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CASE REPORT

Identification of a novel *MYO1D* variant associated with laterality defects, congenital heart diseases, and sperm defects in humans

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Abstract **The establishment of left–right asymmetry is a fundamental process in animal development. Interference with this process leads to a range of disorders collectively known as laterality defects, which manifest as abnormal arrangements of visceral organs. Among patients with laterality defects, congenital heart diseases (CHD) are prevalent. Through multiple model organisms, extant research has established that myosin-Id (MYO1D) deficiency causes laterality defects. This study investigated over a hundred cases and identified a novel biallelic variant of *MYO1D* (NM_015194: c.1531G>A; p.D511N) in a consanguineous family with complex CHD and laterality defects. Further examination of the proband revealed asthenoteratozoospermia and shortened sperm. Afterward, the effects of the D511N variant and another known *MYO1D* variant (NM_015194: c.2293C>T; p.P765S) were assessed. The assessment showed that both enhance the interaction with β-actin and SPAG6. Overall, this study revealed the genetic heterogeneity of this rare disease and found that *MYO1D* variants are correlated with laterality defects and CHD in humans. Furthermore, this research established a connection between sperm defects and *MYO1D* variants. It offers guidance for exploring infertility and reproductive health concerns. The findings provide a critical basis for advancing personalized medicine and genetic counseling.**

Keywords MYO1D; laterality defect; congenital heart disease; sperm defect; β-actin; SPAG6

Introduction

Left–right (LR) asymmetry is a widespread phenomenon in living organisms. Abnormal asymmetry is rare and can result from genetic mutations or environmental factors. The normal arrangement of visceral organs is known as situs solitus, and the inverted arrangement is referred to as situs inversus. Heterotaxia is a term used when subsets of organs show normal or aberrant positioning or morphology. Situs inversus and heterotaxia are collectively termed laterality defects, and they are often accompanied with congenital heart diseases, bronchiectasis, reproductive dysfunction, and other disorders [1]. Advancements in genomics and sequencing technologies have revealed that gene-encoding ciliary components and planar cell polarity (PCP) proteins play a role in these conditions, leading to their classification as ciliopathies [2].

The actin cytoskeleton is closely associated with ciliopathies because it regulates ciliogenesis and PCP [3,4]. Unconventional myosin-Id (MYO1D) functions as an actin-based motor protein and cooperates with the core PCP gene *Vangl2* in the formation of the animal LR axis [5]. Previous research that used various model organisms has established that MYO1D deficiency can cause laterality defects [5–8]. However, evidence on its pathogenicity in humans is lacking.

In this study, we identified a novel biallelic variant of *MYO1D* (NM_015194.2:exon12:c.1531G>A:p.D511N) in a proband from a consanguineous family, who presented with complex congenital heart disease, laterality

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defects, and asthenoteratozoospermia. This *MYO1D* variant and another *MYO1D* variant (NM_015194.2: c.2293C>T:p.P765S) found in an Arab family [9] suggested that *MYO1D* variants are responsible for laterality defects and congenital heart diseases and may play a role in sperm development.

Case report

This study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University, China, and written informed consent was obtained from subjects.

The proband, a 30-year-old man, presented with a complex set of medical conditions, including double outlet right ventricle defect, ventricular septal defect, patent foramen ovale, pulmonic stenosis, mirror image dextrocardia, and heterotaxia (Fig. 1A–1D).

After a genetic analysis, a novel variant in the *MYO1D* gene was identified as a potential candidate. Considering MYO1D's role in ciliogenesis, we assessed the patient's nasal mucociliary and spermatozoal functions. The nasal nitric oxide (nNO) concentration was measured to be 295 ppb, and the nNO production value was 117 nL/min, which exceeded the diagnostic cutoff value of 77 nL/min for primary ciliary dyskinesia (PCD). High-speed video microscopy revealed normal ciliary movement. Computer-aided sperm analysis was employed to assess the sperm function. In accordance with the WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition), sperm motility, progressive motility, and the normal morphology rate were calculated and found to be lower than the reference values, indicating asthenoteratozoospermia (Table 1). In conclusion, aside from heterotaxia and complex congenital heart diseases, the patient also suffered from asthenoteratozoospermia. However, PCD was ruled out based on the evaluation of the nasal mucociliary function and the genetic analysis.

