European Review for Medical and Pharmacological Sciences 2022; 26: 5107-5114

Chest wall deformities and their possible associations with different genetic syndromes

N. ANDREESCU[1,2], A. SHARMA[3,4,5], A. MIHAILESCU[1], C.G. ZIMBRU[1,6], V.L. DAVID[7], R. HORHAT[8], N.R. KUNDNANI[3,9], M. PUIU[1,2], S. FARCAS[1]

[1]Department of Microscopic Morphology, Discipline of Genetics, Genomic Medicine Centre, "Victor Babeș" University of Medicine and Pharmacy, Timisoara, Romania
[2]Regional Center of Medical Genetics Timis, Clinical Emergency Hospital for Children "Louis Turcanu", Timisoara, Romania
[3]Family Physician Clinic, Civil Medical Society Dr Rosca, Teremia Mare, Timis, Romania
[4]Department of Occupational Medicine, Municipal Emergency University Hospital, Arad, Romania
[5]Department of Cardiology, Discipline of Cardio-vascular Rehabilitation, "Victor Babeș" University of Medicine and Pharmacy, Timisoara, Romania
[6]Department of Automation and Applied Informatics, Polytechnic University of Timisoara, Timisoara, Romania
[7]Department of Pediatric Surgery and Orthopedics, "Victor Babeș" University of Medicine and Pharmacy Timisoara, Romania
[8]Department of Functional Science, Discipline of Biophysics, Center for Modelling Biological Systems and Data Analysis, "Victor Babeș" University of Medicine and Pharmacy, Timisoara, Romania
[9]Department of Functional Sciences, Physiology, Centre of Immuno-Physiology and Biotechnologies (CIFBIOTEH), "Victor Babeș" University of Medicine and Pharmacy, Timisoara, Romania

Abstract. – OBJECTIVE: Our primary objective was to identify discrete and syndromic cases of Pectus excavatum (PE) and Pectus carinatum (PC). We also intended to highlight the significance of further genetic exploration in clinically suspected syndromic cases of PC and PE. Pectus excavatum (PE) and Pectus carinatum (PC) are the most common morphological chest wall deformities. Although various hypotheses have been put forth, the pathogenesis of both entities is largely unknown. Clinicians often refer such cases for further genetic evaluation to exclude an associated underlying connective tissue disorder or a syndrome. Additionally, a detailed anamnesis with focused family history and thorough dysmorphological physical examination was done. PE and PC are considered isolated abnormalities if there is the absence of features of other syndromes, eliminating the need for further genetic evaluations. It is believed that the pattern of inheritance of these non-syndromic isolated PE and PC cases with positive family history could be multifactorial in nature. The recurrence risk of such isolated cases is thought to be low. Further diagnostic studies are indicated as PE and PC could be a part of a syndrome. Among the many syndromes, the most common monogenic syndromes associated with PE and PC are Marfan's and Noonan's.

PATIENTS AND METHODS: After obtaining the consent, we compiled a database of the patients who presented with chest wall deformities during the period 2017-2019. We selected 70 cases with PC and PE deformities to identify the discrete and syndromic PC and PE cases. During the study, we perused the cytogenetic and/or molecular analyses, that had been conducted to confirm the clinically suspected syndromic cases. We also scrutinized for the presence of PC and PE cases that are associated with the rare syndrome (s).

RESULTS: Various genetic abnormalities were identified in 28 (40%) of the 70 cases that had been diagnosed with chest wall abnormalities. Along with PE and PC, other thoracic wall abnormalities were also identified, such as the broad chest, bell-shaped thorax, and elongated or enlarged thorax. One case of a rare genetic disorder of Morquio syndrome associated with PC was also identified. Novel (previously unpublished) genomic variants are reported here.

CONCLUSIONS: It is important to delve deeper when encountering cases of PE and PC by conducting a further genetic exploration of such

Corresponding Author: Abhinav Sharma, MD; e-mail: sharma.abhinav@umft.ro

cases to identify syndromic associations that cause other structural and functional disorders, diagnosis of which might be missed during the early developmental period. Early identification of such disorders may help us correcting the defects, slowing the progression of disease processes, and preparing better to deal with the potential outcome.

