid-adolescence. Proper treatment and management is necessary in these patients in order to provide adequate hearing and prevent deterioration of the tympanic membrane. Most patients require myringotomy and tube placement several times throughout their childhood in addition to cleft palate repair.

In addition to Eustachian tube dysfunction, the presence of a cleft palate alters the way the velopharyngeal sphincter muscle works to close off the opening between the nasal passage and the mouth which is important during speech and swallowing. When the muscle incompletely closes off this opening it is termed velopharyngeal incompetence or insufficiency. This leads to an audible escape of air from the nose (nasal emission) when the individual attempts to produce sounds involving a build up of air pressure. Nasal emission results in hypernasal speech which can render a person's speech difficult to understand. Velopharyngeal dysfunction can occur with or without the presence of a cleft palate.

2.7.2. VELOPHARYNGEAL INSUFFICIENCY

Velocardiofacial syndrome is one of the leading causes of cleft palate and velopharyngeal insufficiency (Vantrappen et al., 1998). Velopharyngeal competence is said to be the most important factor in articulation and listener understanding of speech in those with cleft palate. Patients with cleft palate often demonstrate errors in articulation usually due to velopharyngeal incompetence. In the English language, very few sounds, when made correctly, should resonate nasally ("m", "n" and "ng"). Most other consonants are referred to as pressure consonants and require velopharyngeal closure. When air escapes through the nose it can sound like squeaks or snorts and may make speech difficult to understand. This can lead to a decreased quality of life due to the perception that individuals with hypernasal speech may be less intelligent or less attractive than individuals with normal speech. These false perceptions can affect the person's self-esteem and social-life.

Speech and language delay is one of the most consistent features associated with VCFS due in part to velopharyngeal insufficiency. In children without major physical findings, evaluation for

apparent idiopathic speech and language delay often leads to the diagnosis of deletion 22q syndrome. Although it is true that many children with VCFS have decreased IQ levels, the speech and language delay is often associated with underlying VPI and sometimes hearing loss. It has been shown that although speech and language are delayed, language comprehension is often normal when compared to age related levels (Vantrappen et al., 1998). Published reviews report discrepant prevalence data of velopharyngeal dysfunction leading to confusion as to the true prevalence. Two large studies however report similar rates of VPI of 32% and 27% (Ryan et al., 1997 and McDonald-McGinn et al., 1999 respectively). An overt cleft palate is easily recognized at birth but a submucosal cleft of the palate (SMCP) can only be diagnosed with careful examination. Early diagnosis of velopharyngeal insufficiency due to submucosal cleft palate can provide support for early intervention and speech therapy. Speech therapy is an important part of treatment for children with VPI but the accompanying learning disabilities and behavioral problems can complicate progress and have a limiting role in success. Earlier treatment leads to an improved prognosis.

2.8. HYPOTONIA AND VCFS

A history of hypotonia has been reported in velocardiofacial syndrome and may have long-term effects on the development of the individual. Global hypotonia may affect the acquisition of gross motor skills as well as velopharyngeal function and Eustachian tube dysfunction due to hypoplasia of these structures. Although hypotonia is a common finding in infants requiring surgical intervention for a heart defect, it can be found in children with VCFS without a co-existing cardiac defect. Hypotonia is a very non-specific finding in and of itself.

2.9. META-ANALYSIS

Meta-analysis falls under the research category of systematic review and most are based on summary of data published in articles. Some are based on individual patient data. While the latter format is probably more accurate, it is often not an option or is too time-consuming. All meta-analyses follow four basic steps: 1) choosing data sources 2) creating inclusion/exclusion criteria for study selection 3) data abstraction and 4) statistical analysis. It is important to try to choose a good balance for study selection because being too restrictive can limit the ability to generalize results while being too inclusive can weaken the confidence that can be placed in the findings.

The phenotypic variability observed in velocardiofacial syndrome is great and therefore trying to estimate the prevalence of specific traits is confounded by many published papers reporting variable rates of manifestations. Much of the discrepancy may reflect ascertainment bias and small sample sizes. Moreover, many articles publish data on cases which have been previously reported. By combining data from various studies, it is possible to create a larger sample thereby increasing statistical power and, if done carefully, this type of study can minimize or eliminate sample overlaps. Meta-analysis allows us to combine studies in order to reach general conclusions.

Meta-analysis clearly has some advantages over other methods of study but it is not infallible. It can be subjective in that it integrates results which the reviewer feels are combinable. Bias may be introduced depending upon the inclusion or exclusion criteria used to determine the data that will be used. Literature search bias is common as well as not every study may be published in

every database. Publication bias can also be a problem in that significant results are more likely to be published than non-significant results. In addition to the introduction of bias, the examiner may need to make decisions regarding how to interpret and categorize some data as it is not always stated clearly. There is also the potential for data to be reported more than once although efforts are made to avoid this. Finally, limitations exist to every meta-analysis in terms of language of publications (English only in this case) and access to publications.

3. MATERIALS AND METHODS

3.1. STUDY SELECTION

A PubMed database search was performed for articles published in the English language since 1978 using the following search terms: “velocardiofacial syndrome”, “velocardiofacial syndrome and clinical manifestations”, “velocardiofacial syndrome and phenotype”, “velocardiofacial syndrome and behavior”, “velocardiofacial syndrome and cardiac”, “VCFS and clinical manifestations”, VCFS and phenotype”, “VCFS and cardiac”, “DiGeorge syndrome and clinical manifestations”, DiGeorge syndrome and behavior”, “DiGeorge syndrome and phenotype”, “DiGeorge syndrome and cardiac”, “deletion 22q syndrome”, “deletion 22q11.2”, “CATCH22 and clinical manifestations”, and “CATCH22 and phenotype”. Preliminary searches resulted in a maximum of two hundred forty-three articles containing these keywords. Of these two hundred forty-three articles, eighty-seven articles were selected based on relevance to this research in that there was data published on traits of interest.

Articles were obtained and systematically reviewed to determine if there was an overlap in samples or if they included previously published data. This was done by recording the city/country in which the research was conducted and the method by which the samples were obtained. Additionally, all bibliographies were cross-referenced. We also reviewed all authors listed in an attempt to further define any overlaps. When sample overlaps were detected or suspected based primarily on geographic location of sample ascertainment, generally the article with the larger sample size (n) was chosen. On occasion, articles with overlapping samples were used however the specific data was only used once. For example, two articles by Gothelf et al. (2003, 2004) were used, the samples are assumed to be overlapping but one provided palatal data

while the other provided primarily psychiatric data. Every effort was made to ensure that case data was used only once in this analysis.

3.2. DATA COLLECTION

Data of interest that was obtained from each study when available included the year the study was published, total number of cases (n), ascertainment method, location of the study (city, state, country), age range, male to female ratio, type of deletion (de novo or inherited) and prevalence data on the following traits: ADHD, CHD, hypotonia, palatal defects, VPI, SMCP and CP, schizophrenia, any other psychiatric disorder. These nine traits were evaluated as the outcome variables (dependent variables) and the predictor variables were as follows: ascertainment type (see below for coding information), prevalence of males, # of years ago the study was published, age range (see below for categories), prevalence of *de novo* deletions, and the continent on which the subjects were ascertained (Europe or North America).

When summarizing the data, it was noted that some authors reported traits differently than others. Some authors delineated between overt clefting of the palate, submucosal cleft palate, and VPI, whereas other authors simply reported these findings in sum as palatal anomalies. Efforts were made to categorize these as accurately as possible. Referring to Table 9 in the appendix, the "Palatal anomalies" category combines all data categorized as CP, SMCP, and VPI. In some articles, data was given as combinations such as "VPI or CP" and therefore was only documented as "palatal anomalies". The VPI category includes data reported either as VPI or as hypernasal speech since hypernasal speech is generally associated with velopharyngeal insufficiency. When cleft palates were not subcategorized as SCMP or overt CP, they were

documented in total as CP. The category "psychiatric disorder" includes data from the studies regarding any psychiatric diagnosis as stated by the authors of the study and also includes those individuals reported to have schizophrenia.

3.3. EXCLUDED STUDIES

Articles that were not available through the Health Sciences Library System at the University of Pittsburgh were not included in the current study (less than five). Articles reporting data on monosomy 22q due to translocations or other rearrangements were also not included. Furthermore, articles that contained only case reports or family reports that examined less than five families were excluded as well in an attempt to minimize reporting bias. In all, twenty-nine studies met the inclusion criteria (failed to meet the exclusion criteria) and were selected for this study. Of the fifty-seven remaining articles, nineteen were excluded because they reviewed previously published data by other groups, six articles were excluded because they were case or family reports, and sixteen were excluded due to suspected or reported sample overlaps. In addition, some articles were excluded due to obvious ascertainment bias. For example, three articles were excluded because the subjects were ascertained for cleft palate and then the prevalence of the deletion was determined. Only palatal data was provided in these studies. Similarly, four articles were excluded due to subject ascertainment by heart defect, again only cardiac data was presented. Lastly, ten articles were excluded because they ascertained subjects with schizophrenia or psychosis and only the data regarding these diagnoses was provided.

3.4. INCLUDED STUDIES

Data used for this analysis was extracted from the following twenty-nine published articles: Arnold et al., 2001; Botto et al., 2003; Digilio et al., 2003; Driscoll et al., 1992; Du Montcel et al., 1996; Eliez et al., 2000; Feinstein et al., 2002; Ford et al., 2000; Gerdes et al., 1999; Gothelf et al., 2004; Gothelf et al., 2003; Havkin et al., 2000; Hopkin et al., 2000; Kelly et al., 1993; Kerstjens-Frederikse et al., 1999; Kitsiou-Tzeli et al., 2004; Leana-Cox et al., 1996; McDonald-McGinn et al., 1999; McDonald-McGinn et al., 2001; Meinecke et al., 1986; Murphy et al., 1999; Niklasson et al., 2001; Oskarsdottir et al., 2004; Papolos et al., 1996; Ravnan et al., 1996; Ryan et al., 1997; Shprintzen et al., 1978; Shprintzen et al., 1981; and Swillen et al., 1997.

Of note, the Ryan et al., 1997 article states that some subjects were likely previously reported but did not specify how many; therefore it was not possible to determine which studies included here may overlap with this sample. The data provided by this study was included in the current study due the potential importance of including a sample this size.

