An Epidemiologic and Numerical Taxonomic Approach to Multiple Vertebral Segmentation Defects

by

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A Thesis submitted to the Faculty of Graduate Studies of

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ABSTRACT

Skeletal dysplasias involving segmentation defects of the spine and ribs provide a unique nosological challenge. Individuals with multiple vertebral segmentation defects (MVSD) are clinically and radiographically heterogeneous, making genetic counselling for prognosis and recurrence risk difficult.

The goals of our study were to perform epidemiological and radiographical evaluations of MVSD using local cases identified from the Program of Genetics and Metabolism database and to perform numerical taxonomy and discriminant analyses to identify groups of individuals with shared features and to determine which are important differentiating factors between the clusters .

The Manitoba birth prevalence of MVSDs from 1990 to 2003 inclusive was 2.82 per 10,000 total births. One hundred and thirty-one local patients were ascertained. Defects were found at all levels of the spine. Associated malformations were present in 87.8% of cases, and were commonly seen in the craniofacial (61.1%), cardiovascular (43.5%), urinary (38.9%) and gastrointestinal systems (30.5%). Numerical taxonomy based on 110 cases identified seven clusters. Discriminant analysis revealed the variables with the highest discriminating power between the clusters in descending order to be neural tube defects, kidney defects, preaxial involvement of upper limbs, curvature of the spine, ear anomalies, anal anomalies, facial asymmetry and lung anomalies.

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1. INTRODUCTION

1.1 Congenital malformations

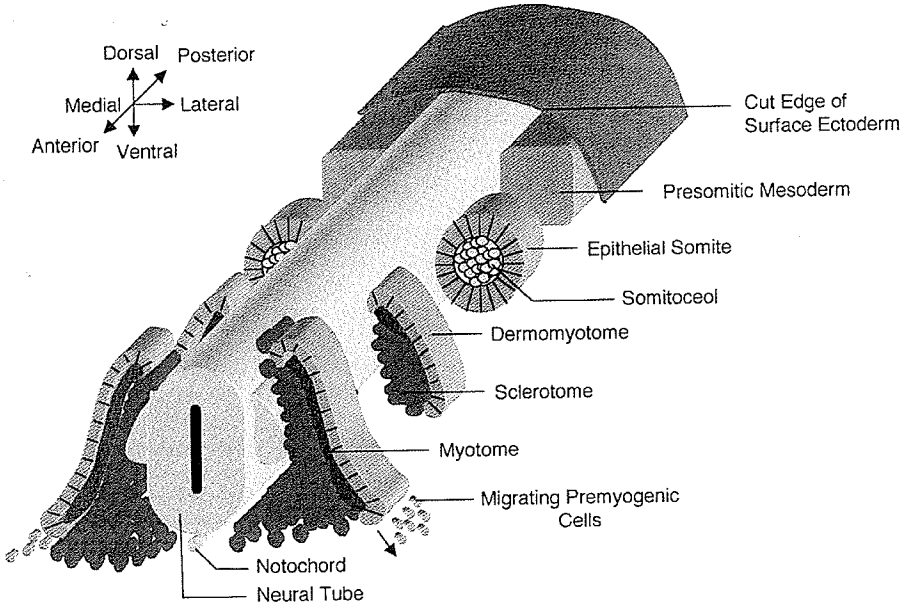
Congenital malformations, occurring in approximately 5% of newborn infants, are a common contributor to childhood morbidity and mortality. Careful evaluation of the frequency, pathogenesis and underlying cause of congenital defects is difficult, especially when they occur in complex patterns of multiple malformations and/or are heterogeneous with respect to etiology. The difficulty in identifying these defects is compounded when the anomaly is not immediately obvious on physical examination, and radiographs or post-mortem examinations are not done. This is certainly the case with respect to the structural defects in the formation and segmentation of the vertebrae that were the focus of this research.

1.2 Development of the vertebrae

1.2.1 Normal development

In humans, the early development of the vertebral column begins with mesenchymal cells, which are drawn to the notochord, forming two columns of tissue known as the paraxial or presomitic mesoderm. Towards the end of the third week of development, the paraxial mesoderm begins to divide into smaller units called somites (Figure 1). These somites continue to form in a cranio-caudal fashion. Thirty-eight pairs of somites form during development days 20-30 post-conception, which is why this time is also known as the somite period of development[1].

Figure 1: Process of somitogenesis in the paraxial mesoderm[2]



A "clock" is thought to control the cycling process that determines the formation of the somites. Each somite differentiates into three different types of tissue: sclerotome, myotome and dermotome. The sclerotome becomes the vertebrae and ribs, while the myotome and dermatome become developing muscle and dermis. At four weeks post-conception, the sclerotomes surround the notochord. The densely packed mesenchymal cells move cranially to become the intervertebral discs. The remaining cells form the centrum of the vertebra. Each vertebral body forms from the cranial and caudal halves of two adjacent sclerotomes. The notochord begins to degenerate as the vertebral body develops around it and it expands laterally to form the gelatinous centers of the intervertebral discs. The mesenchymal cells also form the vertebral arch surrounding the neural tube and the costal processes, which become ribs. At 6 weeks of development, chondrification centres appear in each mesenchymal vertebra and the spinous and transverse processes begin to develop. The ribs also form from mesenchymal cells making up the costal processes of the thoracic vertebrae[1]. Ossification of the vertebrae

begins at about 9 weeks of gestation. This process is well underway by 12 weeks, and, by birth, only the coccyx has not ossified. The final product of this precisely timed process is a segmented spinal column. The spinal column is typically categorized into four levels: cervical, thoracic, lumbar and sacral regions, in addition to the coccyx. There are a set number of vertebral bodies in each region, though these numbers may vary slightly in some individuals. Approximately 95 percent of individuals have seven cervical, twelve thoracic, five lumbar and five sacral vertebrae. The other five percent have one to two more or one less vertebrae[1].

1.2.2 Abnormal development

Vertebral segmentation defects are congenital structural bony defects of the vertebral bodies that occur when there is a deviation from the normal development of the somites as described above. Vertebral defects can result from essentially two events. The first is failure of formation, which may result in a complete or partial absence of vertebrae. Partial failure of a vertebral body to form may result in a hemivertebra (half of a vertebral body) or a wedge vertebra (more well-formed than a hemivertebra, yet is noticeably deficient unilaterally). A sagittal cleft may be present within a vertebral body, resulting in what is known as a butterfly vertebra. As well, a coronal cleft may be present, and is observed as a deficiency between the vertebral body and the posterior processes. The second mechanism in which a vertebral defect may occur is failure of vertebral segmentation. Failure of normal segmentation may result in several types of defects. The first is a block vertebra, which results from the failure of adjacent vertebral bodies to separate. A block vertebra may include two or more vertebral bodies fused together.

Failure of vertebral segmentation may also occur unilaterally, resulting in a vertebral bar. There may also be variations of vertebral formation and segmentation defects occurring together. Table 1 lists the common types of vertebral defects that may be observed.

Table 1: Anomalies of the vertebral bodies[3]

1. Absence
    a. Complete
    b. Partial (hemivertebra)
2. Hypoplasia
    a. Wedge vertebra (Anterior or lateral wedge)
    b. Dorsal hemivertebra
    c. Lateral hemivertebra
    d. Posterior corner hemivertebra
3. Fusion
    a. Complete (block vertebra)
    b. Partial (vertebral bar)
    c. Transitional vertebrae (taking on characteristics of vertebra in adjoining level of spine)
4. Cleft vertebra
    a. Sagittal (butterfly vertebra)
    b. Coronal

1.3. Vertebral segmentation defects

1.3.1 Epidemiology

Vertebral segmentation defects are not common congenital anomalies; as such, their epidemiology has not been extensively studied. Shands and Eisberg studied radiographs of the spine as part of a tuberculosis study in 1955. They examined lower cervical, thoracic and upper lumbar vertebrae, and found the incidence of anomalies to be 0.5 per 1,000 spines[4]. This is an underestimate as Wynne-Davies points out that the most severe segmental defects would have been excluded from this study as result of high

mortality at young age[5]. To the best of our knowledge, only one population-based study has reported birth prevalence data for vertebral segmentation defects. In 1994, Martinez-Frias and colleagues reported that malformations of the spine and ribs were present in 2.51 in 10,000 live births in their Spanish registry, which contained records from 1,052,517 births spanning over 17 years[6]. Their statistic included both individuals with single vertebral and multiple vertebral anomalies. Martinez-Frias and Urioste also reported, based on the Spanish registry, that multiple malformations (at least two vertebrae or at least one vertebra and one rib involved) of the ribs and vertebrae were present in 1.17 in 10,000 live births[7]. A limitation to the prevalence data reported in these studies is that they are likely an underestimate, as terminations of pregnancy and stillbirths were not included. A more precise estimate of prevalence of multiple vertebral segmentation defects (MVSD) including these pregnancy outcomes was not available until this work was initiated. Further study was clearly required to further elucidate the epidemiology of these anomalies.

1.3.2 Classification

Aside from the epidemiologic challenge, skeletal dysplasias involving the spine and/or the ribs also provide a nosological or classification challenge. This group of skeletal dysplasias has been described under various terms, such as spondylocostal dysostosis, spondylothoracic dysostosis, costovertebral dysplasia, Jarcho-Levin syndrome, Klippel-Feil syndrome, and short-trunked dwarfism. This group shares multiple vertebral segmentation defects in common; however, the nature and extent of these defects differ widely as do the associated anomalies. The difficulty in

characterizing these malformations stems from the high degree of phenotypic, as well as genetic heterogeneity within the group. Manifestations of costovertebral segmentation defects range from a normal appearance to severe spine and rib defects leading to respiratory insufficiency and death in infancy. Additional malformations not related to the spine and ribs may be present in some cases.

1.3.2.1 Historical classification

In order to understand the fragments of existing classification systems and how MVSD have come to be known under so many different titles, knowledge of the history of these defects is required. A syndrome characterized by multiple vertebral segmentation defects throughout the spine was first described by Saul Jarcho and Paul Levin in 1938. In their case report, they described two siblings with severe vertebral defects at many levels of the spine[8]. They had greatly shortened spines, scoliosis, and extensive malformations of the entire spine. At the time of this publication, these defects were most closely correlated with the cervical spine fusions characteristic of Klippel-Feil syndrome. Both individuals died in infancy. These cases were quite different from previously reported cases of Klippel-Feil syndrome in that the sibs had involvement of several levels of the spine and were associated with poor survival due to respiratory insufficiency. The constellation of features present in these siblings is now known as spondylocostal dysostosis. Interestingly, the name Jarcho-Levin syndrome is typically associated with different features than originally described in Jarcho and Levin's patients. The main feature of what became known as Jarcho-Levin syndrome is a crab-like configuration of the ribs, with most ribs originating from a similar location on the vertebral column.

Jarcho and Levin did describe their cases as each having a thoracic cage with several ribs fused near the spine and originating from the same location on the vertebral column; however, in the cases now known as Jarcho-Levin syndrome, scoliosis is not severe, in spite of a severely restricted thorax.

In 1968, Rimoin and colleagues proposed the term spondylocostal dysplasia to describe a family with short-trunked dwarfism associated with multiple vertebral and rib malformations[9]. The authors note the difference between the family they reported and previous reports of multiple vertebral segmentation defects, including that of Jarcho and Levin. Autosomal dominant inheritance and normal survival was documented in this family. The members of this family were much more mildly affected than cases previously described. Rimoin and colleagues were the first to identify the problem of clinical and genetic heterogeneity within the group with multiple vertebral segmentation defects. They proposed two groups of disorders: a clinically mild autosomal dominant form (their cases) and a severe autosomal recessive form that result in early death (similar to Jarcho and Levin's cases).

Solomon and colleagues (1978) agreed with the classification proposed by Rimoin and colleagues and documented differentiating factors between the groups: distribution and extent of skeletal anomalies, inheritance and survival[10]. They proposed that the term spondylothoracic dysostosis be applied to those patients with the characteristic crab-like or fan-like configuration of the ribs without intrinsic rib anomalies. The term spondylocostal dysostosis should be used to refer to patients with intrinsic rib changes, regardless of mode of inheritance.

1.3.2.2 Clinical classification

The most recent clinical classification paper was published in 1996[11]. In this evaluation of MVSD, at least three distinct entities were identified from 141 patients (26 were newly described, the remaining 115 were literature cases). The first group included those with the radiographically distinct symmetric crab-like thorax (described by Pérez-Comas and Garcia-Castro in 1974). Vertebral segmentation defects usually involved the entire spine, and other major congenital malformations were usually absent. Death usually occurred before the age of two years due to respiratory insufficiency (19/20 cases). Autosomal recessive inheritance was suggested, and Puerto Rican ethnic background was common (14/20). This group is commonly referred to as the Jarcho-Levin phenotype.

The second group was referred to as spondylothoracic dysostosis. This group was also characterized by autosomal recessive inheritance, but patients in this category did not have the crab-like thorax. Individuals in this group showed variable mortality: either death in infancy or survival into adulthood with minimal symptoms. Associated anomalies were usually the cause of death in lethal cases.

The third and final group described had an autosomal dominant inheritance pattern and is now commonly referred to as spondylocostal dysostosis. These cases have a normal lifespan and very few associated anomalies. They usually present with scoliosis, lower back pain and decreased mobility of the spine. Multiple vertebral segmentation defects and/or rib anomalies are common throughout the spine although they tend not to be as severe as those seen in the previously described groups.

Mortier and colleagues identified the difficulty in classifying seemingly sporadic cases with associated anomalies[11]. They commented that associated anomalies may involve both mesodermally and ectodermally derived structures and noted that the site of vertebral involvement usually corresponds with the body segment in which the associated malformation occurred. They proposed that those cases with associated anomalies likely fall into the spondylothoracic dysostosis category or into a different nongenetic condition. They postulated that the chances for survival are probably more related to pulmonary status and presence of complex heart malformation than to the skeletal anomalies.

1.3.2.3 Associated malformations

Although Mortier and colleagues identified the difficulty in classifying patients with anomalies additional to MVSD, no further extensive research has been undertaken[11]. In their study, they did an evaluation of the anomalies that were observed in their 26 local patients and found that renal agenesis was the most common anomaly, seen in 23% of patients. Kidney anomalies were followed by imperforate anus (19%), limb anomalies (19%), hernias (15%), pelvis anomalies (15%), heart anomalies (11%) and lastly, ambiguous genitalia (4%)[11]. The incidence of anomalies associated with literature cases of MVSD (n=61) had been examined previously in another publication[12]. This study found that 15% of patients also had neural tube defects, 13% had hernias, 10% had anal defects, 8% had lower limb anomalies and finally 7% had urinary anomalies. Interestingly, the study by Mortier et al. did not find neural tube defects to be common, whereas Karnes et al. did not identify heart defects as being present in their study population[11,12].

Several entities involving MVSD and additional anomalies have been described in detail. Those most commonly known are VACTERL (vertebral-anal-cardiac-tracheoesophageal-renal-limb) association, MURCS (mullerian hypoplasia/aplasia-renal agenesis-cervicothoracic somite dysplasia) association, and OAV (oculo-auriculo-vertebral) spectrum. An entity involving MVSD with anal and urogenital anomalies has been described in a Mennonite population and is known as Casamassima-Morton-Nance association (CMN)[13]. Another entity has been described as COVESDEM or costovertebral segmentation defect with mesomelia, and is characterized by short limbs[14].

1.3.2.4 Radiographic classification

Erol and colleagues took a different approach to classification of vertebral segmentation defects in their study published in 2004[15]. They examined 84 cases of vertebral segmentation defects by radiologic screening and specifically quantified the extent of vertebral involvement. The rationale behind this approach was that, in order to identify the etiological factors involved in the development of vertebral anomalies, the patients should be radiographically characterized based on the extent of the disruptions. Based on this study, they proposed three types of segmental malformations: global defects affecting all vertebrae, multiple contiguous defects and multiple single defects resulting from a transient disruption.

