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The prevalence of pathogenic variants in the *BMPR2* gene in patients with the idiopathic pulmonary arterial hypertension in the Russian population: sequencing data and meta-analysis

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Abstract

Background Idiopathic pulmonary arterial hypertension (IPAH) is a rare and severe form of pulmonary hypertension, with a genetic basis most commonly associated with mutations in the BMPR2 gene. However, no genetic testing has been reported for IPAH patients in the Russian population, nor have systematic studies been conducted to assess the frequency of pathogenic variants in this group.

Methods The study cohort included 105 IPAH patients, consisting of 23 males and 82 females, who were managed at the PH care center in Moscow, Russia, from 2014 to 2024. Genetic testing was performed using whole-genome sequencing. Variant identification and annotation were conducted using GATK, DeepVariant, VEP, sv-callers and AnnotSV. A meta-analysis, performed with MOOSE, included 24 studies involving 3124 IPAH patients and 470 P/LP variants. Pathogenicity reassessment was carried out using InterVar, which incorporates ACMG criteria.

Results Analysis of 105 adult IPAH patients in Russia revealed 11 patients (10.48%) as carriers of pathogenic or likely pathogenetic (P/LP) BMPR2 variants. As the result of reassessment, the number of P/LP *BMPR2* variants raised from 394 (59%) to 445 (67%) with 80 pathogenic variants became of uncertain significance, and 152 unclassified variants became P/LP. The meta-analysis of these reevaluated pathogenic variants showed that while the frequency of P/LP variants in our cohort (10.48%) is lower than the overall average of 17.75% from the meta-analysis, the difference is not statistically significant ($p = 0.062$). Additionally, we report three P/LP BMPR2 variants, not reported in literature, with one being structural, and four P/LP variants in TBX4, ATP13A3 and AQP1 genes from 27 IPAH genes in 3 patients.

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Conclusions For the first time, we present the results of genetic testing in IPAH patients from the Russian population. Despite the considerable heterogeneity in the world-wide data, the prevalence of pathogenic BMPR2 mutations in IPAH patients from the Russian population does not significantly differ from the overall average in the meta-analysis. It is crucial to periodically reassess the pathogenicity of published variants, as half of the pathogenic BMPR2 IPAH variants were reclassified as LP or of uncertain significance.

Keywords Bone morphogenetic protein receptor type-2, BMPR2, Genetics, Idiopathic pulmonary arterial hypertension, InterVar, Pathogenic variants, Likely pathogenic variants, Meta-analysis, Pulmonary arterial hypertension, Re-evaluate pathogenicity, Russian population, Whole-genome sequencing

Introduction

Pulmonary hypertension (PH) is a condition characterized by an increased mean pulmonary artery pressure (mPAP) of more than 20 mmHg, assessed by right heart catheterization at rest. One of the types of PH is idiopathic pulmonary arterial hypertension (IPAH), a rare and severe form with a genetic basis. IPAH is marked by pulmonary artery remodeling, which leads to increased pulmonary vascular resistance, right ventricular hypertrophy, and ultimately right heart failure.

If relatives have the same disease, it is referred to as "hereditary pulmonary arterial hypertension" (HPAH) [1]. The inheritance of the disease is autosomal dominant with low penetrance, which varies between males and females. Therefore, the term "IPAH/HPAH" is commonly used to refer to both forms, as they share the same clinical characteristics. Currently, the disease mechanism is associated with defects in genes encoding components of the bone morphogenetic protein receptor type-2 (BMPR2) signaling pathway. To date, 27 genes have been described as potentially involved in the disease [2]. Based on experimental and clinical evidence, the involvement of 12 of these genes is considered definitive, while 6 others have moderate evidence. The remaining genes have either low or disputed evidence.

The most common IPAH-associated pathogenic variants are located in BMPR2 gene. Its frequency varies from study to study in a wide range from 8% [2] to 52% [3], in the latter case, among lung transplant IPAH/HPAH patients [3].

These differences could arise from several factors. They may be related to variations in the severity of IPAH/HPAH within specific groups, reflecting the distinct characteristics of the study populations. Additionally, the frequency of pathogenic variant carriers may be influenced by ethnic or regional factors. Furthermore, we suggest that the use of different methods and programs to assess the pathogenicity of variants in IPAH/HPAH patients could significantly contribute to these discrepancies.

Thus, the aim of the study was to determine the carriage of pathogenic variants of IPAH/HPAH–associated genes, to re-evaluate all published variants of the BMPR2 gene using a modern method considering the current pathogenicity criteria, and to compare the frequency of carriage of pathogenic variants of the BMPR2 gene in the Russian population with that in other studies using meta-analysis.