The patient was born into a consanguineous family, where his parents are cousins and have divorced (Fig. 2A). Whole exome sequencing was performed on the patient, and after data filtration [10], seven variants were retained (Table 2). Among these variants, a novel biallelic variant in the *MYO1D* gene (NM_015194.2: exon12:c.1531G>A:p.D511N) was identified as the causative factor and validated through Sanger sequencing. The patient's parents were found to carry the same heterozygous variant (Fig. 2B). The amino acid sequence of MYO1D around position 511 showed high

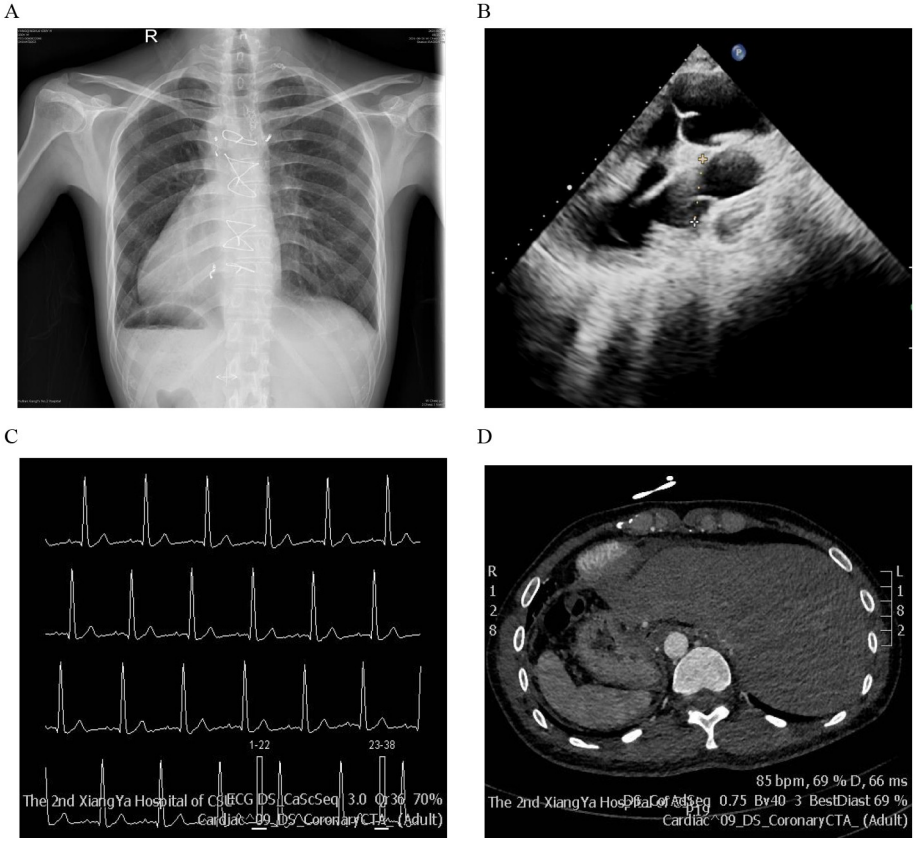

Fig. 1 Clinical presentations of the patient. (A–D) The patient exhibited mirror image dextrocardia, double outlet right ventricle defect, ventricular septal defect, patent foramen ovale, pulmonic stenosis, and heterotaxia.

Table 1 Semen characteristics of the patient with the D511N variant

Semen parameter	Proband	Reference limits
Semen volume (mL)	5.0	1.5
Sperm concentration (10^6/mL)	17.8	15.0
Motility (%)	26.0	40.0
Progressive motility (%)	21.0	32.0
Normal morphology rate (%)	3.3	4.0

conservation across multiple species, indicating its potential functional importance (Fig. 2C).

Bioinformatic analysis (MutationTaster, PolyPhen-2, SIFT, CADD, etc.) indicated that this variant was deleterious (Table 2). The allele frequency of D511N was significantly less than 0.001, and no homozygotes were found in genetic databases. Furthermore, the variant was not detected in 200 unrelated ethnically matched healthy controls or in a cohort of 115 heterotaxia patients.