The most recent paper published on the subject of MVSD by Takikawa and colleagues examined the radiographic and clinical findings of spondylocostal dysostosis[16]. They highlighted the fact that still no clear definition of spondylocostal dysostosis exists and that little information is available regarding the clinical and

radiographic features. They set precedent by defining spondylocostal dysostosis as a congenital spinal disorder involving at least two vertebral anomalies associated with rib anomalies, but without the crab-like configuration of ribs[16]. Their investigation of 30 patients with two or more vertebral defects associated with rib defects yielded the findings that patients had at least four vertebral anomalies, that block vertebrae were related to short stature after 12 years of age, and that short stature was not always present at birth. No previous studies had reported on body height in association with costovertebral anomalies.

1.3.2.5 Cluster analysis classification

In 1986, Ayme and Preus performed a cluster analysis of published cases of spondylocostal dysostosis, spondylothoracic dysplasia and Jarcho-Levin syndrome that utiltized similarities or differences in demographic, clinical and radiological findings[17]. This analysis attempted to distinguish between different presentations of MVSD in such a heterogeneous group. They were successful in identifying two main clusters plus a third cluster of two cases of Robinow syndrome (costovertebral segmentation defects with mesomelia). The severe form of spondylothoracic dysplasia, with autosomal recessive inheritance, severely abnormal chest, respiratory insufficiency and death in infancy was differentiated from the mild form, which included patients with both autosomal recessive and dominant inheritance. These groups are similar to those described by Rimoin and colleagues; however, Ayme and Preus emphasized the genetic heterogeneity that remained within their second group, which provides an opportunity for additional research[9]. Unfortunately, Ayme and Preus fail to discuss their cluster analysis parameters

in depth. Prognosis and recurrence risk remained difficult to determine and counselling a challenge.

1.4 Numerical taxonomy

Numerical taxonomy, also known as cluster analysis, is a classification technique that uses mathematical equations to separate a large heterogeneous group of data points or individuals into smaller, more homogenous subgroups. Numerical taxonomy seeks to separate data into groups where either there is no prior knowledge of grouping or where previous known groupings are ignored. Numerical taxonomy methods were traditionally developed for use in botany and zoology; however, they have also proved to have applications to medical sciences, including the study of congenital malformations. These applications have proven to be particularly useful in the areas of syndrome identification and delineation[18]. For example, one medical researcher, Pinsky, used these techniques to further classify the hand-foot-ectodermal dysplasias[19], while others, Preus and MacGibbon, used these methods to study syndrome delineation in four recognized syndromes[20].

There are two main types of hierarchical clustering techniques: agglomerative and divisive. Agglomerative clustering utilizes the similarities between cases and starts by fusing initial cases until one large group is formed, while divisive clustering identifies the differences between data points and begins with one large group which then divides into two groups and continues on until each data point is separate. Agglomerative clustering is the approach now more commonly used. In this case, the data points consist of individuals in a given population, while the similarities measured are observations of

characteristics or features referred to as attributes. These attributes may be coded either in binary or multi-state form. The compiled information for each individual encodes a complex data matrix.

This data matrix may then be used to quantify the similarities between data points (individuals). This is done through complex computer calculations that create similarity coefficients. Once the coefficients are calculated, individuals are sorted through a strategy that places those with the highest degree of similarity together in a cluster. A graphical representation of the resulting clusters is known as a dendrogram.

Interpretation of the dendrogram is the final step in a numerical taxonomy analysis. The clusters are defined by shared similarities. Both similarities and differences may provide hints as to the underlying biological relationships and may prove to be the first step in identifying the etiological factors responsible for specific traits.

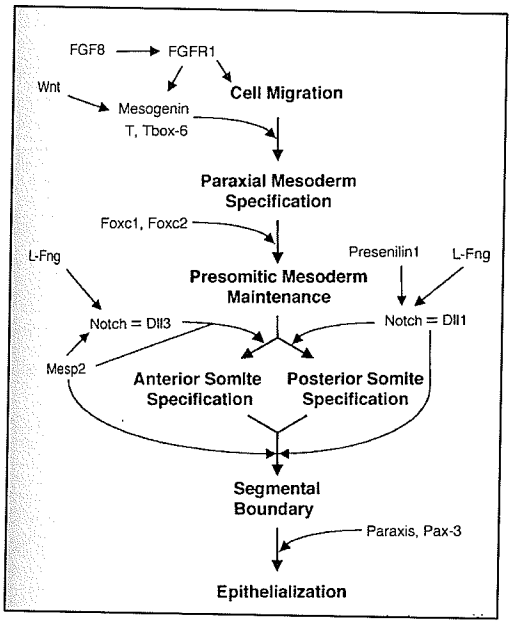
1.5 Molecular genetics of MVSD

1.5.1 Notch signalling

In order to delineate the causes of MVSD, it is important to identify the developmental pathways involved in the formation and segmentation of vertebrae. In the past decade, we have learned much more about the genes involved in this process. The whole process begins with specification of cells in the anterior node that will form the paraxial mesoderm, which is dependent on signaling from wingless-type (Wnt) genes and fibroblast growth factor (Fgf) genes. There are two ongoing processes during somitogenesis: caudal extension of the embryo and the segmentation of the paraxial mesoderm. These processes lead progressively to somite formation. The "clock and

wavefront" model has been suggested for many years and has gained considerable experimental support for explaining both the temporal and spatial regulation of somitogenesis[21]. Downstream targets that influence the presomitic mesoderm include brachyury-T and Tbox6 homeobox transcription factors and mesogenin, while foxhead transcription factors aid in maintaining the presomitic mesoderm. Other key processes are the specification of the anterior and posterior components of the somite and the formation of the boundaries that offset each somite from the presomitic mesoderm and the epithelialization process. A schematic of these interacting processes is depicted in Figure 2.

Figure 2: Gene interactions known to play a role in somitogenesis[2]



The Notch signalling pathway is, at present, the key pathway with a known role in the regulation of somitogenesis. The functions of several genes and their products have been partially elucidated, along with their interactions with other pathway players. Recently, Wnt and Fgf pathways have also been linked to regulating segmentation,

suggesting that a complex interconnected network of several signalling pathways is required for this process.

Notch signaling regulates a wide variety of cell types during specification, patterning and morphogenesis of different tissues and organs in invertebrates and vertebrates. In humans, the Notch genes themselves encode for large transmembrane receptors that recognize two sets of ligands: delta and serrate/jagged. The affinity Notch has for its ligands is mediated by lunatic fringe through a process of differential glycosylation. Upon ligand binding, the Notch receptor undergoes proteolytic cleavage at the membrane level, leading to translocation of the intracytoplasmic domain to the nucleus. This protein, along with the transcription factor RBPjk, activates the cycling genes[22].

All genes initially characterized were found to be targets for the Notch pathway, although recently, a second group of genes in the Wnt pathway was described in mice[23]. As Lunatic Fringe is rhythmically produced, this allows for the oscillation patterns seen in the Notch downstream target genes. As well, Dubrulle and Pourquie described FGF signaling within the presomitic mesoderm[24]. Travelling in a cranio-caudal fashion, decreasing levels of FGF and Wnt signaling would eventually lead to a critical threshold known as the determination front. This is believed to mark a developmental change in the segmentation process. The interaction between the "clock" and these gradients is thought to define the segment size[25]. The retinoic acid pathway has also been shown to have a gradient, running in the opposite (caudal to rostral) direction to the FGF and Wnt gradient[25]. The mechanism in which the segmentation clock and the expression gradients interact remains unknown, although a mechanism suggested includes simply a negative

feedback loop with a long delay due to transcription and translation of downstream genes. Given the complexity of the system controlling MVSD, there are certainly many places where it can be disrupted.

1.5.2 Molecular classification in humans

Most research in this area has examined these pathways in mice. The effects of these signalling pathways and the genes involved in humans are beginning to be elucidated. Several genes have been found to cause these developmental defects in humans; therefore, a molecular classification for MVSD has also been devised.

1.5.2.1 Delta-Like 3 (*DLL3*)

The first gene mutation found to cause MVSD in humans was a mutation in *DLL3*, a Notch pathway ligand. *DLL3* was investigated in humans with MVSD following the discovery of the radiation-damaged *dll3* knockout mouse also known as the "pudgy" mouse, named after its phenotype of having a short thorax as a result of MVSD. *DLL3* mutations were found to be responsible for a specific autosomal recessive form of spondylocostal dysostosis in humans in 1999[26]. This form is known as spondylocostal dysostosis type 1. Controversy surrounds this naming scheme as Martinez-Frias points out that the name is arbitrary and continues to confuse those aiming to classify these entities. She proposes using the molecularly descriptive title of "DLL3-related axial skeletal field defects"[27]. Radiological analysis of individuals with *DLL3* mutations typically reveals abnormal segmentation throughout the spine, with the most dramatic abnormalities seen in the thoracic vertebrae. There is typically overall symmetry of the

thorax. Additional anomalies appear to be rare in the instance of *DLL3* mutations, and there appears to be little clinical heterogeneity between individuals with *DLL3* mutations. Currently, 24 different *DLL3* mutations from 26 families have been identified[28]. From the time that *DLL3* mutations were identified to cause MVSD, other genes from the Notch developmental pathway have been investigated excitedly as potential candidates causing these defects.

1.5.2.2 Mesoderm Posterior 2 (*MESP2*)

One of these candidate genes was *MESP2*[29]. After excluding a *DLL3* mutation in a large consanguineous family, a genome-wide scanning strategy was used, which revealed linkage to 15q21.3-15q26.1. Subsequently a 4 bp duplication in the human *MESP2* (mesoderm posterior 2) gene was identified. On radiographic analysis of the two affected family members homozygous for this mutation, it was noted that there were similarities to the defects seen in patients with mutated *DLL3*. However, the lumbar vertebrae appeared more angular and irregular than those seen in *DLL3* cases. The *MESP2* mutation is also recessive, as the parents of these individuals are heterozygous for the mutation and show no axial skeleton malformations. In the mouse, *Mesp2* plays a key role in somitogenesis by establishing rostro-caudal polarity through the Notch signalling pathway. Until very recently, upon investigating the status of this gene in other patients with MVSD, no further mutations in *MESP2* had been identified. Recently, Cornier et al. suggested that mutations in *MESP2* were the cause of the classical Puerto Rican form of Jarcho-Levin syndrome[30]. This group analyzed 12 Puerto Rican families with Jarcho-Levin syndrome probands and identified a homozygous recessive nonsense

mutation (E103X) in 10 individuals. Three individuals were compound hetergozygous for another nonsense mutation (E230X) or a missense mutation (L125V) as well as the E103X mutation.

1.5.2.3 Lunatic Fringe (*LFNG*):

Lunatic fringe (*LFNG)* was initially identified as a strong candidate gene as the *Lfng* null mouse has a phenotype that includes MVSD. A homozygous mutation in the *LFNG* gene has been identified in one human patient with consanguineous parents to be the cause of spondylocostal dysostosis[31]. This resulted in the discovery of a missense mutation in a highly conserved phenylalanine region close to the active site of the enzyme. The authors determined that this mutation, in the homozygous state, led to improper localization in the cell and the inability to modulate Notch signalling. Their patient presented with MVSD, long slender fingers and camptodactyly of the left index finger.

1.5.2.4 Hairy-and-enhancer of split 7 (*HES7*)

Most recently, a fourth gene causing spondylocostal dysostosis in humans was discovered. A homozygous missense mutation coding for the DNA-binding domain of the Hes7 protein was identified in a male proband with a bell-shaped symmetrical and shortened thorax, lumbo-sacral myelomeningocele, ectopic stenotic anus, hydrocephalus and talipes born to consanguineous Caucasian Mediterranean parents[32]. *HES7*, like *LFNG*, was found to be a direct target of the Notch signalling pathway. In *hes7*-null mice, somites are not properly segmented leading to severely disorganized vertebrae and ribs.

Lfng was found to be expressed continuously throughout the presomitic mesoderm instead of oscillating, suggesting that *hes7* controls the cyclic expression of *lfng*.

1.5.2.5 *JAGGED1* and *NOTCH2*

Mutations in *JAGGED1* and *NOTCH2* genes in the Notch signalling pathway are known to cause Alagille syndrome, an autosomal dominant multi-system disorder[33,34]. Vertebral malformations are often seen in patients with Alagille in the form of butterfly vertebrae, though these vertebral malformations are commonly much less severe than in the cases of typical spondylocostal dysostosis. Many other systems are involved with features including bile duct paucity, congenital cardiac defects, posterior embryotoxon in the eye and typical facial features. As Alagille syndrome is known to be caused by an autosomal dominant mutation, it differs from the other cases of MVSD known to be the result of recessive mutations and may be further away on the disease spectrum.

In spite of the recent advances in the discovery of genes causing spondylocostal dysostosis within the Notch pathway, the areas left unexplored are vast. For instance, Sparrow and colleagues estimate that only 20-25% of all autosomal recessive cases of spondylocostal dysostosis can be explained by the three known genes[31]. This leaves a large gap in the known etiology of these MVSD. Cases with malformations involving other systems in addition to the axial skeleton have not even begun to be explained. As well, the cause of autosomal dominant cases of MVSD, if such an inheritance pattern truly exists, remains largely unknown. There has been a report of one family that exhibited several generations of an autosomal dominant pattern of inheritance of MVSD

that was subsequently determined to be the result of two different *Dll3* mutations running in the family[35]. Both homozygotes and heterozygotes existed within this family leading to confusion when deciphering the mode of inheritance on a clinical basis. It remains to be conclusively determined whether or not there is a causative autosomal dominant mutation.

1.6 Environmental and other factors

There is also some evidence that ethnicity may play a role in the "susceptibility" to having MVSD. Although, there is little data for comparison, it appears that Canadian Inuit skeletons show an unusually high frequency of sagittal cleft vertebrae[36]. It is unclear whether this is due to genetic or environmental factors.

Aside from the genetic unknowns, environmental factors have also been implicated in having an association with MVSD. Excessive maternal consumption of alcohol during pregnancy is known to cause fetal alcohol syndrome (FAS) In one study, as many as 43% of individuals with FAS have cervical vertebral anomalies[37]. Rib anomalies may also be associated. Poorly controlled pregestational diabetes is another factor associated MVSD, in particular lower spine abnormalies, as well as other major congenital malformations[38-40]. Clearly it is important that many factors be examined in order to further delineate the etiologies of this complex group of patients. Classification of these individuals into more homogenous groups is the first step in this process.

1.7 Objectives

The objectives of this study were as follows:

1. To ascertain a population of patients with multiple vertebral segmentation defects and to determine the birth prevalence of these defects in the Manitoba population
2. To describe the demographic and radiographic features of this population
3. To establish a coding system for costovertebral and associated anomalies to allow for consistent scoring of the nature and severity of these defects
4. To carry out a refined cluster analysis incorporating these scores
5. To perform discriminant function analyses to identify an algorithm that could be utilized to differentiate between clusters
6. To evaluate the robustness of the cluster analysis and the algorithm

1.8 Hypothesis

The hypothesis tested in this study was that distinct subgroups, with respect to costovertebral and associated malformations, exist within the Manitoba population of individuals with MVSD, and that these could be identified by detailed coding of the malformations and subsequent cluster analysis. The discriminating variables, those malformations important for differentiating between the clusters, could be determined through discriminant analysis.

