Full text open access online (Since 2001)

PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 24(3): 299-305 (2024)
DOI: 10.31901/24566322.2024/24.03.894

Trio-based Whole Genome Sequencing Arrests de novo Mutations in Pai Syndrome

Jian Ma[1, 2, *], Xiaoxu Liu[3], Chen Hu[1, 2, *], Kun Zhai[1, 2], Lili Yu[1, 2], Denglan Yang[1, 2] and Yongqing Huang[1, 2, *]

[1]Department of Oral and Maxillofacial Surgery, Hospital of Stomatology, The General Hospital of Ningxia Medical University, Ningxia Province, Yinchuan, 750004, P.R. China
[2]Department of Oral and Maxillofacial Surgery, Stomatology College of Ningxia Medical University, Ningxia Province, Yinchuan, 750004, P.R. China
[3]Department of Stomatology, The Affiliated Peace Hospital of Changzhi Medical College, Shanxi Province, Changzhi, 046000, P.R. China

KEYWORDS Pai Syndrome. Clinical Phenotypes. Whole Genome Sequencing. De Novo Missense Mutations

ABSTRACT Pai syndrome (PS) is a multiple phenotypic rare syndrome of cleft lip and palate with an unclear diagnostic criteria and etiology. This research reported the clinical phenotypes for two cases of PS and collected the two trios families. Genomic DNA was extracted from blood and sequenced by whole-genome sequencing (WGS) on two probands and their parents. Variant Effect Predictor (VEP) was utilized to determine causative variants of the patients. Identified causal mutations were further confirmed by Sanger Sequencing. WGS analysis identified 153 single-nucleotide polymorphisms (SNPs) and 69 indels in patient 1, while 165 SNPs and 57 indels were identified in patient 2. After multiple filtrations, the researchers identified four de novo missense mutations (FLG, DHX57, ANXA7, and GOLGA3) in patient 1 and two de novo missense mutations (PRSS23 and USP7) in patient 2. These findings still need to be validated but already provide more information on the genetic basis.

INTRODUCTION

Pai syndrome (PS), detailed for the first time in 1987 (Pai et al. 1987), is a rare congenital midline facial developmental defect, which is characterized by a midline nasal or facial polyps, midline cleft lip (MCL), pericallosal lipoma, and ocular abnormalities (Rudnik-Schöneborn and Zerre 1994; Masuno et al. 1997; Li and Galvin 2018; Pérez et al. 2020). In addition, there are other less typical attributes of Pai syndrome including congenital polyp in mid-anterior alveolar process, midline cleft alveolus, frontal poly, duplicated maxillary frenulum, bifid nose and ventricular septal defect (Debnath et al. 2019; Kocaaga et al. 2023). There is no agreement in the literature for the minimum phenotypic-inclusion criteria. Based on the literature review, Morice et al. proposed a new definition for the PS spectrum: (1) Mandatory diagnosis: Congenital polyp or mass occurring in the nose, mediofrontal skin, or mid-anterior alveolar process; (2) Any one of the following auxiliary diagnoses is sufficient: midline cleft lip, midline alveolar cleft, pericallosal lipoma or interhemispheric lipoma (Morice et al. 2019). The incidence of PS is slightly higher in females and is difficult to identify due to complex phenotypes (Morice et al. 2019). Most PS cases are sporadic and the etiology of this syndrome is not well known, but occasionally it can exhibit family aggregation (Lees et al. 2006). Masuno et al. proposed 46,X,t(X;16)(q28;q11.2) in a female PS case by high-resolution Giemsa banding in 1997 (Masuno et al. 1997). Li and Galvin identified a 1.071-Mb duplication located at the 4q35.2 region using microarray-based comparative genomic hybridization (array CGH), but this particular genetic variant did not exhibit any documented disease association, including a cleft lip or palate (Li and Galvin 2018).

