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ORIGINAL ARTICLE • UPPER LIMB - NERVE GRAFT

Thumb duplication: molecular analysis of different clinical types

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Abstract

Purpose Molecular analysis of different types of thumb duplication and identification of new suspected gene mutations.

Materials and methods In a series of patients operated for polydactyly, DNA was extracted from blood samples collected preoperatively. Among these, the samples of two patients with thumb duplication (Wassel types III and IV) were initially selected for molecular analysis. The method of Clinical Exome Solution was used for the study of the phenotype-involved genes. Next-generation sequencing (NGS) was performed on a NextSeq-500 Platform (Illumina), and Sophia DDM® SaaS algorithms were used for the bioinformatics analysis of the data.

Results In total, 8—including 4 new—mutations were detected in *CEP290* (1 mutation), *RPGRIP1* (2 mutations), *TMEM216* (2 mutations), *FBN1* (1 mutation), *CEP164* (1 mutation), and *MEGF8* (1 mutation) genes. NGS revealed 3 mutated genes in the patient with Wassel III thumb duplication and 5 mutated genes in the patient with Wassel IV duplication. The molecular analysis revealed that the patients had 2 mutated genes in common, but they only shared one common mutation.

Conclusion The new detected mutations are most probably associated with thumb duplication, as they belong to genes with already described mutations causing ciliopathies, often including polydactyly in their phenotype. Recognition of these mutations will be helpful to prenatal diagnosis, operative treatment strategy prediction, and possible future experimental applications in gene therapy.

Keywords Polydactyly · Thumb duplication · Gene · Mutation · Next-generation sequencing · Ciliopathy

Introduction

The development of the human limb bud starts during the end of the fourth week of intrauterine life. Approximately 4 weeks later, an interplay of genes and molecular factors results in the development of a complete set of limbs with a well-defined appearance, function, and a specific number of digits. Proper positional signaling within the three-dimensional structure of the developing limb is of crucial importance for the future cell fate during embryogenesis. Disturbances in these signaling pathways can result in a large number of congenital limb malformations, many of which were already described in the in mythology and antiquity [1–6].

Polydactyly is the most frequently observed congenital hand malformation with a prevalence between 5 and 19 per 10,000 live births [3]. Polydactyly can occur as an isolated disorder, in association with other hand and foot malformations, or as a part of a syndrome, and is usually inherited as an autosomal dominant trait [3]. According to its anatomical location, polydactyly can be generally subdivided into pre- and postaxial forms. Polydactyly may be also classified according the embryological findings (Winter and Tickle classification) [4] or the classification by Temtamy and McKusick widely used among geneticists [5]. Preaxial polydactyly refers to an excess of parts on the radial side of the limb, including thumb duplication, various forms of triphalangeal thumbs and index finger duplication. Thumb

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polydactyly has further been subdivided into seven subtypes by Wassel [6] according to the level of duplication and the presence of triphalangeal thumb.

Some studies have already identified gene mutations associated with thumb duplication [7]. Thus, the comparison of the phenotypic and genetic findings of different forms of preaxial polydactyly is an important step in analyzing and understanding the etiology and pathogenesis of these limb malformations. The identification of new genes responsible for polydactyly is the first step toward expanding our knowledge on the genetic basis of hand disorders, contributing to genetic consultation and future therapeutic developments. In the present study, molecular analysis with next-generation sequencing (NGS) was performed in two patients with Wassel III and IV thumb duplication.

Materials and methods

From a series of patients with different types of hand and foot polydactyly and synpolydactyly that were operated in the Hand and Microsurgery Units of the University and the Iaso Thessalias Hospitals of Larissa, Greece the last 3 years, DNA was extracted from peripheral blood samples for molecular analysis. The study was approved by the institutional review board, and informed consent for the molecular analysis was obtained from the adult patients or the parents of the toddlers. Among the patients with polydactyly, 17 had thumb duplication of various types according to Wassel classification (type I: 1 patient, type II: 3 patients, type III: 2 patients, type IV: 7 patients, type V: 1 patient, type VII: 3 patients).