2. METHODS

2.1 Study design and inclusion criteria

The design of this study was that of a retrospective, population-based evaluation. Ethics approval for the study entitled "Characterization of Spondylocostal/ Spondylothoracic Dysostosis and other Costovertebral Malformations" was obtained from the University of Manitoba Research Ethics Board prior to its commencement (Appendix 1). As this study was population-based, efforts were made to achieve as complete ascertainment of cases of multiple vertebral segmentation defects (MVSD) as possible. In order to be included in the study, each case must have had at least two malformed vertebrae. Thus the minimum inclusion criterion for a case could be the fusion of two vertebrae in the form of one block vertebra or one vertebral bar. The definition of MVSD in the context of this study reflects the inclusion criterion. Cases with a single rib anomaly in addition to one vertebral malformation were excluded.

2.2 Case ascertainment

As no single registry for congenital anomalies existed in Manitoba at the time of this study, complete ascertainment of all patients with MVSD would not likely be feasible. In order to identify as many cases as possible, the Genetics and Metabolism Program database was used as the primary resource for case ascertainment, accompanied to a lesser extent with cases involved in a larger study of neural tube defects, as well as cases seen in the Department of Pediatric Radiology.

Cases to be included in this study were ascertained primarily from the Genetics and Metabolism Program database at the Health Sciences Centre in Winnipeg. The Program in Genetics and Metabolism is the sole genetics referral service in Manitoba and its patient database is therefore an excellent resource for population-based studies. This database contains details of all patients referred from 1990 onward. Prior to 1990, the database is not considered complete, as there is the potential for only one member of each kindred to be recorded. Database queries were completed for the following terms and conditions likely to be associated with MVSD:

- Spondylocostal/thoracic dysostosis
- Jarcho-Levin syndrome
- Costovertebral
- Rib
- Vertebral anomaly
- Butterfly vertebra
- Fused vertebra
- VATER[a]
- VACTERL[b]
- Insulin-dependent and non-insulin dependent diabetes mellitus, gestational diabetes
- Diabetic embryopathy
- Caudal regression syndrome
- Klippel-Feil syndrome
- Hemifacial microsomia/OAV[c] spectrum/Goldenhar syndrome
- Alagille syndrome
- Congenital scoliosis, kyphosis, lordosis
- MURCS association[d]
- DiGeorge syndrome/22q11.2 deletion
- Robinow/COVESDEM[e]
- Neural tube defect (excluding encephalocele, anencephaly, iniencephaly)

[a]vertebral, anal, tracheoesophageal, and radial ray/renal defects, [b]vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects, [c]oculo-auriculo-vertebral, [d]mullerian duct aplasia, unilateral renal aplasia and cervicothoracic somite dysplasia, [e]costovertebral segmentation defects with mesomelia

In addition to the searches of the Genetics and Metabolism database, cases were also obtained from two other sources. First, cases of MVSD seen by Dr. Martin Reed in the Department of Pediatric Radiology were referred to the study. These patients were either located through the Department of Pediatric Radiology database special interest codes: 30.1651 (spondylocostal dysostosis) and 32.1651 (spondylothoracic dysplasia) or were seen over the course of the study period by Dr. Reed.

A third source of ascertainment was Dr. Jane Evans' ongoing study of neural tube defects. Cases of neural tube defects with MVSD were identified and utilized for the study. Duplication of cases obtained by all methods of ascertainment was avoided by cross-referencing dates of birth.

2.3 Data collection

Genetics and Health Sciences Centre charts were reviewed for all cases of MVSD ascertained. Margaret Coggrave previously completed chart review of cases ascertained through the neural tube defect study. Data collected for all cases included demographic information, maternal and family history, pregnancy history, specific radiographic defect details, additional malformations and current health status. Appendix 2 shows the datasheet used to collect information on cases, as well as the rationale for collection of each piece of information and coding of a sample literature case.

When available, radiographs of cases were also reviewed. Details of specific vertebral and rib malformations were recorded (Figure 3). Radiographic report findings were used to complete this datasheet if films were unavailable. All films difficult to

interpret were coded after consultation with Dr. Martin Reed, Head of Pediatric Radiology.

Figure 3: Datasheet utilized to record radiographic findings

Study ID:

Level[1]	Ex'd[2]	Inv'd[3]	Description	Ribs L		Ribs R	
C1							
C2							
C3							
C4							
C5							
C6							
C7							
T1							
T2							
T3							
T4							
T5							
T6							
T7							
T8							
T9							
T10							
T11							
T12							
L1							
L2							
L3							
L4							
L5							
S1							
S2							
S3							
S4							
S5							
Other							

[1]level of spine, [2]examined, [3]involved

☐ Films examined ☐ Radiology report only ☐ Reviewed with Martin Reed

2.4 Analysis of prevalence

All Manitoba cases recorded between 1990 and 2003 inclusive were utilized, along with Manitoba birth data from these years, to calculate the minimum birth prevalence of MVSD. 1990 was used as a cut off as this was the year the Genetics and Metabolism Program began recording all referrals in its computerized database. Birthplace was recorded at the time of chart review and special care was taken to exclude patients with MVSD born outside of Manitoba from this calculation.

2.5 Numerical Taxonomy

One hundred and ten cases of MVSD were utilized for the numerical taxonomy analysis. Using these cases, a variable coding sheet was developed based on associated major or minor malformations that were present in five to 95 percent of the cases. These were deemed to be variables that were most likely to have discriminating power between the clusters. The variables as well as their corresponding data type are seen in Table 2.

Table 2: Variable coding sheet utilized for cluster analysis:

Variable	Type of coding	Details
Central nervous system		
Hydrocephalus	Multi-state	0=absent, 1=unknown/unspecified cause, 2=Dandy-Walker malformation, 3=Arnold-Chiari malformation (excluding those associated with neural tube defects)
Neural tube defect	Multi-state	0=absent, 1=spina bifida +/- tethered cord (excluding occulta), 2=meningocele, 3=myelomeningocele, 4=syringomyelocele, 5=spinal dysraphism, 6=encephalocele, iniencephaly, exencephaly
Cardiovascular		
Simple/complex cardiac defect	Multi-state	0=absent, 1=simple defect, 2-complex defect
Single umbilical artery	Binary	0=absent, 1=present
Respiratory		
Lung abnormality	Multi-state	0=normal, 1=lung lobation abnormality, 2=abnormal trachea/bronchi
Tracheoesophageal fistula +/- esophageal atresia (TEF +/-EA)	Multi-state	0=normal, 1=TEF+EA, 2=TEF-EA
Gastrointestinal		
Anal defect	Multi-state	0=normal, 1=abnormal placement/anal stenosis/unspecified anal defect, 2=imperforate anus
Rectal fistula	Binary	0=absent, 1=present
Other gastrointestinal defect	Binary	0=absent, 1=present
Urogenital		
Positional kidney defect	Multi-state	0=normal, 1=ectopic kidney, 2=horseshoe kidney
Kidney a/dysgenesis	Multi-state	0=normal, 1=unilateral involvement, 2=bilateral involvement (does not include hydronephrosis or ureter involvement)
Obstructive urinary abnormality	Multi-state	0=absent, 1=unilateral, 2=bilateral
Ureter defect	Multi-state	0=absent, 1=unilateral, 2=bilateral
Lower urinary system anomalies	Multi-state	0=absent, 1=bladder defect, 2=urethral defect
Abnormal internal genitalia	Binary	0=absent, 1=present
Abnormal external genitalia	Binary	0=absent, 1=present
Musculoskeletal		
Cervical spine involvement	Binary	0=absent, 1=present
Thoracic spine involvement	Binary	0=absent, 1=present
Lumbar spine involvement	Binary	0=absent, 1=present
Sacral spine involvement	Binary	0=absent, 1=present
Rib involvement	Binary	0=absent, 1=present
Short trunk	Binary	0=absent, 1=present
Inguinal hernia	Binary	0=absent, 1=present
Sternal abnormality	Binary	0=absent, 1=present
Curvature of spine	Multi-state	0=normal, 1=scoliosis, 2=kyphosis, 3=lordosis, 4=kyphoscoliosis
Congenital hip dislocations	Binary	0=absent, 1=present
Lower limb anomaly	Multi-state	0=normal, 1=digital anomaly, 2=long bone involvement
Postitional foot deformity	Multi-state	0=normal, 1=unilateral, 2=bilateral
Preaxial involvement	Multi-state	0=normal, 1=preaxial polydactyly/triphalangeal thumb/dislocated thumbs/widening of webspace, 2=hypoplastic thumbs, 3=absent thumbs, 4=hypoplastic radius, 5=absent radius
Craniofacial		
Cleft palate	Multi-state	0=normal, 1=high arched palate, 2=cleft palate
Cleft lip +/- palate	Multi-state	0=normal, 1=unilateral, 2=bilateral
Skin/hair	Multi-state	0=normal, 1=hirsute/synophrys, 2=hemangioma
Facial asymmetry	Multi-state	0=normal, 1=mild asymmetry, 2=asymmetry
Eye anomaly (coloboma, esotropia)	Binary	0=absent, 1=present
Dysmorphic eye finding (ptosis, epicanthal folds, prominent)	Binary	0=absent, 1=present
Hypertelorism	Binary	0=absent, 1=present

Abnormal palpebral fissures	Multi-state	0=normal, 1=downslanting, 2=upslanting, 3=small
Neck anomaly	Multi-state	0=normal, 1=short neck, 2=webbed neck
Decreased neck mobility	Binary	0=absent, 1=present
Abnormal ear position	Multi-state	0=normal, 1=posteriorly rotated, 2=lowest
Malformed ears	Multi-state	0=normal, 1=pits/tags, 2=dysplasia, 3=microtia, 4=anotia
Nose anomaly	Multi-state	0=normal, 1=flat nasal bridge/thick nasal root, 2=nostril abnormality
Mouth anomaly	Binary	0=absent, 1=present
Micrognathia	Binary	0=absent, 1=present
Abnormal head shape	Binary	0=absent, 1=present
Other		
Hearing loss	Binary	0=absent, 1=present

The matrix of variables in the 110 cases was then subjected to hierarchical cluster analysis using the classification function available[41]. The agglomerative approach to hierarchical cluster analysis attempts to identify groups of cases based on specified variables using an algorithm that starts with each case as a separate group and fuses groups until only one large one remains. The idea is to create as little heterogeneity within groups as possible. For every combination of cases, a similarity or dissimilarity measure is calculated. For this analysis, the similarity method chosen was Squared Euclidian distance. For the clustering strategy, each pair of cases or clusters fused had the minimum increase in the within-group error sum of squares of all possibilities. This method is known as Ward's sorting strategy. Number of clusters was determined manually from the resulting dendrogram, as the SPSS hierarchical cluster analysis does not support the optimal number of clusters function.

The robustness of this analysis was examined by adding 21 cases not originally utilized for the numerical taxonomy analysis, then reinterpreting the data (performing a new cluster analysis). There was no particular method used to select these 21 cases. They were simply those that were ascertained after the initial cluster analysis was performed. The number of cases that were placed in a different cluster in the numerical taxonomy analysis based on the addition of the 21 cases from the original analysis was determined.

These mismatched cases were utilized to examine the robustness of the numerical taxonomy analysis. The more robust the analysis, the fewer mismatched cases would be expected.

2.6 Discriminant function analysis

Upon completion of the numerical taxonomy analysis and assignment of each case to a cluster, discriminant function analysis was performed using SPSS 14.0. Discriminant function analysis is useful for building a predictive model of group membership based on the observed variables for each case. A set of discriminant functions is created based on linear combinations of variables that are the largest discriminators when determining cluster membership. These discriminators are identified through a stepwise procedure that uses a statistic known as Wilks' lambda. At each step of the procedure, the variable that minimizes the overall Wilks' lambda value the most is entered into the equation first. This continues until a stepwise list of all variables is generated. The variables that lower Wilks' lambda the most are considered to have the greatest discriminating power to differentiate between the clusters.

Using the cross-tabulation function of SPSS, the significance of each variable (for example, whether or not there is a significant difference in the frequency of a coded variable in distinct clusters) was determined. Cluster demographics were also described by utilizing this function with the variables not used for the cluster analysis (for example, gender, family history, mortality).

Once the discriminant function equation was formulated, the equation was reapplied to the 110 cases to determine whether each case utilized for the initial cluster

analysis was placed into the appropriate cluster as predicted by the equation. The hallmark of a strong discriminant function equation is one that produces the smallest number of mismatched cases.

3. RESULTS

3.1 Ascertainment of cases

In total, 131 cases of MVSD were ascertained using the established criteria. The majority of these (106) cases were identified through searches of the Program of Genetics and Metabolism database using disease codes for conditions often associated with MVSD. The number of cases identified and their ascertainment source may be seen in Table 3. In addition to the database searches, 12 patients with MVSD were identified through an ongoing neural tube defect study headed by Dr. Jane Evans. Nine additional cases were identified through the Pediatric Radiology database by Dr. Martin Reed. Four were identified as cases of multiple congenital anomalies from previous studies undertaken in our laboratory.

Table 3: Disorder codes or sources of all cases of MVSD in the local population

Disorder (database code) or source	**Number of cases identified**	**Percentage of local population n = 131**
Spondylocostal dysostosis (SCD)	6	4.6
Spondylothoracic dysostosis/ Jarcho-Levin syndrome (STDJL)	3	2.3
Vertebral anomaly (VANOM)	34	26.0
VATER association (VATER)	35	26.7
Rib anomaly (RIBAN)	1	0.76
Scoliosis (SCOLI)	1	0.76
Caudal dysplasia (CAUDR)	1	0.76
Alagille (ALAGI)	2	1.5
Butterfly vertebrae (BUTER)	2	1.5
Hemifacial microsomia (HEMMI)	9	6.9
Klippel-Feil anomaly (KFEIL)	8	6.1
Insulin-dependent diabetes mellitus (IDDM)	2	1.5
Diabetic embryopathy (DIAEM)	1	0.76
22q deletion (22qdel)	1	0.76
Neural tube defect study (NTD)	12	9.1
Pediatric Radiology database	9	6.9
Other studies	4	3.1

Other conditions that were searched for in the Genetics database, but that did not identify any additional patients with MVSD were MURCS (Mullerian hypoplasia/aplasia, renal agenesis and cervicothoracic somite dysplasia) association, DiGeorge syndrome, and Robinow syndrome, also known as COVESDEM (costovertebral segementation defects with mesomelia).

3.2 Epidemiology

3.2.1 Prevalence

The Manitoba livebirth prevalence of MVSD from 1990 to 2003 inclusive was 1.99 per 10, 000 livebirths (95% CI: 1.45-2.67). As there were several stillbirths and terminations of pregnancy in this population, the total birth prevalence was also examined. There were 2.82 per 10, 000 total births (livebirths and stillbirths) during the same time interval (95% CI: 2.17-3.61). These statistics are considered minimum estimates of prevalence, as we cannot be certain of complete ascertainment.

3.2.2 Birth location

One hundred and nineteen of the 131 patients were born in Manitoba, while the others were out-of-province births (mostly from North-Western Ontario). All patients were born in the time period from 1944 to 2006 inclusive, with the majority of individuals born after 1991.

3.2.3 Sex ratio

There were 62 males and 67 females, as well as 2 cases with ambiguous genitalia (both of which were karyotypically male) ($p = 0.7932$).

3.2.4 Ethnicity

Of the population of 131 patients, 98 (74.8%) had their ethnicity recorded. Of those with known ethnicity, 51 (52.0%) were of Aboriginal descent (Inuit, Métis, and First Nations). Only three cases had parents of Inuit descent. Aboriginal status, when specified, was commonly of a First Nations background. Those not of an Aboriginal background were commonly of a mixed European background (34.7% of total population). Other groups represented in this population were Mennonite (5.1%), Filipino (3.1%) and East Indian (2.0%).

3.2.5 Consanguinity

Consanguinity was not reported in the records of 124 individuals (94.7%). Four cases had related parents, all of whom were second-cousins. Three cases had parents who were related, but more distantly (for example, third cousins, fifth cousins, or "distantly related").