Methods

Study cohort

The study cohort consisted of 105 patients with IPAH, including 23 males and 82 females managed in the PH care center from 2014 to 2024. Most of the patients (80%) were from the Moscow region and 87%—ethnic Russians. The diagnosis of idiopathic pulmonary arterial hypertension was verified according to the current guidelines of the European Society of Cardiology for the diagnosis and treatment of pulmonary hypertension (ESC/ERS Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension) [4], by excluding other groups of pulmonary hypertension (associated with left heart disease, lung diseases, chronic thromboembolic pulmonary hypertension) and associated conditions (congenital heart defects, systemic connective tissue diseases, HIV infection, etc.). Hemodynamic inclusion criteria included a mean pulmonary arterial pressure (mPAP) > 25 mmHg, pulmonary vascular resistance (PVR) > 3 Wood units, and a pulmonary artery wedge pressure (PAWP) < 15 mmHg. Following the publication of the ESC/ERS 2022 recommendations, the mPAP threshold was revised to > 20 mmHg, PVR > 2 Wood units, and PAWP < 15 mmHg. In cases of borderline PAWP values, a fluid challenge test was performed to exclude heart failure with preserved ejection fraction (HFpEF). Indeed, there is currently an observed increase in the average age of patients with idiopathic pulmonary arterial hypertension, which is a global trend likely associated with improved disease diagnosis. In the COMPERA registry, the average age of patients with idiopathic/hereditary/drug-induced pulmonary arterial hypertension was 65.9 ± 15.9 years [5].

Baseline characteristics of PAH patient data including age, age of IPAH first symptoms, age at diagnosis, time from IPAH first symptoms to diagnosis, six-minute walking distance, WHO functional class at time of diagnosis, peripheral oedema, mean pulmonary arterial pressure (mPAP), pulmonary vascular resistance (PVR), right atrial pressure (RAP), tricuspid annular plane systolic excursion (TAPSE), NT-proBNP, N-terminal pro-brain natriuretic peptide are presented in Table 1.

Sequencing

Genomic DNA was extracted using the magnetic bead-based sorption method (MGIEasy Magnetic Beads Blood Genomic DNA Extraction Kit, MGI) and subsequently used for the preparation of genomic libraries for sequencing. Library preparation was performed using a PCR-free protocol with enzymatic DNA fragmentation (MGIEasy FS PCR-Free Library Prep Set, 96 reactions (MIX), MGI). Libraries were sequenced using DNBSEQ-T7 (PE150) sequencer technologies following the manufacturer's recommendations.

Table 1 Characteristics of IPAH/HPAH patients (n = 105)

Characteristics	N = 105
IPAH/HPAH, n (%)/n (%)	101(96.2)/4(3.8)
Females/males, n (%)	82/23 (78.1/21.9)
Age, years	50.4 ± 15.56
Age of PAH first symptoms, years	43.4 ± 17.69
Age at diagnosis, years	48.6 ± 16.78
Time from first symptoms to diagnosis, years	5.2 ± 10.76
Six-minute walking distance, m	367.2 ± 120.17
WHO functional class at time of diagnosis	
I, n (%)	2 (1.9)
II, n (%)	25 (23.8)
III, n (%)	74 (70.5)
IV, n (%)	4 (3.8)
Peripheral oedema, n (%)	52 (49.5)
mPAP, mmHg	55.04 ± 15.928
PVR, Wood unit	13.17 (7.7;16.2)
Cardiac index, L/min/m^2	2.48 (1.7;3.0)
RAP, mmHg	8.16 ± 5.354
Positive vasoreactivity test at time of diagnosis, n (%)	13 (12.4)
TAPSE, mm	16.88 (13;20)
NT-proBNP, pg/ml	2373.6 (221.3; 2479.5)

* HPAH, heredity pulmonary artery hypertension; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; TAPSE, tricuspid annular plane systolic excursion; NT-proBNP, N-terminal pro-brain natriuretic peptide

Variant calling and annotation

The pipeline for calling germline variants was implemented as follows. Initially, adapter sequences and low-quality nucleotides were trimmed with Trimmomatic [6]. Subsequently, the reads were aligned to the GRCh38 reference genome, including additional contigs, in accordance with GATK Best Practices, utilizing bwa version 0.7.17 [7]. Following alignment, duplicates, which comprise approximately 1% of the reads due to the PCR-free library preparation protocol, were marked using the GATK MarkDuplicatesSpark version 4.3.0.0 [8]. Finally, germline SNVs and InDels calling was performed with DeepVariant version 1.4.0 [9]. Only variants with "pass" filter were included in the analysis.

Annotation of the identified variants was performed using the VEP tool [10]. The classification of the pathogenicity of genetic variants was conducted in accordance with the recommendations of the American College of Medical Genetics and Genomics (ACMG), utilizing the InterVar tool [11]. After that we performed additional search for the discovered variants in the databases HGMD [12] and ClinVar [13].

The aligned bam files of 105 patients were used for structural variants (SVs) calling using sv-callers [14], a union of four callers: (1) Lumpy, (2) GRIDSS, (3) DELLY, and (4) Manta. Each tool detects SVs based on a combination of coverage, split reads, and assembly, and has unique discrete workflows. Briefly, SV detections were performed independently with each tool. SURVIVOR (v1.0.7) was then used to merge SVs longer than 50 bp, of the same type, within 100 bp of each other, and identify consensus calls as well as eligible SR > 5&PE > 3 metrics. Only SVs that overlapped with the gene set of interest were included in the analysis. Further analysis included pathogenicity annotation using AnnotSV, based on the joint consensus recommendation of ACMG and ClinGen.