Herein, the researchers report two cases of PS with nasal polyps, mid-anterior alveolar process congenital polyp, and midline cleft alveolus. This

Address for correspondence:
Jian Ma, Chen Hu and Yongqing Huang
Department of Oral and Maxillofacial Surgery,
Hospital of Stomatology,
The General Hospital of Ningxia Medical University
No. 804, Southern Street of Shenli,
Yinchuan, Ningxia Province, 750004, P.R. China.
Phone: 86-0951-6743384
E-mail: majianhs310@163.com, huchen216@126.com, yongqinghuang_6510@126.com

study presents two cases and provides a literature review on PS to improve understanding of PS diagnosis. Whole-genome sequencing was used to screen for potential pathogenic genes in these two trios, aiming to explore the genetic basis of PS. Finally, six likely pathogenetic variants were identified.

MATERIAL AND METHODS

Patient 1 was born at term in 2016. Her family history was uneventful. At birth, she was diagnosed with a nasal polyp that started in the nasal septum, a congenital polyp in the mid-anterior alveolar process, a midline cleft in the alveolus, and a dermoid on the right cornea. (Fig. 1A). Neurological examination was normal. Electrocardiogram and chest radiograph in the neonatal period were all normal. The median nasal and alveolar process appendage was removed at one month to ease feeding, which also yielded excellent cosmetic results (Fig. 1B and 1C). Histological examination of the two appendages revealed them to be benign fibroepithelial polypoid mucosa.

Patient 2 was an 18-month-old girl born after an uneventful pregnancy. She presented with four nasal polyps (Three polyps are situated on the dorsum of her nose, with an additional one attached to the nasal septum of her right nostril) and a midline cleft lip and alveolus. Notably, a larger polyp was observed protruding from her alveolar process into her mouth (Fig. 1D). Electrocardiogram revealed T-wave abnormality. The color Doppler echocardiography showed that the child's arterial duct and oval foramen are both in an unclosed state. Cranial magnetic resonance imaging (MRI) showed bilateral frontal-extracerebral space widening. Excision of the nasal and midanterior alveolar process masses was surgically performed when she was three-month-old (Fig. 1E). Histological examination confirmed the lesion to be a benign fibroma. Considering the insufficient organization of the tissue, the small polys on her right nostril was preserved temporarily. Postoperative recovery has been smooth, with an excellent cosmetic result, and without dimpling or formation of hypertrophic scars. The median cleft lip was also corrected simultaneously (Fig. 1F). She underwent a cheiloplasty with linear incision. Her psychomotor development was normal as of her one-year follow-up.

This study was approved by the Medical Ethics Committee of the General Hospital of Ningxia Medical University and obtained the informed consent of the parents. Blood samples from the two patients and their parents were collected, and next-generation WGS was conducted by BGI Co. Ltd (Shenzhen, China) after DNA extraction in 2017, and their parents were taken as controls. Whole-gene capture was conducted using the Agilent 2100 DNA 1000 Kit, followed by high-throughput sequencing on the Illumina HiSeq X Ten sequencer instrument. The Variant Effect Predictor (VEP) was used to annotate, prioritize, predict, and analyze the genotype-phenotype network of the patients' variants. All variants were detected using the Genome Analysis Toolkit (GATK). The researchers further filtered out common variants (minor allele frequency > 5 percent in 1000 Genomes, the HapMap, Wellderly, CG69, ExAC, and some internal database of BGI such as T2D, PVFD, Autism) and variants involved in parents. Several in silico algorithms were utilized for prediction, incorporating various well-known predictors (SIFT, Mutation Taster, LRT, Mutation Assessor, FATHMM, PolyPhen2_HDIV, PolyPhen2_HVAR, PhyloP, GERP_plus, Gerp, SiPhy, PhastCons, GWAWA). Identified de novo causal mutations were further confirmed by Sanger Sequencing.