Key Words:
Chest wall deformities, Pectus excavatum, Pectus carinatum, Genetic analyses, Syndromes.

Introduction

Anterior chest wall abnormalities represent structural abnormalities affecting sternum, ribs, and musculature, which modify the structure and/or the function of the thorax. Pectus excavatum (PE) and pectus carinatum (PC) may occur as discrete defects or in association with genetic syndromes[1]. These defects are usually diagnosed during childhood and in some cases at birth. Patients with these deformities may experience an increase in the severity of their deformities during their adolescent growth period[2-4].

PE accounts for approximately 90% of chest wall deformities. The defect occurs more frequently in Caucasians, with an incidence of 1 per 400 live births and a male predominance (5:1 M/F ratio)[5,6]. The forms of PE can occur either as an isolated non-familial or familial disorder. The pattern of inheritance identified for the non-syndromic familial cases is autosomal dominant[7].

PC is the second most common chest-wall deformity, with an incidence of 6 per 100 live births and a male predominance (4:1 M/F ratio). PC can also occur as an isolated anomaly, or it can be a part of a number of genetic syndromes[8].

There are more than 32 syndromes with PE/PC as a frequent feature[9]. A review by Kotzot and Schwabegger[8] illustrates the most important syndromes with pectus excavatum/carinatum, indicating that the most frequently observed monogenic syndromes with pectus excavatum/carinatum are Marfan Syndrome and Noonan Syndrome, while the chromosomal disorders frequently associated with PE/PC include Turner syndrome.

Patients and Methods

To conduct our study, we compiled the database of patients who presented with chest wall abnormalities to the Regional Center for Medical Genetics Timis, Romania, which is affiliated with the University Emergency Hospital for Children "Louis Turcanu" Timisoara, Romania. Informed consent was obtained from all the patients that were included in the study. A total of 70 patients with chest wall abnormalities were identified during the three-year study period (2017-2019). Genetic testing was performed to confirm the suspected clinical diagnosis.

Cytogenetic Analyses

Cytogenetic analyses were performed using the cells harvested from peripheral venous blood and were cultured for 72 hours, in a PBMax Karyotyping medium (Gibco, Invitrogen Corp, Carlsbad, California, CA, USA). In order to prepare metaphase cells, each sample was exposed to mitotic inhibitors, hypotonic solution (KCl 0.075 M), and fixed (absolute methanol: glacial acetic acid, 3:1). The cell suspension was mounted on the microscope glass slide and air-dried. The samples were processed using the G banding technique. We evaluated 30 metaphases for each patient respecting the pre-established quality protocols.

Fluorescence In Situ Hybridization

Subsequently, we applied the FISH technique using pertinent probes guided by the clinical suspicion or by the karyotype. Fluorescence *in situ* Hybridization (FISH) was performed using the Cytocell Aquarius FISH microdeletion probes kit (Cytocell Inc., Adderbury, Oxfordshire, UK). Slides were immersed in +37ºC 2×SSC (SSC: 20×Saline-sodium citrate buffer) for 30 min and then dehydrated in alcohol 70%, 80% and 100% for 2 minutes each. Slides were denatured at +73ºC (in a pre-warmed water bath) for 2 min and rinsed in ice-cold series of alcohol 70%, 80%, 100% for 2 minutes each. Slides were put on a slide warmer (40ºC), applied 10 µL probe and hybridized overnight at 37ºC. These hybridization slides were then washed in 0.4X SSC 0.3% NP 40 solutions at 73°C for 2 minutes and rinsed in 2X SSC solution. For each case, a minimum of 200 cells was evaluated by using a fluorescent microscope. The best images were captured using the camera mounted on the microscope attached to a computer with karyotyping and FISH software.

Molecular Testing

Genomic DNA isolation was performed according to the protocol provided by the manufacturer, by using the MagCore Automatic Nucleic

Acid Extractor and the MagCore Genomic DNA Whole Blood Kit (RBC Bioscience, New Taipei City, Taiwan). The extracted DNA was eluted into 60 µL of the buffer. DNA concentrations and DNA purity were evaluated by using Qubit® dsDNA HS Assay Kit (Invitrogen Corp. Carlsbad, California, CA, USA) according to the instructions provided by the manufacturer.