3.5. ASCERTAINMENT CODING

In order to compare trait results based on ascertainment of subjects the different types or locations of ascertainment were coded. Studies in which ascertainment was restricted to craniofacial centers were coded as 1 (CC=1), VCFS clinics were coded as 2 (VCFS=2), medical or clinical genetics clinics were coded as 3 (GEN=3), mixed ascertainment was coded as 4 (MIX=4) and those not fitting these categories were coded as 5 (OTHER=5). Category 5 also included studies in which the ascertainment was not clearly specified in the article. The majority of studies were coded as mixed (4) or other (5) (twelve were mixed, eight were other). Four

studies were coded as 1 (CC), four as 3 (GEN) and one as 2 (VCFS). In the "other" category, four articles did not clearly state their ascertainment modes, one study ascertained subjects through behavioral clinics, one study ascertained through a VCFS association in addition to using web advertising (therefore we do not know how these subjects were initially ascertained), one study that evaluated all children in a certain age group though testing laboratories, and finally one study which evaluated the parents of children with VCFS who also were found to have the deletion.

3.6. AGE RANGE CATEGORIES

Very few studies provided the specific age of each subject in conjunction with their phenotypic information for each subject. Most studies however, did provide a range of ages; in others, it was possible to infer the range. In order to perform analyses on this type of data, age categories were created. Because many of the traits of interest are evident at birth or in early childhood with the exception of some behavioral and psychiatric illnesses, the age categories created were based on suggested evidence for childhood to early adolescent onset of these findings in the VCFS population (Feinstein and Eliez, 2000; Papolos et al., 1996; Gerdes et al., 1999). Based on these articles, the assigned categories are in reference to age fifteen years. Those studies including subjects both younger and older than fifteen years make up the "mixed" (m) category. The "older" (o) category means that all subjects are ≥ 15 years of age, and the "younger" (y) category includes subjects under the age of fifteen years. Of the twenty-seven studies that reported age ranges, the majority (18/27) fell into the mixed age range category. Data from seven studies fell into the younger (y) category while only two consisted of populations that were older than the cutoff age.

3.7. STATISTICAL ANALYSIS METHODS

In the twenty-nine studies reviewed, there were a total of 1,588 individuals with a deletion of 22q11.2 described, of which, six hundred-thirty eight (638) were male and six hundred-fifty seven (657) were female. Once all data was extracted from the included studies, the prevalence of each trait per study, the overall prevalence of each trait across all studies, and the 95% confidence intervals were calculated (refer to figure 8 and table 9 in the Appendix). Although not ideal, prevalence data was used in this study because generally the data provided in the articles were presented as such. Predictor variables were classified as either class or continuous variables. Class variables included ascertainment type, age range and continent and the remaining variables (maleness, number of years ago the study was published, and *de novo* deletion status) were classified as continuous. Because the overall prevalence of these variables was provided and used to do the analysis, maleness and *de novo* deletion status were categorized as continuous variables

Statistical analyses were conducted using SAS (statistical analysis system). The general linear model was used to test the association between categorical and continuous predictor variables and outcome variables. From the results, p values, R square values, R, and the number of observations (studies) used for each analysis, were obtained. When the predictor variable was a continuous variable, the coefficient of the estimate was also be obtained. From this regression analysis, we can test whether each predictor variable can significantly account for the differences in prevalence rates between studies. Both weighted and unweighted analyses were performed and an alpha level of 0.05 was chosen as the threshold of significance.

In order to perform a weighted analysis in SAS, a weight variable had to be created. The weight variable was the total number of subjects in a particular study divided by the total number of subjects with the trait of interest. In this manner, a different weight variable was created for each trait since not every study documented every trait. This was then incorporated into the analysis using a "weight" command specific to SAS.

In general, analyses were limited by the small number of studies and by the data presented within each study; thus limiting the ability to draw conclusions even when the finding was significant. Thus, it is important to consider the R squared value for this study due to it relative invariance to sample size. For a summary of unweighted and weighted results, please refer to tables 1 through 6 in the results section.

4. RESULTS

4.1. BY ASCERTAINMENT TYPE

In order to determine the association between ascertainment mode and the prevalence of each trait, the ascertainment modes were categorized as discussed earlier. The majority of study subjects fell into the mixed ascertainment category (MIX=4) with a total of one thousand-one hundred four (1,104) individuals from twelve studies. The next largest category of ascertainment was "other" (OTH=5) with a total of one hundred eighty-three (183) individuals from eight studies. In decreasing size order, next came those ascertained in medical or clinical genetics departments with one hundred sixty-two (162) subjects from four studies. This was followed by those ascertained through craniofacial centers with one hundred three (103) subjects from four studies. Lastly, thirty-six (36) subjects were recruited through a single VCFS center.

Statistical analyses were carried out to determine whether each of the following traits was associated with the mode of ascertainment: ADHD, congenital heart defect (CHD), cleft palate (CP), hypotonia, palatal defects (includes CP, SMCP, VPI), the diagnosis of any psychiatric disorder, submucosal cleft palate (SMCP), schizophrenia, and velopharyngeal insufficiency (VPI). Only the relationship between ascertainment type and ADHD was found to be statistically significant (p=0.02 unweighted and p=0.005 weighted; six observations, n=408). The R- squared value for this relationship, which shows the degree of association between two random variables, was found to be $R^2 = 0.99$. With regards to the remaining outcome variables, the p values range from 0.13 to 0.95 unweighted and 0.14 to 0.88 weighted. R squared values

range from 0.1 to 0.71 unweighted and 0.16 to 0.63 weighted (table 1). Figure 2 depicts the overall prevalence of ADHD in those studies that evaluated individuals for this diagnosis.

Table 1: Unweighted and weighted results by ascertainment type

Data by Ascertainment Type

			Unweighted analysis				**Weighted analysis**		
	# of Studies	# of Cases	R squared	R	p value		R squared	R	p value
ADHD	6	408	0.99	0.99	0.02		0.99	0.99	0.005
CHD	21	1,256	0.16	0.4	0.37		0.27	0.52	0.14
CP	14	1,036	0.23	0.48	0.64		0.21	0.46	0.68
Hypotonia	6	166	0.43	0.66	0.72		0.64	0.8	0.49
Palatal	21	1,117	0.19	0.44	0.46		0.21	0.46	0.4
Psychiatric d/o	9	288	0.65	0.81	0.13		0.58	0.76	0.19
SMCP	10	901	0.71	0.84	0.13		0.63	0.8	0.21
Schizophrenia	4	151	0.1	0.32	0.95		0.23	0.48	0.88
VPI	13	946	0.33	0.57	0.28		0.16	0.4	0.64

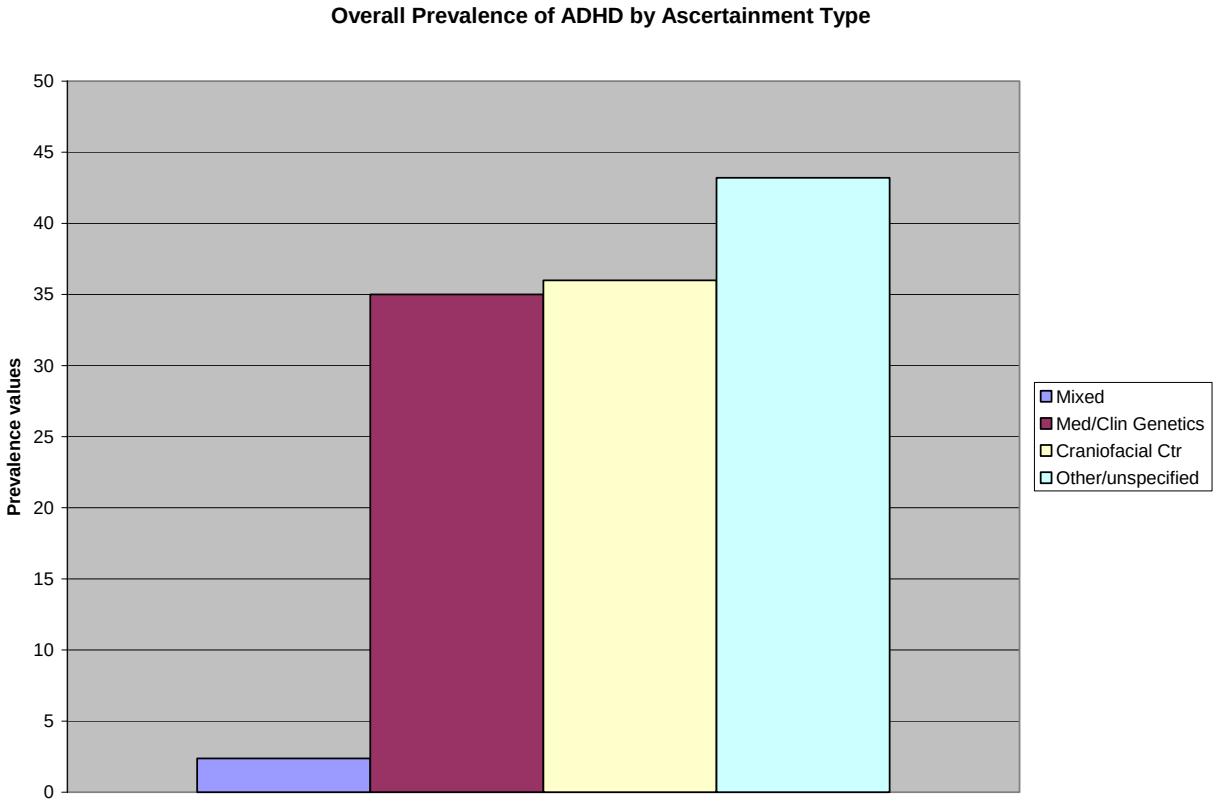

Figure 5: Overall prevalence of ADHD by ascertainment type

P= 0.02 by unweighted analysis and p= 0.005 by weighted analysis; MIX, n=252; GEN, n=20; CC, n=25; OTH, n=111

4.2. BY MALENESS

Analyses were conducted to determine whether the prevalence of males is associated with the prevalence of a particular trait. The outcomes that were evaluated include ADHD, CHD, CP, hypotonia, palatal defects, any psychiatric disorder, SMCP, schizophrenia, and VPI. None of these relationships proved to be significant, p values range from 0.06 to 0.89 in the unweighted analysis and from 0.07 to 0.87 in the weighted analysis. R squared values range from 0.003 to 0.74 unweighted and 0.84 weighted. Further investigation into the relationships between VPI and maleness as well as hypotonia and maleness may be warranted since the unweighted p

values of each approach statistical significance (p=0.06); however, in the weighted analysis, both of these relationships moved away from statistical significance. Refer to table 2 for results.