3.2.6 Pregnancy Outcome

Most cases were live births (107)(81.7%). There were several terminations of pregnancy (14)(10.7%) and stillbirths (10)(7.6%).

3.2.7 Prenatal diagnosis

Sixty-three (48.1%) mothers had ultrasounds during their pregnancy. Of these, 51 cases were identified on prenatal ultrasound screening to have some type of abnormality. Only 9 of those 51 cases had specific abnormalities of the axial skeleton identified through prenatal ultrasound, the earliest of which were observed at 14 weeks. The majority of axial skeleton defects were observed after 20 weeks gestation. The remainder had additional non-vertebral malformations that originally brought these cases to attention prenatally.

3.2.8 Maternal diabetes

The most prevalent type of maternal disease in this population of patients was maternal diabetes. Twenty-two of the 131 cases identified had some form of maternal diabetes (16.8%). Of these cases, 5 mothers had Type 1 diabetes; 10 mothers had Type 2 diabetes or non-type 1; 5 had gestational diabetes and 2 had diabetes of an unspecified nature.

3.2.9 Other maternal disease

Other types of maternal disease were not common in this population. Of note, two mothers had epilepsy; one mother discontinued use of antiepileptic drugs before pregnancy and the other continued with valproic acid use during the pregnancy maintaining good seizure control. One other mother had several immunological disorders (psoriasis and arthritis) in addition to hypertension and was treated with methotrexate and minocycline during the pregnancy.

3.2.10 Maternal exposures

Fifty-nine (45%) cases had documented maternal exposures. The exposures ranged from illness/infection, high fever, prescription drugs and radiation to alcohol, cigarettes, and street drugs. Thirty-six (27.5%) cases were exposed to alcohol; 33 (25.3%) cases were exposed to cigarettes; 4 to street drugs; 3 to fever; 2 to illness/infection and 1 to radiation.

3.2.11 Family history

Eleven individuals in this population of MVSD patients were documented to have a family member with similar anomalies. Two pairs of individuals in the study population were related. In one case, the proband, who was originally thought to have a Klippel-Feil anomaly, gave birth to a child with spondylocostal dysostosis and trisomy 21.

3.2.12 Parental age

Maternal age was documented as part of this study. Of the 131 cases ascertained, maternal age was known in 124 (94.7%). Ages ranged from 15 to 42 years. Ten cases were born to women who were over 35 years of age. The average age of mothers in this population was found to be 26.0 ± 5.7 SD. Paternal age was known in only 67 cases and ranged from 15 to 50 years, with the average age being 29.5 ± 6.4 SD.

3.2.13 Parental height

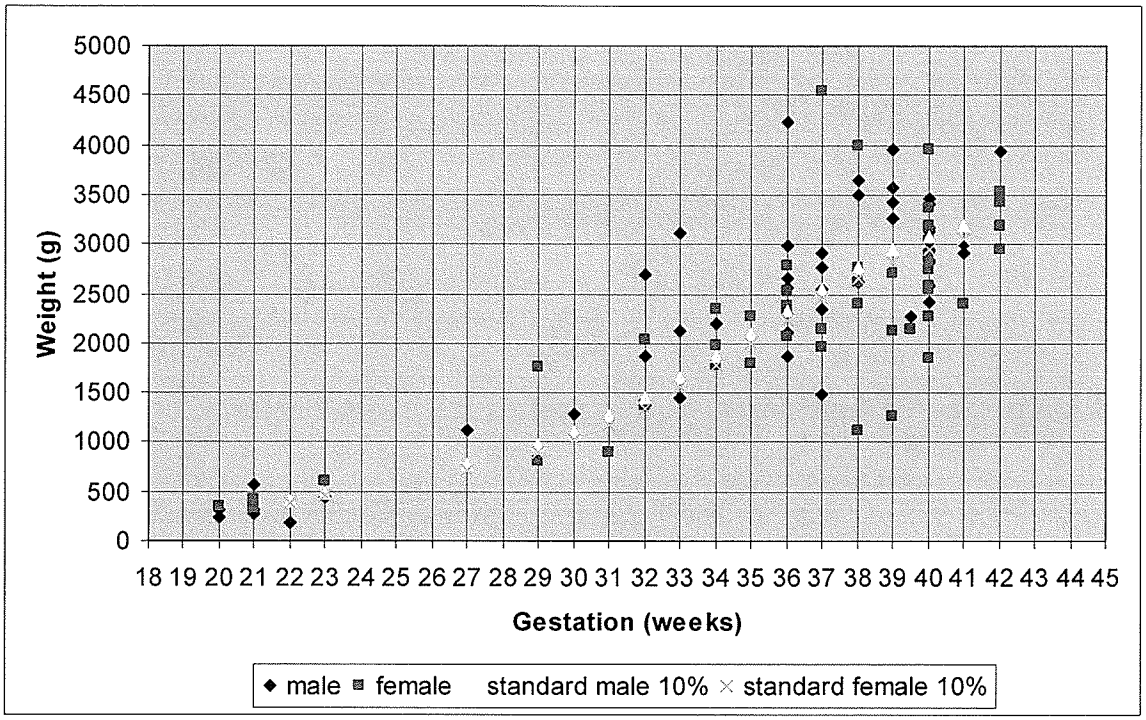
In the majority of records, parental heights were not documented. This is unfortunate given the fact that skeletal dysplasias such as spondylocostal dysostosis, are typically characterized by short stature and may be inherited in an autosomal dominant fashion.

The height of at least one parent was documented for 33 cases; most typically it was the mother's height that was recorded. Most mothers (84.8%) had heights greater than the 10^{th} percentile. Only five cases had maternal heights falling below the 10^{th} percentile. In one case, this is not surprising as the mother was similarly affected with vertebral anomalies. Paternal heights were documented only in 8 cases and all but one was above the 10^{th} percentile.

3.2.14 Birth weight

Birth weight and gestational age data were available for 99 cases. Of these, 41 (41.4%) had normal birth weights (between the 10^{th} and 90^{th} percentiles). Forty-four (44.4%) had birth weights below the 10^{th} percentile. Six cases (6.1%) had birth weights above the 90^{th} percentile; five of these were born to women with diabetes. Eight cases were born before 22 weeks gestation and were excluded from the birth weight statistics. The distribution of birth weights in the local population, as well as the 10^{th} percentile standard Canadian birth weights[42] are shown in Figure 4.

Figure 4: Birth weights of local cases compared to Canadian standard 10th percentile birth weights



3.2.15 Karyotype

Eighty-five of 131 (64.9%) cases had their karyotype documented. Of these, 80 (94.1% of those karyotyped) had a normal karyotype with no detectable deletions, duplications or rearrangements. Five had unbalanced karyotypes: two had 22q11.2 deletions, one had trisomy 21, one had triploidy (69, XXX), and one had a deletion of chromosome 13 (46 XX, del(13)(q22q34)).

3.2.16 Associated malformations

Most (87.8%) of the local population had at least one congenital anomaly in addition to their vertebral malformations. Only 16 of the 131 cases did not have additional malformations. Fourteen of these cases had dysmorphic attributes that were

coded for the cluster analysis. Only two cases did not display any attributes other than the levels of spine affected or ribs affected.

The distribution of associated malformations by body systems is shown in Table 4. Craniofacial abnormalities were the most common. Eighty cases (61.1 %) had at least one craniofacial abnormality. The musculoskeletal system was the next most commonly affected. Fifty-eight cases (44.3%) had a musculoskeletal malformation other than MVSD. Cardiovascular, urinary and gastrointestinal system anomalies were also quite common (43.5%, 38.9% and 30.5% respectively). The reproductive system was the least commonly affected with additional malformations.

Table 4: Birth defects present in cases with MVSD

Defect	Number of cases (%)
Central nervous system	**29 (22.1)**
Neural tube defect	22 (16.8)
Cardiovascular	**57 (43.5)**
Simple cardiac defect	16 (12.2)
Complex cardiac defect	26 (19.8)
Single umbilical artery	23 (17.6)
Respiratory System	**29 (22.1)**
Tracheoesophageal fistula	22 (16.8)
Gastrointestinal	**40 (30.5)**
Anal defect	35 (26.7)
Urinary system	**51 (38.9)**
Upper urinary system	44 (33.6)
Reproductive system	**22 (16.8)**
External genital defects	14 (10.7)
Musculoskeletal system (excluding MVSD)	**58 (44.3)**
Upper limb preaxial involvement	26 (19.8)
Positional foot deformity	20 (15.3)
Craniofacial	**80 (61.1)**
Cleft palate +/- lip	18 (13.7)
Ear a/dysplasia	30 (22.9)
Abnormal ear position	28 (21.4)
Facial asymmetry	16 (12.2)
Eye anomaly	19 (14.5)
Nose anomaly	15 (11.5)
Micrognathia	14 (10.7)

3.2.17 Survival

Of the 107 cases that were liveborn, 81 (75.7%) were alive at the time of this study. Of those who were deceased, 18 (69.2%) died in the first month of life. Four died after the first month, but before the age of two years. Two years was utilized as a cut-off as individuals with the most severe MVSD are commonly deceased before they reach this age [12]. Four children died after the age of two years, including one from an accidental death. The majority (84.6%) of deaths related to anomalies occurred in the first 24 months of life. The ages of those surviving, however, ranged from birth to 61 years. The

survival statistics are estimates, as future follow-up may show that there are in fact more deaths; however, most (65 cases (80.2%)) were over the age of two years at time of last visit/exam; thus we would not expect great disparity between our estimate and a follow-up count. Nine individuals were less than one month of age, and seven were less than two years at the time of their last visit.

3.2.18 Respiratory distress and infections

Of the 81 survivors, 17 (21.0%) had respiratory infections in infancy, 15 (18.5%) had respiratory distress and 8 (9.88%) had both infections and distress. Of the 25 non-survivors (not including those stillborn or terminations of pregnancy and the one case of accidental death), three (12%) had infections, six (24%) had distress and four (16%) had both respiratory distress and infections in infancy. Those in the non-survivor group may not have had as many respiratory infections as the survivor group as they were alive for a comparatively shorter period of time.

3.3 Radiographic evaluation

Each individual in our study population had at least two vertebrae affected or malformed, as defined by our inclusion criteria. Radiographs illustrating the extent of vertebral defects in these individuals are shown in Figure 5 & 6. Sixty-one cases (46.6%) had anomalies of the cervical spine; 99 (75.6%) had thoracic spine anomalies; 41 (31.3%) had lumbar spine defects; and 42 (32.1%) cases had malformations of the sacral spine. Thus a total of 243 regions were involved in those 131 cases. The distribution of cases with cervical, thoracic, lumbar and sacral region involvement very closely approximated that expected based on the relative size of each region ($p = 0.99$). Thus the percentages of patients with each level of spine affected appear to be representative of the distribution of vertebral bodies throughout the spine.

Figure 5: Lateral view of the cervical spine of one local patient with multiple block vertebrae from C2 to C6 and C7 to T1 as well as a hypoplastic posterior arch of C3

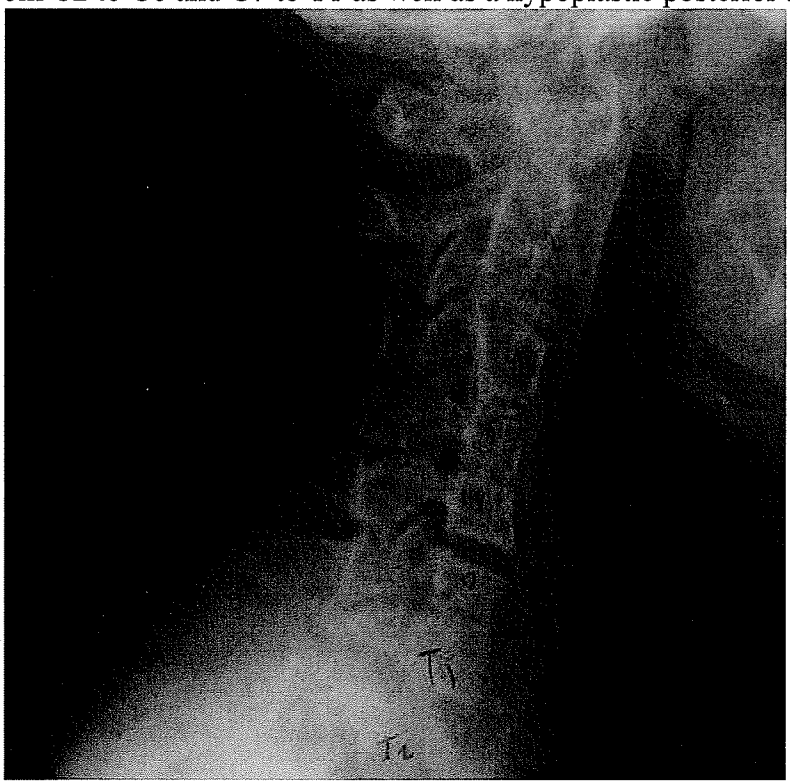
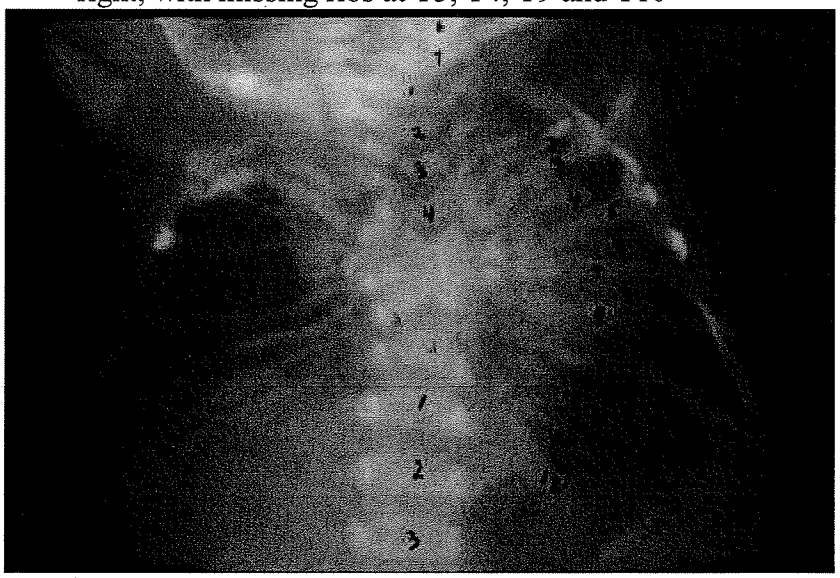


Figure 6: Posterior-anterior view of a local patient with extensive thoracic vertebral involvement including block fusion of T5 to T8, absent T9 and T10, right hemivertebra at T11. Left ribs are fused medially and thickened. Only 8 ribs exist on the right, with missing ribs at T3, T4, T9 and T10



When the number of levels of spine affected was examined, only 5 of 131 cases had all four levels of spine affected. Fifty-one cases had one level affected; 53 cases had two levels; 22 cases had three levels affected. The distribution of vertebral malformations between levels is illustrated in Table 5.

Table 5: Distribution of vertebral malformations throughout the levels of the spine

Levels of spine affected				**Local population**	
Cervical	**Thoracic**	**Lumbar**	**Sacral**	**# Cases**	**% Cases**
■				15	11.5
	■			25	19.1
		■		2	1.53
			■	9	6.87
Subtotal (1 level)				**51**	**38.9**
■	■			26	19.8
■		■		1	0.76
■			■	2	1.53
	■	■		12	9.16
	■		■	3	2.29
	■		■	9	6.87
Subtotal (2 levels)				**53**	**40.5**
■	■	■		8	6.11
■	■		■	4	3.05
■		■	■	0	0
	■	■	■	10	7.63
Subtotal (3 levels)				**22**	**16.8**
■	■	■	■	5	3.87
Subtotal (4 levels)				**5**	**3.87**
Total				**131**	**100**

Of the 131 total cases, 32 did not have information regarding specifically how many vertebral bodies were affected. Of the remaining 99 cases, only a minimum estimate of the average number of vertebral bodies affected could be calculated as not all levels of the spine were visualized in all cases and radiology reports may not be specific as to the number of bodies involved. Given these limitations, the average number of

vertebral bodies affected was a minimum of 5.5 in these cases. A vast range was seen (2 to 19) in the number of vertebral bodies affected per case.