RESULTS

The two cases in this research both exhibit the typical clinical symptoms and meet the diagnostic criteria of Pai syndrome. In total, 153 SNPs and 69 indels were identified in patient 1 and 165 SNPs and 57 indels were identified in patient 2. After frequency filtering and retaining the mutation of coding region, the researchers identified five SNPs (*SMAD4*, *FLG*, *DHX57*, *ANXA7*, and *GOLGA3*) in the first families and five SNPs (*KRTAP5-5*: 1651373, 1651376, 1651391, *PRSS23*, and *USP7*) in the second. These mutations have not been reported in the previous literature of PS. Each locus was validated in six samples using Sanger Sequencing, and four *de novo* missense mutations (*FLG*, *DHX57*, *ANXA7*, and *GOLGA3*) in patient 1 and two *de novo* missense mutations (*PRSS23* and *USP7*) in patient 2 (Tables 1 and 2, Fig. 2) were finally confirmed. No identical gene was found between the two patients.

Table 1: Candidate SNP loci in the first trio

Gene	*Genomelocus*	*Position*	*Ref*	*Alt*	*Protein change*	*Numbers of hazardprediction software*
FLG	1q21.3	152282527	T	G	p.Glu1612Ala	0
DHX57	2p22	39088419	T	C	p.Asn378Ser	4
ANXA7	10q22.2	75147502	C	T	p.Arg103His	6
GOLGA3	12q24.33	133349851	A	T	p.Met1255Lys	5

Ref: Reference base, and Alt: Alternative base

Table 2: Candidate SNP loci in the second trio

Gene	*Genomelocus*	*Position*	*Ref*	*Alt*	*Protein change*	*Numbers of hazardprediction software*
PRSS23	11q14.2	86519270	A	C	p.Leu163%3D	0
USP7	16p3.2	9024228	T	C	p.Ile20Val	2

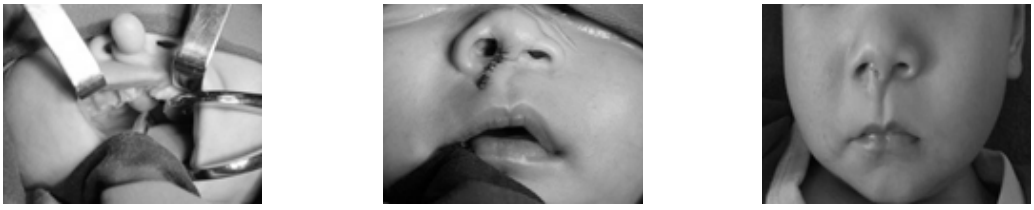
DISCUSSION

PS is a rare midline craniofacial malformation with polyps present at birth, which was first reported by Pai et al. in 1987 (Pai et al. 1987). Its typical clinical manifestation includes midline nasal polyps or oral mucosal polyps, midline cleft lip and lipoma of the central nervous system.

Congenital cutaneous-nasal polyps appear to be the hallmark of PS, as they have been reported in almost all documented cases. The number of these polyps for each case ranges from one to three, and polyps are mostly located in the nostrils. In addition, congenital polyps are also seen in the medial alveolar process mucosa or mediofrontal skin, which is consistent with the cases in this present study. A midline-clefts upper lip was presented in 67 percent of PS patients, with a wide range of severity exhibited, from midline notch of the upper vermillion to a complete cleft extending up to the columella base (Morice et al. 2019). MCL with or without cleft alveolus is the most common facial cleft, followed by bifid nose and duplicated maxillary frenulum (Castori et al. 2007). Cleft alveolus was reported in both cases of the present study, with MCL only reported in Case 2. A pericallosal lipoma or interhemispheric lipoma was found in about 73 percent patients of PS. Intracranial lipomas are usually asymptomatic, and neuropsychological development is generally normal (Patil and Harsh 2017; Tormey et al. 2017; Huckstadt et al. 2018). A PS girl with a corpus callosum lipoma was diagnosed with attention-deficit/hyperactivity disorder (ADHD), but the causality was unknown between ADHD and the lipoma at the corpus callosum. However, another girl with PS developed epilepsy at the age of four years, but a brain MRI performed at four years was normal. In this study's patient, the parents of Case 1 refused to take a cranial MRI, while the patient in Case 2 only showed bilateral enlargement of extracerebral space in the frontal region. Systematic brain imaging is recommended when a patient has congenital midline facial skin polyps or midline facial clefts, as there may be associated with brain disorders. Another common additional feature found in the literature is ophthalmological abnormalities. The typical clinical spectrum of ophthalmological anomalies reported in previous literatures contains corneal leukoma, microcornea, heterochromia iris, conjunctival lipoma, corneal dermoid, and hypertelorism (Demir et al. 2021; Imai et al. 2019; Olivero and Foiadelli 2020). This study's first patient was diagnosed with a corneal dermoid tumor in the right eye during a pediatric ophthalmic examination.

