A POPULATION BASED STUDY OF THE VACTERL ASSOCIATION

A thesis submitted

to

THE FACULTY OF GRADUATE STUDIES
UNIVERSITY OF MANITOBA

In partial fulfillment of the requirements for the
DEGREE OF MASTER OF SCIENCE

by

LEONIE STRANC
DEPARTMENT OF HUMAN GENETICS

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TO MY PARENTS
THANK YOU

ACKNOWLEDGEMENTS

I would like to thank all those who have assisted in the completion of this research, especially Dr. J. A. Evans. Without her this work would not have been possible. She has again demonstrated her versatility and ability to juggle "hats" being both teacher, guide and friend.

I would also like to express my appreciation to the faculty, staff and students of the Department of Human Genetics and the Section of Clinical Genetics for their support. In addition I would like to thank Mary Cheang who understood, and took the time and trouble to bring light to the darker corners of my statistical ignorance. I would also like to thank Ann Whitehurst of the Manitoba Congenital Anomalies Registry and my brother, Chris Stranc, for his help and patience.

The work presented in this thesis was supported in part through a studentship funded by the Manitoba Health Research Council.

ABSTRACT

This study of the VACTERL (Vertebral, Anal, Cardiovascular, Tracheo-Esophageal, Renal and Limb anomalies) association was based on unbiased population data covering Manitoba births from 1979-1983. Numerical classification techniques were applied to generate and delineate more homogeneous subgroups from within this population.

Forty-five individuals were ascertained with anomalies in three or more VACTERL systems and another forty-four had anomalies in two systems. Numerical taxonomy was found to be an effective tool for association analysis, consistently clustering the potential VACTERL cases into five main groups, the first containing individuals with cloacal exstrophy; the second tracheal agenesis association variants; the third representing the caudal regression spectrum; and the last two indicating potential VACTERL subgroups.

VACTERL A represented what has classically been considered to be VACTERL, with a high frequency of imperforate anus, vertebral, renal and genital anomalies. There were no cardiac anomalies and no non-VACTERL

anomalies seen in this group. VACTERL B had a lower incidence of imperforate anus, no tracheo-esophageal fistula or genital anomalies, and all cases had a cardiac anomaly. Overlap between VACTERL B and the facio-auriculo-vertebral spectrum was demonstrated by the presence of such non-VACTERL anomalies as microtia, epibulbar dermoid and branchial cleft.

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1.000 INTRODUCTION

1.001 Case Report.

Baby girl J.F. was born at 38 weeks gestation by vaginal delivery to a 29 year-old gravida 1 para 0 mother whose delivery had been induced because of polyhydramnios. The pregnancy that resulted in this child was unplanned and followed a four year period of infertility. At approximately three weeks gestation Mrs F had "influenza" and sinusitis that was accompanied by a high fever. A course of tetracycline was prescribed. Bleeding was reported in the first trimester. No other medications were taken, or unusual exposures noted during the pregnancy.

Baby F's APGAR scores were 8 at one minute and 10 at 5 minutes. Anal atresia and tracheo-esophageal fistula (TEF) were noted at birth. She weighed 2,795g (45%ile) and had a crown-rump measurement of 30cm (5%ile) and length of 47cm (20%ile) and a head circumference of 34.5cm (50%ile). Upon further investigation the following anomalies were detected: fusion of several vertebral bodies in the lumbar region, absence of distal sacral segments, a recto-vaginal fistula, an ectopic left kidney attached to the lower pole

of the right kidney, and a haemangioma on the right thigh. The heart was enlarged on chest X-ray but no evidence of congenital heart disease was found and the EKG was normal. Radial ray involvement was demonstrated by the proximal implantation of the thumbs and bilateral short first metacarpals. A "diagnosis" of the VACTERL association was made.

Two days after birth, brief seizure activity was noted. No cause could be found but a subsequent EEG was normal and there were no recurrences. The TEF was repaired shortly after birth and the recto-vaginal fistula dilated to allow passage of stool. Her renal function appeared to be within normal limits, even though there did not appear to be a functioning collecting system for the ectopic kidney.

A family history revealed that Mr F. had a sister who had died in childhood with a blind esophageal pouch and an imperforate anus and that Mrs F. was a twin. There did not appear to be any other affected individuals or any other twin pairs in the family and no diabetes or gestational diabetes was noted. J.F. is now six years old and is progressing well. She has undergone numerous surgical proceedures including a colostomy, bilateral reimplantation

of ureters, repair of imperforate anus and recto-vaginal fistula, closure of the colostomy, revision anoplasty, and repeat colostomy. She is at the 10th percentile for height, the 5th percentile for weight and her head circumference is at the 25th percentile. Motor coordination and speech development are normal for her age. Although she has developed a lumbar scoliosis due to the vertebral anomalies, she suffers no neurologic sequelae.

The birth of a child like baby J.F., with multiple congenital anomalies is devastating to a family, especially as in all likelihood, this possibility had not been seriously entertained. Approximately one percent of all newborns have multiple anomalies and only 40% of these can be diagnosed as having specific, recognised syndromes. The other 60% represent conditions about which we know little of etiology (Cohen,1981). A diagnosis is sought not only to better manage the child's problems and determine its prognosis but also for the support of the parents. At this time there are many questions asked, and any answers as to what has happened or why, are invaluable both initially and also later on as the family adjusts and when future pregnancies are considered.

1.100 The Concept of Association.

One specific patterning of multiple congenital anomalies is the "association". An association has been defined as "the nonrandom occurrence in two or more individuals of multiple anomalies not known to be a polytopic field defect, sequence or syndrome." (Spranger et al. 1982). So defined, it is a diagnosis of exclusion. (Actually, this is not a diagnosis but rather a description of a constellation of findings). Associations are seen in over 10% of all patients with multiple congenital anomalies and are not rare, occurring in about 1/1,000 births (Czeizel et al., 1981c).

One of the most outstanding features of associations is that, unlike syndromes or sequences, they are causally non-specific. Initially a statistical concept, it was suggested that associations were merely transitional labels that would be replaced with more accurate syndromal diagnoses once they were better understood, and that associations in themselves had no biologic reality (Spranger et al, 1982). However recent work suggests that they are indeed distinct entities (Opitz et al, Proceedings of the 7th International Congress of Human Genetics, Berlin, (1986), in press).

Inherent in the concept of association is a problem with definition of their boundaries since they are not discontinuous but merge into other associations. They represent "continuous multidimensional spectra with different patterns of anomalies or even single defects at different end points" (Evans, 1985). The approach that this study takes is the use of numerical taxonomy to study a specific association, VACTERL. This seems particularly appropriate since numerical taxonomy, like the concept of association, is also based on a statistical approach. This methodology has the advantage of being able to objectively look at the components of an association.

1.200 The VACTERL Association.

The VACTERL association is a non-random clustering of Vertebral, Anal, Cardiovascular, Tracheo-Esophageal, Renal, and Limb defects (see Appendix 1 for list of abbreviations). Once known syndromes have been ruled out, any individual with defects in three or more of the systems encompassed by VACTERL can be said to have this association. Within each system the scope of anomalies is broad. For example, cardiovascular defects can range from complex congenital heart anomalies such as Tetralogy of Fallot and transposition of the great vessels to

coarctation of the aorta and single umbilical artery (SUA). Similarly, limb anomalies include reduction deformities, pre or post-axial polydactyly, and radial ray anomalies. Only limb deformations such as club foot and congenital hip dislocation are not included in the spectrum. Due to the variety of anomalies that are considered to be potentially a part of this association, the resulting phenotype is necessarily variable.

1.210 Evolution of the VACTERL association.

Combinations of two or more congenital malformations are frequently observed. Indeed, it has been noted that all major malformations occur together more often than would be anticipated by chance alone (Roberts and Powell, 1975). Prior to the recognition of the VACTERL association many of its components were observed to occur together. These included radial dysplasias and esophageal atresia; upper vertebral defects and esophageal atresia; lower vertebral defects and imperforate anus; and esophageal atresia and imperforate anus (Ladd et al., 1934; Kirkpatrick et al., 1965; Kato, 1924; Stevenson, 1972; Harris and Osbourne, 1966).

In 1968, Say and Gerald reported a new "syndrome" with a triad of anomalies including imperforate anus (IA), tracheo-esophageal fistula (TEF), and vertebral anomalies. In a later paper Say et al. (1971a), extended this to include poly- oligodactyly and skeletal anomalies. Quan and Smith's papers (1972, 1973) increased the scope of anomalies, stressed that this represented an association and not a syndrome, and proposed a possible etiology. They proposed the acronym VATER which encompassed Vertebral defects, Anal atresia, Tracheo-Esophageal fistula, Renal and Radial dysplasia. They postulated that this array of anomalies was due to a primary defect of mid-posterior axis mesoderm resulting in abnormal mesodermal differentiation before the thirty-fifth day of gestation.

Since this met a need and a possible pathogenetic mechanism was provided, and also because of Smith's reputation as a dysmorphologist, this became a popular concept. It also stimulated reports of additional cases, many of which seemed to fit into this pattern, and others which also had additional features that were not considered typical, that led to a broadening of the scope of anomalies considered to be a part of the association.

Filippi (1972) noted a high incidence of renal anomalies in this association , Kaufman (1972) emphasized malformations of the gastrointestinal system as a feature and Temtamy and Miller (1974) proposed a (cardio-) vascular component, based on the frequent observation of ventriculo-septal defect (VSD) and SUA in their population. Nora and Nora (1975) again emphasized this cardiovascular component and also pointed out that since ulnar as well as radial limb defects were seen, a more general "limb" category would be more correct and proposed the modification of the acronym to VACTERL (where L stands for limb). Among others, Apold et al. (1976); Eddie et al. (1980); and Sofatzis et al.(1983), noted a possible genital component to the VACTERL association. In 1977, Say et al. (1977a) proposed that congenital eye defects also be included, and in 1984, Briard et al. described a new entity of VACTERL-like anomalies and hydrocephalus.

The four cases with ocular defects that were described by Say et al.(1977a) had relatively minor anomalies; ptosis, strabismus, and myopia rather than severe structural abnormalities and it was felt that these might have been missed as a feature of the association due to their relatively mild nature. This report also presented further evidence that children with the VACTERL

association who survive beyond the neonatal period are not intellectually impaired and that aggressive management is warranted. Another aspect of this association appears to be short stature, with surviving individuals at or below the tenth percentile for both height and weight (Quan and Smith ,1972, Temtamy and Miller, 1974, Say et al., 1977, Bull et al., 1985). In contrast, individuals with VACTERL-type anomalies and hydrocephalus have a very poor prognosis with most not surviving the neonatal period (Briard et al., 1984; Aleksic, 1984).

1.211 Ascertainment of Cases of the VACTERL Association.

The majority of papers on VACTERL have been either case reports or retrospective studies. In the latter group ascertainment of cases was usually through a specific "key" anomaly such as polydactyly, imperforate anus or tracheoesophageal fistula or through a combination of key defects proposed by earlier reports (Filippi, 1972; Temtamy and Miller, 1974; Nora and Nora, 1975).

Say and Gerald's new "syndrome" was based upon 10 cases ascertained through polydactyly. Quan and Smith perceived an even broader association of defects. The seven cases that they presented as examples were selected

by virtue of their having at least two of the five defects that they had decided were key anomalies. In 1974, Temtamy and Miller "defined" the scope of VATER, using data from ten new and twenty four previously reported cases that had been ascertained as having three or more of the key anomalies proposed by Quan and Smith. This approach necessarily limits the investigation of the nature and strength of the interrelationships between the anomalies and can bias perceptions of the association since the opportunity for ascertainment is not equal for all anomalies.

1.220 Overlap Between VACTERL and Other Genetic Conditions

Case reports of known syndromes with VACTERL-type anomalies and "classic" VACTERL cases with atypical features posed a problem, and it soon became apparent that there was considerable overlap between VACTERL and many other conditions (Gossey et al.,1982; Hall, 1979; Mendelburg et al., 1985; Silver et al., 1972). VACTERL-type anomalies are seen with some chromosomal syndromes such as Trisomy 13, Trisomy 18, and 13q- (thumb hypoplasia, microcephaly, eye defect, cardiac anomaly). They are also seen with single gene disorders including Meckel syndrome (encephalocele, polydactyly, polycystic kidneys), Holt-Oram

(upper limb hypoplasia, cardiac anomaly, narrow shoulders), Duane Retraction syndrome and environmentally induced malformations such as those due to amnion disruption and the Thalidomide embryopathy. There is also overlap with the CHARGE spectrum (Coloboma, congenital Heart, Atresia choanae, Retarded mentation and growth, hypoGenitalism, Ear anomalies),(Koletzko et al., 1984); the MURCS association (Mullerian duct aplasia, Renal anomalies, Cervico-thoracic Somite dysplasias),(Duncan et al., 1979); Facio-auriculo-vertebral spectrum (Pashayan et al., 1970), the caudal regression spectrum (Russell et al., 1981) and cloacal exstrophy.

1.230 Patterns of Inheritance.

The majority of VACTERL cases are sporadic with no evidence of genetic or environmental factors or parental age effects although some recurrences have been documented (Auchterlonie and White, 1982; Briard et al., 1984; Sujanski and Leonard, 1983). Families with VACTERL-like anomalies have also demonstrated different modes of inheritance including autosomal recessive and autosomal dominant. This has been further complicated by the variable expressivity among affected individuals.

1.231 Autosomal Dominant Inheritance.

Autosomal dominant inheritance has been seen in the VACTERL-like Townes Brock syndrome which is characterized by anal (atresia, antereposed anus), ear (sensorineural deafness, pinnal malformation, preauricular tags), and thumb anomalies (Townes and Brock, 1972). In 1978, Kurnit et al. reported autosomal dominant transmission of a syndrome of anal, ear, renal and radial malformations. The family studied contained nine individuals with an anal anomaly in varying association with defects of the ears and radial ray anomalies.

Possible autosomal dominant inheritance for the VACTERL association was also postulated by Say et al.(1971b) who described a young girl with anal atresia, hypoplastic thumb, hemivertebrae, and unilateral renal agenesis, whose mother had a possible thumb anomaly and whose maternal grandmother had vertebral and thumb abnormalities. Furhman (1968) reported a boy with anal atresia who had a brother with anal atresia, pre-axial polydactyly and a possible cardiac anomaly. Their mother had isolated pre-axial polydactyly.

1.232 Autosomal Recessive Inheritance.

In 1976 Naveh and Friedman described two sibs, one of whom had anal atresia, renal, cardiovascular and radial ray anomalies and the other, isolated anal atresia. Both sibs were also mentally handicapped. Their parents were apparently normal but they were consanguineous. An autosomal recessive mode of inheritance was thought to be most likely.

In 1983, Sujanski and Leonard reported three sibs with VACTERL-type anomalies and hydrocephalus. The first child, who died at four and a half months of age, had hydrocephalus, tracheo-esophageal fistula, congenital heart disease, renal hypoplasia and bilateral agenesis of the radii and thumbs. The second case was a fetal demise at 21 weeks gestation. Upon autopsy the following findings were noted; tracheo-esophageal fistula, bilobed right lung, hypoplastic kidneys, absent left radius, bilaterally absent thumbs, and bilateral equinovarus deformities. The third affected fetus was diagnosed prenatally with progressive hydrocephalus on ultrasound examination and the pregnancy was terminated. The autopsy revealed dilated ventricles, renal anomalies, absent right thumb and rudimentary left thumb.

These examples only serve to emphasise the heterogeneity within VACTERL. Many of the familial entities may be "private" syndromes that require more precise delineation. However, until this can be accomplished they are most logically grouped with the other VACTERL cases even though the prognosis and recurrence risks may not be the same.

1.233 Possible Environmental Influences.

Progesterone/Estrogen Compounds

In 1975, Nora and Nora proposed a causal relationship between maternal intake of progesterone/estrogen compounds and the VACTERL association. This study was prompted by a heightened awareness of the possible teratogenic effects of common drugs, as evidenced by Thalidomide, and the fortuitous admission to hospital of two individuals with multiple congenital anomalies and documented maternal estrogen exposure. Of their nineteen VACTERL cases that were felt to have reliable drug exposure histories, thirteen were also found to have had maternal exposure to progesterone/estrogen compounds during the vunerable period of embryogenesis. This relationship has recently been refuted by Lammer, Cordero, and Khoury (1986) in a

registry-based, case control study.

Pinsky (1978) discussed this type of teratogenic effect upon the fetus using the autosomal recessive disorder McKusick-Kaufman Syndrome as an example. He suggested that hyperestrogenicity might be responsible for both the dysmorphia and the uterine dysfunction. In that disorder vaginal atresia and uterine hypersecretion in the perinatal period produce hydrometrocolpos. Another common feature of this syndrome is postaxial polydactyly. Imperforate anus is seen occasionally but occurs much more commonly with vaginal atresia if hydrometrocolpos is present than if it is absent.

Maternal Diabetes

The association between maternal diabetes and fetal malformation has been extensively documented. Multiple organ systems are susceptible to the teratogenic effects of poorly controled diabetes, with many studies showing an increase in malformations of the cardiovascular, genitourinary, musculoskeletal and central nervous systems. (Moldsted-Pedersen et al., 1964; Mills, 1982; Soler et al., 1976; Kucera, 1971; Breidahl, 1966; Barr et al., 1983).

The best known of these malformation complexes is that of the caudal regression sequence (Duhamel, 1961) in infants of diabetic mothers (Lenz and Mairer,1964; Rusnak and Driscoll, 1965; Kucera, 1971; Assemany et al., 1972). It occurs in about 1% of infants of diabetic mothers, roughly 200 times more frequently than in infants of non-diabetic mothers (Mills, 1982).

The most severe form of this defect, sirenomelia, is thought to be due to a primary defect in the posterior caudal axis blastema which would result in fusion of the limb buds and absence or incomplete development of the intervening caudal structures. Since both radial hypoplasia and tracheo-esophageal fistula with esophageal atresia have been seen as features of the caudal regression sequence (Williamson, 1970; Grix, 1982) some authors have proposed that the VACTERL association may represent a milder expression of this malformation sequence (Smith et al., 1976).

1.240 PATHOGENESIS.

1.241 Disruption of Early Mesodermal Development.

Quan and Smith (1972, 1973) proposed a common developmental pathogenesis, such as an early mesodermal defect, as the basis for the varied anomalies seen in the VACTERL association. The mesoderm first appears between the ectoderm and the entoderm in the posterior quadrant of the embryonic disc. Intimately associated with the formation of the general mass of the mesoderm is the origin of an axially located cylindrical mass of cells, the notochord.

The mesoderm on either side of the notochord (paraxial mesoderm) becomes thickened and forms the somites which ultimately give rise to the axial and appendicular skeleton. Extending to either side of this region are sheets of lateral mesoderm which are later divided into the somatic and splanchnic mesoderm by the development of the coelom. Derivatives of the somatic mesoderm form the inner lining of the pleura, pericardium, and peritoneum; and those of the splanchnic mesoderm form the heart, visceral pleura, visceral peritoneum, and mesentries. Between the dorsal and lateral mesoderm is a narrow zone;

the intermediate mesoderm. Structures derived from this tissue include the pronephros and mesonephros and the internal and external genitalia.

Thus, an insult to the embryo (whether it be environmental or genetic) could result in a large number of malformations in many different body systems. Malformations of non-mesodermal derivatives that are commonly seen as part of VACTERL are those of the trachea and esophagus, both of which are derived from the entoderm and anal anomalies which may be due to anomalous development of the entodermal cloaca or the anal ectoderm.

1.242 Abnormal Differentiation of the Embryonic Gut.

Duncan and Shapiro (1979) assembled a series of cases ascertained according to the criteria proposed by Quan and Smith. In addition to individuals with "VACTERL" also included were individuals with Hemifacial Microsomia and Sirenomelia. Despite the phenotypic heterogeneity, all cases were felt to be interrelated and possibily caused by the same primary embryologic pathogenetic mechanism: an alteration of the embryonic gut (yolk sac-allantois) occurring in the first two weeks of embryonic life. This they postulated, would explain the large number, and

widespread distribution, of anomalies of the alimentary canal, lower urinary tract, and respiratory tract that were encountered.

1.243 Embryonic Hyperflexion Theory.

Stephens (1981a) examined the association of tracheo-esophageal and anorectal malformations, and was struck by the embryologic similarity between the cloaca and the foregut in that they are both "tubes" that undergo partitioning, the first to form the rectum and urogenital sinus and the second the trachea and esophagus. He proposed the following as a pathogenetic mechanism: at the end of the third week of life, the flat trilaminar embryonic disc folds into a roughly cylindrical embryo. This folding is due to the rapid growth of the embryo, in particular of the neural tube, and occurs in both the longitudinal and transverse planes producing head and tail folds that result in the cranial and caudal regions moving ventrally. During this period while both the cloaca and the foregut are divided into two by the invagination of mesodermal shelves, somite formation of the embryo, rapid elongation and ventral flexion of the head and tail occur. Hyperventroflexion of the embryo could cause urorectal and tracheoesophageal malformations by disrupting this folding

process so that the septa are not properly aligned (Stephens, 1981b). Vertebral anomalies can also be explained by this hyperflexion since the stress it causes in the area of maximum angulation may interfere with the development of the vertebral segments beyond that point.

1.244 Abnormal Neural Tube Development.

Gardner postulated that anomalies of the heart, gastrointestinal tract, kidneys and limbs may be caused by damage to their mesodermal or entodermal analagen by overdistention of the embryonic neural tube. This theory was supported by experimental and clinical evidence of the frequent association between some non-neural anomalies and dysraphic conditions (Gardner and Breuer, 1980; Gardner, 1979). Another similar mechanism was described by Padget (1970), who postulated that the leak of neural tube fluid from a rupture of the neural tube might cause abnormal development in adjacent non-neural tissues. However, the frequency of individuals with VACTERL-type anomalies who also have neural tube defects is low, so it would appear that damage to the neural tube is not the primary cause of this association.

1.245 The Neural Crest Theory.

McCreadie proposed a neuroanatomic basis for the classification of multiple malformations related to phocomelia. Skeletal and internal structures were shown to be anatomically linked through segmental levels of innervation, neurotomes, and so related embryologically to the neural crest. Neurotomes were defined as that part of an embryo related to a segmental level of neural crest. This included both skeletal (sensory) and visceral (autonomic) components (McCreadie, 1976).

Congenital abnormalities within the same or adjacent neurotomes would explain the distribution of defects in the Thalidomide embryopathy and morphologically similar multiple malformation syndromes. Theoretical "syndromes" were predicted using this information and it was expected that upper limb defects would be associated with malformations of the heart, esophagus, trachea, and diaphragm due to involvement of the lower cervical neurotomes and that lower limb defects would be associated with malformations of the rectum, anus, bladder, and external genitalia due to the involvement of the lumbo-sacral neurotomes (McCreadie, 1983).

As at any one time the embryo will contain a range of stages of neural tube and neural crest development (most advanced at the cephalic end), this would provide a spectrum of stages and a potential spectrum of vunerability to any agent that disrupts the normal development of neural crest cells. This theory holds that the susceptible tissue is that of the neural crest and not the mesodermal organs which it supplies.

1.246 The Developmental Field Concept.

The pathogenesis of VACTERL can also be explained using the developmental field concept. A developmental field is that part of the embryo that reacts as a temporally and spatially co-ordinated unit to localized forces of organization and differentiation (Opitz and Gilbert, 1982). The components of a field may be contiguous (monotopic) or more distantly located (polytopic), so a single cause acting on a field will produce a single but complex malformation involving all structures normally derived from that field. The timing of the insult, its strength, and location, as well as genetic susceptibility are also important factors in determining the final constellation of malformations produced. The concept of developmental fields has been supported by work

in many different areas (see "Developmental Field Concept" (in press), or Volume 21(1), Am. J. Med. Genetics, May 1985).

The midline is considered to be a poorly buffered developmental field (Opitz and Gilbert, 1982). This is supported by the association of schisis-type abnormalities such as neural tube defects, oral clefts, omphalocele, and diaphragmatic hernia described by Cziezel (1981b). Midline organs include the heart, uterus, central nervous system, and external genitalia. Tracheo-esophageal fistula, other laryngo-tracheal anomalies, imperforate anus, omphalocele, cleft lip and/or palate as well as malformations of the midline organs are all midline anomalies.

Only the renal and limb defects seen in VACTERL are non-midline anomalies. However, in early fetal development the renal primordia and limb buds are more closely contiguous than at a later stage of development and thus these two systems may also be influenced by the disruptive forces acting on the embryo.

All these theories are potentially capable of explaining the constellation of findings that we see in the VACTERL association but they are not necessarily mutually

exclusive: for example, it is possible to envisage the disruption of a developmental field due to the anomalous morphogenesis of the neurotomes. There is a great need for more experimental work in this area in order to more clearly understand the precise chain of events.

1.300 NUMERICAL TAXONOMY.

1.310 Methodology.

Man has always sought to group similar objects to make them more easily remembered and organised. Taxonomy (the study of classification) has produced many different ways in which objects can be grouped. They are all based on similarities perceived by the observer. However, the relative importance of the chosen criteria may not be universally accepted.

Classification in numerical taxonomy is based on a matrix of resemblances in which taxa or groups are constructed through various techniques designed to disclose and summarize the structure of that matrix. Their value is that they permit delineation of groups in an objective manner. Numerical taxonomy uses mathematical and statistical techniques to classify a large and heterogeneous group of objects into potentially more meaningful subunits. It produces a phenetic relationship, that is, one that is based on overall similarity based on all available characters initially without any weighting.

Numerical taxonomy was first developed in the early 1900s and was initially used in physical anthropology. Its potential to produce faster reliable classifications of objects could not be fulfilled at a time when computerized technology was not yet widely available. The modern era of numerical taxonomy and its rediscovery was initiated independantly by Sokal and Sneath in the 1950s. Since then there has been a rapid increase in the development of different methods and applications of these techniques in such diverse areas as geology, zoology, botany, agriculture, psychology, and the study of birth defects (for further details of numerical taxonomy methodology and application: An Introduction to Numerical Classification, Clifford and Stephenson, 1975; Numerical Taxonomy, Sokal and Sneath, 1973).

The fundamental principles on which this type of classification is based are as follows (modified from Sneath, 1958):

1. The greater the content of information in the taxa of a classification and the more characters on which it is based, the better a given classification will be.

2. A priori, every character is of equal weight in creating taxa.

3. Overall similarity between any two entities is a

function of their individual similarities in each of the characters through which they are being compared.

4. Distinct taxa can be recognised because correlations of characters differ between the groups of organisms under study.

5. Phylogenetic inferences can be made from the taxonomic structures of a group and from attribute correlations, given certain assumptions about evolutionary pathways and mechanisms.

Standard numerical taxonomy techniques attempt to classify individuals in one of two ways: they may either look at the whole population and divide it into smaller, more homogeneous, subgroups (divisive techniques) or they can start by considering the individual subunits and group these together to form larger units (agglomerative techniques). This classification can be either based on the use of single key characters (monothetic classification) or on the basis of several shared characteristics that define the group (polythetic classification). Of the four possible combinations (monothetic divisive, monothetic agglomerative, polythetic divisive, polythetic agglomerative), only monothetic divisive and polythetic agglomerative techniques are currently used.

Both the monothetic divisive and polythetic agglomerative techniques may be used to classify either individuals (normal analysis) or attributes (inverse analysis). Normal or Q analysis looks at the relationship of pairs of attributes over all the individuals that comprise the data set and groups individuals on the basis of their attributes. Inverse or R analysis looks at the relationship of pairs of individuals over all the attributes in the individual/attribute matrix and groups the attributes.

Thus, the goal of taxonomy is to provide groups whose parameters can be defined and be used to classify new individuals in addition to giving us information about the group itself. However, generalizations about groups cannot be made before they have been recognised. Likewise, a group cannot be recognised before the resemblances between the individuals under study are known, and these cannot be estimated before the individuals and their attributes have been examined.

1.311 Selection of Attributes.

Deciding what attributes should be included in the attribute/individual matrix is crucial to the validity of the final groupings. This cannot be decided in a subjective manner. As much data as possible must be obtained about each individual and the frequency of each character be determined for the whole population so that those attributes that are found in a reasonable proportion of the population are included in the matrix.

1.312 Selection of Measures of Similarity.

The next step is the selection of measures of similarity or difference (and in polythetic agglomerative methods, the choice of a clustering strategy). These quantify the resemblance between the elements in the two columns of the data matrix that represent any two individuals or attributes and calculate the degree of similarity between them. The choice of coefficient is largely determined by the form of the data: a different coefficient would be appropriate for continuous data than for quantitative data.

There are four main types of similarity measures; coefficients of association, coefficients of similarity, Information measures and Euclidean distance. Similarity coefficients are derived from a ratio of the number of attributes shared by a pair of individuals relative to the total number of attributes involved in the comparison. Coefficients of association estimate the extent of association between attributes within populations. They may also be used as similarity measures, with the groups being compared in pairs with respect to the set of attributes available.

Information measures determine the diversity within a system. This is done by partitioning groups of individuals and looking at the total variation within the groups. Euclidean distance is a measure of dissimilarity that shows the relative distance between two individuals. Their position is determined with respect to their coordinates which are defined by, and referenced to, a set of Cartesian axes.

Although the end result of the clustering process is determined by the inherent structure and relationships between the cases, it is also appreciably affected by the choice of clustering algorithm. When a variety of

similarity coefficients are used in combination with several clustering methods the robustness and relative frequency with which the same groups are produced provides an estimation of the usefulness of the various coefficients.

1.313 Choice of Clustering Technique.

Monothetic Divisive.

Monothetic divisive techniques divide the initial data set into successively smaller subgroups on the basis of the presence or absence of a certain key attribute. The attribute that divides the group is the one with the largest coefficient of similarity with respect to all others considered. This calculation is repeated after each split to generate the next key attribute. The factor which is stressed in this type of analysis is constancy, since all the members of a particular group must have the key anomaly. A major drawback to this technique is that an individual will be misclassified if the key attribute is not present, even though it is obvious from the overall phenotype that it belongs to that group.

Polythetic Agglomerative.

Polythetic agglomerative techniques calculate the similarity between each individual in the data set, and every other individual in that set, and group together those individuals with the greatest overall similarity. No single attribute is an essential requirement for membership in a group or is sufficient to make an individual a member of that group. This method produces groups with a high content of information which are also more "natural" than those produced by monothetic divisive methods. However, since they are based on overall similarity between all the individuals there is often partial overlap, as two individuals may be similar yet the first may also be similar to other individuals through a different group of shared attributes.

1.314 Nodal Analysis.

A specialized use of the monothetic divisive technique, nodal analysis, was devised by Lambert and Williams (1962) to explore the nature and extent of coincidences between normal and inverse analysis and to establish the central "species-in-habitat" coincidences (in our case this would correspond to anomalies-in-individuals) around which the population under examination may be considered to vary and to isolate the strongest "malformation-individual" clusters. This is accomplished by superimposing the results of a normal and an inverse analysis to form a two-way table so that each cell in the table is defined by both an Q (normal) group of individuals and a R (inverse) group of attributes.

1.315 Definition of Clusters.

Once the analyses have been completed the classification must be checked for obvious misclassifications. This completed, the groups produced should be defined. Since the objective of numerical taxonomy is to improve the classification of variation it follows that once this is accomplished the classification can then be studied in an attempt to determine the cause of

this variability.

1.320 Applications of Numerical Taxonomy.

Numerical taxonomy has been the tool of several investigators studying birth defects. Preus used this approach to more precisely delineate the del(4p) phenotype (1985), Williams Syndrome (1984), Brachmann-De Lange Syndrome (1983), and the Marshall and Stickler Syndromes (1983), and to establish a diagnostic index for Down syndrome (1977). Another example of the use to which it has been put is Pinsky's classification of ectodermal dysplasias (Pinsky, 1977). This technique has also been used by Evans to study heterogeneity within associations (Evans, 1982, 1984). An interesting aspect of numerical taxonomy is that of individuals who obviously do not belong in any of the taxa produced. One such case, included in a study of malformation associations of infants with renal agenesis and/or radial hypoplasia, was consistently misclassified and was hypothesized to represent a new syndrome. Subsequently similar cases have been reported substantiating this claim (Evans, personal communication).

Preliminary studies by Evans (1982, 1984) on the VACTERL association provided the stimulus for the present

endeavour. Through the use of numerical taxonomy on two differently ascertained populations it appeared that the VACTERL association could be divided into distinct subgroups with differing incidences of key anomalies and demographic parameters such as sex ratio, stillbirth and infant death rates.

In the present study a very broad definition of VACTERL-type anomalies was initially determined including ear and genital anomalies and choanal atresia. Only by giving each anomaly an equal opportunity for ascertainment would the true interrelationships become evident. Furthermore, unrepresentative data may well mask true relationships since missing pertinent material cannot be compensated for in the analysis. However, extraneous information should not pose a problem as it would become evident from the analysis that it is not a feature of the association under study. Only once the association has been properly delineated can demographic data be examined to determine the natural history and recurrence risks involved.

1.400 OBJECTIVES

The specific aims of the present study were to:

1. Obtain unbiased population based data on the VACTERL association.

2. Determine the incidence of components of the VACTERL association over a five year period (1979-1983 inclusive).

3. Test different methods of numerical taxonomy and the use of different coefficients to evaluate their use in association analysis.

4. Apply numerical classification techniques to the data to see if more homogenous subgroups can be ascertained.

5. Define key anomalies for the VACTERL association, or for any subgroups generated.

2.000 MATERIALS AND METHODS.

2.100 Ascertainment of Cases.

The initial population included all newborns with a birth defect born in Manitoba over the five year period from 1979 to 1983 inclusive. From this population those individuals with at least one anomaly compatible with VACTERL were ascertained. A broad definition of VACTERL-type anomalies was used (see Table 1.). There were multiple sources of ascertainment including a review of postmortems performed in the Children's Hospital over the study period and a review of charts from the Section of Clinical Genetics. The primary source of data was the Manitoba Congenital Anomalies Registry which, in 1979, began receiving a printout of all newborns with discharge diagnosis codes that fell in the congenital anomalies section (codes 740 to 759) of the International Classification of Diseases, 9th Revision, from the Manitoba Health Services Commission.