The two *MYO1D* variants identified in humans were mapped to the protein domain's structure illustration. The D511N variant was located in the myosin motor domain, just before the actin binding domain, and P765S was situated in the nondomain region between IQ2 and TH1 domains (Fig. 3A).

The protein 3D model was downloaded from the AlphaFold Protein Structure Database, and the positions of 511, 765, and the actin-binding domain of MYO1D received high confidence scores. Protein models with the specific variants were generated using PyMOL. Similar to Alsafwani et al.'study, we observed minor structural changes when the 765th residue position was substituted by serine. However, the substitution of aspartic acid with asparagine at the 511th residue position did not result in any significant differences from the native structure (Fig. 3B). Electrostatic potential maps showed that both variants altered the surface potential (Fig. 3C and 3D). No changes were observed in the actin-binding domain during the structural simulation process.

MYO1D plasmids carrying the D511N and P765S point mutations were constructed and transfected into 293T cells to evaluate the effect of the variants. Western blot analysis revealed that the expression level of the MYO1D protein in the cells transfected with the D511N plasmid was significantly higher than that in the cells transfected with the normal (wild-type) plasmid, indicating that the D511N variant probably led to an overexpression of MYO1D, whereas the P765S variant slightly decreased its expression (Fig. 3E). The

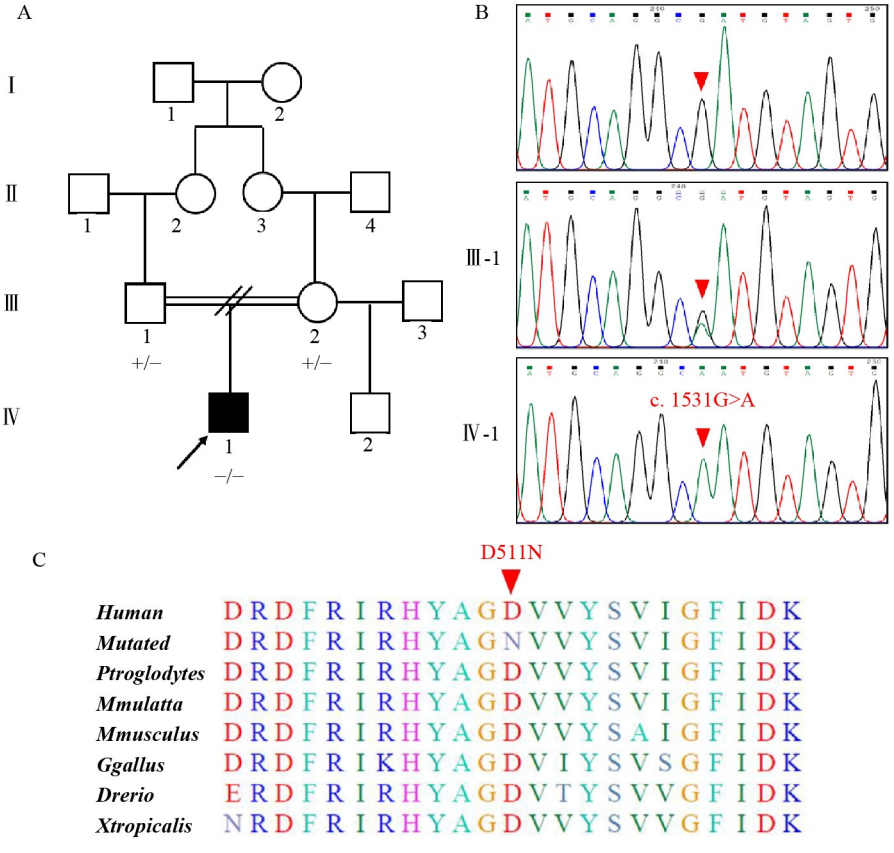

Fig. 2 Genetic analysis identified a novel *MYO1D* variant. (A) Pedigree of a consanguineous family; the patient's parents are divorced. (B) Sanger sequencing diagrams showing a heterozygous variant in individual III-1 and a homozygous variant in the proband (IV-1). (C) The amino acids of MYO1D are evolutionarily conserved.