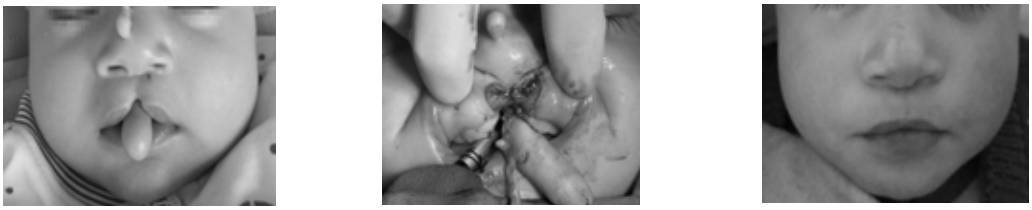
To date, four definitions of PS have been described. Based on the literature review, Morice et al. proposed a new definition for the PS spectrum: (1) a congenital nasal or mediofrontal skin mass and/or a mid-anterior alveolar process polyp (regardless of the pathological diagnosis, that is, hamartoma, dermoid cyst, and/or lipoma) as an obligatory criterion, and at least one of the following criteria: (2) midline cleft of the upper lip and/or mid-

Patient 1



A **B** **C**

Patient 2



D **E** **F**

Fig. 1. The characteristics of the two patients, preoperative (A and D), intraoperative (B and E) and postoperative (C and F) photos

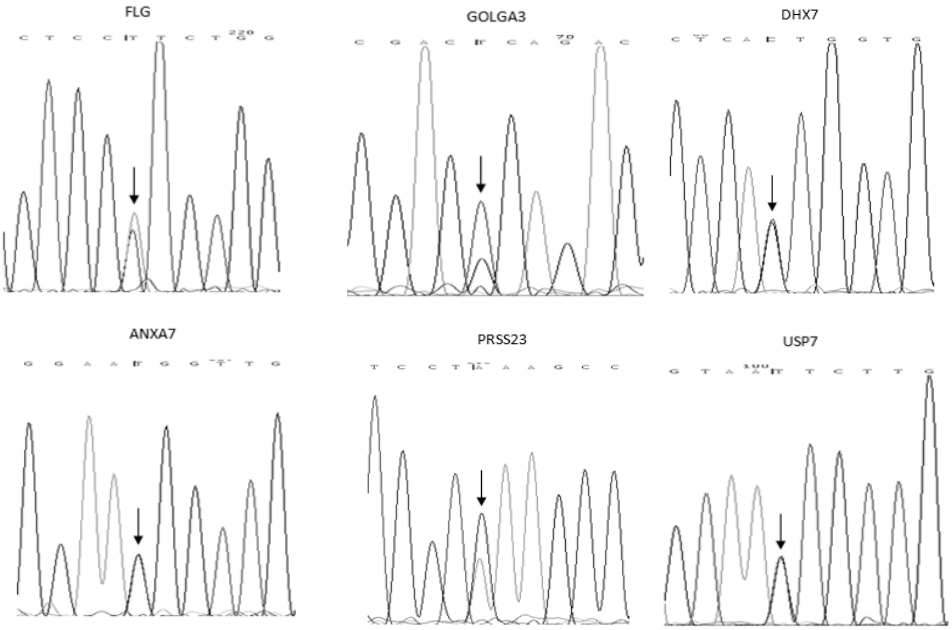


Fig. 2. Sequence results of each SNPs in the six genes

line alveolar cleft, and/or (3) a pericallosal lipoma or interhemispheric lipoma in the case of corpus callosum dysgenesis. Careful physical examination is fundamental for diagnosis of patients with a midfacial mass, independent of whether it is a genetical syndrome. The patients in this present study fulfill all the established diagnostic criteria.