For each individual ascertained, a card was made up documenting name, sex, date of birth, date of death (when applicable), gestation, birthweight, hospital of birth, gravidity and parity of mother, mother's name and age at

Table 1. ICDM Codes Used To Ascertain Individuals With Anomalies Compatible With The VACTERL Association.

VERTEBRAL
756.1 anomalies of the spine
756.2 cervical rib
756.3 other anomalies of the rib and sternum

ANAL
751.2 atresia and stenosis of the large intestine, rectum and anal canal

CARDIOVASCULAR
745.0 common truncus
745.1 transposition of the great vessels
745.2 tetralogy of Fallot
745.3 common ventricle
745.4 ventriculo-septal defect
745.5 atrial-septal defect
745.6 endocardial cushion defects
745.7 cor bioculare
745.8 other anomalies of septal closure
745.9 unspecified defect of septal closure
746.0 anomalies of the pulmonary valve
746.1 tricuspid atresia and stenosis
746.2 Ebstein's anomaly
746.3 congenital stenosis of the aortic valve
746.4 congenital insufficiency of the aortic arch
746.5 congenital mitral stenosis
746.6 congenital mitral insufficiency
746.7 hypoplastic left heart
746.8 other specified anomalies of the heart
746.9 unspecified anomalies of the heart
747.0 patent ductus arteriosus
747.1 coarctation of the aorta
747.2 other anomalies of the aorta
747.3 amonalies of the pulmonary artery
747.4 anomalies of the great veins
747.5 absence or hypoplasia of the umbilical artery
747.6 other anomalies of the peripheral vascular system
747.9 unspecified anomalies of the respiratory system

TRACHEOESOPHAGEAL/RESPIRATORY
750.3 tracheoesophageal fistula, esophageal atresia and stenosis
748.0 choanal atresia
748.2 web of larynx
748.3 other anomalies of the larynx, trachea and bronchus

RENAL
753.0 renal agenesis and dysgenesis
753.2 obstructive defects of the renal pelvis and ureter
753.3 other specified anomalies of the kidney
753.4 other specified anomalies of the ureter
753.8 other specified anomalies of the bladder and urethra

LIMB
755.0 polydactyly
755.2 reduction deformity of the upper limb
755.3 reduction deformity of the lower limb
755.4 reduction deformity unspecified limb
755.52 congenital elevation of the scapula
755.54 Madelung's deformity
755.58 cleft hand, congenital
755.66 other anomalies of the toes

EAR
744.01 absence of the external ear
744.1 accessory auricle
744.23 microtia

GENITAL
752.4 anomalies of the cervix, vagina, and external female genitalia
752.8 other specified anomalies of male genital organs

OTHER
759.0 anomalies of the spleen
759.3 situs inversus

the birth of this child, father's name and age, address, the attending physician or family physician, registry number, anomalies present, outcome (stillbirth, livebirth, neonatal/infant death), and a diagnosis where possible.

2.110 Review of Case and Anomaly Ascertainment.

Autopsy reports from the Children's Hospital of Winnipeg, the Clinical Genetics nosology file, and data gathered by Dr. J. Evans for other studies were reviewed to determine how complete registry ascertainment was and to see why individuals were missed, if indeed any were. These sources were also scrutinized in an attempt to obtain full documentation of all anomalies present, and demographic data. Where this approach did not suffice, a letter was sent to the family doctor for additional information.

One of the cases (from the VACT 3 group) was removed from the analysis once it became evident that it was consistently being misclassified in the analyses due to incomplete anomaly ascertainment. This individual had Trisomy 13 but no autopsy had been performed therefore only the external anomalies were documented. Similarly, data on patent ductus arteriosus was not collected, although it is a common anomaly in a newborn population, because no

criteria could be devised to determine its significance in this population. This problem was also faced with cryptorchidism, but from the literature a reasonable definition was found. It was considered present if one or both testes were undescended in a baby of greater than 35 weeks gestation or with a birthweight of greater than 2,500g (Frey & Raiffer, 1982).

2.200 Incidence.

A total study population of 888 cases was ascertained and was then divided into three groups:

VACT 1: individuals with one anomaly compatible with VACTERL

VACT 2: individuals with anomalies in two systems compatible with VACTERL

VACT 3: individuals with anomalies in three or more systems compatible with VACTERL

Once the cases had been ascertained, any individual with a major structural anomaly of the genitalia (i.e. bifid scrotum, ambiguous external genitalia, uterus didelphus) had this counted as another major system of involvement. This resulted in some individuals in the VACT 3 group having anomalies in three systems classically

considered to be compatible with VACTERL, and others who only had involvement in two but who also had a major genital anomaly.

The first group consisted of 798 individuals, the second of 44, and the third of 45. (The one individual with Trisomy 13 who was excluded from the analysis has been counted in the total number of cases ascertained.) This population of 887 individuals included 71 with a known cause for their malformations: 7 in VACT 2, 11 in VACT 3, and 53 cases in the VACT 1 group (see Table 2). Although these individuals were obviously not true VACTERL cases, they were included since they served as internal controls for the numerical taxonomy. "Known Syndrome" was strictly defined as one where the cause of the malformations, either chromosomal, single gene, or environmental, was known. There were many cases that represented sequences or syndromes of unknown etiology such as cloacal exstrophy, Potter's bilateral renal agenesis-oligohydramnios sequence, and the DiGeorge sequence that were not included in the known group.

Once an alphabetical file of individuals with one or more anomaly compatible with VACTERL was completed a nosologic file was compiled, again using the ICDM codes for

Table 2. Known Syndromes Included in the VACT 1, VACT 2, and VACT 3 Groups.

VACT 1. GROUP		System through which case was ascertained:
CHROMOSOMAL		
Down syndrome	34	(all picked up through cardiac(C))
Trisomy 13	2	(all C)
Trisomy 18	2	(one Tracheal, one C)
Turner syndrome	1	(C)
SINGLE GENE		
Camptomelic dwarfism (?AR)	2	(limb)
Oto-palatal-digital syndrome (XD)	1	(limb)
Laurence-Moon-Beidel (AR)	1	(limb)
Roberts syndrome (AR)	1	(limb)
Congenital porphyria (AR)	1	(renal)
Cerebro-oculo-facial skeletal syndrome (AR)	1	(limb)
ENVIRONMENTAL		
Amniotic disruption sequence	6	(five limb, one C)
Congenital rubella	1	(C)
TOTAL:	53	

VACT 2 GROUP		VACT 3 GROUP	
CHROMOSOMAL		CHROMOSOMAL	
Trisomy 13	2	Trisomy 13	3
Trisomy 18	1	Trisomy 18	1
4p+	1		
Turner syndrome	1	SINGLE GENE	
		Smith-Lemi-Opitz (AR)	3
SINGLE GENE		Meckel syndrome (AR)	1
		Cryptophthalmos (AR)	1
Oculo-dental-digital syndrome (AD)	1		
Pfeiffer syndrome (AD)	1	ENVIRONMENTAL	
		Amnion disruption	2
TOTAL:	7	TOTAL:	11

reference, and divided into yearly intervals. From this the incidence of anomalies for a single VACTERL component, two components, or three or more components was calculated, as were the most common combinations in the latter two groups.

All of the cases in the VACT 1, 2, and 3 groups were classified according to the number of systems involved (ie. 1 through 7: V,A,C,T,R,L,G). The frequencies of each combination of defects (ie. VA, VC, VAC) and the proportion of cases with each combination were calculated. These were compared to the expected number based on the incidence rate of each defect in the Manitoba population (assuming that the anomalies had occured independently).

2.300 Numerical Taxonomy

2.310 Selection of Attributes and Coding

A coding sheet was compiled which represented the eighty-three anomalies that were found in ten percent or more of the combined VACT 2 and VACT 3 groups (see Figure 1.). In addition fourteen demographic variables were coded. The information was represented in binary form (the anomaly was either present or absent) due to the limitations of the computer program used (CLUSTAN 1C; Wishart, 1975). In the anomaly group 1 = present, 0 = absent, 9 = no information. For the demographic data the coding was as follows; female = 1, male = 0; yes = 1, no = 0. Those individuals living in either Winnipeg or Brandon were designated urban dwellers since these were the only two cities in Manitoba with a population exceeding 35,000, as determined over the study period, using data supplied by Statistics Canada.

On the coding sheet, abnormal internal/external genitalia and abnormal radial ray were not all inclusive categories. They were only used when a specific designation was not appropriate (i.e. bifid scrotum, preaxial polydactyly).

Figure 1. VACTERL Coding Sheet.

a.
b. patient identification number
c.

1. tracheoesophageal fistula
2. atresia/hypoplasia of trachea/larynx
3. lung septation abnormalities
4. pulmonary hypoplasia
5. neural tube defects
6. arrhinecephaly/cebocephaly/holoprosencephaly/absent interlobar fissures
7. microcephaly

8. other renal *
9. hydronephrosis and hydroureter
10. polycystic kidneys
11. horseshoe kidneys
12. unilateral renal agenesis
13. bilateral renal agenesis
14. duplex collecting system
15. bladder agenesis/hypoplasia
16. urethral atresia
17. ureteral atresia

18. imperforate anus
19. ectopic anus
20. ectopic/agenesis gallbladder
21. malrotation/malfixation
22. Meckel's diverticulum
23. duodenal/ileal atresia/stenosis/duplication
24. ectopic/hypoplastic liver
25. hypoplastic bowel
26. ectopic/hypoplastic pancreas
27 ectopic/hypoplastic adrenals
28. ectopic/hypoplastic thymus

29. scoliosis
30. skin tags
31. cervico-thoracic-anomalies
32. lumbo-sacral-anomalies
33. extra ribs
34. absent/hypoplastic ribs

35. vaginal atresia
36. uterus didelphus
37. bilateral cryptorchidism
38. bifid scrotum
39. hypospadias

40. other abnormal external genitalia $
41. other abnormal internal genitalia $$
42. omphalocele
43. cloacal/bladder exstrophy
44. rectal fistula

45. patent foramen ovale/atrial septal defect
46. ventricular septal defect
47. persistent left superior vena cava
48. anomalies/abberant origin of subclavians
49. single umbilical artery
50. mitral valve atresia/stenosis
51. pulmonary trunk anomalies **
52. other migration defects #
53. hypoplastic left ventricle
54. right ventricular hypertrophy
55. coarctation of the aorta
56. overiding aorta
57. other aortic trunk abnormalities ##
58. other abnormal vasculature (peripheral) @

59. prominent occiput/dolicocephaly/scaphocephaly
60. low set ears
61. preauricular skin tags
62. dysplastic ears/microtia
63. flat nose/depressed nasal bridge
64. micropthalmia
65. hypertelorism
66. cleft lip and palate
67 cleft palate
68. micrognathia

69. preaxial polydactyly of fingers/toes
70. postaxial polydactyly of fingers/toes
71. other abnormal/hypoplastic radial ray @@
72. positional deformity of the feet
73. reduction deformity/hypoplasia lower limb
74. reduction deformity/hypoplasia upper limb
75. abnormal palmar creases
76. dislocated/subluxable hips

77. membranous/herniated diaphragm
78. lumbo-sacral dimple/skin tag/hair
79. webbed neck/excess nuchal skin
80. wide sutures/ third fontanelle

81. sex
82. birthweight <2,500g
83. gestation <37 weeks
84. maternal age >35 years

85. maternal age <20 years
86. maternal diabetes
87. previous positive family history
88. complications of pregnancy
89. first birth
90. fourth or more birth
91. stillbirth
92. neonatal death/infant death
93. urban/rural
94. previous pregnancy loss

* other renal includes: ectopic kidneys, dysplastic kidney, bladder diverticulae, urachal sinus, fistula between the colon and bladder, ectopic orifice on bladder.

$ other abnormal internal genitalia includes: streak gonads/absent/dysplastic gonads, bifid vagina, septated uterus.

$$ other abnormal external genitalia includes: hypoplastic penis, absent/ambiguous external genitalia, bifid clitoris, bilobed penis.

** pulmonary trunk anomalies include: bicuspid pulmonary valve, infundibular pulmonary stenosis, hypoplastic pulmonary trunk, pulmonary valve stenosis, pulmonary trunk atresia, pulmonary artery atresia, coarctation of the pulmonary artery, abberant origin of pulmonary arteries.

other migration defects include: transposition of the great vessels, tetralogy of Fallot, double outlet right ventricle, common truncus arteriosus, articopulmonary window.

other aortic trunk anomalies include: double aortic arch, right sided aortic arch, aortic valve atresia, hypoplastic aortic arch, subaortic stenosis, vascular ring, bicuspid aortic valve.

@ other abnormal vasculature includes: absent renal arteries and veins, partial anomalous venous return, bilateral double renal arteries, drainage of the inferior vena cava into the superior vena cava by the right azygous vein,persistent left superior vena cava draining into the coronary sinus,abnormal origin of the coronary artery, lower aorta ending as an umbilical artery, no internal iliac artery.

@@ other abnormal radial ray does not include preaxial polydactyly.

2.320 Selection of Coefficients of Similarity.

Two different types of numerical analyses were applied to the VACT 2 and VACT 3 groups: Normal or Q-analysis and Inverse or R-analysis. With each of these analyses two different clustering methods were used; Hierarchy (a polythetic agglomerative technique) and Divide (a monothetic divisive technique). Three different coefficients were used with each of these techniques (figure 2).

Since CLUSTAN has many different coefficients that can be used for this type of study was necessary to do some preliminary analyses to select the most appropriate coefficients to use for this study. Thus twenty-four patients with limb and/or renal defects were analysed using all of the different coefficients and different clustering strategies available with the CLUSTAN program to see which produced the most appropriate and correct groupings. The test group included four individuals with Trisomy 18, four with the Poland sequence, four with Potter sequence and another twelve individuals with VACTERL-type anomalies with or without a renal component. Any coefficient that produced chains of groups that only consisted of one or two individuals or that did not effectively classify

Figure 2. Combinations of Classification Techniques, Clustering Strategies and Similarity Coefficients Applied to the VACTERL Population.

Classification Technique	Program	Similarity Coefficient	Clustering Strategy
Polythetic agglomerative	HIERARCHY	Dice Sorenson	Furthest Neighbour
Polythetic agglomerative	HIERARCHY	Jaccard	Group Average
Polythetic agglomerative	HIERARCHY	Phi	Furthest Neighbour
Monothetic divisive	DIVIDE	Sum of the squareroot of chi-square	N/A
Monothetic divisive	DIVIDE	Optimise Coefficient 40	N/A
Monothetic divisive	DIVIDE	Sum (AD-BC)**2	N/A

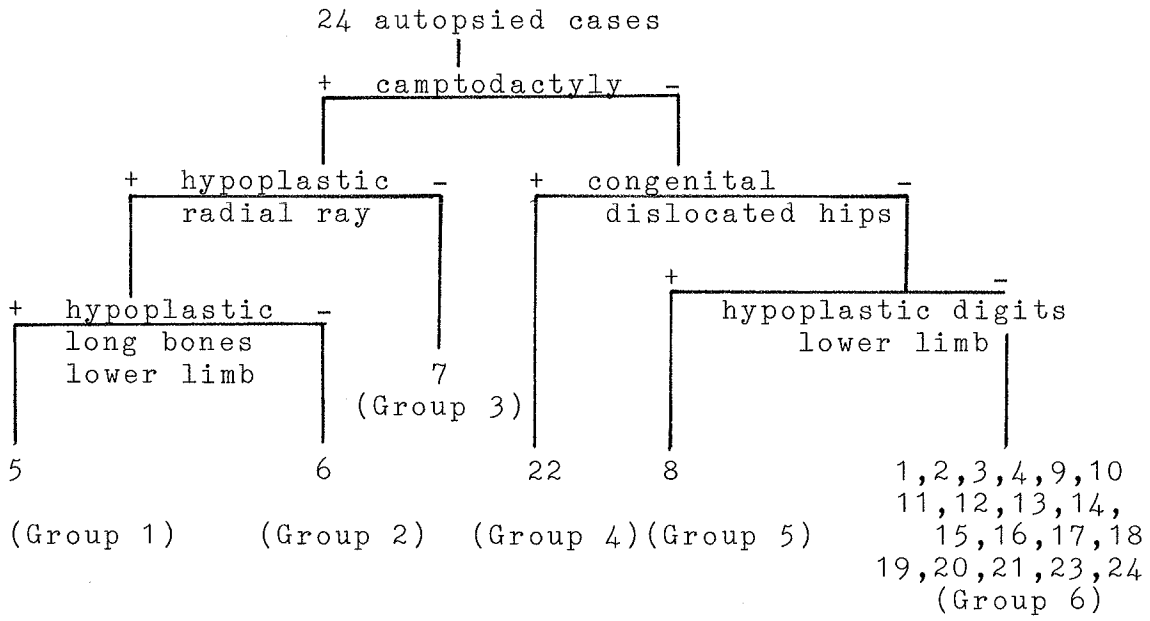
individuals was rejected. Examples of poor groupings are given in Figures 3. and 4.

Figure 3. is an example of the DIVIDE option, a monothetic divisive procedure. The coefficient used gives a poor result since it produces groups with only one member, with the exception of the last group to be formed which contains the residual individuals. It is obvious that one cannot learn anything of significance about the interelationships between the individuals in this population using this coefficient. Figure 4. is an example of the HIERARCHY option, a polythetic agglomerative procedure. It is a relatively good classification since it does group together individuals with similar patterns of anomalies such as Poland's and Potter's sequences. However, it is not ideal as it does not group together the three individuals with Trisomy 18. Previous studies (Evans, 1982) have shown that individuals with this syndrome usually form a very definite group, especially when, as in this case, anomaly ascertainment is complete.

The six coefficients that were chosen were again tested on data from an unselected series of twenty one patients with multiple congenital anomalies chosen at random from autopsied cases that had at least one VACTERL-

Figure 3. Example of a Classification Scheme Produced using the DIVIDE Option.

Association analysis: HIERARCHY
Similarity coefficient: Sum of Chisquares



Group	Case #	Diagnosis
1	5	Trisomy 18
2	6	Trisomy 18
3	7	Trisomy 18
4	22	Caudal regression sequence (CRS)
5	8	Trisomy 18

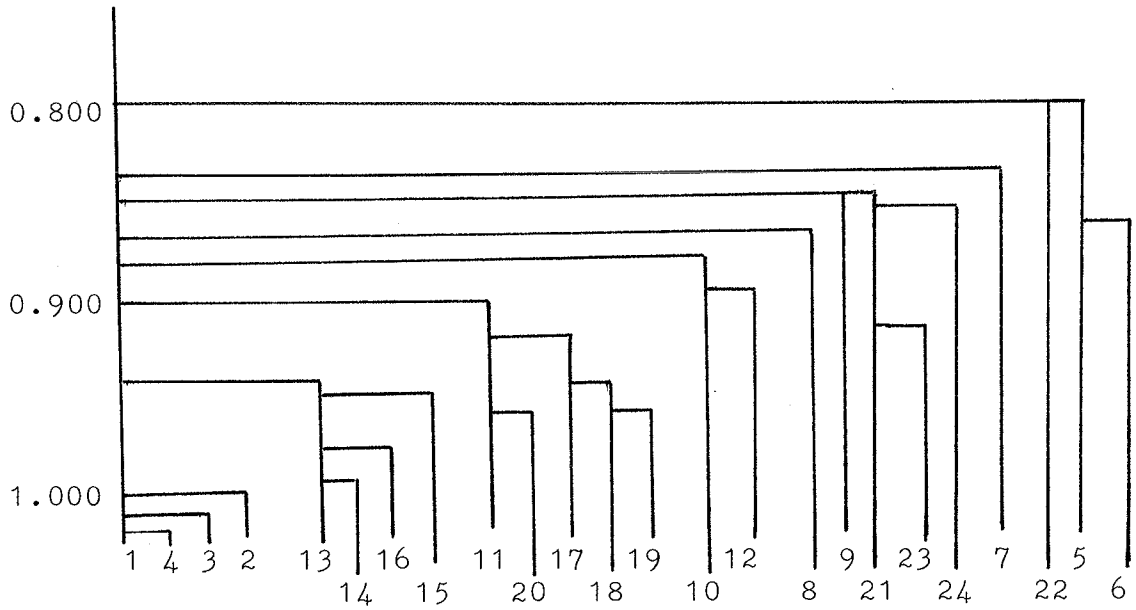
Group	Case #	Diagnosis
6	1	Poland
	2	Poland
	3	Poland
	4	Poland
	9	VWR
	10	VWR
	11	VWR
	12	VWR
	13	Potter S.
	14	Potter S.
	15	Potter S.
	16	Potter S.
	17	VNR
	18	VNR
	19	VNR
	20	VNR
	21	CRS
	23	CRS
	24	CRS

KEY.

CRS: Caudal regression sequence
VWR: VACTERL with renal involvement
VNR: VACTERL without renal involvement

Figure 4. Example of a Classification Scheme Produced using the HIERARCHY Option.

Association Analysis: HIERARCHY
Clustering technique: Group Average
Similarity soefficient: Dice Sorenson



Group	Case #	Diagnosis
1	1	Poland
	4	Poland
	3	Poland
	2	Poland
2	13	Potter
	14	Potter
	16	Potter
	15	Potter
3	11	VWR
	20	VNR
	17	VNR
	18	VNR
	19	VNR
4	10	VWR
	12	VWR
5	8	Trisomy 18
6	9	VWR
	21	CRS
	23	CRS
	24	CRS
7	7	Trisomy 18
8	22	CRS
	5	Trisomy 18
	6	Trisomy 18

like component, to see if the same coefficients produced reasonable groupings on this less well defined population. The results were satisfactory even with this population and produced reasonable and consistent groupings. The coefficients chosen are listed on the following page.

These coefficients are based on coincidences (either positive or negative) in the population as compared to situations where the anomalies are neither both present or absent. This can be thought of in terms of a contingency table (see Figure 5.) where all possible combinations of phase between two anomalies can be taken into account. A positive coincidence is illustrated in cell a, a negative coincidence in cell d and situations where no coincidence is present in cells b and c. The coefficients can be used to determine the similarity between any two anomalies or, as is the case in this study, they can be used as summed statistics that are calculated over all the anomalies/individuals present.

FIGURE 5. SIMILARITY COEFFICIENTS

TWO WAY CONTINGENCY TABLE

ANOMALY 2 \ ANOMALY 1	+	-
+	a (++)	b (+-)
-	c (-+)	d (--)

DICE SORENSON: $\frac{2a}{2a+b+c}$

SIMILARITY RATIO: (JACCARD) $\frac{a}{2a+b+c}$

PHI: $\frac{ad-bc}{(a+b)(a+c)(c+d)(b+d)\ **2}$

SUM OF THE SQUAREROOT OF CHI-SQUARE: $\sum \sqrt{\frac{(ad-bc)**2(a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}}$

OPTIMISE COEFFICIENT 40:

$$Snlogn - \sum_{j=1}^{S} a_j \log a_j + (n-a_j)\log(n-a_j)$$

where s = # binary attributes
n = # elements
j = jth attribute in a elements
a = ath element

SUM(AD-BC)**2: $\sum (ad-bc)^2$

2.330 Choice of Clustering Technique

Clustering strategies are only used with polythetic agglomerative operations such as the HIERARCHY option and not with monothetic divisive operations such as DIVIDE. Furthest neighbour is a single-linkage clustering method; as with all clustering strategies the fusions start with the most similar pairs of individuals. In order to join a group an individual must have a similarity that is equal to the similarity of the group to its furthest member. In the case of group average, the mean distance of the individual to each member of a given cluster is obtained. The individual will fuse with the cluster that gives the shortest mean distance.

The classification techniques, clustering strategies and similarity coefficients shown in Figure 2. were applied in both a Normal and an Inverse analysis to the following groups of patients: VACT 3, VACT 3 excluding knowns, VACT 2, VACT 2 minus knowns, VACT (2+3), VACT (2+3) minus knowns. In all twelve analyses were performed on each group. The resulting dendograms were then checked for misclassifications.

2.400 Nodal Analysis

A nodal analysis was also performed on the VACT 3 minus known group using monothetic divisive techniques and the sum of the square root of chi-squares coefficient. Nodal analysis combines both normal and inverse analyses to establish the existence and importance of coincidences in these analyses. It defines "noda" in the population that represent doubly defined and doubly extracted malformation-patient clusters.

Each patient group identified through the normal analysis is subjected to an inverse analysis and a key anomaly identified. Similarly, the groups identified by the inverse analysis are subjected to a normal analysis to identify key individuals. These two analyses are combined to form a two-way matrix of patients and malformations. The key parameters defined can be used to detect the partial and/or total coincidences of malformations and individuals which account for most of the variance in the population.

2.500 Definition of Key Anomalies and Demographic Data.

A taxonomic key was formed for the VACT 3 minus known group, based on the conserved clusters in the Hierarchy and Divide analyses. The groups that were determined were checked for obvious misclassifications by examining and comparing the anomalies present in each individual against those present in the other members of the group.

Potential problems with this approach include the misclassification of individuals due to incomplete reporting of anomalies, and the inclusion of individuals who are clinically felt to represent known syndromes since karyotypic confirmation of their status was not possible. Once the groups had been reviewed, malformation criteria were established for each.

3.000 RESULTS

3.100 Ascertainment.

Through multiple sources 888 individuals were ascertained who had one or more anomalies compatible with VACTERL. Of these only 12 (26.7% of the final VACT 3 group) were on the registry and had documented involvement of three or more systems. Another 25 cases (55.6% of the final VACT 3 group) were on the registry but the documentation of the anomalies was either incomplete, or had been replaced by a specific diagnosis and so was initially missed. A further 8 cases (17.7% of the final VACT 3 group) were not on the registry but were found through other sources (the individual who was later excluded from the analysis also fell into this group).

Of the cases that comprised the VACT 2 group, 23 (52.3%) were initially ascertained from the registry, 16 (38.1%) were on the registry but documentation of anomalies was incomplete, and 5 (11.4%) were not on the registry but were ascertained from other sources. For keys to the VACT 2 and 3 individuals, see appendices 2. and 3.

3.200 Incidence.

There were a total of 83,893 births in Manitoba over the five year period from 1979 to 1983 (Table 3). The incidence of anomalies for each individual category of the ICDM codes used in this study is given in Table 4. These were calculated over the entire five year period and reported as per 10,000 total births. Thus, they are not strictly incidence figures but rather a birth prevalence.

3.300 System Involvement in the VACT 2 and 3 Groups.

Tables 6. through 10. illustrate the probability of observing our 2, 3, 4, 5, and 6 group combinations by chance alone. For this analysis genital anomalies were excluded as a system of involvement, since ascertainment in the VACT 1 group was incomplete (20 of the 50 documented genital anomalies in the VACT 2 and 3 were added to the nosologic file subsequent to the case reviews). Not all the 89 cases in the combined VACT 2 and 3 groups are represented in these tables since two of the VACT 2 cases only had genital anomalies and involvement in one other system (individuals 12 and 26).

Table 3. Births in Manitoba between 1979 and 1983.

Livebirths in Manitoba	Stillbirths	Year
16,562	241	1979
16,313	211	1980
16,414	234	1981
16,526	163	1982
17,036	193	1983
Total: 82,851	1,042	

Total number of births in Manitoba 1979-1983: 83,893

** Data provided by Statistics Canada

Table 4. Incidence of Index Anomalies determined over the five year period from 1979 to 1983.

Anomaly (number of cases reported)	Incidence (per 10,000 total births)
VERTEBRAL	
756.1 anomalies of the spine (23)	2.74/10,000
756.2 cervical rib (4)	0.48/10,000
756.3 other anomalies of the rib and sternum (17)	2.03/10,000
ANAL	
751.2 atresia and stenosis of the large intestine, rectum and anal canal (56)	5.36/10,000
CARDIOVASCULAR	
745.0 common truncus (8)	0.95/10,000
745.1 transposition of the great vessels (43)	5.13/10,000
745.2 tetralogy of Fallot (18)	2.15/10,000
745.3 common ventricle (9)	1.07/10,000
745.4 ventriculo-septal defect (255)	30.40/10,000
745.5 atrial-septal defect (99)	11.80/10,000
745.6 endocardial cushion defects (13)	1.55/10,000
745.7 cor bioculare (0)	---
745.8 other anomalies of septal closure (2)	
745.9 unspecified defect of septal closure (2)	
746.0 anomalies of the pulmonary valve (14)	1.67/10,000
746.1 tricuspid atresia and stenosis (5)	0.60/10,000
746.2 Ebstein's anomaly (2)	0.24/10,000
746.3 congenital stenosis of the aortic valve (7)	0.83/10,000
746.4 congenital insufficiency of the aortic arch (4)	0.48/10,000
746.5 congenital mitral stenosis (6)	0.72/10,000
746.6 congenital mitral insufficiency (8)	0.95/10,000
746.7 hypoplastic left heart (27)	3.22/10,000
746.8 other specified anomalies of the heart (52)	
746.9 unspecified anomalies of the heart (46)	
747.1 coarctation of the aorta (23)	2.74/10,000
747.2 other anomalies of the aorta (18)	2.15/10,000
747.3 anomalies of the pulmonary artery (49)	5.84/10,000
747.4 anomalies of the great veins (24)	2.86/10,000
747.5 absence or hypoplasia of the umbilical artery (40)	4.77/10,000
747.6 other anomalies of the peripheral vascular system (4)	
747.9 unspecified anomalies of the respiratory system (20)	

TRACHEOESOPHAGEAL/RESPIRATORY		
750.3	tracheoesophageal fistula, esophageal atresia and stenosis (24)	2.86/10,000
748.0	choanal atresia (8)	0.95/10,000
748.2	web of larynx (3)	0.36/10,000
748.3	other anomalies of the larynx, trachea and bronchus (47)	5.60/10,000
RENAL		
753.0	renal agenesis and dysgenesis (41)	4.90/10,000
753.2	obstructive defects of the renal pelvis and ureter (55)	6.56/10,000
753.3	other specified anomalies of the kidney (14)	1.67/10,000
753.4	other specified anomalies of the ureter (13)	1.55/10,000
753.8	other specified anomalies of the bladder and urethra (12)	1.43/10,000
LIMB		
755.0	polydactyly (102)	12.16/10,000
755.2	reduction deformity of the upper limb (39)	4.65/10,000
755.3	reduction deformity of the lower limb (18)	2.15/10,000
755.4	reduction deformity unspecified limb (2)	
755.52	congenital elevation of the scapula (0)	---
755.54	Madelung's deformity (0)	---
755.58	cleft hand, congenital (0)	---
755.66	other anomalies of the toes (15)	
EAR		
744.01	absence of the external ear (4)	0.48/10,000
744.1	accessory auricle (76)	9.06/10,000
744.23	microtia (8)	0.95/10,000
GENITAL		
752.4	anomalies of the cervix, vagina, and external female genitalia (37)	4.41/10,000
752.8	other specified anomalies of male genital organs (24)	2.86/10,000
OTHER		
759.0	anomalies of the spleen (5)	0.60/10,000
759.3	situs inversus (2)	0.24/10,000

Table 5. Rate per Total Births of Selected Anomalies Occuring in the VACTERL "Systems".

AFFECTED SYSTEM	GROUP	No. of cases	Rate (Total births)
Vertebral anomalies *	VACT 1	8	
	VACT 2	11	
	VACT 3	18	
	TOTAL:	37	4.41/10,000
Imperforate anus	VACT 1	13	
	VACT 2	8	
	VACT 3	25	
	TOTAL:	46	5.48/10,000
Total ** Anorectal anomalies	VACT 1	24	
	VACT 2	9	
	VACT 3	27	
	TOTAL:	60	7.15/10,000
Cardio-vascular anomalies	VACT 1	499	
	VACT 2	32	
	VACT 3	37	
	TOTAL:	568	67.71/10,000
Tracheo-esophageal fistula	VACT 1	17	
	VACT 2	2	
	VACT 3	5	
	TOTAL:	24	2.86/10,000

* excludes spina bifida

** includes imperforate anus, ectopic anus, anorectal stenoses.

Table 5. continued...

AFFECTED SYSTEM	GROUP	No. of cases	Rate (Total births)
Total *** laryngo-tracheo esophageal anomalies	VACT 1	66	
	VACT 2	3	
	VACT 3	9	
	TOTAL:	78	9.30/10,000
Renal anomalies	VACT 1	68	
	VACT 2	20	
	VACT 3	31	
	TOTAL:	119	14.18/10,000
Limb anomalies	VACT 1	133	
	VACT 2	12	
	VACT 3	25	
	TOTAL:	170	20.26/10,000

*** includes TEF, hypoplasia of larynx/trachea, laryngomalacia, tracheomalacia, branchial cleft.

The expected values were determined using the rates shown in Table 5. rather than those in Table 4. since the numbers in each category in the latter table cannot be summed to give an accurate estimate of how often malformations occur in a particular system since the same system will be multiply ascertained if it has multiple malformations. In Table 5. affected systems in each of the three groups are singly ascertained. The exact probability quoted was calculated using the Poisson distribution to take into account the very small expected values.

The frequency of anomaly involvement in the different systems varied, with the majority of cardiovascular, laryngo-tracheo-esophageal (including tracheo-esophageal fistula) and renal anomalies appearing in the VACT 1 group and smaller, but roughly equal, numbers of these anomalies reported in the VACT 2 and VACT 3 groups. The number of individuals with documented anomalies of the vertebrae (excluding spina bifida) was similar between the VACT 2 and VACT 3 groups (11 vs 18 respectively), but was obviously underascertained in the VACT 1 group (only eight individuals were reported with vertebral anomalies as the only VACTERL system of involvement). Also of note, both imperforate anus and total anorectal anomalies were found to be considerably less common in the VACT 2 group as

compared to the other two groups.

3.310 Two System Combinations.

The 52 cases with defects in two systems represent 13 times the number expected had they occured together by chance alone. The three most commonly observed two system combinations were CR (16 cases), CL (11 cases), AR (5 cases) and VC (5 cases). The three most commonly expected combinations were CT, CR, and CL. All of these combinations were significant at $p<0.05$.

Other highly significant ($p < 0.05$) two system combinations that were observed included VA, VL, AC, AL, and CT. Only the renal-limb combination was observed but was not significantly more common ($p=0.1888$). There were no individuals with both renal and limb anomalies in the VACT 2 group, wheras 16 of the 45 individuals in the VACT 3 group demonstrated this combination. It would appear from this that the acro-renal field described by Dieker and Opitz (1969) only becomes apparent when other systems are involved.

