

## CASE REPORT

# Split Hand Foot Malformation Syndrome: A Novel Heterozygous FGFR1 Mutation Detected by Next Generation Sequencing

Panayiota Papasozomenou<sup>1,2,\*</sup>, Ioannis Papoulidis<sup>3</sup>, Themistoklis Mikos<sup>4</sup> and Menelaos Zafrakas<sup>1,4</sup>

<sup>1</sup>School of Health and Medical Care, Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece; <sup>2</sup>Fetal Medicine Centre of Kalamaria, Thessaloniki, Greece; <sup>3</sup>Access to Genome, Clinical Laboratory Genetics, Kalamaria, Thessaloniki, Greece; <sup>4</sup>1<sup>st</sup> Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece

**Abstract: Background:** Split-hand/foot malformation syndrome is a rare, clinically and genetically heterogeneous group of limb malformations characterized by absence/hypoplasia and/or median cleft of hands and/or feet. It may occur as an isolated abnormality or it may be associated with a genetic syndrome.

**Case Report:** In the present case, isolated split-hand/split-foot malformation was diagnosed by prenatal ultrasound at 24 weeks in a male singleton fetus, with deep median cleft of the right hand, syndactyly and hypoplasia of phalanges in both hands, and oligodactyly of the right foot. During consultation, the father of the fetus revealed that he also had an isolated right foot dysplasia. The parents chose elective termination and autopsy confirmed prenatal ultrasound findings. Genetic testing of the aborted fetus with QF-PCR analysis for common aneuploidies and array comparative genomic hybridization (aCGH) showed a male genomic pattern, without aneuploidies or chromosomal imbalances. Further investigation with next generation sequencing of 49 clinically relevant genes revealed a novel heterozygous *FGFR1* mutation c.787\_789del (p.Ala263del) in the fetus; the father was heterozygous to the same mutation.

**Conclusion:** A novel heterozygous *FGFR1* mutation causing split-hand/foot malformation syndrome is reported. Accurate genetic diagnosis allowed detailed counseling to be provided to the couple, including the underlying cause, recurrence risks, and detailed management plan with preimplantation genetic diagnosis for future pregnancies.

---

**ARTICLE HISTORY**

---

Received: February 03, 2019

Revised: May 11, 2019

Accepted: May 11, 2019

DOI:

10.2174/1389202920666190530092856

**Keywords:** Split-hand/foot malformation, limb malformations, prenatal diagnosis, next generation sequencing, FGFR1, novel mutation.

## 1. INTRODUCTION

Split-Hand/split-Foot Malformation (SHFM) syndrome, also referred to as ectrodactyly is a rare congenital malformation [1]. SHFM is a clinically and genetically heterogeneous group of limb malformations characterized by absence/hypoplasia and/or median cleft of hands and/or feet, and various mutations seem to be implicated in the SHFM phenotype [2, 3]. The aim of the present study is to present a rare case of isolated split-hand/split-foot malformation diagnosed by prenatal ultrasound at 24 weeks, due to a novel heterozygous *FGFR1* mutation c.787\_789del (p.Ala263del).