Table 2: Unweighted and weighted results by maleness

Data by Maleness									
			Unweighted analysis				Weighted analysis		
	# of Studies	# of Cases	R squared	R	p value		R squared	R	p value
ADHD	5	383	0.43	0.66	0.23		0.71	0.84	0.07
CHD	17	1,183	0.01	0.1	0.68		0.01	0.1	0.68
CP	13	1,000	0.14	0.37	0.22		0.003	0.05	0.87
Hypotonia	5	130	0.74	0.86	0.06		0.67	0.82	0.09
Palatal	17	1,043	0.007	0.08	0.75		0.02	0.14	0.56
Psychiatric d/o	8	263	0.08	0.28	0.51		0.20	0.45	0.26
SMCP	9	865	0.003	0.05	0.89		0.09	0.3	0.42
Schizophrenia	4	151	0.51	0.71	0.28		0.60	0.77	0.22
VPI	12	931	0.31	0.56	0.06		0.04	0.2	0.53

4.3. BY YEARS AGO

Analyses were conducted to determine whether the number of years ago the study data was published could explain the differences in the reported prevalence of the traits of interest. The traits that were included in this portion of the analysis again were ADHD, CHD, CP, hypotonia, palatal defects, any psychiatric disorder, SMCP, schizophrenia, and VPI. An associated was detected between the numbers of years ago a study was published and the prevalence of cleft palate (p=<0.0001, unweighted; p=0.002 weighted) with an R squared value of 0.76 and 0.55 (unweighted, weighted respectively). The direction of the relationship is positive such that as the number of years ago increases, so does the prevalence of cleft palate (Figure 3). In addition, the influence of the number of years ago a study was published on the prevalence of all palatal anomalies was also significant with a p value of 0.03 in both unweighted and weighted analyses (twenty one observations, n=1,117). The R squared value here is 0.44. Significance values for

the other outcome variables range from 0.15 to 0.87 in the unweighted analysis and R squared values 0.007 to 0.42 (table 3). Refer to figure 8 in the appendix to see the distribution of studies published by year.

In addition to the relationships discussed, the weighted analysis revealed others that were significant as well. In reference to ADHD, it was found that the number of years ago a study was published can explain 68% of the differences reported across studies by looking at the R squared value. The p value is 0.04 and the direction of the relationship is negative. This would be expected since as our knowledge of VCFS has increased over the years, there is more data that points to behavioral problems and thus it is being evaluated more frequently. Finally, it was noted that the weighted analysis also revealed a significant association between this particular predictor and VPI (p=0.05, R squared = 0.3) with a negative direction of the relationship.

Table 3: Unweighted and weighted results by number of years ago

			Data by Years Ago						
			Unweighted analysis				Weighted analysis		
	# of Studies	# of Cases	R squared	R	p value		R squared	R	p value
ADHD	6	408	0.37	0.61	0.20		0.68	0.82	0.04
CHD	21	1,256	0.02	0.14	0.51		0.03	0.17	0.46
CP	14	1,036	0.76	0.87	0.0001		0.55	0.74	0.002
Hypotonia	6	166	0.007	0.08	0.87		0.009	0.09	0.86
Palatal	21	1,117	0.23	0.48	0.03		0.23	0.48	0.03
Psychiatric d/o	9	288	0.27	0.52	0.15		0.18	0.42	0.26
SMCP	10	901	0.07	0.26	0.46		0.15	0.39	0.28
Schizophrenia	4	151	0.42	0.65	0.35		0.53	0.73	0.28
VPI	13	946	0.14	0.37	0.20		0.30	0.55	0.05

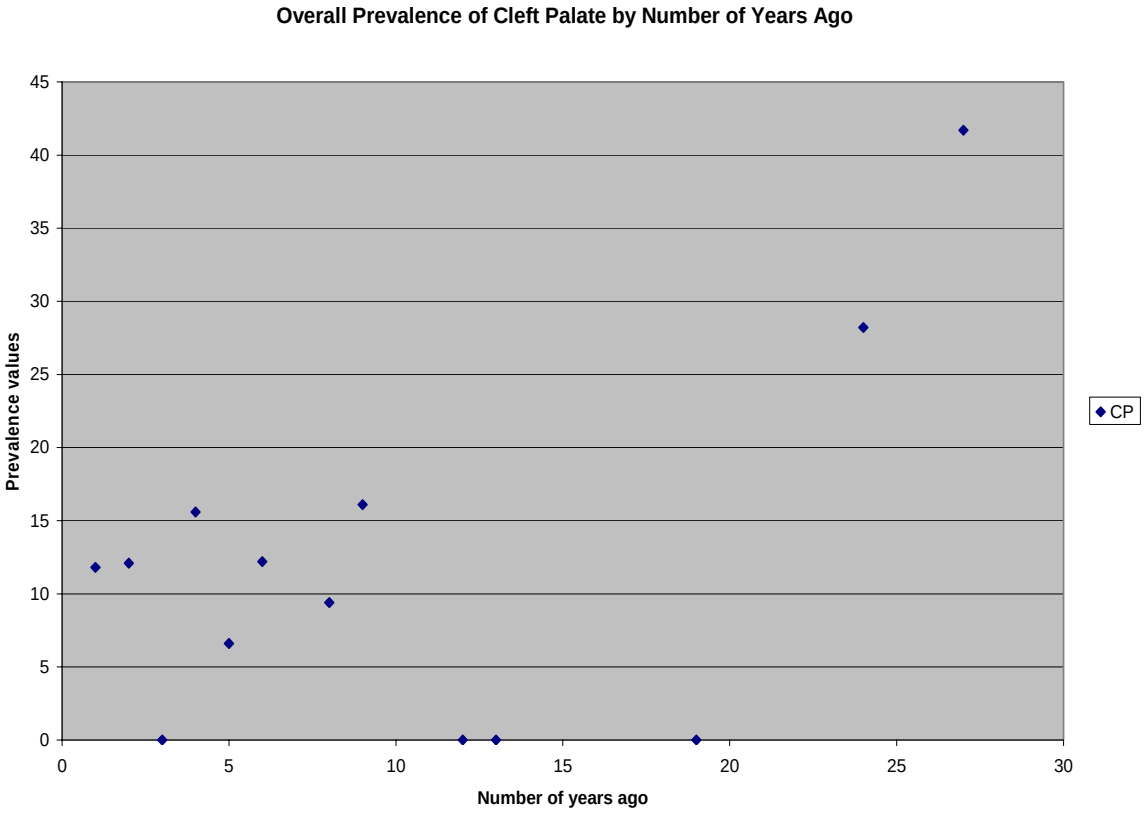

Figure 6: Overall prevalence of cleft palate by number of years ago

Shows the overall prevalence of cleft palate based on number of years ago the study was published; p=<0.0001unweighted; p=0.002 weighted

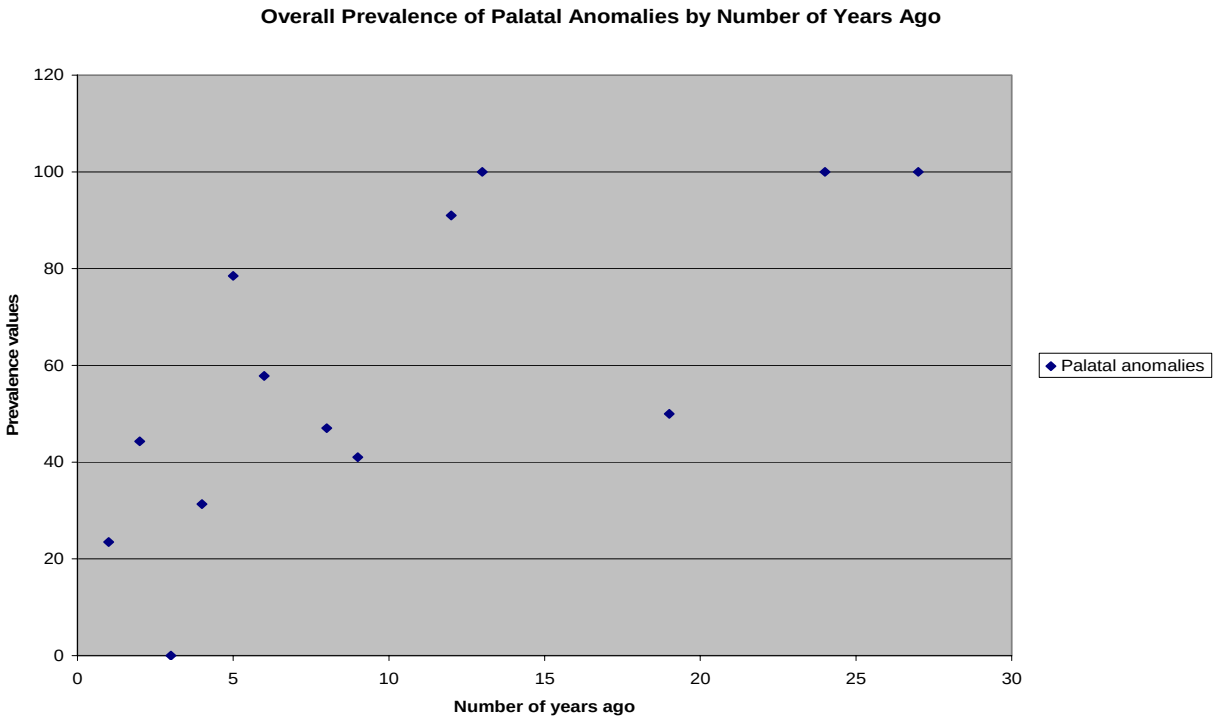

Figure 7: Overall prevalence of palatal anomalies by the number of years ago

Shows the overall prevalence of palatal anomalies based on the number of years ago the study was published; p=0.03 by unweighted and weighted analysis

4.4. BY AGE RANGE

Analyses were conducted to determine whether age influenced the prevalence of traits observed. Two studies did not specify the age range of the subjects used and therefore were eliminated from this portion of the analysis. Again, traits examined included ADHD, CHD, CP, hypotonia, palatal defects, any psychiatric disorder, SMCP, schizophrenia, and VPI. Of the relationships examined, a significant association was found between age range and CHD (p=0.0002 unweighted and p=0.003 weighted; nineteen observations, n=1,230) as well as age range and schizophrenia (p=0.05 unweighted, p=0.03 weighted; four observations, n=151). Please refer to table 4 for the complete results.

