Triphalangeal thumb

a study of a congenital hand malformation

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Triphalangeal thumb

a study of a congenital hand malformation

Triphalangeale duim

een studie van een aangeboren handafwijking

Proefschrift

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to my teachers

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Preface

The most ancient known record of a congenital malformation is a sculpture of a double-headed twin goddess from the neolithic site of Catal Hüyük in southern Turkey, from approximately 6500 B.C.[1]. An Aboriginal rock drawing of similar age from New South Wales, Australia, shows a double headed human figure with six fingers on the right hand, and four fingers on the left. The first *written* records of congenital malformations are left by the ancient inhabitants of Babylonia. The Assyrian clay tablets discovered in the 19th century in a mound near the Tigris river are believed to represent copies of old Babylonian texts. The series dealing with births and congenital malformations are estimated to be approximately 4000 years old. These tablets represent a small part of the so-called omen literature on events used to predict future, and demonstrate remarkable knowledge on congenital malformations. A separate section on one of the tablets deals with the malformations of the arms, hands, and fingers[1].

Congenital malformations have continued to attract attention during the centuries. Next to the curiosity aspect, there was also a practical one to the children born with congenital anomalies. There can be little doubt that in many primitive cultures, deformed children were quickly disposed of. Infanticide was also widespread amongst the early Greeks and Romans. In Rome, the family law as enacted in the "Twelve Tables" (5th century B.C.) gave the father of a congenitally malformed child the right to destroy it[2]. Disposing of deformed children was even advocated by Plato in his Republic. This "habit" reached its height in the ancient Sparta, where the child was disposed of "if it did not, from the very outset, appear made to be healthy and vigorous"[1]. Warkany observed that the Spartans contributed nothing to the world heritage of art and culture - probably because they removed the Spartan equivalents of the tiny babies like Newton, Darwin, Voltaire, Rousseau and Napoleon Bonaparte once were[3].

During the middle ages, christian church influenced the point of view of congenital malformations. It was stated that "monstrous births" were no mistake by God, but that God willed to create them[2]. Later on, a moralizing view was

added to "monstrous births". A belief became widespread in christian countries that malformations resulted from association of human beings with demonic creatures. In pamphlets and broadsheets that represented creatures of "unmistakable satanic origin", malformations as phocomelia, hairy naevi, clubfeet, syndactyly or polydactyly can be recognised[1]. The beliefs in "demonic associations" extended the danger of extermination from the malformed child to the mother, and/or other persons apparently involved[3].

From the 13th century onward, anatomy becomes an accepted science with the first public dissections being performed[4]. Important advances in anatomy and embryology during the Renaissance are made by the great artist - anatomists of the time like Leonardo da Vinci[5].

William Harvey, Physician Extraordinary to King James I of England, educated in Padua, became famous as the man who discovered the circulation of blood[6]. In 1651, he published a less famous, but evenly important part of his work, "De Generatione Animalium", in which he postulated that congenital malformations are caused by an arrest in normal embryonic development[1]. Until the 19th century, knowledge of morphology, embryology and taxonomy of abnormal human and animal development accumulates[7]. Next to human malformation studies, experiments in lower animals are introduced to supplement morphologic investigations. The insight emerges that some malformations are passed from parents to children, while others appeared to be caused by influences from the environment. In 1866 the monk Gregor Mendel published his work on plant hybrids, and described the fundamental laws of heredity. At the beginning of this century, geneticists formulated explanations for non-mendelian inheritance patterns. Teratologists postulated that malformations not only depended upon the nature of the teratogenic agent, but also upon the time of exposure[7]. However, medical sciences at that time were not aware of the applicability of these discoveries, and it lasted a few more decades before this knowledge was used for the prevention, diagnosis and treatment of congenital malformations.

Amongst congenital malformations, hand anomalies have always attracted particular attention. A possible explanation for this "attraction" is that hands are

a unique characteristic of human kind. The anatomic term "manus" comes from Latin "manipulus" - hence man "is he who has hands to manipulate". Aristotle defined hands as "antecedent to, or productive of, all other instruments; organs of investigation instead of locomotion"[8].

Congenital limb malformations are probably older than human kind. Polydactyly[9,10], syndactyly[11], split hand/split foot deformity[12] and reduction defects[13] have been described in various non-human primates. It is recorded that a horse of Julius Caesar, Roman emperor (100 B.C. - 44 B.C.), had "toes" instead of hoofs; in ancient Rome, it was believed to be a sign that the world would one day belong to its master[1]. In the Old Testament a giant with hexadactyly of all four extremities is described[14]. Aristotle also documented redundance and reduction of the fingers, toes, hands, and feet[3]. Ambroise Paré described polydactyly in the 16th century as "superfluous digits which grow by the thumb or little finger, but seldom otherwise"[8]. This was probably the first "classification" of polydactyly into preaxial (or radial) and postaxial (or ulnar) form.

Despite the long history of records, the treatment of congenital hand malformations is a rather young medical discipline. From the beginning of this century, thanks to development of asepsis and anaesthesia, general surgery began to flourish. However, paediatric surgery was not developed until much later[7]. After the discovery of the thalidomide-induced embryopathies during the 1960's, the public awareness was focused on the dangers of maternal drug ingestion for the fetus. A wave of thalidomide induced limb malformations also provided a stimulus for basic research and development of new surgical solutions for difficult problems such as the congenitally malformed hand[15]. The complexity of the treatment of these malformations at young age is determined by the small size of the infant's hands, the different anatomy, the difficult functional evaluation, and the necessary but difficult anticipation on growth processes that continue after surgery. Timing of surgery is another complex issue which mainly depends on the skills and experience of the surgeon[16]. Furthermore, there is the inevitable burden on the parents of "guilt", uncertainty about how to handle the affected child, fear of recurrence in a next pregnancy, and - a wish to understand

"why".

During the past decades, treatment of congenital hand malformations has improved enormously by the development of new surgical techniques and medical technology - both culminating in microsurgery and free tissue transfers. However, management of these malformations includes not only surgery, but also adequate support and guidance of the parents, who will have to support their child. Knowledge of genetic or psychological aspects will further improve the surgeons capacity to regard not only the malformed hand, but also the affected child, and its family as a whole.

This thesis represents a study of different aspects of a rare congenital hand malformation, the triphalangeal thumb (TPT). During the period from 1983 to 1991, 15 children were referred for treatment of this disorder to the Department of Plastic and Reconstructive Surgery of the Sophia Children's Hospital in Rotterdam. Eleven of these children had a positive family history for TPT, and all the families originated from the same small area in the south west part of The Netherlands. Preliminary studies in this region have led to the discovery of a small, isolated population with an extremely high prevalence of this disorder. Clinical, radiological and psychological aspects of the phenotype were studied, and the search for the underlying genetic defect was started in the form of linkage analysis.

Chapter 1.1

General Introduction

Triphalangeal thumb

In The Netherlands, approximately 320 children with congenital hand malformations are born each year[17,18]. Polydactyly is the most frequently observed congenital hand malformation and one of the most frequent congenital disorders in general[19]. It is defined as a duplication of a finger or a part of it. The prevalence of polydactyly with or without an associated malformation varies between 5 and 17 per 10,000 live births[17,18,20]. An explanation for the large variety of recorded prevalences in different studies could be that some geographical areas are inhabited by the clusters of affected families. Isolated (non-syndromic) polydactyly can be generally subdivided in pre- and postaxial polydactyly. Preaxial or radial polydactyly refers to an excess of parts on the radial side of the limb. It describes the so-called duplicated thumbs, as well as various forms of triphalangeal thumbs and index finger duplications. Duplication is a semantically inaccurate term, for in no category of radial polydactyly there is a normal thumb[21]. Preaxial polydactyly has been classified in four types by Temtamy and McKusick[8]:

1. Thumb polydactyly (type I)
2. Polydactyly of a triphalangeal thumb (type II)
3. Polydactyly of an index finger (type III)
4. Polysyndactyly (type IV).

Postaxial polydactyly can be subdivided in type A (fully developed extra ray) and type B (rudimentary extra ray). Postaxial polydactyly is 11 times more frequent among the black population, whereas preaxial polydactyly has a similar incidence in both, the black and the white populations. The defect may be unilateral or bilateral, and hands or feet or both may be affected. These malformations often occur together, or in combination with syndactyly.

Triphalangeal thumb (TPT) is a rare form of preaxial polydactyly, with prevalence in the general population estimated at 1 : 25.000[22]. It is characterized by a long, sometimes finger-like thumb with three phalanges instead of two. The first description of TPT is given by Renaldi Columbi in 1559[23]. Manoiloff reported a Russian family in which this disorder was transmitted for over 2000

years. The ancestors of this family could be traced back to Italy and were "said to have been traced to general Scipian the African (185-129 B.C.), who likewise had six fingers and six toes"[24].

TPT can occur as a feature of a syndrome or sequence, in combination with other malformations of the hands, or an isolated defect. Syndromal associations include congenital heart defects, anorectal malformations, bone marrow dysfunction, onychodystrophy, sensorineural hearing impairment, radial aplasia or hypoplasia and tibial hemimelia[8,25,26]. The Holt-Oram or the hand-heart syndrome is an autosomal dominant disorder characterised by abnormalities of the upper extremity and congenital heart disease. The most frequent cardiac defects are atrial and ventricular septal defects, patent ductus arteriosus and transposition of the great vessels. The Aase syndrome is characterised by anaemia because of dysfunctional red cell precursors, leucopenia, TPT and mild degrees of radial hypoplasia. Fanconi pancytopenia is an autosomal recessive disorder which affects multiple organ systems. Next to the pancytopenia, about 80% of the patients have various thumb malformations or hypoplastic radii. Townes-Brock syndrome comprises anorectal malformations (imperforate anus, anorectal stenosis, ectopic anus), abnormal auricles, urinary tract malformations (renal hypoplasia, vesicoureteral reflux, urethral valves), mild deafness, and thumb anomalies[8,25,26]. In the so-called tibial hemimelia-polysyndactyly-triphalangeal thumb syndrome, TPT is associated with tibial aplasia and various forms of poly- and syndactyly[25,26]. There is a number of "miscellaneous disorders" TPT can be associated with, like absence of pectoral muscle, cleft palate and abnormal sternum, trichorhinophalangeal syndrome type II, trisomy 13, lacrimo-auriculo-dental-digital syndrome, and hypomelanosis of Ito, but these associations are rare[25,26]. TPT is often associated with malformations of the upper extremities, such as pre- or postaxial polydactyly, syndactyly, and split hand deformity. Isolated TPT may occur as a sporadic disorder, but it is usually inherited as an autosomal dominant trait. The underlying genetic defect is unknown.

Many theories were put forward in an attempt to explain the origin of triphalangism of the thumb. Some 19th and early 20th century authors believed

that a normal thumb resulted from a loss of one phalanx in the course of evolution of the first digit, that was originally similar to others. Accordingly, triphalangeal thumbs were seen as an atavistic trait. Other authors believed that the biphalangeal primate thumb resulted from the fusion of the middle and distal phalanges during embryogenesis. This theory was supported by the observation that while the thumb is the shortest digit, it possesses the longest distal phalanx. Following this theory, TPT represents persistence of a middle phalanx owing to failure of a normal fusion process. Yet another hypothesis postulated that TPT can be considered as a duplication of the index finger in association with absence of the thumb[25]. Finally, a theory that the extra phalanx in TPT results from incomplete thumb duplication is also described[25,27].

The treatment of triphalangeal thumbs

From the functional point of view, there are two features which make TPT a challenge for the hand surgeon: its association with hypoplasia of the thenar muscles and subsequent opposition impairment, as well as its abnormal length. The phenotype can vary from opposable TPT - an almost normal looking thumb with a small extra ossicle in the interphalangeal joint, to a fully developed index-like digit instead of a thumb - non-opposable TPT, or the so-called "five-fingered hand". A large spectrum of degrees of severity can be found between these two extremes.

Wood described three skeletal variations of this disorder based on the shape of the extra phalanx: delta, rectangular and full[28]. The shape of the extra phalanx is an important factor in choosing treatment options. The surgical goal is to construct the best thumb possible from all available tissues[21]. According to Wood, five separate surgical problems associated with this disorder can be distinguished[29]:

- association of TPT with other hand malformations, in particular polydactyly, syndactyly, and the cleft hand;
- the narrow first web-space;

- presence of an extra phalanx, often abnormal in shape, and an extra joint;
- frequent position of the thumb in the same plane with other digits;
- hypoplastic thenar muscles.

Several treatment options have been advocated in the past. Bunnell stated that no treatment was necessary for TPT[30]. Some authors recommended amputation of the entire distal phalanx to achieve length reduction[31]. In 1897, Beatson recommended extirpation of the extra triangular phalanx for the opposable TPT[27]. Milch[32] redescribed this technique, and made distinction between treatment principles for children and adults. He considered surgical correction of this deformity contraindicated in adults, as the function may become seriously impaired. Children, on the contrary, have sufficient remodelling capacity of the articular surface after the extirpation of the ossicle, and it is at this early age that surgical measures may be taken.

Wassel pointed out that in cases of complicated preaxial polydactyly, when TPT is associated with an "extra" biphalangeal thumb, better results are obtained by removing the triphalangeal, rather than the biphalangeal digit[33]. Wood observed that the radial (biphalangeal) digit is often hypoplastic in this type of polydactyly[34]. He introduced a refinement of Wassel's technique by transposing the triphalangeal thumb from the ulnar to the radial position. In this technique, the ulnar metacarpal head is impaled on the distal end of the metacarpal of the radial thumb, allowing growth of the newly created thumb to almost correct functional length.

Providing that proximal interphalangeal joint (PIP) is normal, arthrodesis of the distal interphalangeal joint (DIP) can be performed. If the distal interphalangeal joint has a good articular surface, shortening can be obtained by fusing the distal portion of the "middle phalanx" to the proximal stump of the proximal phalanx (PIP arthrodesis). If there is an inclination of the distal joint, sharpening of the "middle phalanx" into a peg and impaling it into the medullary canal of the proximal phalanx allows correction of the angulation deformity and a firm union[16].

For more complicated cases of a rectangular "middle phalanx", Peimer described

a technique of a combined reduction osteotomy. He combined a transversal and a longitudinal osteotomy of the middle and distal phalanx, which included deangulation, ablation of the extra joint and abnormal epiphysis[35]. The results after the 11-year review of this procedure were impressive[31].

However, in the non-opposable TPT, reduction osteotomy or arthrodesis at the level of distal or proximal interphalangeal joint is not sufficient. The reasons are twofold: insufficient length reduction that can be achieved at the PIP or DIP level, and position of the thumb in the same plane with other digits. At the DIP-level, very limited length reduction can be performed because of the presence of the nail bed. At the PIP level more reduction can be achieved, but the excessive length in this type of TPT comes primarily from the extremely long first metacarpal. Furthermore, the proper positioning of the thumb in a plane at approximately 90 degrees towards the other digits, can only be achieved by means of rotation osteotomy of the first metacarpal. Buck-Gramcko described this technique of two osteotomies at two different levels, and recommended it for the so-called transient TPT - an intermediate form between the wedge-shaped middle-phalanx and the five-fingered hand[29]. He suggested that the treatment of the five-fingered hand should consist of the pollicization procedure which is the same as in the index finger pollicization[36].

The principles of these operation techniques more or less reflect theories on the evolution of the primate thumb from the beginning of this century. Galen (2nd century AD) believed that the first metacarpal, which, unlike the other metacarpals has a proximal epiphysis, was not a true metacarpal, but actually represented the proximal phalanx of the thumb[25]. When in the five fingered hand the TPT is pollicized, the proximal phalanx is indeed replacing the first metacarpal. Other operating techniques are based on removing the extra phalanx and the extra joint by means of "fusion" of the proximal and distal phalanx, hereby creating a "normal" endphalanx.

From the 15 patients treated in Rotterdam, seven patients were treated by a single-stage procedure of osteotomies at two different levels: at the DIP and the first metacarpal. The other eight were treated with different techniques, varying

from the resection of the middle phalanx, DIP and PIP arthrodesis, to the transposition of the ulnar first ray into radial position. In particular the technique of reduction osteotomies at two different levels originally described by Buck-Gramcko[29], gives excellent results in a population with large phenotype variability. In Rotterdam, this technique was combined in a number of cases with preoperative metacarpophalangeal pattern (MCPP) profile analysis, which enabled the surgeon to study the length of each individual bone of the hand. This information was valuable in calculating the exact length of the reduction osteotomies prior to the operation.

Clinical and genetic analysis of congenital hand malformations

Probably the oldest scientific method is sheer observation. The next step in the development of the scientific thought would be recording, followed by analysis of the recorded observations. The subsequent step would be classification of the records. Classification means "to arrange systematically"[37]. "Systematically", in turn, implies a method: working according to a plan, not casually or at random. A scientific method is usually dependent on the current level of knowledge, and the level of technological development. Hence, a classification reflects a time during which it was proposed.

Classifications can generally be subdivided into morphological/topographical, pathogenetic, and etiological ones. Classification of a disorder can be a helpful tool in diagnosis, treatment, and counselling.

The long and extensive history of records of congenital hand malformations resulted in a number of descriptive classifications. With the development of medical sciences and new techniques like the X-ray, new perspectives for observations opened up: e.g. studies of skeletal morphology. Classification of thumb polydactyly by Wassel[33] based on osseous anatomy is still widely used among hand surgeons.

An accurate description of a disorder is of crucial importance for a morphological/topographical classification. A good descriptive classification can

elucidate clues about pathogenesis and etiology. However, in disorders with great phenotypic variety such as congenital hand malformations, a problem can arise in finding a classification "midway between one so general that it is valueless, and one so detailed that its use becomes impossible"[16]. A classification of congenital hand malformations most widely used among hand surgeons is the one by Swanson[38]. This classification is based on description of the affected limb elements, both skeletal and soft tissue. Seven categories can be distinguished, according to the parts that have been affected by certain embryologic failures[38]:

I. Failure of formation of parts
II. Failure of differentiation (separation) of parts
III. Duplication
IV. Overgrowth (gigantism)
V. Undergrowth (hypoplasia)
VI. Congenital constriction band syndromes
VII. Generalized skeletal abnormalities.

According to this classification, polydactyly or digit duplication, can be subdivided into radial (preaxial), central, and ulnar (postaxial) polydactyly. The descriptive character of the current classifications of congenital hand malformations reveals their unknown etiology. However, the progress that has been made in the human molecular genetics during recent years, is presently contributing to reclassifications of many complex malformation syndromes. An example of these developments are the craniofacial disorders. Many craniofacial malformations are known by the name of the man credited with describing a particular syndrome[39]. Increasing insight into (molecular) genetic origins of these disorders reveals that several syndromes have a common genetic origin, and that different mutations in the same gene (allelic heterogeneity) can sometimes lead to different phenotypes. Just as intriguing is that a particular syndrome can be caused by mutations in different genes (locus heterogeneity)[40].

Until approximately 15 years ago, molecular analysis of candidate genes in inherited disorders was mainly based on identification and characterisation of

the gene product - the so-called "functional cloning". This requires knowledge of the responsible protein defect, and the purification of the normal protein. However, for the majority of inherited malformations or diseases (approximately 90%), the underlying protein defect is unknown. In recent years, a strategy of genetic mapping of human disorders called "positional cloning" is developed. This strategy makes identification of a gene possible without prior knowledge about its function[41]. The first step is the establishment of the chromosomal location of the gene responsible for a phenotype. The studies of chromosomal rearrangements (translocations or deletions) are a very valuable tool for pointing in the right direction, towards the deficient gene. For example, the first autosomal locus that was found to be responsible for the split hand/split foot malformation, was mapped to chromosome 7q21 after various reports of patients with ectrodactyly associated with chromosomal aberrations in the 7q21 region[42,43,44,45]. When a disease or a phenotype is not associated with chromosomal aberrations, the gene must be "mapped" by means of the so-called linkage analysis. This is a method that allows mapping of genes that are detectable only as clinical phenotypic traits, such as genes underlying the congenital hand malformations. The vast majority of genes underlying genetic disorders falls into this category because neither their biochemical, nor their molecular basis has yet been elucidated. It is often the successful mapping of a disease gene by linkage analysis, that provides the first real evidence that a collection of clinical abnormalities observed in family members actually is due to mutations at a particular, identifiable gene[46].

Genes that are close together on the same chromosome have a tendency to be transmitted together through meiosis. Since two randomly chosen genes are most likely localized on separate chromosomes, or localized far apart on the same chromosome, they are generally transmitted independently. However, the closer two genes are on a chromosome, the higher the probability that they will stay together through meiosis; in other words, they could be "genetically linked". Linkage can be established between two genes, or a gene (DNA sequence coding for at least one protein), and a non-coding sequence of DNA with a well localised

position at a chromosome, which can be used as a marker. When a gene is found to be linked with a DNA marker, the position of a gene at a certain chromosome is localized. At present, genetic linkage maps of the whole genome are available. For use in linkage analysis, a DNA marker must be polymorphic, which means that a high proportion of persons in the general population, and in the families to be studied, has two different alleles (is heterozygous) for this marker. Heterozygosity for the marker is needed in order to be able to follow the transmission of each of the two homologous regions of a pair of chromosomes. Linkage analysis basically correlates segregation of a specific phenotype in a family with that of a well localized polymorphic marker. It has proved to be a tremendously important and powerful tool in medical research[39,46,47].

Finding linkage roughly establishes the position of a disease gene to a specific chromosomal region. Using the available family material and more specific DNA markers for that particular chromosomal region, the "candidate region" is narrowed down. The next step is identifying all functional genes contained within the candidate region. By comparing the sequence of the candidate genes between patients and control individuals, the mutations and the gene that - if mutated - leads to the phenotype in affected family members can be identified[41,46,47]. Positional cloning of a disease gene can be very time consuming and labour intensive. A good example of it is the search for the gene responsible for Huntington's disease. The gene was mapped to chromosome 4p in 1983, but it was identified only 10 years later[48,49].

The two essential requirements for positional cloning of a gene are a clearly defined phenotype in a sufficient large family material to establish linkage, and adequate DNA markers. A requirement for DNA markers can now be easily met. Finding suitable families can be a challenge. One very large kindred, or a group of small families with the same phenotype, will usually provide sufficient material for linkage studies. In the latter case, locus heterogeneity might complicate the analysis, and require a proportionally much larger family material.

In principle, familial congenital hand malformations constitute a group of disorders that are very suitable for linkage analysis. These disorders are often

inherited as simple autosomal dominant traits, and the detection of the phenotype (diagnosis) is clear cut. Accurate phenotype analysis based on clinical examination and radiological investigation can provide important clues about the possible function of the gene under investigation.

In the period from 1993 to 1997, during which this study was performed, a significant amount of genetic factors involved in embryonic limb development is discovered, followed by an increasing amount of studies of the molecular basis of human limb malformations. In 1994 the first gene responsible for an isolated human hand malformation phenotype was localized[50,51] (Chapter 3), and in 1996 the gene responsible for the so-called synpolydactyly was identified[52].