SNP-Array

Single-nucleotide polymorphism (SNP) microarray testing was performed using Illumina HumanCytoSNP-12 v2.1 BeadChip (Illumina Inc, San Diego, California, CA, USA). The DNA samples were adjusted to a concentration of 50 ng/µL. The amplification, tagging, and hybridization were performed according to the manufacturer's protocol. Array slides were scanned on the iScan (Illumina Inc., San Diego, California, CA, USA). The GenomeStudio V2011 software (Illumina Inc., San Diego, California, CA, USA) was used to analyze the genotypes and evaluate the experimental quality.

Targeted NGS

Amplicon sequencing libraries were prepared from 50 ng of DNA per sample according to the Trusight One/Trusight Cardio protocol (Illumina Inc., San Diego, California, CA, USA). We performed the genomic DNA tagmentation, we cleaned up the tagmented DNA and the accumulated DNA, we hybridized the probes and caught hybridized probes; then we performed the second hybridization and the second catch, we cleaned up the cached library, accumulating the enriched library, and finally we cleaned up the accumulated enriched library according to the instructions provided by the manufacturer. The pooled libraries were sequenced on a microflow cell with V3 chemistry on a MiSeq instrument (Illumina Inc., San Diego, California, CA, USA). After de-multiplexing and generation of FASTQ files, sequence alignment to the reference genome and sequence quality filtering was performed using the Illumina MiSeq Reporter v2.6 platforms. The sequences were aligned with BWA, and variant calling was performed with GATK using the human reference sequence hg19/GRCh37. Variant calling was performed on the variant call format (VCF) output files by evaluating the coverage (the number of times that a targeted variant is read during the sequencing), and the quality score (Q-score; the estimated probability of the base call being wrong). VCF files were further subjected to annotation using ANNOVAR with the dataset dbNSFP 35a[10,11].

Statistical Analysis

We performed descriptive statistical analysis of the study population to examine the incidences of syndromic cases, the major abnormalities exhibited by those patients, as well as genotype-phenotype correlations. Contingency tables and Chi-square test were used to detect statistical significance for categorical data. Statistical significance was considered for $p<0.05$.

Results

Genetic abnormalities were identified in 28 cases (40%) of the 70 patients that were diagnosed with chest wall abnormalities. However, in some patients the genetic diagnosis was not completed, either due to variants of unknown significance (VUS) or heterozygous variant identified in autosomal recessive disorders (in the child with short-rib thoracic dysplasia). Novel (previously unpublished) genomic variants are reported here. Along with PE and PC, other thoracic wall abnormalities were also identified, such as the broad chest, bell-shaped thorax, and elongated or enlarged thorax.

Pectus Excavatum

PE was diagnosed in 16 cases (57.14%) with a male to female ratio of 5:3. By comparison to the other chest wall abnormalities in our study, PE was associated mainly with Marfan syndrome (7

Table I. Abnormalities in patients with pectus escavatum and Marfan syndrome.

Genetic disorder (gene)	Other abnormalities	Genetic testing
Marfan syndrome *FBN1*	• Skeleton abnormalities: arachnodactyly, articular hyperlaxity, flat foot, arm span > height • Facial dysmorphism: up slanting palpebral slant • Abnormal height • Other abnormalities: mitral valve prolapse, aortic dilatation, myopia	*P5: c.4691_4695del* *P9: c.7606G>A* *P10: c.7343G>A* *P16: c.6379+1G>C* *P18: c.5743C>T*, *P23: c.7180C>T*,

Table II. Abnormalities in patients with pectus excavatum and Noonan syndrome.