Two patients with Wassel III and IV thumb duplication were initially selected for NGS molecular analysis. The female patient with right thumb Wassel III duplication (Fig. 1a, b) was referred at the age of 18 months, and the male patient with right thumb Wassel IV duplication (Fig. 2a, b) was referred at the age of 9 months. Pediatric evaluation confirmed the absence of anomalies in other systems in both patients.

Total genomic DNA was extracted from peripheral blood of the patients. QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to achieve high molecular genomic DNA, following standard procedures according to the manufacturer's instructions. Extracted DNA was used as template for the subsequent genotypic analysis.

The method of Clinical Exome Solution (sequencing of 4493 genes so far associated with human diseases) was used for the study of the phenotype-involved genes, on a NextSeq-500 Platform (Illumina). Sophia DDM® SaaS algorithms were used for the bioinformatics analysis of data. The Clinical Exome Solution (CES) (SOPHiA GENETICS) consists of more than 100,000 individually designed probes and spans 11 Megabases (Mb) of target region covering more than 4493 genes with known inherited disease-causing mutations. In the Illumina platform, the amplified sequencing features are generated by bridge PCR and after immobilization in the array, all the molecules are sequenced in parallel. During the sequencing process, each nucleotide is recorded through imaging techniques and is then converted into base calls.

Results

The female patient with right thumb Wassel III duplication (Fig. 1a, b) was operated at the age of 20 months with excision of the ulnar thumb and reconstruction using corrective osteotomies, tendon realignment, and soft tissue and skin reconstruction (Fig. 1c, d). At the time of the latest follow-up at the age of 4 years, the girl had a functional thumb with normal sensation and motion (extension, abduction, and adduction) apart of a small lack of flexion of the interphalangeal joint (Fig. 1e).

The male patient with right thumb Wassel IV duplication (Fig. 2a, b) was operated at the age of 12 months with excision of the radial thumb and osteotomies of the first metacarpal and proximal phalange (Fig. 2c, d). One year later, at the age of 2 years, the patient underwent a second surgical procedure for correction of radial deviation of the remaining thumb (Fig. 2e, f), with osteotomy of the proximal phalange (Fig. 2g, h). At the time of the latest follow-up at the age of 4 years, the boy had a functional thumb with good alignment (Fig. 2i, j).

In total, 8—including 4 new—mutations, were detected by NGS analysis in the genes *CEP290* (1 mutation), *RPGRIP1* and *TMEM216* (two mutations in each one), *FBN1* (1 mutation), *CEP164* (1 mutation), and *MEGF8* (1 mutation) (Fig. 3).

More specifically, NGS revealed 3 mutated genes in the patient with Wassel III thumb duplication as follows: (a) the substitution c.1639 G>T (p. Ala547Ser) (rs10151259) in *RPGRIP1* gene, (b) an insertion of adenine (c.43_243_1 insA) in *TMEM216* gene (new mutation), and (c) the A>G nucleotide substitution (c.490) in *FBN1* gene (new mutation). In the other patient with Wassel IV duplication, the following mutations were identified: a) a duplication of adenine in exon 45 of *CEP290* gene (c.6264dupA, p. Leu2089) (new mutation), b) two nucleotide substitutions in *RPGRIP1* gene, c.1639 G>T, p.Ala547Ser (rs10151259), and c.685 G>A, p.Ala229Thr (rs174707), (c) an insertion of adenine in *TMEM216* gene, c.432-11 432-10 insA (rs11382548), (d) a nucleotide substitution G>C c.8249 in *MEGF8* gene, and e) a nucleotide substitution T>A c.548 in *CEP164* gene (new mutation). Both patients carried the same mutation in