It is to be expected that in the near future, the majority of genes involved in the etiology of congenital hand malformations will be localized and identified. These developments will probably lead to the establishment of new, pathogenetic and etiological classifications, as a supplement to the current morphological ones, and will improve the genetic counselling of families in which these malformations, or the associated complex malformation syndromes, occur.

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Chapter 1.2

Genetics of limb development and congenital hand malformations

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Submitted

Introduction

One in approximately 626 newborns has a congenital malformation of the upper limb[1]. These malformations can occur isolated, in combination with other hand and/or foot anomalies, or as part of a syndrome. The etiology can be subdivided into environmental and genetic causes. A well known example of an environmental cause is the wave of thalidomide induced hand malformations that occurred in the 1960s.

Malformations caused by a genetic defect can be subdivided into three categories:

- single gene disorders (Mendelian inheritance patterns),
- chromosomal abnormalities, and
- multiple gene disorders (polygenic inheritance)[2].

A majority of the congenital hand malformations are single gene disorders. Usually, there are no large chromosomal abnormalities like translocations or deletions which can be visualized with various cytogenetic techniques, and can point towards a chromosomal localization of the deficient gene. When no chromosomal aberrations are associated with a disease or a phenotype, the deficient gene must be "mapped" by means of the so-called linkage analysis. In linkage analysis, DNA markers with known chromosomal localizations are used to find a mutated gene in the affected families. Unfortunately, this technique is only suitable for gene searches in large kindreds. The modern techniques of molecular genetics have been reviewed by Mulliken and Warman[3] as they apply to craniofacial disorders. The same molecular genetic techniques are applicable to congenital hand malformations as well.

Until recently, little was known about the etiology and pathogenesis of congenital hand malformations, which is reflected in present classifications based on descriptions of morphology, or osseous anatomy. However, this era is coming to an end. During the past decennium, vertebral limb has become a model system for studying developmental mechanisms and pattern formation during embryogenesis. Pattern formation is the term used to describe the emergence of

spatial biological organization during development. Mechanisms involved in the control of pattern formation include cell-cell communication, control of cell growth, and tissue differentiation. Studies of the developing limb, facilitated by its accessibility and large size, have resulted in now classical models for vertebrate pattern formation[4].

Recent developments in molecular biology have contributed to the identification of a significant amount of genes and molecular factors involved in limb morphogenesis and pattern formation. Unravelling the mechanisms of limb development has broadened evolutionary insights. In addition, several genes involved in the etiology of human hand malformations have been localized or identified. Localization indicates that a gene is mapped to a chromosome, but its nucleotide sequence remains to be determined. Congenital hand malformations that have been mapped so far, include preaxial polydactyly[5,6], split hand/split foot[7,8,9], and brachydactyly type C[10]. Identification indicates that the nucleotide coding sequence of a gene is determined and that a mutation in this sequence has been demonstrated to be responsible for the phenotype in affected individuals. The first and only gene identified so far to be responsible for a human hand malformation phenotype, is the so-called HOXD13 responsible for synpolydactyly[11].

The purpose of this article is twofold: to supply an overview of the genetics of limb development, and to summarize the discoveries in the genetics of human hand malformations. Because of the significance of recent advances in molecular biology of limb development and their relationship with normal and pathologic limb morphogenesis, limb development and patterning will be discussed first.

Limb Development and Patterning

The upper limb bud in humans appears at 26-28 days after the fertilization, and the lower limb bud follows approximately two days later. A majority of the factors involved in limb patterning are the same for upper and lower extremities, which explains the often observed overlap in phenotypes of the affected hands

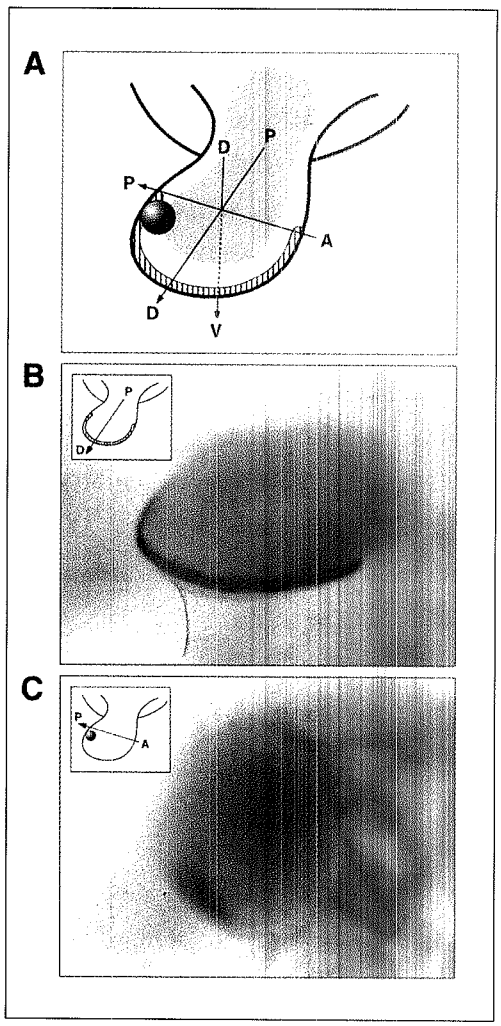
and feet. Digits in the upper limb become distinguishable at 41-43 days, and are fully separated approximately 10 days later. In the lower limb these events occur at 44-46 days and 54-56 days, respectively. During a period of 25 days, an interplay of genes and complex embryological processes have created a limb with the right amount of digits, the right appearances and functions at the right place. It has been recognized that positional information within the three-dimensional "coordinate system" of the growing limb is of crucial importance for the future cell fate during embryogenesis. At present, a number of signals that control patterning along each of the three axes, have been identified[12]. For reasons of clarity, each of the three axes will be discussed separately, followed by a short outline of the function of the so-called "Hox" genes. Hox genes are the "key-genes" involved in control of the morphogenesis of the developing embryo.

The three axes system

Proximo-Distal axis, Apical Ectodermal Ridge and Fibroblast Growth Factors

The vertebrate limb bud develops from the lateral plate mesoderm[13]. A yet unknown trigger initiates local proliferation of mesodermal cells at the appropriate level of the flank. The rapidly dividing mesodermal cells induce the ectodermal cells along the rim of the limb bud to form a so-called Apical Ectodermal Ridge (AER)[14] (fig 1a). The AER is responsible for the outgrowth of the limb bud along the proximo-distal axis and consists of pseudo-stratified epithelium which is maintained by the underlying mesoderm. Cells from the subectodermal zone underneath the AER, the so-called "progress zone"[15], have a high mitotic activity which leads to the growth of the bud in both lateral and distal directions. As growth proceeds, cells located in the proximal parts of the progress zone, "leave" this zone. Experimental evidence suggests that, by this time, they have received their "positional identities"; in other words, patterning information is acquired in the progress zone[15].

Fig 1:



A) The three axes of the developing limb.
***P-D** indicates the proximo-distal axis - from the humerus to the digits. The Apical Ectodermal Ridge (AER) is responsible for the outgrowth of the limb along this axis (represented at the distal border of the limb bud).*
***A-P** indicates the antero-posterior axis, from the thumb to the little finger. Patterning of the limb along this axis is controlled by the Zone of Polarizing Activity (ZPA), represented at the posterior border of the limb bud.*
***D-V** indicates the dorso-ventral axis, from the dorsum to the palm of the hand. The grey area at the dorsum of the limb bud represents the area where the "Wnt-7a" gene is expressed.*

B) The upper limb of the mouse embryo, 9,5 days post-coitum, with expression of the Fibroblast Growth Factor 8 in the Apical Ectodermal Ridge.

C) The upper limb of the mouse embryo, 9,5 days post-coitum, with expression of the Sonic Hedgehog in the Zone of Polarizing Activity.

As the limb bud grows, differentiation becomes apparent, initially of the most proximal structures (primordia of the humerus), followed by the differentiation of the primordia of the more distal structures (radius, ulna, wrist bones and digits)[16]. Experimental ridge removal from the growing limb bud results in truncation of the limb. The proximo-distal level at which the truncation occurs is dependent on the time of the ridge removal[16]. Human transversal limb mal-

formations are probably caused by disturbances in differentiation along the proximo-distal axis.

The function of the AER can be replaced by the application of members of the Fibroblast Growth Factor (FGF) family. There are at least nine FGF "family members"[17]. Three of them, FGF-2, FGF-4 and FGF-8 (fig 1b) are expressed in the AER[18]. It has been demonstrated that local application of beads soaked in FGF-4[16] and FGF-2[19] can substitute the ridge function, following ridge removal.

Remarkably, beads soaked in FGF-1, FGF-2, and FGF-4 placed in the flank of chick embryos induce formation of ectopic limb buds. These limb buds form an AER and develop into complete additional limbs, suggesting that local FGF production could be the required signal for the initiation of a limb bud formation[20]. However, FGF-1, FGF-2, and FGF-4 are not expressed at the right time or the right place to be the candidates for the real "limb inducer". Expression of the FGF-8 is detected in the prelimb ectoderm *before* a morphologically distinct AER is formed, making FGF-8 an excellent candidate for the initiation of limb bud outgrowth[18].

Antero-Posterior Axis, Zone of Polarizing Activity, Retinoic Acid and Sonic Hedgehog

Patterning of the limb along the antero-posterior (AP) axis is controlled by the Zone of Polarizing Activity (ZPA)[21], a highly "specialized" mesenchyme region at the posterior border of the growing limb bud. When this region is transplanted to the anterior border of another limb bud, it will induce symmetrical mirror-image duplications of the normal limb elements reflected about the AP axis. This property is called the "polarizing activity"[4,21,22]. These classic transplantation experiments produced animal phenotypes that mimic the human phenotype of the ulnar mirror hand.

It has been hypothesized that patterning along the AP axis is controlled by a signalling molecule, or a morphogen, that is released from the ZPA, and forms a gradient across the early limb bud. A high concentration of the ZPA morpho-

gen, which can be found along the posterior border of the limb bud, would give rise to digits with posterior character, and tissues exposed to the lower levels of the ZPA morphogen would develop into more anterior digits[23]. A concept of concentration dependent signal from the ZPA was supported by the finding that the extent of digit duplications is proportional to the number of transplanted ZPA cells[24]. At first, retinoic acid was thought to be the ZPA morphogen[25]. Retinoic acid shows a "natural" gradient across the AP border of the limb bud: it is present in higher concentrations along the posterior border of the limb bud[26], and a bead soaked in appropriate concentrations of retinoic acid implanted in the anterior margin of the limb bud mimics exactly the mirror-image duplications observed with the ZPA grafts[27]. However, evidence accumulated that retinoic acid does not fit into the "morphogen model". Retinoic acid acts by a mechanism dependent on absolute concentration, rather than on graded distribution. Polarizing activity in the limb fails to correlate with retinoic acid levels[4]. It appears that, rather than being the endogenous signal, retinoic acid can induce an ectopic ZPA[28]. Sonic hedgehog, a secreted protein, is now identified as the most likely ZPA morphogen (fig 1c). The expression of Sonic hedgehog colocalizes spatially and temporally with the ZPA[29]. The relationship between Sonic hedgehog and retinoic acid is a bit more clarified by the finding that retinoic acid has the ability to induce the expression of Sonic hedgehog in the tissue of the anterior limb bud[30].

For the maintenance of a functional ZPA, an intact AER is required[31]. FGF-4, which is produced by the AER, is supporting a functional ZPA by maintaining the expression of Sonic Hedgehog[31,32]. In addition, Sonic hedgehog protein produced by the ZPA, can induce FGF-4 within the AER by a positive feedback loop[33]. The feedback loop suggests a mechanism by which outgrowth and patterning along the PD and AP axes of the limb can be coordinately regulated. It appears that patterning and outgrowth of the developing limb are regulated by two major signalling centers, the AER and ZPA.

A recent study of expression patterns of genes involved in limb patterning in the mutated "limbless" chicken embryos possibly opens a new chapter in limb

development research[34]. The authors have found that antero-posterior polarity is established in the initial limb bud prior to the expression of Sonic hedgehog, apical ridge genes (FGF's), or dorso-ventral asymmetry. The authors propose that gene expression in the emerging limb bud is established by axial influences on the limb field. This means that there is still a "missing piece", an yet unknown factor initiating antero-posterior polarity *before* the AER and ZPA appear.

Dorso-Ventral Axis and "Wnt-7a"

The so-called Wnt gene family encodes a group of secreted signalling molecules that play a role in patterning during limb embryogenesis. It has been suggested that Wnt-7a could play a role in specification of dorso-ventral polarity in view of its specific expression in the dorsal ectoderm (fig 1a), and in no other regions of the limb bud[35,36]. Construction of the so-called "knock-out" mice, or mice lacking Wnt-7a activity, indicated that Wnta-7a is the "dorsalizing" signal[37]. The mutant mice develop *dorsal* footpads, flexor tendons on both, ventral and dorsal side of the digits, and accordingly, abnormal "dorsal" flexion. Interestingly, the phenotype is more severe distally than proximally. Furthermore, many mutant mice lack posterior digits, demonstrating that Wnt-7a also plays a role in antero-posterior patterning[37]. The signals from the dorsal ectoderm, where Wnt-7a is expressed, appear to be necessary to maintain Sonic hedgehog expression and formation of the posterior skeletal elements. All three axes (proximo-distal, antero-posterior and dorso-ventral) appear to be closely linked by their respective signalling molecules FGF-4, Sonic hedgehog and Wnt-7a, during limb patterning and outgrowth[38].

Hox genes

The so-called homeobox genes represent a gene family that subdivides the early embryo into fields of cells with the potential to become specific tissues and organs[39]. This gene family is named after the "homeotic mutation" - a mutation

which causes a body part to be replaced with a structure normally found elsewhere on the body. The first homeotic mutation was described in 1894 by W. Bateson[40]. In 1948, it was discovered by Lewis that homeotic transformation can be caused by a mutation in a single gene, and assumed that a mutation affected one of the "master genes" that control function of many other "subordinate" genes. In 1984, the first Drosophila gene with a homeobox motif was discovered[41]. The homeobox motif is a DNA sequence that is found in different development-controlling genes; in other words, this DNA sequence is said to be conserved. This conserved DNA region can be found in the genes of different species. The homeobox encodes a sequence of 60 amino acids that is very similar in the protein products of most of the homeotic genes. The function of the homeodomain is to recognize and bind to the "subordinated" genes that are regulated by the homeotic genes[39].

It is now clear that homeotic genes play an important role in the vertebrate development. The proteins encoded by these genes can differ greatly from one another, except at the highly conserved homeodomain. All vertebrates have four homeobox complexes, each located on a separate chromosome. These complexes probably arose by duplication and divergence from a common ancestral cluster in invertebrates[40]. The order of the homeobox genes in a cluster (and on a chromosome), corresponds directly to where the genes are expressed.

The term "Hox" always indicates a gene from one of the Hox gene clusters. It has been agreed to use different written nomenclature for different species: Hox indicates mouse, and HOX human genes. There are 38 vertebrate Hox genes, organized in Hoxa, Hoxb, Hoxc and Hoxd complexes. Each complex contains different subsets of paralogous genes, indicating that some members of a cluster were not duplicated during the evolutional events that led to the formation of multiple complexes[42]. Homeoboxes are also found in a number of other developmentally important genes located outside of the clusters - these genes usually have other names, but also carry the "family" name of Homeobox genes. It is not (yet) clear how many homeobox containing genes we have.

Homeobox genes appear to play an important role in the maintenance of the

proper interactions between the AER and the underlying mesoderm of the progress zone. Cells of the progress zone express various combinations of Hox proteins at different times or positions in the zone[43], while the expression of one of the Homeobox genes, the so-called "En-1" gene, is restricted to the ectodermal ridge[44]. These findings suggest a function in the "communication" of the ectodermal and mesodermal compartments of the developing limb.

Activation of the Hox genes during limb morphogenesis follows distinct spatial and temporal patterns. Analysis of these patterns suggests that the expression of each of the Hoxa and Hoxd genes is regulated in three independent phases. Each of these phases is associated with the specification and patterning of one of the proximo-distal segments of the limb (upper arm, lower arm and hand)[45]. Various models suggest that the genes from the Hoxd complex regulate the positional identity along the AP axis of the limb bud[46]. The Hoxd genes are expressed at the posterior/distal tip of the developing limb, overlapping the ZPA. These expression patterns divide the embryonic limb bud into five sectors along the AP axis, suggesting that there might be an evolutionary constraint on developing more than five morphologically "different" digits. Each of the five sectors with different Hoxd code correlates with the anlage of individual digits[47], making Hoxd genes excellent candidates for hand malformations like poly- and syndactyly.

Considerably less is known about the expression and regulation of the Hoxb and Hoxc genes even though it appears that expression of some members of these complexes is restricted to either fore or hind limb bud[45]. It is possible that morphological differences between the upper and lower extremity can be correlated with these expression patterns.

Genetics of congenital hand malformations

Even though the classification of congenital hand malformation by Swanson[48] is widely used by hand surgeons, we will refer to the classification given by Temtamy and McKusick[2]. This classification is generally used in clinical genetic

studies, and experimental molecular biology. Furthermore, it is applicable on both, the upper and the lower extremity. Until 1994, no isolated human hand malformation phenotypes have been assigned to a specific gene, or gene locus. During the past few years, the explosive development in research towards genetic factors involved in limb development, is followed by an increasing amount of studies of human limb malformations. At present, a number of genes responsible for human hand malformation phenotypes is localized, and the gene responsible for the so-called synpolydactyly, has now been identified[11] (table 1).

Polydactyly

According to location, polydactyly can be divided in radial or preaxial polydactyly, ulnar or postaxial polydactyly, and central polydactyly. It is not clear whether central polydactyly represents a separate entity. The prevalence of polydactyly with or without an associated malformation varies between 5 and 17 per 10,000 live births[49,50,51].

Temtamy and McKusick define two types of postaxial and four types of preaxial polydactyly. Postaxial polydactyly is subdivided in type A (fully developed extra ray) and type B (rudimentary extra ray). The four types of preaxial polydactyly are defined as follows:

Type I or thumb polydactyly: duplication of one or more of the skeletal components of a biphalangeal thumb;

Type II or polydactyly of a triphalangeal thumb;

Type III or polydactyly of an index finger;

Type IV or polysyndactyly: both preaxial polydactyly and syndactyly are cardinal features of this phenotype, but syndactyly never occurs without polydactyly[2].

Type I has further been divided in six subtypes, depending on the level of a duplication considering bony anatomy[52]. This type of preaxial polydactyly is usually sporadic, often unilateral, and less frequently associated with thenar hypoplasia then the other three types. Type II, type III and type IV are usually

inherited as autosomal dominant traits.

In 1994 two independent studies reported linkage of two different phenotypes of preaxial polydactyly to chromosome 7q36 - namely the triphalangeal thumb and complex polysyndactyly[5,6]. The phenotype in the family with triphalangeal thumbs varied between opposable and non-opposable triphalangeal thumbs, indicating a common genetic origin of these two phenotypic variants[5,53]. Linkage of complex polysyndactyly to the same chromosomal region suggested that these two phenotypes could be caused by different mutations in the same gene (allelic heterogeneity), or by mutations in two different, but closely linked genes (locus heterogeneity). Presence of different degrees of postaxial polydactyly in both phenotypes brought up the question whether this gene (these genes) also could be responsible for isolated postaxial polydactyly. However, studies of seven Dutch Caucasian kindreds with isolated postaxial polydactyly type A or B showed no linkage with the locus on chromosome 7q36[54], suggesting that pre-and postaxial polydactyly have different genetic origin.

Linkage studies of preaxial polydactyly type I will show whether all different types of preaxial polydactyly can be traced down to the same chromosomal region on 7q36.

Synpolydactyly

Synpolydactyly is defined as syndactyly of the third and fourth fingers as well as syndactyly of fourth and fifth toes, associated with polydactyly of the same fingers and toes. It is usually inherited as an autosomal dominant trait[2].

Linkage studies in a large kindred with synpolydactyly (SPD) or syndactyly type II phenotype[55,56], mapped the SPD gene to a locus on chromosome 2q31[57]. The authors speculated that a mutation in one of the members of the HOXD cluster is likely to be responsible for this phenotype, in view of the expression of these genes in the distal limb bud. Indeed, a mutation in HOXD13 gene was demonstrated to be responsible for this phenotype[11]. The mutation appeared to be caused by an expansion of a polyalanine stretch in the amino-terminal of the HOXD13

gene. The mutation was not located in the homedomain, but in the non-DNA binding part. It has been suggested that alanine stretches in genes could play a critical role in modulating the activity of homeoproteins. A very interesting feature of the alanine repeats in HOXD13 is that they are not present in Hoxd13 of fish, suggesting that insertion of alanine repeats in the amino-terminal of Hox proteins might have played a role in the acquisition of new characteristics, in this case limbs from fins[11,58].

Analysis of the amino acid alignment of the Hoxd13 in humans and chickens revealed a high degree of homology between the two species with the most differences located at the amino-terminal of the gene. This is probably, in evolutionary terms, the "youngest" part of the gene, and also a part where the mutation causative for the SPD phenotype is located[59].

Meanwhile, two other kindreds with SPD phenotype have been reported to be caused by the mutations in the same gene[59]. The phenotypes of the reported SPD kindreds, heterozygous and homozygous[11,55,56], correspond well with the phenotype reported in the mouse model with targeted deficiency in the Hoxd complex[60]. The major features of the heterozygous phenotype are syndactyly and digit duplications. Branching of the metacarpals involved in digit duplications is observed. The much more severe homozygous phenotype comprises short hands and feet, complete cutaneous syndactyly of all four limbs, polydactyly, loss of normal tubular shape of the carpal, metacarpal and phalangeal bones resulting in polygonal structures, and bone fusions.

The mouse Hoxd genes are expressed as a series of overlapping domains ("Russian dolls") in the limb bud, which is suggestive of a role in the specification of the digit pattern[61]. If we assume that the interplay of Hox genes indeed controls digit identity[42], a mutation, or rather a kind of a "genetic hick-up", is likely to cause duplications of one of the digits (polydactyly), or disturbances in patterning and separation of the digits (syndactyly).

Hoxd-13 is the last gene to be activated during limb development, and its expression is restricted to the most posterior region of the limb bud, which corresponds with the finding of poly- and syndactyly of postaxial rays in SPD

phenotypes. It is not likely that the expansion of the alanine tract observed in the SPD patients causes a loss of function of the HOXD13 protein, but probably results in a protein with an altered function[58]. As cooperative interactions between HOX proteins are very important, a mutation in one member of the family is likely to involve the function of other members too.

SPD phenotypes show considerable overlap with polysyndactyly, or preaxial polydactyly type IV phenotype. Both disorders are characterized by the presence of poly- and syndactyly. However, an important difference between the synpolydactyly and preaxial polydactyly phenotypes is the thumb involvement. No subjects from the reported kindreds affected with SPD phenotypes show thumb polydactyly. Hopefully, genes responsible for pre- and postaxial polydactyly will be cloned in the near future and their functional analysis will provide explanations for the overlapping phenotypes.