Genetic disorder (gene)	Other abnormalities	Genetic testing
Noonan syndrome *SOS1*	• Cranio-facial malformations: up slanting palpebral, low set ears, epicanthus • Flat foot • Pulmonary stenosis • Pterygium coli • Short stature	*SOS 1* *P4: c.512T>C,* *P8: c.1322G>A*
Noonan syndrome *PTPN 11*	• Cranio-facial malformations: brachiocephalic, narrow forehead, down slanting palpebral slant, low set ears, hypertelorism, epicanthus • Short stature • Cardiovascular abnormalities: pulmonary stenosis, atrial septal defect, aortic insufficiency and aortic valve dysplasia • Other abnormalities: pterygium coli, umbilical hernia, "café au lait" spots	*PTPN 11* *P12: c.923A>G* *P17: c.1504T>G* *P25: c.188A>G* *P27: c.236A>G*

cases: 43.7%, p=0.72) and Noonan syndrome (6 cases: 37.5%, p=0.75). The remaining two male patients with PE were diagnosed with Chromosome 22q11.2 duplication syndrome and distal arthrogryposis, along with other abnormalities (Tables I, II, III).

Pectus Carinatum

PC was reported in 7 (10%) of the 70 cases included in the study: 3 cases (42.86%, p=0.99) presented with Marfan syndrome and each of the other 4 cases (14.28%) were either diagnosed with Noonan syndrome, Down syndrome, DiGeorge or Morquio syndrome (Table IV).

Other Thoracic Wall Abnormalities

Other thoracic wall abnormalities were diagnosed in 5 (17.24%) of the 28 cases. In a female case of Noonan syndrome, along with low stature and pterygium coli, the broad chest was observed. Bell-shaped thorax was diagnosed in the female case of short ribs syndrome and the male case of trisomy 21 syndrome. Elongated thorax was present in the 15q duplication syndrome case and the enlarged shape, along with other abnormalities, was present in the Morquio syndrome case (Table V).

Discussion

PE and PC are the most commonly encountered thoracic wall abnormalities. As mentioned in the literature, their presence can be discrete, or they can occur as a part of various syndromes[6]. It is important to establish the etiology of chest wall deformities, since their association with ge-

Table III. Abnormalities in other disorders associating pectus excavatum.

Genetic disorder (gene)	Other abnormalities	Genetic testing
Chromosome *22q11.2* duplication syndrome (1 case) (M: F=1:0)	• Cranio-facial abnormalities: down slanting palpebral slant, short philtrum • Intellectual disabilities • Obesity	*P14: dup 22q11.2-21.1*
Marfan like phenotype	• Diabetes mellitus • Increased creatine phosphokinase (CPK)	*FKRP* *P6: c.148A>G /c.985G>A*
Distal arthrogryposis (1 case) (M: F=1:0)	• Microcephaly • Scoliosis • Palpebral ptosis • Anterior rotation of pinna • Severe Intellectual disabilities	*P18: TPM2: c.826 C>G*

Table IV. Other genetic disorders associated with pectus carinatum.

Genetic disorder (gene)	Other abnormalities	Genetic testing
DiGeorge syndrome (M: F=1:0)	• Facial dysmorphism • Moderate Intellectual disabilities • Low stature • Immunodeficiency • Hypocalcemia	*P6: del22q11*
Noonan syndrome (M: F=0:1)	• Low stature • Atrial septal defect (ostium secundum) • Heart failure (NYHA II/III)	*P20: PTPN11: c.922A>G*
Down Syndrome (M: F=0:1)	• Plagiocephaly, brachydactyly, single transverse palmar crease, • Facial abnormalities: up slanting palpebral slant, pseudo-macroglossia, low insertion of pinna • Moderate Intellectual disabilities, short stature	*P2: 47XY+21*
Morquio syndrome (M: F=0:1)	• Skeleton abnormalities: kyphosis, dwarfism, genu valgum, "bullet shape" vertebrae, bilateral coxa valga, left coxo-femoral luxation • Rough facial features • Short stature • Intellectual disabilities • Umbilical hernia • Hypermetropia • Atrial septal defect • Aortic coarctation	*P27: GALNS: c.871G>A*
Marfan syndrome	• Cranio-facial malformations: microcephaly, dolichocephaly, micrognathia and hypoplasia of middle face, dental abnormalities • Skeleton abnormalities: articulate hyperlaxity, kyphoscoliosis, winged scapula • Cardio-vascular abnormalities: aortic dilatation, pulmonary ectasia • Abnormal height • Myopia • High arched palate	*P11: FBN1:c 6740A>G* *P15: FBN1: c.7168dup* *P24:FBN1: c.1155_1165del*

netic disorders, such as connective tissue defects, may result in a different outcome and long-term prognosis when surgically treated[12].