Table 6. Two System Combinations

COMBINATION	OBSERVED	EXPECTED	EXACT PROBABILITY**
VA	2	0.03	0.0004
VC	5	0.25	0.0000
VT	0	0.03	0.9705
VR	0	0.05	0.9512
VL	4	0.07	0.0000
AC	3	0.41	0.0076
AT	0	0.06	0.9418
AR	5	0.09	0.0000
AL	2	0.12	0.0064
CT *	3	0.53	0.0146
CR *	16	0.81	0.0000
CL *	10	1.15	0.0000
TR	0	0.11	0.8958
TL	0	0.16	0.8521
RL	1	0.24	0.1888
TOTAL:	51		

* indicates commonest expected combinations

** The exact probability indicates the probability of seeing the observed value by chance alone. For example, the probability of observing, by chance, 16 individuals with cardiac and renal anomalies (when the expected number is 0.81) is zero.

3.320 Three or More System Combinations.

The 35 cases with defects in three or more systems represent 4,704 times the number expected had they occurred together by chance alone. All the values for the 3, 4, 5, and 6 system combinations were highly significant.

In the VACT 3 minus known group 14 cases (41%) had anomalies in 3 systems, 11 (32%) had anomalies in 4 systems, 5 (15%) had anomalies in 5 systems, and 4 cases (12%) had anomalies in six systems.

The most commonly observed 3 system combinations were ACR (6 cases), ACL (3 cases), VAC (2 cases), and CRL (2 cases). The expected values for all of these combinations were so small that one would not anticipate to see them occur in this population. Based on our population incidence figures and the derived expected values, the only one of the three most commonly anticipated combinations that was not seen was the Cardiac-Tracheal-Limb grouping.

The most commonly observed four system combinations were ACTR (3 cases) and VCRL (3 cases). All of the observed combinations were highly significant. Since it was one of the combinations with the largest expected value,

the ACTL combination might have been seen in our population, but did not occur. The most commonly observed five system combination was VACRL (4 cases). Again, all observed combinations were highly significant, as was the six system combination, VACTERL, that was seen.

Table 7. Three System Combinations Observed.

COMBINATION	OBSERVED	EXPECTED	EXACT PROBABILITY
VAC	2	1.79x10-4	0.0000
VAR	1	3.75x10-5	0.0000
VAL	1	5.36x10-5	0.0000
VCR	1	3.55x10-4	0.0000
VCT	1	2.33x10-4	0.0002
VCL	1	5.08x10-4	0.0005
ACL *	3	8.23x10-4	0.0000
ACR	6	5.76x10-4	0.0000
ATR	1	7.91x10-5	0.0001
CRL *	3	1.63x10-3	0.0000
TOTAL:	20		

* indicates most commonly expected combinations.

NOTE: CTL combination not present, expected value 1.07x10-3

Table 8. Four System Combinations Observed

COMBINATION	OBSERVED	EXPECTED	EXACT PROBABILITY
VCRL	3	7.20x10-7	0.0000
ACRL *	1	1.17x10-6	0.0000
ACTR	3	5.36x10-7	0.0000
CTRL *	2	1.52x10-6	0.0000
TOTAL:	9		

* indicates most commomly expected combinations.

NOTE: ACTL combination not seen, expected value 7.65x10-7

Table 9. Five System Combinations Observed

COMBINATION	OBSERVED	EXPECTED	EXACT PROBABILITY
ACTRL	1	1.09x10-09	0.0000
VATRL	1	7.07x10-11	0.0000
VACRL	4	5.15x10-10	0.0000
TOTAL:	6		

Table 10. Six System Combinations Observed

COMBINATION	OBSERVED	EXPECTED	EXACT PROBABILITY
VACTRL	1	2.60x10-13	0.0000

3.400 Effect of Ascertainment Anomaly on the VACT 3 minus Knowns Group.

The effect of the ascertainment anomaly on the frequency of associated malformations was determined for the VACT 3 minus knowns group (see Table 11). This demonstrated that the perception of an association such as VACTERL was significantly influenced by the chosen ascertainment anomaly. For example, 66.7% of individuals with tracheal anomalies also have an anal anomaly, whereas only 16.0% of individuals with an anal anomaly also have a tracheal anomaly.

Table 11. Effect of Ascertainment Anomaly on the Frequency of Associated Malformations. (Derived Using VACT 3 Minus Known Group, n=34).

ASCERTAINMENT ANOMALY

	V	A	C	T	R	L
V	-----	36.0%	37.9%	50.0%	41.7%	50.0%
A	64.3%	-----	69.0%	66.7%	75.0%	68.8%
C	78.6%	80.0%	-----	83.3%	83.3%	87.5%
T	21.4%	16.0%	17.2%	-----	20.8%	18.8%
R	71.4%	72.0%	69.0%	83.3%	-----	62.5%
L	57.1%	44.0%	50.0%	57.1%	41.7%	-----

NOTE: A = total anal anomalies
T = total tracheal anomalies

EXAMPLE: 66.7% of individuals with tracheal anomalies in the VACT 3 minus known group also have an anal anomaly, whereas only 16.0% of individuals with an anal anomaly also have a tracheal anomaly.

3.500 Numerical Taxonomy

The following graphs and hierarchies give examples of the results of the numerical taxonomy. For all but one of the groups examined (VACT 3 plus known), only two of the coefficients/clustering techniques that were applied to this poulation are presented in order to minimise inter-coefficient variation when comparing the analyses i.e. VACT 2 plus knowns versus VACT 2 minus Knowns. In the Divide analyses this coefficient is the sum of the square root of chi-squares, and in the Hierarchy analyses the clustering technique is Group Average and the coefficient Jaccard (similarity ratio).

3.510 VACT 3 PLUS KNOWN ANALYSES PRESENTED.

NORMAL DIVIDE

COEFFICIENT: SUM(AD-BC)**2
SUM OF THE SQUARE ROOT OF CHI-SQUARES
OPTIMISE COEFFICIENT 40

TOTAL: 3 ANALYSES

NORMAL HIERARCHY

CLUSTERING TECHNIQUE: (COEFFICIENT)
- FURTHEST NEIGHBOUR (DICE SORENSON)
- GROUP AVERAGE (PHI)
- GROUP AVERAGE (SIMILARITY RATIO)

TOTAL: 3 ANALYSES

INVERSE DIVIDE

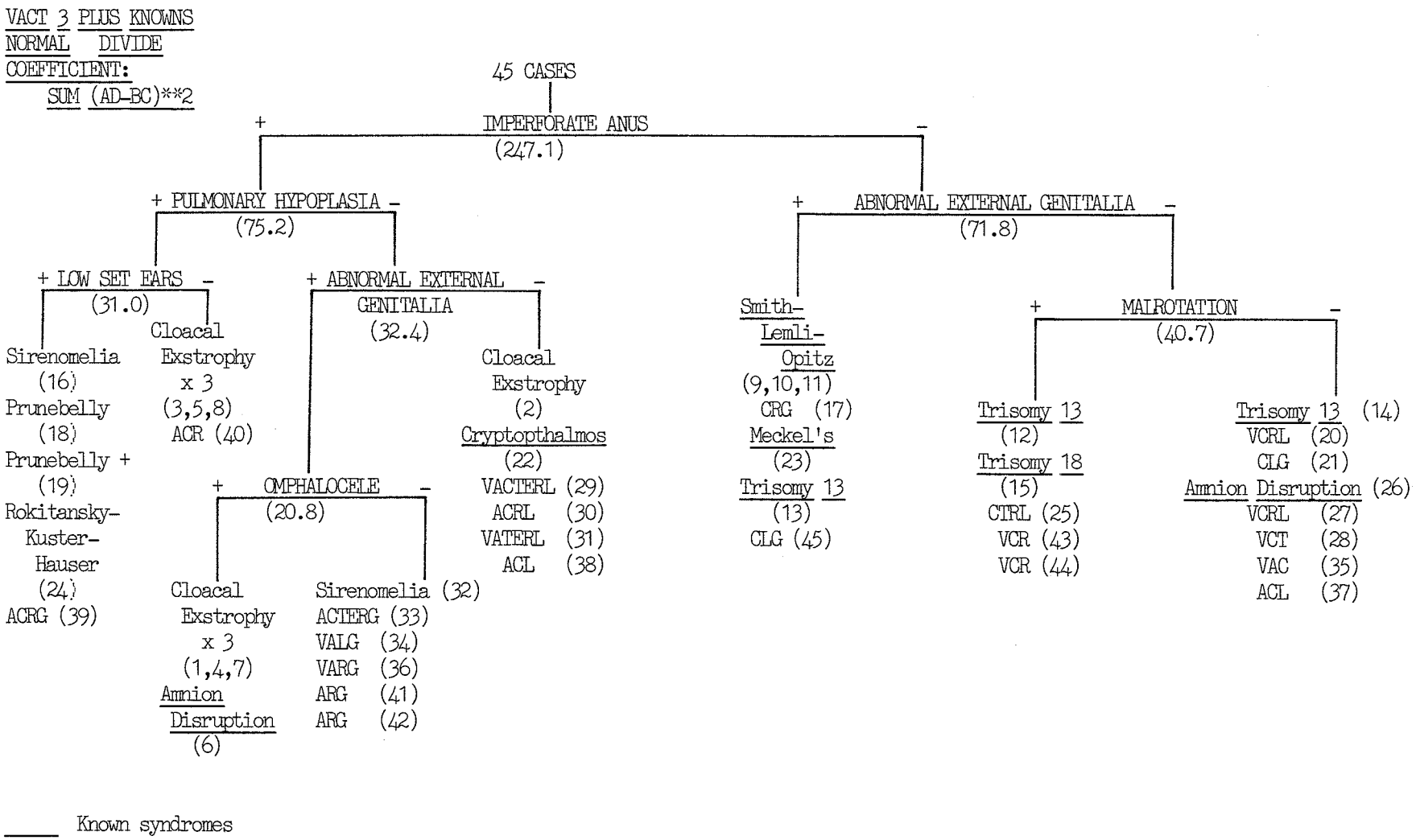
COEFFICIENT: SUM OF THE SQUARE ROOT OF CHI-SQUARES

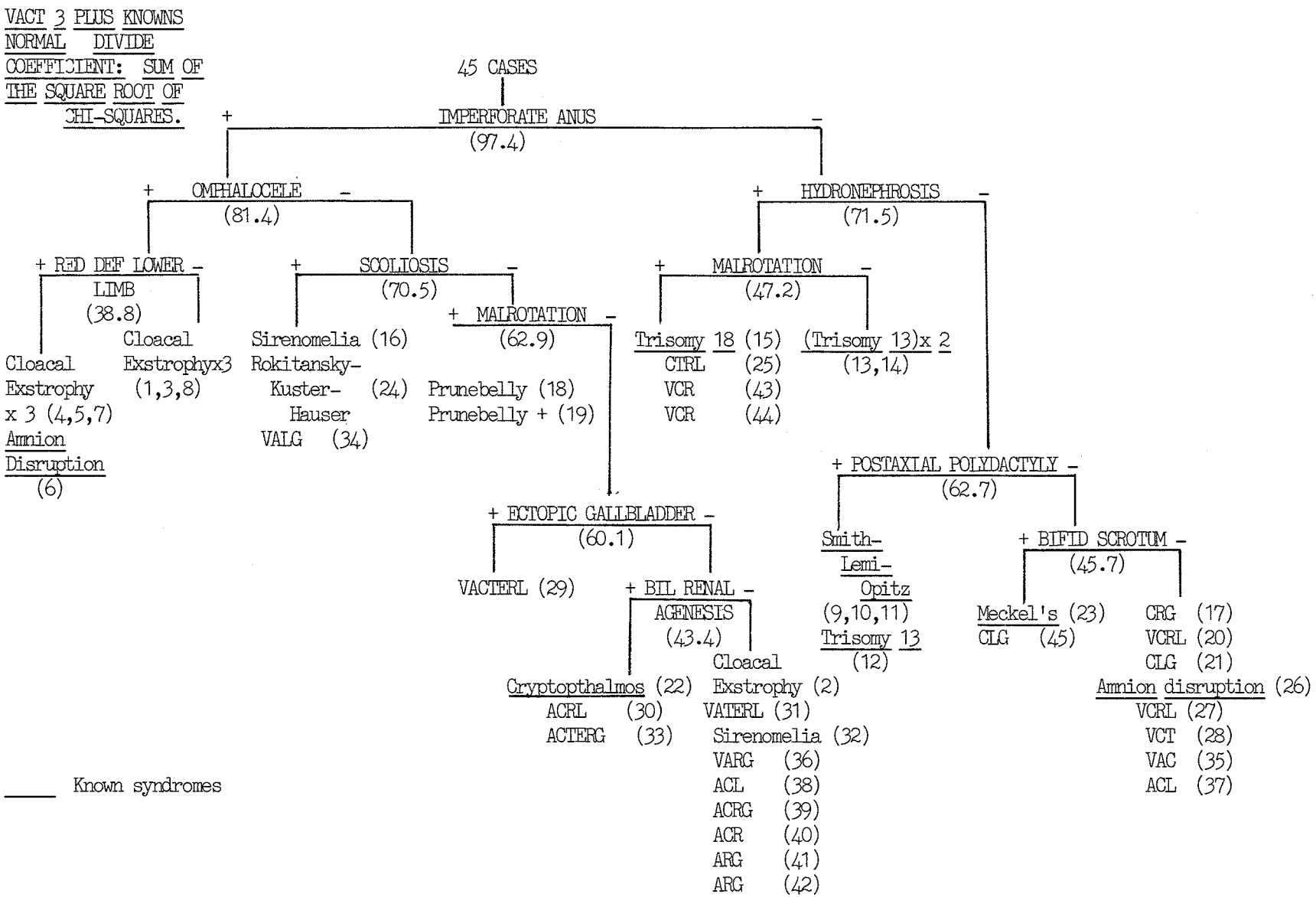
TOTAL: 1 ANALYSIS

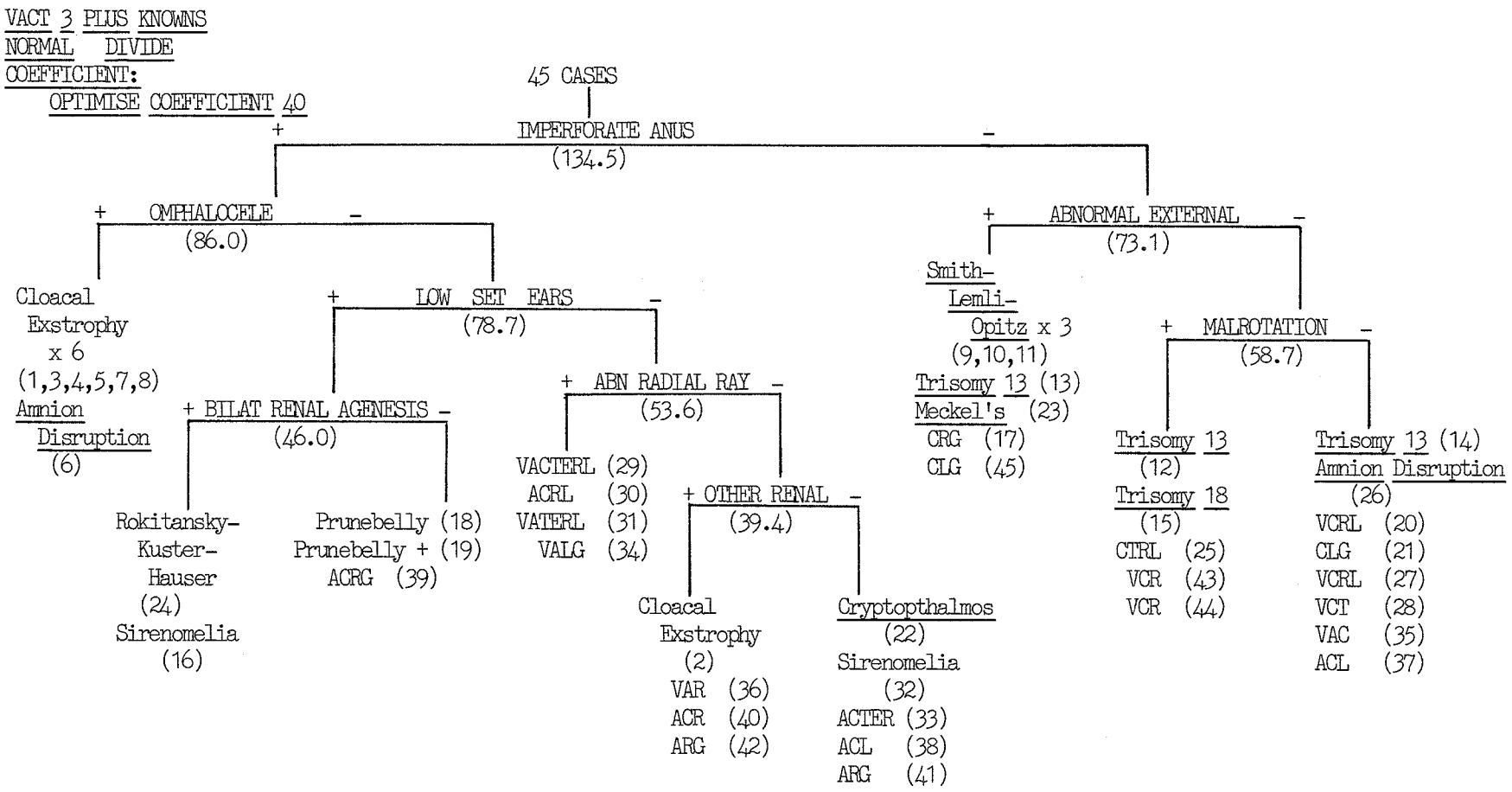
INVERSE HIERARCHY

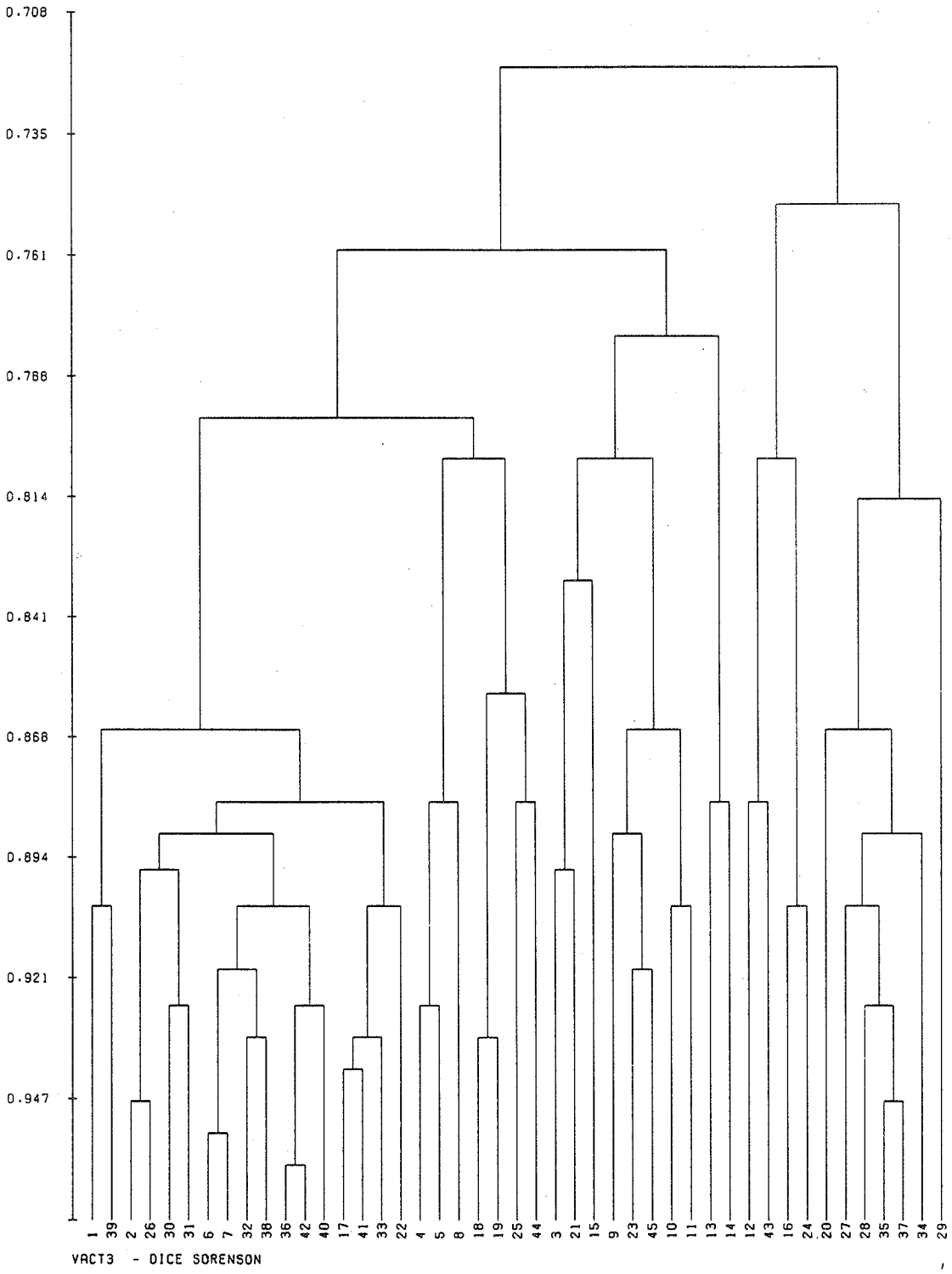
CLUSTERING TECHNIQUE: (COEFFICIENT)
- GROUP AVERAGE (SIMILARITY RATIO)

TOTAL: 1 ANALYSIS


VACT 3 PLUS KNOWNS
NORMAL DIVIDE
COEFFICIENT:
SUM (AD-BC)**2
45 CASES
IMPERFORATE ANUS
(247.1)
+
-
+ PULMONARY HYPOPLASIA -
(75.2)
+ ABNORMAL EXTERNAL GENITALIA -
(71.8)
+ LOW SET EARS -
(31.0)
+ ABNORMAL EXTERNAL GENITALIA -
(32.4)
Sirenomelia (16)
Prunebelly (18)
Prunebelly + (19)
Rokitansky-Kuster-Hauser (24)
ACRG (39)
Cloacal Exstrophy x 3 (3,5,8)
ACR (40)
+ OMPHALOCELE -
(20.8)
Cloacal Exstrophy x 3 (1,4,7)
Amnion Disruption (6)
Sirenomelia (32)
ACTERG (33)
VALG (34)
VARG (36)
ARG (41)
ARG (42)
Cloacal Exstrophy (2)
Cryptopthalmos (22)
VACTERL (29)
ACRL (30)
VATERL (31)
ACL (38)
Smith-Lemli-Opitz (9,10,11)
CRG (17)
Meckel's (23)
Trisomy 13 (13)
CLG (45)
+ MALROTATION -
(40.7)
Trisomy 13 (12)
Trisomy 18 (15)
CTRL (25)
VCR (43)
VCR (44)
Trisomy 13 (14)
VCRL (20)
CLG (21)
Amnion Disruption (26)
VCRL (27)
VCT (28)
VAC (35)
ACL (37)
Known syndromes


VACT 3 PLUS KNOWNS NORMAL DIVIDE COEFFICIENT: SUM OF THE SQUARE ROOT OF CHI-SQUARES.
45 CASES
IMPERFORATE ANUS (97.4)
OMPHALOCELE (81.4)
HYDRONEPHROSIS (71.5)
RED DEF LOWER LIMB (38.8)
SCOLIOSIS (70.5)
MALROTATION (47.2)
MALROTATION (62.9)
Cloacal Exstrophy x 3 (4,5,7) Amnion Disruption (6)
Cloacal Exstrophyx3 (1,3,8)
Sirenomelia (16) Rokitansky-Kuster-Hauser (24) VALG (34)
Prunebelly (18) Prunebelly + (19)
Trisomy 18 (15) CTRL (25) VCR (43) VCR (44)
(Trisomy 13)x 2 (13,14)
POSTAXIAL POLYDACTYLY (62.7)
ECTOPIC GALLBLADDER (60.1)
Smith-Lemi-Opitz (9,10,11) Trisomy 13 (12)
BIFID SCROTUM (45.7)
VACTERL (29)
BIL RENAL AGENESIS (43.4)
Meckel's (23) CLG (45)
CRG (17) VCRL (20) CLG (21) Amnion disruption (26) VCRL (27) VCT (28) VAC (35) ACL (37)
Cryptopthalmos (22) ACRL (30) ACTERG (33)
Cloacal Exstrophy (2) VATERL (31) Sirenomelia (32) VARG (36) ACL (38) ACRG (39) ACR (40) ARG (41) ARG (42)
Known syndromes


VACT 3 PLUS KNOWNS
NORMAL DIVIDE
COEFFICIENT:
OPTIMISE COEFFICIENT 40
45 CASES
IMPERFORATE ANUS
(134.5)
+
-
+ OMPHALOCELE -
(86.0)
+ ABNORMAL EXTERNAL -
(73.1)
Cloacal Exstrophy x 6
(1,3,4,5,7,8)
Amnion Disruption
(6)
+ LOW SET EARS -
(78.7)
Smith-Lemli-Opitz x 3
(9,10,11)
Trisomy 13 (13)
Meckel's (23)
CRG (17)
CLG (45)
+ MALROTATION -
(58.7)
+ BILAT RENAL AGENESIS -
(46.0)
+ ABN RADIAL RAY -
(53.6)
Rokitansky-Kuster-Hauser
(24)
Sirenomelia
(16)
Prunebelly (18)
Prunebelly + (19)
ACRG (39)
VACTERL (29)
ACRL (30)
VATERL (31)
VALG (34)
+ OTHER RENAL -
(39.4)
Trisomy 13
(12)
Trisomy 18
(15)
CTRL (25)
VCR (43)
VCR (44)
Trisomy 13 (14)
Amnion Disruption
(26)
VCRL (20)
CLG (21)
VCRL (27)
VCT (28)
VAC (35)
ACL (37)
Cloacal Exstrophy
(2)
VAR (36)
ACR (40)
ARG (42)
Cryptopthalmos
(22)
Sirenomelia
(32)
ACTER (33)
ACL (38)
ARG (41)
Known syndromes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0.788
0.814
0.841
0.868
0.894
0.921
0.947
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VACT3 - DICE SORENSON

VACT 3 PLUS KNOWNS
NORMAL HIERARCHY
COEFFICIENT: FURTHEST NEIGHBOUR (DICE SORENSON)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE CASES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Cloacal exstrophy (1), ACL (39), Cloacal Exstrophy (2), Amnion disruption (26), ACRL (30), VATERL (31), Amnion disruption (6), cloacal exstrophy (7), sirenomelia (32), ACL (38), VARG (36), ACL (? Trisomy 13, 38), ARG (42), ACR (40), CRG (17), ARG (41), ACTERG (33), Cryptopthalmos (22).

GROUP 2.

Cloacal exstrophy x 3 (4,5,8).

GROUP 3.

Prunebelly (18), prunebelly + (19), CTRL (Tracheal agenesis, 25), VCR (? Trisomy 13, 44).

GROUP 4.

Cloacal exstrophy (3), CLG (Private syndrome, 21), Trisomy 18 (15).

GROUP 5.

Smith-Lemli-Opitz x 3 (9,10,11),Meckel's (23), CLG (Private syndrome, 45).

GROUP 6.

Trisomy 13 x 2 (13,14).

GROUP 7.

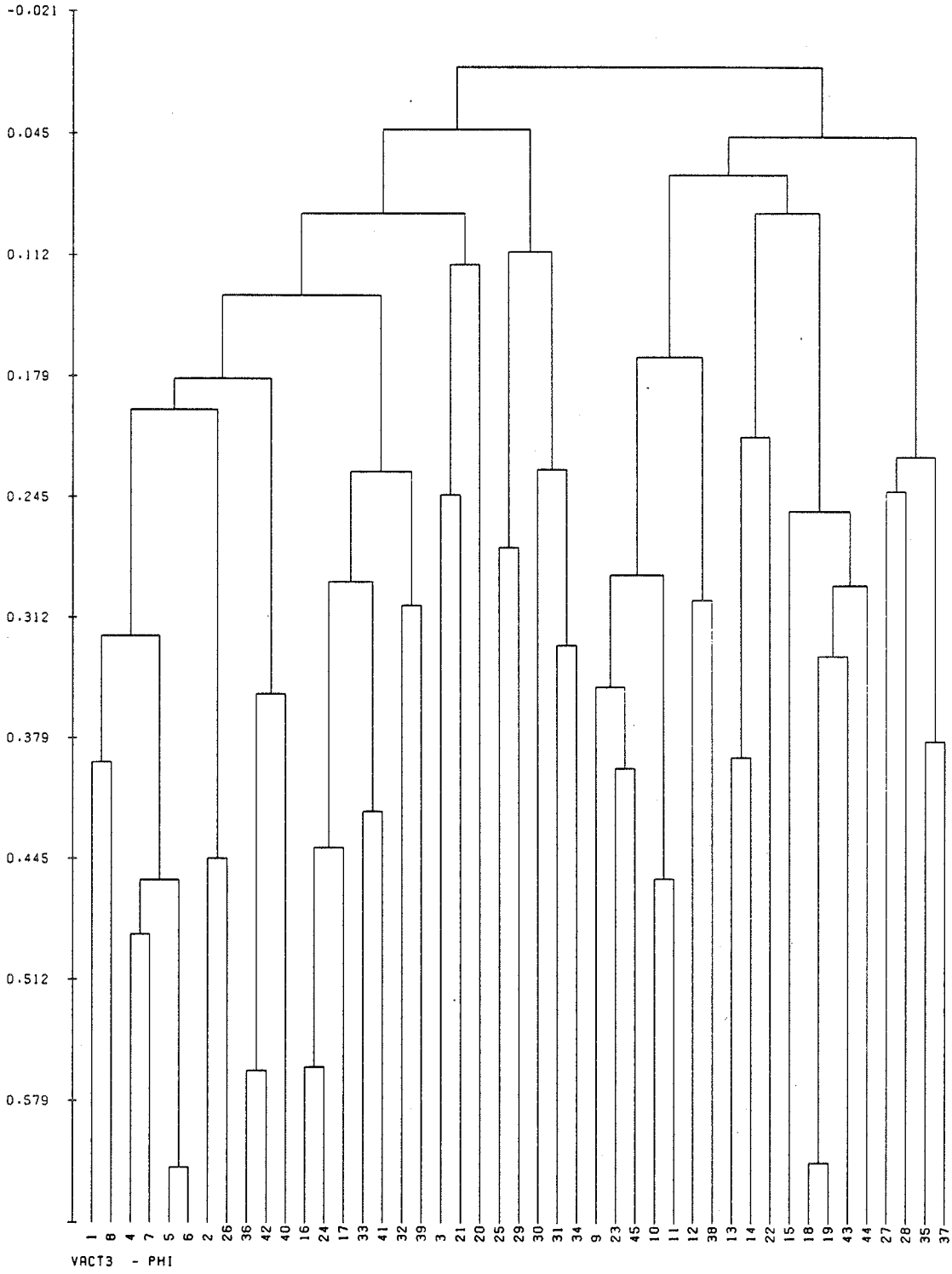
Trisomy 13 (12), VCR (43), Sirenomelia (16), Rokitansky-Kuster-Hauser (24).

GROUP 8.

VCRL (Private syndrome, 20), VCRL (27), VCT (28), VAC (35), ACL (37), VALG (34).

GROUP 9.

VACTERL (29)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0.112
0.179
0.245
0.312
0.379
0.445
0.512
0.579
1 8 4 7 5 6 2 26 36 42 40 16 24 17 33 41 32 39 3 21 20 25 29 30 31 34 9 23 45 10 11 12 38 13 14 22 15 18 19 43 44 27 28 35 37
VACT3 - PHI

VACT 3 PLUS KNOWNS
HIERARCHY NORMAL
GROUP AVERAGE (PHI)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE CASES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Cloacal exstrophy x 5 (1,8,4,7,5), Amnion disruption (6), Cloacal exstrophy (2), VARG (36), ARG (42), ACR (40).

GROUP 2.

Sirenomelia (16), Rokitansky-Kuster-Hauser (24), CRG (17), ACTERG (33), ARG (41), Sirenomelia (32), ACRG (39).

GROUP 3.

Cloacal exstrophy (3), CLG (Private syndrome, 21).

GROUP 4.

VCRL (Private syndrome, 20)

GROUP 5.

CTRL (Tracheal agenesis, 25), VACTERL (29)

GROUP 6.

ACRL (30), VATERL (31), VALG (34).

GROUP 7.

Smith-Lemli-Opitz x 3 (9,10,11), Trisomy 13 (12), Meckel's (23), CLG (Private syndrome, 45), ACL (? Trisomy 13, 38).

GROUP 8.