Split hand/split foot

Split hand/split foot malformation (SHSF), also termed ectrodactyly, is characterized by the absence of the central digital rays, deep median cleft and syndactyly of the remaining digits. Typical and atypical categories are recognized[2]. Atypical cases are usually sporadic. The majority of the familial cases are inherited in an autosomal dominant fashion, but autosomal recessive[62] and X-linked[7] inheritance have been described. The most frequent syndromic association of SHSF is the EEC (ectrodactyly, ectodermal dysplasia and cleft lip/palate) syndrome[2]. Both the isolated and syndromic form of this disorder show great phenotypic variety. SHFM is genetically heterogenous - until now three different loci have been reported to play a role in the etiology of this disorder. The first autosomal locus is termed SHFM1, and is localized at chromosome 7q21[8]. Various authors have attributed with reports of patients with ectrodactyly in whom chromosomal aberrations of 7q21 region were described[63,64,65]. An X-linked locus termed SHFM2 has been mapped at Xq26[7]. A second autosomal locus termed SHFM3 has been mapped to chromosome 10q24-25[9,66].

Table 1. *Genes responsible for human congenital hand malformations that have been localized or identified.*

Disorder	Location	Gene	Reference
Preaxial Polydactyly type II and III	7q36	?	Heutink et al.[5]
Preaxial Polydactyly type IV	7q36	?	Tsukurov et al.[6]
Synpolydactyly (Syndactyly type II)	2q31	HOXD13	Muragaki et al.[11]
Split hand/split foot (SHFM1) Autosomal Dominant	7q21	DSS1?	Scherer et al.[8] Crackower et al.[67]
SHFM2 X-linked	Xq26	?	Faiyaz-ul-Haque et al.[7]
SHFM3 Autosomal Dominant	10q24-25	?	Nunes et al.[9] Gurrieri et al.[66]
Brachydactyly type C	12q24	?	Haws et al. [68]

Several genes from the critical chromosomal region of the SHFM1 gene have been investigated as candidate genes. A gene called DSS1 (for deleted in the split hand/split foot SHFM1 region) from the same chromosomal region has now been identified[67]. The DSS1 gene encodes a highly conserved acidic polypeptide with no similarity to any known proteins. Expression analysis of the murine homolog Dss1 reveals expression in the limb bud, facial primordia and skin. The authors propose that reduced expression of this gene during human embryogenesis could explain phenotypes observed in SHFM1 patients, but also in patients with EEC syndrome. However, whether DSS1 is "the SHFM1 gene" remains uncertain in view of the fact that no mutations have been detected so far in any SHFM1 patient[68].

Brachydactyly type C

Brachydactyly comprises a vast group of malformations with shortening of the digits as a common characteristic. Brachydactyly type C (Haws type) is also characterized by hyperphalangism. It is usually inherited as an autosomal dominant trait[2]. In 1963 Haws described a large Mormon kindred affected with this disorder[67]. Using linkage analysis in the members of this same large kindred, the gene for brachydactyly type C is mapped to human chromosome 12q24[68]. Future developments around this gene will be interesting to follow, particularly in view of the hypersegmentation, and hand growth impairment in the affected individuals.

Even though tremendous advances have been made in this field during the past years, the story of genetic basis of congenital hand malformations is far from complete. Hand surgeons can give a considerable contribution by reporting affected families which are suitable for linkage analysis. It is not to be expected that prenatal diagnosis will become a routine for couples at risk of getting a child with a hand malformation. However, increasing knowledge in this field increases our insights into the molecular basis of congenital hand anomalies, and functional analysis of these gene families reveals their roles in pattern formation and (normal) embryogenesis. In the future, genetic classifications of congenital disorders will arise as a supplement to the current morphological ones, and perhaps provide the explanations for the great phenotypic variability and overlapping phenotypes.

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Chapter 1.3

Backgrounds and aims of the study

During the period from 1983 to 1991, 15 children were treated for triphalangeal thumbs (TPT) at the Department of Plastic and Reconstructive Surgery of the Sophia Children's Hospital in Rotterdam. Eleven of these children had a positive family history for this disorder, and all the families originated from the same small area in the south west part of The Netherlands. Eleven patients with the same (rare) disorder, originating from the same area and treated within a span of approximately seven years triggered our curiosity, and preliminary studies were started. The aim was to estimate the prevalence of TPT in that particular area, to explore the phenotypic variation, and discriminate between possible environmental and/or genetic causes behind it.

The local general practitioners made it clear that TPT in that area was inherited as an autosomal dominant trait. We soon realised, even without performing genealogical studies, that all different phenotypes that were observed during this period, were probably due to mutations in a single gene. This opened possibilities for further research. Even though at that time enormous progress was being made in the research concerning the factors involved in (vertebral) limb development, not a single isolated human hand malformation phenotype had been assigned to a specific gene or gene locus.

In collaboration with one of the local general practitioners, a clinical genetic and genealogical investigation was initiated. Phenotype analysis of triphalangeal thumb and associated malformations in this patient population are described in **Chapter 2**. A genealogical search for a common ancestor was performed by using the population and census records, and civil registration in the municipal archives. At present, all affected families are brought back to one common ancestor couple, that lived approximately 200 years ago. In the course of phenotypic and genealogical studies, we realised that these families were very suitable for linkage analysis. Linkage analysis in this family material mapped the TPT gene to chromosome 7q36 (**Chapter 3**). This linkage study confirmed that all phenotypic variants of TPT observed in these kindreds probably shared a common genetic origin. Furthermore, it was the first time that a human gene solely involved in the pathologic morphogenesis of the hand was localised. Present research in our

group is focusing on the identification of the TPT gene.

Not only do congenitally malformed hands have an anatomy which is different from normal, but this anatomy shows also large phenotype variability, sometimes even within one patient. An important determinant of hand anatomy is the hand skeleton. The length of the hand bones plays an important role in the surgical treatment. Metacarpophalangeal pattern (MCPP) profile analysis is a method for studying the osseous morphology of the individual hand, based on bone length measurements. In **Chapter 4** the MCPP pattern profile which appears to be specific for the triphalangeal thumb, is described together with the possible applicability of this method in the treatment of congenital hand malformations where abnormal osseous length is involved.

In the course of the "field-studies", various members of the affected families who participated in our research, mentioned a large psychological impact this disorder apparently had on some affected individuals. Furthermore, two young mothers reported that their affected children, contrary to their non-affected siblings, used to put literally everything in their mouth - until the age of seven and eight, respectively. Both children had severe opposition impairment and were not (yet adequately) surgically treated. Apparently, these two children were using their mouth as a tactile organ. These observations prompted us to investigate this part of the TPT phenotype.

Hand function plays an important role in the development of a child. Through the motor and perceptual tasks of the hands, the infant develops knowledge about his environment, learns, explores and begins to communicate. A congenital hand malformation influences both, the executive and perceptual function of the hand. In order to explore the influence of an isolated congenital hand malformation on the psychomotor development of a child, an exploratory, observational study on 18 children with triphalangeal thumbs was performed. These observations are presented in **Chapter 5**.

Finally, in **Chapter 6**, the results of this study and future perspectives are discussed.

Chapter 2

Phenotypic analysis of triphalangeal thumb and associated hand malformations

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Introduction

Congenital hand anomalies have a prevalence at birth of about 5 per 1000[1]. For The Netherlands, the best estimate is 16/10,000[2] (nationwide approximately 300 affected births per year). The most frequently observed hand malformation is post- or preaxial (thumb) polydactyly with prevalences from 7 to 14 per 10.000 live births[2-4].

Many attempts have been made to develop an adequate classification system for preaxial polydactyly. The most widely accepted classifications among clinicians are the two by Wassel[5] and Swanson[6], both based on bone anatomy. The classification of preaxial polydactyly according to Temtamy and McKusick is widely used among clinical geneticists and defines the following subtypes[7]:

Type I preaxial polydactyly which comprises various degrees of duplications of biphalangeal thumbs. It is usually sporadic, unilateral and not associated with thenar anomalies.

Type II and type III are two different forms of triphalangeal thumbs, opposable and non-opposable, respectively.

Type IV (polysyndactyly) is usually associated with feet anomalies and resembles the type of limb deformity seen in Greig cephalopolysyndactyly syndrome.

Pre-axial polydactyly can occur as an isolated anomaly or as part of several complex congenital malformation syndromes. The defect may be unilateral or bilateral, and hands or feet or both may be affected. Sporadic as well as familial occurrence has been described, with autosomal dominant inheritance as the most likely mode of transmission[8].

Triphalangeal thumb (TPT), a long finger-like thumb with three phalanges instead of two, is usually regarded as a subtype of preaxial polydactyly. Lapidus indicates the prevalence of TPT at 1:25.000[9]. In the series of Iowa University, TPT represents 3% of congenital malformations of the upper extremities[10]. TPT is occasionally seen as a sporadic disorder, and more often as an autosomal

dominant familial trait. It is therefore probable that the overall prevalence of this disorder is very low in large populations, but may peak in areas with affected families.

Recently, we identified 11 possibly related probands and their families (Dutch, caucasian), in which the expression of thumb anomalies ranged from an opposable triphalangeal thumb to a triphalangeal index-like digit instead of a thumb, including two extra hypoplastic rays radial to the "thumb" (septadactyly).

In this paper we describe our findings in the families of six of these 11 probands with respect to pattern of inheritance and variability of clinical phenotype, function and morphology. A comparison with similar conditions reported during the past 10 years is provided. The usefulness of the currently available classifications and the potential significance of gene localisation and identification in order to understand the pathogenesis of complex hand malformations will be discussed.

Patients, materials and methods

Through a review of medical records of the Department of Plastic and Reconstructive Surgery of the University Hospital Rotterdam-Dijkzigt/Sophia concerning patients with congenital hand malformations, we ascertained 11 probands with TPT, all of whom had been referred for primary or secondary plastic surgery. The review concerned a period of referrals during the years 1983-1991. Each of these 11 probands had a strikingly similar phenotype, a strongly positive family history of similar hand malformations suggestive of autosomal dominant inheritance, and all their parents had their origin in a demographically and geographically small region in the south-west part of The Netherlands. This raised the hypothesis that all patients and their affected relatives might have one and the same genetic disorder. Therefore we initiated, in collaboration with one of the family physicians of this community (P.S.) a clinical genetic and genealogical investigation.

The families of the 11 probands were contacted through the family physician and

one of us (J.Z.) with a request for cooperation in the investigation. Full written information about the protocol was provided and informed consent was obtained from each participant. The research protocol was approved by the Medical Ethics Committee of the Erasmus University Rotterdam and Academic Hospital Rotterdam (Project no. 92.1031)

Standardized forms were used for:

- Medical history of each participant including a check-list for teratogenic exposures during pregnancy, congenital anomalies and previous surgery,
- Family history, of each affected/unaffected relative; maternal and paternal, including a check-list for parental consanguinity, congenital malformations, hereditary and/or chronic diseases; family histories from different members of the same family were cross checked to increase validity of the information, and supplemented with available photographs on which hand morphology of the ancestors was verified.
- Physical examination, including a check-list for general and specific abnormalities of hand, feet and craniofacial malformations.

In principle, both parents of each patient underwent complete examination in order to evaluate the possibility that a patient might have inherited the putative gene defect from the spouse of the affected parent or from both parents.

Persons were regarded as affected when the following criteria were fulfilled:

- Triphalangeal thumb or biphalangeal partly duplicated thumb alone, or in combination with any of the following:
- Preaxial extra ray and/or postaxial polydactyly type B
- Syndactyly between digits III and/or IV and/or V.

A person displaying postaxial polydactyly only was not considered to be affected.

All patients were seen by one of us (J.Z.). Ten patients and ten apparently unaffected first degree relatives were also seen by a clinical geneticist (D.L.), in order to evaluate the possibility that TPT was part of a more complex malformation syndromes. Of most of the patients, clinical photographs were taken and reviewed (D.L., J.Z.). In addition, the registry of the Clinical Genetics Center Rotterdam covering the South-West region of the Netherlands was screened for

potentially related congenital malformations, diagnosed in patients from this area.

Results

According to the family histories, 186 out of a total of 346 subjects, were presumably affected and 160 presumably non-affected, with a sex-ratio among affected subjects of 91/95 (M/F).

So far, we had the opportunity to examine the families of six of the 11 probands. By means of genealogical studies we were able to link the families of three of these probands and their affected relatives to each other. These three interrelated families will be refered to as family A (fig.1). The ancestors of the other probands all originated from the same village. 124 subjects distributed over the four families of the six probands have been personally examined by the investigators. 60 persons out of this group were affected, 38 were non-affected, and 26 were (healthy) partners of affected subjects.

Figure 1: ***Family A.*** *Three initial probands are indicated by arrows. [-] indicates that the family member was examined by one of the authors personally.*

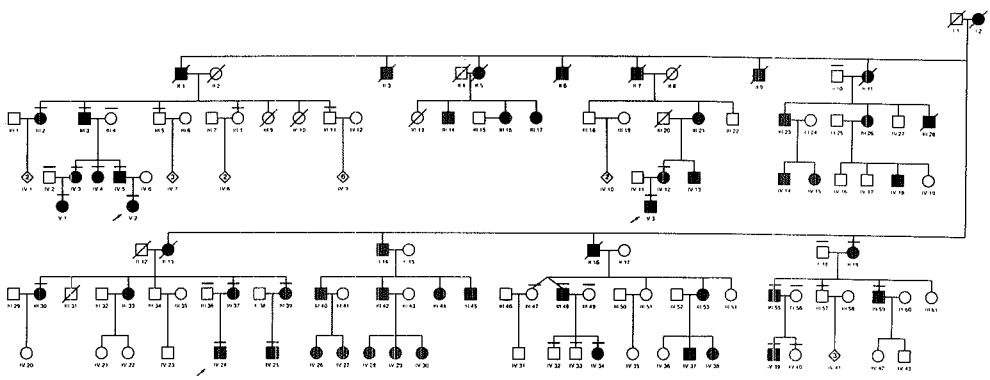
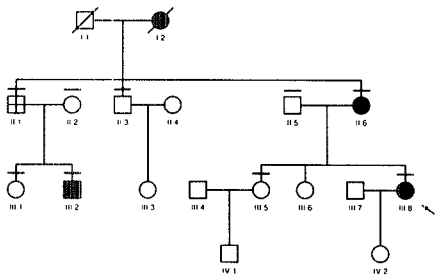


Figure 2:



Family B. Proband is indicated by an arrow. [-] indicates that the family member was examined by one of the authors personally. II:1 had only unilateral rudimentary postaxial polydactyly of the hand (see text for further description and discussion about variable expressionand reduced penetrance).

Among the examined couples, there was no known consanguinity. The inheritance pattern is clearly autosomal dominant with almost complete penetrance. The variability of the expression of this disorder could not be related to the sex of the subject or the sex of the affected transmitting parent. Male to male transmission was observed in 11 out of 60 affected parent - child pairs (table 1).

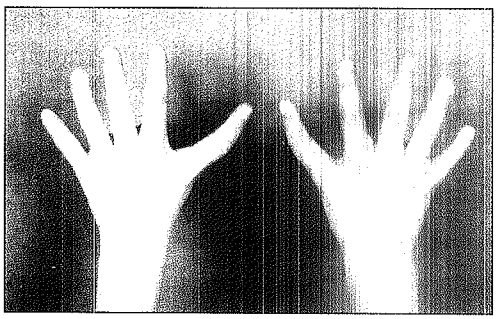
	Family A	Family B	Family C	Family D	Subtotal
Male to male	2	4	4	1	11
Male to female	1	5	6	0	12
Female to male	7	3	4	1	15
Female to female	9	5	6	2	22
Subtotal	19	17	20	4	60

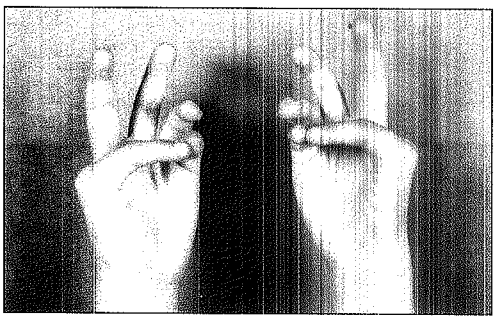
Table 1: *Parent-offspring couples by gender combinations among present families*

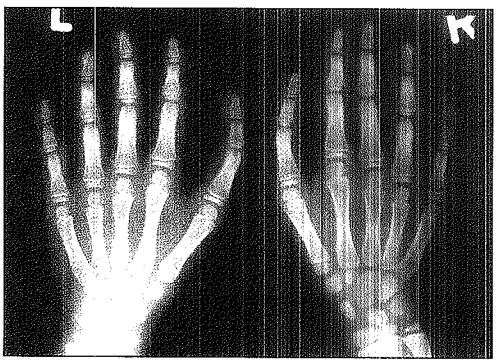
The phenotype varied between the following two extremes: Subject X, of the age of 41, had an ulnar deviation in the interphalangeal joint of her both thumbs, based on an extra delta-shaped phalanx. Thenar musculature was normally developed as well as the first web. She had normal opposition function in both hands.

Her son, subject Y, of the age of 21, had bilateral triphalangeal index-like digits instead of normal thumbs, both in the same plane as other hand digits. On both hands, additional hypoplastic digits were present, resembling rudimentary thumbs which have been surgically removed at an early age. The maximum

Figure 3:







Photographs and roentgenograph of the hands of proband 1, aged 7, after amputation of the preaxial extra rays, correction of cutaneous syndactyly between digits IV and V of the left hand, and before thumb surgery. Notice the indexlike appearance of the thumb, the narrow first web-space, pseudo-opposition,hypoplastic thenar muscles, and on the roentgenograph the absence of the sesamoid bones and two epiphyses of the first metacarpal.

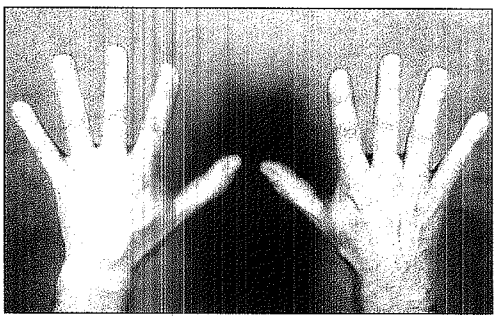
distance between the distal finger pads of the "thumb" and the index finger was only 5 cm on both hands, indicative of a severe narrowing of the first web.

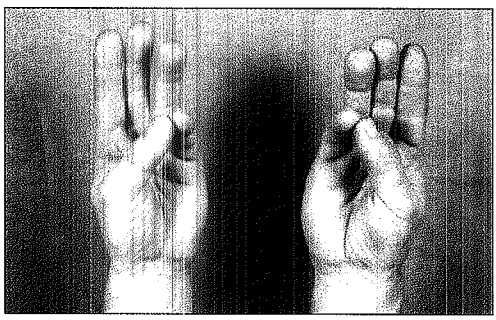
The thenar muscles of both hands were hypoplastic. This patient was only capable of "pseudo-opposition", performed by the adductor and both flexor muscles of the thumb. Both hands showed cutaneous syndactyly between digits IV and V over the full length. On both fifth digits there was rudimentary post-axial polydactyly. He also had cutaneous syndactyly between his fourth and fifth toes on both feet. No photographs of this patient are shown because he just underwent the fifth surgery session on his hands at the time we saw him.

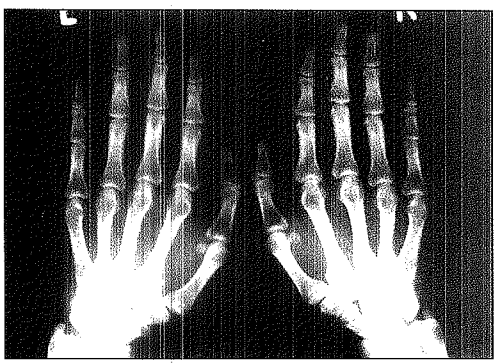
The expression in most other patients varied between these two extreme forms.

A feature common to all patients is the presence of a triphalangeal thumb. A delta-shaped extra phalanx is usually associated with less outspoken thenar hypoplasia, normal first web, normal position of the thumb and good thumb function.

Figure 4:



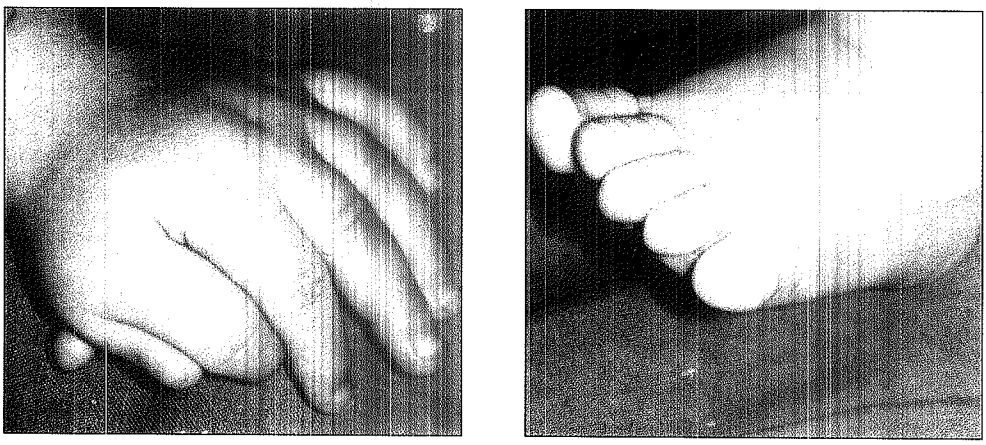




Photographs and roentgenograph of the hands ofthe uncle of proband 1 showing slight ulnar clinodactyly in the interphalangeal joints of the thumbs, almost normal thenar and normalopposition, and on the roentgenograph sesamoid bones and two small delta phalanges in boththumbs (arrows).

A rectangular extra phalanx (index-like thumb) is often associated with more severe thenar hypoplasia, narrow first web, thumb in the same plane as other hand digits, and defective opposition. On X-ray examination, thumbs with an delta-shaped extra phalanx usually showed at least one sesamoid bone at the level of the metacarpophalangeal joint, suggesting the presence and development of at least some thenar muscles, whereas thumbs with a rectangular extra phalanx had an digit-like appearance,

Figure 5:



Photographs of the left hand and foot of proband 2, aged 15 months, before surgery, showing index-like triphalangeal thumb with two hypoplastic preaxial extra rays and duplication of the fifth toe.

usually without sesamoid bones (fig. 3 and 4). In both types of TPT the epiphysis of the first metacarpal was seen either distally or proximally, and sometimes on both sites.

Most subjects were symmetrically affected, with sometimes small differences in expression only, for example in the degree of thenar hypoplasia, the presence or absence of pre- and/or post-axial polydactyly, the degree of syndactyly, or duplication of the fifth toe.

In only one person (subject II:1 from family B), there was a discrepancy between the phenotype and the genotype as derived from information from the pedigree. This male person, age 55 years, had an affected mother as well as affected offspring. On examination, he had only a rudimentary post-axial polydactyly on his left hand in the form of a wart with a diameter of 3mm on the lateral border of his middle phalanx. No other abnormalities were found.