The molecular basis of PS remains unknown and only two mutations have been delineated. Masuno et al. reported a 13-year-old girl with Pai syndrome, characterized by a cleft of midline upper lip, a pedunculated skin masses in the nasal septum, frontal bone protrusion, hypertelorism, short and downward sloping palpebral fissures, short stature, and delayed mental development. Chromosome analysis of her peripheral blood lymphocytes revealed the presence of de novo reciprocal translocations at chromosomes 46, X, t (X; 16) (q28; qll. 2). Another discovery has been in the form of a 1.071-Mb duplication at 4q35.2 by microarray-based comparative genomic hybridization, but the variant had no documented disease association, including cleft lip or palate. Given that clinical diagnosis of PS remains uncertain, genetic analysis using WGS is recommended to uncover potential genomic defects underlying PS.

In the present study, the researchers report two cases of a rare syndromic cleft lip or palate (SCL/P) associated with midfacial mass and/or interhemispheric lipoma. To identify the underlying genetic defect, the researchers utilized WGS to delve into potential causative genes and finally identified six de novo mutations (*FLG*, *DHX57*, *ANXA7*, *GOLGA3*, *PRSS23*, and *USP7*). WGS can theoretically detect all sequence variations in coding and noncoding regions, enhancing result reliability. Furthermore, widely used algorithms like SIFT and Mutation Taster have pinpointed mutations causing deleterious effects and protein structure changes. This appears to be the initial one to identify the genetic causes of PS in patients of China utilizing the WGS approach.

FLG, located on chromosome 1q21.3, consists of three exons and produces a protein associated with intermediate filaments, responsible for bundling keratin-intermediate filaments in the mammalian epidermis. Dysregulated expression of this protein is linked to various keratinizing disorders like ichthyosis vulgaris (Smith et al. 2006; Hoober and Eggink 2022). *DHX57* is a protein-coding gene, related to nucleic-acid binding and helicase activity. Another study found that genetic variants near *MLST8* and *DHX57* affected the epigenetic age of the cerebellum (Lu et al. 2016). *ANXA7* spans approximately 34 kb in the genome with 14 coding exons. The ANXA7 protein belongs to the annexin family of calcium-dependent phospholipid-binding proteins and exhibits various characteristics such as voltage-sensitive calcium channel activity, ion selectivity, and membrane fusion (Meng et al. 2020). It is a tumor suppressor gene, which can be expressed in hereditary spherocytosis, prostate, and other cancers (Ling et al. 2021). GOLGA3 plays a role in glycosylation and the transportation of proteins and lipids within the secretory pathway, which is associated with smallpox. *PRSS23* encodes a serine protease that belongs to a member of the trypsin family, with which diseases are associated including exudative vitreoretinopathy and cutaneous malignant melanoma. PRSS23 has a homologous proteases PRSS35, which has been associated with cleft lip/palate in humans (Letra et al. 2010). The USP7 gene encodes a deubiquitinating enzyme in the peptidase C19 family that functions with components of the MAGEL2/TRIM27 ubiquitin ligase complex, regulating target proteins like p53 and WASH (crucial for endosomal protein recycling). Mutations in this gene are associated with a neurodevelopmental disorder characterized by intellectual disability, epilepsy, autism spectrum disorder, aggressive behavior, hypogonadism, and hypotonia (Hao et al. 2015). Some studies have confirmed that USP7 can mediate the regulation of activities and functions of many proteins in cells, including tumor-suppressor proteins, DNA-repair proteins, immune-response proteins, viral proteins, and epigenetic regulators. *USP7* also plays an important role in the development and progression in lots of diseases (Bhattacharya et al. 2018) In addition, *USP7* can positively regulate Hh signaling by modulating Gli ubiquitination and stabilization. Hh signaling plays an essential role in animal development and tissue homeostasis, and its dysregulation can cause many congenital diseases and cancers (Zhou et al. 2015). Hh signaling also promotes deubiquitination and stabilization of PHF8, which is involved in various pathological disease processes, leading to diseases such as X-linked intellectual disability (XLMR) and tumorigenesis (Sobering et al. 2022). Simultaneously, among patients with the PHF8 mutation, some cases exhibit craniofacial abnormalities characterized by cleft lip and palate. In addi-

tion to CL/P, XLMR also shows some rare clinical malformations- such as steep forehead, widened Nose Bridge, prominent supraorbital ridge, and upward slanting palpeblar fissures-which partially overlap with those of PS (Laumonnier et al. 2005).