Previous studies have shown PE to be an inherited disorder, mainly through an autosomal dominant transmission, however, other patterns of transmissions such as autosomal recessive, X-linked inheritance, or multifactorial are also possible. Due to the co-existence of other abnormalities such as long arms, legs, and fingers, high-arched palate, scoliosis, flexibility, flat feet, mitral valve prolapse, heart arrhythmia, and childhood myopia, pedigree analysis suggests a possible association of PE with connective tissue disorders[13].

Recent studies[13,14] have found correlations between PE and heritable connective tissue disorders such as Marfan, Ehlers-Danlos, Poland, ASS (mitral valve prolapse, not progressive aortic enlargement, skeletal and skin alterations) phenotype. These associations are confirmed by biochemical and immunohistochemical analyses[13,14] showing abnormalities in the structure, distribution, and arrangement of type-2 collagen in costal cartilage, abnormally high levels of zinc, and low levels of magnesium and calcium or disturbance of collagen synthesis. Other findings[13,15] include disorders in endochondral ossification and alterations in the metabolism of acid mucopolysaccharides. These modifications are associated with altered biomechanical properties of costal cartilage and an impaired response to tension, compression, and bending during fetal breathing activity, crying spells, respiratory obstruction, or hiccups[16].

In our study, from 28 cases of genetic abnormalities, 16 (57.14%) were associated with PE

Table V. Other thoracic wall abnormalities.

Chest deformity	Genetic disorder	Other abnormalities	Genetic testing
Broad chest (1 case)	Noonan like phenotype (M: F=0:1)	• Pterygium coli • Short stature	*P1: CACNA1C c.1531C>T*
Bell-shaped thorax	Short ribs syndrome (M: F=0:1)	• Hydrocephaly • Skeletal dysplasia • Respiratory failure • Generalized hypotonia • Bilateral hydronephrosis	*P13: DYNC2H1:* c.195+7T>C
	Down syndrome (M: F=1:0)	• Cranio-facial abnormalities: plagiocephaly, up slanting palpebral slant, epicanthus, ogival palate, low-inserted pinna, • Intellectual disability, short stature, single transverse palmar crease, Sandal gap deformity	*P20: 47XX+21*
Elongated thorax	15q duplication (M: F=0:1)	• Kyphosis • Severe intellectual disability • Upper limbs stereotypes • Divergent strabismus • Dental dystrophia	*P22: dup 15q13.3*
Enlarged thorax	Morquio syndrome (M: F=0:1)	• Other skeletal abnormalities: dysplasia, L2 hemivertebra, long bones spondilo-epiphyseal dysplasia • Short stature • Generalized muscular hypotrophy • Hypermetropy • Mitral valve prolapse	*P26: GLB1: c.425G>T /c.176G>A*

and 7 (25%) with PC. The two thoracic wall abnormalities are listed in almost 328 syndromes, according to the dysmorphology database, but in the majority of the cases, PC and PE are associated with Marfan and Noonan syndrome[6,8]. Medical literature mentions[6,8] asymmetric PE or PC as the most specific skeletal feature for the Marfan syndrome. With an autosomal dominant pattern of inheritance, it is caused by a variant in the Fibrillin 1 gene on the long arm of chromosome 15[6]. For instance, two heterozygote fukutin related protein *(FKRP)* variants were found in one patient who had *pectus excavatum* associated with limb girdle muscle dystrophy of this gene are characterized by abnormal O-glycosylation of the alpha-dystro glycan, which plays an important role in linking the intracellular skeleton with the extracellular matrix. Although implicated in a wide range of neurological disorders, variant in *FKRP* gene identified in the patient in our study group was associated only with pectus *excavatum*[17].

In Noonan syndrome, due to the variants in various genes in the RasMAPK pathway and autosomal pattern of inheritance, the anterior chest wall is deformed by superior PC and inferior PE[6]. Among the 16 cases of PE, we identified 8 (50%) cases of Marfan syndrome, 5 (31.2%) cases of Noonan syndrome, and one case (6.25%) each of Chromosome 22q11.2 duplication syndrome and distal arthrogryposis. Wilson et al[18] reported one case of PE in 44 patients diagnosed with DiGeorge syndrome. Along with anteverted slouched shoulders, and inflexible back, PE was mentioned in patients with distal arthrogryposis[19].