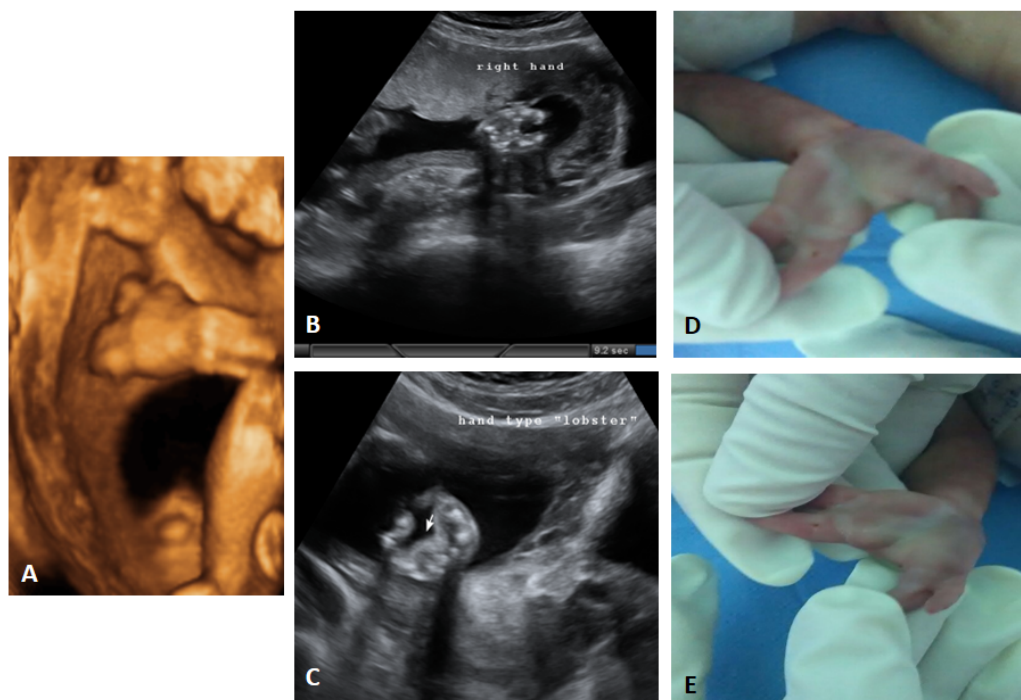
## 2. CASE REPORT

A 30-year-old white Caucasian woman, G1, P0 was referred at 24 weeks' gestation due to a possible fetal limb abnormality; "aplasia of the middle finger of the right hand

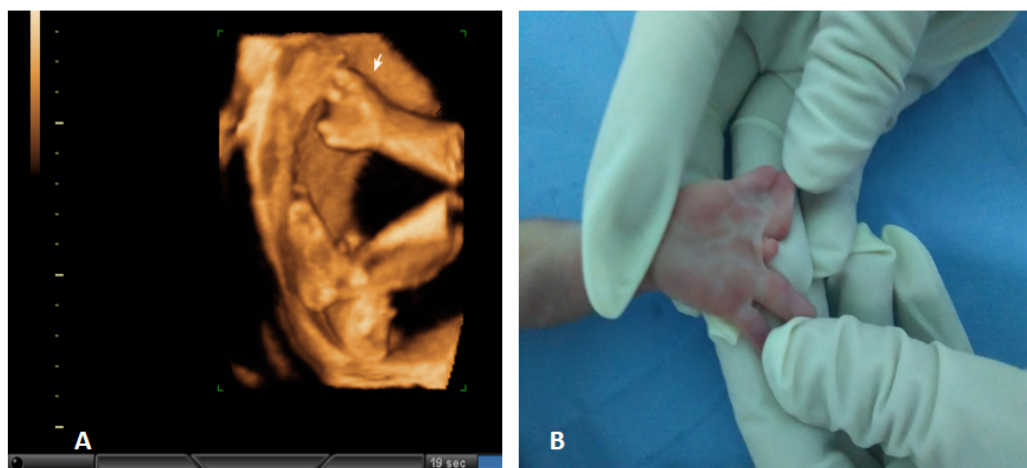
of the fetus" was reported in a recent second-trimester anomaly scan, while two previous anomaly scans at 19 and 21 weeks were reported as "normal". The woman's past medical history was uneventful. Our level II examination revealed a male singleton with ectrodactyly, manifested by deep median cleft of the right hand, resulting from the absence of the central digital rays. Syndactyly and hypoplasia of phalanges were found in both hands (Figs. 1-3), as well as oligodactyly of the right foot and a two-vessel umbilical cord. Hence, isolated split-hand/split-foot malformation (SHFM) was diagnosed and amniocentesis was offered after detailed counseling regarding the syndrome, its prognosis and the role of genetic analyses. During consultation, the father of the fetus revealed that he had an isolated right foot dysplasia, with big toe syndactyly, exadactyly and syndactyly of 4<sup>th</sup> and 5<sup>th</sup> digit (Fig. 4). The parents chose elective termination at 24 weeks' gestation and genetic analysis of the fetus; the post-mortem gross features of the fetus were identical to those of antenatal ultrasound. QF-PCR analysis for detection of common aneuploidies of chromosomes 13, 18, 21, X and Y (ABI<sup>®</sup>PRISM 3130xl, Life Technologies) and array comparative genomic

---

\*Address correspondence to this author at the Ethnikis Antistaseos 81, 55134 Kalamaria, Thessaloniki, Greece; Tel: +30-2310444364; Fax: +30-2310222498; E-mail: [yiota248@yahoo.com](mailto:yiota248@yahoo.com)