Selection of genes for analysis

IPAH gene list included 27 genes that are divided into five category: definitive (BMPR2, ACVRL1, ATP13A3, CAV1, EIF2AK4, ENG, GDF2, KCNK3,KDR, SMAD9, SOX17, and TBX4), moderate (ABCC8, GGCX, and TET2), limited (AQP1, BMP10, FBLN2, KLF2, KLK1, and PDGFD), disputed (BMPR1A, BMPR1B, NOTCH3, SMAD1, and SMAD4), and unknown (TOPBP1) [2].

Selection of articles for meta-analysis

For meta-analysis, we selected studies that reported data on the frequency of pathogenic and likely pathogenic variants in the *BMPR2* gene among patients with IPAH. A total of 561 articles were initially identified in the

PubMed database using the following search query: "(idiopathic pulmonary arterial hypertension OR pulmonary arterial hypertension OR PAH OR IPAH) AND (*BMPR2* OR bone morphogenetic protein receptor type 2)." After a preliminary screen based on abstracts, 144 articles were selected for further consideration. Following a comprehensive full-text review, 81 studies were included in the analysis. Of those, 48 articles contained relevant data on the frequency of *BMPR2* variants, while detailed variant descriptions were provided in only 24 of the selected studies (Fig. 1).

A meta-analysis of the frequency of pathogenic variants was conducted in accordance with the MOOSE (Meta-Analysis of Observational Studies in Epidemiology) guidelines [15]. The Freeman-Tukey transformation was used to calculate the pooled weighted proportion under both the fixed and random effects models. Heterogeneity of the model was assessed using the Q statistic and the I2 index. In cases of substantial heterogeneity ($I2 > 75\%$), the random effects model was applied, while the fixed effects model was used for lower heterogeneity levels. For the Q-statistic, a $p < 0.1$ was considered to indicate heterogeneity. The presence of publication bias was assessed using the Egger's test and Begg's test.

Results

Discovered pathogenic and likely pathogenic variants in IPAH/HPAH patients in the Russian population.

In the cohort of 105 patients with IPAH/HPAH nine SNVs and one structural deletion were identified in the *BMPR2* gene (NM_001204.7) as pathogenic or likely pathogenic based on ACMG criteria. All variants are heterozygous. Seven of ten variants have been previously reported in dbSNP and literature. A subsequent analysis of these seven variants in HGMD and ClinVar confirmed their pathogenicity. All variants had a "Disease-causing mutation" status associated with "Pulmonary hypertension, primary" in the HGMD database and P or P/LP with 2-star review status for rs863223426, rs1060502581, rs137852751, rs1085307151