The clinical findings in our families are summarized in table 2.

Table 2: *Phenotype analysis among presented families*

	Family A	Family B	Family C	Family D	Total
TPT	19/19	17/17	20/20	3/4	59/60
Thenar hypoplasia	13/19	17/17	17/20	2/4	49/60
Pre-axial extra ray	11/19	16/17	14/20	2/4	43/60
Postaxial polydactyly	9/19	8/17	10/20	1/4	28/60
Syndactyly	2/19	2/17	0/20	1/4	5/60
Postaxial foot polydactyly	1/19	1/17	0/20	0/4	2/60
Foot syndactyly (IV-V)	1/19	1/17	0/20	0/4	2/60

Note: In family D person II:1 who only had rudimentary postaxial polydactyly is included in the patient denominator assuming that he is obligate carrier of the TPT gene in view of TPT in his mother and his son. For further description see text.

Discussion

The currently described families show a consistent phenotype of opposable or non-opposable TPT, and varying expressions of extra radial ray(s), rudimentary postaxial polydactyly, and cutaneous syndactyly. All initially identified probands originated from the same geographically and demographically small region and the families of three of them could be linked to each other, suggestive of a common gene mutation.

The pattern of inheritance is clearly autosomal dominant with (almost) complete penetrance and variable expression, and without evidence for imprinting. Male to female ratio is almost equal to one. Table 1 shows that the transmitting parent was more often a female than a male. This can be explained by the fact that in three out of four presented families, the transmitting parent of the oldest generation was a female with much larger number of offspring than in subsequent generations.

According to our present diagnostic criteria, Patient II-1 from family B was not affected, showing no clinical or radiographical signs of TPT. However, in the

pedigree he was an obligate TPT-gene carrier, implying that the penetrance of the TPT-gene is not 100% but slightly less, with postaxial polydactyly in patient II-1 from family B as a coincidental finding. Alternatively we have to consider isolated postaxial polydactyly as a forme fruste expression of this gene mutation. Future studies on more families with TPT may clarify this question.

Table 3a: *Qualitative phenotypic comparison of presented and previously reported families*

	Merlob	Nicolai	Warm	Miura	Radhakrishna	Present
TPT	+++	++	+++	+++	+++	+++
Thenar hypoplasia	-	+	+++	++	?	+++
Preaxial extra ray	-	++	++	++	+++	++
Postaxial polydactyly	-	++	-	+++	-	++
Syndactyly	-	+++	-	++	-	+
Preaxial foot polydactyly	++	-	-	+	++	-
Postaxial foot poly- and/or syndactyly	-	+	-	-	-	+

Note: - absent, + present, ++ prominent, +++ predominant feature

Table 3b: *Quantitative phenotypic comparison of presented and previously reported families*

	Merlob	Nicolai	Warm	Miura	Radhakrishna	Present,
TPT	3/3	?/7	3/3	2/2	?+/71	59/60
Thenar hypoplasia	0/3	?/7	2/3	2/2	?+/71	49/60
Pre-axial extra ray	0/3	7/7	3/3	1/2	61/71?	43/60
Postaxial polydactyly	0/3	7/7	0/3	2/2	0/71	28/60
Syndactyly	0/3	7/7	0/3	2/2	0/71	5/60
Preaxial foot polydactyly	2/3	0/7	0/3	1/2	21/71?	0/60
Postaxial foot poly- and/or syndactyly	0/3	?/7	0/3	0/2	0/71	4/60

Note: nr. of patients with indicated symptom/ nr. of patients examined by reporting authors

During the past, several other families with TPT and associated hand malformations similar as observed in our families have been reported[11-18]. However, none of these clinical phenotypes completely match with each other and with ours. There are remarkable differences with respect to the severity of each symptom as

well as their frequency among affected individuals. In table 3, the clinical phenotypes of 5 families reported during the past 10 years are compared with each other and with the families reported in this paper.

In the family described by Merlob et al.[15], the three examined family members had an opposable TPT, which in two of the members was associated with duplication of the big toes. This phenotype, apart from the absence of preaxial extra rays, is very much alike the phenotype in an Indian family reported by Radhakrishna et al.[12]. It included TPT, preaxial (radial) extra ray and duplication of the big toes. Especially the latter malformation was not observed in any of 60 affected individuals of our families. The fact that preaxial extra rays at the hands was not observed in the family by Merlob may be due to chance since they reported on three affected relatives only.

Warm et al.[14] described a family with non-opposable TPT, which in two out of three family members was associated with a preaxial extra ray. The phenotype is compatible with that of our families, even though Warm et al. did not observe postaxial polydactyly, syndactyly, or postaxial polydactyly of the feet, since this may have been due to chance. These three features were observed only with low frequencies in our patients, and their family report consisted of only three examined affected individuals.

Nicolai et al.[11] and Miura et al.[13] reported families with complex hand anomalies consisting of syndactyly, polydactyly and TPT, resembling Haas's malformation. These two families and the presently reported four families share the same characteristics, but, apart from a triphalangeal thumb, there is a clear difference with respect to the most predominant symptom. Severe syndactyly and postaxial polydactyly were noticed in all of seven patients described by Nicolai et al, whereas these malformations were much milder in our families and observed with low frequencies only. A comparison with the family reported by Miura is hampered by the smal size of their family, but it is remarkable that preaxial foot polydactyly was present in one of their two patients, but was not observed in any of our 60 patients.

The classification of congenital hand malformations is usually based on clinical

appearance or skeletal morphology but is frequently complicated by the coexistence of different types of malformations, like polydactyly, syndactyly, and thumb hyperphalangism in the TPT-families discussed here. Usually the most prominent or most frequent malformation is used as a lead for classification of complex malformations. However, the application of the currently available classification systems is complicated by the large variation in expression and the considerable overlap between apparently different complex hand malformation syndromes. This makes it difficult to draw conclusions about the extent of genetic heterogeneity among the previously and currently reported TPT-families. Nevertheless, the fact that the phenotypic spectrum is very consistent among our currently reported four families - together with their common geographic and demographic origin, strongly supports the hypothesis that all affected individuals examined by us sofar share the same genetic defect.

Although the differences in expression patterns between the currently and previously reported families could be explained by genetic (allelic or locus) heterogeneity, the variable expression within each family indicates the role of additional genetic or environmental factors. This suggests that the TPT-gene is a regulatory gene involved in the development of the hand during embryogenesis. Localisation of the disease gene(s) by positional cloning strategies and identification of the gene(s) envolved and the mutations causing TPT will help to answer these questions and may contribute to the establishment of a new, etiological and pathogenetic classification of complex hand malformations, as a supplement to current morphological classifications.

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NOTE: Pedigrees of all families are available on request.

Chapter 3

The gene for triphalangeal thumb maps to the subtelomeric region of chromosome 7q

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Pre-axial polydactyly or congenital deformities of the first digital ray of the hand can occur as an isolated anomaly, in association with other abnormalities of the hand or as a component of complex developmental disorders[1].

Tentamy and McKusick classified isolated, non-syndromic polydactyly on a anatomical basis into five separate entities[2]:

- Postaxial polydactyly
- Thumb polydactyly (type I)
- Polydactyly of a triphalangeal thumb (type II)
- Polydactyly of the index finger (type III)
- Polysyndactyly (type IV)

Other morphologically based classifications have been proposed[2,3], but a classification based on the genetic components determining the different phenotypes is as yet impossible.

A triphalangeal thumb (TPT) is a long, finger-like thumb with three phalanges instead of two. Familial occurrence has been described, with an autosomal dominant mode of inheritance. Prevalence has been estimated to be 1 in 25.000[4]. The clinical presentation of TPT can vary from an opposable thumb with a delta-shaped extra phalanx to an non-opposable index like digit instead of a thumb. TPT can occur as an isolated congenital defect in association with other anomalies of the hand and/or feet, or as part of a syndrome[1].

The underlying developmental defect for TPT is unknown but must involve disturbance of the formation of the anterior-posterior axis of the developing forelimb. Embryological studies on the development of the forelimb bud in vertebrates indicate that regulation of several homeobox genes or genes regulating programmed cell death are involved in the shaping of the final hand and foot[5-10].

Treatment varies from excision of the extra (delta) phalanx in the simple cases of triphalangeal thumbs to "double osteotomy" which is a pollicization resembling procedure for the most extensive cases.

In this paper we report the results of a linkage study in two large pedigrees where TPT segregated as an autosomal dominant disorder with apparently complete

penetrance.

Family studies

To localize the gene for TPT we have ascertained two Dutch caucasian kindreds with TPT from a relatively isolated population. This population has an estimated prevalence for TPT of 1 in 1000. Within these families the expression of thumb anomalies in different family members is highly variable and ranges from an opposable thumb with a delta phalanx, to an extreme form of pre-axial polydactyly with a triphalangeal index-digit instead of a thumb (fig 1), two extra hypoplastic rays radial to the "thumb" (septadactyly), hypoplastic thenar muscles and, occasionally, syndactyly between the fourth and the fifth ray. Following the classification of Tentamy and McKusick[2] the anomalies in all affected individuals could be diagnosed as polydactyly of a triphalangeal thumb (type II) and/or polydactyly of the index finger (type III).

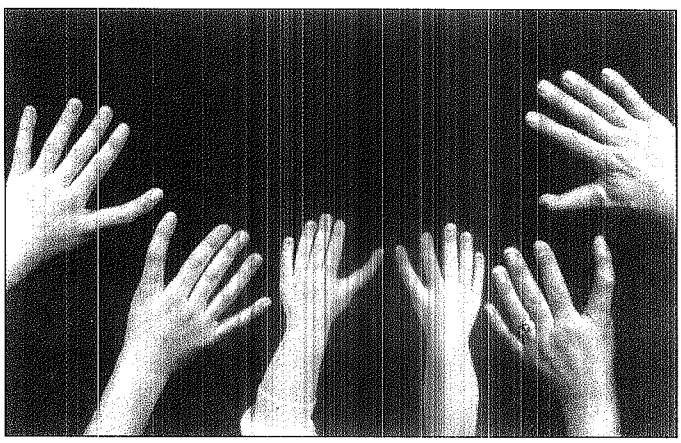

Fig 1.
Cases with triphalangeal index-digit instead of a thumb in a three generation family. Hands of grand-mother on the right, mother on the left and child in between.

Blood samples from 70 family members including 34 affected persons were obtained with informed consent (fig 2). The trait was never transmitted by unaffected family members. Male to male transmission was observed in seven cases ruling out X-linked inheritance. Transmission of TPT in these families was consistent with an autosomal dominant mode of inheritance with apparently

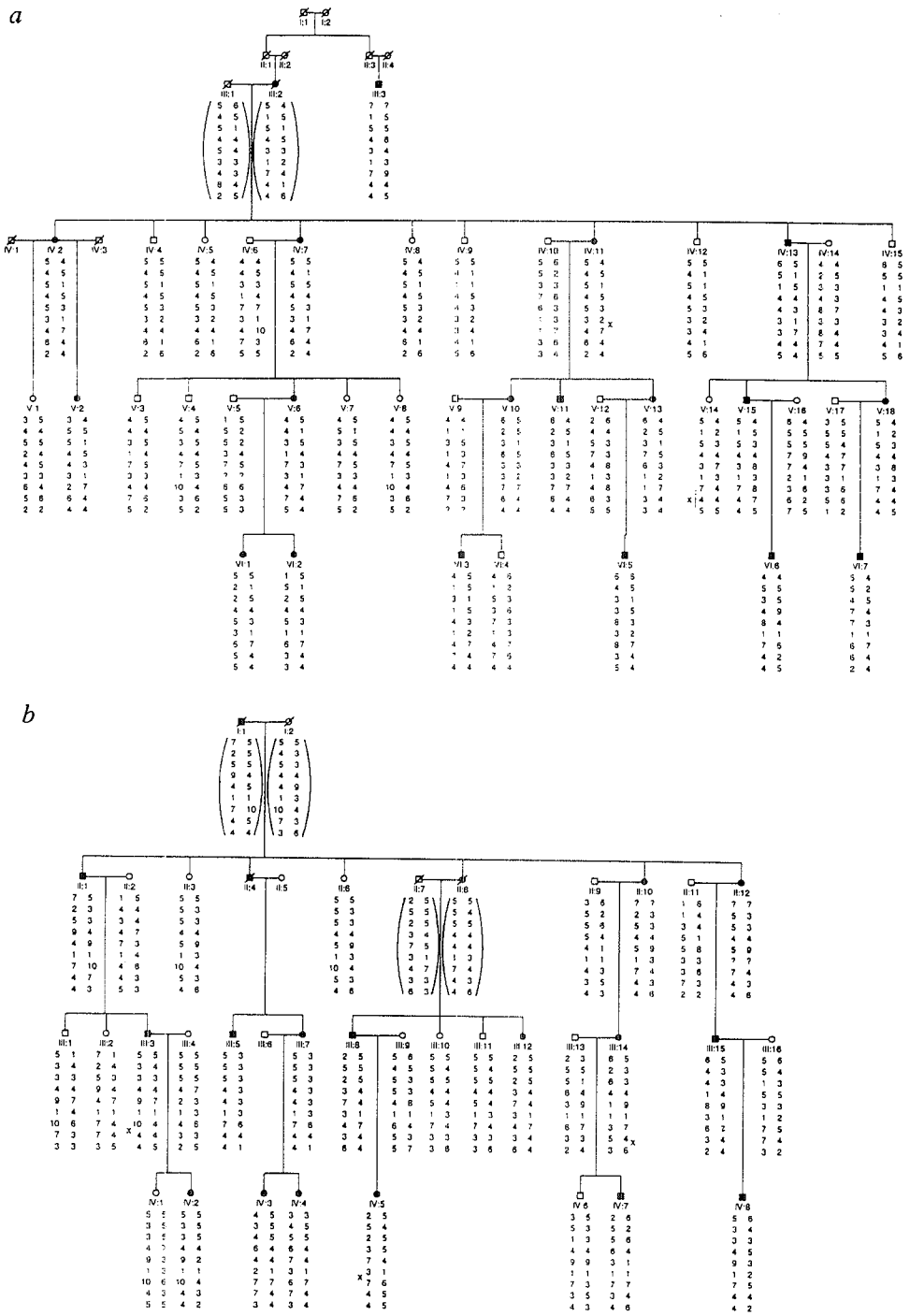
a
b

Fig 2. Pedigrees of two TPT families. A. Family 1 B. Family 2.
The roman numbers represent the generations. The arabic numbers identify the individuals. The affected individuals are indicated by a filled symbol. Haplotypes are presented for 9 chromosome 7q markers ordered according to their chromosomal localization. pter-D7S495-D7S498-D7S505-D7S483-AFM205va3-AFM211xc3-D7S550-D7S559-D7S594-qter. Key recombination events are indicated by x. | indicates that the exact position of the recombination event could not be determined.

complete penetrance. A common ancestor of the two families could not be identified so far. Detailed information on these families will be given elsewhere.

Linkage studies

Initially linkage studies were started with family 1 which was informative enough to detect linkage by itself. When lod scores greater than one were found in two-point linkage analyses, neighboring markers were included in the analysis.

Our initial focus was on possible candidate genes. A number of genes have been implicated in the formation of the forelimb. Several homeobox genes are expressed in the developing limb bud. *Hox-4* has been proposed as a gene involved in patterning of the anterior-posterior axis of the developing forelimb. An intragenic marker for *Hox-4*[11] yielded strong evidence against tight linkage ($Z=-17.633$ at $\Theta= 0.0$). Markers known to be located close to seven other homeobox genes were also tested but none of them yielded an indication for linkage. Subsequently we started a systematic genome search with polymorphic microsatellite markers evenly distributed over the human autosomes. In total 126 microsatellite DNA polymorphisms were analyzed on family 1. Significant evidence for linkage was obtained with several markers on the most distal part of chromosome 7q (table 1). These positive findings were confirmed by testing chromosome 7 markers in a second family with TPT (fig 2). The summed lod score for both families reached a value of $Z_{max}=12.609$ at $\Theta=0.0$ with marker *D7S559*. Table 1 summarizes the pair wise lod scores for both families of

Table 1. *Pairwise lod score of chromosome 7q markers at various recombination distances* [1].

		recombination fraction (cM)						
Marker		0.00	0.01	0.05	0.10	0.20	0.30	0.40
D7S495	Family 1	-17.821	-10.111	-5.140	-2.935	-1.033	-0.285	-0.031
	Family 2	-1.796	-1.711	-1.444	-1.189	-0.791	-0.479	-0.219
	Total	-19.617	-11.822	-6.584	-4.124	-1.824	-0.764	-0.250
D7S498	Family 1	-11.647	-4.590	-1.362	-0.005	0.942	0.995	0.575
	Family 2	-2.625	-2.438	-1.872	-1.389	-0.728	-0.322	-0.103
	Total	-14.272	-7.028	-3.234	-1.394	0.214	0.675	0.472
D7S505	Family 1	-7.749	-1.196	0.404	0.970	1.168	0.933	0.514
	Family 2	-1.667	-1.558	-1.206	-0.901	-0.489	-0.237	-0.099
	Total	-9.416	-2.754	-0.802	0.069	0.679	0.696	0.415
D7S483	Family 1	-2.397	1.239	2.283	2.434	2.086	1.423	0.635
	Family 2	-0.355	-0.299	-0.154	-0.057	-0.001	0.030	0.051
	Total	-2.752	0.940	2.129	2.377	2.085	1.393	0.686
205va3	Family 1	0.051	2.049	2.632	2.683	2.310	1.661	0.819
	Family 2	-2.958	-2.747	-2.009	-1.363	-0.629	-0.253	-0.076
	Total	-2.907	-0.698	0.623	1.320	1.681	1.408	0.743
211xc3	Family 1	-5.295	2.902	3.461	3.463	2.936	2.052	0.915
	Family 2	0.090	0.103	0.136	0.150	0.136	0.094	0.043
	Total	-5.205	3.005	3.597	3.613	3.072	2.146	0.958
D7S550	Family 1	6.565	6.518	6.233	5.756	4.581	3.172	1.520
	Family 2	-0.464	-0.078	0.592	0.892	0.940	0.667	0.290
	Total	6.101	6.440	6.825	6.648	5.521	3.839	1.810
D7S559	Family 1	7.302	7.181	6.686	6.043	4.671	3.169	1.503
Family 2	5.307	5.216	4.842	4.354	3.299	2.128	0.864	
Total	12.609	12.397	11.528	10.397	7.970	5.297	2.367	
D7S594	Family 1	8.043	7.911	7.372	6.673	5.179	3.532	1.684
Family 2	2.184	2.695	3.002	2.910	2.382	1.633	0.751	
Total	10.227	10.606	10.374	9.583	7.561	5.165	2.435	

[1] Markers are arranged according to their chromosomal localization from pter to qter.

chromosome 7q markers against TPT at various recombination distances.

Multipoint linkage and haplotype analysis on chromosome 7q

In order to determine the most likely position of the TPT gene, relative to the chromosome 7q markers, we ordered the markers listed in table 1 on the basis of information obtained from several sources. The position of markers *D7S495, D7S498, D7S505, D7S483, D7S550* was obtained from the Genethon map_{12}. The relative position of AFM205va3, AFM211xc3 and *D7S559* within the Genethon map was obtained from unpublished genotypings on CEPH reference panels performed by Genethon and Dr. J.L. Weber (pers. comm.). The position of marker *D7S594* in this map has not yet been determined. Its physical localization is approximately 40 kb from the telomere repeat of chromosome 7q and no other microsatellite markers could be isolated from the remaining sequences towards the telomere[13]. The construct a preliminary map including *D7S594* of the 7q region. *D7S594* was mapped against *D7S550* and *D7S559*. We could place *D7S594* between these two markers and 2 cM distal from *D7S559*.

Multipoint analysis was performed with *D7S550, D7S559* and *D7S594*. (Fig. 3) This yielded a maximum lod score of 15.652 at *D7S550*. In the multipoint analysis the candidate region for the TPT locus could not be determined. This is possibly caused by the fact that no genotypings are available from a number of individuals

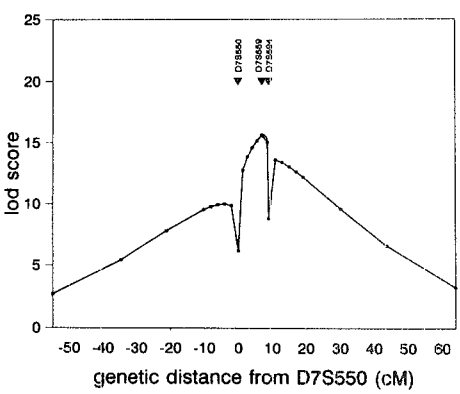

Fig 3. *Multipoint analysis for TPT versus three markers of chromosome 7. Relative position of the markers is indicated by their D numbers. Recombination fractions were converted to centimorgans using Kosambi's map function.*

in the first generations of the pedigrees. We therefore performed haplotype analysis with the markers listed in table 1. This analysis revealed several recombination events. One recombination event between marker AFM211xc3 and *D7S550* in family 1 (individual IV:11) and one event in family 2 (individual IV:5) place the TPT locus distal from AFM211xc3. A recombination event in family 2 between *D7S550* and *D7S559* places the TPT locus distal from *D7S550* (individual III:3). The unaffected individual V:14 in family 1 receives the haplotype associated with TPT except for *D7S559* and *D7S594* also suggesting a localization distal from *D7S550*. A recombination event in individual III:14 in family 2 places TPT proximal from *D7S594*.

Discussion

Triphalangeal thumb as an isolated feature or in association with other anomalies of the hand and/or feet has been reported to segregate in an autosomal dominant mode[14-16]. This was confirmed in the two families reported here. In these two families strong evidence for linkage of TPT with markers located at the subtelomeric region of chromosome 7q was found with a maximum lod score of 12.609 at *D7S559*. This lod score was raised to 15.652 in the multi point analysis. Based on haplotype analysis of chromosome 7q35-qter markers the TPT locus could be placed between *D7S550* and *D7S594*. The exact size of the candidate region could not be determined since *D7S594* has not been incorporated into the linkage maps that are available from Genethon or the Cooperative Human Linkage Center. Individual V:14 from family 1 shows no clinical signs of TPT, even after X-ray examination of the hands and feet, but has inherited the disease haplotype between marker *D7S495* and *D7S559*. This individual could be a case of non-penetrance. Penetrance for TPT is usually regarded as complete, therefore there is a high probability that individual V:14 has a recombination between *D7S559* and *D7S594*. For the linkage analysis we used a conservative penetrance value of 0.95 and this is the reason that this individual was not regarded as a recombinant in the analysis. In order to reduce

the candidate region for the TPT gene we are in the process of ascertaining additional families with TPT so that positional cloning of the gene involved can be undertaken.