The types of anomalies seen were also variable. Specific information available regarding the type of radiographic anomalies was available for 115 cases. Fusions, in the form of block vertebrae, were the most commonly seen radiographic feature and were present in 57 cases (49.6%). Hemivertebrae were also common: 55 cases (47.8%). Butterfly vertebrae (sagittal clefts) were seen in 39 cases (33.9%). At least one vertebra was absent in 27 cases (23.5%). Vertebral bars or unilateral fusions were seen in 17 cases (14.8%). Vertebrae were hypoplastic in 16 (13.9%). Extra vertebrae were seen in only 7 cases (6.1%). The least commonly seen radiographic anomaly was the coronal cleft. Coronal clefts were present in only 4 cases (3.5%).

3.4 Numerical taxonomy

One-hundred and ten cases were utilized for the numerical taxonomy or cluster analysis portion of this study. Each of these cases had, at a minimum, one of the 46 attributes previously listed and used for analysis. The dendrogram resulting from the numerical taxonomy analysis showed 7 distinct groups or clusters.

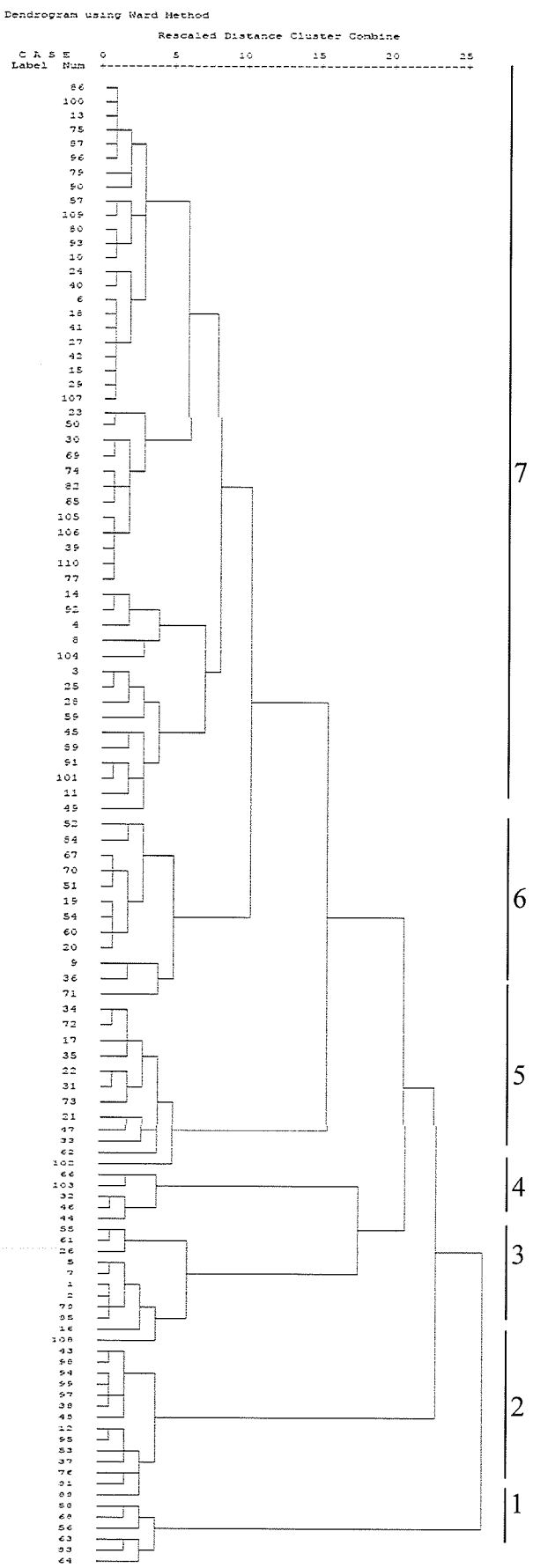


Figure 7: Dendrogram illustrating the results of the numerical taxonomy analysis of 110 cases with MVSD

A Pearson's Chi square statistic was computed for each of the 46 variables in order to determine if the pattern of distribution of the malformations among the clusters was statistically significant (Table 6)[41].

Table 6: Results of Pearson's Chi square analysis for each attribute

Attribute	Chi-square p-value
Central nervous system	
Hydrocephalus (not associated with NTD)	0.647
Neural tube defect	<0.001**
Cardiovascular	
Cardiac defect	< 0.001**
Single umbilical artery	<0.001**
Respiratory	
Lung abnormality	0.039*
Tracheoesophageal fistula +/- esophageal atresia	0.031*
Gastrointestinal	
Anal defect	<0.001**
Gastrointestinal defect	0.022*
Rectal fistula	0.005**
Urogenital	
Positional kidney defect	0.313
Renal a/dysplasia	<0.001**
Obstructive urinary defect	0.030*
Ureter defect	<0.001**
Lower urinary system anomaly	<0.001**
Internal genital defect	0.529
External genital defect	0.012*
Musculoskeletal	
Cervical spine defect	<0.001**
Thoracic spine defect	0.485
Lumbar spine defect	0.020*
Sacral spine defect	0.014*
Rib anomaly	0.031*
Short trunk	0.002**
Sternal abnormality	0.501
Curvature of spine	<0.001**
Inguinal hernia	0.128
Lower limb defect	0.001**
Preaxial involvement	<0.001**
Hip dislocation	0.714
Craniofacial	
Cleft palate	0.626
Cleft lip + palate	0.345
Positional foot defect	0.062
Skin/hair abnormality	0.243
Facial asymmetry	<0.001**
Eye anomaly (for example, cataracts, micropthalmia, esotropia)	0.431
Dysmorphic eye abnormality (for example, ptosis, epicanthal folds, hypotelorism)	0.148

Hypertelorism	0.830
Abnormal palpebral fissures	0.039*
Neck anomaly	0.028*
Neck immobility	<0.001**
Positional ear anomaly	0.648
Structural ear anomaly	<0.001**
Nose anomaly	0.947
Mouth anomaly	0.568
Micrognathia	0.120
Abnormal head shape	0.728
Other	
Hearing loss	0.422

*Significant at $p \leq 0.05$ **Significant at $p \leq 0.01$

Cluster 1 was the most unique or dissimilar to the other groups. There were four females and two males in this cluster. The striking feature in this group is that all six cases had preaxial involvement of the upper limbs. This involvement tended towards a more severe presentation. Four cases had an absent radius, while the two other cases had a hypoplastic radius and absent thumbs respectively. Five cases had heart defects (4 of these complex heart defects, 1 simple). Several of the cases had a tracheoesophageal fistula without esophageal atresia (50%), imperforate anus (50%) and unilateral renal defect (50%). These defects in combination are indicative of VATER association. This was, in fact, the diagnosis assigned to all cases in this cluster. Dysmorphic facial features were not commonly reported in this group. There were no cases that exhibited facial asymmetry; however, half did have microtia. Only one case had a lower limb anomaly in addition to the upper radial ray anomaly. Four of these cases were alive at the time of the study. The other two died in the first month of life.

Cluster 2 consisted of 14 individuals (8 females, 6 males), all with structural ear involvement. Eight cases (57%) had an affected cervical spine. Eight cases (57%) also had facial asymmetry. Four of these cases also had eye anomalies. Other anomalies, especially those affecting caudal structures, were extremely uncommon in this cluster. It

is likely that the majority of these cases fall into the spectrum of defects involved in OAV spectrum. Eight cases were clinically diagnosed with OAV spectrum, while two were given the diagnosis of VATER, one did not have a precise diagnosis, though it was suspected to be along the spectrum of VATER/OAV spectrum/SCD and one had sacral agenesis, only two were not given diagnoses. Of the liveborn cases in this cluster, 12 were alive at the time of the study. Only one case had died in the first month of life.

Cluster 3 was made up of 11 cases (5 females, 6 males). Each case had a degree of spinal curvature. Three of the five total cases where all four levels of spine are affected fell into this group. Malformations of the ribs were present in all but one case. Decreased neck mobility was a complaint in this group. A common diagnosis in this group was spondylothoracic or spondylocostal dysostosis, which is typically associated with severe curvature of the spine. Of the 10 live born cases in this cluster, 8 were alive at the time of the study. One case was deceased before the age of two years. Another case was deceased, though the cause of death was an accident.

Cluster 4 consisted of only five cases (3 females, 2 males). All patients in this category had neural tube defects (NTD), which tended to be severe. Three of the five cases had heart defects. Obstructive urinary complications were common. Of the three liveborn cases in this cluster, one was deceased in the first month of life and the other two were surviving at the time of this study.

Cluster 5 was made up of 12 cases (5 females, 7 males). All cases had bilateral renal involvement. As such, many also had ureter defects. All but one case had thoracic spine anomalies. Ten of the 12 cases had rib anomalies. Eight cases (66.7%) had single umbilical arteries. Seven cases (58.3%) had anal anomalies. The members of this group

tended to have many caudal-type defects. Heart and tracheoesophageal defects were not common in this group, with two and one cases respectively. Several cases of VATER, as well as one case of urorectal septum malformation sequence make up this group. Of the four liveborn cases in this cluster, three were deceased in the first month of life and one was alive at the time of the study.

Cluster 6 consisted of 12 cases (6 females, 6 males). Of these, all 12 had anal defects. Eight of the 12 had the more severe form of anal anomaly: imperforate anus. Nine cases (75%) had sacral anomalies. Eight (66.7%) cases had tracheoesophageal defects. Five cases (41.7%) had heart defects. There were three cases with unilateral kidney involvement. This group appears to be etiologically similar to cluster 5, although the occurrence of renal and ureter anomalies was not as high. Of the 11 liveborn individuals in this cluster, only 5 were surviving at the time of study. Four were deceased in the first month of life; one was deceased before the age of two years and one after two years.

Cluster 7 was the largest and most heterogeneous group, consisting of 50 individuals (28 females, 22 males). Thoracic and cervical spine malformations were common in this group (62% and 78%). Twenty-three cases (46%) had neck anomalies. Eighteen cases had heart defects (36%). Sixteen cases (32%) had either cleft palate or cleft lip and palate. The diagnoses given to patients in this group were variable. It is interesting to note that 10 of 11 cases of Klippel-Feil syndrome were placed in this group; although they did not form a distinct subgroup within this cluster, most tended to be closer to the less differentiated (leftmost) side of the cluster; these cases had few major associated anomalies. The two cases located closer to the more differentiated (rightmost)

side had multiple associated major malformations. As well, both cases of Alagille syndrome were placed in this group, directly adjacent to each other. Of the 48 live births in this cluster, 40 were surviving at the time of this study. Five cases died before the age of one month, two died after that time but before the age of two years, and one died after the age of two years.

The robustness of the numerical taxonomy analysis was examined by using an additional 21 cases of MVSD, along with the original 110 MVSD cases then reinterpreting the data. The influence of the addition of the 21 cases produced only four mismatches within the groups. To clarify, four (3.6%) cases were placed in different groups using 131 cases for the numerical taxonomy analysis than when only 110 cases were utilized to run the analysis.

One case (cluster I.D. 65) was originally placed in group 2, only to be placed in group 7 once the additional cases were used. This case had eye anomalies, which are more common in group 7, although ear anomalies (present in the form of microtia in this case) are common to group 2.

A second case (I.D. 66) was placed in group 6 when the additional cases were utilized, instead of where it originally placed in group 4. This case had a neural tube defect, characteristic of group 4. A heart defect, lung abnormality, tracheoesophageal fistula, ureter anomaly, lower limb anomaly and preaxial involvement of the upper limbs summarize this case. A hallmark of group 6 was anal anomalies, which this case does not have. The combination of the above listed anomalies may have predisposed this case, regardless of the presence of neural tube defect and absence of anal anomaly, to being classified into group 6.

The third mismatched case (I.D. 71) was placed in group 7 instead of group 6; however, this case is just on the cusp between the two groups. This case will be further examined after the discriminant analysis with respect to misclassifications.

The final mismatched case (I.D. 59) was shown to be in group 5 instead of 7 when the additional cases were used for analysis. With the combination of bilateral kidney and anal anomalies, it is not surprising that this case was predicted to be part of group 5. This case will also be examined in after the discriminant analysis misclassifications.

3.5 Discriminant analysis

The stepwise statistics leading to the formulation of the regression equation are listed in Table 7 below. For each step, the attribute that minimized the overall Wilks' λ statistic was chosen. The eight variables with the highest discriminating power are listed in descending order: neural tube defects, kidney a/dysplasia, preaxial involvement of the upper limbs, curvature of the spine, ear anomalies, anal anomalies, facial asymmetry and lung anomalies.

Table 7: Stepwise statistics of attributes in the regression equation and Wilks' λ

Step	Attribute	Wilks' λ
1	Neural tube defect	.242
2	Kidney a/dysplasia	.069
3	Preaxial involvement of upper limbs	.019
4	Curvature of spine	.005
5	Ear anomalies	.002
6	Anal anomalies	.001
7	Facial asymmetry	.001
8	Lung anomalies	.001

The distribution of the discriminating variables among the clusters is shown in Table 8.

Table 8: Distribution of discriminating attributes among clusters

Attribute	**Cluster n (%)**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Neural tube defect	0 (0)	1 (7.1)	1 (9.1)	5 (100)	0 (0)	1 (8.3)	1 (2)
Kidney a/dysplasia	3 (50)	0 (0)	3 (27.3)	2 (40)	12 (100)	2(16.7)	6 (12)
Preaxial involvement of upper limbs	6 (100)	1(7.1)	3 (27.3)	1 (20)	0 (0)	4 (33.3)	9 (18)
Curvature of spine	3 (50)	4 (28.6)	11 (100)	2 (40)	0 (0)	2 (16.7)	13 (26)
Ear anomalies	3 (50)	14 (100)	1 (9.1)	0 (0)	1 (8.3)	1 (8.3)	9 (18)
Anal anomalies	3 (50)	1 (7.1)	1 (9.1)	0 (0)	7 (58.3)	12 (100)	8 (16)
Facial asymmetry	0 (0)	8 (57.1)	4 (36.4)	1 (20)	0 (0)	0 (0)	3 (6)
Lung anomalies	2 (33.3)	3 (21.4)	0 (0)	2 (40)	1 (8.3)	0 (0)	1 (2)

The linear discriminant functions used to derive each cluster are shown in Table 9. This table gives an overview of which variables are positively or negatively associated with each cluster.

Table 9: Classification function coefficients for each attribute (listed in stepwise order) in each group

	Group						
Attribute	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Neural tube defect	4.492	2.856	1.869	24.593	-6.206	.054	.103
Kidney a/dysplasia	-3.547	-4.244	-.510	-9.778	14.920	-.344	.216
Preaxial involvement of upper limbs	10.973	1.146	1.707	5.690	-2.421	1.311	.634
Curvature of spine	3.250	3.412	12.349	2.885	-1.238	.202	1.096
Ear anomalies	4.173	6.947	.468	.986	-.890	.247	.575
Anal anomalies	.400	-.297	-1.102	-3.947	2.066	4.093	.317
Facial asymmetry	2.540	6.226	6.752	6.357	-1.882	-.020	.678
Lung anomalies	4.953	6.455	5.082	12.049	-3.690	-1.289	.431
Constant	-31.129	-16.694	-23.011	-55.141	-17.806	-5.837	-2.329

Fisher's linear discriminant functions

The regression equation generated from the discriminant analysis was reapplied to the 110 cases of MVSD to determine if their predicted cluster membership, based on the 8 discriminating attributes, would match their cluster assignment according to the numerical taxonomy analysis. This process showed that 101 cases or 91.8% of the original grouped cases were correctly classified. The misclassified cases are indicated in Table 10.