Table 4: Unweighted and weighted results by age range

Data by Age Range			Unweighted analysis				Weighted analysis		
	# of Studies	# of Cases	R squared	R	p value		R squared	R	p value
ADHD	6	408	0.003	0.55	0.91		0.06	0.24	0.63
CHD	19	1,230	0.66	0.81	0.0002		0.64	0.8	0.003
CP	14	993	0.13	0.36	0.48		0.03	0.17	0.85
Hypotonia	6	166	0.14	0.37	0.47		0.11	0.33	0.52
Palatal	19	1,091	0.1	0.32	0.44		0.06	0.24	0.61
Psychiatric d/o	9	288	0.38	0.62	0.24		0.49	0.7	0.13
SMCP	10	901	0.03	0.17	0.64		0.31	0.56	0.09
Schizophrenia	4	151	0.9	0.95	0.05		0.94	0.97	0.03
VPI	12	946	0.09	0.3	0.66		0.17	0.41	0.44

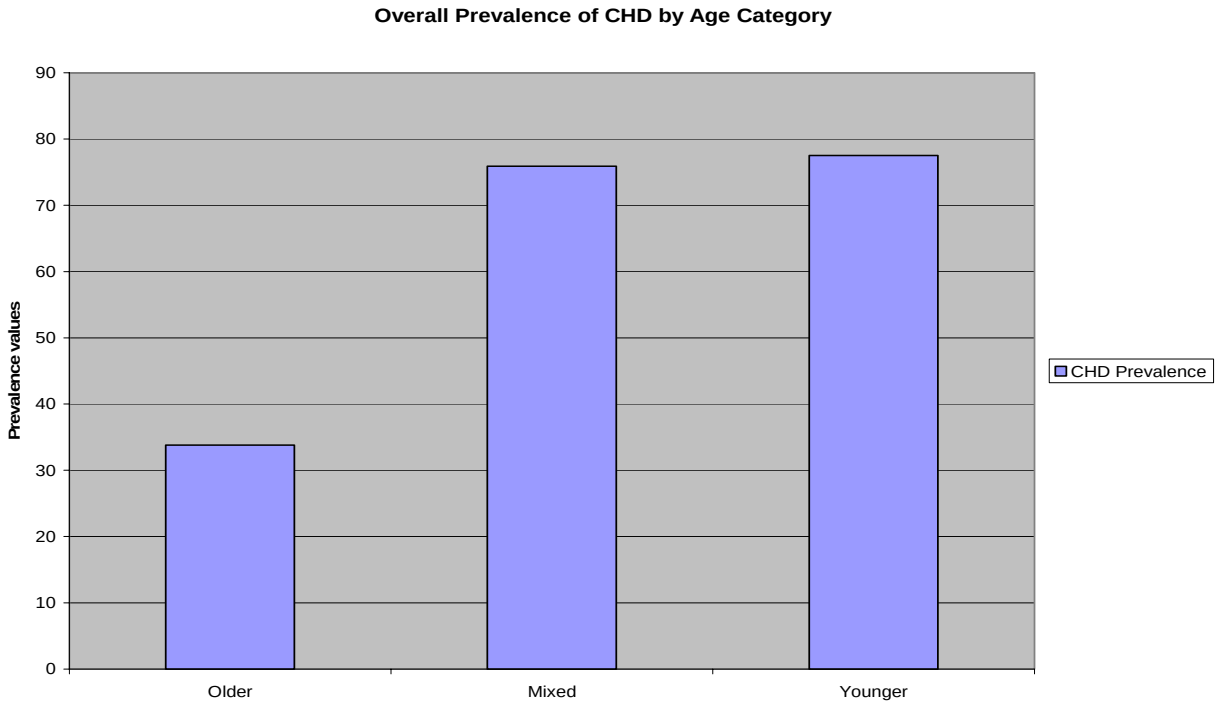

Figure 8: Overall prevalence of CHD by age category

p=0.0002 by unweighted analysis, p=0.03 by weighted analysis; Older (2 observations, n= 65) Mixed (13 observations, n= 1,094) Younger (4 observations, n=71)

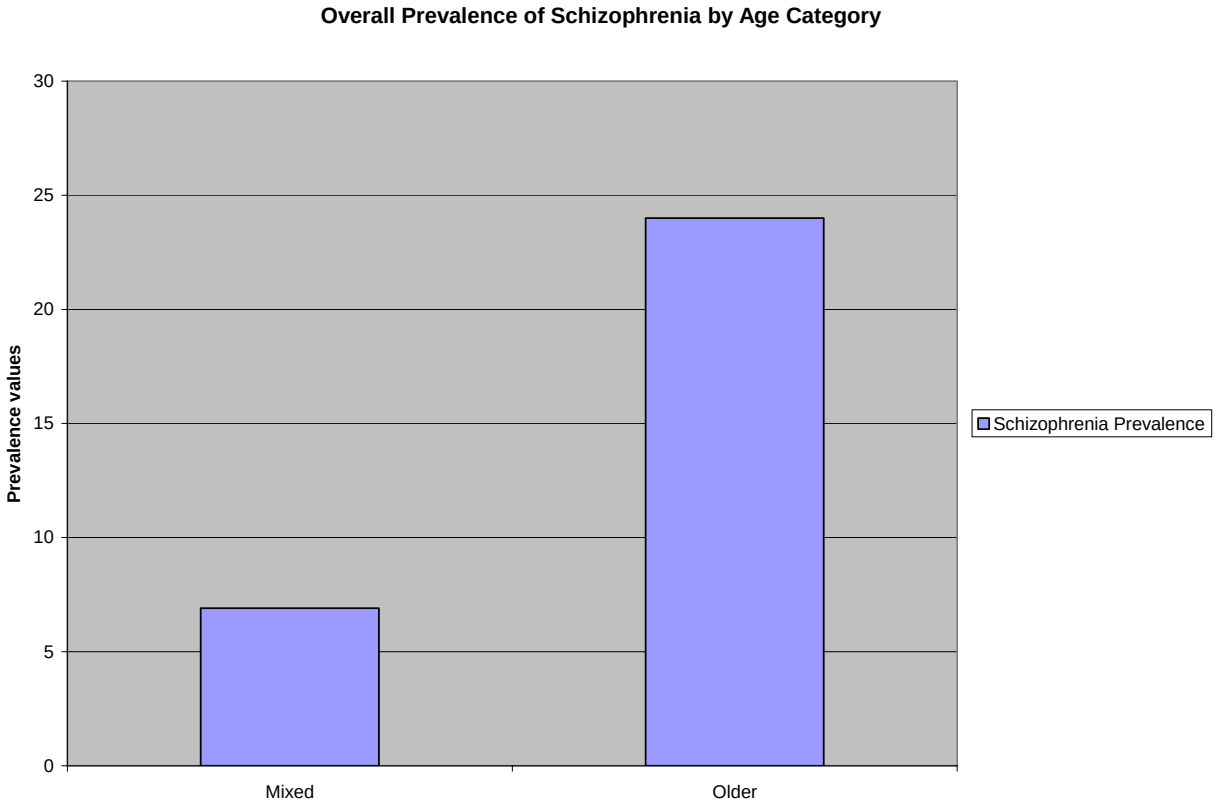

Figure 9: Overall prevalence of schizophrenia by age category
p=0.05; Mixed (3 observations, n=101), Older (1 observation, n=50)

4.5. DE NOVO DELETION

Analyses were conducted to determine whether the prevalence of *de novo* deletions is associated with the prevalence of the traits observed. Traits examined included ADHD, CHD, CP, hypotonia, palatal defects, the diagnosis of any psychiatric disorder, SMCP, schizophrenia, and VPI. Statistical significance was reached for the relationship between the prevalence of a submucosal cleft palate and prevalence of *de novo* deletions with p=0.02 in the unweighted analysis and p=0.01 weighted (three observations, n=580). The R squared value for these results is 1.0 in both the weighted and unweighted analyses. The analysis could not be carried out for ADHD, hypotonia and schizophrenia since not enough studies provided data on each of these

traits in addition to deletion information. The remaining analyses had p values ranging from 0.08 to 0.85 and R squared values of 0.02 to 0.57 unweighted. In the weighted analysis, significance was also reached for cleft palate (p=0.05) whereas it was not significant in the unweighted analysis Refer to Table 5 below for the aforementioned results.

Table 5: Unweighted and weighted results by deletion type

Data by *de novo* Deletion Type

			Unweighted analysis				Weighted analysis		
	# of Studies	# of Cases	R squared	R	p value		R squared	R	p value
ADHD	2	272	*	*	*		*	*	*
CHD	6	716	0.16	0.4	0.43		0.43	0.66	0.16
CP	6	649	0.57	0.75	0.08		0.67	0.82	0.05
Hypotonia	1	40	*	*	*		*	*	*
Palatal	5	612	0.17	0.41	0.48		0.33	0.57	0.31
Psychiatric d/o	3	96	0.47	0.68	0.52		0.67	0.82	0.39
SMCP	3	580	1.0	1.0	0.02		1.0	1	0.01
Schizophrenia	1	20	*	*	*		*	*	*
VPI	4	600	0.02	0.14	0.85		0.12	0.65	0.66

4.6. BY CONTINENT

Geographical location can sometimes provide insight into variation in prevalence rates of phenotypic traits and therefore was chosen as a predictor variable. All data collected for this research came from study populations in either Europe or North America. Only two studies used samples that overlap both of these continents and therefore were not used in this portion of the analysis (Meinecke et al., 1986 and Kelly et al., 1993). A total of seven hundred eighteen (718) subjects were described from the North American continent while eight hundred fifty-one (851) European individuals were described. Significance was not observed in evaluation of any relationship between the traits of interest and the geographical location in the unweighted analysis (p values ranged from 0.09 to 0.73; R squared from 0.04 to 0.36). The relationship

between hypotonia and geographical location could not be tested because there were no European studies that reported data on hypotonia. The weighted analysis did reveal significant association between palatal anomalies and the geographical location (p=0.02; R squared=0.28) (refer to Figure 7). Table 6 summarizes this data.