In our study, PC was reported in 7 patients, 3 (42.86%) cases of Marfan syndrome and one case (14.28%) each of Noonan syndrome, Down syndrome, DiGeorge, and Morquio syndrome were identified. PC is the second most common chest-wall deformity, and it mostly occurs in patients with Marfan or Noonan syndrome[20]. This type of thoracic wall deformity has also been

reported in cases with DiGeorge syndrome[21]. Eight patients in our study group presented thoracic wall abnormalities associated with Noonan syndrome. The majority (6 of them) presented with *PTPN11* variants; a gene implicated in the RAS/mitogen-activated protein kinase (MAPK) pathway. It encodes a ubiquitous non-receptor tyrosine phosphatase, and its mutations are mainly associated with pulmonary stenosis, short stature, and thoracic deformities[22]. In 2 of the 8 patients with Noonan syndrome, an *SOS1* gene variant was identified. *SOS1* encodes a guanine nucleotide exchange factor responsible for stimulating the conversion of RAS to its active form. These mutations tend to be mainly associated to ectodermal abnormalities, with the absence of cognitive deficits[23]. In our study, for one patient with Noonan like phenotype and broad chest, the panel sequencing for cardiovascular disease did not identify a variant for the phenotype; however, an incidental finding was reported in *CACNA1C* gene associated with Brugada Syndrome that might identify a cardiovascular risk for the patient. Furthermore, one case presented with Morquio syndrome associated PC, a rare autosomal recessive disorder that results in the deficient activity of the enzyme N-acetylgalactosamine-6-sulfatase.

Conclusions

Since anterior wall chest deformities may be associated with a genetic disorder or syndrome, a thorough family history taking, along with appropriate pediatric and genetic investigations, can provide valuable information. Information derived from such a multi-level approach can influence clinical decision-making and can result in positive implications, both from a therapeutic and prognostic standpoint.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Acknowledgements
These authors contributed equally as Co-first authors: Nicoleta Andreescu and Abhinav Sharma. We thank Dr. Adela Chirita-Emandi for her involvement in clinical patient management.

Funding
No funding was received.

Ethics Approval
Ethics approval was obtained from all concerned authorities before initiating the study.

Informed Consent
Informed written consent was obtained from all patients enrolled in the study.

ORCID ID
Nicoleta Andreescu: 0000-0002-2647-4234; Abhinav Sharma: 0000-0002-0865-0054; Alexandra Mihailescu: 0000-0002-7401-0549; Cristian Zimbru: 0000-0002-2652-0931; Vlad Laurentiu David: 0000-0003-4295-7487; Nilima Rajpal Kundnani: 0000-0002-2824-7182; Maria Puiu: 0000-0002-4078-2831. Raluca Horhat: 0000-0002-3079-2139.

References

1) Burjonrappa S, Sosulski AB, Hsieh L. A Review of Current Treatment Trends in Pediatric Chest Wall Abnormalities. J Pediatr Neonatal Care 2017; 6: 18-22.
2) Jaroszewski D, Notrica D, McMahon L, Steidley DE, Deschamps C. Current management of pectus excavatum: a review and update of therapy and treatment recommendations. J Am Board Fam Med 2010; 23: 230-239.
3) Desmarais TJ, Keller MS. Pectus carinatum. Curr Opin Pediatr 2013; 25: 375-381.
4) David VL, Stanciulescu MC, Horhat FG, Sharma A, Kundnani NR, Ciornei B, Stroescu RF, Popoiu MC, Boia ES. Costal cartilage overgrowth does not induce pectus-like deformation in the chest wall of a rat model. Exp Ther Med 2022; 23: 146.
5) Fokin AA, Steuerwald NM, Ahrens WA, Allen KE. Anatomical, histologic, and genetic characteristics of congenital chest wall deformities. Semin Thorac Cardiovasc Surg 2009; 21: 44-57.
6) Cobben JM, Oostra RJ, van Dijk FS. Pectus excavatum and carinatum. Eur J Med Genet 2014; 57: 414-417.
7) Blanco FC, Elliott ST, Sandler AD. Management of congenital chest wall deformities. Semin Plast Surg 2011; 25: 107-116.
8) Kotzot D, Schwabegger AH. Etiology of chest wall deformities--a genetic review for the treating physician. J Pediatr Surg 2009; 44: 2004-2011.
9) Jones KL JM, Campo M del. Smith's recognizable patterns of human malformation. 7th edition. 7th ed. Philadelphia, PA: Elsevier Saunders; 2013; 998.
10) Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010; 38: 164.