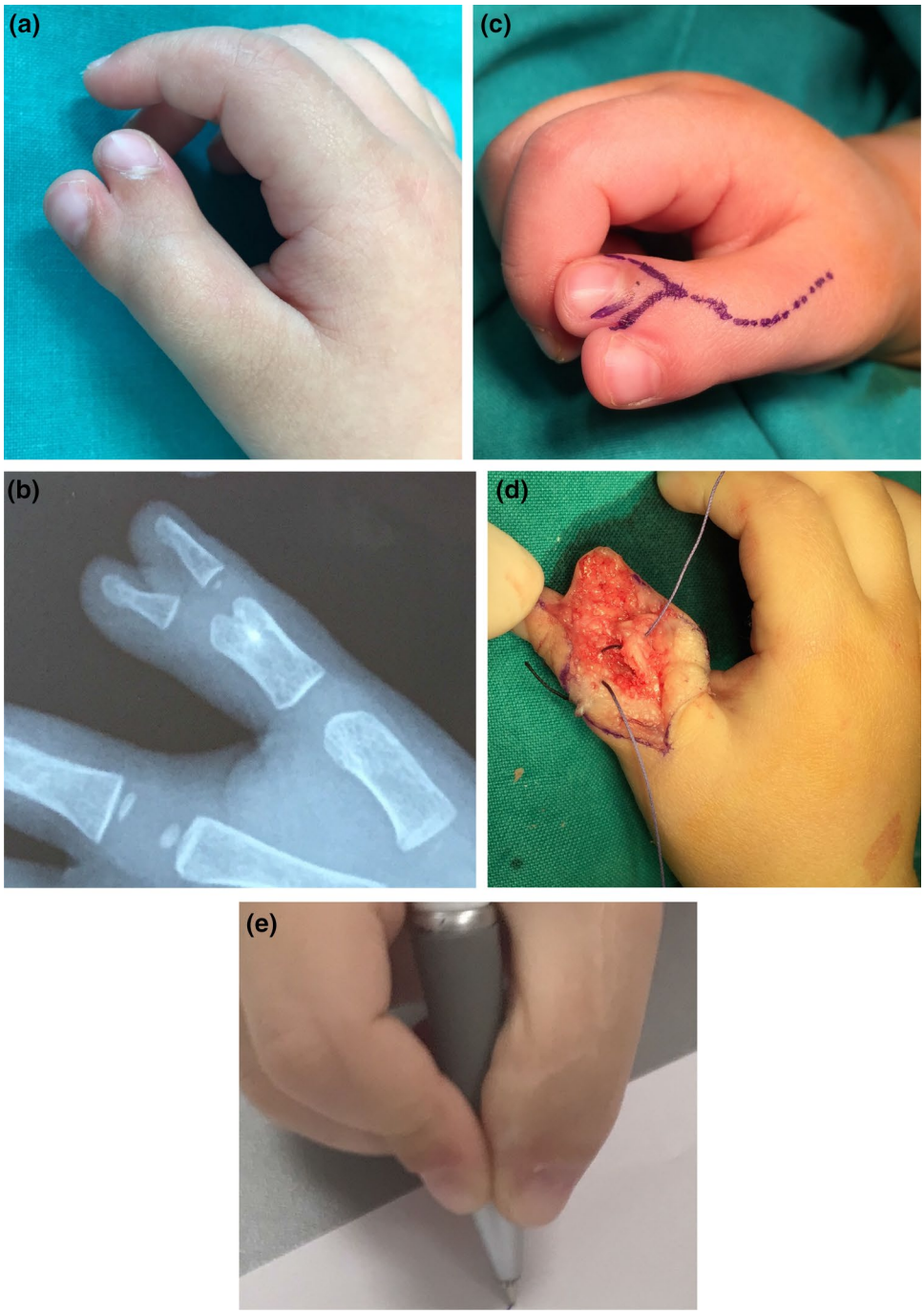

Fig. 1 **a** Patient with Wassel III right thumb duplication, **b** preoperative radiograph, **c** intraoperative photograph depicting the incision and the duplicated thumb that will be excised, **d** intraoperative photograph depicting the longitudinal osteotomy and reconstruction of the proximal phalange, **e** Postoperative result at the age of 4 years

RPGRIP1 gene c.1639 G>T, while each patient had a different mutation in another common gene (*TMEM216*).