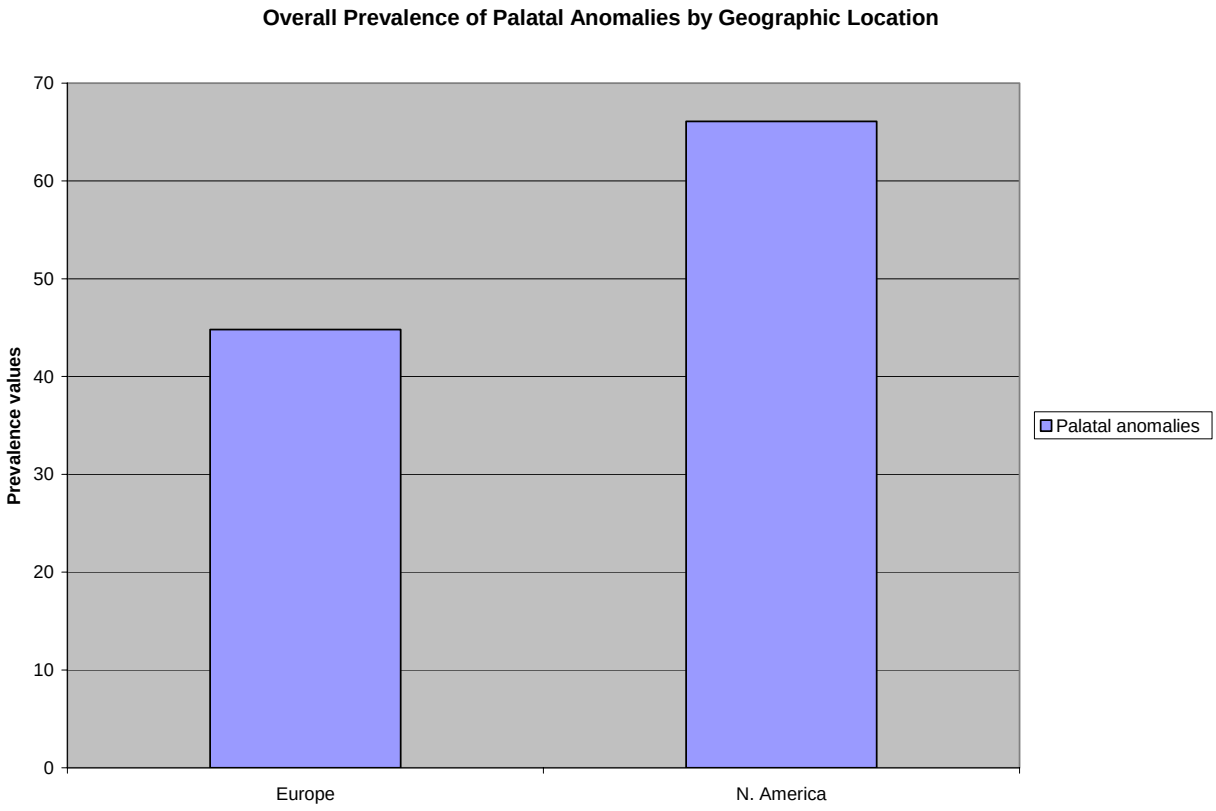

Figure 10: Overall prevalence of palatal anomalies by geographic location

Europe: overall prevalence = 44.8% (8 observations, n=638); N. America: overall prevalence = 66.1% (11 observations , n=460)

Table 6: Unweighted and weighted results by geographic location

Data by Geographical Location

			Unweighted analysis				Weighted analysis		
	# of Studies	# of Cases	R squared	R	p value		R squared	R	p value
ADHD	6	408	0.12	0.35	0.51		0.31	0.56	0.25
CHD	19	1,237	0.04	0.2	0.43		0.01	0.1	0.71
CP	14	1,207	0.05	0.22	0.42		0.14	0.37	0.18
Hypotonia	5	158	*	*	*		*	*	*
Palatal	19	1,098	0.16	0.4	0.09		0.28	0.53	0.02
Psychiatric d/o	9	268	0.1	0.32	0.42		0.10	0.32	0.4
SMCP	9	893	0.24	0.49	0.18		0.35	0.59	0.1
Schizophrenia	4	151	0.36	0.6	0.40		0.31	0.56	0.44
VPI	12	938	0.01	0.1	0.73		0.03	0.17	0.57

5. DISCUSSION

This meta-analysis investigated explanations for the variation in reported prevalence data across studies for various manifestations commonly seen in individuals with Velocardiofacial syndrome. Although the data is limited and many studies do not report on the same manifestations, thereby decreasing the sample sizes with which to analyze, the information remains useful. Furthermore, other limiting factors include the uncertainty in sample overlap in the Ryan et al. (1997) study and the inability to obtain complete data due to barriers such as language and resource limitations.

In addition to summarizing the published data, attempts were also made to not only explain the differences by identifying modifiers, but also to identify gaps in the current knowledge of this syndrome. It is hoped that this information may provide insight into prospective research ideas. Mode of ascertainment or recruitment of study subjects can often create a bias in reporting; thus in this study the author intended to investigate the hypothesis that ascertainment mode would be significantly associated with the prevalence of various traits. The ascertainment category MIX (4) has the greatest number of study subjects, thereby making it difficult to draw conclusions on individual modes of ascertainment. Nevertheless, although the category MIX has a large sample size associated with it, it represents the lowest reported prevalence of ADHD. This finding was further evaluated and it is noted that although only one study from this ascertainment category actually reported data on ADHD, though it was a large European study of five hundred fifty eight subjects (Ryan et al., 1997) of which two hundred fifty two (ages 3-18) were evaluated for behavioral and psychiatric problems. Six individuals (2.38%) were diagnosed with ADHD, though the specific diagnostic criteria were not provided. This figure is in stark contrast to other

reports. An earlier report by Papolos et al. (1996) reported a prevalence of ADHD of 36% and has since been corroborated by several other studies that cite a range of 35% to 46% (Arnold et al., 2001; Gothelf et al., 2004; Niklasson et al., 2001; and Feinstein et al., 2002). This finding by Ryan et al. is interesting and should be further investigated as to why the great discrepancy in prevalence. Explanations may include variability in the way in which the individual were diagnosed. ADHD is difficult to diagnose in that some children's behaviors vary with depending upon the setting they are in. In addition, although mixed ascertainment may have been the largest category, a breakdown of the specific ascertainment sources may provide additional information though it is not commonly provided. The association between other predictor variables and ADHD were assessed as well and only the predictor "number of years ago" in the weighted analysis was significant. This would be expected since behavioral disorders were not known to be associated with this syndrome when it was initially being studied. Of note is that it could not be determined whether or not ADHD could be associated with the origin of the deletion (*de novo* or familial) as only two studies reported both of these findings. Those studies ascertaining through either craniofacial centers or medical/clinical genetics departments report data similar to other published rates of approximately 35% (Arnold et al., 2001; Papolos et al., 1996). The "other" category reports a somewhat higher overall prevalence of ADHD (43.2%) likely due to the fact that this category includes one study (n=51) that ascertained an unspecified portion of its subjects through a behavioral genetics clinic. Further research is required to determine the true prevalence of ADHD in individuals with deletion 22q.

Another hypothesis is that gender and age would have an influence on the presence or development of manifestations such as psychiatric illness. Therefore, maleness and age range

were evaluated separately to see if they are associated with the prevalence rates of behavioral/psychiatric illness. The current study did not find any significant association between the prevalence of males and any of the behavioral dependent variables tested such as ADHD, psychiatric illness or schizophrenia. This is in contrast to what is found in the literature on studies of ADHD in the general population. In these articles, it is commonly suggested that ADHD has a higher prevalence rate in males than females (Biederman and Faraone, 2004). There are several possibilities why this might be. One explanation is that because young girls often present with less hyperactivity (i.e. primarily the inattentive form) than boys therefore, it is possible that the parents may be less likely to seek intervention services. Therefore, it is possible that boys may be diagnosed earlier or more frequently thus explaining the gender differences reported by some. Secondly, it may be that because those reports on individuals in the general population are more likely to be on an unbiased group of subjects, it would be less likely that the subjects have the same underlying cause for their lack of attention control. In children with VCFS, it has been shown that the type of attentional problems in these children is non-verbal, which differs from children with ADHD in the general population. The underlying cause in VCFS is possibly related to *COMT* gene activity and the dysregulation of dopamine. Because there is a common underlying defect (the deletion) in both genders in this syndrome, it may then affect them the same way such that we would not see a gender difference. However, small sample size limits our ability to draw a conclusion that there is no association between maleness and the prevalence of ADHD because the current meta-analysis only includes six articles that evaluated the prevalence of ADHD in the VCFS population.

In terms of schizophrenia and the prevalence of any psychiatric disorder, again there was not a significant association with the prevalence of males and these outcomes. This however, may not be as surprising for similar reasons as those discussed for ADHD. In the literature, based on schizophrenia in the general population, there is not a gender difference in terms of the lifetime incidence only in the age of onset.

Age range was found to have a significant influence on the prevalence of schizophrenia ($p=0.0498$). As discussed earlier, age categories m, o, and y (mixed, older = ≥15y, younger = <15y) were based on data suggesting an earlier age of onset of behavior problems and/or psychiatric disorders in the VCFS population. In the general population there is a distinct difference in age of onset of schizophrenia in particular between the genders that is often considered a hallmark of the illness. Although this difference exists and has been documented in almost all cultures where the illness has been studied, the onset typically occurs during the reproductive years in both sexes (adolescence to menopause). It has been hypothesized that the protective anti-dopaminergic properties of estrogen may influence the onset of schizophrenia because although men and women have a comparable lifetime risk of schizophrenia, women tend to have a later onset (Hafner, 2003; Halbreich and Kahn, 2003). The average age of onset in men, as characterized by the first psychotic episode, is in their early twenties and in women occurs in the late twenties. In the VCFS population however, though debatable, there are some studies that suggest that the age of onset is earlier (Usiskin et al., 1999).

It would be interesting to learn if this pattern of discrepant onset age between the sexes holds true in the VCFS population. In addition, it would be valuable to learn whether or not the gender

differences in onset age are related to differing vulnerabilities of each sex and again whether or not that would hold true in those with VCFS since they would likely have the same underlying cause for their schizophrenia.

Although significance was reached in the current research between age range and schizophrenia, only four observations were considered in the analysis as only four studies reported data on both schizophrenia and age range. Furthermore, only data in the "mixed" and "older" categories was available with very small total sample sizes (n=101 and n=50 respectively; see figure 6). This limits our ability to draw conclusions based on this data. In this case however, the R squared value is important to consider. The R squared value is 0.9 and 0.94 in the unweighted and weighted analyses respectively meaning that 90-94% of the differences in the prevalence of schizophrenia between studies can be attributed to the age range. This is not a surprising finding however since we would expect the prevalence of schizophrenia to increase as age increases. This is exactly what is shown in Figure 6; the "older" population has a greater prevalence than the "mixed" population. Moreover, an important factor to address regarding mental illness is the issue of cognitive impairment in individuals with VCFS as the prevalence of psychiatric illness is assumed to be higher in those with LD in the general population (without VCFS), though good estimates are not available. Studies report that learning disabilities or cognitive impairment is nearly universal in those with VCFS (Driscoll et al., 1992; Shprintzen, 2000).