Table 10: Discriminant analysis classification results

Study ID	Cluster ID	Original Assigned cluster	New predicted cluster		Study ID	Cluster ID	Original Assigned cluster	New predicted cluster
80	64	1	1		66	51	6	6
103	83	1	1		88	70	6	6
79	63	1	1		83	67	6	7*
72	56	1	1		104	84	6	6
84	68	1	1		67	52	6	6
74	58	1	1		63	49	7	7
109	88	2	2		12	11	7	7
101	81	2	2		123	101	7	7
96	76	2	2		112	91	7	7
50	65	2	2		110	89	7	7
69	53	2	7*		58	45	7	7
117	95	2	2		75	59	7	5*
13	12	2	2		35	28	7	6*
62	48	2	2		30	25	7	7
51	38	2	2		3	3	7	7
119	97	2	2		126	104	7	6*
121	99	2	2		9	8	7	6*
116	94	2	2		4	4	7	7
120	98	2	2		114	92	7	7
56	43	2	2		15	14	7	7
130	108	3	3		97	77	7	7
18	16	3	3		132	110	7	7
105	85	3	3		52	39	7	7
98	78	3	3		128	106	7	7
2	2	3	3		127	105	7	7
1	1	3	3		81	65	7	7
8	7	3	3		102	82	7	6*
5	5	3	3		93	74	7	7
32	26	3	3		85	69	7	7
77	61	3	3		38	30	7	7
71	55	3	3		65	50	7	7
57	44	4	4		27	23	7	7
59	46	4	4		129	107	7	7
40	32	4	4		36	29	7	7
125	103	4	4		17	15	7	7
82	66	4	4		55	42	7	7
124	102	5	5		34	27	7	7
78	62	5	5		54	41	7	7
41	33	5	5		20	18	7	7
61	47	5	5		6	6	7	7
23	21	5	5		53	40	7	7

92	73	5	5	29	24	7	7
39	31	5	5	11	10	7	7
24	22	5	5	115	93	7	7
44	35	5	5	100	80	7	7
19	17	5	5	131	109	7	7
90	72	5	5	73	57	7	7
42	34	5	5	111	90	7	7
89	71	6	7*	99	79	7	7
49	36	6	6	118	96	7	7
10	9	6	6	108	87	7	7
22	20	6	6	95	75	7	6*
76	60	6	6	14	13	7	7
70	54	6	6	122	100	7	7
21	19	6	6	107	86	7	7

The most common misclassification occurred between groups 6 and 7. There were 5 cases (cluster I.D.s: 8, 28, 75, 82, 104) that were actually placed in group 7 when the predicted group membership was group 6. Each of these 5 cases had anal anomalies, one of the 8 most powerful discrimating factors, and an attribute present in all cases predicted to be in group 6. Two of the 5 cases (I.D.s: 8, 82) had no other anomalies that were part of the algorithm. Two (I.D.s 28, 75) had preaxial involvement of the upper limbs, which is consistent with cases in group 6. One of the two cases (I.D. 28) with preaxial involvement also had unilateral kidney involvement and facial asymmetry likely making this a difficult case to classify within the scope of the algorithm. One last case had curvature of the spine in addition to imperforate anus, which may have led to the classification discrepancy.

Two of the 9 total misclassified cases were predicted to be part of group 7 when they were actually placed in group 6 (I.D.s 67, 71). These two cases both had anal anomalies, though not as severe as imperforate anus, which is most common in group 6.

They also had curvature of the spine, which was commonly seen in groups 3 and 7. Anal anomalies were not common in group 3.

One misclassified case (I.D. 59) was identified as belonging to group 7 when the predicted group membership was group 5. This case was unique in that 5 of the 8 top discriminating attributes were present. Because of the combination of bilateral kidney and anal anomalies, it is not surprising that this case was predicted to be part of group 5. The difficulty in determining which group this case belonged to is likely the reason it was placed in group 7, the most heterogeneous group.

The final misclassified case was a group 2 case predicted to be in group 7 (I.D. 53). The only discriminating attribute present in this case was ear anomaly. Many cases placed into group 2 also had facial asymmetry, but this case did not.

4. DISCUSSION:

4.1 Epidemiology

For many years, individuals with MVSD have proved to be a nosological challenge. This has been complicated by the fact that MVSD are not common defects and may also be confounded by the fact that these anomalies are not usually immediately obvious at birth. Many are found as a result of further investigations due to observation of other anomalies. Additionally, the literature surrounding MVSD is confusing; as many different names have been used to describe these defects that seemingly fall along the same spectrum. Individuals with MVSD typically differ with respect to level of spine involved, severity of the defect and associated malformations. Various attempts have been made to evaluate inheritance patterns, and radiographic and clinical phenotypes. The largest study surrounding MVSD reported in the literature to date, which was published over 10 years ago, examined only 26 new patients as well as 115 cases from previously published cases[11]. To our knowledge, our retrospective study of 131 local patients is the largest epidemiologic evaluation of MVSD to date.

4.1.1 Prevalence

The birth prevalence calculated from our local study was compared to other statistics regarding the prevalence of MVSD. Martinez-Frías and Urioste calculated the birth prevalence of multiple vertebral segmentation defects to be 1.17 per 10,000 livebirths (95% CI: 0.96-1.41) from the Spanish Collaborative Study of Congenital Malformations (ECEMC) Registry[7]. In contrast, the Manitoba birth prevalence of MVSD

is significantly higher, at 1.99 per 10,000 livebirths (95% CI: 1.45-2.67), than that of the Spanish registry data. Several explanations may account for this disparity between statistics. There may be differences regarding the detection of anomalies and termination of an affected pregnancy in Spain relative to care and options in Manitoba leading to more affected fetuses being detected and aborted; therefore a lower prevalence of affected livebirths. The most likely explanation is that the length of time for ascertainment differs in the two studies. The Spanish registry examined newborn infants during the first three days of life to identify any congenital anomalies. Vertebral anomalies, especially in the absence of other congenital anomalies, may go largely undetected during this time frame. As our study examines all cases referred to the Genetics and Metabolism Program, irrespective of age at diagnosis, it is not surprising that our birth prevalence is higher. The Manitoba birth prevalence of MVSD is lower, however, than the prevalence of vertebral anomalies found by Shands and Eisberg in their study (5 per 10,000 affected)[4]. This could be due to the fact that their method of ascertainment was examining all radiographs to determine the number of individuals with vertebral defects (including single defects). Some individuals they picked up using this method, with isolated vertebral defects, would not necessarily have come to clinical attention. Therefore, an ascertainment bias existed in our study as such individuals with isolated vertebral defects, in the absence of other malformations, would not have been referred to the Genetics and Metabolism Program and would not have been included in this study.

4.1.2 Sex ratio

There were approximately equal numbers of males and females in this study born with MVSD, which is consistent with what has been reported in the literature[10]. This suggests that X-linked recessive mutations are not likely a cause of MVSD. Again, this is not surprising as the modes of inheritance proposed for these defects have all been autosomal dominant, recessive or sporadic to date.

4.1.3 Ethnicity

Ethnic background, where available, was recorded for this population given the known predilection of some forms of spondylocostal dysostosis to be increased in certain groups, for example, in those of Puerto Rican ancestry[43]. We did not observe any individuals of Puerto Rican descent in this study; although one individual presented with the typical crab-like configuration of the ribs we would expect to see from this ethnic descent; however, her ethnicity was reported as East Indian.

There have also been literature reports of cases with the combination of MVSD and urogenital anomalies in the Mennonite population. One group initially published a report of two male infants born to a consanguineous Mennonite couple with severe MVSD and anal and urogenital anomalies[13]. For this reason, we evaluated each of the 5 cases with Mennonite listed as the ethnic background. Only one of the five had a urogenital anomaly (posteriorly-situated anus with absent uterus and fallopian tubes).

A potential unanticipated finding with respect to the ethnic background of individuals included in this study was that the majority, where ethnicity was listed, were of Aboriginal descent. This group included individuals of Inuit, Métis and First Nations background. The minimum percentage of individuals of Aboriginal descent, assuming that the entire subgroup where ethnic background was not available was not Aboriginal, was 39%. According to the most recent Canada Census (2001) for which results are available, only 13.6% of Manitobans identify with an Aboriginal group, though the Aboriginal birth rate is likely higher than the percentage of population. Of the 150,040 Aboriginal people living in Manitoba in 2001, 18,000 were between the ages of 0-4 years[44]. This comprises approximately 24.4% of Manitoba live births per year. There is likely an over-representation of individuals of Aboriginal descent (39%) in our study of MVSD; however, without reliable statistics as to the number of births that would be considered Aboriginal, the exact magnitude is uncertain. As well, there are many challenges to determining the appropriate reporting of Aboriginal status.

Several hypotheses could be proposed for why this apparent over-representation of individuals of Aboriginal descent is present. Type 2 Diabetes is more common in this population, especially within certain Aboriginal communities[45]. As an association has been suggested between diabetes and axial skeletal malformations, it would not be surprising to observe an increase of individuals of Aboriginal descent in our population. It has been proposed that certain Aboriginal populations, for example, the Inuit, may have an inherent genetic predisposition to developing vertebral anomalies as bioarchaeology has shown a high incidence of axial skeleton malformations that predate the high frequency of diabetes[36].

4.1.4 Prenatal diagnosis

Prenatal diagnosis of MVSD has not often been reported. The report of the first successful ultrasound detection of Jarcho-Levin syndrome was published in 1987[46]. The authors reported a couple with a previously affected infant with a crab-like configuration of ribs due to multiple rib and vertebral defects. In a subsequent pregnancy, the fetus was found to have a severely deformed spine and incomplete ribcage when ultrasound examination was performed at 20 weeks gestation. More recently, ultrasound detection of Jarcho-Levin syndrome has been reported as early the end of the first trimester [47-49]. In our study, of the 63 mothers who had ultrasounds during their pregnancy, only 9 of those had specific axial skeleton anomalies identified, the majority of which were observed after 20 weeks.

4.1.5 Inheritance

The focus of many papers on spondylocostal dysostosis has been to attempt to delineate the patterns of inheritance of these defects and to then classify them by such patterns. What has not been especially emphasized in the literature is that most cases are seemingly sporadic, occurring in the family for the first time in the proband. Only 7 individuals in our study were the product of consanguineous relationships, which were either second cousins or more distantly related family members. These cases are suspicious for autosomal recessive inheritance. Only one case of vertical transmission (mother to daughter) of MVSD was seen in our study, suggesting autosomal dominant

inheritance; however, this was further complicated by a diagnosis of trisomy 21 in the child.

4.1.6 Maternal Diabetes and exposures

Pregestational maternal diabetes has classically been associated with lower spine involvement such as caudal dysplasia or sacral agenesis[38, 40]. For this discussion, it should be noted, that the differentiation between Type I and Type II diabetes in our population may be complicated by the presence of a unique population of Oji-Cree individuals who have a mutation in *HNF-1α* that has been linked to very early onset Type II diabetes that could be misclassified as Type I diabetes[45]. Gestational diabetes is not usually seen in association with congenital anomalies, as it does not develop until after the first trimester of pregnancy, well after somitogenesis leading to the formation of normal vertebral segments has occurred. It is possible that the 5 cases of gestational diabetes observed were actually yet to be diagnosed pregestational diabetes. These cases may also be coincidental, or more likely, other factors are involved. Confounding factors such as obesity are reported to play a role in the pathogenesis of gestational diabetes[50].

Poor maternal control of diabetes was reported in several of our cases. Poor control during pregnancy is more likely to be associated with congenital malformations. Several groups have reported that malformation rates are increased in infants of diabetic mothers with higher HbA1c levels[38-40]. Maternal diabetes is an important risk factor for MVSD. As the rates of congenital malformations have been reported to be decreased in infants of diabetic mothers with good control during pregnancy, preventative strategies

should aim for strict monitoring of blood glucose levels of women who are planning a pregnancy.

Maternal exposures must also be considered as a confounding factor when examining our data, particularly alcohol consumption during pregnancy. Cervical spine fusions are known to occur in patients with fetal alcohol syndrome[37]. In our local population, 27.5% of cases had reportedly been exposed to alcohol. Of these, 55.6% had cervical spine involvement. Of cases not reported to have been exposed to alcohol during pregnancy, only 42.1% had cervical spine involvement. Alcohol consumption during pregnancy may be acting as a confounding factor, leading to a slightly increased number of upper spine malformations; however, in our study, the difference in cervical involvement between those exposed to alcohol and those not exposed during pregnancy was not shown to be statistically significant ($p=0.1023$) .

4.1.7 Associated malformations

The literature examining malformations observed in association with MVSD has been extremely limited. With the exception of Mortier et al.'s study in which 26 new patients were evaluated with respect to their associated malformations, the only other publications relating to MVSD and associated malformations have been case reports. Mortier and colleagues report that the most commonly associated anomalies in descending order were kidney defects (23%), imperforate anus (19%), limb defects (19%), pelvic anomalies (15%), hernias (15%) heart defects (11%) and ambiguous genitalia (4%)[11]. In general, our study identified a higher frequency of these associated malformations. We found that 34% had upper urinary system (kidney) defects, 34% had

limb defects, 32% had heart defects, 20% had imperforate anus (IA), although 27% had any type of anal defect (including IA), 11% had defects of the external reproductive system, though not necessarily ambiguous genitalia.

Mortier et al. suggest that the site of vertebral involvement usually corresponds to the body segment in which the associated malformation occurs[11]. We found this was not tightly observed in our study population. We observed that 71% of those with heart defects had thoracic spine involvement, 65% of those with clubfoot had lumbar/sacral involvement, and 48% of those with renal anomalies have lumbar and sacral involvement. The highest degree of correlation between the level of spine involved and the associated anomaly was seen in cases with anal defects, where 80% had lumbar/sacral involvement.

4.2. Radiographic evaluation

Until very recently, few published papers have reported detailed radiographic evaluation of individuals with MVSD. A 2004 study identified 81 cases with congenital cervical, thoracic or lumbar vertebral defects and scored them with respect to the location and type of anomalies present[15]. They found that 30% of their cases had defects that were limited to a single level. This is comparable to our observation that 32% of our population had single level defects present in those three levels.

Another study by Takikawa et al. identified 30 patients with more than two vertebral anomalies associated with rib anomalies (fusion ± absence) without symmetric crab-like chest[16]. They found that anomalies of the thoracic spine were found in all cases. Most cases had at least four vertebral anomalies. In contrast to this study, only 75.6 % of

our cases had thoracic spine anomalies. This could reflect the differences in inclusion criteria between the two studies. By excluding individuals without rib anomalies, individuals with only multiple lower spine anomalies could be missing from the previous study's ascertainment. Our study's focus; however, was MVSD while their focus was spondylocostal dysostosis, which by definition, should have rib abnormalities. The average number of vertebral bodies affected, at a minimum, calculated by our study was 5.5. This is slightly higher than the four anomalies reported by Takikawa et al. The discrepancy between the two studies likely results from fewer levels of spine studied in the published study. They examined vertebral levels C5 to S3, whereas our study examined as many vertebral levels as were available through reports or radiographs.

Interestingly, only one case in our entire study exhibited the "typical" Jarcho-Levin radiological phenotype with a crab-like configuration of ribs. This phenotype has often been seen in patients of Puerto Rican descent (23/47 cases (49%) of STD described in medical literature[43]). Our patient, as previously mentioned, was of East Indian descent.