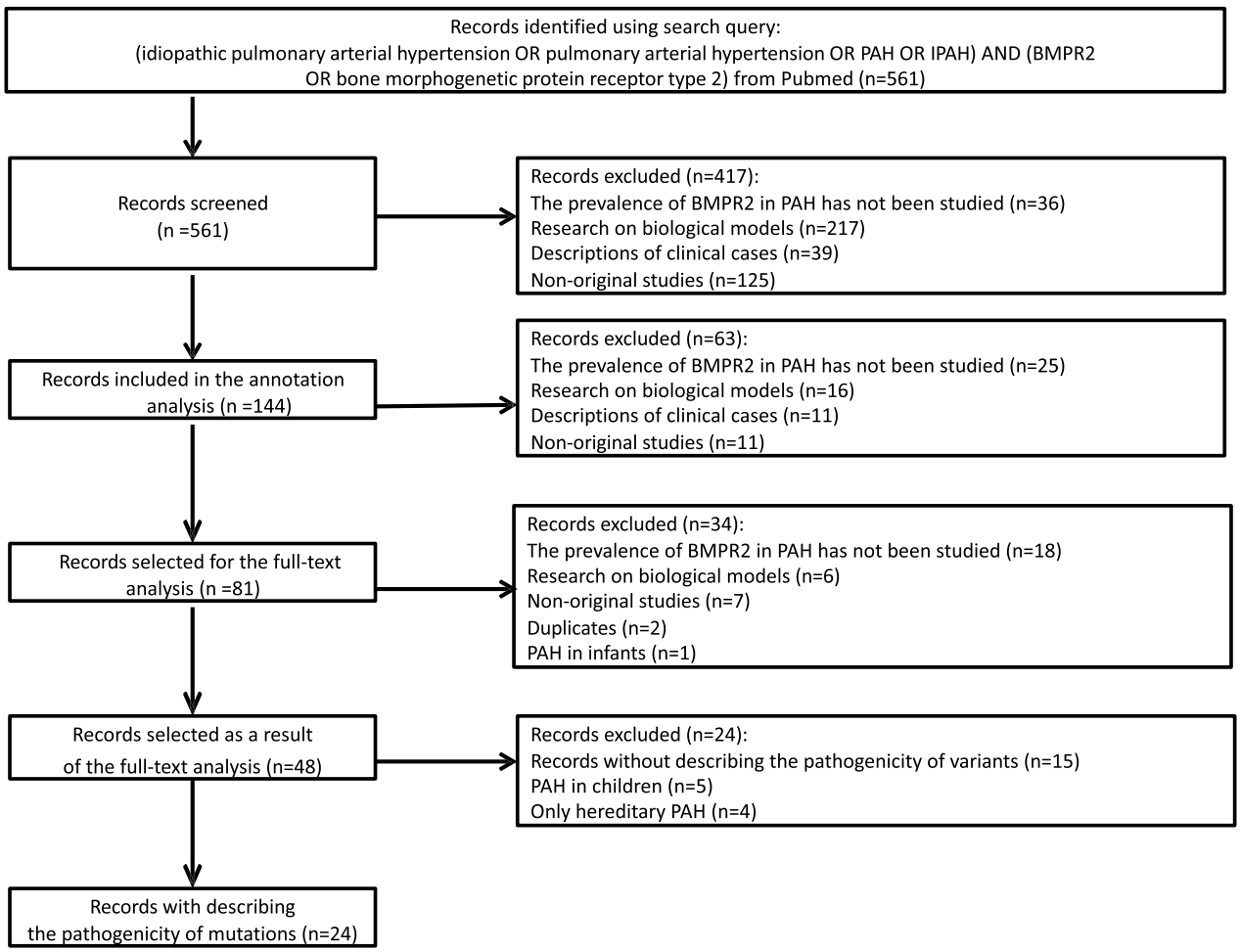

Fig. 1 Article selection pipeline for inclusion in the meta-analysis

Table 2 Pathogenic and likely pathogenic variants of IPAH/HPAH genes

Pts No	Gene	Chr	Pos	Id	Ref	Alt	TYPE	HGVSc	HGVSp	ACMG	HGMD	ClinVar
1,2	*BMPR2*	2	202467648	rs863223426	A	G	Missense variant	c.377A > G	p.Asn126Ser	LP	DM	P/LP
3	*BMPR2*	2	202520195	rs1060502581	C	T	Stop gained	c.961C > T	p.Arg321Ter	P	DM	P
4	*BMPR2*	2	202530820	rs137852751	C	T	Stop gained	c.994C > T	p.Arg332Ter	P	DM	P
5	*BMPR2*	2	202532663	rs946132834	C	T	Stop gained	c.1207C > T	p.Gln403Ter	P	DM	P
6	*BMPR2*	2	202518992	NA	TGA	T	Frameshift variant	c.793_794delGA	–	LP	NA	NA
7	*BMPR2*	2	202377512	rs1085307151	G	A	Stop gained	c.38G > A	p.Trp13Ter	P	DM	P
8	*BMPR2*	2	202532715	rs1085307325	G	A	Missense variant	c.1259G > A	p.Cys420Tyr	LP	DM	P
9	*BMPR2*	2	202513718	NA	G	A	Splice acceptor variant	c.419-1G > A	–	P	NA	NA
10	*BMPR2*	2	202552773	rs137852746	C	T	Missense variant	c.1471C > T	p.Arg491Trp	LP	DM	P
11	*BMPR2*	2	202514389	NA	G*	–	SV (del)	g.202514389_202517603del	–	P	NA	NA
12	*ATP13A3*	3	194430969	NA	CT	C	Frameshift variant	c.2597_2598delinsG	p.Gln866ArgfsTer2	LP	NA	NA
13	*AQP1*	7	30922075	NA	G	T	Missense variant	c.394G > T	p.Gly132Cys	LP	NA	NA
	TBX4	17	61467644	NA	AC	A	Frameshift variant	c.536_537delinsA	p.Phe181LeufsTer47	LP	NA	NA
14	*TBX4*	17	61482932	rs1603256040	C	T	Stop gained	c.1054C > T	p.Arg352Ter	P	NA	P

Pts: patients; ACMG 2015: The American College of Medical Genetics and Genomics guidelines

and rs137852746, and 1-star for rs946132834 and rs1085307325 status in ClinVar (Table 2 and Fig. 2).

Four P/LP variants were also identified in other genes from the IPAH gene list (Table 2). All variants are heterozygous. 2 in the gene TBX4 (NM_001321120.2), 1 in the gene ATP13A3 (NM_001367549.1) and 1 in the gene AQP1 (NM_198098.4). Three patients were identified as carriers of these variants.

The clinical data of patients with identified pathogenic or likely pathogenic variants in the 27 IPAH genes are presented in Table 3.

Notably, no significant differences were observed in the overall clinical profiles of these patients. However, individuals with these variants exhibited significantly lower cardiac output (3.08 ± 0.88 L/min, 4.9 ± 1.96 L/min, p = 0.002), cardiac index (1.8 ± 0.41 L/min/sq m vs 2.6 ± 1.04 L/min/sq m, p = 0.005), and higher PVR (21.0 ± 10.67 Wood unit Vs 12.0 ± 8.52 Wood unit, p = 0.002).

Reevaluation *BMPR2* gene pathogenicity with ACMG criteria and InterVar software tool

Before conducting the meta-analysis to compare the frequencies of IPAH-associated genes in the Russian population with those in global populations, we first decided to reassess the pathogenicity of the published variants. The studies included in the analysis used heterogeneous methods for determining P/LP variants. To standardize the approach, we applied a single pathogenicity assessment tool, InterVar, to all studies included in the meta-analysis. The meta-analysis focused solely on the BMPR2 gene, as we did not detect a significant number of P/LP variants in other genes, likely due to the limited sample size.