Table 2 Candidate variants after WES data filtration and pathogenicity predictions

Gene	Genotype	Variant	CADD	SIFT	Polyphen2	MutationTaster	FATHMM	PROVEAN	MetaSVM	MetaLR	M-CAP
PKD1L2	hom	NM_052892.3:NA:c.1998-2A>C	19.96	–	–	D	–	–	–	–	–
MYO1D	hom	NM_015194.2:exon12:c.1531G>A:p.D511N	23.3	D	P	D	D	D	D	D	D
GAB4	hom	NM_001037814.1:exon6:c.1141G>T:p.G381C	21.8	D	P	D	T	N	T	T	T
CMYA5	hom	NM_153610.4:exon2:c.8872G>T:p.A2958S	27.8	D	D	D	T	N	T	T	D
SAMD9	hom	NM_001193307.1:exon2:c.1246G>T:p.V416L	25.3	D	D	D	T	N	T	T	T
PHKA1	hom	NM_001122670.1:exon16:c.1685T>G:p.I562S	25	D	P	D	D	D	D	D	D
SLFN14	hom	NM_001129820.1:exon4:c.2556dup:p.T853Hfs*28	22.6	–	–	–	–	–	–	–	–

D, deleterious; P, possibly damaging; T, tolerated; N, neutral.

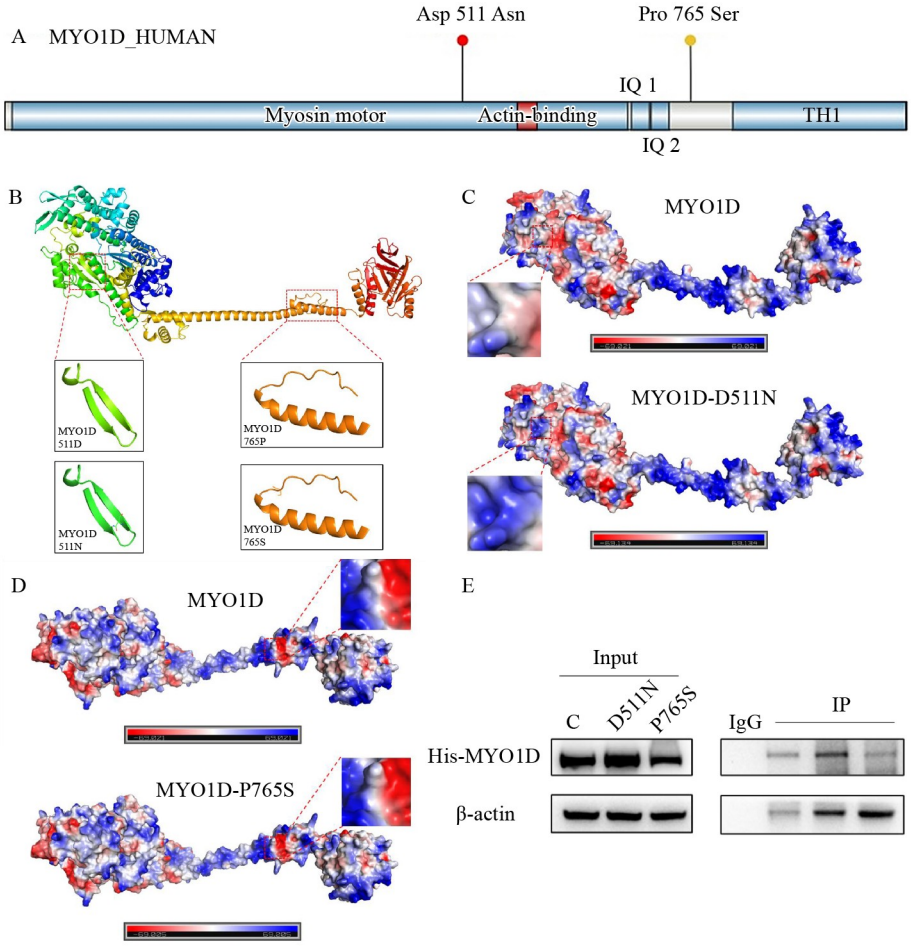

Fig. 3 Bioinformatic and co-immunoprecipitation analyses. (A) Mapping of the two identified *MYO1D* variants in humans to the protein domain's structure illustration. (B) The D511N variant showed no effect on the protein structure, whereas P765S resulted in minor structural drifts. (C, D) Both variants caused changes in the surface potential of the protein. (E) The D511N variant significantly enhanced the expression level of MYO1D, whereas the P765S variant slightly decreased its expression. Co-immunoprecipitation analysis demonstrated that both *MYO1D* variants (D511N and P765S) exhibited increased binding with β-actin.