CONCLUSION

In summary, to the researchers' knowledge, this is the first report to use WGS to explore the genes involved in PS. The researchers identified six novel mutations in two patients (*FLG*, *DHX57*, *ANXA7*, *GOLGA3*, *PRSS23*, and *USP7*). These findings expand the understanding of the correlation between the spectrum of PS mutations and phenotypes. Even though it is difficult to draw definitive conclusions from only these two patients, accumulated reports could gradually enrich the dataset so that the identification of causative mutations and subsequent early-gene screening could be expected.

RECOMMENDATIONS

In this study, the researchers presented two cases of Pai syndrome exhibiting typical characteristics, including median cleft lip with or without cleft palate, midfacial mass, and/or interhemispheric lipoma. The trio samples were detected using WGS to investigate potential causative genes, ultimately revealing six de novo mutations (FLG, DHX57, ANXA7, GOLGA3, PRSS23, and USP7). It is important to note that we have only made preliminary identifications of genes and mutations that may be associated with Pai syndrome. Further validation through population studies and functional experiments is necessary to confirm these results.

ACKNOWLEDGMENTS

We appreciate all those who made this study possible, particularly these two families of the patients who willingly collaborated with us.

FUNDING INFORMATION

This research was supported by National Natural Science Foundation of China (81600853 and 81960197), Key Research and Development Program of Ningxia province (2021BEG02043 and 2022BEG03159).

CONFLICT OF INTEREST STATEMENT

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

All data in this study are available to interested researchers upon request by contacting with the corresponding author.

REFERENCES

Bhattacharya S, Chakraborty D, Basu M, Ghosh MK 2018. Emerging insights into HAUSP (USP7) in physiology, cancer and other diseases. *Signal Transduction and Targeted Therapy,* 3: 17.

Castori M, Rinaldi R, Bianchi A, Caponetti A, Assumma M, Grammatico P 2007. Pai syndrome: First patient with agenesis of the corpus callosum and literature review. *Birth Defects Res A Clin Mol Teratol,* 79(10): 673-679.

Debnath KK, Kanapaty Y, Yong DJ, Chandran S, Bakri A 2019. 'Pai Syndrome' with anterior alveolar polyp: A variant of a rare clinical entity. *The Medical Journal of Malaysia,* 74(3): 229-230.

Demir CI, Yasar EK, Bayrak BY, Isik H, Alagöz M 2021. First report of Pai syndrome with upper alveolar cavernous hemangioma: Diagnosis and surgical treatment. *J Craniofac Surg,* 32(1): e31-e32.

Hao YH, Fountain MD, Jr., Fon Tacer K, Xia F, Bi W, Kang SH et al. 2015. USP7 acts as a molecular rheostat to promote WASH-dependent endosomal protein recycling and is mutated in a human neurodevelopmental disorder. *Molecular Cell,* 59(6): 956-969.

Hoober JK, Eggink LL 2022. The discovery and function of Filaggrin. *International Journal of Molecular Sciences,* 23(3): 1455.

Huckstadt V, Heis Mendoza ME, Moresco A, Obregon MG 2018. Pai syndrome: Two new cases with unusual manifestations. *Archivos argentinos de pediatria,* 116(2): e336-e340.

Imai Y, Kure S, Nara C, Takagi N, Tachi M 2019. Mental development and surgical prognosis of Pai Syndrome: A case report and review of the literature. *Cleft Palate Craniofac J,* 56(2): 273-279.