Other hereditary hand malformations that have been mapped on the human genome include Greig cephalopolysyndactyly syndrome (GCPS) on chromosome 7p13[17], Fanconi Anemia (FA) of which one gene is localized on chromosome 20q[18], and Holt Oram syndrome (HOS) for which chromosomal regions 14q23-24.2[19], 20p13, and 20q13.2[20] are candidate regions based on de novo structural rearrangements in sporadic patients. In all these syndromes preaxial hand malformations may occur, but as part of a complex malformation syndrome (GPCS, HOS) or as part of a multisystem disorder (FA). The gene for GCPS was recently identified as a zinc-finger gene[17]. To our knowledge, the TPT in the presently reported families represents the first in which a gene for isolated hand malformations including preaxial polydactyly has been mapped to one of the human chromosomes. Identification and characterization of the gene defect and studies of the expression pattern during embryonic development may help clarify questions about the role of this gene in other developmental processes than those in hands.

The regulation of differentiation of the developing forelimb is a complex process (see ref. 9,10 for reviews). One of the key elements involved in the formation of the separate digits is the apical ectodermal ridge (AER). Numerous transplantation experiments in vertebrates indicate that disturbance of the AER can lead to the formation of an abnormal number of digits. In the AER and in the adjacent mesoderm, homeobox genes as well as genes regulating programmed cell death, growth factors or receptors are expressed. Disruption of any of these genes might potentially interfere with normal differentiation. In what way these genes interact and which genes are involved in the formation of the five digits is still largely unknown. *Hox-4*, a homeobox gene complex is involved in the regulation of differentiation along the anterior-posterior axis of the developing limb (9,10 and references therein). The more 3' genes in this complex are expressed earlier then the more 5' genes and also have a more proximal

expression boundary, therefore *Hox-4* is a good candidate for regulation of the development of the five digits. We excluded linkage of TPT and the *Hox-4* complex therefore this gene complex is not directly responsible for TPT. It is, however, quite possible that the TPT gene product interacts with *Hox-4* and/or other genes by regulating their expression, resulting in a fine tuning of the differentiation process in the developing limb. Interestingly the engrailed-2 (*En-2*) gene is localized in the candidate region[21,22]. This is a homeobox gene thought to be involved in regulating differentiation on the dorsal-ventral axis of the developing limb[23]. Whether or not this gene is also involved in regulating differentiation along the anterior-posterior axis remains to be investigated. In *in vitro* studies the *Drosophila En* protein can act as a specific repressor of activated transcription[24]. Disruption of such a gene could lead in overgrowth of the AER or mis-regulation of programmed cell death resulting in an abnormal number of digits in the hand or foot.

On mouse chromosome 5 the *En-2* gene maps very close to the *hemimelic extra-toes (Hx)* gene and the *hammer toe (Hm)* gene[25,26]. *Hx* and *Hm* mutations cause skeleton defects of all four limbs. The dominant mutation *Hx* causes abnormalities that include preaxial polydactyly and hemimelia[27], whereas the semidominant mutation *Hm* causes the failure of the webbing between the toes to undergo normal regression during development, and is characterized by syndactyly between digits 2 to 5, resulting in the strong flexion of the second phalanx of digits on all four feet[28]. These mouse mutants *Hx* and *Hm* are not allelic but are located very close to each other as one recombination has been observed in 3664 offspring of two crosses[29]. The *En-2* gene and the human homologous of the *Hx* and the *Hm* gene are candidate genes for the disease gene in the families studied.

Between affected individuals within a single family there are large differences in expression of the phenotype. Also there are differences in the phenotypes of some of the families that have been reported in the literature. For example in the two families reported here, pre-axial polydactyly is a predominant feature, in contrast with the family reported by Nicolai and Hamel[14] where despite the

presence of triphalangeal thumbs, post-axial polydactyly and syndactyly were the most prominent features. The complex nature of limb differentiation makes it likely that this variation is the result of modifying genes.

Cloning of the TPT gene and its functional characterization will help us to understand the underlying etiology of congenital malformations of the hand and the processes that are involved in development of the limbs. The localization of the TPT gene is the first step in the process of isolating the responsible gene. To our knowledge this is the first human gene localized that is involved solely in the pathologic morphogenesis of the hand and feet. The localization of the TPT locus is also the first step in the development of a classification of polydactylies on a genetic basis. An intriguing question is whether other, sporadic as well as familial, forms of polydactyly and other hand and foot malformations are variations in the expression of the same gene defect, the result of different mutations in the same gene, or due to defects in genes localized elsewhere in the human genome.

Methodology

Family studies

Blood was obtained from 70 members of two large TPT kindreds. Diagnosis was made by means by physical examination. Thirty four family members were affected, twenty females and fifteen males. The defect was bilateral in all affected individuals. Between family members the large variability in expression of the disorder could not be related to the gender of a patient or the sex of the affected parent.

Expression varied from ulnar deviation in the interphalangeal joint of the thumb based on an delta-shaped extra phalanx, to triphalangeal index like digits instead of the thumbs, associated with thenar hypoplasia, narrow first web, additional hypoplastic digits radial to the thumb, soft tissue syndactyly between the fourth and the fifth digit and polydactyly of the fifth toe.

DNA studies

Genomic DNA was isolated from peripheral blood as described by Miller et al[30]. 315 microsatellite markers evenly distributed on the human chromosomes were selected. Oligonucleotides for amplification were obtained with a grant from the Netherlands Organization for Scientific Research (N.W.O).

Microsatellite markers were amplified in multiplex reactions and analyzed essentially as described by Weber and May[31].

Additional oligonucleotide primers were labeled during synthesis with Fluorescein Amidite (FluorePrime, Pharmacia, Sweden). PCR products were resolved according to size by denaturing gel electrophoresis (5,5 % Hydrolink, 40 W) using an A.L.F. automated sequencer (Pharmacia LKB Biotechnology AB). Data were analyzed with the Fragment Manager software package version 1.00 (Pharmacia LKB Biotechnology AB).

Linkage analysis

Pairwise lod scores were calculated for each family using the MLINK program of the LINKAGE package (version 5.1)[32] assuming TPT to be an autosomal dominant disease with a gene frequency of 0.001 and a conservative penetrance estimate of 95%. Mutation rate was set at zero and equal recombination rates between males and females were assumed. Marker allele frequencies were kept equal. Calculation of pair-wise lod scores with allele frequencies calculated from individuals marrying in into the TPT kindreds did not substantially alter results (<10%).

Multipoint analysis was performed between the TPT locus and three loci mapping to the subtelomeric region of chromosome 7q (pter-*D7S550-D7S559-D7S594*-qter) using the LINKMAP program with sex-average recombination fractions of 0.070 and 0.020 in the respective intervals. In the analysis Kosambi's map function was used.

Acknowledgments

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Chapter 4.1

MetaCarpoPhalangeal Pattern (MCPP) Profile Analysis in a family with Triphalangeal Thumb

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Introduction

Triphalangeal thumb (TPT) is a developmental disorder characterized by a long, sometimes finger-like thumb, with three phalanges instead of two. TPT is rare, and usually inherits as an autosomal dominant trait, although sporadic cases have been described[1]. The underlying genetic defect is probably situated in one of the regulator genes involved in the differentiation of the developing limb. The gene for TPT has recently been localized on chromosome 7q36 by means of linkage analysis in two large Dutch family pedigrees in which TPT was inherited as an autosomal dominant disorder with almost complete penetrance and variable expression[2]. Further clinical, molecular-genetic and genealogical study of the original two families revealed that they are connected to each other and both part of a single large kindred. As part of the project examining the etiology and different phenotypic variations of this disorder, we performed metacarpophalangeal pattern profile analysis in one of the two kindreds in which linkage analysis was performed.

The metacarpophalangeal pattern (MCPP) profile analysis is a method of measuring the length of each of the 19 tubular bones of the hand on the X-ray, and comparing this length with a standard of the normal population according to age and sex. This method is used to detect absolute as well as proportional alterations in the length of the hand bones in various birth defects and the pattern profile appears to be specific for several congenital malformation syndromes[3]. The osseal configuration of the hands in TPT patients is studied. Specific MCPP profiles in this family with TPT are described, in concordance with different clinical phenotypes. For comparison of the affected and non-affected individuals from this family with individuals with a different genetic background, the investigated population was augmented with two sporadic TPT examples and with 44 individuals randomly selected from the normal population.

Material and methods

Clinical data and detailed analysis of the phenotype in the investigated family are described elsewhere[4]. MCPP profile analysis was performed on the radiographs of 16 hands from 13 affected persons, and of 12 hands from 12 non-affected siblings from the same family with TPT (fig 1). When on clinical and X-ray examination variation in phenotype between left and right hand was noticed, metacarpophalangeal pattern profiles were determined for both radiographs (n = 3). In case the phenotype of the two hands showed no differences between each other, MCPP profile of only one hand was used for further analysis. Ages at the time of the radiological investigation ranged from 8 to 74 years. All the affected individuals from this family were proven to be gene carriers by DNA analysis[2]. The phenotype in affected individuals varied between

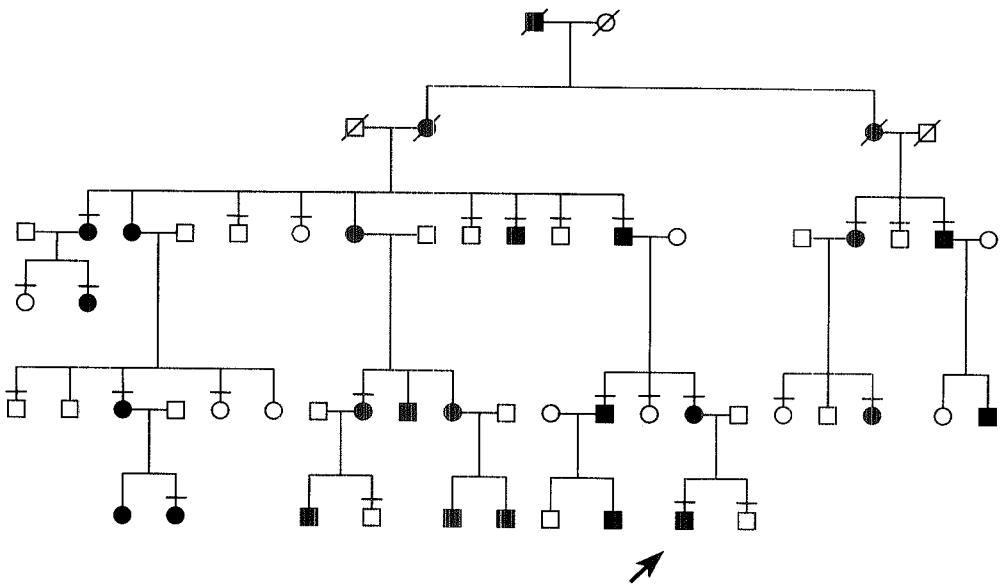


Figure 1: *Family with TPT. [-] indicates that the individual was included in the MCPP analysis. The proband is indicated by an arrow.*

non-opposable and opposable TPT. A single case of non-penetrance in which a carrier of a TPT gene had rudimentary unilateral postaxial polydactyly is included in the latter group.We used a type of MCPP analysis called the Q-score analysis[5]. In a Q-plot (graphic illustration of the Q-score), the percentage of the pathological lengthening and/or shortening of the individual bones of the hand can be directly read from the Y axis.

Radiographic measurements were obtained with a digitizer. Length measurements with epiphysis were used for all the 19 (20 in case of TPT) metacarpal and phalangeal bones and the Q-scores were determined for all the X-rays according to the method described by Dijkstra[5]. Measurements of the extra phalanx of the thumb were not included in the plot because of lack of the appropriate reference values. In order to compare the affected individuals with other individuals with the same disorder, but different genetic background, one sporadic patient with TPT and one published TPT radiograph[6] were included in the analysis. Furthermore, in order to compare the non-affected individuals from the investigated family with individuals from population at large, radiographs from 44 individuals randomly taken from the general population were also measured and analyzed.

The profiles of the obtained Q-scores will be discussed below.

In this study we have applied a new method of normalisation of the bone length measurements resulting in the so-called P-scores. These P-scores are independent of the absolute scale factor, and therefore the P-score is a descriptor of the *shape* of a length profile. The method of this normalisation has been described elsewhere[7]. One profile was represented by the score P_i, i = 1,...,19, 1 to 19 being the 19 measurements in one hand. Such a vector may be regarded as a point in a 19-dimensional space, each dimension representing one of the hand bones. If it is true that a set of P_i scores is representative of a syndrome, then points representing measurements of patients with the same syndrome will cluster together in this 19-dimensional space. Points corresponding to measurements of patients with different syndromes will lie far apart.

Even though it is difficult to imagine point distributions in spaces with a

dimensionality higher than three, procedures exist to map a set of points in a high dimensional space onto a plane, such that the interpoint distances are preserved as nearly as possible[8]. Procedure NLMAP in ISPAHAN is such a procedure[9]. This procedure was used to map the "Normal" and "Pathological" configurations onto a two-dimensional plot (fig 8). The class "Normal" consisted of the unaffected TPT family members (n=12) and the randomly selected individuals from the general population (n=44). The class "Pathological" consisted of the affected family members (n=13), one sporadic TPT patient, and one radiographic image of a TPT patient taken from a book[6].

Results

The relative lengths of the 19 bones and the profile of the Q-plot in the non-affected persons did not differ from the randomly selected individuals from the general population (fig 2). The Q-plots from the affected individuals were divided into three different subgroups according to the severity of the phenotype.

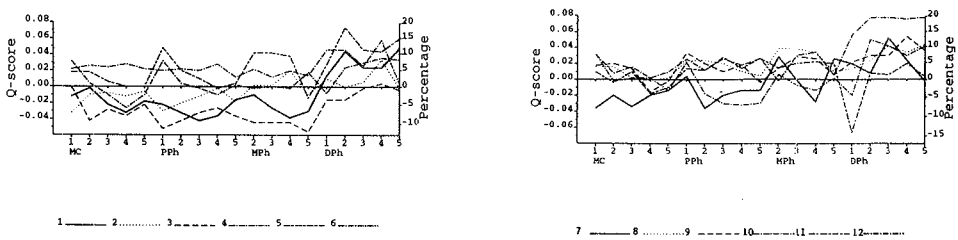


Figure 2: *Q-scores of the 12 non-affected members of the family. The numbers on the X-axis represent the hand bones. They are listed as Metacarpal bones 1 to 5 (MC1 to MC5), Proximal phalanges 1 to 5 (PPh1 to PPh5), Middle Phalanges 2 to 5 (MPh2 to Mph5) and Distal Phalanges 1 to 5 (DPh1 to DPh5). The numbers on the Y-axis represent the Q-scores for each particular bone. The zero line represents the mean of the population. Patients 1 to 6. Patient 7 to 12. The 44 individuals from the general population are not presented as there were no differences between the profiles of these individuals and the 12 non-affected members of the investigated family.*

Severity of the osseous pathology corresponded with the degree of functional impairment. The most severe phenotype was observed in the group of patients with the so called "non-opposable" TPT (fig 3). These patients have a rectangular extra phalanx in the thumb which resembles an index finger ("five fingered hand") and have no, or hypoplastic sesamoid bones - which corresponds with hypoplastic thenar muscles. None of these patients was capable of making a "pinch grip" because they had no normal opposition function. In children with non-opposable TPT, extensive thumb surgery is required at an early age to

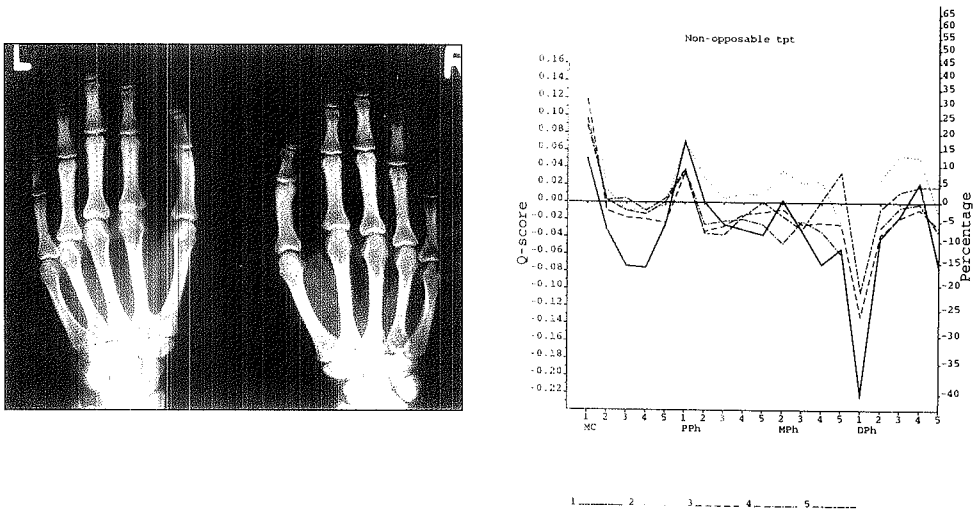
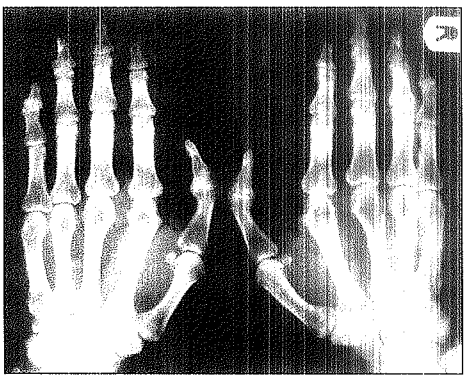


Figure 3: *X-ray and Q-plots of the hands of the individuals with "non-opposable" TPT. Notice the index-like appearance of the thumb and the percentage of the excessive length in the first metacarpals. Measurement of DPh1 in patient number 2 is lacking because of performed distal interphalangeal joint arthrodesis in the past.*

develop (reasonably) normal hand function.

The mildest phenotype was observed in the group of patients with the "opposable TPT" (fig 4). On the X-ray the thumb shows a normal configuration with a delta shaped extra phalanx in the interphalangeal joint. The sesamoid bones of the thumb (anchor places of the thenar muscles) were normally

developed as were the thenar muscles. These patients have an almost normal performance on examination of hand function. One patient from this family showed no clinical or classic radiographical signs of TPT, except for a unilateral



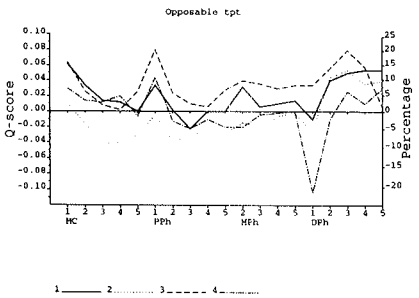
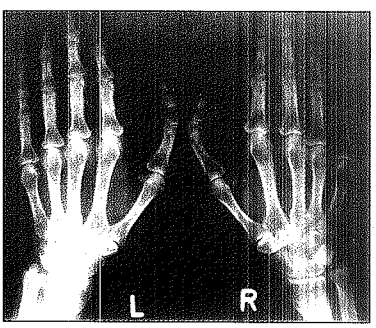


Figure 4: *X-ray and Q-plots of the individuals with "opposable" TPT. Notice the normal appearance of the hand skeleton and the mild presence of the profile.*

rudimentary *post*axial polydactyly. However, this patient was an obligate gene carrier in the pedigree and the only case of reduced penetrance, as confirmed by DNA analysis[2,4].

Finally, the MCPP plots of the patients whose thumbs showed both characteristics of the thumb and the index-finger on X-ray examination were classified as "intermediate form TPT" and are shown in fig 5.



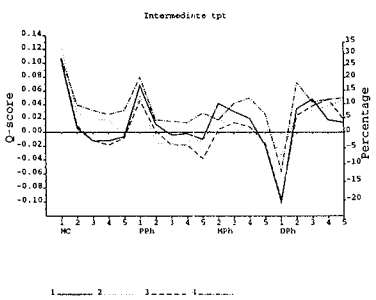


Figure 5: *X-ray and Q-plots of the individuals with "intermediate" TPT.*

On analysis of the Q-plots it appeared that all affected persons have a systematic lengthening of the first metacarpal and first proximal phalanx, and a systematic shortening of the distal phalanx of the thumb. The profile of the plots was very consistent in different phenotypic variations of this disorder, only the relative level of the "peaks" in the thumb measurements correlated with the severity of the disorder.

One sporadic TPT patient and one reproduction of an X-ray with TPT from a book[6] were included in the analysis and both showed the above described profile (fig 6 and 7). This profile appears to be characteristic for TPT in general and not only for this family.

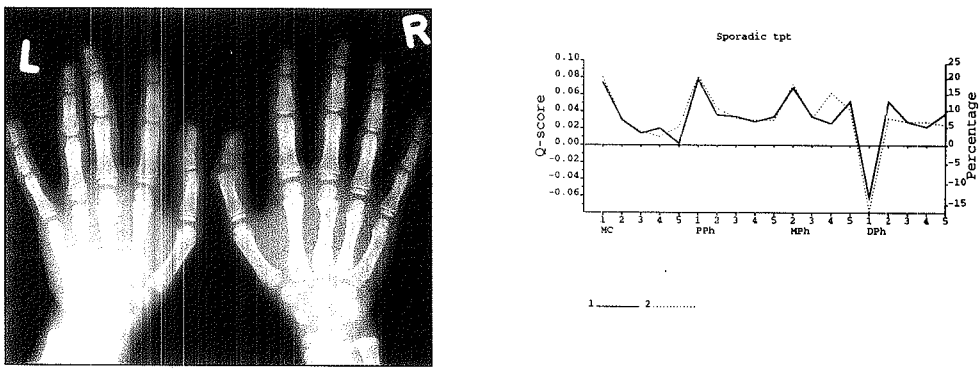
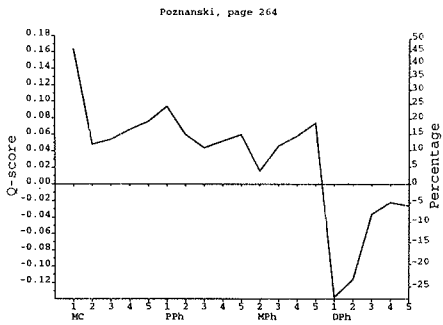
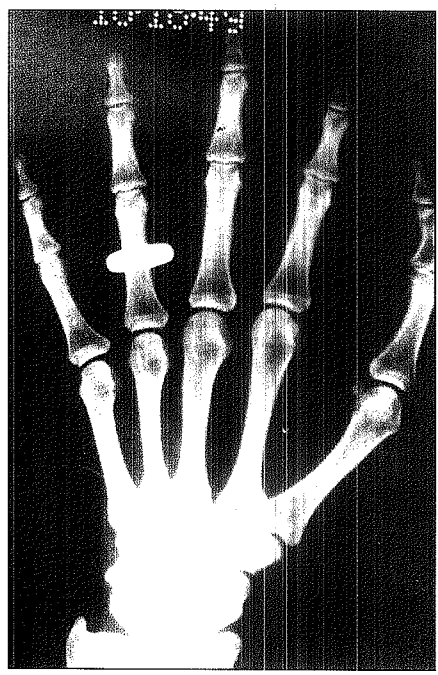


Figure 6: *X-ray and Q-plots of the hands of a patient with sporadic TPT.*

After the normalisation of the Q-scores was carried out as described above, the P-scores[7] were used for the non-linear mapping procedure. The "pathological" profiles of the affected individuals grouped in the upper right quadrant of fig 8, whereas the cluster of "normal" individuals remained in the left lower quadrant. Four points representing MCPP plots of affected persons came in the vicinity of the "normal" cluster. Two of them belong to the same person. All three persons have the "opposable" form of TPT with a very mild phenotype,

Figure 7: *Reproduction of the X-ray from a book[6] and a Q-plot.*





one of them being the only case of non - penetrance of the TPT gene in this family. One plot of a normal individual came in the vicinity of the "affected" cluster. This probably reflects a coincidental finding that this individual had brachyphalangy of the distal phalanx of the thumb.