interaction of MYO1D with actin fibers is essential for its function [6]. Co-immunoprecipitation experiments were conducted to assess this interaction [11], and they revealed that the D511N variant did not affect the interaction of MYO1D with β-actin, but the P765S variant significantly enhanced the binding to β-actin (Fig. 3E). These findings suggest that the D511N and P765S variants exhibited a gain-of-function characteristic. However, given the recessive inheritance pattern of *MYO1D* variants, our hypothesis leans toward a loss-of-function pathogenic mechanism. The two variants potentially affect MYO1D proteins by reducing their

motor functionality, suppressing ATPase activity, disrupting their interaction with calmodulin chains, and other effects. The increase in expression and the intensified interactions might be compensatory reactions.

The spermatozoal length of the patient was generally 10 μm shorter than that of the control group, which is in accordance with the cilia phenotype observed in *myo1d* knockout zebrafish [5] (Fig. 4A–4C). Spermatozoa with a length larger than 50 μm were rarely found in the patient's semen (Fig. 4C). Furthermore, functional analysis of sperm motility in the patient revealed a considerable reduction in progressive motility compared with the control group.

Sperm-associated antigen 6 (SPAG6), a causative gene of severe asthenoteratozoospermia, interacts with MYO1D and facilitates its translocation to the plasma membrane [12,13]. The ability of these variants to bind SPAG6 is consistent with their ability to bind β-actin. The D511N variant demonstrated a similar binding capacity as the native protein. The P765S variant showed a stronger binding capacity than the native proteins (Fig. 4D).

These findings establish a connection between sperm defects and *MYO1D* variants, thus providing valuable new clues for exploring infertility and reproductive health concerns.

Discussion

MYO1D deficiency causes laterality defects in zebrafish, frog, and *Drosophila* models [5–8]. In humans, the first clinical evidence was from a heterotaxy patient in an Arab consanguineous family, where genetic analysis identified a homozygous variant (c.2293C>T) in the *MYO1D* gene [9]. This patient, similar to ours, suffered from complex congenital heart disorders and heterotaxy. These findings strongly suggest a conservative role of MYO1D in breaking LR symmetry [6].

Clinically, cardiac diseases are prevalent in patients with heterotaxy [12]. Large-scale forward genetic screening in mice has unveiled the central role of cilia in congenital heart disease and established a correlation between LR asymmetry and cardiac development [13]. Studies on mice further revealed that the sonic hedgehog (SHH) signaling transduced by cilia coordinates LR patterning, heart looping, and differentiation of the heart tube and regulates subsequent events of heart development, including outflow tract septation and formation of the atrioventricular septum [12,14]. In zebrafish and *Xenopus*, knockdown of *myo1d* leads to short cilia in the LR organizer (LRO), and although the movement of short cilia may be normal, the LRO flow is altered [5,8]. These changes in cilia length can result in aberrant SHH signaling transduction, which may underlie defective cardiac jogging in zebrafish and congenital heart defects in humans [5,12,15].

Male infertility is also a primary phenotype of ciliopathy [16]. In addition to the structural components in cilia, the actin cytoskeleton contributes to spermatogenesis by cell polarity [17]. Studies have shown strict spermatid apico-basal polarity and Sertoli cell polarity during spermatogenesis, and altered polarity can cause