4.3 Numerical taxonomy

Numerical taxonomy has previously been utilized by others in our research group to evaluate complex disorders with heterogeneous phenotypes[51,52]. We approached our local population of MVSD in a similar fashion. The benefit in performing this numerical taxonomy analysis is that evaluating the resulting clusters may be the first step in identifying how these defects are associated, or the common link between them. The benefit of utilizing this type of analysis is that any preconceived associations are not

taken into account and all features, whether considered potentially important or not, were used if present in sufficient numbers.

We identified seven distinct clusters from the numerical taxonomy analysis. The first or right-most cluster (cluster 1) on the resulting dendrogram was considered to be the most unique from the rest. The attribute that all cases shared in this cluster was preaxial involvement of the upper limbs. Upper limb anomalies have not been reported in individuals with spondylocostal or spondylothoracic dysostosis as commonly as lower limb anomalies, although they are common in VACTERL association. Of Mortier et al.'s 26 new patients examined for associated malformations, only one had an upper limb anomaly in the form of an absent thumb[11].

All individuals in cluster 2 had ear anomalies, and many presented with other anomalies that fit the clinical picture of OAV spectrum. A study by Tsirikos and McMaster found that the prevalence of OAV-associated conditions in patients with congenital spine malformations was 2%[53]. Our study identified 12 individuals with OAV, 8 of which were classified in this cluster. The 12 individuals made up 9% of this population of individuals with MVSD. The discrepancy between the two studies could be the result of the broader inclusion criteria in the Tsirikos and McMasters study, as they utilized cases where only a single vertebral anomaly was present.

Ignacio et al. identified the difficulty in attempting to classify individuals with both severe axial skeleton anomalies and OAV spectrum, especially given that these conditions are considered to have different inheritance patterns[54]. They favour the concept that OAV spectrum is a polytopic developmental field defect and therefore, may lead to a variety of anomalies and a wide range of severity[55].

The common attribute to all cases in cluster 3 was curvature of the spine. This cluster most closely represented the cases of spondylocostal dysostosis that have been previously reported in the literature[11]. A variety of associated malformations were identified in these cases, though no particular pattern was identified. Four cases were identified as having facial asymmetry, which may further the idea proposed by Ignacio et al. that the presentation of these axial skeleton-type defects are on a spectrum[54].

Neural tube defects (NTD) were present in all cases in cluster 4. Spina bifida has been previously reported in several cases of spondylocostal and spondylothoracic dysostosis[10, 56, 57]. Wynne-Davies first suggested the idea that MVSD and NTD are etiologically related, [58] and several studies have examined the validity of this association. One determined that the risk for open NTD in sibs was the same regardless of whether the proband had an open NTD or occult spinal dysraphism with MVSD[59]. Another compared the number and distribution of vertebral anomalies in a group of individuals with MVSD without apparent NTD and a group of individuals with spina bifida cystica and found that the distribution of anomalies was not statistically different[60]. A recent evaluation, utilizing a proportion of MVSD cases from this study found that 30.8% of patients with MVSD had a NTD while 8.0% of NTD cases had MVSD[61]. The overlap in phenotype of MVSD and NTD is relatively clear, although the underlying relationship between somitogenesis and neural tube closure which results in these defects remains largely unknown. Etus et al. discussed the concept that a gene defect resulting in an effect at the time when the two somitic columns form around the neural tube could explain such an association[62]. Until very recently, NTD had not been reported in any human case

where a Notch pathway gene mutation had been identified. The discovery of the *HES7* mutation in an individual with MVSD and a lumbo-sacral myelomeningocele provides more concrete evidence of a shared etiology for some proportion of individuals with MVSD and NTD.

Bilateral renal involvement was the main characteristic of cluster 5, with all cases being affected. Many cases also had single umbilical arteries and anal anomalies. A case described by Murr et al. had features very similar to those seen in this cluster[63]. They described an infant male with MVSD, imperforate anus, vesicorectal fistula, bilateral renal dysplasia, sacral agenesis, single umbilical artery, pulmonary hypoplasia, scoliosis and hexadactyly of the left thumb. They noted that their case closely resembled cases of CMN syndrome. In their report though, the two individuals they describe did not have renal anomalies as seen in Murr's patient or in the cases in this cluster. As well, there were no known individuals of Mennonite background.

Anal anomalies were the hallmark of cluster 6. This cluster most closely resembles VATER association, as imperforate anus, tracheoesophageal fistula and renal anomalies were present in this group. The renal anomalies were unilateral in nature as compared to the bilateral renal involvement of cluster 5. This cluster does not appear to closely resemble CMN syndrome in spite of the presence of anal anomalies, as there are more rostral anomalies than are typically seen in individuals with CMN.

As the most heterogeneous group, cluster 7 consisted mostly of cases that lacked the presence of multiple attributes. However, there are smaller subgroups within this large cluster. This is identified by the proximity of many of the cases of Klippel-Feil

syndrome to each other and the two cases of Alagille syndrome being adjacent to each other in another subgroup.

The numerical taxonomy analysis was determined to be adequately robust as the addition of 21 cases to the original analysis produced only 4 (3.6%) mismatches within the groups. The regression equation created by the discriminant analysis correctly classified 91.8 of the cases when predicting cluster membership for the same 110 individuals that were utilized in creating the equation. This equation could now be useful for classifying further cases of MVSD.

4.4 Proposed etiological mechanisms

Recently, research in the area of MVSD has almost exclusively been focused on the Notch pathway. It is clear that the genes involved in this pathway are essential for the proper regulation of somitogenesis leading to normal axial skeleton development; however, mutations in these genes explain only a fraction of all MVSD. Other mechanisms have been proposed, such as the idea of a primary developmental field defect (DFD)[64]. Axial mesodermal dysplasia complex (AMDC), which encompasses a broad spectrum of malformations in mesenchymally-derived tissues, has been thought to result from such a DFD. Both VACTERL association and OAV spectrum, among others, have been described as AMDC disorders[65,66]. The theory proposes that defects result from failure of mesodermal cell migration during early blastogenesis early in development. As mesoderm gives rise to cartilage, bone, connective tissue, muscles, heart, blood, kidneys, gonads and genital ducts among others, the spectrum of defects may involve any or all of these tissues[1], and these are representative of the tissues in

which associated malformations were seen in our local cases. The pattern of resulting defects is thought to be considered representative of the time the causal agent or disruption was present. Maternal diabetes has been proposed as such a causal agent.

Disruption of embryonic blood flow has also been proposed as a causal agent. A review of current etiological models for OAV spectrum concluded that the theory that a mechanism for development of branchial arch anomalies is when there is a disruption of the orderly development of the facial circulatory system[67]. The further delineation of these theories may, in time, begin to explain the etiology of MVSD cases that cannot be explained by the identification of gene mutations.

4.5 Classification

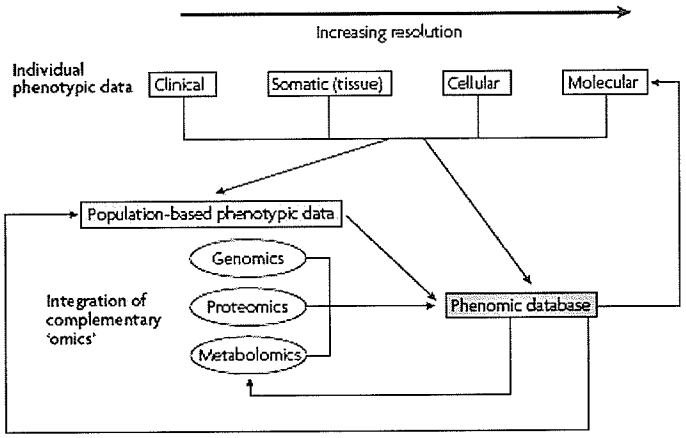
Existing classification systems, as discussed earlier, have been devised based on clinical, molecular or radiographic approaches. A recent classification system proposed by Offiah and colleagues divides MVSD into several categories[68]. The first, under the title of generalized MVSD includes both defined (spondylocostal/spondylothoracic dysostosis) and undefined (all others). The second category is regional (for the level of spine). Again, these may be defined (in cases of associations or syndromes such as Alagille, VATER, VACTERL, OAV, CHARGE) and undefined (all others). This new classification approach is not beneficial, as it would place many of our cases into the undefined category, once again, leaving these cases unclassified. Our study has sought to begin with the entire group of MVSD and observe which categories result from a numerical taxonomy analysis using detailed description of individual phenotypes. We observed many individuals with a similar diagnosis clustering together; however,

invariably, there remains phenotypic overlap as different clusters contain individuals with the same clinical diagnosis. Our study has identified features that are most likely to distinguish these clusters from each other. In cases where features may be associated with more than one syndrome or association, it is useful to be able to determine which feature is the most important for classification. The discriminant analysis regression equation is exceedingly useful for this purpose

4.6 Approaching complex disorders

Traditionally there have been two basic ways to approach complex disorders, such as MVSD. The first approach was the "top-down" approach of linking phenotypes with genotypes, which was achieved, for example, by researching potential candidate genes. The second approach was a "bottom-up" approach, which began with gene changes identified, for example, by a genome scan, and then linked them to particular disease phenotypes. Though these approaches have been very useful for delineating the causes of single-gene disorders, it has become evident that more delineation of the connections among genes, their products, intermediate phenotypes and clinical presentations is required to tease out the causes of complex disorders. This delineation of connections has been referred to as "phenomics"[69].

Figure 8: Schematic to illustrate the many aspects involved in phenomics and the role this research plays in this larger picture[70]



The study of phenomics has already proved to be very useful for understanding the variable clinical presentations of several disorders, the best example being in the case of laminopathies[71]. The systematic mapping of the different phenotypic features of laminopathies, all with identified mutations in the LMNA gene, identified correlations between the position of the gene mutation and the clinical features seen. This is complicated by the fact that the same LMNA mutation may be responsible for different phenotypes. This is why such careful methodical delineation of individual phenotypic data, as well as population-based phenotypic data is important. The evaluation and careful delineation of this phenotypic data at both the individual and population level was the focus of our research of MVSD. This aspect, as well as how this is involved in the larger picture of phenomics, is illustrated in Figure 8.

The careful construction of our database with detailed demographic, radiographic and phenotypic data may serve as a tool for others requiring such specific data. From a

molecular perspective, this data will be useful for quickly and efficiently identifying a target human population to test as new mutations are discovered in animal models. This should increase the speed of the time from the discovery of a mutation in one individual or family to the time of multiple individuals, seemingly sporadic cases on their own, being diagnosed and subsequently being offered appropriate genetic counselling and targeted prenatal testing for future offspring if applicable.

5. SUMMARY AND FUTURE DIRECTIONS

This study consisted of three main parts. The first part was the ascertainment of a population of patients with MVSD and the determination of birth prevalence and demographic and radiographic features of this population. With the ascertainment of 131 individuals with MVSD, this was the largest retrospective population-based evaluation known to date. The Manitoba birth prevalence was found to be significantly higher than the only other comparable study of birth prevalence of MVSD. Most of the local population had at least one associated malformation in addition to MVSD. These malformations were commonly seen in the craniofacial, musculoskeletal, cardiovascular or urinary systems. Radiographically, the minimum number of vertebrae affected was 5.5, and these anomalies were typically seen in all levels of the spine.

The second part of this study dealt with establishing a coding system for anomalies and carrying out the numerical taxonomy analysis incorporating these scores. Numerical taxonomy identified seven clusters of cases. Cluster 1 (n=6) was the most unique; all cases had preaxial involvement of the upper limbs and many had anomalies along the VATER spectrum. Cluster 2 (n=14) cases all had structural ear involvement and anomalies consistent with OAV spectrum. Cases in cluster 3 (n=11) all had a degree of spinal curvature; many in this group were diagnosed with spondylocostal dysostosis. Cluster 4 (n=5) cases all had severe NTD. Cluster 5 (n=12) cases had bilateral renal involvement. Cluster 6 (n=12) cases all had anal defects, as well as other anomalies along the VATER spectrum. Cluster 7 (n=50) was the largest, most heterogeneous group, which included cases of Klippel-Feil and Alagille syndrome.

The final part of this study was the utilization of discriminant function analysis to identify an algorithm useful for differentiating between the clusters previously identified. The variables identified in this equation were NTD, kidney a/dysplasia, preaxial involvement of the upper limbs, curvature of the spine, ear anomalies, anal anomalies, facial asymmetry and lung anomalies.

For the future directions, the next step in this study would be to test the local population of individuals with MVSD for mutations in the Notch pathway genes already identified. This would require a new study and Research Ethics Board approval. The most appropriate individuals to begin testing would be those belonging to cluster 3. The phenotype of these individuals appeared to be similar to phenotypes of individuals reported to have Notch pathway gene mutations. Additionally, careful consideration of the clinical findings within clusters identified by the numerical taxonomy analysis could be undertaken to propose new candidate genes to be tested for in this local population.

REFERENCES

1. Moore KL, Persaud TVN. The Developing Human: clinically oriented embryology. Elsevier, 2008.

2. Rawls A, Rhee JM. Inborn errors of development: the molecular basis of clinical disorders of morphogenesis. Epstein CJ, Erickson RP, Wynshaw-Boris A (eds.) Oxford University Press Inc., 2004; 120-132.

3. Macpherson RI, Genez B. Pediatric Skeletal Radiology. Reed MH (ed.) Williams and Wilkins, 2007; 209-304.

4. Shands AR, Eisberg HB. The incidence of scoliosis in the state of Delaware: a study of 50,000 minifilms of the chest made during a survey for tuberculosis. J Bone Joint Surg Am 1955; 37A:1243-1249.

5. Wynne-Davies R. Heritable disorders in orthopaedic practice. Blackwell, Oxford, 1973.

6. Martinez-Frias ML, Bermejo E, Paisan L et al. Severe spondylocostal dysostosis associated with other congenital anomalies: a clinical/epidemiologic analysis and description of ten cases from the Spanish registry. Am J Med Genet 1994: 51: 203-212.

7. Martinez-Frias ML, Urioste M. Segmentation anomalies of the vertebras and ribs: a developmental field defect: epidemiologic evidence. Am J Med Genet 1994; 49:36-44.

8. Jarcho S, Levin PM. Hereditary malformation of the vertebral bodies. Bull Johns Hopkins Hosp. 1938; 62:216-226.

9. Rimoin DL, Fletcher BD, McKusick VA. Spondylocostal dysplasia: A dominantly inherited form of short-trunked dwarfism. Am J Med 1968; 45:948-953.

10. Solomon L, Jimenez RB, Reiner L. Spondylothoracic dysostosis: report of two cases and review of the literature. Arch Pathol Lab Med 1978; 102:201-205.

11. Mortier GR, Lachman RS, Bocian M et al. Multiple vertebral segmentation defects: analysis of 26 new patients and review of the literature. Am J Med Genet 1996; 61:310-319.

12. Karnes PS, Day D, Berry SA et al. Jarcho-Levin syndrome: four new cases and classification of subtypes. Am J Med Genet 1991; 40:264-270.

13. Casamassima AC, Casson Morton C, Nance WE et al. Spondylocostal dysostosis associated with anal and urogenital anomalies in a Mennonite sibship. Am J Med Genet 1981; 8:117-127.

14. Wadia RS, Shirole DB, Dikshit MS. Recessively inherited costovertebral segmentation defect with mesomelia and peculiar facies (Covesdem syndrome): A new genetic entity? J Med Genet 1978; 15: 123-127.14.

15. Erol B, Tracy MR, Dormans JP et al. Congenital scoliosis and vertebral malformations: characterization of segmental defects for genetic analysis. J Pediatr Orthop 2004; 24:674-682.

16. Takikawa K, Haga N, Maruyama T et al. Spine and rib abnormalities and stature in spondylocostal dysostosis. Spine 2006; 31:E192-E197.