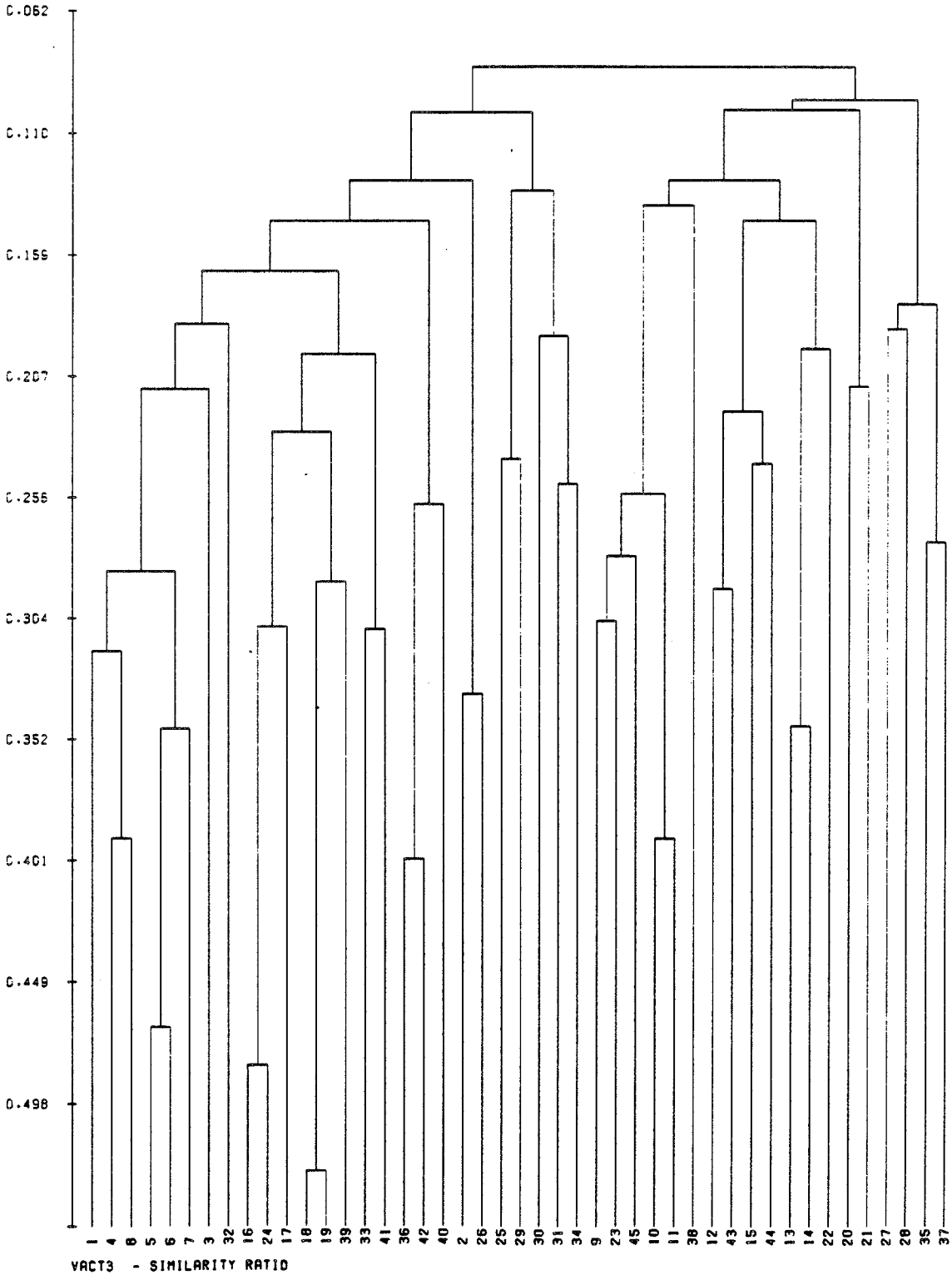
Trisomy 13 x 2 (13, 14), Cryptopthalmos (22).

GROUP 9.

Trisomy 18 (15), Prunebelly (18), Prunebelly + (19), VCR (43), VCR (? Trisomy 13, 44).

GROUP 10.

VCRL (27), VCT (28), VAC (35), ACL (37).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0.256
0.304
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0.401
0.449
0.498
1 4 8 5 6 7 3 32 16 24 17 18 19 39 33 41 36 42 40 2 26 25 29 30 31 34 9 23 45 10 11 38 12 43 15 44 13 14 22 20 21 27 28 35 37
VACT3 - SIMILARITY RATIO

VACT 3 PLUS KNOWNS
NORMAL HEIRARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE CASES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Cloacal exstrophy x 4 (1,4,8,5), amnion disruption (6), cloacal exstrophy x 2 (7,3), sirenomelia (32).

GROUP 2.

Sirenomelia (16), Rokitansky-Kuster-Hauser (24), CRG (17), ACRG (Prunebelly, 18), ACTERG (Prunebelly +, 19), ACRG (39), ACTERG (33), ARG (41).

GROUP 3.

VARG (36), ARG (42), ACR (40).

GROUP 4.

Cloacal exstrophy (2), amnion disruption (26).

GROUP 5.

CTRL (tracheal agenesis, 25), VACTERL (29), ACRL (30), VATERL (31), VALG (34).

GROUP 6.

Smith-Lemli-Opitz (9), Meckel syndrome (23), CLG (Private syndrome, 45), Smith-Lemli-Opitz x 2 (10,11), ACL (? Trisomy 13, 38).

GROUP 7.

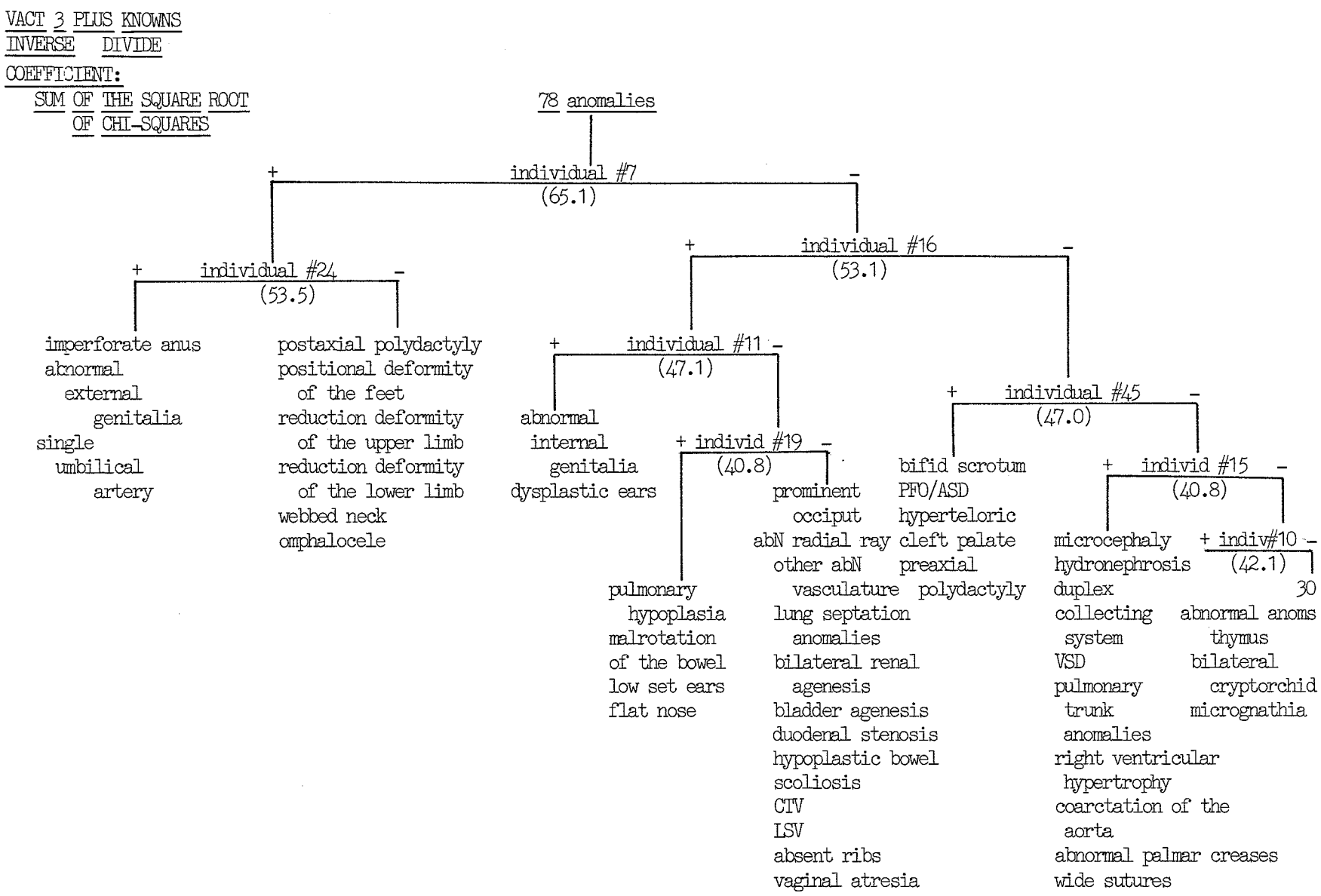
Trisomy 13 (12), VCR (43), Trisomy 18 (15), VCR (? Trisomy 13, 44), Trisomy 13 x 2 (13,14), cryptopthalmos (22).

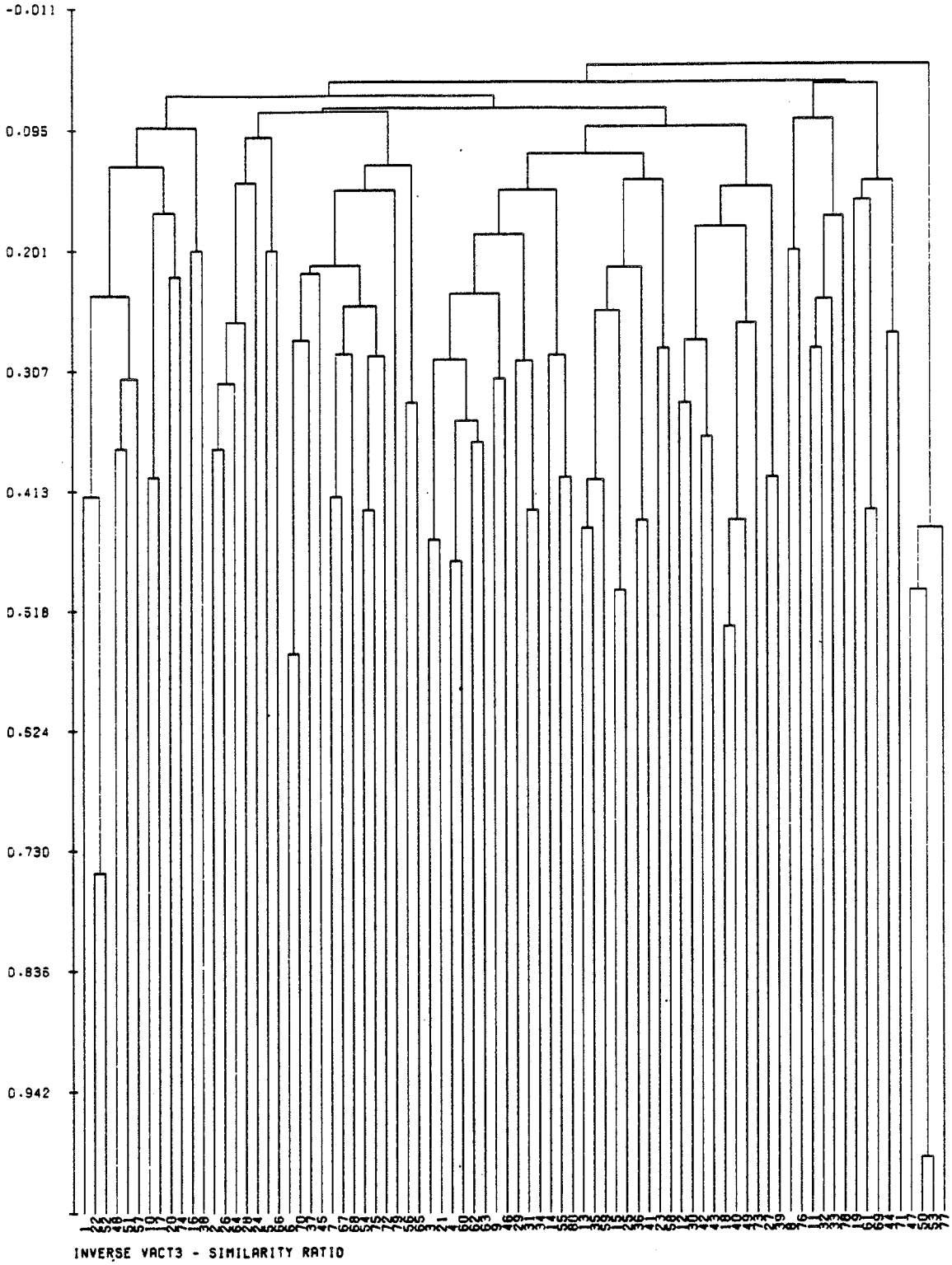
GROUP 8.

VCRL (Private syndrome, 20), CLG (Private syndrome, 21).

GROUP 9.

VCRL (?Goldenhar, 27), VCT (?Turner, 28), VAC (35), ACL (37)


VACT 3 PLUS KNOWNS
INVERSE DIVIDE
COEFFICIENT:
SUM OF THE SQUARE ROOT OF CHI-SQUARES
78 anomalies
individual #7
(65.1)
individual #24
(53.5)
individual #16
(53.1)
imperforate anus
abnormal external genitalia
single umbilical artery
postaxial polydactyly
positional deformity of the feet
reduction deformity of the upper limb
reduction deformity of the lower limb
webbed neck
omphalocele
individual #11
(47.1)
individual #45
(47.0)
abnormal internal genitalia
dysplastic ears
individ #19
(40.8)
bifid scrotum
PFO/ASD
hyperteloric
cleft palate
preaxial polydactyly
individ #15
(40.8)
pulmonary hypoplasia
malrotation of the bowel
low set ears
flat nose
prominent occiput
abN radial ray
other abN vasculature
lung septation anomalies
bilateral renal agenesis
bladder agenesis
duodenal stenosis
hypoplastic bowel
scoliosis
CTV
LSV
absent ribs
vaginal atresia
microcephaly
hydronephrosis
duplex collecting system
VSD
pulmonary trunk anomalies
right ventricular hypertrophy
coarctation of the aorta
abnormal palmar creases
wide sutures
indiv#10
(42.1)
30
abnormal anoms thymus
bilateral cryptorchid
micrognathia


-0.011
0.095
0.201
0.307
0.413
0.518
0.624
0.730
0.836
0.942
INVERSE VACT3 - SIMILARITY RATIO

VACT 3 PLUS KNOWNS
HIERARCHY INVERSE
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE ANOMALIES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Tracheo-esophageal fistula, Meckel's diverticulum, other migration defects, anomalies of the subclavians, pulmonary trunk anomalies, other aortic trunk anomalies, polycystic kidneys, ureteral atresia, ectopic gallbladder, reduction deformity upper limb, urethral atresia, bifid scrotum.

GROUP 2.

Hypoplastic larynx, abnormal pancreas, micropthalmia, abnormal thymus, abnormal liver, neural tube defect, cleft lip and palate.

GROUP 3.

Arrhinecephaly, postaxial polydactyly, bilateral cryptorchid, PFO/ASD, microcephaly, cleft palate, micrognathia, right ventricular hypertrophy, abnormal palmar creases, positional deformity of feet, webbed neck, overiding aorta, hyperteloric.

GROUP 4.

Lung septation abnormalities, malrotation of the bowel, pulmonary hypoplasia, low set ears, dysplastic ears, flat nose, hydronephrosis, VSD, scoliosis, cervico-thoracic vertebral anomalies, absent ribs, duplex collecting system, coarctation of the aorta, widely separated sutures.

GROUP 5.

Bilateral renal agenesis, vaginal atresia, prominent occiput, bladder agenesis, hypoplastic bowel, uterus didelphus, abnormal internal genitalia, duodenal stenosis, other abnormal vasculature.

GROUP 6.

Unilateral renal agenesis, skin tags, omphalocele, cloacal exstrophy, imperforate anus, abnormal external genitalia, single umbilical artery, reduction deformity lower limbs, adrenal anomalies, hypospadias.

GROUP 7.

Other renal, dislocated hips, horseshoe kidney, lumbo-sacral vertebral anomalies, extra ribs, L.S. dimple.

GROUP 8.

Ectopic anus, urethral atresia, preaxial polydactyly, rectal fistula, abnormal radial ray.

GROUP 9.

Persistent left superior vena cava, mitral valve atresia, hypoplastic left ventricle, membranous diaphragm.

Although we were primarily interested in the VACT 3 minus known group it was first necessary to establish the relative merits of the different clustering methods and coefficients that were applied to this population. This was accomplished by looking at the syndromes of known etiology, such as Trisomy 13 and Smith-Lemli-Opitz, and sequences of unknown etiology like cloacal exstrophy and prunebelly, to see if they were recognized and formed distinct clusters (as evidenced by the Normal analyses), and to see if the anomaly groupings also reflected those expected for these syndromes (shown in the Inverse analyses).

3.520 Hierarchy versus Divide

The Hierarchy and Divide options take very different approaches to the same data set. The Divide analyses produce "tighter" groupings since only those individuals/anomalies that are found to have a high degree of similarity are shown in the individual/anomaly clusters. The remainder (who do not bear such a strong resemblance to the clustered cases/anomalies) are all clumped together in the last group to be formed.

Hierarchy classifies all anomalies or individuals. In order to do this it places an individual/anomaly with the

group that it most strongly identifies with. Thus the groups will become "less tight" as other individuals/anomalies that are not as strongly related will be included since they must be placed somewhere. Looking at the graphical representation of the group structure allows us to better appreciate the strength of the association, since the more strongly the individuals/anomalies are related the earlier (closer towards the horizontal axis of the hierarchy scale) they join together. For example, in all three of the Hierarchy inverse analyses anomalies 50 and 53 are very strongly associated. These codes correspond to mitral valve atresia and hypoplastic left ventricle, and confirm an already well documented association. To judge the strength of the asociation one must consider the way in which the groups are formed in each analysis as well as the extent to which these groups are conserved between all the analyses.

The Divide analysis documents key anomalies/individuals that generate the most variation in the population and which sucessively divide it into the final groupings observed. It may appear that Divide is a more useful tool than Hierarchy however, it will misclassify individuals if they do not possess the key anomaly necessary for membership in a group. This is not

problematic when the population under study is well defined since it is easy to recognise cases that have been misclassified, but there is a potential risk of misinterpreting clusters of cases of unknown etiology since even "obvious" misclassifications may pass unrecognized. The normal Hierarchy avoids this pitfall since it compares all the anomalies/individuals and groups them according to their degree of overall similarity.

Similarly, the Divide option is of limited value in producing malformation groupings: these are more subjective than those produced by Hierarchy since only those anomalies that are seen in the Key individual that separates a group, and that have not been previously assigned, are potentially available to be included in a cluster.

Hierarchy assigns each anomaly to a group, but again some of the malformations seen in a cluster may be only very weakly correlated. As with the Divide analyses, each anomaly may only appear once in the classification. This somewhat limits our understanding of the nature of the associations since, if there are two malformations commonly seen with an anomaly i.e. imperforate anus and renal anomalies, imperforate anus and abnormal external genitalia, only the stronger of the two associations will

be represented. To overcome this nodal analysis can be used.

Based on the above features of the Hierarchy and Divide options, it was decided that for the analysis of our population the Divide option would be used to generate key anomalies/individuals and the Hierarchy option would be used for anomaly/individual groupings. Nodal analysis was used with the VACT 3 minus known group to look at the malformation groupings at a greater depth. However, it must be remembered that if the inherent similarity between the individuals/anomalies is high, then different clustering methods or coefficients should still give similar clusters. This was indeed evidenced in our VACT 3 plus known analysis with both the Hierarchy and Divide options and the different coefficients.

3.530 Evaluation of the Different Coefficients Used.

To determine which of the three coefficients used with the Hierarchy and Divide options would be presented, the clusters produced (in the Normal analyses) by each were examined to see how effectively syndromes of known etiology and sequences of unknown etiology were clustered, and also how "tight" these groupings were i.e. were they found as a

distinct group or as part of a much larger, more heterogenous group.

In the Hierarchy option, Dice Sorenson (Furthest Neighbour) was rejected since the first group produced was very large and heterogeneous to the detriment of the subsequent groups that were determined. It also did not group the cases of cloacal exstrophy as effectively as the other two analyses. Both Group Average (Similarity ratio) and Group Average (Phi) produced very similar groupings.

Similarity ratio recognized and clustered individuals with Smith-Lemli-Opitz, Trisomy 13, and cloacal exstrophy. Also, individual #2, an atypical example of cloacal exstrophy, was identified as such and not included in that cluster.

The clusters produced with Phi were not as specific for the individuals with cloacal exstrophy, excluding one case (#3) while including the atypical case (#2), and were also not as effective as Similarity Ratio for grouping the individuals with Trisomy 13.

Fifty percent of the individuals in the VACT 3 group were consistently grouped together in all three analyses:

(4,5,8) cloacal exstrophy; (2,26) cloacal exstrophy, amnion disruption; (16,24) sirenomelia, Rokitansky-Kuster-Hauser; (18,19) Prunebelly; (36,40,42) VAR, ACR, ARG; (30,31) ACTRL, VATERL; (9,10,11,23,45) Smith-Lemli-Opitz x 3, Meckel syndrome, CLG; (13,14) Trisomy 13; (27,28,35,37) VCRL, VCT, VAC, ACL.

The same procedure was repeated for the normal Divide analyses. The sum of the squareroot of chisquares was chosen as the single best coefficient since it appropriately grouped the trisomies, Smith-Lemli-Opitz, cloacal exstrophy, prunebelly, and also recognized the individual with Meckel syndrome as separate.

Sum (AD-BC)**2 was not as effective at grouping the trisomic individuals or those with cloacal exstrophy, and the individual with Meckel was grouped together with the cases of Smith-Lemli-Opitz as was one case of Trisomy 13. Optimise coefficient 40 also did not group the trisomic individuals as well as the sum of the squareroot of chisquares coefficient, nor did it recognise the individual with Meckel as distinct. Otherwise, this analysis produced groupings that were very similar to those produced using the sum of the squareroot of chisquares coefficient.

3.531 VACT 3 Plus Known Analyses Using the Sum of the Square root of Chi-squares and Similarity Ratio.

The normal Hierarchy produced appropriate groupings with individuals having Trisomy 13 and Smith-Lemli-Opitz clustering together in discrete groups. Furthermore, recognized sequences of unknown etiology such as cloacal exstrophy and prune belly also clustered together.

The malformation groupings in the Inverse Hierarchy reflect the strength of the "syndrome" groupings: Groups 2. and 3. gives a Trisomy 13 picture. Although these groups are spatially very closely related (on the graphical representation), the anomalies in group 2. are slightly atypical for Trisomy 13, perhaps being more strongly influenced by individual 25 with the tracheal agenesis association (who was one of the Key individuals determined by the Inverse Divide).

Group 4. shows the oligohydramnios sequence with associated renal anomalies, Group 5. is strongly influenced by the caudal regression spectrum and the Rokitansky-Kuster-Hauser Sequence. The anomalies in Group 6. are commmonly found associated with the cloacal exstrophy sequence. Group 1. shows a VACTERL-type picture with tracheal, renal, genital, cardiac and limb anomalies.

Key anomalies (determined by the Normal Divide analysis) in this group were imperforate anus, omphalocele, reduction deficiency of the lower limb, scoliosis, hydronephrosis, malrotation, ectopic gallbladder, bilateral renal agenesis, postaxial polydactyly and bifid scrotum.

From the Inverse Hierarchy (Similarity ratio) analysis the strongest associations were determined as follows (in order of declining strength): mitral valve atresia and hypoplastic left ventricle; Meckel's Diverticulum and other cardiac migration defects; arrhinecephaly and postaxial polydactyly; bladder agenesis and hypoplastic bowel; and imperforate anus and other abnormal external genitalia. Of these, the first and the last two groupings are not unexpected, nor is the association of arrhinecephaly and postaxial polydactyly which reflects the frequency and strength of this finding in Trisomy 13. Only the association of Meckel's Diverticulum and other migration defects was not anticipated.

Of the key individuals recognized, three had known syndromes (#11 and 10 with Smith-Lemli-Opitz, and #15 with Trisomy 18) and five were unknowns. It is interesting that the "knowns" were important in the lower portion of the key and not in the initial divisions.

3.540 VACT 3 Minus Knowns.

Analyses Presented:

NORMAL HIERARCHY

NORMAL DIVIDE

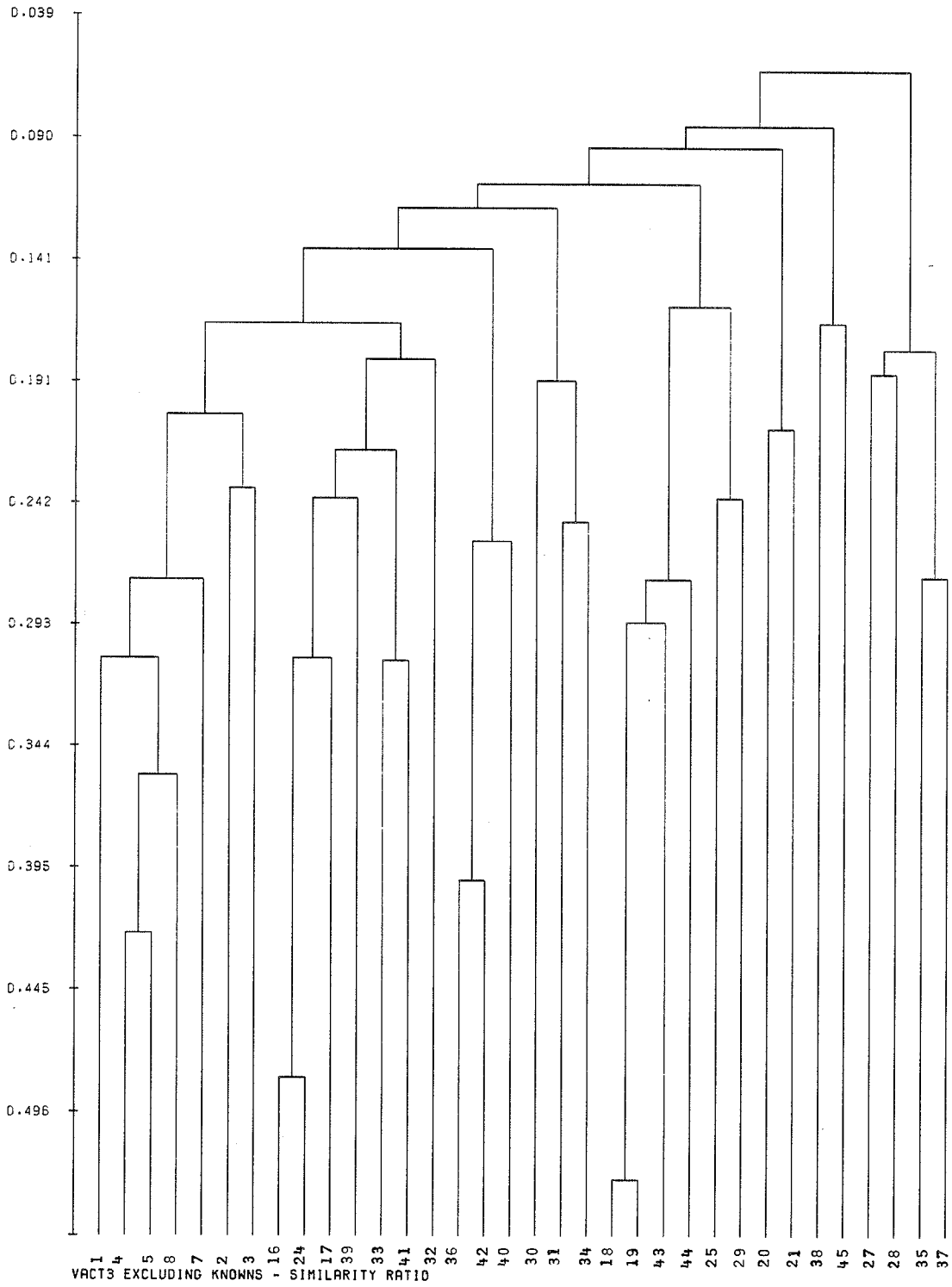
INVERSE HIERARCHY

INVERSE DIVIDE

NODAL ANALYSIS

The normal Hierarchy and Divide analyses are presented to show the clustering of the "unknowns" and the key anomalies that influence these groupings. The inverse Divide analysis shows those individuals in the VACT 3 minus knowns group that generate the most variation while the Inverse Hierarchy describes the anomaly groupings. These will be compared to the individual/anomaly groupings found in the VACT 3 plus known and the VACT 2 minus known analyses.

As in the Normal Hierarchy analysis of the VACT 3 plus known group, individuals with cloacal exstrophy fall into one distinct group (Group 1). No other cases were included in this cluster. Group 2. consists of seven individuals that fit into the caudal regression spectrum of


0.039
0.090
0.141
0.191
0.242
0.293
0.344
0.395
0.445
0.496
1 4 5 8 7 2 3 16 24 17 39 33 41 32 36 42 40 30 31 34 18 19 43 44 25 29 20 21 38 45 27 28 35 37
VACT3 EXCLUDING KNOWNS - SIMILARITY RATIO

VACT 3 MINUS KNOWNS
NORMAL HIERARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE CASES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Cloacal exstrophy x 7 (1,4,5,8,7,2,3).

GROUP 2.

VACRLG (Sirenomelia, 16), Rokitansky-Kuster-Hauser (24), CRG (Renal agenesis, 17), ACRG (39), ACTERG (33), ARG (41), VACRLG (Sirenomelia, 32).

GROUP 3.

VARG (36), ARG (42). ACR (40).

GROUP 4.

ACRL (30), VATERL (31), VALG (34).

GROUP 5.

ACRG (Prunebelly, 18), ACTERG (Prunebelly +, 19), VCR (43), VCR (? Trisomy 13, 44), CTRL (Tracheal agenesis,25), VACTERL (29).

GROUP 6.

VCRL (Private syndrome, 20), CLG (Private syndrome, 21).

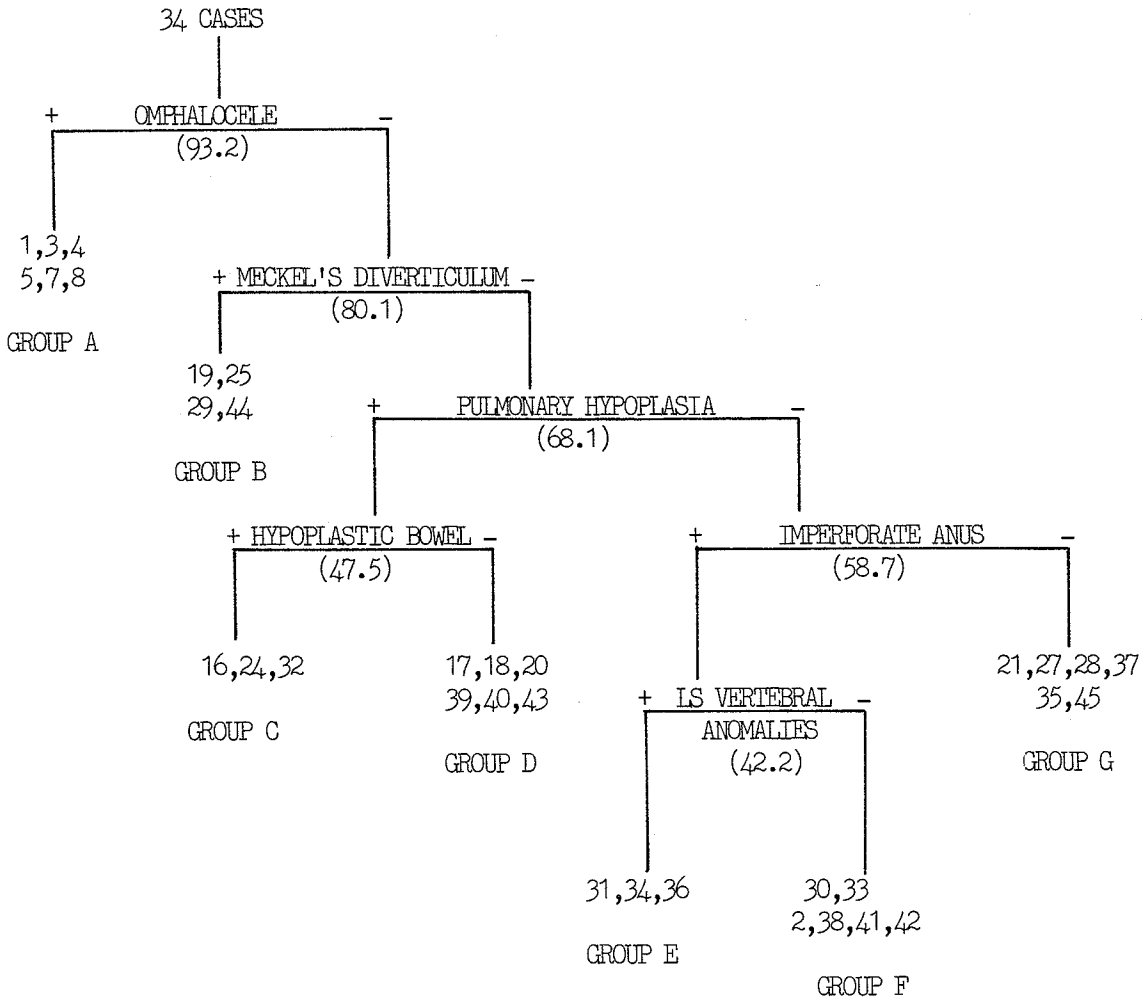
GROUP 7.