The literature analysis identified 665 unique variants of the BMPR2 gene (Supplemental files 1, 2). For each variant we included pathogenicity evaluation as it was presented in the corresponding study, along with the variant type. The distribution of reassigned P/LP variants of *BMPR2* gene over genomic regions is presented in Fig. 2. To overcome heterogeneity in methods for pathogenicity assessment in various studies, for all variants we utilized the same program InterVar, which is the widely used and most cited tool for pathogenicity assessment based on ACMG criteria. Out of 342, initially defined as pathogenic (without mixed classification) variants, only 87 (25.4%) remain pathogenic, 172 (50.3%) were reclassified as likely pathogenic and 80 (23.4%) as variants of uncertain significance. 3 (0.9%) pathogenic variants were even moved to likely benign category. All 230 previously unclassified variants were reclassified to all categories as pathogenic, likely pathogenic, uncertain significance, likely benign and benign. As the result of reassessment, we raised the number of P/LP *BMPR2* variants from 394 (59%) to 445 (67%). The distribution of *BMPR2* gene variants, identified from the literature, over categories before and after reassessment is given in Fig. 3.

Meta-analysis of the prevalence of pathogenic variants of *BMPR2* gene in IPAH/HPAH patients

The final meta-analysis included 24 studies that evaluated the frequency of pathogenic variants in the *BMPR2* gene among patients with IPAH/HPAH (full list with summary is presented in Table 4). Additionally, the analysis included the results from our sequencing data of 105 IPAH/HPAH patients from the Russian population with 11 patients being identified as carriers of pathogenic or likely pathogenic variants.

The final analysis included data from 3124 patients with IPAH/HPAH. Due to significant heterogeneity in the data, as indicated by the Q statistic of 100.4 and I2 value of 76.10%, a random-effects model was applied for the final frequency estimation. After reassessment the number of pathogenic variants' carriers decreased in 19 studies, increased in one study [29], and not changed in 4 studies. The data heterogeneity index ($I^2 = 69.02\%$) allowed to use the fixed effects model. The proportion of patients whose carrier frequency of pathogenic variants was revised comprised 21.9% (18.97–25.15). No systematic error in data selection was recorded.

All analyzed studies fell within the confidence interval of the funnel plot, although asymmetry in the frequency distribution was observed (Fig. 4). In our cohort, the frequency of pathogenic or likely pathogenic variants was [10.48% (5.34–17.97)]. Overall average frequency from the meta-analysis was [17.75%, (14.88–20.72)], though the difference was not statistically significant (p = 0.062). No evidence of selection bias was detected based on Egger's test (p = 0.661) and Begg's test (p = 0.851). Here we conclude that the frequency of *BMPR2* gene variants in the Russian population is not statistically different from those reported for other populations.

Discussion

Thus, we provide the first report on the prevalence of P/LP variants in genes associated with IPAH/HPAH in the Russian population. The patients included in the study were managed at Moscow PAH expert center, and the ethnic composition of the cohort was typical of the Russian population. The prevalence of PAH varies widely, ranging from 8 to 50 cases per 1 million people, with approximately one-third of these cases being IPAH patients [38]. The estimated number of IPAH/HPAH patients in Moscow, with a population of about 13 million, ranges from 35 to 217. Therefore, we believe that our study sample of 105 patients with IPAH/HPAH,