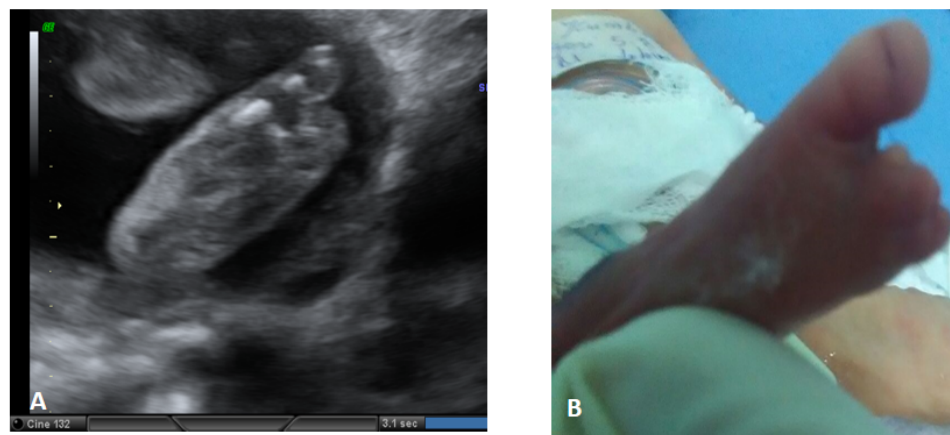
**Fig. (1).** Right fetal hand malformation characterized by oligodactyly (only four fingers present; absence of the middle finger), syndactyly of the thumb and the index finger, and deep central cleft or lobster claw hand. **A:** Prenatal 3D-ultrasound image of the right hand. **B** and **C:** Prenatal 2D-ultrasound images of the right hand. **D** and **E:** Autopsy images of the right hand.



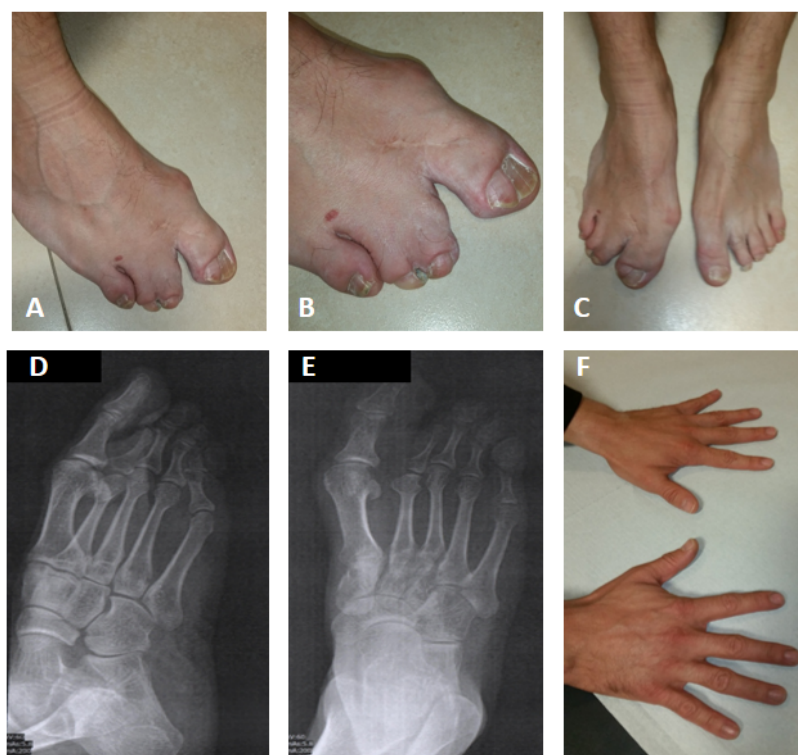
**Fig. (2).** Left fetal hand malformation characterized by oligodactyly (only four fingers present; absence of the middle finger) and syndactyly of the thumb and the index finger; there was no central cleft. **A:** Prenatal 3D-ultrasound image of the left hand. **B:** Autopsy image of the left hand.