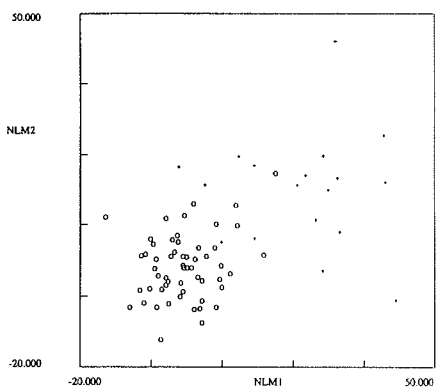


Figure 8: *Non-linear mapping procedure of the plots. [0] represents the plots of the normal individuals and [+] represents the plots of the affected individuals.*

Discussion

Triphalangeal thumb can be roughly divided, on the basis of the functional impairment, into an opposable, an intermediate, and a non-opposable category. However, the recent linkage study in TPT families[2] shows that different forms of TPT may represent phenotypic variations of a single gene disorder. The underlying defect for TPT remains to be discovered, but probably involves disturbance in formation of the antero-posterior axis of the developing limb bud.

In MCPP profile analysis, the configuration of the hand can be studied. Normally, the profile remains more or less the same for one individual throughout his or her life[3,6]. Within a syndrome there is usually some variation in the form of a profile. A particular profile is found in a number of congenital malformation syndromes. For most of the congenital malformation syndromes there is no consistent profile between independent individual patients[3,5,6].

MCPP plot analysis of the X-rays of all affected persons from the investigated family with TPT shows a consistent profile. The amount of lengthening or shortening of metacarpals and phalanges varies with the severity of the phenotype - the patients with non-opposable thumb have a larger percentage of excessive length of the first metacarpal and the first proximal phalanx than the patients with the opposable TPT. Likewise, the percentage of shortening of a distal phalanx of the thumb was smaller in the latter group. This can be explained by the presence of a fully developed mid-phalanx (not included in the plot) in the non-opposable TPT. The typical shape of the profile was also present in the only case of non-penetrance where clinical examination and X-ray of the hands have failed to show any abnormalities. However, because all the measured values fitted in the variation range of the normal population, it would have been difficult to recognize it if analyzed without comparison with other affected family-members.

A characteristic profile emerged also in the sporadic patient and in the reproduction of one TPT radiograph from a book. This suggests that the above described profile is specific for TPT and could be used as a helpful diagnostic tool in the

syndromes which include TPT. However, more research should be done to investigate the characteristic profiles of the syndromal TPT like in Holt-Oram syndrome, Townes-Brocks syndrome, Fanconi pancytopenia syndrome, etc.

An attempt was made to investigate whether MCPP analysis is adequate to discriminate a pattern profile of an affected individual from an non-affected relative. Because of the small size of the investigated population, as usually is the case with (familial) congenital malformations, it was decided not to investigate this by means of rigid statistical analysis. Instead, the potential of MCPP analysis as a diagnostic tool in this family was examined by means of exploratory pattern recognition techniques. The applied procedure indicates that profiles of individuals affected by the TPT syndrome cluster together. This cluster is well separated from the cluster formed by profiles of non-affected family members and normal individuals. Four patients with opposable TPT's lie closer to the cluster of normal individuals, confirming the mild expression of the phenotype in this group.

A characteristic profile that emerges from the MCPP plots of the members of a family with TPT is based on the measurements of abnormal lengths in the thumb bones. Clinical observation of an "index-like" thumb in the patients with non-opposable TPT suggests an underlying differentiation problem between the thumb and index-finger during limb morphogenesis. MCPP analysis confirms this by finding up to 50% excessive length in the first metacarpal in patients with non-opposable TPT.

More studies of the patterns that emerge in abnormal phenotypes of human limb malformations will be necessary in the future. During the past few years several genes responsible for congenital limb disorders have been mapped to different chromosomes in the human genome. However, their function, their role in limb development, and especially their interactions remain to be discovered. One of the late events during limb embryogenesis is the morphogenesis of the distal skeleton. Studies of the skeletal morphology have the potential, together with molecular genetic studies, to bring new insights into molecular mechanisms controlling developmental "fates" in abnormal genotypes.

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Chapter 4.2

The role of MetaCarpoPhalangeal Pattern (MCPP) Profile Analysis in the treatment of Triphalangeal Thumbs; description of a method and a case-report

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Introduction

Morphogenesis of the distal skeleton is a late event during limb development and can be affected by genetic factors, environmental factors, or both. Growth alterations which occur as a result of these influences can involve a single bone, a digital ray, or a group of bones.

At the beginning of this century, radiographic measurements of the hand bones were introduced. It was recognized that hand length correlates with stature. In forensic medicine, estimates of stature are still obtained from radiographically determined metacarpal length. The hand bones are small in patients with dwarfism and large in those with gigantism. Furthermore, it has been noticed that various congenital malformation syndromes are associated with specific hand morphology or changed proportions in the hand (Poznanski, 1984).

However, the evaluation of an abnormality is largely dependent upon the knowledge of the normal anatomy and anatomical variations. Length of the hand bones in a normal individual can vary with race, sex and familial background. In order to study hand morphology in different disorders, it is necessary to obtain standard values in a comparable population, and develop a method of discriminating between normal variation and a malformation.

During the first half of this century, different methods of radiographic measurements of the hand skeleton were developed (Achard, 1902; Parish, 1966). In the 1970s the first statistical data on length of the hand bones for children of all ages and adults became available from White American (Garn et al, 1972), Hungarian (Gefferth, 1972) and Venezuelan populations (Arias and Larralde, 1980). The normal values for Japanese (Matsura and Tadashi, 1989) and Nigerian population (Odita et al, 1991) followed.

In 1972, the metacarpophalangeal pattern (MCPP) profile analysis was developed by Poznanski et al. The MCPP profile analysis is a method of measuring the length of each of the 19 tubular bones of the hand on an postero-anterior X-ray, and comparing this length with the standard of the normal population according to age and sex. The results of this analysis can be visualized in the form of a plot.

This so-called "MCPP-plot" represents a graph of relationships between the lengths of metacarpal bones and phalanges.

Normally, the MCPP profile remains more or less the same for one individual throughout life. In most normal as well as bilaterally affected individuals there is little asymmetry between the left and right hand, so that it is sufficient to use one hand for the measurements (Poznanski, 1984). Familial similarities can be observed in normal families as well as in those with an inherited malformation. MCPP profile analysis has proved to be a valuable method to detect absolute as well as proportional alterations in the length of the hand bones in various birth defects, and the pattern profile appears to be specific for several congenital malformation syndromes (Poznanski, 1984).

As part of a project examining different phenotypic variants of triphalangeal thumb (TPT), we performed MCPP analysis on a family in which TPT was inherited as an autosomal dominant trait. The specific profile that emerged in this family and that appears to be specific for this disorder has been described elsewhere (Zguricas et al, 1997).

The purpose of this paper is to report on the possible use of MCPP profile analysis in the treatment of congenital hand deformities and specifically in the triphalangeal thumb.

Method

Radiographic measurements of each of the 19 tubular bones of the hand (Fig 1a) were obtained with a digitizer from an postero-anterior X-ray of the hand. We used the type of MCPP analysis called the Q-score analysis (Dijkstra and Venema, 1991; 1992). The Q-score is defined as "the ^{10}logarithm of the quotient of the length of the hand bone from a patient and the reference length for that bone":

$Q = {}^{10}\log (\text{bone length}_i / \text{reference length}_i)$; i = the bone number

The graphic illustration of a Q-score analysis is called a Q-plot (Fig 1b).

The numbers on the X-axis represent the hand bones listed from the first metacarpal (MC1) to distal phalanx 5 (DPh5), and the numbers on the Y-axis

represent the results of comparison of measured length of each particular bone, with the reference length for that bone. The zero line represents the mean of the population. In the Q-plot, the percentage of the (pathological) lengthening and/or shortening of the individual bones of the hand can be directly read from the (right) Y axis.

It is not the exact height of the curve which is most important, but the **profile** which results from the individual lengthening or shortening of the bones. Characteristic profiles for triphalangeal thumb are represented in Figure 2. The extra phalanx of the triphalangeal thumb is always measured, but not included in the plot because of lack of the appropriate reference values.

Our operative treatment of the bones in triphalangeal thumbs is by two reduction osteotomies: one at the level of the distal interphalangeal joint (DIP), and one at the metacarpal level. The purpose of the DIP reduction osteotomy and subsequent arthrodesis is to remove the supernumerary joint and phalanx. This is achieved by excision of the distal part of the middle phalanx, the proximal part of the distal phalanx and the distal epiphysis. Fusion is achieved by K-wire osteosynthesis and transosseous suture or wiring.

The metacarpal osteotomy has two uses. If the first digit has a normal "thumb" position, it is used for length reduction. When the first digit is more "index-like", a wedge shortening-osteotomy of the first metacarpal is performed with a rotation of 90° and abduction of approximately 35°, to reach the position in which the pulp of the thumb faces the fourth finger after fixation. Fusion is achieved by K-wire osteosynthesis and transosseous wiring.

It is not considered wise to do osteotomies at three different levels in one digital ray, and osteotomy at the level of proximal phalanx could impair tendon gliding, so the proximal phalanx is left intact.

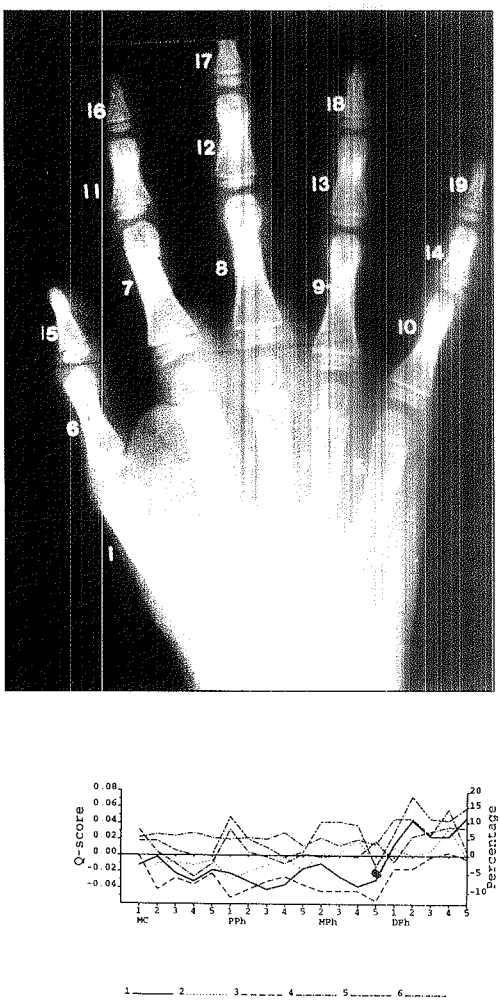


Fig 1

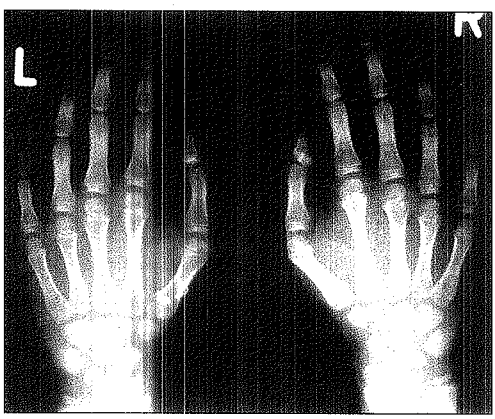
(A) Tubular bones of the hand, numbered from 1 to 19. The first to fifth metacarpals are numbered 1 to 5, the proximal phalanges 6 to 10, the middle pha langes 11 to 14 and the distal phalanges, 15 to 19.

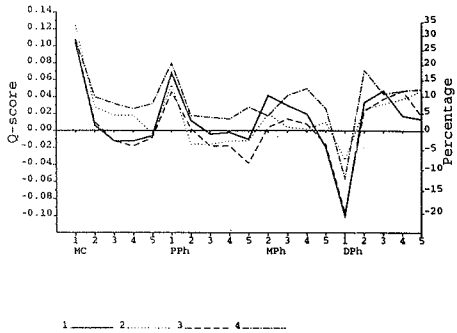
(B) Profiles of six different hands from six normal individuals; the numbers on the X-axis represent the numbers of the hand bones; the numbers on the Y-axis represent the percentage difference between the measured length of each individual bone from a patient and the reference length for that bone. The zero line represents the mean for the population. Note that there is no clear profile which can be distinguished and that all the measured values fluctuate within the normal range.

The required length of the reduction osteotomy in these patients is usually estimated from the basis of the thumb tip at the level of the proximal interphalangeal (PIP) joint of the indexfinger. MCPP
profile analysis was performed on the postoperative X-rays of five patient treated for triphalangeal thumbs a few years ago in our department in the Sophia Children's Hospital in Rotterdam. Even though clinically and on the X-ray the results appeared satisfactory, the characteristic profile for TPT on the

postoperative Q-plots was the same. The first metacarpal of the patient in Figure 3 is still 20% too long, the proximal phalanx is unchanged and the distal phalanx is 30% too short.

Fig 2 *MCPP profiles of four different patients with triphalangeal thumbs.*





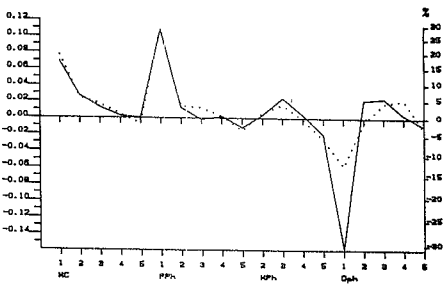
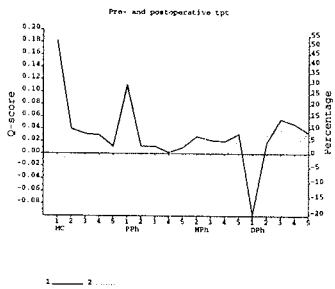


Fig 3 *(A) Postoperative X-ray and (B) Postoperative MCPP profile of a patient treated without MCPP profile analysis before operation. Note that the profile is similar to that in Figure 2.*

Case report

We present a case in which MCPP analysis was taken into account before operation. Pre- and postoperative X-rays and pre- and postoperative plots of the hand

of this patient are shown in Figure 4. On the preoperative Q-plot, the first metacarpal has 50% excess length, the proximal phalanx of the thumb is 30% too long and the distal phalanx of the thumb is 20% too short. At operation, the "missing" 20% of the distal phalanx is provided from the extra (mid)phalanx by means of reduction osteotomy and subsequent DIP arthrodesis. The required length reduction at the metacarpal and at the DIP level was calculated from the plot and measured bone lengths. In order to compensate for the extra length in the proximal phalanx, an exaggerated rotation-abduction osteotomy of the first metacarpal was done in an attempt to create an optimal thumb for this particular hand. The postoperative plot shows 65% reduction in length of the first metacarpal; on the plot, the first metacarpal is now 15% too short. However, this compensates for the extra length in the proximal phalanx, which remains intact. The newly created distal phalanx has become 25% too long, partly because of a slight degree of malunion at the level of DIP arthrodesis.



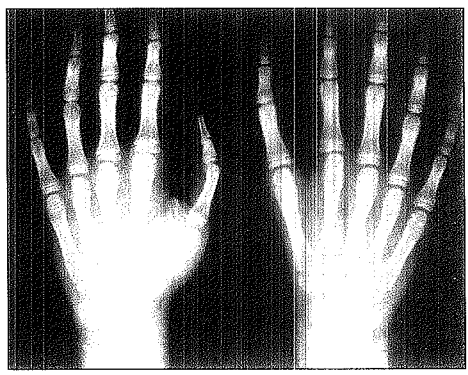


Fig 4 *(A) MCPP profiles of the left hand before (number 1) and after (number 2) surgery.*

(B) X-ray of both hands of a patient, left hand after surgery .

Discussion

Classification of congenital disorders plays an important role in diagnosis,

treatment, and understanding of aetiology. The large and confusing number of classifications of congenital hand malformations illustrates the level of our knowledge. Many patients with congenital hand malformations present with such variation in phenotype that they may be candidates for several, or none of the defined classification groups.

The usefulness of MCPP analysis in this group can be twofold: to increase the diagnostic precision, and analyse the length of the individual bones in the affected hand before operation.

This technique is already used in clinical genetics and dysmorphology as a helpful diagnostic tool. Distinct pattern profiles have been described for a number of syndromes, among others: Holt-Oram syndrome (Poznanski et al, 1972), trichorhinophalangeal syndrome (Felman and Frias, 1977), Rubinstein-Taybi syndrome (Hennekam et al, 1990). Furthermore, MCPP analysis has been used in diagnosing acrocephalosyndactyly syndromes even in patients with few clinical signs of the disorder, although it could not discriminate between the different types of those syndromes (Escobar and Bixler, 1977; Kaler et al, 1982). At least four of the acrocephalosyndactyly syndromes - namely Crouzon, Jackson-Weiss, Pfeiffer and Apert, have the same genetic origin (Heutink et al, 1995). It seems that a common genetic origin can give rise to similar MCPP profiles even when clinical phenotypes are different. MCPP analysis and charting profiles of a few family members on the same plot for comparison, can provide a way of differentiating between sporadic cases of an malformation and less penetrant familial traits (Miura, 1984; Miura and Suzuki, 1984).

Another potential application of MCPP profile analysis is the calculation of the required reduction in length of an affected digital ray before operation.

Guidelines for the length of the individual bones of the hand can be found in tables of reference values for the normal population (Garn et al, 1972; Poznanski, 1984). However, these are based on measurements in normal hands. Even though it may not be obvious, the hand morphology, or proportions in a congenitally malformed hand, may be significantly different from the normal.

A general rule in the treatment of triphalangeal thumbs is that the length of a

normal thumb should not exceed the proximal interphalangeal (PIP) joint of the index finger. This can be used as a guide to the length of the whole ray, but it does not provide a guide for the length of individual bones. The phenotypical variations in these patients, sometimes even between the left and right hand of one patient, makes an accurate guide desirable. The length of the first metacarpal shows the greatest variability. As it is also the longest of the bones involved, "gain" from appropriate length reduction in this bone is the greatest. Slight inaccuracy in the length of the newly created distal phalanx is inevitable because of the presence of the nailbed, which often hampers the "ideal" DIP arthrodesis.

MCPP analysis provides an insight into the individual architecture of a hand with a congenital disorder. Instead of imprecise estimations of lengths of bony parts in the malformed hand, the percentage abnormal length can be directly read from the Q-plot and calculated for each bone. This information enables the surgeon to make decisions about the required reduction in length, and to make an exact plan before the operation. Furthermore, as there is usually a considerable gap between operations on the hands in bilaterally affected patients, the problem of creating two symmetrical hands is solved.

MCPP profile analysis is a cheap and a simple procedure. The necessary equipment consists of a personal computer with the necessary software, printer, plotter and a digitizer. A Q-plot of one patient takes less than 5 minutes to produce.

To investigate the possible future role of this method in congenital hand surgery, larger groups of patients with different hand malformation syndromes should be studied.

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Chapter 5

Psychomotor development of children with Triphalangeal Thumbs: an exploratory study

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Submitted

Introduction

One in approximately 626 newborns has a congenital malformation of the upper limb[1]. They can occur as an isolated malformation, in combination with other hand and/or foot anomalies, or as part of a syndrome[2]. Growing insight into the genetic and embryologic basis of the (human) limb malformation phenotypes is in contrast with relatively little knowledge about the effects of congenital hand function impairment on the psychomotor development of the affected child.
In order to outline the backgrounds of this study, a brief overview of the evolutionary development of the human hand, followed by a short outline of the role of the hand function in the normal development of a child, will be given.

The hand in phylogenetic perspective

All primates have pentadactylous, convergent hands. Convergence and divergence of the digits make grasping and holding of the objects against gravity possible[3]. Hand morphology of the great apes and man show a newly developed functional domain: an opposable thumb. From here on, the power grip and precision grip can be distinguished[4]. Variation of hand function in primates is a matter of the length, and the degree of divergence and opposability of the thumb[3].
In humans, hand function can generally be divided into executive and perceptual function. In both functions, the hand can be considered to have two parts: the thumb and the rest of the hand. The thumb, or the "Lesser hand" as Albinus called it, is the cornerstone of all the skilled procedures the hand is capable of[5]. Furthermore, an opposable thumb enables us to use the hand as a unique organ for the perception of form, which could have been an important drive in cerebral development and hominization. The human hand can encompass an endless amount of sets of data regarding the shape of an object. Vice versa, our hands can be used for shaping of forms non-existent in the

environment which introduces a new quality: abstraction[6].

Another important development which took place during hominization is the development of speech. Some anthropologists speculate that precision movements of the human hands have had a semantic role in development of gestural language. A shift from the gestural language system to a vocal one involved tremendous changes in the structural anatomy of the vocal tract, neuronal networks and central nervous system. However, a peculiar association of right-handedness and left-hemisphere dominance for both language skills, and manual precision handling in humans, suggest a close relationship of hand function and speech[7].

Hand function and the psychomotor development of the child

During the first month of life, an infant holds his hands in tight fists that clench on contact. At two to three months voluntary grasp starts to develop. At five months, palmar grasp is developed, and at seven months opposition is being exercised. A child starts to crawl at approximately 10 months, which increases the opportunities for exploration. At this age, tripod pinch and tip pinch are well developed. At 18 months, the basis for manipulative prehension is present[1,8,9].

Closely intertwined with the development of motor functions, is the development of our hands as organs of perception. Of particular importance is the haptic perception - also defined as "active touch". Where passive touch involves only the excitation of the receptors in the skin and underlying tissues, haptic perception deals with size and shape of the objects through manual and in-hand manipulation[10].

This first developmental phase, from 0 to 24 months of age, is according to Piaget the sensory-motor phase. The stepwise development of motor skills leading to hand-eye coordination enables the child to explore and master the world in a practical way, by way of daily exercise; hence, it is the phase of "practical intelligence". Accurate manipulation confers to the young child a sense of virtuosity and pride. This enhances the infant's will to perform ("funkionslust"), and

triggers the mother's pleasure in her child[11]. Furthermore, the exploratory play of the first years of life plays an important role in the interactions with the environment, and in interpersonal contacts[12].

Fine motor skills as well as haptic perception of the hands are both depending on good opposability. Limitations in motor or perceptual processing could affect not only the motor performance, but also the child's ability to learn. Manipulative tasks require the greatest interaction of cognitive and motor capacities. This is also reflected in the fact that most intelligence tests require demonstration of fine motor skills to complete the performance component of the test battery[13].