Discussion

It is acknowledged that the combination of movements and the spatial rotation of the thumb granted humans advanced manipulation capabilities [8], and therefore, all deformities and dysfunctions of the thumb must be diagnosed and treated early to prevent loss if the fine competence. Concerning the optimal timing of surgical treatment of thumb duplication, many factors should be taken into account including the dimensions of the thumb that should be adequate for multiple soft tissue and bone procedures including osteotomies, the appearance of ossification centers, the length of the procedure and the risks of general anesthesia, the cortical learning and the establishment of patterns of hand function. Thus, some authors recommend the age of 6–9 months as ideal for surgical intervention,

Fig. 2 **a** Patient with Wassel IV right thumb duplication, **b** preoperative radiograph, **c** intraoperative photograph depicting the incision and the duplicated thumb that will be excised, **d** intraoperative photograph depicting the reconstruction of the first metacarpal and proximal phalange, **e** 1 year postoperatively the operated thumb has radial deviation, **f** postoperative radiograph depicting the radial deviation of the operated thumb, **g** second surgical procedure for correction of radial deviation of the remaining thumb with osteotomy of the proximal phalange, **h** intraoperative result with aligned thumb, **i** at the age of 4 years, after 2 surgical procedures, the patient has an aligned and functional thumb, **j** radiographic result 1 year after the second surgical procedure

before fine motor skills have developed with the abnormal anatomy, while others believe that the child should reach the age of 12–24 months to reduce the risks of anesthesia [9–11]. Recently, it has been proposed that the timing of the procedure should be based on the Wassel type of duplication and the appearance of thumb ossification center, and according to this study, the ideal age for the procedure ranges from 1 to 2.5 years [12].

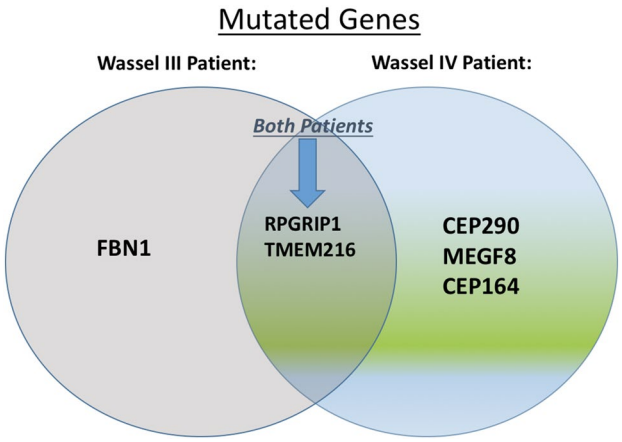

Fig. 3 Mutated genes identified in the two patients with thumb duplication

The Wassel type also plays a significant role at the operative strategy that will be followed by the hand surgeon. Different reconstruction methods and combinations of procedures are used, depending on the type and the level of duplication [11, 13, 14]. Studies in the literature determine the frequency of different methods used for treating duplicated thumbs and found that the vast majority of cases (85%) were treated with resection of the extra thumb and reconstruction–alignment of the remaining through the use of osteotomies and ligament reconstructions. Simple ablation alone (5%), the Bilhaut–Cloquet procedure (8%), pollicization (1%), and the on-top plasty (1%) are rarely used [15]. Preoperative discussion and planning must consider the possibility of multiple surgeries and the likelihood of inferior thumb function as well as size. In polydactyly, a reoperation rate of up to 25% is reported, with most reoperations performed because of residual or subsequent deformity [16]. Risk factors for thumb duplication reoperation are type IV thumb duplication, preoperative "zigzag" deformity, and radially deviated thumb elements at presentation [17].