Kocaaga A, Bildirici Y, Yimenicioglu S, Karademir-Arslan NC, Vural C, Yildirim E 2023. Case report: Pai syndrome with multiple ventricular septal defect and without cleft palate. *Clin Neurol Neurosurg,* 236: 108045.

Laumonnier F, Holbert S, Ronce N, Faravelli F, Lenzner S et al. 2005. Mutations in PHF8 are associated with X linked mental retardation and cleft lip/cleft palate. *Journal of Medical Genetics,* 42(10): 780-786.

Lees MM, Connelly F, Kangesu L, Sommerlad B, Barnicoat A 2006. Midline cleft lip and nasal dermoids over five generations: A distinct entity or autosomal dominant Pai syndrome? *Clinical Dysmorphology,* 15(3): 155-159.

Letra A, Menezes R, Fonseca RF, Govil M, McHenry T et al. 2010. Novel cleft susceptibility genes in chromosome 6q. *Journal of Dental Research*, 89(9): 927-932.

Li E, Galvin JA 2018. Ophthalmic abnormalities of Pai syndrome: A case report and review of literature. *Ophthalmic Genetics*, 39(2): 282-285.

Ling F, Zhang H, Sun Y, Meng J, Sanches JGP et al. 2021. AnnexinA7 promotes epithelial-mesenchymal transition by interacting with Sorcin and contributes to aggressiveness in hepatocellular carcinoma. *Cell Death Dis*,12(11): 1018.

Lu AT, Hannon E, Levine ME, Hao K et al. 2016. Genetic variants near MLST8 and DHX57 affect the epigenetic age of the cerebellum. *Nature Communications*, 7: 10561.

Masuno M, Imaizumi K, Fukushima Y, Tanaka Y, Ishii T et al. 1997. Median cleft of upper lip and pedunculated skin masses associated with de novo reciprocal translocation 46,X,t(X;16)(q28;q11.2). *Journal of Medical Genetics*, 34(11): 952-4.

Meng K, Lu S, Yan X, Sun Y, Gao J, Wang Y et al. 2020. Quantitative mitochondrial proteomics reveals ANXA7 as a crucial factor in mitophagy. *J Proteome Res*, 19(3): 1275-1284.

Morice A, Galliani E, Amiel J, Rachwalski M et al. 2019. Diagnostic criteria in Pai syndrome: Results of a case series and a literature review. *Int J Oral Maxillofac Surg*, 48(3): 283-290.

Olivero F, Foiadelli T 2020. Pai syndrome: A review. *Childs Nerv Syst*, 36(11): 2635-2640.

Pérez CG, Carrera Fernández S, Rodríguez D'Aquila A 2020. Nasal and lip polyps: Pai syndrome. *Acta otorrinolaringologica Espanola*, 71(3): 195-196.

Pai GS, Levkoff AH, Leithiser RE Jr. 1987. Median cleft of the upper lip associated with lipomas of the central nervous system and cutaneous polyps. *Am J Med Genet*, 26(4): 921-924.

Patil SB, Harsh S 2017. Lipoma of Columella with septal extension in Pai syndrome: report of a rare case. *BMC Ear Nose Throat Disord*, 17: 2.

Rudnik-Schöneborn S, Zerres K 1994. A further patient with Pai syndrome with autosomal dominant inheritance? *Journal of Medical Genetics*, 31(6): 497-498.

Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE et al. 2006. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nature Genetics*, 38(3): 337-342.

Sobering AK, Bryant LM, Li D, McGaughran J, Maystadt I, Moortgat S et al. 2022. Variants in PHF8 cause a spectrum of X-linked neurodevelopmental disorders and facial dysmorphology. *HGG Advances*, 3(3): 100102.

Tormey P, Bilic Cace I, Boyle MA 2017. Ocular dermoid in Pai Syndrome: A review. *Eur J Med Genet*, 60(4): 217-219.

Zhou Z, Yao X, Li S, Xiong Y et al. 2015. Deubiquitination of Ci/Gli by Usp7/HAUSP regulates hedgehog signaling. *Developmental Cell*, 34(1): 58-72.

Paper received for publication in November, 2023
Paper accepted for publication in May, 2024