The malformed hand

Consequences of a congenital hand malformation on executive hand function can be well investigated by means of thorough clinical examination. The influence of a hand disorder on the haptic perception is more difficult to assess and the consequences for the development even more so. A majority of congenital hand disorders will impair the child's fine motor skill performance, and thus can influence a variety of activities: from personal hygiene and getting dressed, to performance at school, in play and communication. It is the impairment of the fine motor skill performance and its influence on the development that we are interested in.

Since a couple of years, our departments are conducting research towards etiology and pathogenesis of triphalangeal thumb (TPT) and associated hand malformations. Triphalangeal thumb is a part of the expression ray of radial polydactyly. It is a rare disorder, and it is usually inherited as an autosomal dominant trait. Almost all phenotypic variants of TPT are associated with a certain degree of thenar hypoplasia, which makes this disorder particularly interesting for the hand surgeon. We performed an observational study on 18 children with TPT. The purpose of our study is to explore the influence of this isolated congenital hand disorder, and in particular opposition impairment, on the psychomotor development of a child. We tried to achieve this by means of hand function

examination, a semi-structured interview with the mother considering the development of her child, the so-called "Hand test"[14], and the "Child Behavior Checklist" (CBCL)[15,16].

Patients, materials and methods

Eighteen children with triphalangeal thumbs were investigated. All children and their families participated in our earlier study on the genetic basis of triphalangeal thumbs and associated hand malformations[17,18]. This study is approved by the Medical Ethics Committee of the Erasmus University and University Hospital Rotterdam (Project no. MEC 118.150/1992/57).
The age of the subjects at the time of examination varied from three to sixteen years. Six children were not surgically treated for their hand malformation. Nine of the investigated children were surgically treated at our department at the Sophia Children's Hospital, and three children were treated elsewhere. As it would surpass the scope of this paper, the kind of operative procedure, the number of procedures, and the time of surgery are not evaluated.
The investigative procedure was composed of the following parts:

Hand function examination

- prehension testing: cylinder grip, ball grip, lateral pinch, tip pinch and palmar pinch[1,4], adjusted for age (Fig 1);
- precision handling and complex hand actions: painting, drawing, writing and cutting shapes with scissors[1,19], adjusted for age.

Semi-structured interview with the mother

All mothers were asked the same questions about the developing manipulative skills of their child from infancy up to present, of which a written report was

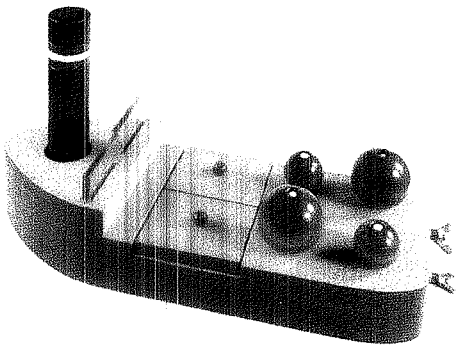


Figure 1. *The "Hand-boat" is developed by the "Group Hand Therapy for Congenital Defects of the Upper Extremity" and is being used in the University Hospitals and Rehabilitation Centers in The Netherlands for hand examination of children from two to ten years of age. The following grips can be observed at play: cylinder grip (chimney), ball grip (large and small ball) , tip pinch ("take the hatches off"), lateral pinch ("take off the windows"), and palmar pinch (supporting bars for the balls). Manipulation is tested by butterfly nuts at the back of the boat ("take off the motor").*

made. This report was then "scored" for the following five items:

- late speech development and/or speech therapy;
- prolonged "mouthing";
- prolonged "oral fase";
- preferences for certain types of clothing or shoes;
- presence or absence of crawling.

The reasons these five items were chosen to focus on are the following:

- late speech development and/or speech therapy could indicate a relationship between impairment of fine motor skills and speech development;
- prolonged "mouthing" and prolonged "oral fase" can indicate compensatory use of the mouth as an organ for haptic perception;
- preferences for certain types of clothing or shoes, or rather avoidance of it (for example jeans, shoes with laces, or shirts with buttons), can be used as an estimate of the fine motor skills used in every-day-activities;
- presence or absence of crawling was introduced after a few interviewed mothers had made a spontaneous report of the fact that their affected children had never crawled.

Hand test

Children above the age of six were submitted to a Hand Test. The Hand Test is a diagnostic technique used in psychiatry and clinical psychology. The Hand Test consists of ten cards approximately three by five inches in size, and is based on the use of hand pictures as a projective medium (Fig 2)[14]. The subject is asked to tell what the hands on the cards are doing. In this way, the subject must "project". It is assumed that action tendencies will be projected into the pictures of hands, since the hands are crucial for interacting with, and relating to the external world.

Figure 2. *Card number 1 from the Hand Test*

The answers given during the hand test can be classified in four different categories: interpersonal, environmental, maladjustive and withdrawal responses. The *"interpersonal"* and *"environmental"* responses are considered as normal, and are reporting on interactions with other human beings and inanimated objects. Normal subjects give approximately the same number of "interpersonal" and "environmental" responses.

"Maladjustive" responses represent difficulty in successfully carrying out various action tendencies because of inner weakness and/or external prohibition. It usually indicates distress arising from a failure to achieve need satisfaction.

"Withdrawal" responses represent a more severe reaction to life's problems.

Withdrawal responses usually indicate withdrawal from real situations, and varying degrees of inappropriate behavior. The "withdrawal" responses can manifest themselves in three different ways:

- a "*failure*" to give any answer what so ever;
- in a so-called "*descriptive*" response with short descriptions of the hand picture, but without comment on intended action;
- "*bizarre*" response which is pathognomonic for psychosis.

The "maladjustive" and "withdrawal" responses usually do not occur in normal scores, even though occurrence of one or two does not necessarily imply mental pathology[14].

All the answers as well as time needed to produce an answer are recorded. "Average Initial Reaction Time" or AIRT is calculated and is used as an estimate of the time needed to organize and verbalize a perception[14].

Child Behavior Checklist (CBCL)

Both parents were asked to fill in this standardized questionnaire designed to report the behavior of children as assessed by their parents[20,21,22]. The questionnaire consists of 20 competence items and 120 problem items. The competence items are designed to measure the child's positive skills. In the problem section of the CBCL questions are asked on a broad range of problem behavior. The parents are requested to rate the problem items on a three-point scale: 0 if the item is not true for their child, 1 if the item is somewhat or sometimes true, and 2 if the item is very true or often true. A total problem score is computed by summing all the ratings. The higher the score, the more problematic the child's behavior.

The CBCL was translated into Dutch, and the good reliability and discriminative validity established by Achenbach[15] were confirmed for the Dutch translation[16].

All children and participating family members were seen by one of the authors (JZ, DR). The investigative procedure of the children was recorded with a video-camera.

Results

The investigated population is heterogenous concerning the age of the subjects, and the number of hospital admissions, surgical treatments and complications. The population is homogenous concerning the type of congenital hand malformation, and its genetic background[17].

All children were affected bilaterally. Based on the degree of thenar hypoplasia, opposition impairment, clinical and X-ray appearance of the thumb, affected children were "classified" into one of the following three categories: opposable TPT, intermediate TPT and non-opposable TPT. The thumbs of children with opposable TPT have the appearance of a normal thumb with slight clinodactyly in the interphalangeal joint. On the X-ray, there is a small, delta shaped extra phalanx in the interphalangeal joint. The sesamoid bones of the thumb (an-chor places of the thenar muscles) are normally developed, as are the thenar muscles (Fig 3).

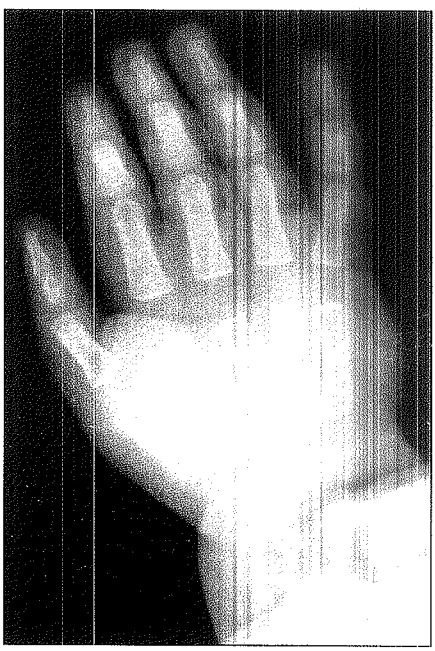
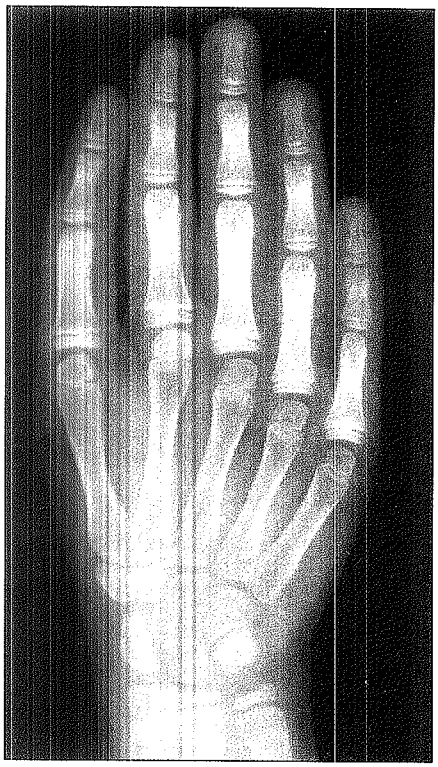


Figure 3. *X-ray of a hand of a child with opposable triphalangeal thumb. Thenar muscles are well developed and there is no opposition impairment.*

The most severe phenotype was observed in the group of patients with the so called non-opposable TPT. These patients have a fully developed, rectangular extra phalanx in a thumb that resembles an index finger ("five fingered hand"). These children have no, or hypoplastic sesamoid bones - which corresponds with hypoplastic thenar muscles. Furthermore, these children have a

Figure 4. *X-ray of a hand of a child with non-opposable triphalangeal thumb. Thenar muscles are hypoplastic and there is only "pseudo-opposition".*



narrow first web space, and the thumb is placed in the same plane as the other digital rays (Fig 4). Finally, patients whose thumbs showed both characteristics of the thumb and of the index-finger on clinical and X-ray examination, and had partial thenar hypoplasia were classified as intermediate TPT (Fig 5)[23].

Six children were classified as opposable TPT, five as intermediate TPT and seven as having the non-opposable form of this disorder. None of the children from the "opposable group" were surgically treated.

Hand function examination

Hand function examination of the children with opposable TPT revealed no significant disturbances of the hand function. Thenar muscles were well developed in all subjects and there was no opposition impairment. All types of pinch and grip were available and complex hand actions were smoothly performed.

All children with intermediate and non-opposable TPT's were surgically treated, and all were examined at least one year after surgery, except one patient who was examined six months after surgery. These children showed no function impair-

ment with respect to the powergrips. Cylinder grip, ball grip and lateral pinch were also present. However, palmar pinch and tip pinch were often replaced by lateral pinch, especially in the latter group. Two children with intermediate TPT and all children with non-opposable TPT showed some difficulties in handling scissors and writing.

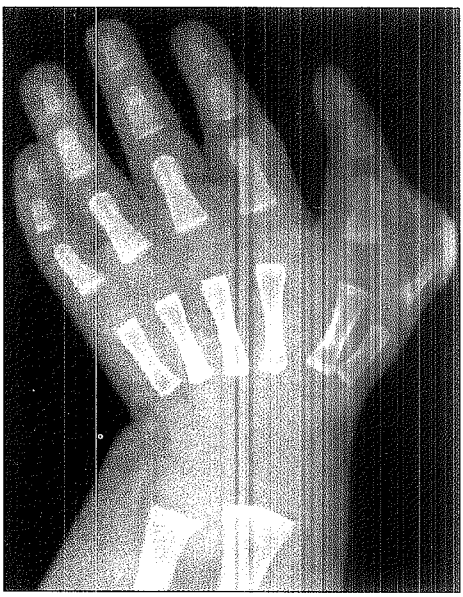


Figure 5. *X-ray of a hand of a child with intermediate triphalangeal thumb. There is partial hypoplasia of the thenar muscles and varying degrees of opposition impairment*

Semi-structured interview with the mother

The results from the semi-structured interview taken from the mother are summarized in Table 1. The majority of the children with opposable TPT were reported to have had late speech development (first words after 24 months) and/or speech therapy. Using mouth as an organ for haptic perception after the age of two was observed in none of the children with opposable TPT, but in three out of five children with intermediate, and in three out of seven children with non-opposable TPT. The children with prolonged oral fase were evenly distributed over the three groups. All children with clothing preferences have intermediate or non-opposable TPT. Finally, children that were reported never to

Table 1: *Maturational problems and immature behavior in TPT children. Signs ¥, ◊ ,* , • indicate siblings.*

sex (M=male, F=female) and age at examination	problems with dressing, like jeans and/or laces	mouth for haptic perception > 2y.	presence of crawling	speech therapy or late speech development	thumb sucking, long dummy or bottle
OPPOSABLE TPT					
M 8 years ¥			yes	first words at 2,5 years	still thumbsucking
M 10 years ¥			yes	first words at 18 months	still thumbsucking
M 9 years			yes	speechtherapy	
F 9 years ◊			yes		
M 7 years ◊			yes	speechtherapy	still thumbsucking
M 4 years ◊			yes	talks at 3 years	
INTERMEDIATE TPT					
M 8 years	jeans		yes	first words at 4 years	dummy > 5 years
M 8 years		+	yes	speechtherapy	dummy > 5 years
M 13 years	jeans, laces		no		dummy > 5 years
F 10 years *	jeans, laces	+	yes		still thumbsucking
M 15 years *	jeans, laces	+	yes	speechtherapy	
NON-OPPOSABLE TPT					
M 4 years			no		
M 10 years	jeans, laces	+	yes	first words at 2 years	
M 3 years			no		
F 14 years •			no		thumbsucking until 13 years of age
F 16 years •			no		thumbsucking until 9 years of age
F 5 years	jeans	+	yes		still thumbsucking
M 4 years	laces	+	yes		

Table 2: *Four types of responses during the Hand Test (14). The Hand Test consists of ten cards with hand pictures. Interpersonal and environmental answers are considered as normal. Maladjustive and withdrawal responses usually do not occur in normal scores. In particular the "wihdrawal" responses are indicative of lack of internal strength (14).*

sex (M=male, F=female) and age at examination	number of interpersonal responses out of 10 cards	number of environmental responses out of 10 cards	number of maladjustive responses out of 10 cards	number of whithdrawal responses out of 10 cards
OPPOSABLE TPT				
M 8 years	3	3	0	4 (failure)
M 10 years	2	8	0	0
M 9 years	3	5	0	2 (descriptive)
F 9 years	4	4	0	2 (failure)
INTERMEDIATE TPT				
M 8 years	6	3	0	1 (descriptive)
M 8 years	4	2	0	4 (failure)
M 13 years	6	3	0	1 (failure)
F 10 years	5	3	0	2 (descriptive)
M 15 years	9	0	0	1 (failure)
NON-OPPOSABLE TPT				
M 10 years	5	3	0	2 (descriptive)
F 14 years	3	3	0	2 (descriptive) 2 (failure)
F 16 years	5	2	0	2 (descriptive)

have crawled also have intermediate or non-opposable TPT.

Hand test

Twelve children older then six were submitted to a Hand Test. The results are summarized in Table 2. Note that no "maladjustive" responses were recorded. The most interesting result from the Hand test is the high amount of "withdrawal" responses in the investigated population. Two types of "withdrawal" responses occurred: failures to give a scorable response whatsoever to a particular card, and descriptive responses. The latter represent a feeble, "safe" reaction to reality. Both types of answers can occur in a normal population, but are found to be specific for organic disorders[14]. Furthermore, most of the examined children had a long "Average Initial Reaction Time" (high AIRT). The high AIRT implies difficulty with the presented situation. The subject is threatened by the cards and must take time to absorb the stimuli, recover and erect suitable defences to neutralize the threat.

Child Behavior Checklist (CBCL)

Parents of all children provided Child Behavior Checklists (CBCL). The scoring procedure for Dutch CBCLs is described by Verhulst et al. in 1996[16]. Both the scores from the competence scales and the scores from the problem scales were within the normal range for all investigated children. Because of the small sample size, no further statistical analysis was performed.

Discussion

An exploratory, observational study of the role of congenital hand dysfunction on the psychomotor development of the affected child is performed on 18 children with triphalangeal thumbs. The small sample size, the variety of the sample composition with respect to age and previous medical history, and lack of

standardized procedures for this type of studies, made it necessary to chose for a broad approach. Even though the size of the investigated population does not allow to draw any "firm" conclusion, some interesting observations have been made.

The hand function examination revealed that none of the investigated children had functional problems with respect to the power grip(s). This finding is in concordance with the clinical and intraoperative observations that the extrinsic muscles and musculus *adductor pollicis* are well developed, and that the "anatomical substrate" of this disorder is localised on the radial side of the thumb. When in 1962 Landsmeer introduced the term "precision handling", he observed that in the power grip the thumb is *adducted* in both the metacarpophalangeal and the carpometacarpal joints, while in the precision grip the thumb is *abducted* in both joints[18].

The children with intermediate and non-opposable TPT showed varying degrees of dysfunction with respect to the precision handling. The most important underlying anatomical substrate in these groups is aplasia, or hypoplasia of the thenar muscles. This muscle group is of crucial importance for the rotatory movements in both the carpometacarpal, and metacarpophalangeal joint. It is rotation of the thumb in the opposing position that is required for almost any hand function. These rotatory movements are responsible for the great flexibility of our hands as fine instruments and their adaptability as instruments for perception of shape.

A semi-structured interview with the mother provided information on individual child development. From Table 2 it appears that six out of twelve children with functional problems still have, or have had aversion towards jeans (buttons) and shoe laces. The same group of children was using their mouth as an organ for haptic perception after the age of two. In an infant, the hands and the mouth are potential sources of haptic information. Early haptic discrimination using the mouth is seen at one month, and haptic discrimination using the hands appears at one to two months of age. Starting from 6 months, when opposition begins to develop, mouthing is usually replaced by manual

manipulation. By one year, mouthing gradually begins to disappear. Prolonged mouthing could be a sign of immature somatosensory system[10]. In the investigated population, it probably indicates a compensation mechanism for lack of opposition, and inability of the infant's hands to provide sufficient haptic "input". A mother of one child with non-opposable TPT provided an illustrative example: her eldest (affected) son she used to call "little vacuum-cleaner" because he used to put literally everything in his mouth until the age of eight (at this age surgery was performed). This in contrast to her youngest son (not affected), who used his mouth only during the first few months of his life.

Five children from the non-opposable group and one child from the intermediate group were reported by their mothers never to have crawled. Some authors suggest that early crawling experience can influence later development of sensory and motor systems of the body and general motor skill development[24]. Other studies propose that the development of crawling can be seen as one of the prerequisites for the development of cognition[25]. A relatively large proportion of non-crawling infants in our investigated population could be a coincidental finding. Even though it is tempting to speculate on the possible relationship of a congenital hand anomaly and non-crawling, the sample size does not allow to draw any conclusions.

When we look at the reported language development, it appears that five children with a very mild disorder with no measurable functional consequences have had speech therapy and/or late language development. This observation is more difficult to explain only from the functional point of view. However, in most of the congenital malformations there is no collinearity between the "anatomical substrate" of the malformation and the functional disability it can cause. A full meaning of the word phenotype trespasses the boundaries of the body and involves the ways one is dealing with it. At this point, anatomical substrate of a disorder, individual coping mechanisms, and environmental influences come together.

From ancient times, congenital malformations have attracted interest and provoked different speculation on their etiology and/or meaning. Probably the

oldest records are from Babylonia, estimated to be over 4000 years old. Babylonian priests considered congenital malformations as omens for events to come. In other cultures, new theories and beliefs developed. One of these theories is the one of "maternal impressions" in which events encountered by the pregnant woman can cause congenital malformations. This theory probably gave way to the concept of congenital malformations as punishment by the gods, which is still very vivid in many countries, including Europe and United States[26]. This could provide a partial explanation for the feelings of guilt that most mothers of sporadically affected children express.

The same mechanism may also be present in affected families, although it follows a slightly different path. The question of "guilt" which inevitably arises when an affected child is born, need not be "asked": clearly, the affected parent is "responsible" for passing "it" on. Many affected parents who participated in our research showed an extreme interest in the possibilities to "extinct" a congenital disorder. Two affected mothers with one affected child each, "confessed" that they have had a sterilisation procedure performed after the birth of their first (affected) child. Apparently, the burden on them in their childhood was so heavy, that they could not bear the idea of a possibility of giving birth to another affected child.

Because of the important role the hands play in daily living and communication, congenitally different hands will always attract attention. One mother that participated in our research expressed regret that her child was not born with a heart defect instead, since such a defect would not have been visible.

As children tend to "mirror" themselves in their parents, it is possible that they "inherit" a part of their reaction patterns. If the affected parent has not yet learned to accept his hand problem, he/she may not be able to teach the affected child to handle the daily confrontations with his or her congenital "difference", which will, eventually, influence the coping capacity of the affected child itself.

An illustrative example of projection of the parents fears into their child is that of a child with bilateral preaxial extra rays in the form of floating thumbs. The child's mother was afraid the child could "suck off" his floating thumbs and

swallow them. The floating thumbs were surgically removed at the age of three months - until then, the child wore gloves firmly tied up around its wrists.

The Hand Test was used to focus on the role of the hands as an instrument of interaction with the outside world and other human beings. Analysis of the results from the Hand test suggests that dealing with hands and hand actions, poses difficulties for the investigated subjects. The most striking feature among the results is the high amount of "withdrawal" responses which can be subdivided in "failure" and "descriptive". According to the Hand Test Manual, "failure" responses, especially if two or more occur in a single protocol, are the best indicators of "organic" disorders. "Descriptive" responses are the next best indicators. The guarding behaviour during this part of interview in combination with the high "Average Initial Reaction Time" suggests that most of the children from the investigated population are experiencing the hand stimuli as threatening.

Finally, the Child Behavior Check List (CBCL) was used to assess child psychopathology. Even though our observations so far suggest specific developmental difficulties at the level of fine motor skills and language development, the Child Behavior Checklist (CBCL) results indicate that this group of TPT children show no signs of psychopathology compared with normative children of the same age and sex. CBCLs provide a quantitative measure of parents remarks of children's problem behaviours.

The absence of psychopathology in the examined TPT children is in accordance with our general judgment: the investigated children and their parents are good natured and optimistic and they tend to minimize their daily problems. Only the focused interview with the mother, with particular emphasis on psychomotor development in infancy, the actual hand function examination, and the Hand Test as a global mirror of the child's inner view of the hand with all its modalities, give a sharper clinical picture of a TPT child.