hybridization (aCGH; microarray 8X60K G3 ISCA V2; CytoGenomics software; Agilent Technologies) revealed a male genomic pattern, without aneuploidies or chromosomal imbalances. The following 49 clinically relevant genes were analyzed by using next generation sequencing: *BHLHA9*, *BMP2*, *BMPRI1B*, *BTRC*, *CC2D2A*, *CDH3*, *CEP290*, *CHSY1*, *DLX5*, *DLX6*, *ESCO2*, *FAM58A*, *FBLN1*, *FBXW4*, *FGF10*, *FGFR1*, *FGFR2*, *FGFR3*, *FMN1*, *GDF5*, *GJA1*, *GLI3*, *GNAS*, *GREM1*, *HOXA13*, *HOXD13*, *IHH*, *KIF7*, *LMBR1*, *LRP4*, *MGP*, *MKS1*, *NOG*, *PTHLH*, *RECQL4*, *ROR2*, *RPGRIPL*, *SALL1*, *SHFM1*, *SHH*, *SOX9*, *TBC1D24*, *TBX15*, *TMEM216*,

*TMEM67*, *TP63*, *WNT10B*, *WNT3*, *WNT7A*. This analysis led to the detection of a novel heterozygous *FGFR1* mutation c.787\_789del (p.Ala263del). There were no suspicious variants detected in the other 48 relevant genes tested. Targeted sequencing of *FGFR1* in both parents showed that the father was heterozygous to the same mutation, whereas the mother was not, suggesting a 50% risk of recurrence. The following sequences have been used for targeted sequencing: *FGFR1* GENE REF SEQ: NM\_023110.2: c.787\_789del; NP\_075598: p.Ala263del. The Sanger sequencing results of the mutation and control are presented in Fig. (5). Given



**Fig. (3).** Right fetal foot malformation characterized by oligodactyly, with only the big toe present. **A:** Prenatal 2D-ultrasound image of the right foot. **B:** Autopsy images of the right foot.



**Fig. (4).** Paternal feet and hands. **A and B:** Paternal right foot malformation after surgery: Initially hexadactyly; an ectopic extra toe (only a bony part without a nail) between the 1<sup>st</sup> and 2<sup>nd</sup> metatarsal bone was removed surgically; the surgical scar is visible. There is also syndactyly of 4<sup>th</sup> and 5<sup>th</sup> toe and syndactyly of big toe and the 2<sup>nd</sup> toe, as well as aplasia of the last phalange of the second toe. **C:** Paternal feet: right foot malformation and normal left foot. **D and E:** X-rays of the right foot malformation. **F:** Normal paternal hands.