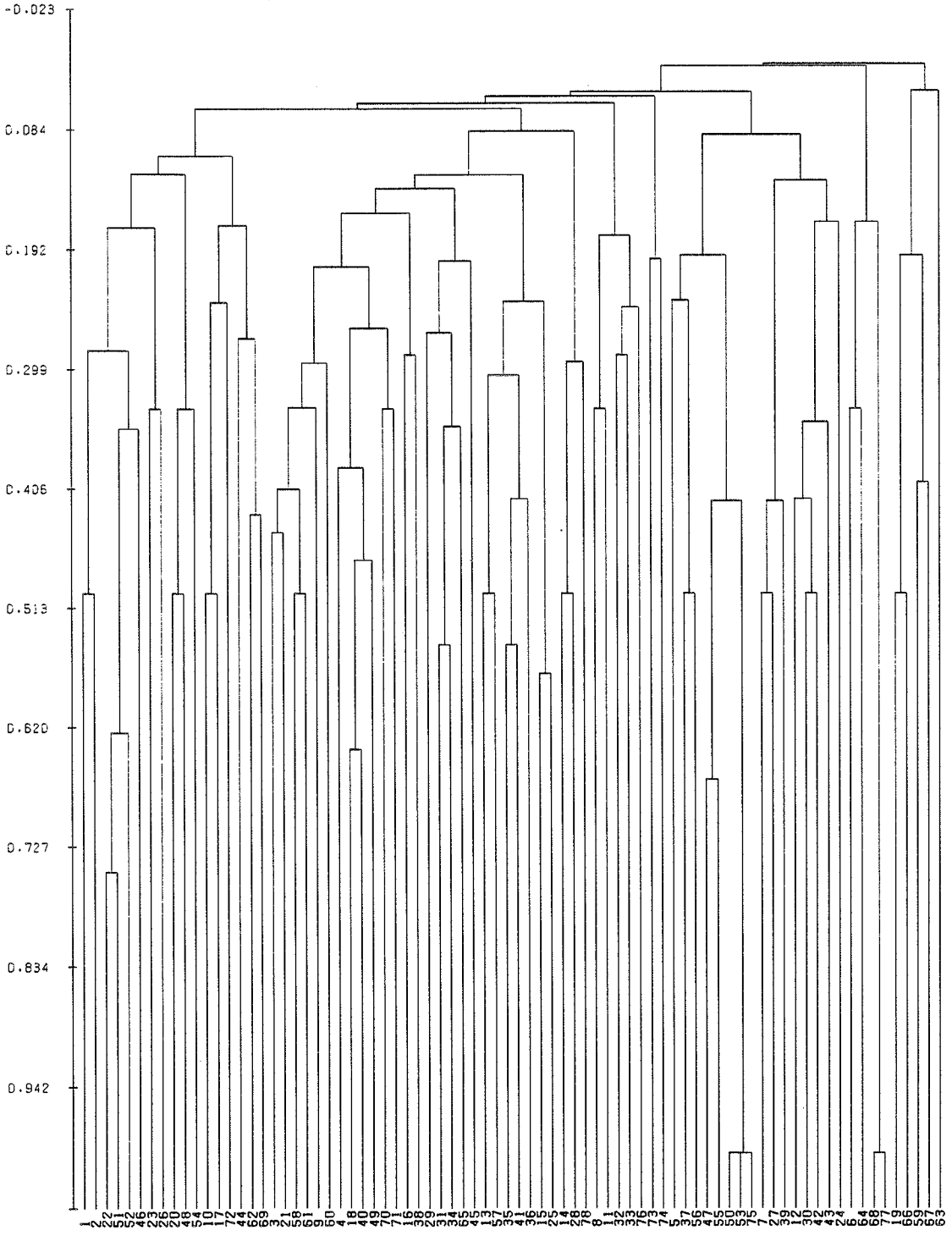
ACL (? Trisomy 13, 38), CLG (Private syndrome, 45).

GROUP 8.

VCRL (?Goldenhar,27), VCT (?Turner,28), VAC (35), ACL (37.)

VACT 3 MINUS KNOWNS
NORMAL DIVIDE
COEFFICIENT: SUM OF
THE SQUARE ROOT OF
CHI SQUARES





INVERSE VACT3 EXCLUDING KNOWNS - SIMILARITY RATIO

VACT 3 MINUS KNOWNS
INVERSE HIERARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE ANOMALIES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Tracheo-esophageal fistula, hypoplastic larynx, Meckel's diverticulum, pulmonary trunk anomalies, other migration defects, VSD, duodenal stenosis, abnormla pancreas, ectopic gallblader, anomalies of the subclavian arteries, right ventricular hypertrophy, polycystic kidneys, ureteral atresia, reduction deficiency upper limb, rectal fistula, micropthalmia, abnormal radial ray.

GROUP 2.

Lung septation abnormalities, malrotation of the bowel, low set ears, flat nose, hydronephrosis, dysplastic ears, pulmonary hypoplasia, imperfortae anus, abnormal external genitalia, single umbilical artery, positional deformity feet, reduction deficiency lower limb, urethral atresia, bifid scrotum, scoliosis, cervico-thoracic vertebral anomalies, absent ribs, cleft palate, PFO/ASD, bilateral renal agenesis, prominent occiput, vaginal atresia, abnormal internal genitalia, uterus didelphys, bladder agenesis, hypoplastic bowel.

GROUP 3.

Duplex collecting system, abnormal thymus, wide sutures.

GROUP 4.

Other renal, horseshoe kidneys, lumbo-sacral vertebral anomalies, extra ribs, L.S. dimple.

GROUP 5.

Abnormal palmar creases, dislocated hips.

GROUP 6.

Neural tube defect, bilateral cryptorchid, other abnormal vasculature, persistent left superior vena cava, other aortic trunk anomalies, mitral valve atresia, hypoplastic left ventricle, membranous diaphragm.

GROUP 7.

Microcephaly, adrenal anomalies, hypospadias, unilateral renal agenesis, skin tags, omphalocele, cloacal exstrophy, abnormal liver.

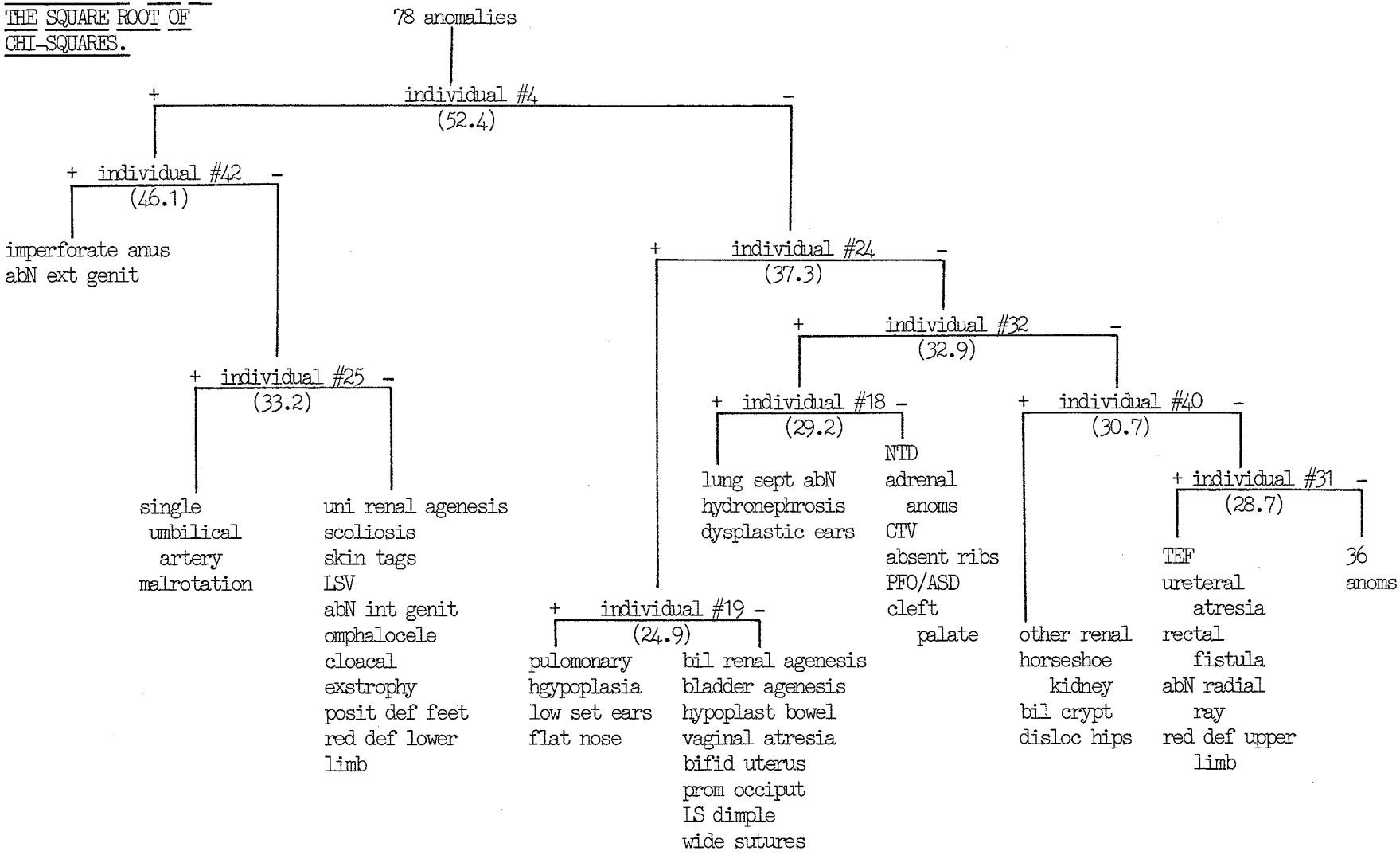
GROUP 8.

Arrhinecephaly, cleft lip and palate, postaxial polydactyly, webbed neck.

GROUP 9.

Ectopic anus, micrognathis, preauricular tags, preaxial polydactyly, hyperteloric.

VACT 3 MINUS KNOWNS
INVERSE DIVIDE
COEFFICIENT: SUM OF
THE SQUARE ROOT OF
CHI-SQUARES.



malformations. It includes two individuals with sirenomelia and one with Rokitansky-Kuster-Hauser sequence.

In this analysis, as in all other analyses, individual #16 who has sirenomelia is considered to be much more similar to the individual (#24) with Rokitansky-Kuster-Hauser sequence than to the other sirenomelic individual (#32). This illustrates several interesting points, that of incomplete reporting of minor anomalies, and the creation of an artificial homogeneity due to a combination of the latter, intrinsic limitations of the coding sheet, and the "logic without interpretation" approach of the program.

Both individuals 16 and 24 have identically coded renal and genital anomalies (bilateral renal agenesis, absent vagina, and other abnormal external genitalia), individual 32 had a different renal anomaly, namely bilateral multicystic kidneys, and as he was male, he was unable to present with vaginal atresia. Thus due to the somewhat different phenotype of individual #32, the different genital anomalies present, and the lack of a specific category in the coding sheet relating to limb fusion (due to its low frequency in our population), he appears to be less similar to the other sirenomelic individual than is individual #24.

Another contributing factor to this perceived decreased similarity can be attributed to the incomplete reporting of minor anomalies. Neither single umbilical artery nor low set ears are reported to have been present in individual #32 yet both of these are very likely to have been present (especially the single umbilical artery). Although these are not anomalies of major consequence to the individual they may be of considerable importance in the analyses, with low set ears being chosen as the key/differentiating anomaly in a Divide analysis to split off individuals who fall into the caudal regression or oligohydramnios spectrums.

Group 3 consists of three individuals who all have anal and renal anomalies and at least one other system affected. In two of the cases this involves a genital anomaly. Group 4. is composed of three individuals. The number of affected systems is very varied in this cluster with 3, 4 and 5 system involvement. These individuals all have anal and limb anomalies and a high frequency of renal involvement (two of the three cases). This group can be differentiated from Group 3. by the limb involvement and the lack of genital malformations.

Group 5. consists of two individuals who present with

a prunebelly picture, two cases with vertebral, cardiac and renal anomalies, an individual with tracheal agenesis and one with anomalies in all six of the VACTERL systems. The first two cases are very similar (see the graphical representation) as are the last two cases (they join to form a sub-cluster before they join the main body of the group). All these individuals in this group have cardiac and renal anomalies as well as a high frequency of tracheal (3/5) and anal (3/5) anomalies.

Group 6. is composed of only two cases both of which are considered to represent "private" syndromes that have yet to be delineated. Group 7. also consists of two individuals with cardiac and limb involvement. The cases in Group 8. all possess a cardiac anomaly and most (3/4) also have vertebral anomalies.

Five main anomaly groupings were evident in the inverse Hierarchy analysis: group 2. represented the caudal regression and oligohydramnios spectrums seen in individuals 32,16,24,18,19,and 39. The anomalies in Group 7. reflected the strong cloacal exstrophy grouping with cloacal exstrophy, omphalocele, unilateral renal agenesis, skin tags and microcephaly.

Anomalies such as arrhinecephaly, cleft lip and palate, and postaxial polydactyly that were seen in Group 8. strongly resembled those seen with Trisomy 13. However, it is very important to remember that only those individuals where the diagnosis was unequivocal or confirmed by karyotype analysis were considered as "known" syndromes. Thus some of the observed malformation patterns in the minus knowns groups may still reflect known syndromes such as Trisomy 13. Both individuals 38 and 44 (VACT 3) have findings clinically compatible with Trisomy 13 but unfortunately cytogenetic confirmation was not possible.

From its postion on the graphical representation, Group 1. can be seen to be a very strong grouping, on a par with Group 2. (anomalies seen in the caudal regression and oligohydramnios spectrums). It represents classic VACTERL-type anomalies such as tracheo-esophageal fistula, cardiac anomalies (including VSD), polycystic kidneys, other abnormal radial ray anomalies/reduction deficiency of the upper limb, duodenal stenosis, and micropthalmia.

The Key anomalies generated by the normal Divide analysis were imperforate anus, omphalocele, Meckel's Diverticulum, pulmonary hypoplasia, hypoplastic bowel, and

lumbo-sacral vertebral anomalies. Only two of these anomalies (omphalocele and imperforate anus) were common to both the VACT 3 plus known and the VACT 3 minus known analyses.

The patient clusters generated by the Normal Divide were essentially identical for Groups A,B,C,E, and G. Both Groups D and F were the last groups to be formed at either end of the two "chains" produced (i.e. they contained individuals with none, or only the initial, key anomaly) and so were not expected to be as specific.

There were nine key individuals determined by the inverse Divide analysis. Four of these came from the caudal regression-oligohydramnios cluster (individuals #16,24,18,19,32) and three were also key individuals in the VACT 3 plus known inverse Divide (individuals # 16,19,24). In both the VACT 3 plus knowns and the VACT 3 minus known analysis the individual who first split the population into two subgroups had cloacal exstrophy. It was not, however, the same individual in both analyses (individual #4 in the VACT 3 minus known group and individual # 7 in the VACT 3 plus known group), indicating that it was not the specific individual but rather the overall pattern of anomalies in cloacal exstrophy that was significant in the population.

A large number of key individuals determined by the Inverse Divide had anomalies compatible with caudal regression, indicating a strong, separate identity for individuals with anomalies in this spectrum from other members of the VACT 3 group.

The strongest associations determined by the Inverse Hierarchy are (in order of decreasing strength): mitral valve atresia, hypoplastic left ventricle, membranous diaphragm; imperforate anus and other abnormal external genitalia; Meckel's Diverticulum, pulmonary trunk anomalies, and other cardiac migration defects; and postaxial polydactyly and webbed neck. These are very similar to their counterparts ascertained for the VACT 3 plus known group. The exception to this is the postaxial polydactyly/webbed neck combination, it was only observed once in the VACT 3 minus known group, in an individual who might possibly have Trisomy 13 (individual #38).

3.550 Nodal Analysis of the VACT 3 Minus Known Group.

When nodal analysis was performed on the 34 cases and 80 anomalies considered in our VACT 3-Known population, 34 patients and 43 anomalies were represented in the final analysis. The two-way matrix of 70 cells derived from 7 patient groups and 10 malformation groups is shown in Fig. 6. The records cluster in less than 11% of the cells and only 9 cells are complete in both directions. (All other cells can be disregarded from the analysis). The major subdivision parameters are shown as solid arrows in the margins and as solid circles joined by solid lines. Not all the groups had enough residual variation for a statistically significant parameter to be generated. However, a weaker parameter can be chosen by selecting the key malformation or individual responsible for the establishment of the original group. These are shown as open arrows and as open symbols joined by broken lines.

Malformation patient clusters are represented by enclosed boxes. Cells defined in both directions are referred to as noda and represent the strongest coincidences of malformations and patients. If the cell is defined in only one direction, the coincidence is weaker and these are referred to as subnoda. Both noda and

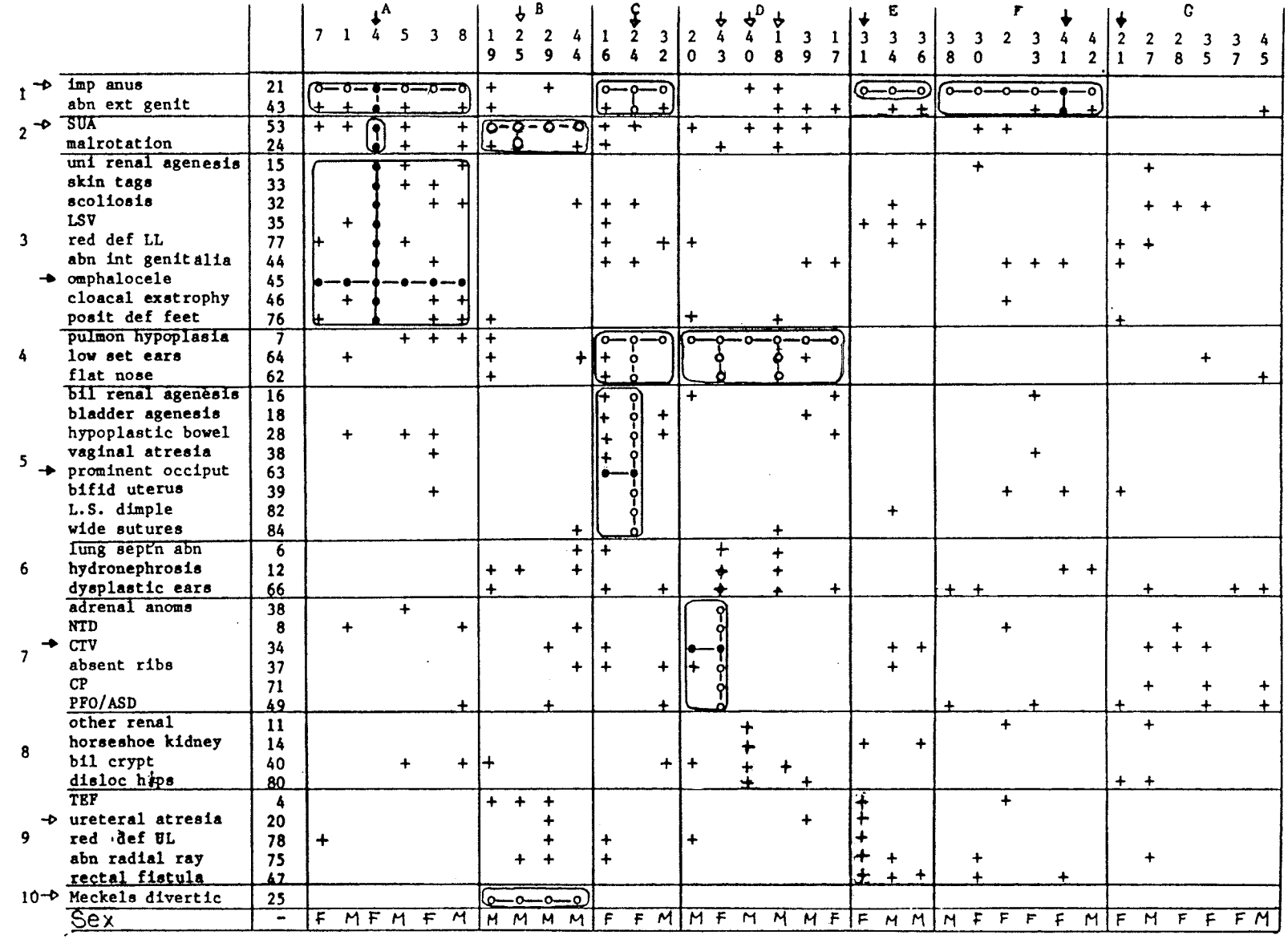


Figure 6. Nodal Analysis

subnoda may be either major or minor depending on the strength of the subdivision parameter used to define them.

The initial division of the normal analysis was on the basis of omphalocele. This separated those individuals with cloacal exstrophy (except for one atypical case who had no omphalocele) from the others (Group A). This a very strong group and clearly distinct from the others.

Group B was based on a relatively weak clustering. It must be kept in mind that this analysis is based on the Normal Divide, with the groups presented in the order that they appear in that analysis. Thus, the group is considered by the Normal Divide analysis to be second only in importance to the cloacal exstrophy picture seen in Group A. Also, the definition of this group becomes stronger if individual 44 is removed (as she would indeed have been if the clinical diagnosis of Trisomy 13 had been confirmed), with a much higher proportion of concurences in cell B9. Upon reviewing the specific anomalies present in members of this group, it was thought to represent variation of the tracheal agenesis association (Evans et al., 1985).

Groups C and D were relatively strong clusters with

pulmonary hypoplasia as a key anomaly, and other features of the oligohydramnios sequence, such as low set ears, present. Group C included two sirenomelic individuals and one with Rokitansky-Kuster-Hauser sequence. Cell C2 should also be complete in one direction, but was not since no mention was made of a missing umbilical artery in one of the sirenomelic individuals (#32). This group represented the most severe expression of the caudal regression sequence. Group D represented a milder form, with a very high incidence of single umbilical artery but the renal anomalies were less severe (with 3/6 as "other renal"). Four of the six cases in this group had oligohydramnios reported as a complication of pregnancy. Of the two that did not, one patient had anencephaly that was accompanied by polyhydramnios and the other was the infant of a diabetic mother.

Group E, a relatively weak grouping, had imperforate anus and reduction deformities of the lower limbs as key anomalies. This was felt to represent a VACTERL subgroup, since, in addition to imperforate anus and limb reduction deformities, a high incidence of other classic VACTERL-type anomalies such as cervicio-thoracic vertebral anomalies, horseshoe kidney, abnormal radial ray, abnormal external genitalia, lumbo-sacral dimple, and rectal

fistula were present.

Although it was a relatively strong group (based on a major subnoda), Group F had only imperforate anus as a key anomaly, indicating the importance of this anomaly (as perceived by the analysis) in differentiating between the relatively weaker and more heterogenous cases that form the last two groups. Other commonly found malformations included abnormal external genitalia, abnormal internal genitalia and ASD.

Group G consisted of six individuals. There were no key anomalies. This was the last group produced by the normal analysis of the raw data matrix and as such contained all those cases that had not been previously assigned a group. A description of the common anomalies in the groups defined by the nodal analysis is presented in Table 12.

Table 12. Malformation-Patient Clusters Derived from the Nodal Analysis fo the VACT 3 minus Known Group.

Group	# Cases	Key Anomalies	Other Common Anomalies (>50%)
A	6	Omphalocele Imperforate anus	Abnormal Ext Genit Single Umbilical Artery Skin tags Malrotation of Bowel Uni Renal Agenesis Scoliosis Cloacal Exstrophy Red Def Lower Limbs Positional def feet
B	4	Single Umbilical Artery Meckel's Diverticulum	Imperforate anus Malrotation of Bowel Hydronephrosis Tracheo-esophageal fistula Absent Ribs Abnormal Radial Ray
C	3	Imperforate Anus Abnormal Ext Genitalia Pulmon Hypoplasia	Abnormal Int Genitalia Flat Nose Bil Renal Agenesis Scoliosis Low set ears Bladder agenesis Hypoplastic bowel Vaginal atresia (2/2) Prominent Occiput
D	6	Pulmonary Hypoplasia	Imperforate anus Abnormal Ext Genit Single Umbilical Artery Low Set Ears Hydronephrosis
E	3	Imperforate Anus	Abnormal Ext Genit (2/3) LSV Horseshoe kidney CTV Abnormal Radial Ray Rectal Fistula Red Def Lower Limb

Table 12 continued.....

F	6	Imperforate anus	Abnormal Ext Genit Abnormal Int Genit
G	6	--	ASD CTV Cleft palate Scoliosis Dysplastic Ears

3.560 VACT 2 Analyses.

Analyses Presenteed

VACT 2 Plus Knowns

NORMAL	DIVIDE
NORMAL	HIERARCHY
INVERSE	DIVIDE

VACT 2 Minus Knowns

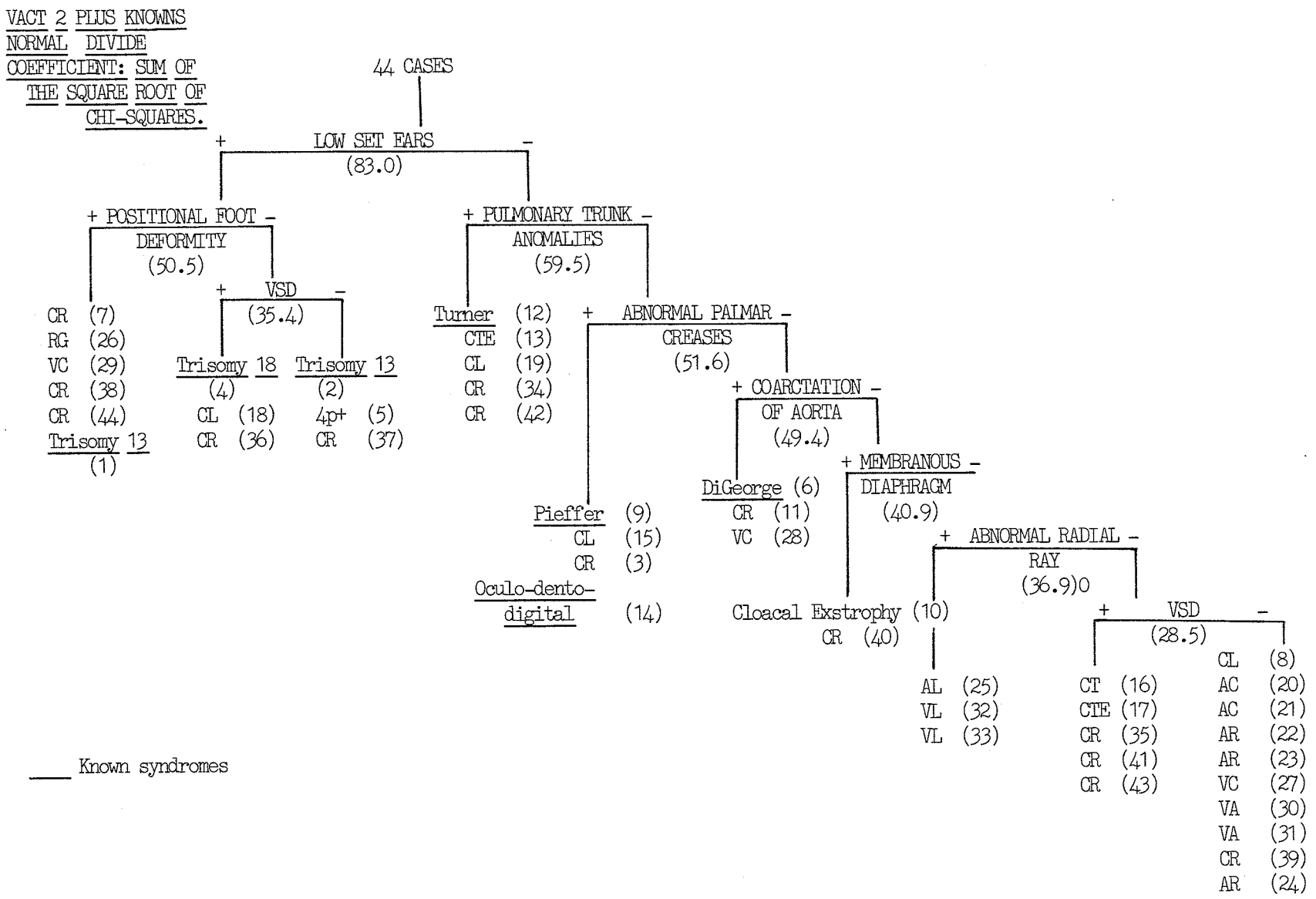
NORMAL	DIVIDE
INVERSE	DIVIDE
INVERSE	HIERARCHY

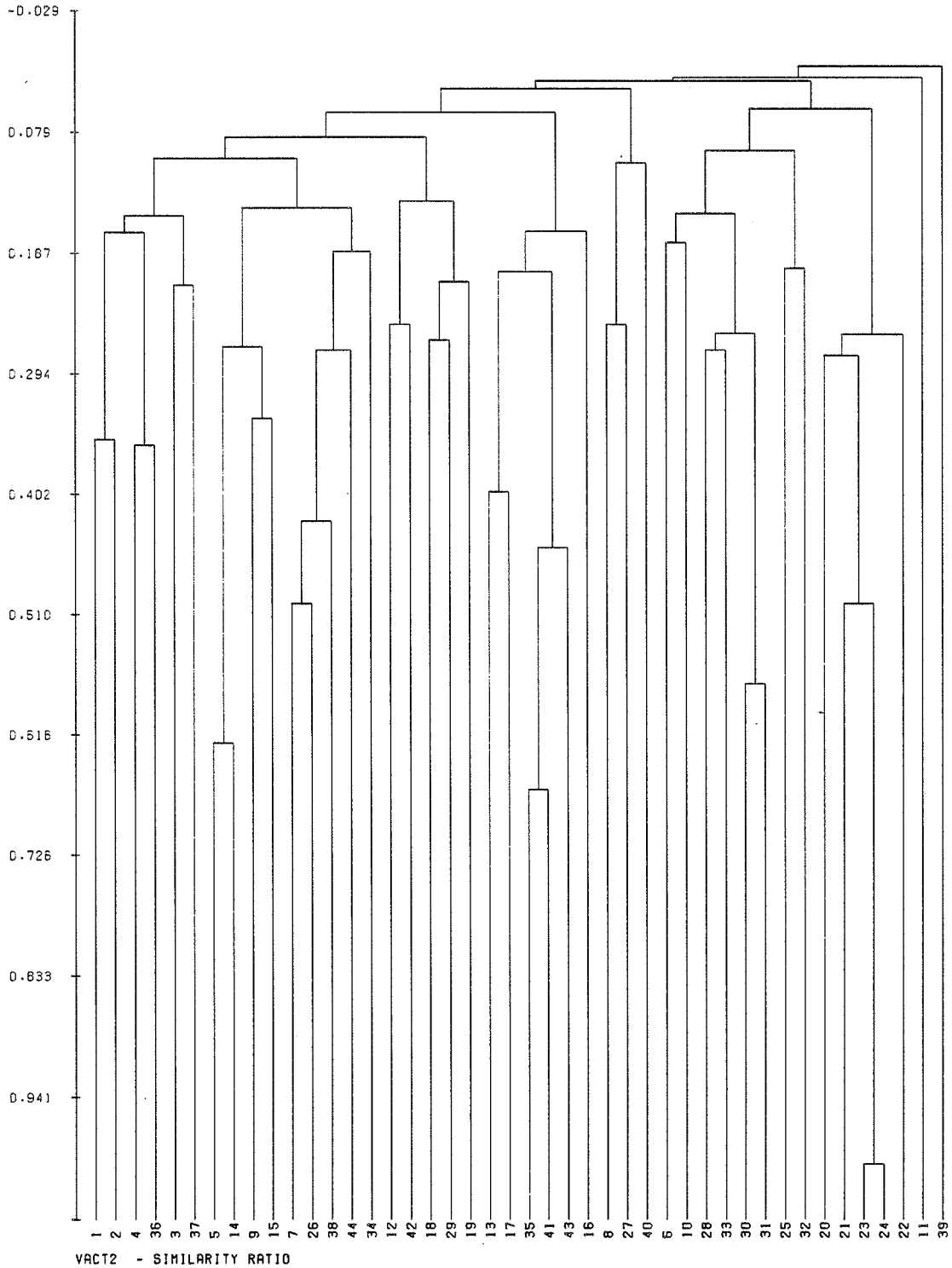
The Normal Divide and Hierarchy analyses are presented to show the clustering of known syndromes in this group and the key anomalies that affect these groupings. The inverse Divide shows those individuals in the VACT 2 group that generate the most variation in anomalies. By comparing these groupings in the VACT 2 minus known group and those in the VACT 3 minus known group we wish to determine if the VACT 2 minus known group represents a less severe expression of the VACTERL association or whether it is distinct.

3.561 VACT 2 Plus Knowns.

As with the VACT 3 plus known analyses, the Normal Hierarchy effectively recognises those individuals with Trisomy 13 and groups them together. Groups 4, 7, and 6 also show a high degree of homogeneity; Group 4. contains 6 individuals with either a tracheal or renal anomaly associated with a cardiac malformation. Group 6. predominantly involves individuals with vertebral anomalies, and Group 7. is composed of individuals with anal malformations.

Two individuals, cases number 11 and 39, are separated out from the rest of the analysis, forming groups of one. Individual number 11 was thought to have Turner's Syndrome as she had neck webbing, coarctation of the aorta, small ovaries, a horseshoe kidney, and a hypoplastic thymus, but was considered by the analysis to be very different from the confirmed case (individual # 12). Both had anomalies compatible with the clinical diagnosis of Turner's syndrome including neck webbing and aortic stenosis, but the other cardiac and genito-urinary anomalies were different in both. Case number 39 was a liveborn girl with aortic atresia and dysplastic kidneys.


VACT 2 PLUS KNOWNS
NORMAL DIVIDE
COEFFICIENT: SUM OF
THE SQUARE ROOT OF
CHI-SQUARES.
44 CASES
+ LOW SET EARS -
(83.0)
+ POSITIONAL FOOT -
DEFORMITY
(50.5)
+ VSD -
(35.4)
+ PULMONARY TRUNK -
ANOMALIES
(59.5)
CR (7)
RG (26)
VC (29)
CR (38)
CR (44)
Trisomy 13
(1)
Trisomy 18
(4)
CL (18)
CR (36)
Trisomy 13
(2)
4p+ (5)
CR (37)
Turner (12)
CTE (13)
CL (19)
CR (34)
CR (42)
+ ABNORMAL PALMAR -
CREASES
(51.6)
+ COARCTATION -
OF AORTA
(49.4)
+ MEMBRANOUS -
DIAPHRAGM
(40.9)
+ ABNORMAL RADIAL -
RAY
(36.9)0
+ VSD -
(28.5)
Pieffer (9)
CL (15)
CR (3)
Oculo-dento-
digital (14)
DiGeorge (6)
CR (11)
VC (28)
Cloacal Exstrophy (10)
CR (40)
AL (25)
VL (32)
VL (33)
CT (16)
CTE (17)
CR (35)
CR (41)
CR (43)
CL (8)
AC (20)
AC (21)
AR (22)
AR (23)
VC (27)
VA (30)
VA (31)
CR (39)
AR (24)
___ Known syndromes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0.618
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0.833
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1 2 4 36 3 37 5 14 9 15 7 26 38 44 34 12 42 18 29 19 13 17 35 41 43 16 8 27 40 5 10 28 33 30 31 25 32 20 21 23 24 22 11 39
VAR12 - SIMILARITY RATIO

VACT 2 PLUS KNOWNS
NORMAL HIERARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE CASES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Trisomy 13 x 2 (1,2), Trisomy 18 (4), CR (36), CR (?Meckel/Trisomy 13, 3) CR (37).

GROUP 2.

4p+ (5), Oculo-Dental-Digital (14), Pfieffer syndrome (9), CL (?Miller Dieker, 15), CR (renal agenesis, 7), RG (26), CR (38), CR (44), CR (34).

GROUP 3.

Turner syndrome (12), CR (42), CL (18), VC (29), CL (19).