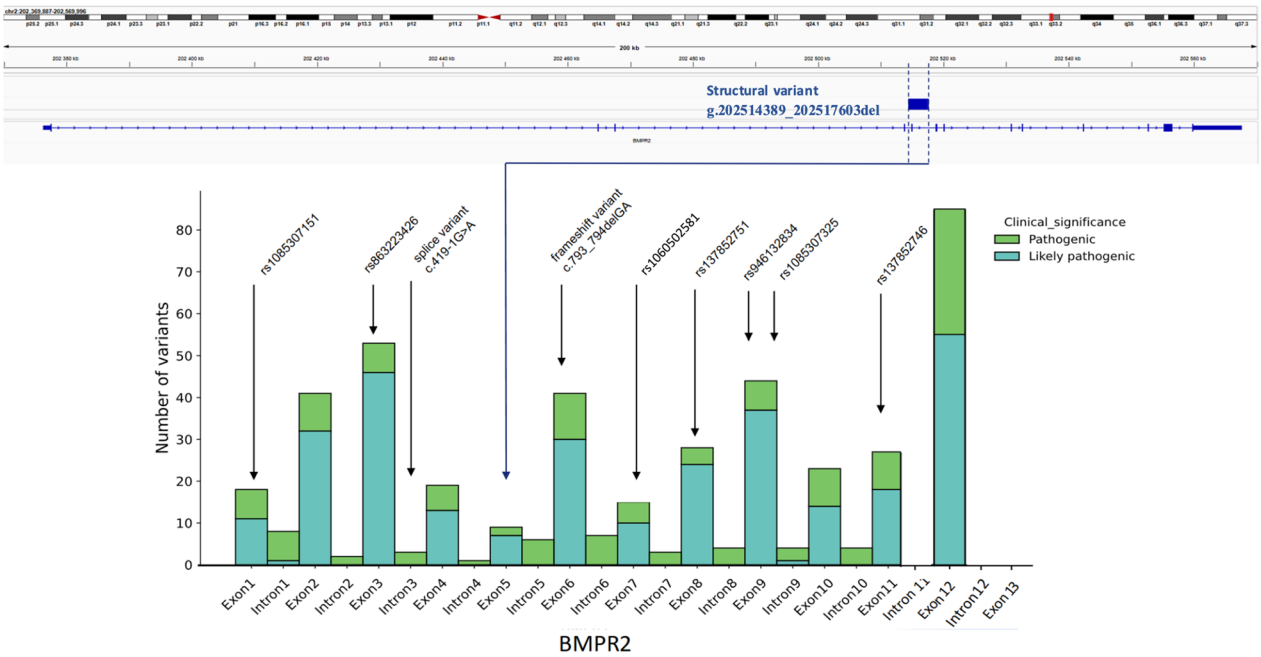

Fig. 2 Distribution of the *BMPR2* gene variants over genomic regions. The distribution of unique variants from the literature after InterVar reassessment. Only pathogenic (146) and likely pathogenic (299) variants within the *BMPR2* gene (NM_001204.7) are presented. Additionally, positions of 10 pathogenic and likely pathogenic variants detected in our cohort are indicated

80% of whom are local residents, is representative. The clinical characteristics of the genotyped patients were comparable with the data of observational studies—ASPAIRE [39], HOPE [40], USPHSR [41] by the age of the included patients, the proportion of women, and the distribution by functional classes.

The *BMPR2* is the primary gene associated with the development of pulmonary arterial hypertension. The presence of pathogenic variants in the *BMPR2* among patients with PAH is typically associated with an earlier onset of the disease and more pronounced hemodynamics and respiratory impairments [42]. The data of our study partially coincides with the data of this meta-analysis. Patients with P/LP variants of *BMPR2* had higher pulmonary resistance and lower cardiac output and cardiac index. The absence of differences in the age of IPAH diagnosis, pulmonary artery pressure level and some other parameters may be due to the relatively small cohort in our study.

Currently, genetic testing and counseling are indicated for patients with an established diagnosis of IPAH/HPAH [4]. Relatives of the proband with P/LP variants of *BMPR2* have a higher risk of the disease. According to the DELPHI-2 study data, if asymptomatic carriers of BMPR2 gene variants are identified, an annual comprehensive examination for at least two years may be recommended. The authors of this study suggest that this approach can help facilitate earlier disease diagnosis and the timely initiation of treatment [43].

However, the significance of the *BMPR2* gene is not limited to direct causality in relation to IPAH. The literature suggests that IPAH is oligogenic, meaning it is influenced by multiple genetic factors rather than being caused by a single gene mutation [44, 45]. Carrying multiple genetic variants can influence the progression of the disease, which is why it is crucial to understand the frequency of pathogenic variant carriers within each population. The analysis of sequencing data for the *BMPR2* gene revealed that the overall prevalence of pathogenic and likely pathogenic variants in our cohort was 10.48% (11 out of 105 patients). Of these, only 7 out of the 10 variants had been previously described in the literature.

Three variants—frameshift variant c.793_794delGA, splice acceptor variant c.419-1G > A and structural deletion g.202514389_202517603del – have not been previously reported in literature (Table 2). Two nucleotide deletion c.793_794delGA induces a frameshift in the region of 6th exon, affecting key positions essential for protein function. Exon 6 is situated in the N-terminal part of the serine-threonine kinase domain, which is made up of conserved subdomains, including exons 6 through 11. This region plays a key role in ATP-binding and contains specific patterns of conserved amino acids. Mutations within this area can disrupt signaling

Table 3 Characteristics of carriers of pathogenic and likely pathogenic variants of the IPAH/HPAH genes

Pts No.	Sex	Age	Time from IPAH first symptoms to diagnosis, months	WHO functional class at time of diagnosis	MPAP, mmHg	PVR, WU	CI, L/min/m^2	Vasoreactivity test at time of diagnosis	Family history of PAH	PAH treatment effect	Follow- up time, outcome
1	F	61	9	3	77	18.8	2.1	Negative	No	Yes	9 months, alive
2	F	33	1	3	43	11.9	1.7	Negative	No	Yes	5 months, alive
3	F	47	18	3	53	12.6	2.0	Positive	No	No	8 months, death
4	F	64	12	1	71	48	1.0	Negative	No	Yes	12 months, death
5	F	37	18	2	71	24	1.6	Negative	No	Yes	9 years, alive
6	F	36	1	2	71	19	2.3	Negative	Yes	Yes	9 years, alive
7	M	25	36	2	64	12.9	2.2	Negative	No	Yes	6.5 years,, alive
8	F	29	71	3	NA	NA	NA	Negative	No	Yes	5 month, alive
9	F	55	45	3	60	24.8	1.3	Negative	No	Yes	4 years, alive
10	M	38	5	3	62	16.9	1.6	Negative	No	Yes	1.5 years, alive
11	M	20	48	3	74	20.6	1.7	Negative	No	No	4 years, death
12	M	43	18	2	69	12.2	2.3	Negative	No	Yes	4 years, alive
13	F	6	NA	3	NA	NA	NA	NA	No	Yes	41 years, alive
14	F	28	1	2	57	3.8	8.1	Positive	No	Yes	1 year, alive