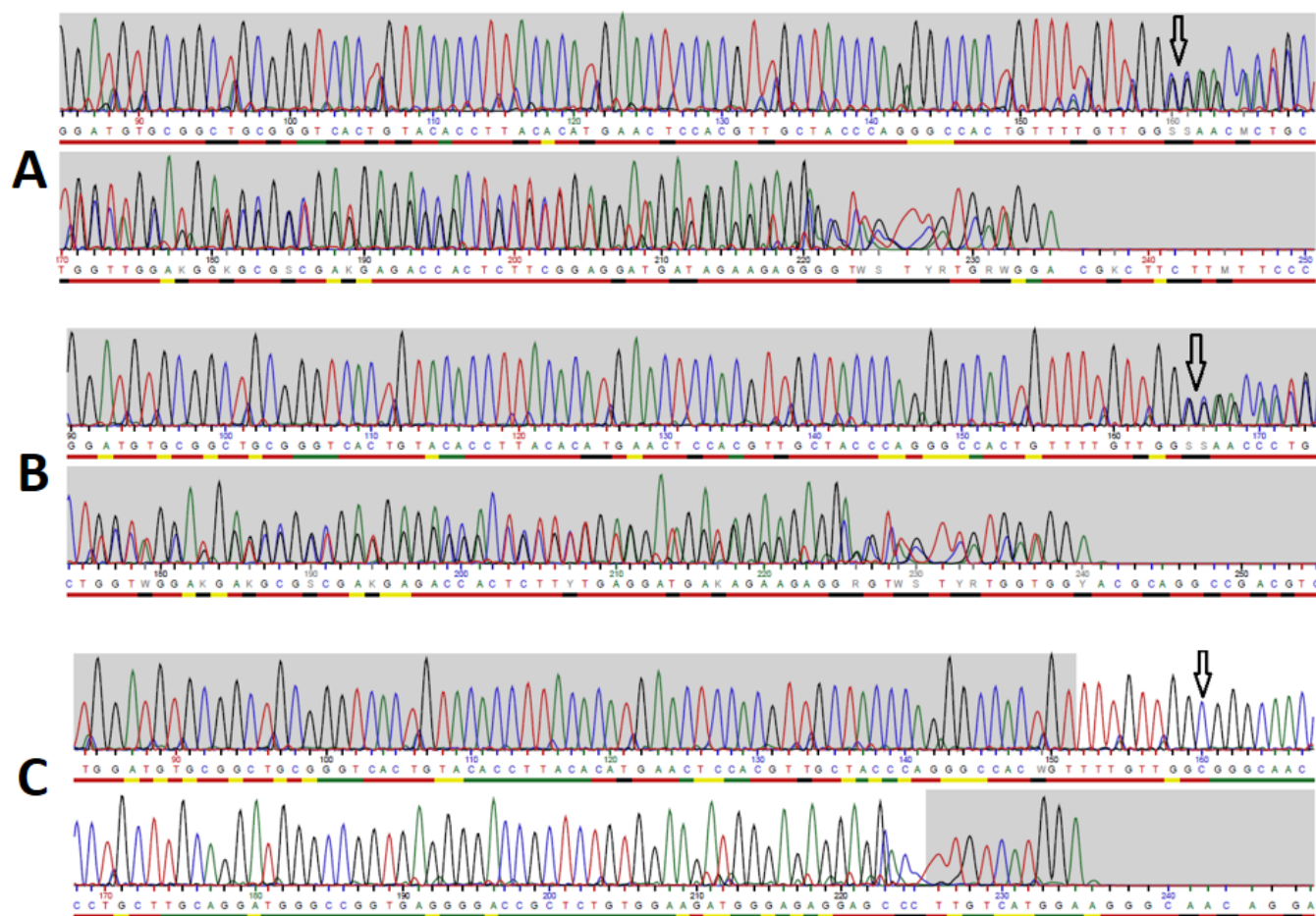
these findings, the option of IVF and preimplantation genetic diagnosis for selection of unaffected embryos in future pregnancies was discussed fully with the couple.

### 3. DISCUSSION

Split-Hand/split-Foot Malformation (SHFM) syndrome, also referred to as ectrodactyly is a rare congenital malformation; it occurs in 1:18,000 liveborn infants and it accounts for 8%-17 % of all limb malformations [1]. SHFM is a clinically and genetically heterogeneous group of limb malforma-

tions characterized by absence/hypoplasia and/or median cleft of hands and/or feet [2, 3].

SHFM may occur as an isolated abnormality or it may be associated with a genetic syndrome. Current evidence suggests that a relatively large number of mutations are implicated in the SHFM phenotype. Most commonly SHFM seems to be inherited in an autosomal dominant fashion with reduced penetrance; while in a typical autosomal dominant inheritance an affected parent would have a 50% chance of passing the genetic defect to his/her off-



**Fig. (5).** Sanger sequencing results. **A:** Embryo heterozygous to the novel *FGFR1* mutation c.787\_789del (p.Ala263del) mutation. **B:** Father of the embryo heterozygous to the same mutation. **C:** Mother of the embryo normal.

spring in each pregnancy, with “reduced penetrance” the person who inherits the underlying genetic defect, may never develop the condition [4-10]. More rarely, other forms of inheritance have been reported, including autosomal-recessive, X-linked, chromosomal deletions and chromosomal duplications [11, 12]. Sporadic cases with isolated SHFM most likely result from *de novo* mutations; in such cases, the recurrence risk for the patient’s sibling is very low, while for the patient’s offspring is as high as 30%-50 % [2]. Given that inheritance of SHFM is associated with reduced penetrance, including skipped generations, variable phenotypic expression, non-Mendelian inheritance and gender bias with anomalous segregation ratios in offspring of affected males [3, 6-8, 10, 12, 13], genetic counseling in such cases is rather complex and challenging. Hence, genetic counseling in SHFM should be based on a panel of clinically relevant genetic testing, in order to be adequately informative and reliable. In the present case, accurate genetic diagnosis, allowed focused genetic and reproductive counseling, including detailed discussion of the option of *in vitro* fertilization and preimplantation genetic diagnosis for embryo selection in future pregnancies.