In a study by Feinstein et al. (2002) that examined twenty-eight individuals with VCFS and twenty-nine age and cognitively matched controls found that although both groups had high rates of psychiatric disorders and behavioral problems, there was no significant difference between the

two groups. The question remains, are the rates of psychosis different between those with VCFS and those without VCFS with comparable cognitive impairment?

Another interesting finding in regards to maleness as a predictor, is the trend that was noted with palatal anomalies. Although none of the relationships were found to be significantly associated, all four outcomes (CP, SMCP, VPI, and all palatal anomalies) have a negative relationship with the predictor variable. This indicates that as the prevalence of males increases, the prevalence of these palatal findings decrease implying that these findings could potentially be more common in female subjects with VCFS.

Palatal anomalies were analyzed several ways. As mentioned earlier, they were first broken down into individual findings, cleft palate, submucosal cleft palate, and velopharyngeal insufficiency, and then analyzed together as a whole. Cleft palate prevalence was found to be significantly associated with the number of years ago the study was published ($p = < 0.0001$) with an R-squared value of 0.76 and a positive estimate of the coefficient. This finding implies that cleft palate prevalence was reported to be higher in the past than what it is currently. This finding is striking because one would not expect the reported prevalence of cleft palate to change over time since this is one of the clinical findings that is typically detectable at birth or very early in childhood depending upon the extent of the cleft. Additionally, there has been little evidence to suggest that the incidence of cleft palate has changed over the last twenty-thirty years. One explanation may be that the two articles that were published the greatest number of years ago (refer to Figure 3) were both published by Shprintzen et al. (1978, 1981) who first described the syndrome. He makes note in his first article that cleft palate is one of the unifying features in

this first cohort of patients (though not all subjects had a cleft palate); thus ascertainment or reporting bias may have played a role as this may have been a feature in children that were more likely to bring them to attention and therefore be evaluated for such a syndrome. The aforementioned articles published by Shprintzen et al. report prevalence rates of cleft palate of 41.6% and 28.2% (1978 and 1981 respectively), which are the highest of the data used for this meta-analysis. The remaining twelve articles cite prevalence data that ranges from 5.5% to 16.1% in the last eleven years. Lastly, it is possible that the reported decreased prevalence of cleft palate in more recent years may represent decreased fitness for various reasons. In those individuals ascertained for cleft palate may have a more severe palatal defect which may also mean that they have a more severe phenotype in general including greater cognitive impairment. There also has been evidence that some individuals with a cleft palate may have issues of self-esteem and therefore may be less likely to reproduce. It is also possible that individuals who are aware of their risks to pass on the deletion to the next generation may choose not to reproduce.

Similarly, when evaluating the influence of the number of years ago on the combination of all palatal anomalies, it too was found to be significant in both the weighted and unweighted analysis ($p=0.03$). The R-squared value is 0.23 with a positive estimate of the coefficient indicating that as the number of years ago increased, so did the number of palatal anomalies therefore; the prevalence data being reported today is lower than that which was reported in the past. The overall prevalence of all palatal anomalies was 54.1% (range of 16.7% to 100%) from twenty-one observations (total $n=1,117$).

It is well established that the majority of all patients carrying a deletion at 22q11.2 have a sporadic or *de novo* deletion. There has been a lot of research conducted in this area in attempt to define any phenotypic differences there may be between these individuals and those with a familial deletion. There has been little evidence for specific differences between these groups. In the current research, a significant association was identified between the predictor variable "*de novo* deletion" and the presence of a SMCP (p=0.01, three observations, n=580) with a positive estimate of the coefficient; however due to limited data, the true significance of this result is uncertain. Here again, the R squared value may be more relevant and was found to be very high (R^2= 0.999), thus stating that 99-100% of the variation of the prevalence of SMCP between studies can be accounted for by the prevalence of a *de novo* deletion. The prevalence range reported in the three studies was 5% to 16.7% with an overall prevalence of 6.6% (mean prevalence of 10.01%). The largest study, which evaluated four hundred ninety-six subjects found the lowest prevalent rate.

Similarly, a significant association was identified between prevalence of a *de novo* deletion and the prevalence of cleft palate (p=0.5, 6 observations/studies); again due to a small number of studies included, it is valuable to look at R squared (0.57 unweighted and 0.67 weighted). More than half of the differences between studies can be accounted for by the prevalence of a *de novo* deletion (positive relationship).

Analyses looking for factors that may suggest an association with the prevalence of CHD were conducted; only age range had a significant association with this finding (p=0.0002 unweighted, p= 0.003 weighted). This finding is interesting because by looking at Figure 5, one can see that

the highest overall prevalence (77.5%) was in the "younger" category (<15 years of age) and this category also has a very small n value (n=71). The "older" age category (≥ 15 years) has the lowest overall prevalence (33.8%, n=65). The largest age category ("mixed", n=1,094) has an overall prevalence similar to that of the younger group, 75.9%. Because the sample sizes here are very different, it is difficult to draw any conclusions, but it can be hypothesized that some of these differences are real. For example, we could argue that the younger category would be more likely than the older group to have a heart defect since it is more likely that older individuals may have died if they had one. The mixed category is more difficult to explain in that it includes individuals ranging in age from birth to fifty-two years of age. It is possible that there are individuals in this category with less severe defects and therefore improved survival rates as CHD is the primary cause of early mortality in VCFS.

One study (Cohen et al., 1999) compared the clinical phenotype in adults and children by reviewing case reports on one hundred twenty-six adults and comparing their findings with a study by Ryan et al. (1997) which evaluated five hundred fifty-eight subjects, the majority of which were under the age of eighteen years (89%). They found that adults with deletion 22q11.2 had fewer heart defects which supports the proposed explanation. Other studies that examined affected parent-child pairings have reported similar results in which the transmitting parent is less likely to have a heart defect (Ryan et al., 1997; Leana-Cox et al., 1996).

In the unweighted analysis, the geographical location (Europe versus North America) was not found to be significantly associated with any of the outcome variables. However, with the weighted analysis, the relationship with combined palatal anomalies became significant

(p=0.02). Please refer back to Figure 7 for a graphical depiction. A possible explanation for this result may be that there is ascertainment bias in the two samples. All of the Europe studies reporting on palatal anomalies ascertained their subjects through either "mixed" ascertainment or by those that fall into the "other" category. In the studies conducted in North America, the ascertainment modes are a combination of all possibilities therefore; it is possible that the increase in palatal findings as a whole may be due to this.

As was alluded to earlier, in studies that are underpowered such as many of the analyses above, it is sometimes better to not emphasize p values but to focus on an value that is not so dependent upon the size of the sample. In this case, we could focus more on the R squared values that were calculated. By choosing an arbitrary cutoff of 50%, we would see that there are many more outcomes which seem to have an association with predictor variables.

Ascertainment type would seem to be associated with ADHD, psychiatric disorders, hypotonia and SMCP as all of the R squared values here are ≥ 0.50 in either the weighted or unweighted analysis. Continuing with this, we could also note that maleness accounts for greater than 50% of the differences between studies for ADHD, hypotonia, and schizophrenia. This is interesting because as is noted in the general population, there are gender differences in the occurrence or diagnosis of ADHD. In this study, the p value was not found to be significant and the direction of the relationship here is positive saying that as the prevalence of males increase, so does the prevalence of ADHD. In contrast, in the general population, we do not see a gender difference in the prevalence of schizophrenia, but here we see that there is a negative direction of the

relationship between maleness and schizophrenia. This difference may be accounted for by the possible differences in age of onset which have not been fully delineated.

The number of years ago a study was published would also appear to be associated with an outcome that was not considered to be significant by the p value (p=0.28). The number of years ago accounts for greater than 50% of the differences in the reported prevalence of schizophrenia in the weighted analysis. Interestingly, the relationship is positive suggesting that as the number of years ago increases, so does the prevalence of schizophrenia. Since behavioral and psychiatric disorders were not known to be a part of the syndrome originally, this finding is unexpected. Perhaps the first estimates were high due in part to VCFS and in part to chance, which prompted more research on larger samples revealing a prevalence that was somewhat lower than what was originally thought. Other relationships with a possible association by the R squared value are with number of years ago and ADHD and cleft palate (R squared = 0.76 and 0.55 unweighed and weighted analysis) though both of these were also significant by p value.

Again, considering R squared values alone, there appears to be an association between age range and CHD as well as age range and schizophrenia using the arbitrary cutoff of 50%. The R squared value for age range and CHD is 0.64 and 0.66 (unweighted, weighted respectively) and for age range and schizophrenia, R squared = 0.9 and 0.94 (unweighted, weighted respectively). Again, with schizophrenia, the association with age range would be an expected finding since as an at-risk person gets older, their likelihood to be diagnosed would increase. In both relationships, these associations are also significant by p value.

In regards to the prevalence of *de novo* deletions, the analyses demonstrated that this predictor accounts for greater than 50% of the between study differences in the prevalence of cleft palate, SMCP and any psychiatric disorder. The relationships between *de novo* deletions and cleft palate and SMCP were also found to be significant by p value, however the R squared value supports this further since the number of studies (observations) was small for both. In fact, the R squared value was 0.999 or 1.0 for SMCP and de novo deletions stating that nearly 100% of the differences in SMCP prevalences between studies can be attributed to the prevalence of *de novo* deletions. In terms of the between study differences in the prevalence of any psychiatric disorder, 67% (in the weighted analysis) can be accounted for by the increase in prevalence of *de novo* deletions though this was not a significant relationship by p value. Again, a small sample number of observations were used in this analysis therefore, the true meaning is difficult to determine. Finally, there were no R squared values found to be greater than 0.5 in the analyses using continent as the predictor variable. It is interesting that the prevalence of palatal anomalies was found to be associated with this predictor variable by p value ($p=0.02$) with a moderately sized number of observations, yet the R squared value using the arbitrary cutoff of 0.5 does not support that there is a strong association. The R value would be 0.53 and by most accounts this would however be considered a moderate association. This demonstrates that depending upon how one interprets the data, can come to differing conclusions.