Pts: patients; Age: Age of IPAH first symptoms, years; CI: Cardiac index: F: female; LP: Likely pathogenic; MPAP: mean pulmonary arterial pressure; P: pathogenic; PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; WU: Wood unit: WHO: World Health Organization

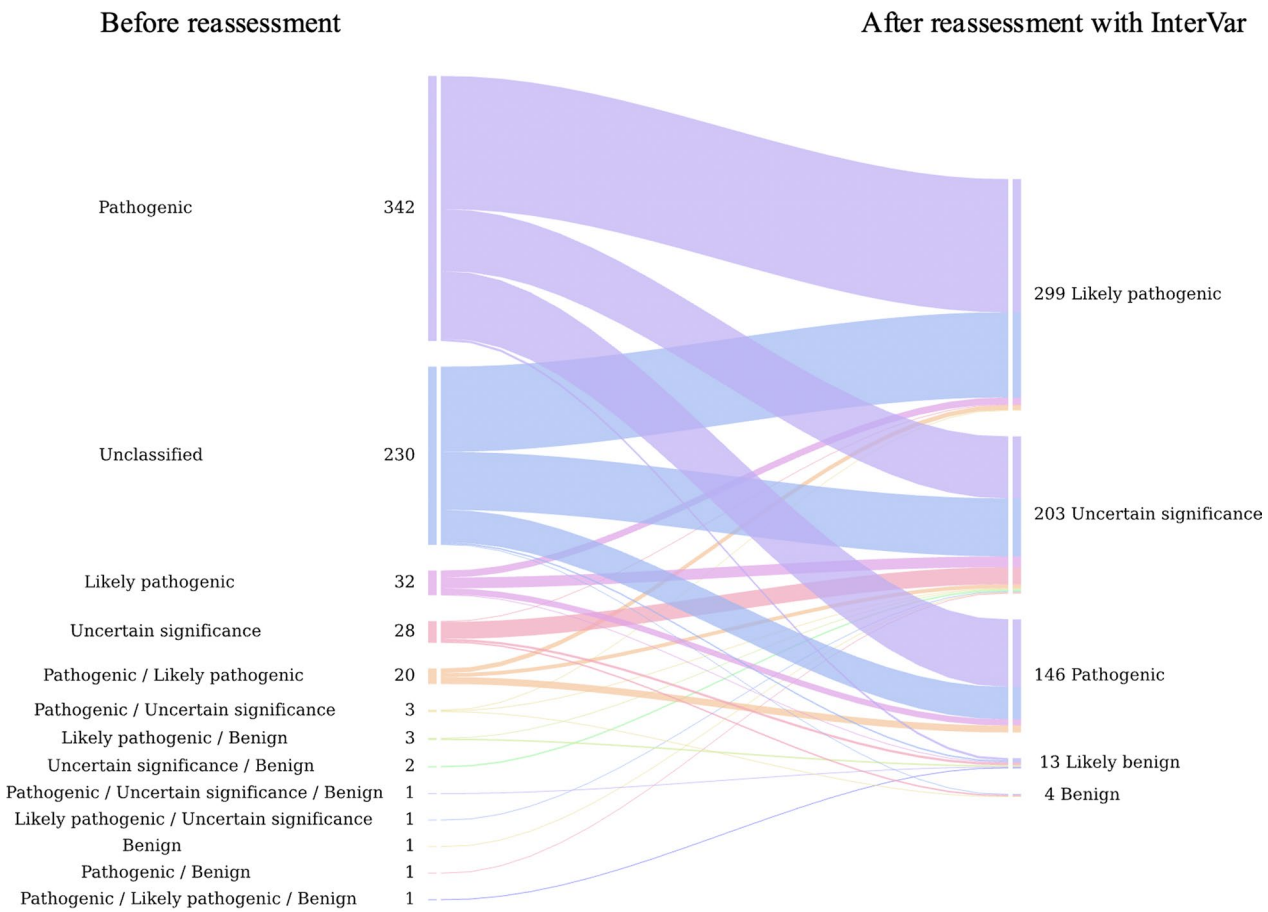

Fig. 3 Reassessment of pathogenicity of variants in the *BMPR2* gene. The distribution of 665 unique variants in the *BMPR2* gene with pathogenicity assessment presented by authors in the literature before and after our reassessment according to the ACMG criteria. Some variants had mixed classifications before re-evaluation, either because they were identified in multiple studies with different assessments or because authors within an individual study were unable to classify the variant conclusively. Some variants reported in the literature do not have a definitive pathogenicity classification and are thus labeled as "Unclassified."