GROUP 4.

CTE (?DiGeorge, 13), CTE (17), CR (35), CR (41), CR (43), CT (16).

GROUP 5.

Hemifacial microsomia (8), VC (27), CR (40).

GROUP 6.

DiGeorge (6), cloacal exstrophy (10), VC (28), VL (33), VA (30), VA (31), AL (25), VL (32).

GROUP 7.

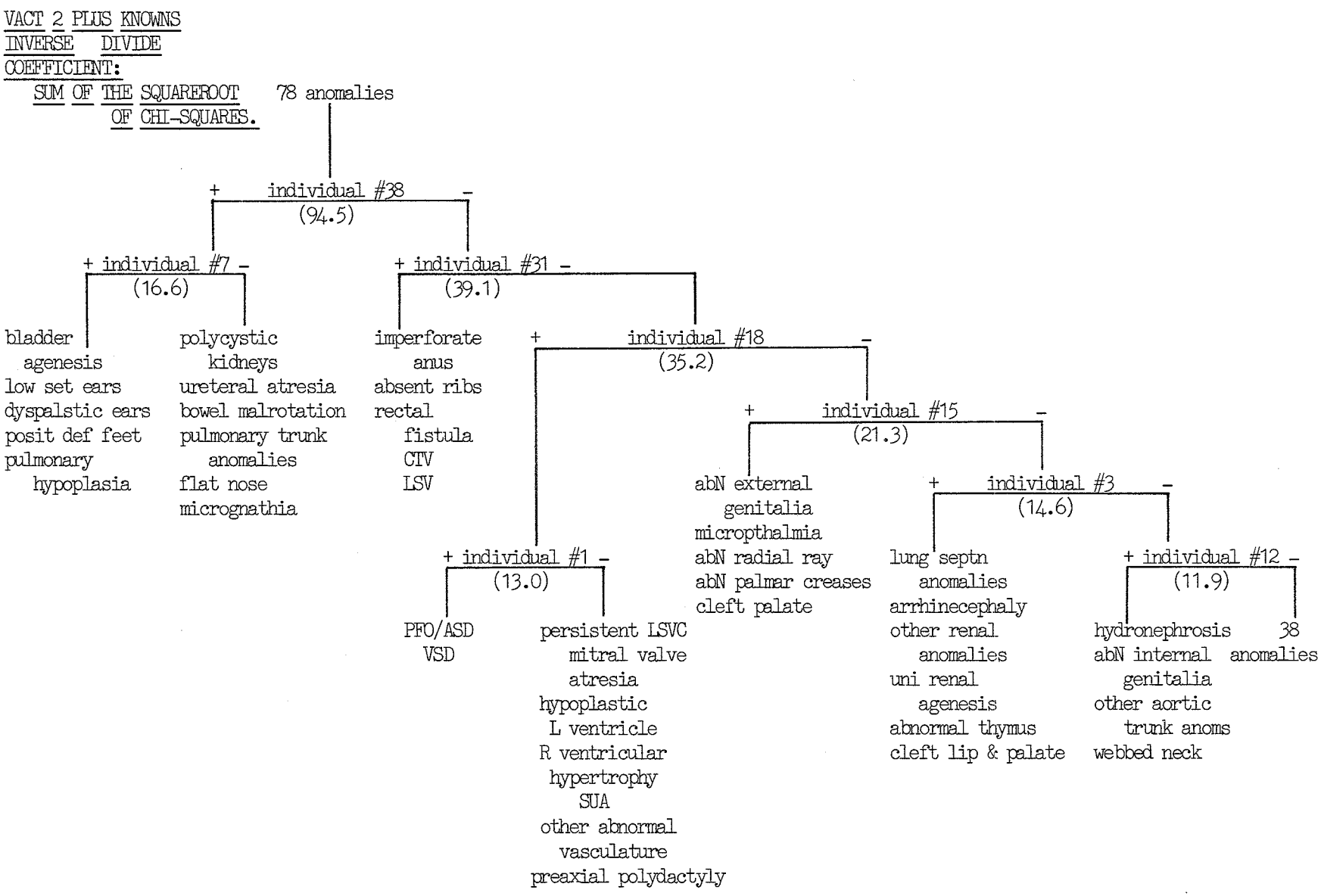
AC (20), AC (21), AR (23), AR (24), AR (24).

GROUP 8.

CR (? Turner syndrome, 11)

GROUP 9.

CR (39)


VACT 2 PLUS KNOWNS
INVERSE DIVIDE
COEFFICIENT:
SUM OF THE SQUAREROOT OF CHI-SQUARES.
78 anomalies
+ individual #38 -
(94.5)
+ individual #7 -
(16.6)
+ individual #31 -
(39.1)
bladder agenesis
low set ears
dyspalstic ears
posit def feet
pulmonary hypoplasia
polycystic kidneys
ureteral atresia
bowel malrotation
pulmonary trunk anomalies
flat nose
micrognathia
imperforate anus
absent ribs
rectal fistula
CTV
LSV
+ individual #18 -
(35.2)
+ individual #15 -
(21.3)
+ individual #1 -
(13.0)
abN external genitalia
micropthalmia
abN radial ray
abN palmar creases
cleft palate
+ individual #3 -
(14.6)
PFO/ASD
VSD
persistent LSVC
mitral valve atresia
hypoplastic L ventricle
R ventricular hypertrophy
SUA
other abnormal vasculature
preaxial polydactyly
lung septn anomalies
arrhinecephaly
other renal anomalies
uni renal agenesis
abnormal thymus
cleft lip & palate
+ individual #12 -
(11.9)
hydronephrosis
abN internal genitalia
other aortic trunk anoms
webbed neck
38 anomalies

The key malformations (determined by the Normal Divide) were low set ears, positional foot deformity, pulmonary trunk anomalies, abnormal palmar creases, coarctation of the aorta, membranous diaphragm, other abnormal radial ray, and VSD. There was no overlap between the "key" anomalies in the VACT 3 plus knowns or VACT 3 minus knowns. This tends to support the hypothesis that individuals with only two anomalies compatible with VACTERL may be distinct from true VACTERL cases but this can only be explored further by considering the VACT 2 minus known group since the bias introduced by the known syndromes will have been removed.

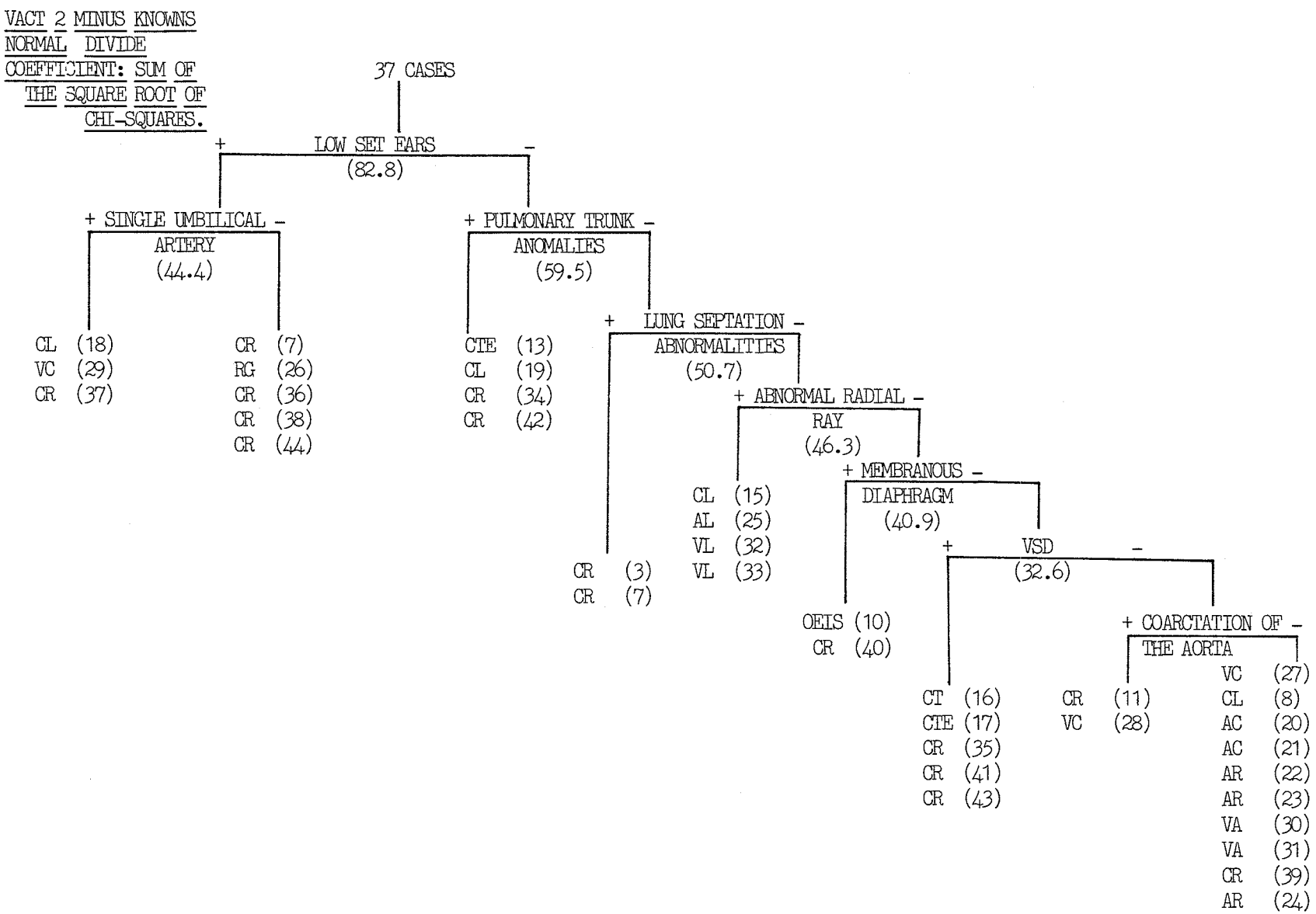
Only one of the key individuals distinguished by the Inverse Divide represented a known syndrome (#4, Trisomy 18). The other eight cases that generated the greatest variability in the population were unknowns.

3.562 VACT 2 Minus Knowns.

The Inverse Divide and Hierarchy are presented to look at the malformation groupings and the individuals in the VACT 2 minus known group that sequentially generate the most variation. The Normal Divide is included so that the key anomalies for this group can be compared to those seen in other groups where the known syndromes have been excluded.

Seven distinct groups were determined from the Inverse Hierarchy analysis. Of these, only two represented well recognised entities: Group 5. described a combination of the Potter's and Oligohydramnios spectrums, and Group 3. described anomalies compatible with those seen in Trisomy 13 (as in the VACT 3 group there was an individual who presented with a phenotype compatible with Trisomy 13, but where confirmation of this was not available).

The strongest anomaly associations (from the Inverse Hierarchy) and the Key anomalies (from the Normal Divide) were compared to those found in the VACT 3 minus Known and the VACT (2+3) minus known groups. Both the VACT 2 minus known and the VACT (2+3) minus known groups had key anomalies determined by the Normal Divide that were more


VACT 2 MINUS KNOWNS
NORMAL DIVIDE
COEFFICIENT: SUM OF
THE SQUARE ROOT OF
CHI-SQUARES.
37 CASES
+ LOW SET EARS -
(82.8)
+ SINGLE UMBILICAL ARTERY -
(44.4)
+ PULMONARY TRUNK ANOMALIES -
(59.5)
+ LUNG SEPTATION ABNORMALITIES -
(50.7)
+ ABNORMAL RADIAL RAY -
(46.3)
+ MEMBRANOUS DIAPHRAGM -
(40.9)
+ VSD -
(32.6)
+ COARCTATION OF THE AORTA -
CL (18)
VC (29)
CR (37)
CR (7)
RG (26)
CR (36)
CR (38)
CR (44)
CTE (13)
CL (19)
CR (34)
CR (42)
CR (3)
CR (7)
CL (15)
AL (25)
VL (32)
VL (33)
OEIS (10)
CR (40)
CT (16)
CTE (17)
CR (35)
CR (41)
CR (43)
CR (11)
VC (28)
VC (27)
CL (8)
AC (20)
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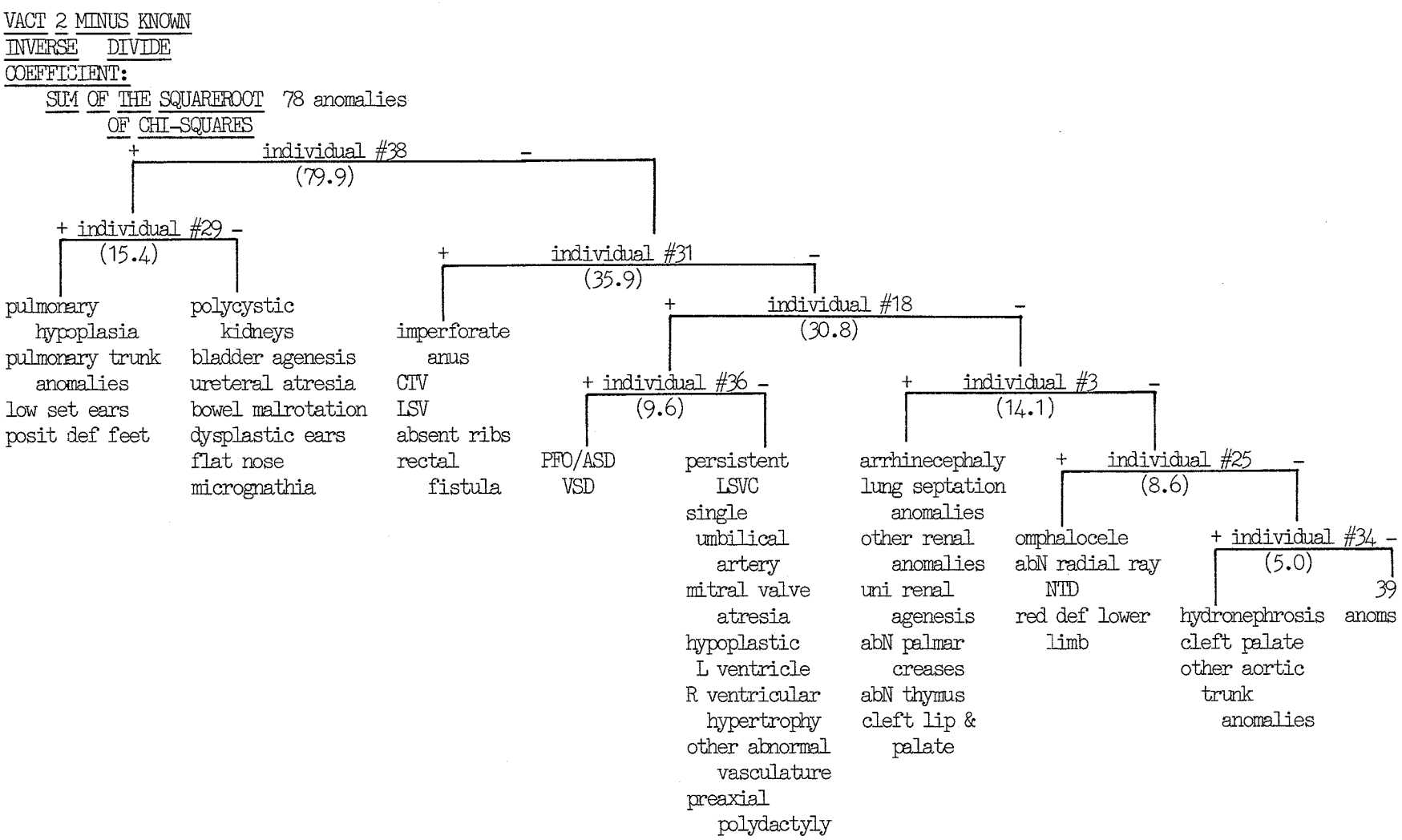
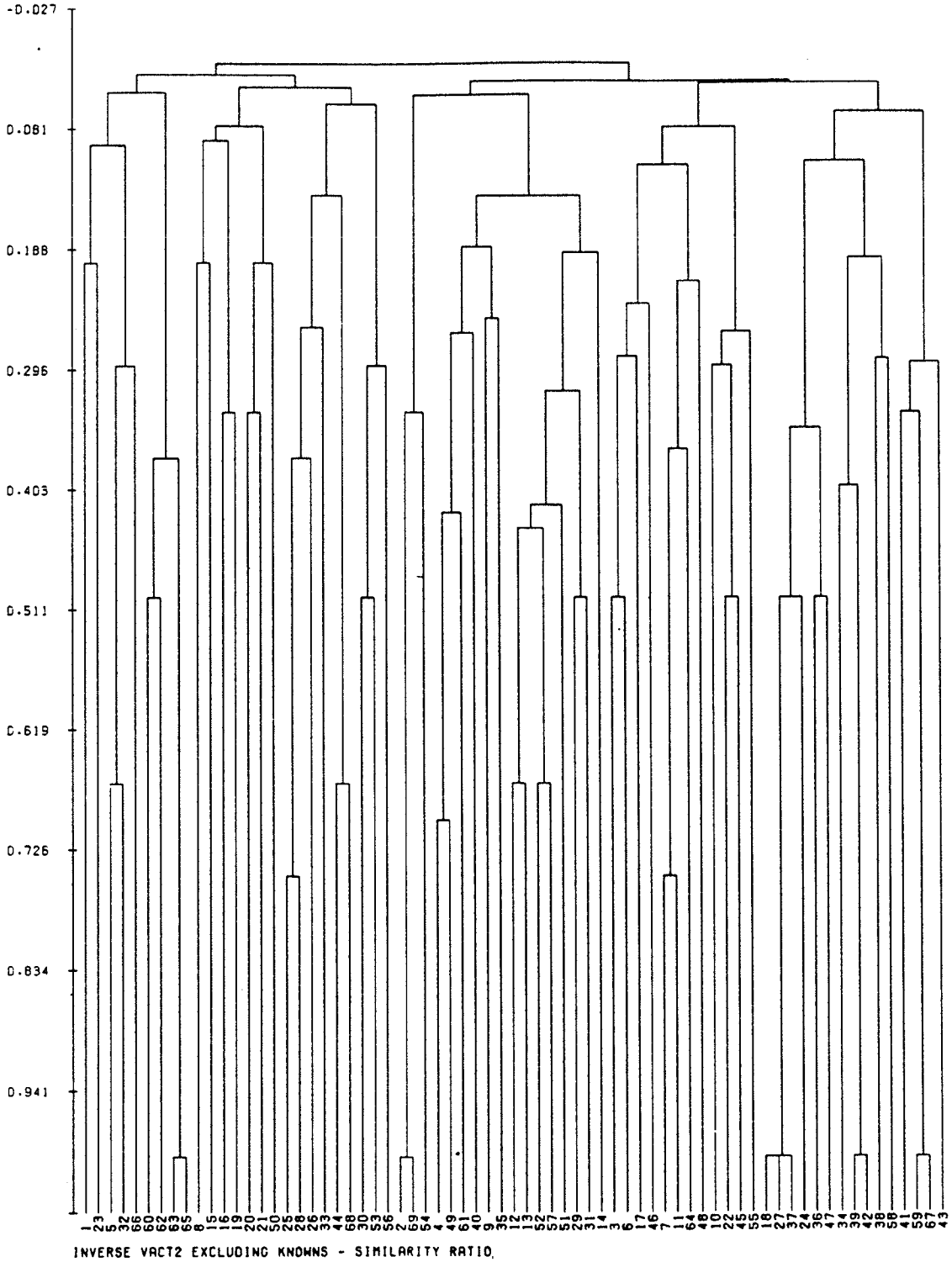
AR (22)
AR (23)
VA (30)
VA (31)
CR (39)
AR (24)


VACT 2 MINUS KNOWN INVERSE DIVIDE COEFFICIENT:
SUM OF THE SQUAREROOT OF CHI-SQUARES
78 anomalies
individual #38
(79.9)
+
-
individual #29
(15.4)
pulmonary hypoplasia
pulmonary trunk anomalies
low set ears
posit def feet
polycystic kidneys
bladder agenesis
ureteral atresia
bowel malrotation
dysplastic ears
flat nose
micrognathia
individual #31
(35.9)
imperforate anus
CTV
LSV
absent ribs
rectal fistula
individual #18
(30.8)
individual #36
(9.6)
PFO/ASD
VSD
persistent LSVC
single umbilical artery
mitral valve atresia
hypoplastic L ventricle
R ventricular hypertrophy
other abnormal vasculature
preaxial polydactyly
individual #3
(14.1)
arrhinecephaly
lung septation anomalies
other renal anomalies
uni renal agenesis
abN palmar creases
abN thymus
cleft lip & palate
individual #25
(8.6)
omphalocele
abN radial ray NTD
red def lower limb
individual #34
(5.0)
hydronephrosis
cleft palate
other aortic trunk anomalies
39 anoms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1 23 5 32 66 60 62 63 65 8 15 16 19 20 21 50 25 28 26 33 44 68 30 53 56 29 69 54 4 49 61 40 9 35 12 13 52 57 51 29 31 14 3 6 17 46 7 11 64 48 10 22 45 55 18 27 37 24 36 47 34 39 42 38 58 41 59 67 43
INVERSE VAC72 EXCLUDING KNOWNS - SIMILARITY RATIO

VACT 2 MINUS KNOWNS
INVERSE HIERARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE ANOMALIES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Tracheo-esophageal fistula, abnormal thymus, neural tube defect, omphalocele, membranous diaphragm, abnormal radial ray, reduction deficiency lower limb, reduction deficiency upper limb, dislocated hips.

GROUP 2.

Hydronephrosis, imperforate anus, ectopic anus, duodenal stenosis, abnormal liver, abnormal pancreas, preauricular tags.

GROUP 3.

Cervico-thoracic vertebral anomalies, absent ribs, lumbo-sacral vertebral anomalies, rectal fistula, coarctation of the aorta, webbed neck, abnormal external genitalia, micropthalmia, cleft palate.

GROUP 4.

Hypoplastic larynx, widely spaced sutures, hyperteloric.

GROUP 5.

Pulmonary hypoplasia, low set ears, positional deformity feet, pulmonary trunk anomalies, polycystic kidneys, VSD, bilateral renal agenesis, bladder agenesis, flat nose, micrognathia, microtia, bifid uterus, abnormal internal genitalia, ureteral atresia.

GROUP 6.

Lung septation abnormalities, arrhinecephaly, malrotaion of bowel, other aortic trunk anomalies, other renal anomalies, unilateral renal agenesis, abnormal palmar creases, prominent occiput, horseshoe kidney, adrenal anomalies, overiding aorta, cleft lip and palate.

GROUP 7.

Meckel's diverticulum, extra ribs, anomalies of the subclavian arteries, scoliosis, persistent left superior vena cava, ather abnormal vasculature, PFO/ASD, mitral valve atresia, hypoplastic left ventricle, single umbilical artery, preaxial olydactyly, other migration defects, postaxial polydactyly, L.S. dimple, right ventricular hypertrophy.

consistent with "classic" VACTERL type anomalies (i.e. bilateral renal agenesis, abnormal radial ray, VSD) than did the VACT 3 group. This strong identity with VACTERL-type anomalies would tend to support the hypothesis that the VACT 2 minus known cases do represent milder forms of the VACTERL association.

There were five very strong anomaly associations determined by the Inverse Hierarchy: Meckel's Diverticulum, extra ribs, anomalies of the subclavian arteries; mitral valve atresia, hypoplastic left heart; postaxial polydactyly, lumbo-sacral dimple; hypoplastic larynx, widely spaced sutures; and reduction deficiency of the upper limb, dislocated hips.

Other strong associations included neural tube defects and omphalocele; cervico-thoracic anomalies and absent ribs; coarctation of the aorta and webbed neck; pulmonary hypoplasia and low set ears; bilateral renal agenesis and bladder agenesis. Many of these combinations were also seen in the other analyses, and are not unexpected. Others such as the hypoplastic larynx and widely spaced sutures are singled out not because these combinations are seen together so commonly, but rather because of their conccurance in a single individual.

Half of the key individuals in the VACT 2 minus known Inverse Divide are the same ones found in the Inverse Divide of the VACT 2 plus knowns group, but none are found in the VACT (2+3) minus known analysis. The key individuals are in themselves not intrinsically important but they do reflect those cases which produce the greatest variation in the population. It does however, indicate that the VACT 2 individuals do not have as great an impact in the combined analyses as do the VACT 3's. Indirectly, this also supports our hypothesis that the VACT 2 group is a milder expression of the VACTERL association since any obviously "different" or unusual cases should be singled out in this analysis.

3.570 VACT (2 + 3) Plus Knowns.

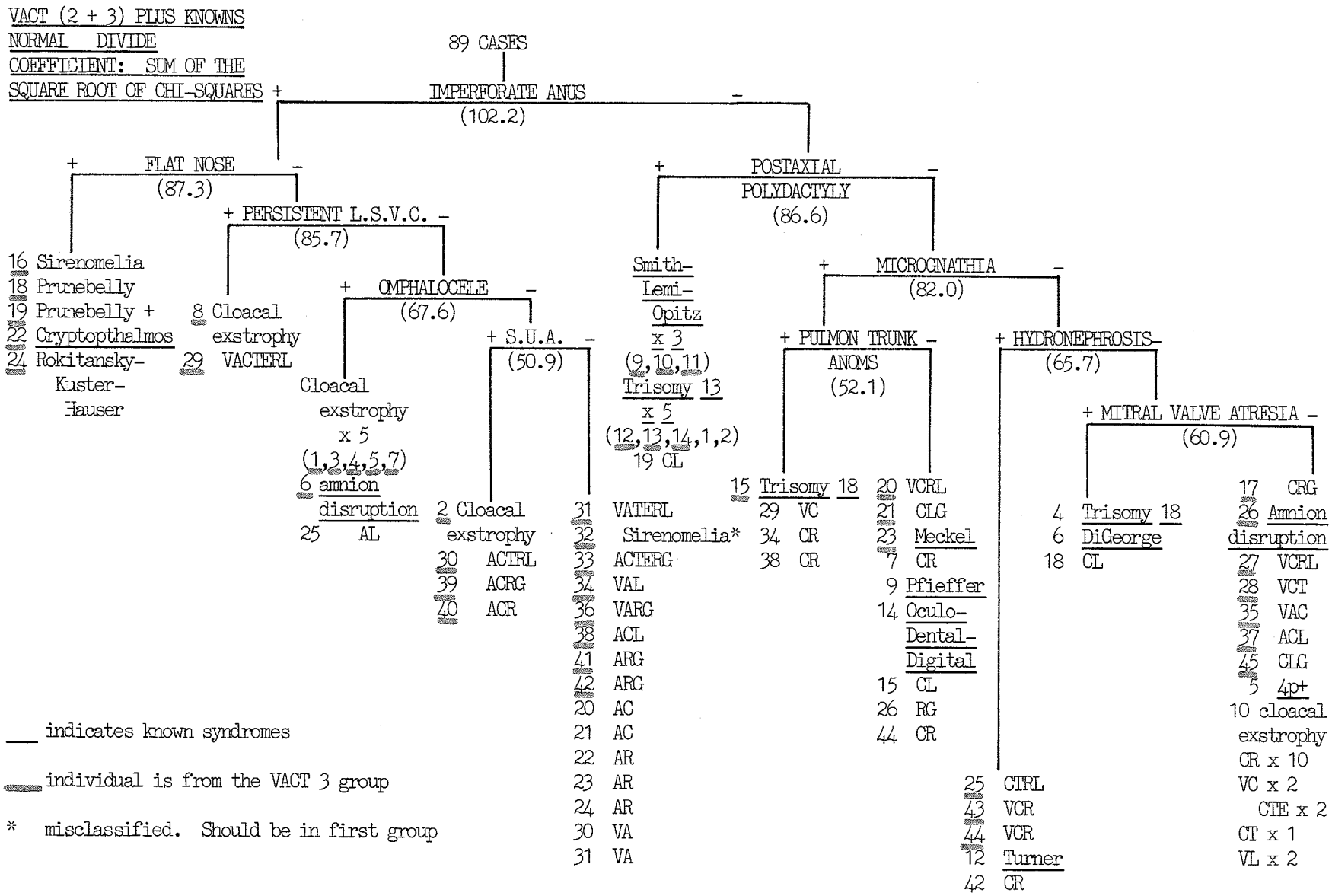
Analyses Presented:

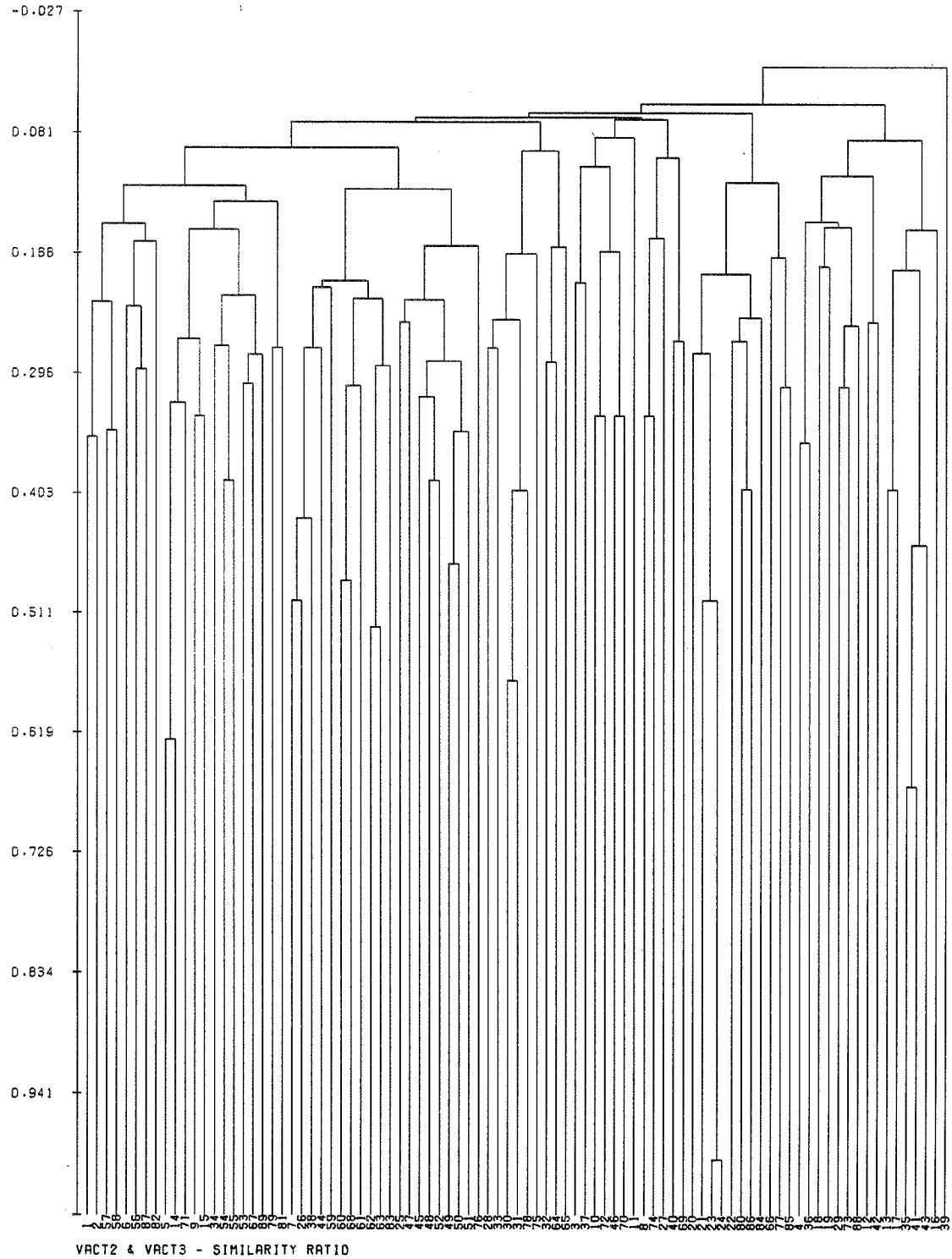
NORMAL DIVIDE

NORMAL HIERARCHY

These analyses were performed to see if the known syndromes that were common to both groups i.e. Trisomy 13, Trisomy 18 and sequences of unknown etiology such as cloacal exstrophy would group together, demonstrating the effectiveness of taxonomic methods on a more heterogenous population, and the influence of incomplete anomaly documentation or variable expressivity on the clusters produced.

The Normal Hierarchy analysis recognized the strong resemblance between those individuals with Trisomy 13 and Smith-Lemli-Opitz, clustering them together in Groups 1. and 2. respectively. Although in both clusters other individuals were included in whom this diagnosis had not been made (but did include at least one individual #82, who clinically appeared to have Trisomy 13). Group 3, the caudal regression and oligohydramnios spectrums, were


VACT (2 + 3) PLUS KNOWNS
NORMAL DIVIDE
COEFFICIENT: SUM OF THE
SQUARE ROOT OF CHI-SQUARES
89 CASES
IMPERFORATE ANUS
(102.2)
+
-
FLAT NOSE
(87.3)
+ PERSISTENT L.S.V.C. -
(85.7)
+ OMPHALOCELE -
(67.6)
+ S.U.A. -
(50.9)
POSTAXIAL POLYDACTYLY
(86.6)
+ MICROGNATHIA -
(82.0)
+ PULMON TRUNK ANOMS -
(52.1)
+ HYDRONEPHROSIS -
(65.7)
+ MITRAL VALVE ATRESIA -
(60.9)
16 Sirenomelia
18 Prunebelly
19 Prunebelly +
22 Cryptopthalmos
24 Rokitansky-Kuster-Hauser
8 Cloacal exstrophy
29 VACTERL
Cloacal exstrophy x 5
(1,3,4,5,7)
6 amnion disruption
25 AL
2 Cloacal exstrophy
30 ACTRL
39 ACRG
40 ACR
31 VATERL
32 Sirenomelia*
33 ACTERG
34 VAL
36 VARG
38 ACL
41 ARG
42 ARG
20 AC
21 AC
22 AR
23 AR
24 AR
30 VA
31 VA
Smith-Lemi-Opitz x 3
(9,10,11)
Trisomy 13 x 5
(12,13,14,1,2)
19 CL
15 Trisomy 18
29 VC
34 CR
38 CR
20 VCRL
21 CLG
23 Meckel
7 CR
9 Pfieffer
14 Oculo-Dental-Digital
15 CL
26 RG
44 CR
25 CTRL
43 VCR
44 VCR
12 Turner
42 CR
4 Trisomy 18
6 DiGeorge
18 CL
17 CRG
26 Amnion disruption
27 VCRL
28 VCT
35 VAC
37 ACL
45 CLG
5 4p+
10 cloacal exstrophy
CR x 10
VC x 2
CTE x 2
CT x 1
VL x 2
___ indicates known syndromes
___ individual is from the VACT 3 group
* misclassified. Should be in first group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VAC72 & VAC73 - SIMILARITY RATIO

VACT (2 + 3) PLUS KNOWNS
NORMAL HIERARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE CASES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Trisomy 13 x 4 (1,2,57,58)**, amnion disruption (6), Trisomy 13 (56), VCR (87), ACL (82).