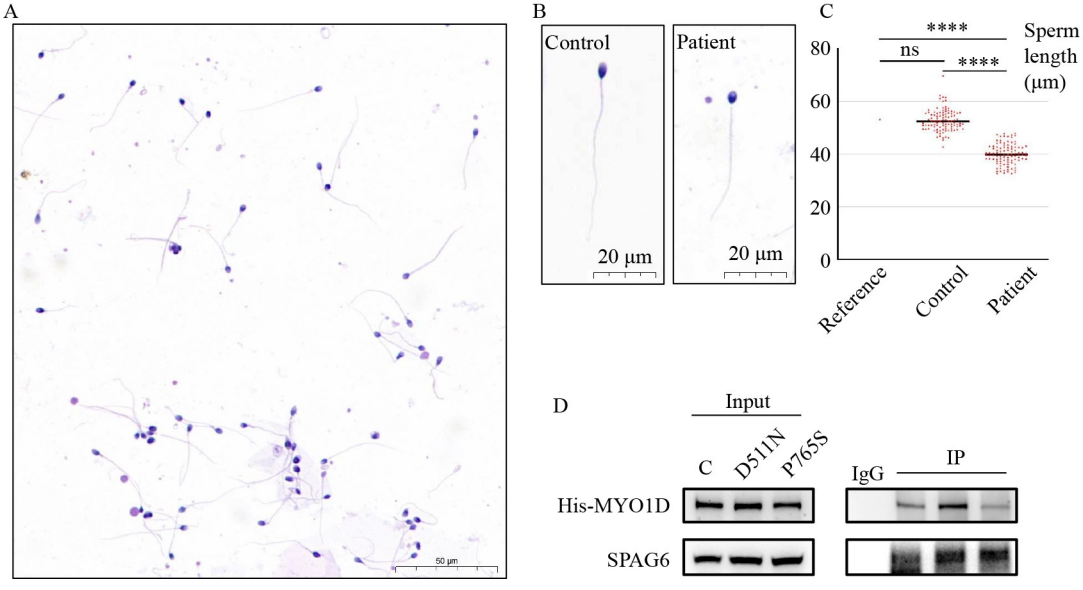

Fig. 4 MYO1D variants associated with sperm defects. (A) The length of the patient's sperms is shorter than 50 μm. (B) Comparison of a typical sperm from a control participant and a sperm from the patient. (C) Comparative analysis revealed a significant sperm length defect (n = 101). ****, $P < 0.0001$. (D) Co-immunoprecipitation analysis demonstrated that the MYO1D variants D511N and P765S exhibited increased binding with sperm-associated antigen 6.

asthenoteratozoospermia and multiple morphological abnormalities of the sperm flagella (MMAF) [17]. For instance, SPAG6 deficiency results in planar cell polarity (PCP) defects and hearing loss in mice, and in humans, homozygous *SPAG6* variants can induce nonsyndromic asthenoteratozoospermia with severe MMAF [18,19]. In *Drosophila* larvae, Myo1d overexpression induces polarized reorganization of epithelial cells toward the dextral orientation [6]. Furthermore, in zebrafish, Myo1d can functionally interact with the core PCP component Vangl2 to shape a productive LRO flow [5]. These results illustrate that MYO1D regulates PCP, and MYO1D deficiency is a potential cause of male infertility. Notably, the patient's sperm was dramatically shorter than the reference and normal sperm of the control subjects, a result that agrees with the findings of the model research and provides strong evidence that MYO1D supports spermatogenesis.

Airway cilia are the main "9+2" motile cilia, and they are frequently disordered and accompanied with heterotaxia and sperm defects. In this study, the patient exhibited reduced sperm motility, but the motility of the airway cilia remained normal. This discrepancy might be due to MYO1D's role as an extra-ciliary component, unlike cilia structural genes, such as DNAH10 [20,21]. MYO1D deficiency does not directly affect the symmetric side-to-side beating of airway cilia; instead, it disrupts the rotational movement of sperm [22]. Furthermore, a shortened sperm length can potentially interfere with the development of the sperm tail, a critical factor for mobility [23].

Conclusions

In conclusion, this study demonstrated that biallelic variants in *MYO1D* are associated with laterality defects, congenital heart defects, and sperm defects in humans.

Acknowledgements

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Compliance with ethics guidelines

Conflicts of interest Zhuangzhuang Yuan, Xin Zhu, Xiaohui Xie, Chenyu Wang, Heng Gu, Junlin Yang, Liangliang Fan, Rong Xiang, Yifeng Yang, and Zhiping Tan declare no conflict of interest.

The study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University, and the study was performed in accordance with the ethical standards stated in the 1964 *Declaration of Helsinki* and its later amendments or comparable ethical standards. Informed consent was obtained from all patients included in the study.

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