In the present study, aCGH was advised since ectrodactyly may be sometimes associated with certain genetic de-

fects, including ectrodactyly ectodermal dysplasia cleft lip/palate syndrome (EEC) and Cornelia de Lange syndrome. SHFM may be either an isolated finding or part of a syndrome, such as EEC, acro-dermato-ungual-lacrimal-tooth syndrome, lacrimo-auriculo-dento-digital syndrome, CHARGE sequence (coloboma of the eye, heart defects, atresia of the nasal choanae, growth and/or developmental retardation, genital and/or urinary abnormalities, and ear abnormalities and deafness), VACTERL association (vertebral anomalies, anal atresia, cardiovascular anomalies, trachea-esophageal fistula, renal and/or radial anomalies, limb defects), mental retardation and sensorineural deafness [1].

In the present study, a panel of clinically relevant genes was analyzed by next generation sequencing of DNA extracted from the aborted fetus; this panel included genes known to be implicated in various fetal genetic syndromes with associated hand-foot malformations. This extensive analysis yielded a novel heterozygous mutation of the Fibroblast Growth Factor Receptor 1 (*FGFR1*) gene: c.787\_789del (p.Ala263del). According to the public genetic databases and to the best of our knowledge, this is the first time that this mutation is found in association with the SHFM. Mutations in the *FGFR* genes have been also asso-

ciated with the Pfeiffer syndrome, the Jackson-Weiss syndrome, the Antley-Bixler syndrome, and with osteoglyphonic dysplasia, which are all characterized by craniosynostosis and limb deformities. The Pfeiffer syndrome in particular is genetically heterogeneous; some cases are linked to mutations of *FGFR1*, which maps at chromosome 8p11.22-p12, while mutations of the *FGFR2* gene, which maps at chromosome 10q25-q26, have been also reported [14]. In the present case, prenatal ultrasound examination did not confirm craniosynostosis and this supports the contribution of ultrasound examination in establishing diagnosis [15].

## CONCLUSION

In conclusion, a novel heterozygous *FGFR1* mutation c.787\_789del (p.Ala263del) causing split-hand/foot malformation syndrome is reported. This study demonstrates the clinical utility of autopsy combined with molecular analysis by using clinically relevant new generation exome sequencing in order to identify the underlying genetic cause in a structurally abnormal fetus. Specific genetic findings may provide parents with an accurate explanation regarding the cause of a certain developmental abnormality, delineate the recurrence risks, and may help in drawing a detailed management plan for subsequent pregnancies.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the procedures performed in the present study were conducted in accordance with the protocol outlined by the Research Committee of the Alexander Technological Educational Institute of Thessaloniki, Greece.

## HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Informed consent was obtained from both parents for anonymous publication of the case and accompanying images.

## AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in Genbank at [https://www.ncbi.nlm.nih.gov/nuccore/nm\\_023110.2](https://www.ncbi.nlm.nih.gov/nuccore/nm_023110.2), reference number [NM\_023110.2].

## FUNDING

None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Declared none.