6. CONCLUSIONS

The purpose of this study was to seek explanations for the differences in the reported prevalence rates of the various findings examined here; to more accurately estimate the prevalence of each of the nine traits examined; to provide insight into future research; and to improve the ability for genetic counselors and clinicians to provide more appropriate services and offer appropriate resources.

Differences in prevalence rates between studies in the literature were found to be as large as a 93% difference for psychiatric disorders and VPI and 83% for palatal findings. This meta-analysis was conducted in an attempt to explain some of these discrepancies by analyzing how several predictor variables may be associated with the prevalence of different outcomes. The predictor variables chosen were 1) ascertainment mode 2) maleness (prevalence of males) 3) number of years ago each study was published 4) age range 5) prevalence of *de novo* deletions and 5) continent or geographical location. The nine traits of which the prevalence was evaluated for were: ADHD, congenital heart defects, cleft palate, submucosal cleft palate, velopharyngeal insufficiency, palatal anomalies in combination, any psychiatric disorder, schizophrenia, and history of hypotonia. Overall prevalence rates are as follows: ADHD 17.2%, CHD 73.5%, cleft palate 11.6%, submucosal cleft palate 17.0%, velopharyngeal insufficiency 35.1%, any palatal anomaly 54.1%, any psychiatric disorder 34.4%, schizophrenia 12.6%, and hypotonia 64.5%.

Mode of ascertainment was found to be associated with the prevalence of ADHD, although the analysis included only six observations. Ascertainment was not significantly associated with any other findings. Surprisingly, maleness was not associated by p value with the prevalence of any

of the traits examined however it nears significance for the prevalence of ADHD and VPI. The number of years ago a study was published was associated with the reported differences in prevalence between studies with regards to ADHD, cleft palate, palatal anomalies, and VPI. Age range was significantly associated with the differences between studies on congenital heart defects and schizophrenia. The prevalence of *de novo* deletions was significantly associated with the prevalence of cleft palate and a submucosal cleft palate. Lastly, geographical location was associated with the prevalence of palatal anomalies, with North America reporting higher prevalence rates than Europe though with a smaller sample size.

Because so many of the current analyses used a small number of observations, it is difficult to draw conclusions. However, using R squared values and being aware of which analyses have the smaller sample sizes, it is possible to provide insight in future research opportunities. Future studies using much larger sample sizes of individuals with VCFS, especially in regards to ADHD, schizophrenia and psychiatric disorders in general will be extremely beneficial. The prevalence of these findings was much higher than that in the general population. Studies regarding the etiology of these behavioral findings in the general population too, will be beneficial to that population and likely to the VCFS population as well. Dopamine regulation has long been hypothesized to play a role in schizophrenia, and there is now evidence that the *COMT* gene commonly deleted in individuals with VCFS, also functions to regulate dopamine activity. Moreover, further research into the age of onset of the behavioral and psychiatric disorders will be important especially if we find that the onset is earlier in the VCFS population as a fewer number of years would be available for early intervention services and preventive

treatment if and when it becomes available. Psychiatric and behavioral problems are a significant public health burden as most psychiatric illnesses are lifelong, debilitating diseases.

An area where the above analyses could be improved is to conduct analyses involving multiple variables simultaneously. For instance, it would be interesting to evaluate both ascertainment as well as age range when looking for explanations in the reported prevalence differences for psychiatric disorders. Again due to the limitations in sample sizes and number of observations, this would be difficult to do until more data is available.

At this time, it is still difficult to provide patients and families accurate prognoses. Palatal findings are certainly significant in this illness as approximately 54% were found to have some type of such anomaly. VPI is difficult to diagnose in very early life and may not present a problem until the child begins to speak or has delayed speech. If this speech delay is not addressed immediately, the child will lose valuable time that he/she could have been receiving targeted speech therapy. If we know that a child has VPI or is susceptible to it having a diagnosis of VCFS, speech therapy could potentially start around that time that speech should normally begin rather than waiting for the delay to occur. There is evidence as well that hypotonia is an underlying factor in VPI, thus children with VCFS with hypotonia, without a cleft, may be at a higher risk to have VPI and therefore speech problems.

Although not discussed in the current study, learning disabilities and cognitive impairment is significant in this population. More specifically, children with VCFS tend to demonstrate a different type of learning difficulty compared to those with LD in the general population.

Children with VCFS tend to have a non-verbal learning disorder such that their verbal IQ is greater than their performance IQ. Non-verbal learning disability affects motor skills, social skills, and executive functioning skills (involving attention). Knowing that these difficulties are likely to present themselves during childhood would make obtaining intervention services as early as possible beneficial to their future success.

Services that should be offered or provided for these families as mentioned already include early intervention for motor skills associated with hypotonia, speech therapy for speech delays due to VPI and/or cleft palate and cognitive interventions for learning disabilities. A great place for parents or family members to look for support is an organization called Velo-Cardio-Facial Syndrome Educational Foundation. This group offers listings for various areas of the United States and also abroad and is meant to educate and provide support to those dealing with someone with this syndrome. There is also a quarterly publication available through the Children's Hospital of Philadelphia called "22q and you", a newsletter for patients, families and professionals as well. These and additional resources are listed below.

To locate a genetic counselor:
National Society of Genetic Counselors
www.nsgc.org/resourcelink.asp

Chromosome Deletion Outreach, Inc
P.O. Box 724
Boca Raton, FL 33429
561-395-4252
www.chromodisorder.org

Genetic Professional Societies (provides a listing of societies and contact information)
www.kumc.edu/gec/prof/soclist.html

Velo-Cardio-Facial Syndrome Educational Foundation
(http://www.vcfsef.org/SupportGroups/index.htm

The Children's Hospital of Philadelphia
The Clinical Genetics Center
One Children's Center
34th and Civic Center Boulevard
Philadelphia, PA 19104
215-590-2920
lunny@email.chop.edu

National Institutes of Health
National Library of Medicine
8600 Rockville Pike
Bethesda, MD 20894
www.nlm.nih.gov

APPENDIX

$$\left[\ P - 1.96\sqrt{P\,\frac{(1-P)}{N}}\ \right] \text{ to } \left[\ P + 1.96\sqrt{P\,\frac{(1-P)}{N}}\ \right]$$

Figure 11: Calculation for 95 percent confidence interval of a proportion

P= proportion, N= total number of subjects/observations

Table 7: Table of p values

	ADHD	CHD	CP	Hypotonia	Palatal	Psych	SMCP	Schizo	VPI
Ascertain type	0.0158	0.37	0.6358	0.716	0.4566	0.1306	0.1277	0.9465	0.2829
Male	0.2258	0.6812	0.2151	0.0611	0.7494	0.5105	0.8885	0.2847	0.058
Years ago	0.1977	5058	<0.0001	0.8682	0.0287	0.1485	0.4587	0.3513	0.2014
Age range	0.9147	0.0002	0.4789	0.4724	0.4378	0.2423	0.6434	0.0498	0.6557
de novo	*	0.4339	0.0848	*	0.4838	0.5181	0.0168	*	0.8498
Continent	0.5092	0.4285	0.4199	*	0.0853	0.4161	0.1788	0.4006	0.7257

* indicates no data available

Table 8: Direction of the relationship between predictor and outcome variables

	Unweighted Analysis				Weighted Analysis		
	Male	**Years Ago**	**De novo**		**Male**	**Years Ago**	**De novo**
ADHD	positive	negative	*		positive	negative	*
CHD	positive	positive	negative		negative	positive	negative
Cleft palate	negative	positive	positive		positive	positive	positive
Hypotonia	negative	positive	*		negative	positive	*
Palatal	negative	positive	positive		positive	positive	positive
Psychiatric D/O	negative	positive	positive		negative	positive	positive
SMCP	negative	positive	positive		positive	positive	positive
Schizophrenia	negative	positive	*		negative	positive	*
VPI	negative	positive	positive		negative	positive	positive

* indicates no data available

Table 9: Overall prevalence ranges, prevalence data, and confidence intervals

Outcome	Prevalence range	Mean Prevalence	95% ci	Overall Prevalence	95% ci
ADHD	2.38 - 46.4	34.12	17.16, 51.08	17.2	13.5, 20.8
CHD	6.7 - 84.6	66.33	58.20, 74.45	73.5	71.0, 75.9
Cleft palate	5.6 - 41.7	14.56	9.04, 20.08	11.6	9.6, 13.5
Hypotonia	25 - 100	60.83	29.55, 92.10	64.5	57.2, 71.7
Palatal	16.67 - 100	59.9	46.72, 73.08	54.1	51.2, 57.0
Psychiatric D/O	3.3 - 96.0	40.71	17.68, 63.74	34.4	28.9, 39.9
SMCP	5.04 - 84.62	39.31	15.27, 63.35	17	14.5, 19.4
Schizophrenia	3.3 - 24	11.29	-2.89, 25.48	12.6	7.3, 17.9
VPI	6.67 - 100	43.42	25.28, 61.56	35.1	32.1, 38.1

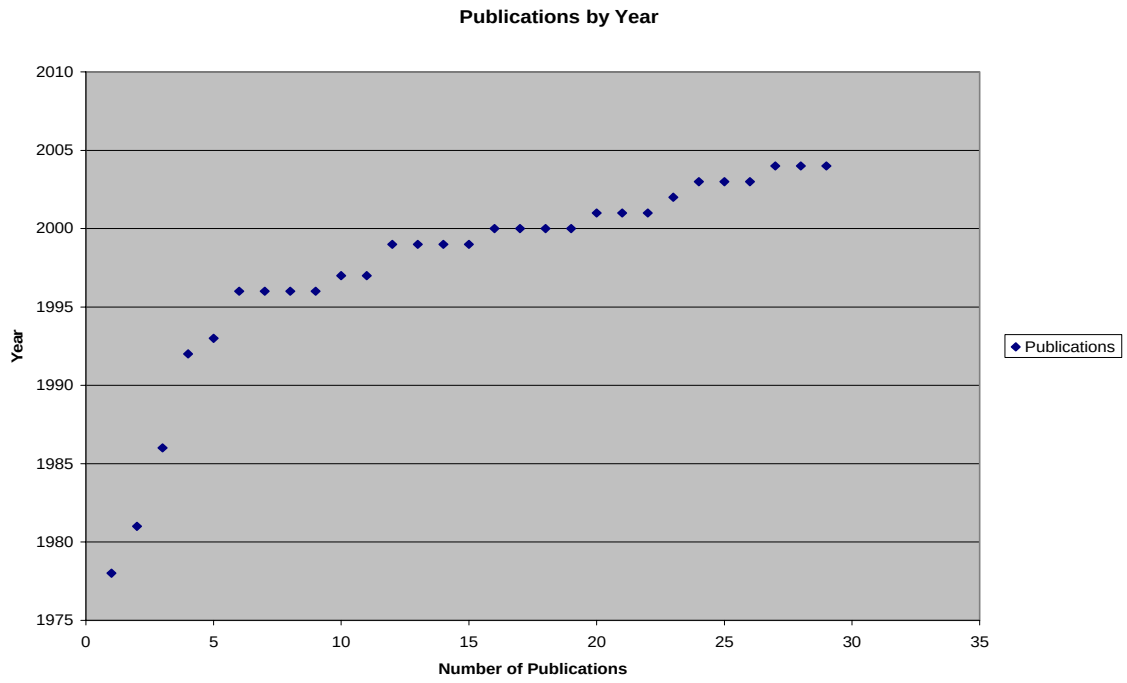

Figure 12: Number of publications by year

Table 10: Overall prevalence and sample size by ascertainment type

Overall prevalence, (# of observations), (n)