Considering the previously described parameters (timing and type of surgery and reoperation potential), the prediction of the Wassel type of thumb duplication by isolating the responsible gene mutation could offer the clinician and the patient's family useful information about the future treating strategies. We have isolated 8 mutations, including 4 new detected, in 6 genes. There are references in the literature that mutations of these genes are responsible for a family of diseases called ciliopathies, which often include polydactyly [18]. Ciliopathies comprise a group of disorders associated with genetic mutations encoding defective proteins, which result in either abnormal formation or function of cilia. Cilia are components of almost all vertebrate cells. Their dysfunction can manifest as a constellation of features that include characteristically retinal degeneration, renal disease, and cerebral anomalies. Additional manifestations include congenital fibrocystic diseases of the liver, diabetes, obesity, and skeletal dysplasias such as polydactyly. Ciliopathic features have been associated with mutations in over 40 genes [18].

RPGRIP1 gene normally encodes a photoreceptor protein that interacts with retinitis pigmentosa GTPase regulator protein. Mutations of the gene are associated with Leber congenital amaurosis (ciliopathy) [19]. The *TMEM216* gene encodes the transmembrane domain-containing protein 216, and mutations at this locus have been associated with Meckel–Gruber syndrome Type 2 and Joubert syndrome (ciliopathies) [20]. The FBN1 gene provides instructions for a large protein called fibrillin-1. Mutations of this gene are associated with acromicric syndrome and Marfan syndrome, which can include polydactyly in their phenotype [21]. The CEP290 gene provides instructions for a protein that is present in many types of cells, the centrosomal protein 290. Other studies suggest that it plays an important role in cell structures called centrosomes and cilia. Several mutations of *CEP290* gene have been identified in syndromes associated with abnormal cilia [22]. The MEGF8 gene encodes the multiple EGF-like domains 8 protein, whose function is unclear but may be involved in cell processes such as cell adhesion and protein interaction. It is also suspected that the MEGF8 protein plays a role in the normal shaping (patterning) of many parts of the body during embryonic development. Mutations in the MEGF8 gene have been found to cause Carpenter syndrome, a condition characterized by irregular skull formation, finger and toe abnormalities, and many other features [23]. The *CEP164* gene encodes the centrosomal protein 164, involved in microtubule organization, DNA damage response, and chromosome segregation. The encoded protein is required for assembly of primary cilia and localizes to mature centrioles. Defects in this gene are a cause of nephronophthisis-related ciliopathies [24].

Next-generation sequencing analysis was initially applied in two patients with a less common (Wassel III) and a very common (Wassel IV) type of thumb duplication. In the patient with Wassel III duplication, 3 mutated genes were revealed, while in the patient with Wassel IV duplication NGS uncovered 5 of the aforementioned mutated genes. Although the patients had 2 mutated genes in common (*RPGRIP1* and *TMEM216*), they only shared one common mutation in *RPGRIP1* gene. Thus, it seems that this common *RPGRIP1* mutation is associated with radial polydactyly but probably does not play a significant role in the Wassel type differentiation. Based on the NGS results, which revealed that *FBN1* gene mutation was only detected in Wassel III patient and *CEP290*, *MEGF8* and *CEP164* gene mutations only in Wassel IV patient, these mutations could be correlated with the respective radial polydactyly phenotypes. The expansion of the analysis to more patients with Wassel III and IV types, as well as the identification of

the aforementioned or different mutations to other Wassel types of thumb duplication, will reinforce the correlation of each identified mutation to the respective phenotype.

In this study, we report clinical as well as genetic NGS findings in 2 patients with thumb duplication. These mutated genes could be considered as responsible for causing thumb duplication, Wassel III or IV. The expansion of our knowledge related to the mutations causing different thumb duplication types using NGS analysis will contribute to prenatal diagnosis, operative treatment strategy prediction, and potential future applications in gene therapy.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical standards This study was approved by the institutional review board and follows the ethical principles for medical research involving human subjects of the World Medical Association Declaration of Helsinki.

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