GROUP 2.

4p+ (5), oculo-dental-digital (14), VCRL (?Goldenhar, 71), Pfieffer syndrome (9), CL (?Miller Dieker, 15), CR (34), Smith-Lemi-Opitz x 3 (54,55,53), Meckel syndrome (67), CLG (89), VAC (79), ACL (81).

GROUP 3.

CR (7), RG (26), CR (38), CR (44), Trisomy 18 (59), VACRLG (Sirenomelia, 60), Rokitnsky-Kuster-Hauser (68), CRG (61), ACRG (Prunebelly, 62), ACTERG (Prunebelly, 63), ACRG (83).

GROUP 4.

AL (25), cloacal exstrophy x 5 (47,45,48,52,49), amnion disruption (50), cloacal exstrophy (51), ACTERG (Sirenomelia, 76).

GROUP 5.

VC (28), VL (33), VA (30), VA (31), VAL (78), VATERL (75), VL (32), VCRL (64), CLG (65).

** Numbers in brackets are identification numbers for the individuals. Numbers 1-44 indicate individuals from the VACT 2 group. Numbers 45-89 indicate identification numbers for the VACT 3 group i.e. individual #45 corresponds to individual #1 in the VACT 3 key.

GROUP 6.

CR (?Meckel's/Trisomy 13, 3), CR (37), cloacal exstrophy (10), VCT (?Turner syndrome, 72), cloacal exstrophy (46), amnion disruption (70), CR (?Turner syndrome, 11).

GROUP 7.

Hemifacial microsomia (8), ACTRL (74), VC (27), CR (40), CTRL (Tracheal agenesis, 69).

GROUP 8.

AC (20), AC (21), AR (23), AR (24), AR (22), VARG (80), ARG (86), ACR (84), Cryptopthalmos (ATRG, 66), ACTERG (77), ARG (85).

GROUP 9.

Trisomy 18 (CR, 4), CR (36), CL (18), CL (19), VC (29), VACTERL (73), VCR (88), CG (12), CR (42).

GROUP 10.

CTE (?DiGeorge, 13), CTE (17), CR (35), CR (41), CR (43), CT (16).

GROUP 11.

CR (39).

another highly conserved cluster in all the analyses. One sirenomelic individual (#32, VACT 3), who in previous analyses was included as a member of this group was excluded (this individual was missclasified in the normal Divide due to the absence of "flat nose").

The individuals with cloacal exstrophy did not form as distinct a cluster as in previous analyses. The majority (6/8) clustered together in Group 4. The two that did not were lacked some of the most commonly associated anomalies found in cloacal exstrophy, one (#2 VACT 3) not having an omphalocele and the other (#10 VACT 2) lacking an imperforate anus (Both of these cases were also separated from the main cluster of individuals with cloacal exstrophy in the normal Divide analysis for these reasons).

Both Groups 5. and 8. stimulated interest since they appeared to represent possible field defects: Group 5. was notable for its very high frequency of vertebral and limb defects (8/9 and 6/9 respectively), however, this did not prove to be a particularly strong association since in only five of the cases were the defects concordant (there was both vertebral and limb involvement) for both systems.

Group 8. had a very high frequency of anal and renal

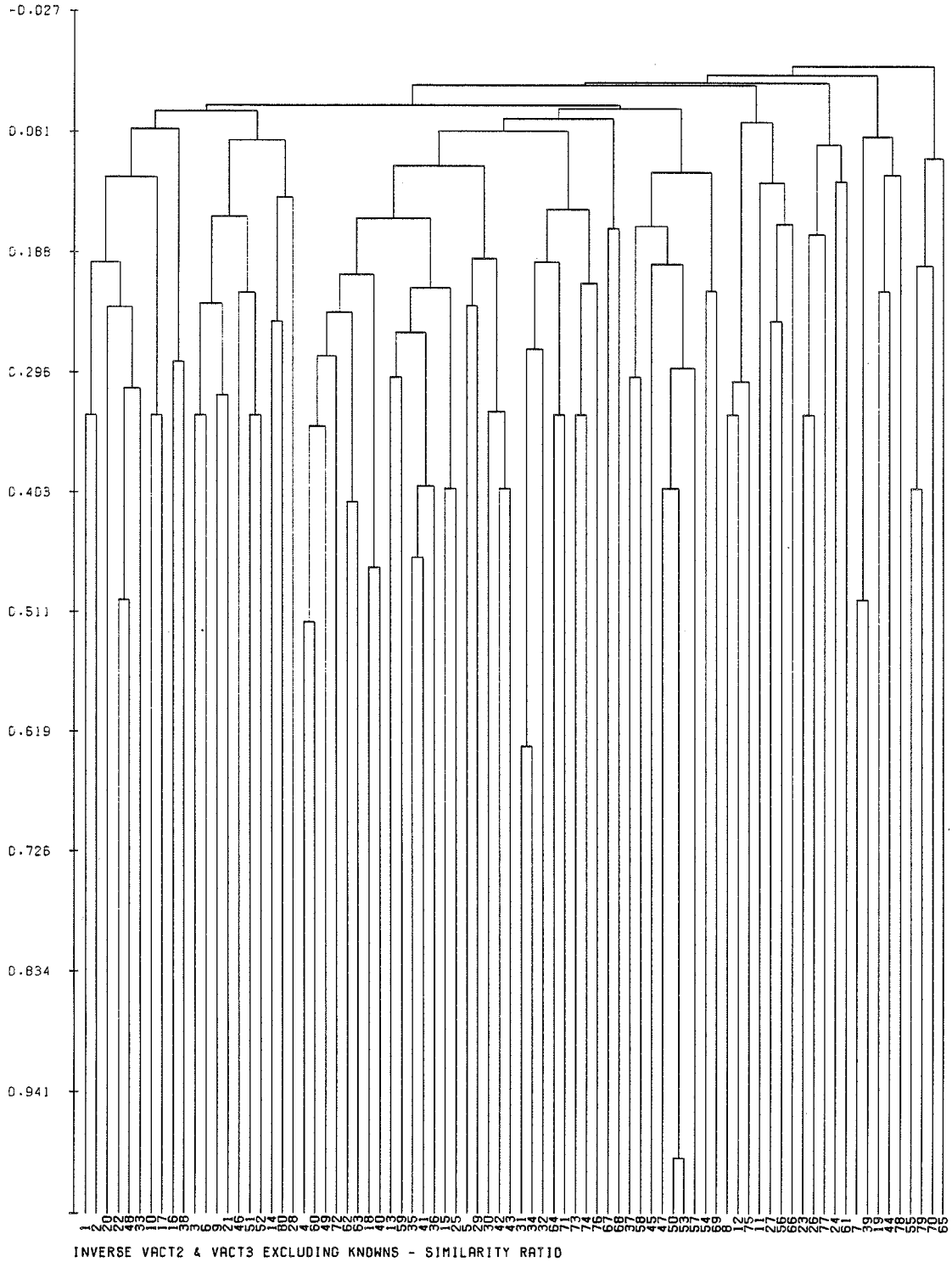
anomalies (11/11 and 9/11 respectively). Only two of the eleven cases were not concordant, showing this to be a much stronger association.

3.580 VACT (2 + 3) Minus Knowns.

Analyses Presented:

INVERSE	HIERARCHY
NORMAL	DIVIDE
NORMAL	HIERARCHY

As in the VACT 2 minus knowns group, the inverse Divide and Hierarchy are presented. Our aim was to see which, if any, of the malformation groupings that were found in this analysis were also seen in the VACT 3 minus knowns and the VACT 2 minus knowns group. Recognizable malformation groupings seen in the Inverse Hierarchy included a combined caudal regression and oligohydramnios spectrum (Group 3.), cloacal exstrophy (Group 4.) and Turner's syndrome (Group 11.). This last group again illustrates how patterns of malformations that we see in recognized syndromes are distinguished in the analysis from other possible anomaly combinations, even though there may be only one or two individuals in the population that may tentatively fit this diagnosis.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0.511
0.619
0.726
0.834
0.941
INVERSE VACT2 & VACT3 EXCLUDING KNOWNS - SIMILARITY RATIO

VACT (2 + 3) MINUS KNOWNS
INVERSE HIERARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE ANOMALIES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

Tracheo-esophageal fistula, hypoplastic larynx, ectopic gallbladder, Meckel's diverticulum, anomalies of the subclavian arteries, extra ribs, polycystic kidneys, ureteral atresia, urethral atresia, bifid scrotum.

GROUP 2.

Lung septation abnormalities, arrhinecephaly, hydronephrosis, malrotation of the bowel, VSD, pulmonary trunk anomalies, other migration defects, duplex collecting system, widely spaced sutures, abnormal thymus.

GROUP 3.

Pulmonary hypoplasia, low set ears, single umbilical artery, positional deformity feet, dysplastic ears, flat nose, imperforate anus, abnormal external genitalia, bilateral renal agenesis, prominent occiput, vaginal atresia, abnormal internal genitalia, bifid uterus, bladder agenesis, hypoplastic bowel.

GROUP 4.

Neural tube defect, scoliosis, skin tags, omphalocele, cloacal exstrophy.

GROUP 5.

Cervico-thoracic vertebral anomalies, absent ribs, lumbo-sacral vertebral anomalies, micropthalmia, abnormal radial ray, reduction deformity lower limbs, reduction deformity upper limbs, dislocated hips.

GROUP 6.

Cleft palate, micrognathia.

GROUP 7.

Bilateral cryptorchid, other abnormal vasculature, PFO/ASD, persistent left superior vena cava, mitral valve atresia, hypoplastic left ventricle, other aortic trunk anomalies, right ventricular hypertrophy, preaxial polydactyly.

GROUP 8.

Other renal anomalies, unilateral renal agenesis, abnormal palmar creases, horseshoe kidney, adrenal anomalies, overiding aorta, cleft lip and palate.

GROUP 9.

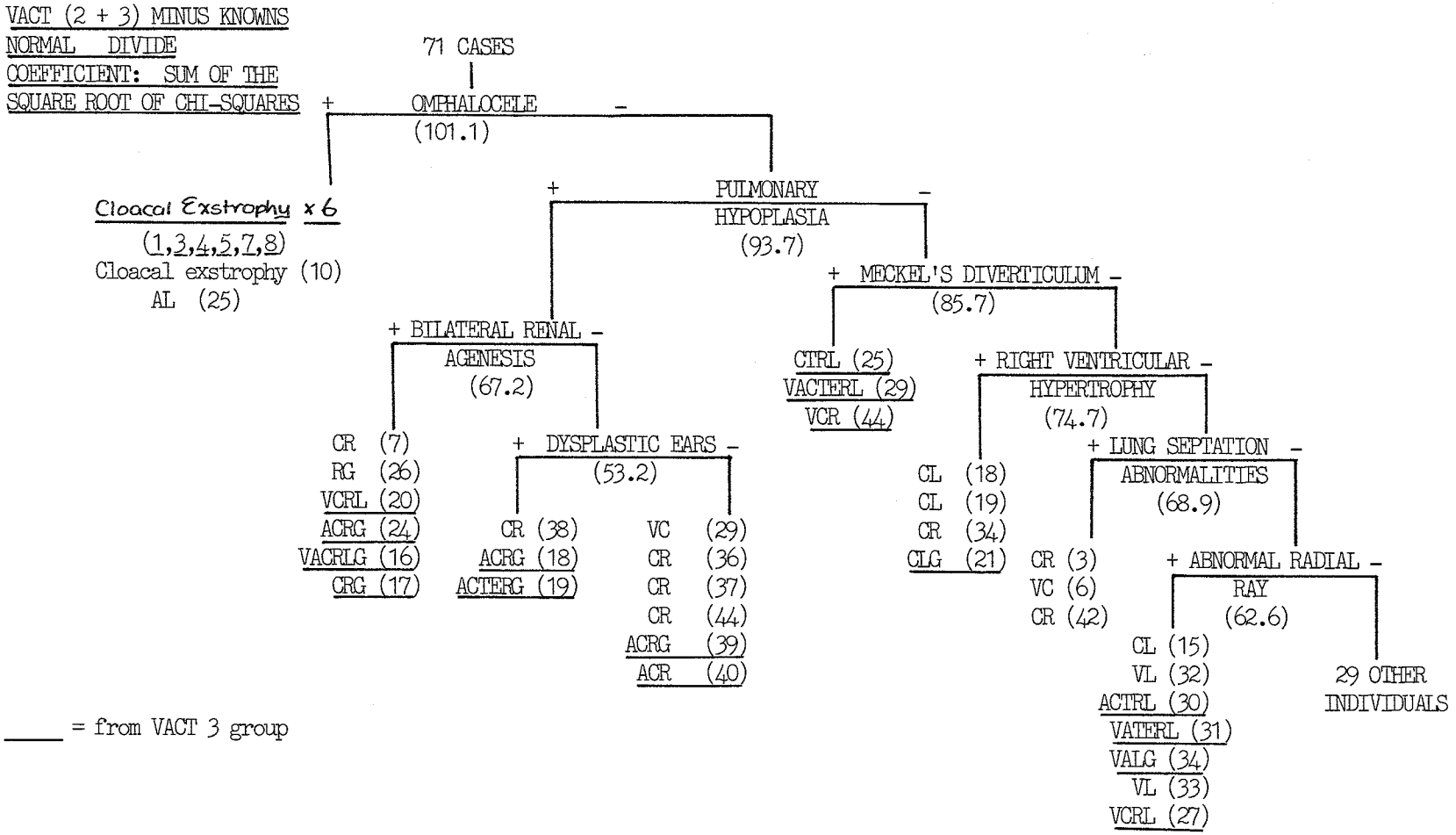
Duodenal stenosis, abnormal pancreas, membranous diaphragm, abnormal liver, preauricular tags.

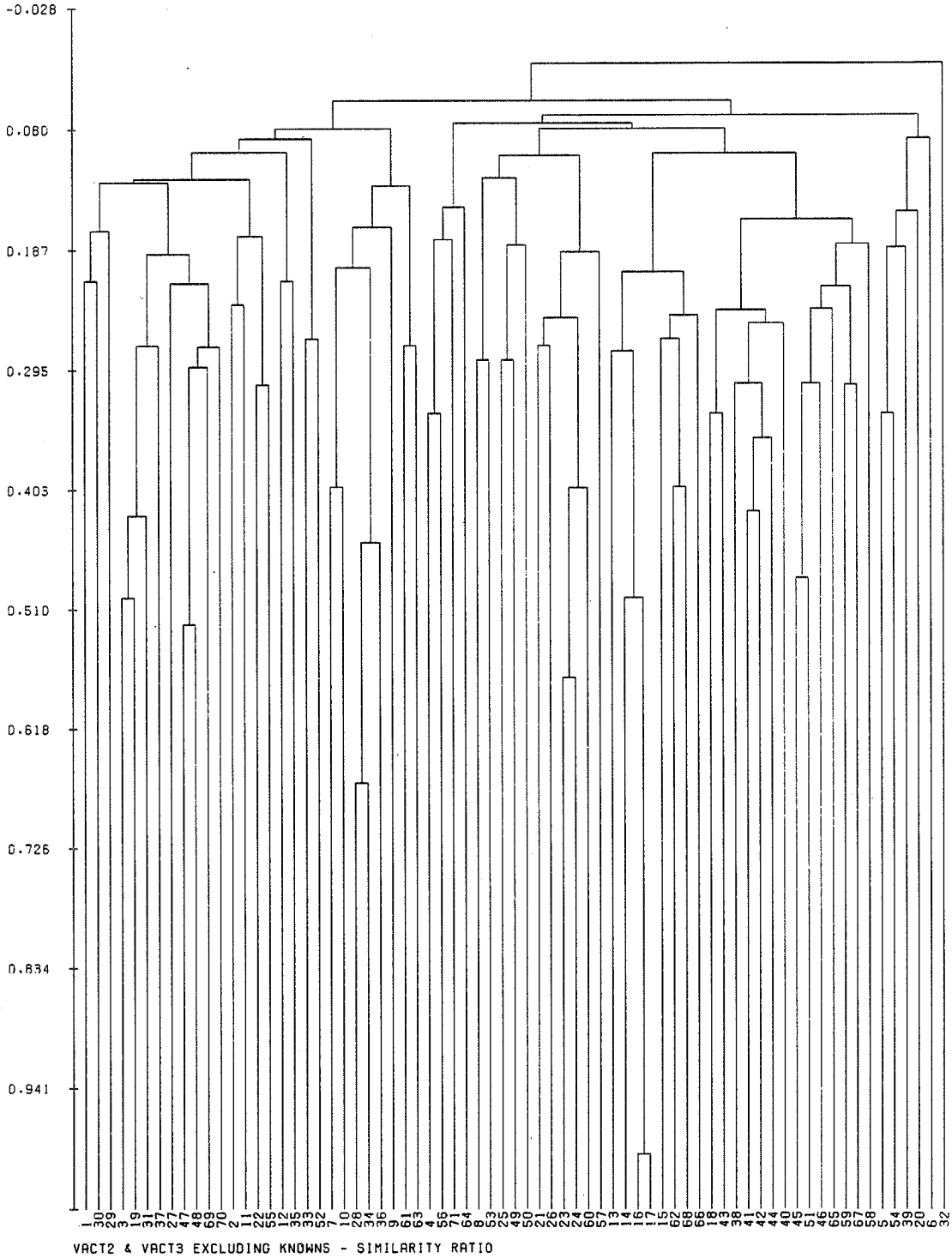
GROUP 10.

Microcephaly, hypospadias, ectopic anus, rectal fistula, L.S. dimple.

GROUP 11.

Coarctation of the aorta, webbed neck, postaxial polydactyly, hypertelorism.


VACT (2 + 3) MINUS KNOWNS
NORMAL DIVIDE
COEFFICIENT: SUM OF THE
SQUARE ROOT OF CHI-SQUARES
71 CASES
OMPHALOCELE
(101.1)
+
-
Cloacal Exstrophy x 6
(1,3,4,5,7,8)
Cloacal exstrophy (10)
AL (25)
PULMONARY
HYPOPLASIA
(93.7)
MECKEL'S DIVERTICULUM
(85.7)
BILATERAL RENAL
AGENESIS
(67.2)
CTRL (25)
VACTERL (29)
VCR (44)
RIGHT VENTRICULAR
HYPERTROPHY
(74.7)
CR (7)
RG (26)
VCRL (20)
ACRG (24)
VACRLG (16)
CRG (17)
DYSPLASTIC EARS
(53.2)
CL (18)
CL (19)
CR (34)
CLG (21)
LUNG SEPTATION
ABNORMALITIES
(68.9)
CR (38)
ACRG (18)
ACTERG (19)
VC (29)
CR (36)
CR (37)
CR (44)
ACRG (39)
ACR (40)
CR (3)
VC (6)
CR (42)
ABNORMAL RADIAL
RAY
(62.6)
CL (15)
VL (32)
ACTRL (30)
VATERL (31)
VALG (34)
VL (33)
VCRL (27)
29 OTHER
INDIVIDUALS
_____ = from VACT 3 group



VACT2 & VACT3 EXCLUDING KNOWNS - SIMILARITY RATIO

VACT(2 + 3) MINUS KNOWNS
NORMAL HEIRARCHY
GROUP AVERAGE (SIMILARITY RATIO/JACCARD)

(GROUPS ARE NUMBERED FROM THE LEFT SIDE OF THE PAGE TO THE RIGHT WITH THE CASES IN THE ORDER IN WHICH THEY APPEAR IN THE DENDOGRAM)

GROUP 1.

CR x 4 (1,30,29,3), RG (19), CR x 3 (31,27,37), ACRG (47), ACTERG (48), VCR (70)

GROUP 2.

VC (2), CL (11), VC (22), VACTERL (55)

GROUP 3.

CL (12), CR (35)

GROUP 4.

CR (33), CTRL (52)

GROUP 5.

CTE x 2 (7,10), CR x 3 (28,34,36), CT (9), VAC (61), ACL (63)

GROUP 6.

CL (4), ACTRL (56), CLG (71), ACL (64)

GROUP 7.

CL (8), VCRL (53), VL (25), VCRL (49), CLG (50).

** Numbers in brackets are identification numbers for the individuals. Numbers 1-37 indicate individuals from the VACT 2 group. Numbers 38-71 indicate identification numbers for the VACT 3 group. Cases are numbered consecutively with Known syndromes excluded i.e. #1 is individual #3 on the VACT 2 key.

GROUP 8.

VC (21), VL (26), VA x 2 (23, 24), VALG (60), VATERL (57)

GROUP 9.

AC x 2 (13,14), AR x 3 (16,17,15), VARG (62), ARG (68), ACR (66),

GROUP 10.

AL (18), ACLG (43), VACG (38), VACRLG (412), ACRLG (42), ACRG (44), ACG (40), VACRLG (45), ACRG (51), CRG (46), ACRG (65), ACTERG (59), ARG (67), VACRLG (58).

GROUP 11.

VC (5), VCT (54), ACRG (39), VC (20).

GROUP 12.

CR (6)

GROUP 13.

CR (32)

The key anomalies in the VACT (2+3) minus known analysis (as determined by the Normal Divide) also include many "classic" VACTERL anomalies such as bilateral renal agenesis, right ventricular hypertrophy, and other abnormal radial ray.

The Normal Hierarchy analysis showed very distinct anomaly groupings: eleven of twelve individuals in Group 1. had both cardiac and renal anomalies and in the majority of cases (9/12) these were the only systems involved. Almost half of the individuals in Group 5. had cardiac and tracheal anomalies. This represented by far the largest number of individuals with this combination found in any group (1/3 of all individuals with both cardiac and tracheal anomalies), and also accounted for all cases where only cardiac and tracheal anomalies were present.

Groups 6. and 7. both demonstrated very high incidences of cardiac and limb involvement (4/4 and 4/5 respectively). Interestingly, Group 6. had no cases with vertebral anomalies whereas in Group 7. three of the five individuals had a vertebral anomaly. Groups 8. and 11. also had strong vertebral involvement, particularly Group 8. where all individuals had a vertebral anomaly, and 4/6 demonstrated the vertebral/anal combination. Three of the

four individuals in Group 11. had vertebral anomalies but in each case this was accompanied by a cardiac anomaly, and never an anal malformation.

Individuals in Group 9. demonstrated a strong association between anal and renal anomalies, with six of the eight individuals having both. All individuals in this group had an anal anomaly. Group 10. had a high frequency of both anal (13/14), cardiac (11/14) and genital (13/14) anomalies. The anal/cardiac/renal combination was also present in half of the individuals in this group.

Although this analysis supported the cardiac/renal and anal/renal fields, it confirmed that the renal/limb combination (as suspected from the system combination data) or vertebral/limb combination (as seen from the VACT (2 + 3) plus known analyses) were relatively weak.

3.600 Further Definition of the VACTERL Association.

3.610 Demographic Data from the VACT 3 Minus Known Group.

Since our VACT 3 minus known group fulfilled the "standard" criteria necessary for an individual to be said to "have" the VACTERL association (a combination of three or more anomalies in any of the following systems; vertebral (not including spina bifida), anal, cardiovascular, tracheo-esophageal, renal and limb (excluding deformations)), the demographic data and frequency of anomalies found in this group were examined (see Figures 7. and 8.).

The mean maternal age did not differ from the Manitoba average, but a large proportion of the cases (61.2%) were first births. Contrary to expectation there was no male preponderance. Thirty percent of the VACT 3 minus known group were stillbirths and another forty percent were neonatal deaths. No infant deaths were recorded. This supports previous findings that, in spite of their multiple malformations, children with VACTERL who do not die shortly after birth have a good prognosis with aggressive therapy.

Figure 7. Characteristics of VACTERL Cases (Based on VACT 3 Minus Known Group (n=34)

Incidence	#	%
Males	18	52.9
Females	16	47.1
TOTAL	34	100.0

Maternal Age Specific Rates (Yrs)

	#	%
<20	5	14.7%
20-24	14	41.2%
25-20	10	29.4%
30-35	5	14.7%
35+	0	00.0%

Mean age 24.3 standard deviation 4.7

Birth Order Specific Rates

Birth Order	# of Cases	%
1	21	61.2%
2	7	20.1%
3	2	5.9%
4+	4	11.8%

Secular Trends

Year	# of Cases	Weighted Score *
1979	3	8.8%
1980	8	23.2%
1981	13	37.9%
1982	4	12.1%
1983	6	18.1%

* Score is proportionally adjusted to take into account yearly fluctuations in the number of total births.

Figure 7 cont.....

Birthweight

		#	%
Less than	2,500g	22	64.7%
Greater than	2,500g	12	35.3%

Gestational Age

		#	%
Less than (or equal to)	37 weeks	18	52.9%
Greater than	37 weeks	16	47.1%

Correlation between low birthweight and prematurity

Birthweight	Gestation	# cases	%
<2,500g	<37 weeks	15	44.1
<2,500g	>37 weeks	6	17.6
>2,500g	>37 weeks	11	32.4
>2,500g	<37 weeks	2	5.9

Mortality	#	%
Stillbirth	10	29.4%
Neo-natal death:		
survival <1 hour	3	
survival <1 day	6	
survival <1 week	5	
Total:	14	41.2%
Infant death	0	00.0%
Surviving	10	29.4%

Residence	#	%
Urban	21	61.8%
Rural	13	38.2%

Figure 8. Birth Defects Found in >10% of VACTERL Cases (Based on VACT 3 Minus Known Group, N=34)

Anomalies of the Musculoskeletal System

	%	(# Cases)
Vertebral	41.2	(14)
LSV	17.6	(6)
CTV	26.5	(9)
Limb anomalies	47.1	(16)
Polydactyly	20.6	(7)
Reduction-- upper limb	20.6	(7)
Reduction-- lower limb	23.5	(8)
Reduction-- Total	38.2	(13)
Abnormal Radial Ray (total)	32.4	(11)
Absent ribs	23.5	(8)
Scoliosis	29.4	(10)
Neural Tube Defect	17.6	(6)
Omphalocele	14.7	(5)
Dislocated hips	14.7	(5)
Positional deformity feet	23.5	(8)

Anomalies of the Genitourinary System

	%	(# Cases)
Anal Atresia	67.6	(23)
Renal Anomalies	70.6	(24)
Unilateral Renal Agenesis	11.8	(4)
Bilateral Renal Agenesis	17.6	(6)
Renal Agenesis Total	29.4	(10)
Hydronephrosis	23.5	(8)
Hypoplastic bowel	17.6	(6)
Bladder Exstrophy	17.6	(6)
Rectal Fistula	11.8	(4)

Figure 8. continued....

	%	(# Cases)
Abnormal Genitalia --External	52.9	(18/34)
Abnormal Genitalia --Internal	32.4	(11/34)
Vaginal atresia	37.5	(6/16)
Uterus didelphus	31.3	(5/16)
Cryptorchid bilateral	27.8	(5/18)
Bifid scrotum	27.8	(5/18)

Anomalies of the Cardiovascular System

	%	(# Cases)
Cardiovascular total:	85.3	(29)
VSD	23.5	(8)
ASD	26.5	(9)
SUA	50.0	(17)

Anomalies of the Alimentary System

	%	(# Cases)
Meckel's Diverticulum	11.8	(4)
Bowel malrotation	26.5	(9)

Anomalies of the Respiratory System

	%	(# Cases)
TEF	14.7	(5)
Tracheal Anomalies	11.8	(4)
Pulmonary hypoplasia	35.3	(12)
Lung septation abnormalities	11.8	(4)

Figure 8. continued....

Craniofacial Anomalies

	%	(# Cases)
Low set ears	26.5	(9)
Dysplastic ears	29.4	(10)
Flat nose	17.6	(6)
Cleft palate	14.7	(5)

Interestingly, the frequency of tracheo-esophageal fistula in members of this group was relatively low (15%). This was notable since TEF had been considered one of the most important ascertainment anomalies in many studies. By contrast, genital anomalies which were not regarded as a particularly important part of the spectrum were seen in 53% of the cases. The highest frequency of a single anomaly was that for imperforate anus, which occured in 67% of the group. Although renal anomalies occured in 70% of the population, they were very heterogenous: the most common anomaly, hydronephrosis, was seen in only 24% of cases.

Radial ray anomalies were seen in 69% (11/16) of the individuals that had a limb anomaly. Other non-VACTERL anomalies that were found at a relatively high frequency in this population were pulmonary hypoplasia (35%), and cleft palate (15%).

3.620 Generation of a Taxonomic Key for the VACT 3 Minus Known Group.

A taxonomic key was constructed for this group based on the most highly conserved clusters of individuals determined from the Normal Divide and Hierarchy analyses (Figure 9). The resulting key stresses the constant nature of the groups that were repeatedly obtained in the different analyses, since this reflects a high degree of intrinsic structure. The frequency of system involvement in the groups distinguished by the key can be found in Table 13 and a more specific description of the types of defects in Tables 14 and 15. The demographic data for each group is listed in Table 16.

The taxonomic key for the VACT 3 minus knowns group recognised five groups within this population. The population was initially divided on the basis of omphalocele and/or cloacal exstrophy. There were seven individuals in Group A. This group had a low frequency of vertebral anomalies (2/6) and the two that were observed were extra ribs in the one instance and hypoplastic innominate in the other. Other non-VACTERL anomalies of the vertebral column were also present at a relatively high frequency: 3/6 individuals had a lumbo-sacral meningocele, and one other case demonstrated a widening of the lumbo-

FIGURE 9. VACT 3 MINUS KNOWNS KEY

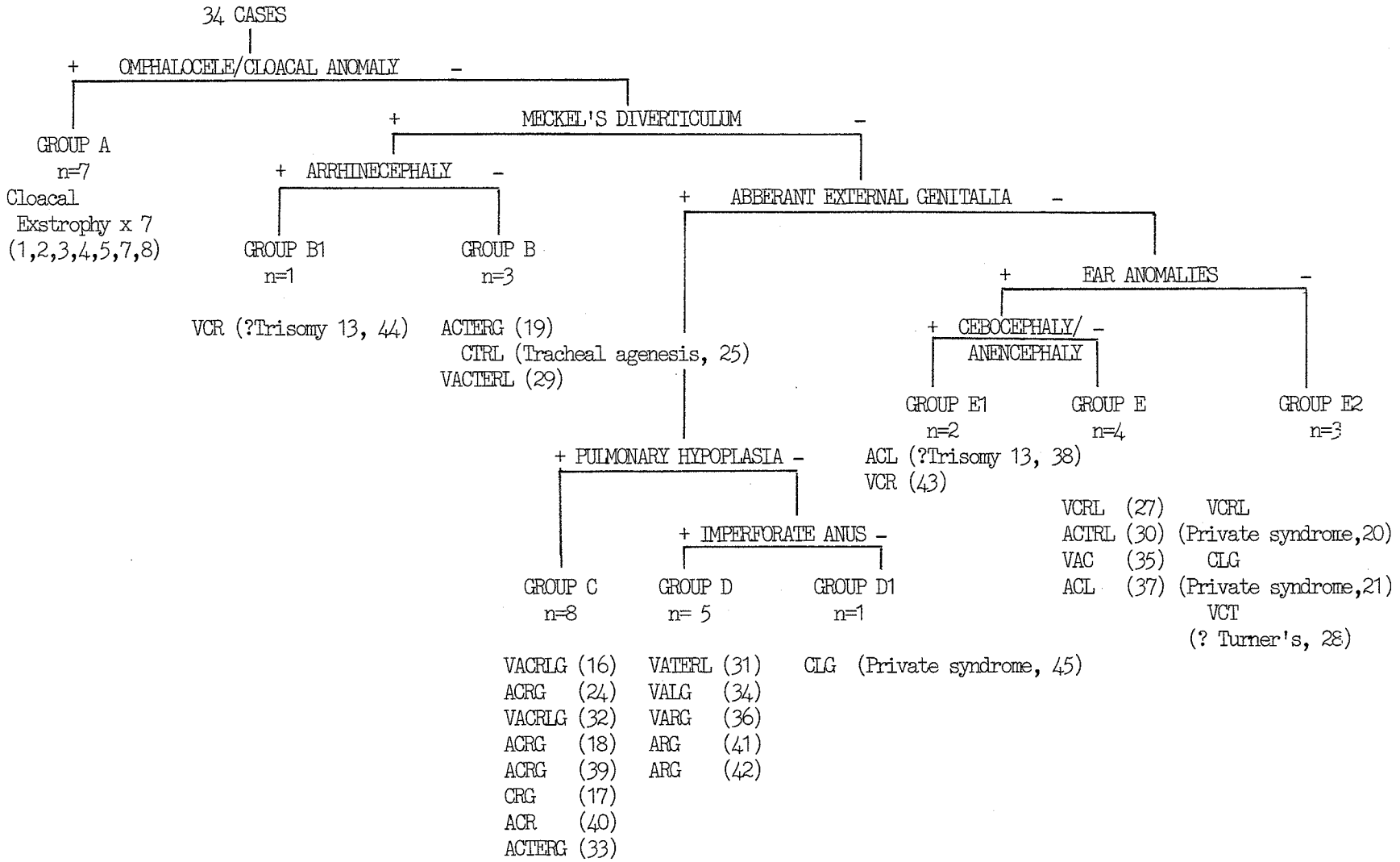


Table 13. The Frequency of System Involvement in the Five Main Groups Determined by the VACT 3 Minus Known Taxonomic Key.