17. Ayme S, Preus M. Spondylocostal/spondylothoracic dysostosis: the clinical basis for prognosticating and genetic counseling. Am J Med Genet 1986; 24:599-606.

18. Evans J. Genetic Disorders, Syndromology and Prenatal Diagnosis. Persaud TVN (ed.), pp. 139-160 MTP Press Limited, Lancaster,1982).

19. Pinsky L. The polythetic (phenotypic community) system of classifying human malformation syndromes. Birth Defects Orig Artic Ser 1977; 13:13-30.

20. Preus, M, MacGibbon B. An application of numerical taxonomy to the classification of syndromes. Birth Defects Orig Artic Ser 1977; 13:31-38.

21. Cooke J, Zeeman EC. A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. J Theor Biol 1976; 58:455-476.

22. Pourquie O, Kusumi K. When body segmentation goes wrong. Clin Genet 2001; 60:409-416.

23. Aulehla A, Wehrle C, Brand-Saberi B et al. Wnt3a plays a major role in the segmentation clock controlling somitogenesis. Dev Cell 2003; 4: 395-406.

24. Dubrulle J, Pourquie O. Fgf8 mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. Nature 2004; 427: 419-422.

25. Diez del Corral CR, Olivera-Martinez I, Goriely A et al. Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. Neuron 2003; 40:65-79.

26. Turnpenny PD, Bulman MP, Frayling TM et al. A gene for autosomal recessive spondylocostal dysostosis maps to 19q13.1-q13.3. Am J Hum Genet. 1999: 65: 175-182.

27. Martinez-Frias ML. Segmentation anomalies of the vertebras and ribs: one expression of the primary developmental field. Am J Med Genet A 2004; 128:127-131.

28. Turnpenny PD, Alman B, Cornier AS et al. Abnormal vertebral segmentation and the notch signaling pathway in man. Dev Dyn 2007; 236:1456-1474.

29. Whittock NV, Sparrow DB, Wouters MA et al. Mutated MESP2 causes spondylocostal dysostosis in humans. Am J Hum Genet 2004; 74:1249-1254.

30. Cornier AS, Staehling-Hampton K, Delventhal KM et al. Mutations in the MESP2 gene cause spondylothoracic dysostosis/Jarcho-Levin syndrome. Am J Hum Genet 2008; 82:1334-1341.

31. Sparrow DB, Chapman G, Wouters MA et al. Mutation of the LUNATIC FRINGE gene in humans causes spondylocostal dysostosis with a severe vertebral phenotype. Am J Hum Genet 2006; 78:28-37.

32. Sparrow DB, guillen-Navarro E, Fatkin D et al. Mutation of Hairy-and-enhancer-of-split-7 in humans causes spondylocostal dysostosis. Hum Mol Genet 2008; 17: 3761-3766.

33. Li L, Krantz ID, Deng Y et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat Genet 1997; 16: 253-251.

34. McDaniell R, Warthen DM, Snachez-Lara PA et al. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. Am. J Hum Genet 2006; 79:169-173.

35. Whittock NV, Ellard S, Duncan J et al. Pseudodominant inheritance of spondylocostal dysostosis type 1 caused by two familial delta-like 3 mutations. Clin Genet 2004; 66:67-72.

36. Merbs CF. Sagittal clefting of the body and other vertebral developmental errors in Canadian Inuit skeletons. Am. J Phys Anthropol 2004; 123:236-249.

37. Smith DF, Sandor GG, MacLeod PM et al. Intrinsic defects in the fetal alcohol syndrome: studies on 76 cases from British Columbia and the Yukon Territory. Neurobehav Toxicol Teratol 1981; 3: 145-152.

38. Greene MF, Hare JW, Cloherty JP et al. First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. Teratology 1989; 39:225-231.

39. Kitzmiller JL, Gavin LA, Gin GD et al. Preconception care of diabetes; Glycemic control prevents congenital anomalies. JAMA 1991: 265:731-736.

40. Ylinen K, Aula P, Stenman UH et al. Risk of minor and major fetal malformations in diabetics with high haemoglobin A1c values in early pregnancy. Br Med J (Clin. Res. Ed) 1984; 289:345-346.

41. SPSS 14.0. 2005. Chicago, SPSS Inc.
Ref Type: Computer Program

42. Kramer MS, Platt RW, Wen SW et al. A new and improved population-based Canadian reference for birth weight for gestational age. Pediatrics 2001; 108:E35.

43. Cornier AS, Ramirez N, Arroyo S et al. Phenotype characterization and natural history of spondylothoracic dysplasia syndrome: a series of 27 new cases. Am J Med Genet 2004; 128A:120-126.

44. Statistics Canada. 2001 Census of Canada,http://www12.statcan.ca/english/census01/index.cfm.2007.
Ref Type: Electronic citation

45. Sellers EA, Triggs-Raine B, Rockman-Greenberg C et al. The prevalence of the HNF-1alpha G319S mutation in Canadian aboriginal youth with type 2 diabetes. Diabetes Care 2002; 25:2202-2206.

46. Tolmie JL, Whittle MJ, McNay MB et al. Second trimester prenatal diagnosis of the Jarcho-Levin syndrome. Prenat Diagn 1987; 7:129-134.

47. Eliyahu S,Weiner E, Lahav D et al. Early sonographic diagnosis of Jarcho-Levin syndrome: a prospective screening program in one family. Ultrasound Obstet Gynecol 1997; 9:314-318.

48. Kauffmann E, Roman H, Barau G et al. Case report: a prenatal case of Jarcho-Levin syndrome diagnosed during the first trimester of pregnancy. Prenat Diagn 2003; 23:163-165.

49. Hull AD, James G, Pretorius DH. Detection of Jarcho-Levin syndrome at 12 weeks' gestation by nuchal translucency screening and three-dimensional ultrasound. Prenat. Diagn 2001; 21: 390-394.

50. Martinez-Frias ML, Frias JP, Bermejo E et al. Pre-gestational maternal body mass index predicts an increased risk of congenital malformations in infants of mothers with gestational diabetes. Diabet Med 2005; 22:775-781.

51. Evans JA, Greenberg CR, Erdile L. Tracheal agenesis revisited: analysis of associated anomalies. Am J Med Genet 1999; 82:415-422.

52. Elliott AM, Evans JA. Genotype-phenotype correlations in mapped split hand foot malformation (SHFM) patients. Am J Med Genet 2006; 140A:1419-1427.

53. Tsirikos AI, McMaster MJ. Goldenhar-associated conditions (hemifacial microsomia) and congenital deformities of the spine. Spine 2006; 31:E400-E407.

54. Ignacio RJ, Palacios J, Lapunzina P. Severe axial anomalies in the oculo-auriculo-vertebral (Goldenhar) complex. Am J Med Genet 1993; 47:69-74.

55. Opitz JM. Developmental field theory and observations--accidental progress? Am. J. Med. Genet. Suppl 1986; 2: 1-8.

56. Moseley JE, Bonforte RJ. Spondylothoracic dysplasia--a syndrome of congenital anomalies. Am J Roentgenol Radium Ther Nucl Med 1969: 106:166-169.

57. Pochaczevsky R, Ratner H, Perles D et al. Spondylothoracic dysplasia. Radiology 1971; 98:53-58.

58. Wynne-Davies R. Congenital vertebral anomalies: aetiology and relationship to spina bifida cystica. J Med Genet 1975; 12:280-288.

59. Carter CO, Evans KA, Till K. Spinal dysraphism: genetic relation to neural tube malformations. J Med Genet 1976: 13: 343-350.

60. Lendon RG, Wynne-Davies R, Lendon M. Are congenital vertebral anomalies and spina bifida cystica aetiologically related? J Med Genet 1981; 18:424-427.

61. Evans JA, Drescher SJ. Multiple vertebral segmentation anomalies and neural tube defects. Proceedings of Greenwood Genetics Center 2007; 26(62).
Ref Type: Abstract

62. Etus V, Ceylan S, Ceylan S. Association of spondylocostal dysostosis and type I split cord malformation. Neurol Sci 2003; 24: 134-137.

63. Murr MM, Waziri MH, Schelper RL et al. Case of multivertebral anomalies, cloacal dysgenesis, and other anomalies presenting prenatally as cystic kidneys. Am J Med Genet 1992; 42:761-765.

64. Opitz JM. Blastogenesis and the "primary field" in human development. Birth Defects Orig Artic Ser 1993; 29: 3-37.

65. Russell LJ, Weaver DD, Bull MJ. The axial mesodermal dysplasia spectrum. Pediatrics 1981; 67:176-182.

66. Stewart FJ, Nevin NC, Brown, S. Axial mesodermal dysplasia spectrum. Am J Med Genet 1993; 45: 426-429.

67. Hartsfield JK. Review of the etiologic heterogeneity of the oculo-auriculo-vertebral spectrum (Hemifacial Microsomia). Orthod Craniofac Res 2007; 10:121-128.

68. Offiah AC, Alman B, Cornier AS et al. Introducing a new classification system for congenital scoliosis and segmentation disorders of the spine.
http://www.icvas.org . 2007.
Ref Type: Electronic Citation

69. Schork NJ. Genetics of complex disease: approaches, problems, and solutions. Am J Respir Crit Care Med 1997; 156:S103-S109.

70. Zbuk KM, Eng C. Cancer phenomics: RET and PTEN as illustrative models. Nat. Rev Cancer 2007; 7:35-45.

71. Hegele RA, Oshima J. Phenomics and lamins: from disease to therapy. Exp. Cell Res 2007; 313, 2134-2143.

APPENDICES

Appendix 1: Research ethics board approval

UNIVERSITY
of MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

PI26-770 Bannatyne Avenue
Winnipeg, Manitoba
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Tel: (204)789-3255
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APPROVAL FORM

Principal Investigator: Dr. Jane Evans

Protocol Reference Number: E95:197A
Date of Approval: November 10, 2004
Date of Expiry: November 10,2005

Protocol Title: **"Characterization of Spondylocostal/Spondylothoracic Dysotosis and other Costovertebral Malformations"**

The following is/are approved for use:

- **Approval to conduct anonymous chart review portion of study submitted October 19, 2004 (per letter received November 10,2004)**

The above was approved by Dr. Ken Brown, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter received November 10, 2004. The Research Ethics Board is organized, and operates according to Health Canada/IH Good Clinical Practices, Tri-Couneil Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 *of the Food and Drug Regulations.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Ken Brown, MD, MBA
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary
Telephone: (204) 789-3255/ **Fax:** (204) 789-3414

Appendix 2: MVSD data extraction form with example from literature

Variable	Rationale	Example Case (Rimoin et al., 1968, Case 1)
Study ID#		
Family Characteristics		
Maternal age/paternal age	Maternal age > 35 yrs. is associated with chromosomal abnormalities (ie. Non-disjunction). Increased paternal age is associated with new dominant mutations.	Paternal age ~28-29 yrs. Maternal age not reported
Consanguinity	Costovertebral malformations have been well documented in cases where parents are related. Knowing whether parents are related may help to determine inheritance patterns.	Nonconsanguineous
Ethnicity	These defects tend to be more prevalent in certain ethnic groups, for example, Puerto Rican population	Caucasian
Family History (Family members with costovertebral malformations or other anomalies)	This may help to determine inheritance patterns.	Paternal grandmother and great grandfather said to be identically affected (could not be examined)
Affected Sibs	This may help to determine inheritance patterns.	None
Chronic Maternal Disease	For example, diabetes, alcoholism. These may indicate other factors responsible for the observed malformations.	None reported
Reproductive History	Previous pregnancies (stillborn, spontaneous abortion (SA), termination of pregnancy (TOP)) may indicate familial inheritance; subsequent pregnancies used to determine recurrence risk.	2 normal sibs

Parental Heights	Short stature is a feature of SCD/STD (short trunk).	Father 59 in.(<5th percentile) Father's upper to lower segment ratio 0.9 (abnormal - decreased)
Details of Pregnancy/Birth		
Type of Birth	For example, livebirth, stillbirth, TOP before 20 weeks, at 20 wks +. This is important to know prenatal mortality.	Livebirth
Date of Delivery	To identify any temporal clusters of malformations and calculate prevalence rates and changes with time.	Not reported
Birth Order	To examine whether birth order has an effect on the nature and/or severity of the malformations.	Not reported
Gender	To determine sex ratios and to evaluate whether penetrance/severity is altered between sexes (M, F, indeterminate, unknown).	Male
Birth Weight	To determine abnormal fetal development.	7 lb 9 oz. (50th percentile)
Birth Length	A key feature of SCD/STD is short stature.	18 in. (5th percentile)
Length of Gestation	Abnormalities in length of gestation may provide alternative explanations for associated anomalies (eg. prematurity).	Not reported
Last menstrual period	To allow calculation of length of gestation.	Not reported
Expected date of delivery	To allow calculation of length of gestation.	Not reported
Prenatal Diagnosis		Not reported
First Prenatal Diagnosis Technique	Ultrasound, maternal AFP, other biochemical testing, amniocentesis, CVS, other, unknown.	Not reported
Gestational age at prenatal diagnosis	To determine gestational age at which malformations may be detected.	Not reported

# of fetuses in pregnancy	If >1, are others affected?	Not reported
Birth order in multiple births (if applicable)	To determine whether birth order affects nature or severity.	N/A
Prenatal vitamin supplementation	Lack of vitamins has shown associations with other major malformations. (For example, neural tube defects with a lack of folate).	Not reported
Pregnancy Complications	To determine other possible causes of malformations.	None reported
Maternal exposures (examples: drugs, alcohol, smoking, x-rays), and when exposure(s) occurred	Any exposures, especially during the early weeks of pregnancy may be important and may be causative of malformations. Somitogenesis occurs at approximately the 3rd week after conception, so exposures around this time are particularly important for this study.	None reported
Clinical Information		
Radiographic data	This provides the specific nature of rib/vertebral malformations and associated skeletal anomalies Examples: Hemivertebrae, block vertebrae, butterfly vertebrae, bifid ribs, crab-like thorax conformation, spina bifida, incomplete segmentation of ribs, abnormal bones, clavicle anomaly, absent atlas, odontoid anomaly.	-3rd thoracic vertebra downward-anomalies of segmentation (sagitally cleft or butterfly vertebrae) -minimal scoliosis to right at 4th and 10th vertebrae and to left at 6th vertebra -11 ribs on right, 12 on left -left 4th and right 6th thoracic vertebrae, pedicles and ribs hypoplastic -small hemivertebra on right between 2nd and 3rd lumbar segments -no coccyx -mild anomalies of sacral segments -iliac wings -minor coxa valga
Malformations – major and minor	To determine syndromic diagnoses and commonly associated malformations.	Minor equinovarus

Syndromes/Associations	Examples: MURCS, VACTERL.	None
Karyotype	Known chromosomal anomaly.	Normal male karyotype
Respiratory infections in infancy	Respiratory compromise is the most frequent cause of death in the more severe forms of SCD/STD. The association of complications with specific costovertebral patterns may help to determine prognosis.	None, asymptomatic except for short stature
Survival (if applicable, age at death)	To determine whether the nature of malformations indicates a specific survival period (for example, crab-like thorax is associated with poor prognosis).	Alive
Current Status		
Age at last examination	These are required to determine growth parameters and to indentify abnormal development.	5 yr. 9 mo.
Height		40 in. (<5th percentile)
Weight		42 lbs (25th percentile)
Head circumference		21 in. (85th percentile)
Upper to Lower segment ratio (U:L)	Individuals with SCD/STD often have lower upper to lower segment ratios (a result of the short-trunk).	1.00 (abnormal - decreased)
Developmental Status (normal/delayed motor, normal/delayed cognitive, normal/delayed general)	These are important to make a syndromal diagnosis/prognosis.	Normal

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