The complexity of the factors involved and difficulty in finding the "palpable parameters" to measure when investigating this aspect of hand malformations, hampers research in this field. The observed phenomena could be congenital, a

consequence of congenital hand disfunction, or caused by hospital admissions. Factors at play include the age at which surgical correction is performed, the number of operations and hospitalisations, occurrence of complications and the way they were treated, and last but not least, interference with school. The quality of the parent-child relationship is another crucial factor.

Fine hand function plays an important role in the motor, social and psychological development of a child. Even though no psychopathology is recorded among the investigated children, our observations indicate specific developmental difficulties at the level of manipulative motor skills and language development. This emphasizes the importance of timing surgery as early as possible, and keeping long follow-ups of the child and its parents. A malformed child affects the whole family. It is important for all those involved in the treatment of children with congenital hand malformations to know the factors that are at play. Developmental domains like fine prehension and daily manipulative skills, (precursors of) language, writing, drawing etc. deserve particular attention. Furthermore, immature behaviour patterns (e.g. oral fixations) not in tune with the child's age might need further attention. The treatment of congenital hand malformations should not include only surgery and hand therapy, but should begin after birth, and end only after the growth and development are fulfilled.

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Chapter 6

General Discussion

The phenotype in the kindreds with triphalangeal thumb described in this thesis, can be classified as preaxial polydactyly type II, and preaxial polydactyly type III according to Temtamy and McKusick[1] (Chapter 1.1). Consistency of the phenotype among the affected family members together with their geographical origin, suggested that the described phenotypic variants share common genetic background (Chapter 2). Using linkage analysis in this family material, the TPT gene was localized at the tip of the long arm of chromosome 7 (Chapter 3).

An American study of a large Dutch kindred in which the affected members exhibited preaxial and postaxial polydactyly, as well as syndactyly of the upper and lower limbs, assigned this phenotype classified as polysyndactyly, or preaxial polydactyly type IV according to Temtamy and McKusick[1], to the same chromosomal region of chromosome 7 as the TPT gene[2]. Genealogical and DNA analysis demonstrated no connection of this kindred with the "Rotterdam families". This finding suggested that the two phenotypes could be caused by different mutations in the same gene (allelic heterogeneity), or by mutations in two different, but closely linked genes (locus heterogeneity). Even though the affected individuals from the "American study" also had TPT, the phenotype in this population was more pronounced at the ulnar, or postaxial side of the hand, with postaxial polydactyly, and marked syndactyly of the ulnar digital rays. The predominant feature in our patient population was TPT, often associated with preaxial extra digits. However, in almost 50% of the affected individuals it was associated with rudimentary postaxial polydactyly and/or syndactyly of the hands and/or feet. In view of this finding, and the fact that TPT and polysyndactyly are possibly caused by mutations in the same gene, the question arose whether this gene also could be responsible for isolated postaxial polydactyly, and isolated syndactyly. No evidence of linkage of isolated postaxial polydactyly, or isolated syndactyly, with the locus at chromosome 7q36 was found in our group. Linkage analysis of isolated postaxial polydactyly by means of genomic search is currently under way. However, these preliminary results already point out that preaxial and postaxial polydactyly have a different genetic background.

Meanwhile, two more studies linked the phenotype of triphalangeal thumbs in

two affected families - one of North American, and one of Indian origin, to the gene locus on chromosome 7q36[3,4]. Current research in our group localized three more slightly different TPT phenotypes in families with different ethnic background to the same locus (unpublished results). All these phenotypes can be classified as preaxial polydactyly type II or III[1]. Preaxial polydactyly type I refers to duplications of *biphalangeal* thumbs at various levels. Contrary to other types of preaxial polydactyly, this disorder is more often seen sporadically, than as a familial trait. We hope in the near future to be able to demonstrate whether this type of thumb polydactyly also can be related to the locus at 7q36.

Recently, linkage analysis was performed in our group on a three generation Cuban family affected with bilateral non-opposable triphalangeal thumbs, and preaxial polydactyly of both feet. Interestingly, one girl from this family showed bilateral tibial aplasia in addition to the preaxial polydactyly of all four extremities. The phenotype in this family is linked with chromosome 7q36 (unpublished results). This finding raises a number of questions concerning the function of the TPT gene. Next to tibial aplasia, the above mentioned patient also showed a mild degree of radial hypoplasia on the X-ray. Linkage of this phenotype with the 7q36 locus implies that the TPT gene could be involved in the pathogenesis of both, radial and tibial aplasia or hypoplasia. Tibial aplasia or hypoplasia without associated duplication of the fibula, but with preaxial polydactyly of the toes and fingers, is known as a rare autosomal dominant trait with variable penetrance and expressivity[1]. It is worthwhile to test such families for linkage of this disorder with chromosome 7q36. Radial aplasia or hypoplasia occurs only sporadically, which limits the possibilities for genetic research at this moment. However, once the TPT gene is cloned, patients with this malformation can be examined for mutations in the TPT gene.

Recently it was demonstrated that mutations in the human HOXD13 gene are associated with synpolydactyly (SPD) phenotype[5]. This malformation is defined as syndactyly of the third and fourth fingers as well as syndactyly of fourth and fifth toes, associated with polydactyly of the same fingers and toes[1]. It is usually inherited as an autosomal dominant trait. Another two kindreds with the same

phenotype were reported to be caused by the mutations in the same gene[6]. The phenotypes of the reported SPD kindreds, homozygous and heterozygous[7,8], correspond well with the recently reported phenotype in mice with targeted deficiency in the Hoxd complex[9]. When this disorder is compared with preaxial polydactyly, there is a remarkable overlap of the phenotypes. Both disorders are characterized with the presence of poly- and syndactyly. However, the most striking difference between the synpolydactyly, and preaxial polydactyly phenotypes, is the thumb involvement. No subjects from the reported kindreds affected with SPD phenotypes show thumb polydactyly. In the "Derbent kindred"[8] affected with SPD, only one subject has been reported to have duplication of halluces. Description of this phenotype is in concordance with the expression of the Hoxd13 gene in the chick limb bud[10]. Hoxd13 is expressed during late developmental stages in the embryonic upper and lower limb, from the posterior margin of the limb anteriorly to the condensing cartilage of digit two in the wing, and digit one in the leg[10]. This finding provides a possible explanation for the presence of double halluces, and absence of thumb polydactyly in patients affected with synpolydactyly. It also provides a suggestion that the TPT gene is involved in the "shaping" of the preaxial limb border. Overlap in spatial and temporal activation patterns could provide an explanation for overlapping phenotypes.

At present, the critical region for the TPT gene has been narrowed down to approximately one million base pairs. This area contains multiple genes, and these genes are considered candidate genes for TPT. Functional and mutation analysis of these genes is currently underway. Once the TPT gene is identified, functional analysis can be performed in the spontaneous mouse mutants, the so-called *Hemimelic extra-toes (Hx)*, and *Hammer toe (Hm)* mouse, which show great similarity with the "Rotterdam" and "American" preaxial polydactyly phenotypes, respectively. The *"Hx"* mouse has preaxial polydactyly of all four extremities, bilateral tibial aplasia, and mild radial hypoplasia. This phenotype shows great resemblance with the phenotype of the proband of the Cuban TPT family, and a more severe phenotype than preaxial polydactyly type II and III

described in the "Rotterdam" TPT families. The *"Hm"* mouse has marked syndactyly of postaxial digits, and resembles the phenotype of polysyndactyly, or preaxial polydactyly type IV, reported in the American study. Both mutations are localised at the mouse chromosome 5, in a region that is syntenic with human chromosome 7q. In our group, studies of human genotypes and phenotypes are combined with studies of the genotypes and phenotypes in these two spontaneous mouse models. As already mentioned, preaxial and postaxial polydactyly appear to be separate genetic entities, which suggests that different genes are involved in the definition of the patterning and outgrowth of the radial (preaxial) and ulnar (postaxial) border of the limb. The TPT gene could be one of the "old" genes involved in the outlinement of the preaxial portion of the limb, which acquired new function(s) during the evolution. Homo sapiens is the only species which developed a fully opposable thumb. Association of TPT with thenar hypoplasia and opposition impairment suggests involvement of this gene in the development of thenar musculature. Future functional analysis of the TPT gene, and comparison of the amino acid alignment of this gene in humans and mouse could bring interesting evolutionary insights.

It has been known for a long time that programmed cell death (PCD), or apoptosis, plays an important role in sculpting different parts of the body. Separation of the embryonic digits is a well studied example[11,12,13]. Furthermore, PCD is involved in the cavitation process during joint development[11]. In the studies of mouse limb development, PCD occurs on the preaxial and postaxial margins of the hand/foot plate during four to five days - from day 10 to day 15[12]. A possible explanation for the presence of PCD on the pre- and postaxial margins of the hand- and footplates is that PCD contributes to the prevention of formation of the extra digits. It has been demonstrated that the two above mentioned mouse phenotypes have altered patterns of PCD[14,15]. In the *"Hx"* mouse, PCD which normally occurs at the preaxial margin of the hand/foot plate, fails to occur. Subsequently, the preaxial portion of the AER in these mice remains in an abnormally thickened, proliferative state, which may be the basis for prolonged preaxial outgrowth. However, the signals that regulate PCD

patterns in normal and abnormal limb embryogenesis are not (yet) known. It is possible that the TPT gene is involved in the regulation of PCD patterns in the developing limb.

Genes underlying congenital hand malformations are influencing morphogenesis of the distal skeleton. The hand skeleton is an important determinant of the hand anatomy. In view of the enormous phenotypic variety of hand malformations, a systemic and standardized approach to the analysis of the individual osseous configuration is indicated. MCPP profile analysis proved to be a valuable method to detect absolute as well as proportional alterations in the length of the hand bones in various birth defects and the pattern profile appears to be specific for several congenital malformation syndromes[16]. A characteristic profile occurred in all examined patients with TPT, and even emerged in the only case of non-penetrance that was discovered during this study (Chapter 4). This profile suggests that the triphalangeal digit in various types of TPT represents various degrees of differentiation between a thumb and an index finger. It further implies, together with the clinical observations, that the underlying genetic defect must be involved in the differentiation of the digits along the antero-posterior axis between the index finger and the thumb, and at the moment that the "genetic identity" of the thumb would normally be defined. The finding of a characteristic profile in different forms of TPT suggests that MCPP profile analysis could be used as a helpful diagnostic tool in complex syndromes which include TPT. However, more MCPP profile studies of syndromes with TPT should be done. It will be interesting to await the future comparisons of genotype/phenotype correlations of isolated TPT, with TPT that occurs as part of a syndrome.

Studies of the skeletal morphology have the potential, together with molecular genetic studies, to bring new insights into molecular mechanisms controlling developmental "fates" in abnormal genotypes. Furthermore, as the percentage of excessive or reduced length of each individual bone of the hand can be read from the MCPP plot, this method may be helpful in planning surgical treatment in a

number of congenital hand malformations when abnormal osseous length is involved.

A congenital hand malformation influences both, the executive and perceptive function of the hand. The faces and the hands provide the first impressions exchanged in personal communication. Perhaps this can explain that there is also a psychological aspect of congenital (hand) malformations, next to the somatic and functional one. In somatic medicine, there is usually a computable factor correlating the amount, and severity of risk factors, and chances of developing a disease. This correlation is usually linear. Psychological principles however, do not follow these rules. Accordingly, severity of a malformation does not necessarily correlate with the effect it can have on the individual life. During the study of the psychomotor development of the children with TPT, a few interviews with mothers of children affected with TPT revealed interesting individual experiences. It appeared that, next to the function impairment, subjective psychological factors play a role in the adjustment to a congenital hand malformation.

Even though several forms of TPT can severely impair hand function, this disorder can be well treated surgically. The ideal objective of reconstructive surgery (in TPT) would be to provide a hand that is normal in function and appearance. The former is often nearly possible, but the latter is frequently impossible[17]. An important factor in the acceptance of a congenital disorder is the subjective experience of it by the child and its parents. The visibility of a disorder, or a scar after surgery, was often experienced as a burden by the parents interviewed during this study, which indicates some difficulties in acceptance and adjustment processes (Chapter 5) . It is possible that the dynamics of the psychological processes involved differ between familial and sporadic cases. More research should be done towards the influences of different congenital hand malformations on the psychomotor development of a child. The best practical help that can be offered to the parents and the affected child, next to adequate surgery, is explanation. This should include explanations of etiology, pathogenesis, incidence, consequences for hand function, and how to make optimal use of

all available structures.

Studies of different aspects of TPT presented in this thesis are applicable to the majority of congenital hand malformations. Undoubtfully, in the near future, the majority of the (genetic) factors involved in the normal and abnormal limb development will be discovered. Future research will focus on the functional analysis of these genes and the molecular factors involved, but also on their interactions. These insights will hopefully give way to the formulation of new, pathogenetic and etiological classifications as a supplement to the current descriptive ones. Ideally, classifications will be formulated which can be used by both, the fundamental scientists and clinicians. Such "common language" would facilitate communication and exchange of valuable data and observations. Only with joint efforts from the "fundamental" and "clinical" plane will the story of the etiology, pathogenesis, prevention, diagnosis and treatment of congenital (limb) malformations once be complete.

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Summary

This thesis represents a study of different aspects of one congenital hand malformation, the triphalangeal thumb (TPT). Triphalangeal thumb is a rare form of preaxial polydactyly which can be seen as an isolated disorder, in association with other hands or feet malformations, or as a part of a syndrome. It is usually inherited as an autosomal dominant trait, although sporadic occurrence is also described. The main feature of this malformation is the presence of an extra phalanx in the thumb, and association with different degrees of thenar hypoplasia. Thenar muscles enable us to bring our thumbs opposite to the other digits, which is the basis of all precision movements the human hand is capable of. Phenotypic presentation can vary from an opposable, almost normal looking thumb, to a so-called five fingered hand where no opposition is possible.

During the period from 1983 to 1991, 15 children were treated for TPT at the Department of Plastic and Reconstructive Surgery of the Sophia Children's Hospital in Rotterdam. Eleven of these children had a positive family history for this disorder, and all the families originated from the same small area in the south west part of The Netherlands. The patients and their affected relatives had a strikingly similar phenotype varying from an opposable thumb with a delta-shaped extra phalanx to an non-opposable index-like digit instead of a thumb, sometimes associated with preaxial extra ray, rudimentary postaxial polydactyly, and cutaneous syndactyly between the fourth and the fifth digit. Consistency of the phenotype among the families, together with their common geographic and demographic origin, strongly suggested that all affected individuals shared the same genetic defect. The prevalence of the TPT in this population was estimated to be at least 100 times higher than in the general population, which provided a very good opportunity for an attempt to localise and identify the disease-gene. Using the strategy of linkage analysis, we have localised the TPT gene to chromosome 7q36. The underlying developmental defect still has to be discovered. In view of its phenotypic presentation, the TPT gene is probably involved in the

differentiation of the preaxial (radial) digits along the antero-posterior axis of the developing hand.

In order to examine skeletal morphology in different phenotypic variations of this disorder, we performed metacarpophalangeal pattern (MCPP) profile analysis on the X-rays of 13 affected persons, and 12 unaffected sibs from the same family with TPT. A characteristic profile occurred in all affected individuals, based on the individual lengthening or shortening of the thumb bones. Comparison of the affected and non-affected individuals from this family with individuals with a different genetic background, suggests that the described profile is specific for TPT and could be used as a helpful diagnostic tool in syndromes which include TPT. Furthermore, we used MCPP analysis in determining the length of the reduction osteotomy before surgery for TPT was performed. The percentage of excessive or reduced length of each individual bone of the hand can be read from the MCPP plot and appeared to be helpful in calculating a more accurate length for the newly created thumb.

A congenital hand malformation influences all functional domains of the hand. In order to explore the influence of an isolated congenital hand malformation on the psychomotor development of a child, an exploratory, observational study on 18 children with TPT was performed. Even though our observations suggest specific developmental difficulties at the level of fine motor skills and language development, the investigated children showed no signs of behavioral psychopathology. However, the important role of the (fine) hand function for the motor, social and psychological development of the child is an argument to time surgery as early as possible, and to follow the affected child until growth and development are fulfilled.

Samenvatting

Dit proefschrift is een studie van verschillende aspecten van een aangeboren handafwijking, de zogenaamde triphalangeale duim (TPD). Triphalangeale duim is een zeldzame vorm van preaxiale polydactylie die als een geïsoleerde afwijking kan optreden, in combinatie met andere hand en/of voetafwijkingen, of als onderdeel van een syndroom. Het erft meestal over als een autosomaal dominante aandoening, sporadische gevallen zijn echter ook beschreven. De belangrijkste kenmerken van deze afwijking zijn de aanwezigheid van een extra phalanx in de duim, en verschillende graden van thenar hypoplasie. Thenar spieren zijn verantwoordelijk voor de duim oppositie, of het vermogen om de duim tegenover de andere vingers te brengen. Dit vormt de basis voor alle precisie handelingen die de menselijke hand kan uitvoeren. Het fenotype kan variëren van een opponeerbare, bijna normaal uitziende duim, tot de zogenaamde vijf-vingerige hand waarbij geen oppositie mogelijk is.

Gedurende de periode van 1983 tot 1991, werden 15 kinderen voor TPD behandeld op de afdeling Plastische en Reconstructieve Chirurgie van het Sophia Kinderziekenhuis te Rotterdam. Elf van deze kinderen hadden een positieve familie anamnese voor dezelfde afwijking, en alle families waren afkomstig uit hetzelfde gebied in Zuid West Nederland. De patiënten en hun aangedane familieleden hadden een vergelijkbaar fenotype variërend van opponeerbare duim met delta-vormige extra phalanx, tot niet opponeerbare wijsvinger-achtige duim, soms in associatie met een preaxiale extra straal, rudimentaire postaxiale polydactylie, en cutane syndactylie tussen de vierde en de vijfde straal. Consistentie van het fenotype in de aangedane families alsmede hun gemeenschappelijke geografische en demografische oorsprong, suggereert dat alle aangedane individuen eenzelfde gendefect delen. Naar schatting is de prevalentie van de TPD in deze populatie zeker 100 keer hoger dan in de algemene bevolking, hetgeen goede mogelijkheden bood voor gen- lokalisatie en identificatie. Gebruik makend van de zogenaamde "linkage" analyse is het TPD

gen gelokaliseerd op chromosoom 7q36. De onderliggende moleculaire afwijking moet nog ontdekt worden. Gezien de fenotypische presentatie is het waarschijnlijk dat het TPD gen betrokken is in de differentiatie van de preaxiale (radiale) stralen langs de antero-posteriore as van de ontwikkelende hand.

Om de ossale morfologie in verschillende fenotypische variaties van deze afwijking te kunnen onderzoeken, hebben we de zogenaamde "Metacarpophalangeal pattern" (MCPP) profiel analyse uitgevoerd op de Röntgen foto's van 13 aangedane individuën en 12 niet aangedane broers en zussen uit dezelfde familie met TPD. Een karakteristiek profiel werd gezien bij alle aangedane personen, gebaseerd op de individuele verlenging of verkorting van de duimbotten. Vergelijking van de aangedane én de niet aangedane leden van deze familie met de aangedane en niet aangedane personen met een andere genetische achtergrond, suggereert dat dit profiel specifiek is voor TPD, en dat bij de beoordeling van syndromen met TPD MCPP analyse als een additief diagnostisch middel gebruikt kan worden. MCPP analyse kan ook gebruikt worden om de lengte van de reductie-osteotomie te bepalen. De afwijking van de lengte van de individuele handbotten kan van de MCPP plot als een percentage afgelezen worden.

Een aangeboren handafwijking beïnvloedt alle handfuncties. Om de invloed van een dergelijke afwijking op de psychomotore ontwikkeling van het kind te kunnen onderzoeken, hebben we een exploratieve, observationele studie uitgevoerd op 18 kinderen met TPD. Onze observaties suggereren specifieke ontwikkelingsmoeilijkheden op het gebied van fijne motoriek en taalontwikkeling. Geen van de onderzochte kinderen vertoonde tekenen van psychopathologisch gedrag. De belangrijke rol van de (fijne) handfunctie voor de motore, sociale en psychologische ontwikkeling van het kind is een argument om de chirurgische correctie zo vroeg mogelijk uit te voeren, en het aangedane kind te vervolgen totdat groei én ontwikkeling zijn voltooid.

Nawoord

In 1990 maakte ik een afspraak bij Dr. Hovius van de afdeling Plastische en Reconstructieve Chirurgie in het AZR, omtrent een mogelijk onderzoek. Dr. Hovius vertelde dat er in een vrij korte tijd veel kinderen met triphalangeale duim in het SKZ geopereerd waren, die allemaal uit dezelfde regio kwamen en dat we dat misschien eens verder moesten uitzoeken. Na wat rondbellen kwam ik in contact met huisarts Pieter Snijders die bevestigde dat deze afwijking in zijn praktijk veel voorkwam. Hij bood zijn medewerking aan.

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CURRICULUM VITAE

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Stellingen

behorende bij het proefschrift

Triphalangeal thumb

a study of a congenital hand malformation

van J. Zguricas

Rotterdam, 15 mei 1997

1. Pollicizatie-operatie reflecteert in haar naam en techniek een deel van de evolutieleer ten aanzien van de mens-apen duim.

(dit proefschrift)

2. MCPP profiel analyse heeft het potentieel om fenotypische kenmerken bloot te leggen die anderszins niet waarneembaar zijn.

(dit proefschrift)

3. Fenotype omvat meer dan het lichaam alleen.

(dit proefschrift)

4. Ontwikkeling bestaat uit vallen en opstaan; opstaan is het leermoment.

5. De basis van elke goede relatie is wederzijds respect.

6. Het wordt steeds moeilijker zich aan de indruk te onttrekken dat medische fondsen wetenschappelijk onderzoek naar een niet-dodelijke aandoening als "not done" beschouwen. Deze opvatting gaat voorbij aan het belang van de kwaliteit van het leven, en geeft het onderzoek binnen een vak als plastische chirurgie een sombere prognose.

7. In Nederland zou meer geld beschikbaar zijn voor onderzoek, als het bureaucratische apparaat dat zich bezig houdt met de verdeling ervan, wat minder voortreffelijk in elkaar zou zitten.

8. In de hedendaagse maatschappij, waarin communicatie zo belangrijk is, wordt geneeskunde ook een communicatie-wetenschap.

9. Een belangrijke taak van iedere leraar is de voorbeeld functie, zowel in positieve als in negatieve zin; de leerling dient zich bewust te zijn dat beide voorbeelden binnen een persoon kunnen bestaan.

10. In de anonimiteit van het verkeer wordt veel onthuld over de persoonlijkheid achter het stuur.

11. Een chirurg moet als een inbreker zijn: de weefsels binnendringen, zijn werk doen, en vervolgens verdwijnen zonder sporen achter te laten.

(Dr J. De Boer)

12. The skill of the hand lies in the brain and it is here that dexterity and adroitness (and clumsiness) originate. The hand is a mirror of the brain, therefore there can be no such combination as dextrous hands and clumsy brains.

(John R. Napier, "Hands")

13. Een goede dokter is allereerst een goed mens.

(Johan Zguricas)