## REFERENCES

- [1] Czeizel, A.E.; Vitéz, M.; Kodaj, I.; Lenz, W. An epidemiological study of isolated split hand/foot in Hungary, 1975-1984. *J. Med. Genet.*, **1993**, *30*(7), 593-596.
- [2] Blattner, A.; Huber, A.R.; Röthlisberger, B. Homozygous nonsense mutation in *WNT10B* and sporadic split-hand/foot malformation (SHFM) with autosomal recessive inheritance. *Am. J. Med. Genet.*, **2010**, *152A*(8), 2053-2056.
- [3] Klopocki, E.; Lohan, S.; Doelken, S.C.; Stricker, S.; Ockeloen, C.W.; Thiele, S.; de Aguiar, R.; Lezirovitz, K.; Mingroni, N.R.C.; Jamsheer, A.; Shah, H.; Kurth, I.; Habenicht, R.; Warman, M.; Devriendt, K.; Kordass, U.; Hempel, M.; Rajab, A.; Mäkitie, O.; Naveed, M.; Radhakrishna, U.; Antonarakis, S.E.; Horn, D.; Mundlos, S. Duplications of *BHLHA9* are associated with ectrodactyly and tibia hemimelia inherited in non-Mendelian fashion. *J. Med. Genet.*, **2012**, *49*(2), 119-125.
- [4] Duijf, P.H.; van Bokhoven, H.; Brunner, H.G. Pathogenesis of split-hand/split-foot malformation. *Hum. Mol. Genet.*, **2003**, *12*(Suppl\_1), R51-R60.
- [5] Scherer, S.W.; Poorkaj, P.; Allen, T.; Kim, J.; Geshuri, D.; Nunes, M.; Soder, S.; Stephens, K.; Pagon, R.A.; Patton, M.A.; Berg, M.A.; Donlon, T.; Rivera, H.; Pfeiffer, R.A.; Naritomi, K.; Hughes, H.; Genuardi, M.; Gurrieri, F.; Neri, G.; Lovrein, E.; Magenis, E.; Tsui, L.C.; Evans, J.P. Fine mapping of the autosomal dominant split hand/split foot locus on chromosome 7 band q21.3-q22.1. *Am. J. Hum. Genet.*, **1994**, *55*(1), 12-20.
- [6] Nunes, M.E.; Schutt, G.; Kapur, R.P.; Luthardt, F.; Kukulich, M.; Byers, P.; Evans, J.P. A second autosomal split hand/split foot locus maps to chromosome 10q24-q25. *Hum. Mol. Genet.*, **1995**, *4*(11), 2165-2170.
- [7] Gurrieri, F.; Prinos, P.; Tackels, D.; Kilpatrick, M.W.; Allanson, J.; Genuardi, M.; Vuckov, A.; Nanni, L.; Sangiorgi, E.; Garofalo, G.; Nunes, M.E.; Neri, G.; Schwartz, C.; Tsiouras, P. A split hand-split foot (SHFM3) gene is located at 10q24-25. *Am. J. Med. Genet.*, **1996**, *62*(4), 427-436.
- [8] Raas-Rothschild, A.; Manouvrier, S.; Gonzales, M.; Farriaux, J.P.; Lyonnet, S.; Munnich, A. Refined mapping of a gene for split hand-split foot malformation. *J. Med. Genet.*, **1996**, *33*(12), 996-1001.
- [9] Ianakiev, P.; Kilpatrick, M.W.; Toudjarska, I.; Basel, D.; Beighton, P.; Tsiouras, P. Split-hand/ split-foot malformation is caused by mutations in the *p63* gene on 3q27. *Am. J. Hum. Genet.*, **2000**, *67*(1), 59-66.
- [10] Boles, R.G.; Pober, B.R.; Gibson, L.H.; Willis, C.R.; McGrath, J.; Roberts, D.J.; Yang-Feng, T.L. Deletion of chromosome 2q24-q31 causes characteristic digital anomalies: case report and review. *Am. J. Med. Genet.*, **1995**, *55*(2), 155-160.
- [11] Ahmad, M.; Abbas, H.; Haque, S.; Flatz, G. X-chromosomally inherited split-hand/split-foot anomaly in a Pakistani kindred. *Hum. Genet.*, **1987**, *75*(2), 169-173.
- [12] Faiyaz-Ul-Haque, M.; Zaidi, S.H.; King, L.M.; Haque, S.; Patel, M.; Ahmad, M.; Siddique, T.; Ahmad, W.; Tsui, L.C.; Cohn, D.H. Fine mapping of the X-linked split-hand/split-foot malformation (SHFM2) locus to a 5.1-Mb region on Xq26.3 and analysis of candidate genes. *Clin. Genet.*, **2005**, *67*(1), 93-97.
- [13] Jarvik, G.P.; Patton, M.A.; Homfray, T.; Evans, J.P. Non-Mendelian transmission in a human developmental disorder: split hand/split foot. *Am. J. Hum. Genet.*, **1994**, *55*(4), 710-713.
- [14] Dicke, J.M.; Piper, S.L.; Goldfarb, C.A. The utility of ultrasound for the detection of fetal limb abnormalities-a 20-year single-center experience. *Prenat. Diagn.*, **2015**, *35*(4), 348-353.
- [15] Schell, U.; Hehr, A.; Feldman, G.J.; Robin, N.H.; Zackai, E.H.; de Die-Smulders, C.; Viskochil, D.H.; Stewart, J.M.; Wolff, G.; Ohashi, H.; Price, R.A.; Cohen, M.M.; Muenke, M. Mutations in *FGFR1* and *FGFR2* cause familial and sporadic Pfeiffer syndrome. *Hum. Mol. Genet.*, **1995**, *4*(3), 323-328.