	Craniofacial Centers =1	VCFS Clinic = 2	Medical/Clinical Genetics Clinics = 3	Mixed Ascertainment = 4	Other or unspecified = 5
ADHD	36, (1), (25)	*	35, (1), (20)	2.38, (1), (252)	43.2, (3), (111)
CHD	69.2, (3), (78)	*	65.7, (2),(102)	76.2, (12), (994)	53.7, (5), (82)
Cleft palate	19.6, (3), (56)	5.56, (1), (36)	14.3, (2), (84)	11.0, (5), (776)	11.9, (3), (84)
Hypotonia	46.5, (2), (43)	100, (1), (36)	50, (1), (40)	70.0, (2),(47)	*
Palatal anomalies	66.1, (3),(56)	97.2, (1), (36)	63.1, (2), (84)	51.0, (10), (869)	55.6, (5),(72)
Psychiatric D/O	96.0, (1), (25)	*	60, (1), (20)	23.4, (3), (141)	29.4, (4),(102)
SMCP	72.0, (2), (25)	92.0, (1), (36)	15.5, (2), (84)	11.6, (4), (724)	15.6, (1), (32)
Schizophrenia	*	*	10, (1), (20)	16.3, (2), (80)	7.8, (1), (51)
VPI	57.1, (3), (56)	*	38.9, (1), (72)	33.0, (6), (784)	34.2, (3), (34)

*indicates no data available

Table 11: Overall prevalence data and sample size by age category

Overall prevalence, (# of observations), (n)

	Mixed (m)	Older (o)	Younger (y)
ADHD	15.9, (5), (383)	*	36, (1), (25)
CHD	75.9, (13), (1,094)	33.8, (2), (65)	77.5, (4), (71)
Cleft palate	11.8, (10), (930)	13.33, (1), (15)	8.8, (3), (91)
Hypotonia	57.0, (4), (90)	*	74.0, (2), (76)
Palatal anomalies	54.6, (12), (919)	35.4, (2), (65)	50.5, (5), (107)
Psychiatric D/O	24.7, (5), (194)	38.5, (2), (65)	89.7, (2), (29)
SMCP	14.0, (8), (853)	*	70.8, (2), (48)
Schizophrenia	6.9, (3), (101)	24.0, (1), (50)	*
VPI	36.0, (9), (869)	66.67, (1), (15)	17.0, (2), (47)

* indicates no data available

Table 12: Overall prevalence data and sample size by geographic location

Overall prevalence, (# of observations), (n)

	North America	Europe
ADHD	39.7, (3), (73)	12.2, (3), (335)
CHD	72.3, (10), (510)	74.6, (9), (727)
Cleft palate	14.0 (9), (439)	9.9, (5), (597)
Hypotonia	66.5, (5), (158)	*
Palatal anomalies	66.1, (11), (460)	44.8, (8), (638)
Psychiatric D/O	49.3, (3), (75)	29.1, (6), (213)
SMCP	33.2, (7), (365)	5.7, (2), (528)
Schizophrenia	6.0, (2), (50)	15.8, (2), (101)
VPI	38.2, (8), (406)	33.0, (4), (532)

* indicates no data available

Table 13: Summary of study data

Study	Year	N	Ascertain	continent	age	M:F	Familial	FamilialP	Denovo	DenovoP
Arnold	2001	20	3	NA	m	9:11	3.00	0.05	17.00	0.05
Botto	2003	43	4	NA	y	21:22				
Digilio	2003	15	5	EU	o	5:10	15.00	0.21	72.00	0.21
Driscoll	1992	15	5	NA						
Du Montcel	1996	12	4	EU	y					
Eliez	2000	4	5	EU	y	2:2				
Feinstein	2002	28	5	NA	m	18:10				
Ford	2000	35	1	NA	m	18:17				
Gerdes	1999	40	3	NA	y	19:21	5.00	0.10	35.00	0.10
GothelfB	2004	51	5	EU	m	33:18				
GothelfC	2003	40	4	EU	m	28:12				
Havkin	2000	36	2	NA	y					
Hopkin	2000	12	3	NA	y	5:7	6.00	0.03	6.00	0.03
Kelly	1993	11	4							
Kerstjens	1999	90	3	NA	m	45:45	7.00	0.19	69.00	0.19
KitsiouTzeli	2004	17	4	EU	m	14:3	3.00	0.04	14.00	0.04
LeanaCox	1996	12	5	NA	m	3:9				
McDonald-McGinnA	1999	250	4	NA	m	120:130				
McDonald-McGinnB	2001	30	4	NA	m	14:16				
Meinecke	1986	8	4		m	6:2				
Murphy	1999	50	4	EU	o	15:35				
Niklasson	2001	32	5	EU	m	13:19				
Oskarsdottir	2004	35	4	EU	m					
Papolos	1996	25	1	NA	y					
Ravnan	1996	31	1	NA	m	15:16				
Ryan	1997	558	4	EU	m	197:202	79.00	0.29	37.00	0.29
ShprintzenA	1978	12	1	NA	m	4:8				
ShprintzenB	1981	39	4	NA	m	15:24				
Swillen	1997	37	5	EU	m	19:18	12.00	0.09	25.00	0.09

P= prevalence

Table 14: Summary of study data by palatal anomalies

Study	CP	CP N	SMCP	SMCP N	VPI	VPI N	Palatal	Palatal N
ShprintzenB	11.00	39.00	28.00	39.00	26.00	39.00	39.00	39.00
Arnold								
Botto	5.00	43.00			6.00	43.00	11.00	43.00
Digilio	2.00	15.00			10.00	15.00	10.00	15.00
Driscoll					1.00	15.00	15.00	15.00
Du Montcel							4.00	12.00
Eliez					2.00	4.00	2.00	4.00
Feinstein								
Ford	1.00	13.00	11.00	13.00	12.00	13.00	12.00	13.00
Gerdes								
GothelfB								
GothelfC							10.00	12.00
Havkin	2.00	36.00	33.00	36.00			35.00	36.00
Hopkin	1.00	12.00	1.00	12.00			2.00	12.00
Kelly							10.00	11.00
Kerstjens	11.00	72.00	12.00	72.00	28.00	72.00	51.00	72.00
KitsiouTzeli	2.00	17.00			2.00	17.00	4.00	17.00
LeanaCox							3.00	6.00
McDonald-McGinnA	20.00	181.00	29.00	181.00	62.00	181.00	111.00	181.00
McDonald-McGinnB								
Meinecke			2.00	8.00	2.00	8.00	4.00	8.00
Murphy							13.00	50.00
Niklasson	5.00	32.00	5.00	32.00			10.00	32.00
Oskarsdottir								
Papolos								
Ravnan	5.00	31.00			8.00	31.00	13.00	31.00
Ryan	47.00	496.00	25.00	496.00	161.00	496.00	233.00	496.00
ShprintzenA	5.00	12.00	7.00	12.00	12.00	12.00	12.00	12.00
Swillen	3.00	37.00						

CP=cleft palate
SMCP=submucosal cleft palate
VPI=velopharyngeal insufficiency
N=number of subjects examined

Table 15: Summary of study data by behavioral traits

Study	Schizo	Schizo N	Psych	Psych N	ADHD	ADHD N
ShprintzenB						
Arnold	2.00	20.00	12.00	20.00	7.00	20.00
Botto						
Digilio			4.00	15.00		
Driscoll						
Du Montcel						
Eliez			2.00	4.00		
Feinstein					13.00	28.00
Ford						
Gerdes						
GothelfB	4.00	51.00	4.00	51.00	21.00	51.00
GothelfC						
Havkin						
Hopkin						
Kelly						
Kerstjens						
KitsiouTzeli						
LeanaCox						
McDonaldMcGinnA						
McDonaldMcGinnB	1.00	30.00	1.00	30.00		
Meinecke						
Murphy	12.00	50.00	21.00	50.00		
Niklasson			20.00	32.00	14.00	32.00
Oskarsdottir						
Papolos			24.00	25.00	9.00	25.00
Ravnan						
Ryan			11.00	61.00	6.00	252.00
ShprintzenA						
Swillen						

Schizo=schizophrenia
Psych=any psychiatric disorder

Table 16: Summary of study data – other traits

Study	CHD	CHD N	Hypotonia	Hypotonia N
ShprintzenB	33.00	39.00	29.00	39.00
Arnold				
Botto	35.00	43.00		
Digilio	1.00	15.00		
Driscoll	8.00	15.00		
Du Montcel	10.00	12.00		
Eliez	3.00	4.00		
Feinstein				
Ford	27.00	35.00		
Gerdes			20.00	40.00
GothelfB				
GothelfC	8.00	12.00		
Havkin			36.00	36.00
Hopkin	7.00	12.00		
Kelly	8.00	11.00		
Kerstjens	60.00	90.00		
KitsiouTzeli	12.00	17.00		
LeanaCox	8.00	11.00		
McDonald-McGinnA	164.00	222.00		
McDonald-McGinnB				
Meinecke	4.00	8.00	2.00	8.00
Murphy	21.00	50.00		
Niklasson				
Oskarsdottir	28.00	35.00		
Papolos				
Ravnan	18.00	31.00	10.00	31.00
Ryan	435.00	545.00		
ShprintzenA	9.00	12.00	10.00	12.00
Swillen	24.00	37.00		

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