11) Liu X, Wu C, Li C, Boerwinkle E. dbNSFP v3.0: A One-Stop Database of Functional Predictions and Annotations for Human Nonsynonymous and Splice-Site SNVs. Hum Mutat 2016; 37: 235-241.

12) Golladay ES, Char F, Mollitt DL. Children with Marfan's syndrome and pectus excavatum. South Med J 1985; 78: 1319-1323.

13) Creswick HA, Stacey MW, Kelly RE, Jr., Gustin T, Nuss D, Harvey H, Goretsky MJ, Vasser E, Welch JC, Mitchell K, Proud VK. Family study of the inheritance of pectus excavatum. J Pediatr Surg 2006; 41: 1699-1703.

14) Rupprecht H, Hümmer HP, Stöss H, Waldherr T. Pathogenesis of chest wall abnormalities--electron microscopy studies and trace element analysis of rib cartilage. Z Kinderchir 1987; 42: 228-229.

15) Serafin J, Swiatkowski J, Majkusiak R, Nowakowski P. 40-year experience in surgical treatment of congenital chest deformations--ethiopathogenesis, operative techniques and clinical results. Acta Chir Orthop Traumatol Cech 2003; 70: 207-213.

16) Feng J, Hu T, Liu W, Zhang S, Tang Y, Chen R, Jiang X, Wei F. The biomechanical, morphologic, and histochemical properties of the costal cartilages in children with pectus excavatum. J Pediatr Surg 2001; 36: 1770-1776.

17) Ortiz-Cordero C, Azzag K, Perlingeiro RCR. Fukutin-Related Protein: From Pathology to Treatments. Trends Cell Biol 2021; 31: 197-210.

18) Wilson DI, Burn J, Scambler P, Goodship J. DiGeorge syndrome: part of CATCH 22. J Med Genet 1993; 30: 852-856.

19) Schrander-Stumpel CT, Höweler CJ, Reekers AD, De Smet NM, Hall JG, Fryns JP. Arthrogryposis, ophthalmoplegia, and retinopathy: confirmation of a new type of arthrogryposis. J Med Genet 1993; 30: 78-80.

20) Colombani PM. Preoperative assessment of chest wall deformities. Semin Thorac Cardiovasc Surg 2009; 21: 58-63.

21) Rosa RFM, Rosa RCM, Krumenauer RCP, Varella-Garcia M, Paskulin GA. Anterior laryngeal membrane and 22q11 deletion syndrome. Braz J Otorhinolaryngol 2011; 77: 540.

22) Athota JP, Bhat M, Nampoothiri S, Gowrishankar K, Narayanachar SG, Puttamallesh V, Farooque MO, Shetty S. Molecular and clinical studies in 107 Noonan syndrome affected individuals with PTPN11 variants. BMC Med Genet 2020; 21: 50.

23) Lepri F, De Luca A, Stella L, Rossi C, Baldassarre G, Pantaleoni F, Cordeddu V, Williams BJ, Dentici ML, Caputo V, Venanzi S, Bonaguro M, Kavamura I, Faienza MF, Pilotta A, Stanzial F, Faravelli F, Gabrielli O, Marino B, Neri G, Silengo MC, Ferrero GB, Torrrente I, Selicorni A, Mazzanti L, Digilio MC, Zampino G, Dallapiccola B, Gelb BD, Tartaglia M. SOS1 variants in Noonan syndrome: molecular spectrum, structural insights on pathogenic effects, and genotype-phenotype correlations. Hum Mutat 2011; 32: 760-772.