GROUP	#	V	A	C	TE	R	L	G
A (cloacal exstrophy)	7	0.3	1.0	1.0	0.0	0.6	0.4	0.9
B (Tracheal agenesis variant)	3	0.3	0.3	1.0	1.0	1.0	0.7	0.3
C (Caudal regression)	8	0.3	0.9	1.0	0.1	0.8	0.3	1.0
D (VACTERL A)	5	0.6	1.0	0.0	0.2	0.8	0.4	0.8
E (VACTERL B)	4	0.5	0.8	1.0	0.0	0.5	0.8	0.0

Table 14. Types of Defects Seen in the Groups Generated by the VACT 3 Minus Known Taxonomic Key.

GROUP	#	V	A	C	TE	R	L	G
A	7	Lumbo-sacral vertebral x 2	Imperforate anus x 7	Single umbilical artery x 6**	———	Unilateral renal agenesis x 3 Horseshoe kidney x 1	Reduction Deformity Limb x 3	Abnormal External Genitalia x 7
B	3	Cervico-thoracic vertebral/extra ribs x 1	Imperforate anus x 2	Complex congenital heart anomalies x 3 Single umbilical artery x 3	TEF x 1 Tracheal agenesis/ broncho-esophageal fistula x 1 Broncho-esophageal fistula/hypoplastic distal trachea x 1	Hydronephrosis x 2 Cystic/dysplastic x 1	Abnormal radial ray x 2	Small phallus x 1
C	8	Absent/hypoplastic ribs x 2 LSV/CTV x 1	Imperforate anus x 6 Rectal stenosis x 1	Single umbilical artery x 6 Hypoplastic heart x 2	TEF x 1	Bilateral renal agenesis x 4 Polycystic kidneys x 2 Horseshoe kidney x 1 Hydronephrosis x 1	Fused lower limbs x 2	Abnormal external genitalia x 8
D	5	LSV X 3 CTV x 2 (rib x 2)	Imperforate anus x 5 recto-vaginal fistula x 4	———	TEF x 1	Horseshoe kidney x 2 Hydronephrosis x 2	Abnormal radial ray x 2	Abnormal external genitalia x 4
E	4	CTV x 2 (rib x 1)	Imperforate anus x 1 Ectopic anus x 2	VSD x 3 SUA x 1 ASD x 1	———	Unilateral renal agenesis x 2	Extra/missing thumbs x 3	———

Table 15. Non-VACTERL Anomalies Seen in VACTERL B.

Anomaly	# of cases	VACT 3 case Number
Microtia	2	30, 37
Dysplastic auricles	2	27, 37
low set ears	1	35
Hearing loss	2	30, 37
Pre-auricular tags	2	30, 37
Epibulbar dermoid	1	30
Microstomia	1	27
Micrognathia	1	35
Micropthalmia	2	27, 30
Branchial cleft	2	27, 30
Cleft palate	2	27, 35

Table 16. Demographic Data for Groups Generated using the Taxonomic Key.

	GROUP				
	A	B	C	D	E
Number in group	7	3	8	5	4
SEX RATIO (M:F)	3:4	3:0	3:5	3:2	1:3
% BTWT <2,500g	85.7	100.0	75.0	20.0	25.0
% GEST'N <37 wks	42.9	100.0	62.5	00.0	75.0
% S.G.A.* (<10%)	57.1	00.0	62.5	20.0	25.0
% FIRST BIRTH	50.0	66.7	62.5	40.0	75.0
% > 4TH BIRTH	14.3	33.3	00.0	20.0	00.0
Mean Birthweight(g)	2004	1763	1861	3318	2864
Standard Deviation	530	405	v big	v big	719
Mean Gestation	37.0	31.3	35.1	39.0	36.5
Standard Deviation	3.4	5.7	5.6	1.6	2.5
Mean Maternal Age	24.0	26.0	25.4	24.0	20.0
Standard Deviation	5.7	7.0	4.6	4.0	4.5
% STILLBIRTHS	28.6	33.3	37.5	00.0	00.0
% PERI MORTALITY	71.4	68.7	62.5	00.0	00.0
% SURVIVING	00.0	00.0	00.0	100.0	100.0
TWINS	1	1	0	0	0

* Small for gestational age (less than the 25th percentile)

NOTE:
Group B: 2/3 pregnancies complicated by polyhydramnios.
1/3 pregnancies complicated by oligohydramnios.
Group C: 4/8 pregnancies complicated by oligohydramnios.
1/8 complicated by bleeding and placenta previa.
3/8 oligohydramnios probably present: bilateral renal agenesis + flat nose, low set ears.

sacral spinal column.

None of the individuals had a tracheo-esophageal fistula and the commonest cardiac anomaly was single umbilical artery, which was reported in 5 of the six cases. In the sixth case it was not commented upon as being either present or absent. Renal anomalies were present in three of the six cases and in each case the anomaly was unilateral renal agenesis. Four of the cases had limb anomalies which were in all instances, reduction deformities. In all but one case an omphalocele was present, and every member had an imperforate anus and abnormal external genitalia. Other common non-VACTERL malformations included cloacal anomalies (5/6), club foot (4/6) pulmonary hypoplasia (3/6), scoliosis (3/6), and bowel malrotation (3/6).

Four individuals formed group B. Of these, one (#44) did not, on the basis of anomaly patterns, appear to belong with the other three. Clinically, this individual had the phenotype associated with Trisomy 13 but chromosome confirmation was not obtained. The other individuals in this group all had tracheal anomalies of some sort and were thought to represent variants of the tracheal agenesis association. The tracheal anomaly varied, in one instance it was a tracheo-esophageal fistula, in another tracheal

agenesis with a broncho-esophageal fistula and in the third a broncho-esophageal fistula and hypoplasia of the distal trachea.

Two of the three individuals had imperforate anus, and all three had renal anomalies (2 with hydronephrosis and 1 with a multicystic, dysplastic kidney), complex cardiac anomalies and single umbilical artery. Both cases with limb involvement had radial ray anomalies. Neither vertebral anomalies nor genital anomalies occured frequently in this group. The only other common anomalies to be seen with this group were Meckel's Diverticulum (3/3), bowel malrotation (2/3) and other gut anomalies including agenesis of the gallbladder and duodenal stenosis.

Group C. consists of eight individuals that represent the caudal regression spectrum. Included in this group were two cases of sirenomelia and four with bilateral renal agenesis including one of the sirenomelic individuals. Vertebral anomalies were present in only two of the eight cases. Seven of the eight had imperforate anus and all eight had a cardiac anomaly. In most instances this was single umbilical artery (5/8), but two cases of cardiac hypoplasia were also documented. Renal anomalies were

present in every individual and each individual had some abnormality of the external genitalia. Only the two sirenomelic individuals had limb anomalies. Also of note was the very low occurence of tracheal anomalies with only one case of tracheo-esophageal fistula. The only other common non-VACTERL anomaly was pulmonary hypoplasia which occured all eight individuals.

Group D. initially consisted of six individuals, however, when the specific anomalies were examined, one (#45) did not appear to fit even though he had the correct key anomalies. He was placed into a separate group, using imperforate anus as the key anomaly for the division. This individual was one of the three in the VACT 3 group that was felt to have a private syndrome.

In the taxonomic key the "key" anomalies chosen are to some extent arbitrary. They reflect an anomaly that is present in all members of that group and not in other individuals. The emphasis is on conserving the individual blocks that remained constant in the analyses.

Among the five cases that formed Group D. there was a relatively high frequency of cervico-thoracic and lumbo-sacral vertebral anomalies (3/5) and all members of this

group had an imperforate anus. The frequency of renal (4/5) and genital (4/5) malformations was also high. Of note, there were no cardiac anomalies in this group, only one tracheo-esophageal fistula, and no non-VACTERL anomalies present. Two individuals had limb involvement and in both cases this was due to a radial ray anomaly. This group was felt to represent a VACTERL subgroup, and will subsequently be refered to as VACTERL A.

Group E. was divided into three subgroups. E1 contained two individuals, one of whom clinically appeared to have Trisomy 13 and the other who had anencephaly and multiple other anomalies including hydronephrosis, absent ribs and a cleft palate. E2 consisted of three individuals, two of whom were felt to have "private" syndromes which have yet to be fully delineated, and the third appeared to have Turner syndrome, but unfortunately chromosome confirmation was not available.

The remaining four individuals formed our second VACTERL group, VACTERL B. This group had a higher frequency of cervico-thoracic vertebral anomalies (2/4) than did VACTERL A, and only one individual had imperforate anus but two others had anal ectopia.

Unlike VACTERL A there were no tracheal-esophageal fistulae or genital anomalies present and all members had a cardiac anomaly (in VACTERL A no cardiac anomalies were present). As in VACTERL A, when limb involvement was present (3/4), it was a radial ray anomaly. There were several interesting non-VACTERL anomalies present: microtia or dysplastic ears were present in three of the four cases (with the fourth having low set ears); a branchial cleft was documented in two of the four cases, and in one of those an epibulbar dermoid was also present. It would appear that VACTERL A represents what is classically considered to be VACTERL, with a high frequency of vertebral, anal, renal, and genital anomalies and no non-VACTERL present, and that VACTERL B is a VACTERL variant that clearly shows overlap with the facio-auriculo-vertebral spectrum.

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4.000 Discussion

4.110 Ascertainment of Individuals.

As was stated in the introduction, for meaningful results using numerical taxonomy techniques, unbiased data must be used. Biases can be introduced through incomplete ascertainment of either individuals or of anomalies. Either could result in a skewed perception of the association since the results would be based on an incomplete "picture" of the anomalies involved.

The ideal method of collecting data on both the incidence and type of malformations and the number of individuals with malformations would be to examine all newborns over a specified time period and follow them over a number of years. Although this is a retrospective study we believe that the ascertainment of cases is essentially complete: any individual who was not on the registry or who was initially missed and had multiple malformations is very likely to have been referred to the Children's Hospital (the largest of the two hospitals in the province equiped to deal with such cases) or to have come to the attention of the Clinical Genetics Section (the only such facility in the province). Finally, many infants with

anomalies that are born (both in Winnipeg and in rural Manitoba) with anomalies and die in the neonatal period are sent for autopsy to the Pathology Department of the Children's Hospital.

4.200 Ascertainment of Anomalies.

The main disadvantage of many retrospective studies are that they only give complete ascertainment for those malformations that are visible at birth or that have clinical consequences in the newborn period (and are correctly recorded). Even following post mortem not all anomalies present may necessarily be documented; such minor malformations as low set ears and abnormal palmar creases may not be commented upon (this depends upon the thoroughness of the pathologist who performs the procedure). The importance of accurate and detailed anomaly assessment for this type of work cannot be overstated. It is especially evident in the numerical taxonomy when apparently minor anomalies figure prominently in the formation of clusters.

In the present study there is at least a three year "grace period" for the documentation of anomalies not initially recognised: any hospital discharge diagnoses

documenting malformations pertaining to these individuals during the first year of life are passed on to the Congenital Anomalies Registry. Also, current hospital charts and autopsy reports were reviewed when available.

4.210 Incidence of Anomalies.

Figure 10. compares our incidence figures for some of the index anomalies in our study to others reported in the literature. This is certainly not an exhaustive list since it was difficult to find comparable data and so only commonly reported anomalies which are likely to be picked up in the newborn period were used.

The figures from this study tend to be slightly higher. There are many factors which may influence this including short term changes in the incidence of malformations (Leck and Millar, 1963), geographic and racial differences, case finding methods or surveillance level, and inclusion criteria (Newman, 1985). It is difficult to assess the relative contributions of each to the differences seen, but the different definitions of the study population between the various studies is felt to be a major factor: few studies actually report the birth prevalence (a figure per x total births), but instead

Figure 10. Comparison of incidence figures obtained in the present study to those reported in other studies.

Anomaly	Incidence in present study (/10,000 total births)	Incidence reported in other studies
TEF	2.86	1.1/10,000 total births range: 4.9-0.4/10,000 (Stevenson et al., 1966) 1.8-3.8/10,000 Livebirths (Leck & Millar, 1963)
Imperforate Anus	5.5	2/10,000 live births (Minor, 1967) 2.8-3.1/10,000 total births* (Leck & Millar, 1963) * excludes sirenomelia 1.7/10,000 total births Range: 0.7-7.6/10,000 (Stevenson et al., 1966)
VSD	30.4	20-35/10,000 total births (Czeizel & Meszaros 1979) 21.7/10,000 total births (Heinonen, 1976)
ASD	11.8	10.0/10,000 births (Fraser & Hunter,1979) 7.3/10,000 total births (Heinonen, 1976)
RENAL AGENESIS (uni and bil)	4.9	1.6-3.4/10,000 total births @ (Leck & Millar,1963) 2.5-3.3/10,000 total births @ (Potter, 1965) @ only bilateral agenesis
SUA	4.8	45/10,000 total births (Aushuler & Reginald, 1975) 72/10,000 live births (Bryan & Kohler,1974)

record the number of cases seen per x livebirths. This would result in lower figures than those obtained in our study since all stillbirths with multiple malformations would be excluded.

Rates for anomalies such as TEF, VSD, and ASD are similar to those reported in the literature and the figure for renal agenesis does not appear to be inappropriate. However, it does include both unilateral and bilateral renal agenesis and dysgenesis (the ICDM code for renal agenesis does not distinguish between them) and unilateral renal agenesis is often not discovered until later in life. Though the incidence of both unilateral and bilateral renal agenesis is known for the VACT 3 and the VACT 2 groups, the majority of cases of unilateral renal agenesis were not on the registry and came to light at autopsy or as a result of the patient being investigated for other anomalies. Thus, it would not be unreasonable to assume that unilateral renal agenesis may well be underreported in the VACT 1 group.

Single umbilical artery is very obviously underrepresented in our population with an incidence of 4.6/10,000 total births compared to an estimated incidence of about 0.72% of total births (Bryan and Kohler, 1974).

However, it is often not considered a major malformation and usually does not come to attention unless other more serious malformations are present. Comparing our incidence figures to those obtained in studies looking at a specific anomaly(s) obviously introduces a bias because of the difference in surveillance level between a population based reporting of anomalies and an in depth study of a particular anomaly.

The present study reports a higher incidence of imperforate anus (5.4/10,000 total births) than expected from the literature. Aside from previously documented factors, this discrepancy is in part due to the description of the category under which it is classified in the ICDM system: "atresia and stenosis of the large intestine, rectum and anal canal". This is a broad definition including ectopic anus, anal stenosis and other anal anomalies such as multiple intestinal atresias as well as imperforate anus. Though the cases of imperforate anus in the VACT 3 and VACT 2 groups are well documented this is not necessarily so for the VACT 1 group.

In 1983 Khoury et al. carried out a population based study of the VACTERL association on data from the Metropolitan Atlanta Congenital Defects Program. They

looked at the interrelationships between the six main systems that they considered to be components of the VACTERL association; vertebral defects, anal atresia, TEF, renal anomalies, cardiovascular anomalies and limb anomalies (except for such minor anomalies as club foot, or congenital hip dislocation if these were the only limb anomalies present).

In comparison to their data, our incidence of imperforate anus was still high (68% versus 40%) and fewer cases of tracheo-esophageal fistula (15% versus 24%) were seen. It appeared that our figures for total limb and renal anomalies were also lower. However, upon looking at the distribution and rate of particular anomalies it became evident that the renal and limb figures agreed very closely except for an apparently lower incidence of polycystic kidneys in our population and a potentially lower number of individuals with radial ray anomalies.

4.300 System Involvement in the VACT 2 and VACT 3 Groups.

All of the combinations of three or more systems were significant (although some that might have been expected to be seen were not present in our population). This finding was not surprising since the chance of seeing any

particular combination by chance decreases as the number of systems involved increases.

The distribution of tracheal anomalies (including tracheo-esophageal fistula) in our VACT 2 and VACT 3 populations was somewhat unexpected: tracheal anomalies were not seen as part of a two system combination except in three cases where the second system of involvement was cardiac. The CT combination was expected to be one of the three most commonly observed two system groupings, as were the CTL and ACTL combinations in the three and four system groupings, which suggests a global underepresentation of tracheal anomalies with any combination of VACTERL systems. This in itself suggests that tracheo-esophageal fistula is not an ideal anomaly for the ascertainment of individuals "with" VACTERL.

As was previously commented upon, the acro-renal field was not found to be present unless other systems were also involved. However, in this population the field is very weak even when other anomalies are present as evidenced by the VACT (2 + 3) minus knowns Normal Hierarchy analysis which failed to show a distinct clustering of individuals with renal and limb malformantions in addition to other anomalies.

4.400 Choice of Ascertainment or "Key" anomaly.

As can be seen from Table 11., the choice of ascertainment anomaly can greatly influence the perception of the degree of involvement of anomalies or systems in an association and so will also greatly influence the designated phenotypic spectrum of that association. For example, if the key anomaly in our definition of VACTERL was tracheo-esophageal fistula, 50% of our "VACTERL" cases would have had a vertebral anomaly (see appendix 3), and 100% would have had a renal anomaly. Conversely, if anal malformations were designated to be the key anomalies then we would see only 36% of cases with associated vertebral malformations, and 72% with renal anomalies.

This type of ascertainment bias was present in the cases presented by Say and Gerald (1968) and Say et al. (1971). Their preliminary report of a polydactyly/imperforate anus/vertebral anomalies "syndrome" was based on patients ascertained through imperforate anus and polydactyly. A later paper presented an expanded version of the initial "syndrome". It included poly-oligodactyly and skeletal (mainly vertebral) anomalies and was based on nine patients that had been ascertained through imperforate anus and "hand" malformations and was accompanied by a review of "similar" cases in the

literature. It was not documented how the "similar" cases were ascertained, but other malformations that are now considered to belong to the VACTERL spectrum were also seen, but were not recognized as such because of their low frequency (i.e., TEF 3/27, agenesis of kidney 2/27, congenital heart anomalies 1 /27 with three other individuals noted as probably having malformations of the heart).

Quan and Smith noted the occurence of an even broader association of five defects (Vertebral and Anal defects, TEF, Radial and Renal dysplasia). However, in their study, rather than chosing a key anomaly(s), they considered any individual with three or more of the five defects, allowing the extent of the spectrum to be better seen. Since then the scope of VACTERL has become rather large, overlaping with many other syndromes and associations.

The number of systems that must be involved for an individual to be considered to "have" VACTERL will also influence the incidence of the anomalies seen. When Temtamy and Miller (1974) looked at VACTERL using tracheo-esophageal fistula as the index anomaly they found a much higher frequency of cardiac defects and radial ray dysplasias among individuals with three or more VACTERL

defects as compared to those individuals with only tracheo-esophageal fistula and one other anomaly (70% and 30% for the former and 40% versus 0% for the latter).

An effort to more narrowly and precisely define the VACTERL association was undertaken by Cziezel and Ludanyi (1983, 1985). In their most recent paper (1985), they recognised two groups: "true-VACTERL" and "VACTERL-like". VACTERL cases were defined as concurrences of three or more of the following six well-defined congenital anomalies: V = vertebral anomalies (absence of vertebrae, fusion of spine, supernummary vertebrae, excluding spina bifida cystica). A = anal atresia with or without fistula. C = cardiac anomalies not including anomalies of the great vessels. TE = tracheo-esophageal fistula with or without esophageal atresia. R = renal agenesis, dysplasia, hypoplasia and some other specified anomalies. L = radial-type reduction defects or polydactyly. A major feature of this category was that other major non-VACTERL anomalies must not be present.

The VACTERL-like group involved the occurence of three or more closely or broadly defined VACTERL-type anomalies with other major non-VACTERL anomalies present. This group was felt to encompass several multiple congenital anomaly associations or syndromes and thus have

a more heterogenous origin than the VACTERL group.

These groupings were felt to be of limited value when deciding how to approach our population since the specific anomalies which must be present(or absent) for any individual to gain membership to a particular group were arbitrarily chosen. Thus the differences that they reveal may be artificial.

4.500 Numerical Taxonomy.

4.510 Hierarchy and Divide.

Intuitively, both the Hierarchy and Divide option should produce roughly the same clusters since they are both dealing with the same population. However, there are as has been mentioned, some very basic differences that affect the interpretation of various aspects of each analysis.

As the Divide option produces a key and all the individuals are in a particular group by virtue of their possessing the key character, it is particularly vunerable to misclassifying individuals who obviously belong on the basis of overall phenotype but who lack this anomaly.

Malformation groupings produced by this analysis also tend to be more subjective than those produced by Hierarchy. However, Divide is useful in producing tighter anomaly/individual clusters, and the Key anomalies/individuals that are identified indicate where the greatest variability resides. The key individuals/anomalies determined by Divide are essential for the nodal analysis, which not only produces groups that

have been doubly defined, but also overcomes a limitation present in both Hierarchy and Divide, and allows an anomaly to be included in more than one group. In this way other strong anomaly associations can be seen and not just the strongest grouping.

4.520 The role of Key anomalies/individuals.

The key anomalies and individuals produced by the Divide analysis allow us to see which factors are given the greatest weight in the Divide analysis. Beyond that these key anomalies/individuals do not have an intrinsic importance. What is important is the classification that is produced and not the mechanism. It is for this reason that the final summary of the data for the VACT 3 minus knowns was presented in the form of a taxonomic key which was based on the groups that were conserved in the Hierarchy and Divide analyses. As has been previously stated, if there is a high degreee of intrinsic structure to the population under study the clusters that are produced by various methods will be similar. This has indeed been demonstrated in both the VACT 3 minus known and the VACT 3 plus known analyses.

The key anomalies produced generally fall into two categories; those that are produced by a positive association, and those produced by virtue of a negative association. Examples of these include omphalocele, flat nose, and membranous diaphragm. Omphalocele generated a very strong positive association because it was so common among individuals with cloacal exstrophy. Flat nose was again a very common anomaly among individuals with oligohydramnios but it was not the malformation primarily responsible for the problem but was instead secondary to it. Membranous diaphragm was a relatively rare malformation and by virtue of this rarity, and the associated strong negative association that it generated, became a key anomaly.

4.530 How effective is Numerical Taxonomy in Association Analysis?

Both Hierarchy and Divide analyses are reasonably good at separating out individuals with known syndromes, as long as the anomaly documentation is adequate, the concern was that faced with a more heterogeneous group the results would be equivocal. However, seventy four percent of the VACT 3 minus known group were found to be conserved in four groups, with only 9 individuals placed in different

clusters by the different analyses.

When the original sheets on the nine individuals who were not consistently classified were studied, it became apparent that three of the cases in this group were felt to represent private syndromes, and another three fit the phenotype of a known syndrome (but cytogenetic confirmation was not possible): two were thought to have Trisomy 13 and the other one Turner's syndrome. (As we had wished to get as comprehensive a picture as possible of VACTERL only the confirmed cases were removed as known syndromes).

It was reassuring that the analyses recognised the separate identity of these individuals from other members of the VACT 3 minus known group. Thus only three of the 34 cases (8.8%) with multiple congenital abnormalities and an unknown etiology were not regularly placed into the same group by the different analyses (cases 40,43, and 33).

4.540 The VACTERL A and VACTERL B Subgroups.

The two VACTERL subgroups that were generated were clearly different not only from the other three groups that were distinguished but also from each other: VACTERL A had much more severe caudal involvement, with all members of the group having imperforate anus, four of the five having a major genital anomaly (2 x bifid scrotum, 1 x hypospadias, 1 x septated uterus & fused labia minora) and most having much more complex vertebral anomalies than seen in the VACTERL B group. No cardiac anomalies were seen in VACTERL A and the renal anomalies tended to be dysplastic (horseshoe kidney and hydronephrosis), rather than the renal agenesis that was seen in VACTERL B. The limb anomalies in both groups always involved the radial ray, however they differed between the two groups with positional anomalies seen in VACTERL A (bilateral proximal implantation of thumbs, unilateral trigger thumb), and duplication/deficiency malformations in VACTERL B (absent thumbs, thumb hypoplasia, and preaxial polydactyly).

VACTERL B had a much stronger craniofacial component, with many anomalies overlapping with the facio-auriculo-vertebral spectrum. Vertebral anomalies were much less complex, and in both cases found in the cervico -thoracic

region; anal anomalies were milder, with only one case of imperforate anus and two instances where the anus was anteroposed. Cardiac anomalies were found in all individuals and were primarily VSD (x 3), although single umbilical artery and ASD were seen. No tracheal or genital anomalies were noted but a large number of additional craniofacial malformations, such as microtia/dysplastic ears, branchial cleft, and micropthalmia were present.

These types of branchial arch malformations have been noted to occur at a much higher frequency in diabetic mothers (Johnson and Fineman, 1982), and though family history is scanty in both VACTERL groups, one of the cases in VACTERL B was known to be the infant of a diabetic mother.

The two groups also differed in birthweight and length of gestation, with 20% of the VACTERL A group being small for gestational age, but none premature (mean gestation: 39 weeks) and 25% of the VACTERL B group being small for gestational age but 75% were premature (mean gestation 36.5 weeks). Given the 100% mortality present in the other two groups, and the good prognosis reported in VACTERL subsequent to aggressive therapy, it was gratifying to see 100% survival in both VACTERL groups. The mean

maternal age was slightly higher for the VACTERL A group (24 years +/-4) than for the VACTERL B group (20+/-4.5) and 75% of the cases in VACTERL A were first births as compared to 40% in VACTERL B.

4.541 Comparison of VACTERL A and VACTERL B to previously determined subgroups.

Two other studies by Evans (1982, 1984), approached the question of homogeneity in the VACTERL association using numerical taxonomy. The first study consisted of 25 individuals of whom 12 had been ascertained through multiple sources using renal and limb anomalies as key anomalies, and an additional 13 patients with three or more components of the VACTERL association. Three strongly defined groups were distinguished; caudal regression, and VACTERL 1 and 2. VACTERL 1 was characterized by a high frequency of cervico-thoracic anomalies, unilateral renal agenesis, and an absence of cardiac and tracheal anomalies. VACTERL 2 was characterized by a high frequency of cervico-thoracic, lumbo-sacral, cardiac anomalies and a high incidence of tracheo-esophageal fistula. No renal agenesis was reported and only one case of renal ectopia.

These groups agree fairly closely with those produced by the present study. VACTERL 2 is very similar to VACTERL A except for the high frequency of cardiac anomalies noted, and aside from the lack of cardiac anomalies in VACTERL 1 it too is very similar to VACTERL B. The differences between the two studies are probably due to the way in which the population was originally ascertained.

A second study by Evans looking at malformation patterns within VACTERL acknowledged the problems faced when attempting to find recurrent patterns or relationships between anomalies when using biased ascertainment. In an attempt to overcome this multiple sources of ascertainment and a broader definition of anomalies compatible with VACTERL were used. Three main VACTERL groups were identified. One was characterized by imperforate anus and a high incidence of lumbo-sacral vertebral anomalies and the other two lacked imperforate anus and were characterized by VSD and cervico-thoracic vertebral anomalies respectively. In the present study VACTERL A certainly had high frequencies of both imperforate anus and lumbo-sacral vertebral anomalies, and VACTERL B seemed to combine the characteristics of the latter two groups described by Evans, having a low frequency of imperforate anus (although other anal ectopia was present) and a higher

frequency of both VSD and cardiac anomalies.

Despite the different ways in which the populations in these three studies were ascertained, the comprability of the VACTERL subgroups generated implies that the interrelationships determined are relatively strong.

4.550 Is the VACT 2 Group a milder expression of VACTERL?

It had been proposed that individuals with anomalies in only two systems compatible with VACTERL represented a milder form of the same association (Evans, 1984). The relationships within the VACT 2 minus known group support this hypothesis, although the VACT 2 group is necessarily more heterogenous, overlapping with many other conditions.

From the VACT (2 + 3) minus known Normal Hierarchy analysis it appears that in most cases the VACT 2 and VACT 3 individuals cluster separately, but this was expected because of the large number of additional anomalies in the VACT 3 minus known group that maintain its identity from the VACT 2 group.

5.000 SUMMARY

1. Numerical taxonomy was found to be an effective tool for association analysis, providing complete anomaly documentation was available and analyses were checked for obvious misclassifications.

2. Using a broad definition of VACTERL and numerical taxonomy five main groups were found in our VACT 3 minus known population: cloacal exstrophy, tracheal agenesis association variants, caudal regression, and two VACTERL groups: VACTERL A and VACTERL B.

3. VACTERL A represented a caudal variant with complex vertebral anomalies (both LSV and CTV), imperforate anus, renal dysplasia, and positional abnormalities of the thumbs. A high frequency of genital anomalies was also seen, but no cardiac or other non-VACTERL anomalies were observed in this group.

4. VACTERL B demonstrated significant craniofacial involvement with overlap with the facio-auriculo-vertebral spectrum. Vertebral anomalies were mainly cervico-thoracic; renal agenesis was observed; limb anomalies involved duplication and deficiency of the thumb; and

cardiac anomalies were found at a very high frequency. When anal anomalies occured, they were ectopias rather than atresias. No genital anomalies were present.

5. Differential mortality was seen in the five groups: the VACTERL groups had 100% survival, whilst the caudal regression, tracheal agenesis variants and cloacal exstrophy had 100% mortality.

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7.000 APPENDIX

List of Abbreviations Used in Text.

VACTERL	Vertebral, Anal, Cardiovascular, Tracheo-esophageal, Renal and Limb anomalies.
VSD	ventriculo-septal defect
ASD	atrial-septal defect
SUA	single umbilical artery
PFO	patent foramen ovale
NTD	neural tube defect
LSV	lumbo-sacral vertebral anomalies
CTV	cervico-thoracic vertebral anomalies
TEF	tracheo-esophageal fistula
Red Def L. Limb	reduction deficiency of the lower limb
Red Def U. Limb	reduction deficiency of the upper limb
AbN ext genit	abnormal external genitalia
AbN int genit	abnormal internal genitalia
Persistent LSVC	persistent left superior vena cava

KEY FOR INDIVIDUALS IN VACT 3

IDENTIFICATION NUMBER	DIAGNOSIS	"KNOWN" SYNDROME	SEX	AFFECTED SYSTEMS
1	Cloacal exstrophy TWIN "A"	---	M	VAC----G
2	Cloacal exstrophy	---	F	-AC--R-G
3	Cloacal exstrophy	---	F	-AC----G
4	Cloacal exstrophy	---	F	VAC--RLG
5	Cloacal exstrophy	---	M	-AC--RLG
6	Amnion Disruption	KNOWN	M	-AC--RLG
7	Cloacal exstrophy	---	F	-AC---LG
8	Cloacal exstrophy	---	M	-AC--R-G
9	Smith-Lemi-Opitz	KNOWN	M	-----RLG
10	Smith-Lemi-Opitz	KNOWN	M	--C---LG
11	Smith-Lemi-Opitz	KNOWN	F	--C---LG
12	Trisomy 13	KNOWN	F	V-C---LG
13	Trisomy 13	KNOWN	M	--CT-RLG
14	Trisomy 13	KNOWN	M	V-C--RL-
15	Trisomy 18	KNOWN	F	--C--RLG
16	Sirenomelia	---	F	VAC--RLG
17	-----	---	F	--C--R-G
18	Prunebelly	---	M	-AC--R-G
19	Prunebelly +	---	M	-ACTER-G
20	Private syndrome	---	M	V-C--RL-
21	Private syndrome	---	F	--C---LG
22	Cryptopthalmos	KNOWN	M	-A-T-R-G
23	Meckel Syndrome	KNOWN	M	--C--RLG
24	Rokitansky-Kuster Hauser	---	F	-AC--R-G
25	Tracheal agenesis	---	M	--CT-RL-
26	Amniotic disruption	KNOWN	F	--C--RL-
27	? Goldenhar	---	M	V-C--RL-
28	? Turner Syndrome	---	F	V-CT----
29	TWIN "B" -----	---	M	VACTERL-
30	-----	---	F	-AC--RL-
31	-----	---	F	VA-TERL-
32	Sirenomelia	---	M	VAC--RLG
33	-----	---	F	-ACTER-G
34	-----	---	M	VA----LG
35	-----	---	F	VAC-----
36	-----	---	M	VA---R-G
37	-----	---	F	-AC---L-
38	? Trisomy 13	---	M	-AC---L-
39	-----	---	M	-AC--R-G
40	-----	---	M	-AC--R--
41	-----	---	F	-A---R-G
42	-----	---	M	-A---R-G
43	-----	---	F	V-C--R--
44	? Trisomy 13	---	F	V-C--R--
45	Private syndrome	---	M	--C---LG

KEY FOR INDIVIDUALS IN VACT 2.

IDENTIFICATION NUMBER	DIAGNOSIS	"KNOWN" SYNDROME	SEX	AFFECTED SYSTEM
1	Trisomy 13	KNOWN	M	--C---L-
2	Trisomy 13	KNOWN	F	--C---L-
3	?Meckel or Trisomy 13	---	M	--C--R--
4	Trisomy 18	KNOWN	F	--C--R--
5	4p+	KNOWN	M	V-----L-
6	DiGeorge	KNOWN	M	V-C-----
7	----------	---	F	--C--R--
8	Hemifacial Microsomia	---	M	--C---L-
9	Pfieffer syndrome	KNOWN	F	-A----L-
10	Cloacal exstrophy	---	F	V-C-----
11	?Turner syndrome	---	F	--C--R--
12	Turner syndrome	KNOWN	F	--C----G
13	?DiGeorge	---	M	--CTE---
14	Oculo-Dental-Digital	KNOWN	M	V-----L-
15	?Miller Dieker	---	M	--C---L-
16	---------	---	F	--CT----
17	---------	---	F	--CTE---
18	---------	---	F	--C---L-
19	---------	---	M	--C---L-
20	---------	---	F	-AC-----
21	---------	---	F	-AC-----
22	---------	---	M	-A---R--
23	---------	---	M	-A---R--
24	---------	---	M	-A---R--
25	---------	---	F	-A----L-
26	---------	---	F	-----R-G
27	---------	---	F	V-C-----
28	---------	---	F	V-C-----
29	TWIN "A" ---------	---	M	V-C-----
30	TWIN "B" ---------	---	F	VA------
31	---------	---	M	VA------
32	---------	---	M	V-----L-
33	---------	---	M	V-----L-
34	---------	---	M	--C--R--
35	---------	---	F	--C--R--
36	---------	---	M	--C--R--
37	---------	---	M	--C--R--
38	---------	---	F	--C--R--
39	---------	---	F	--C--R--
40	---------	---	M	--C--R--
41	---------	---	M	--C--R--
42	---------	---	M	--C--R--
43	---------	---	F	--C--R--
44	---------	---	F	--C--R--