by interfering with ATP-binding and affect the protein function [46]. There is no available information on frequency of the c.793_794delGA variant, therefore, the InterVar defined this variant as likely pathogenic. The splice acceptor variant c.419-1G > A is located within a cryptic splice site in the third intron with a splice distance of − 1. Splicing prediction tools, including SpliceAI and MaxEntScan, indicate a significant impact of this variant on splicing, suggesting complete disruption of the existing acceptor site. Additional in silico tools, such as CADD, DANN, and FATHMM-MKL, also classify this variant as pathogenic. There is no information on the frequency of the variant. An alternative substitution (T instead of A in our study) at the same position (c.419-1G > T) was reported in the study of Zhu et al. [47] in a patient with PAH. This variant (c.419-1G > T) is recorded in the HGMD as a disease-causing mutation for pulmonary hypertension, primary. According to the combination of factors, the InterVar classified the variant c.419-1G > A as pathogenic. The heterozygous structural variant (deletion) of 3214 bp in length in the *BMPR2* gene (NC_000002.12:g.202514389_202517603del) has not been previously reported and, based on AnnotSV predictions, is classified as pathogenic. This deletion completely deletes the 5th exon of the gene and significant regions of surrounding introns. Moreover, this deletion overlaps with the previously described deletion in the dbVar database, identified in a patient with PH: nsv4682448 (NC_000002.12:g.202514864_202514989del).

We also identified three additional carriers of four pathogenic variants in the ATP13A3, AQP1, and TBX4 genes in our group, with one patient carrying two P/LP variants. However, due to the small sample size, it is not possible to draw reliable conclusions about the frequencies of these variants within the Russian

Table 4 Studies included in the meta-analysis

No	Age group	n	Age, years	Diagnosis	Revisiting the number of patients with mutations in *BMPR2*		Country	Refs.
					Before	After		
1	Adults	69	50 ± 20	IPAH	5	3	Taiwan	[16]
2	Adults	45	46 ± 15	IPAH + HPAH	8	7	Taiwan	[17]
3	Adults	201	47 ± 17	IPAH	20	18	Germany	[18]
4	Adults and children	46	29.5 ± 7	IPAH	4	2	Saudi Arabia	[1]
5	Adults	126	49 ± 16	IPAH	29	27	Netherlands	[19]
6	Adults and children	331	28 ± 11	IPAH	81	66	China	[20]
7	Adults	191	30.7 ± 10.6	IPAH	37	34	China	[21]
8	Adults	13	33	IPAH	1	1	Lebanon	[22]
9	Adults	127	42.84 ± 15,22	IPAH	24	13	Spain	[23]
10	Adults	81	39.0 ± 13.4	IPAH + HPAH	14	11	USA	[24]
11	Adults	28	49 ± 16	IPAH	7	3	Spain	[25]
12	Adults	275	40 ± 15	IPAH + HPAH	82	57	China	[26]
13	Adults and children	106	11.1 ± 10.6	IPAH + HPAH	28	19	China	[27]
14	Adults	73	42.7 ± 19.8	IPAH	16	14	Korea	[28]
15	Adults	82		IPAH	15	27	Netherlands	[29]
16	Adults	8	42 ± 9	IPAH	1	1	Turkey	[30]
17	Adults	217		IPAH + HPAH	45	37	China	[31]
18	Adults	37	50 ± 13	IPAH + HPAH + PAH-CHD + PVOD + PAH-HHT	15	9	Germany	[32]
19	Adults	43		IPAH	6	6	Japan	[33]
20	Adults and children	40	30.4 (6–59)	IPAH	14	14	Japan	[34]
21	Adults and children	516		IPAH + HPAH	151	114	France	[35]
22	Adults	117		IPAH + HPAH	37	34	Japan	[36]
23	Adults and children	44	28.5 ± 11.5	IPAH + HPAH	23	19	France	[3]
24	Adults and children	203		IPAH	31	27	France	[37]
25	Adults	105	56.9 ± 14.47	IPAH + HPAH	NA	11	Russia	Own data

population. Future studies will help refine the population frequencies of pathogenic variants in these genes and other IPAH-associated genes.

Reevaluation of the BMPR2 gene pathogenicity using ACMG criteria and InterVar software demonstrated that comparing the frequency of pathogenic variant carriers across different research groups requires a standardized approach to pathogenicity assessment. It is evident that the accumulation of data on the molecular genetic mechanisms of disease development will, in the future, clarify the functional significance of the identified variants. This underscores the importance of regularly reevaluating pathogenicity. To establish an irrefutable association between disease development and the gene, it must be reassessed at least three years after being assigned evidence level as "definitive" [48].

The most extensive data in the literature regarding the frequency of pathogenic variants is available for the BMPR2 gene. We attempted to compare the data from our cohort with findings from other studies; however, the reported frequencies of pathogenic variants in the BMPR2 gene vary significantly across different studies.

Differences in the frequency of detecting P/LP variant carriers may be linked to variations in the clinical characteristics of the cohorts analyzed. A meta-analysis was conducted to aggregate the literature data. Additionally, differences in frequency could be attributed to the methods used to assess the pathogenicity of the identified variants. To evaluate the pathogenicity of the variants, we applied the criteria established by the American College of Medical Genetics and Genomics (ACMG) in collaboration with the Association for Molecular Pathology (AMP) in 2015. These guidelines incorporate 28 distinct factors that facilitate the classification of genetic variants as "pathogenic," "likely pathogenic," "variants of uncertain significance (VUS)," "likely benign," or "benign." These criteria consider various parameters, including the

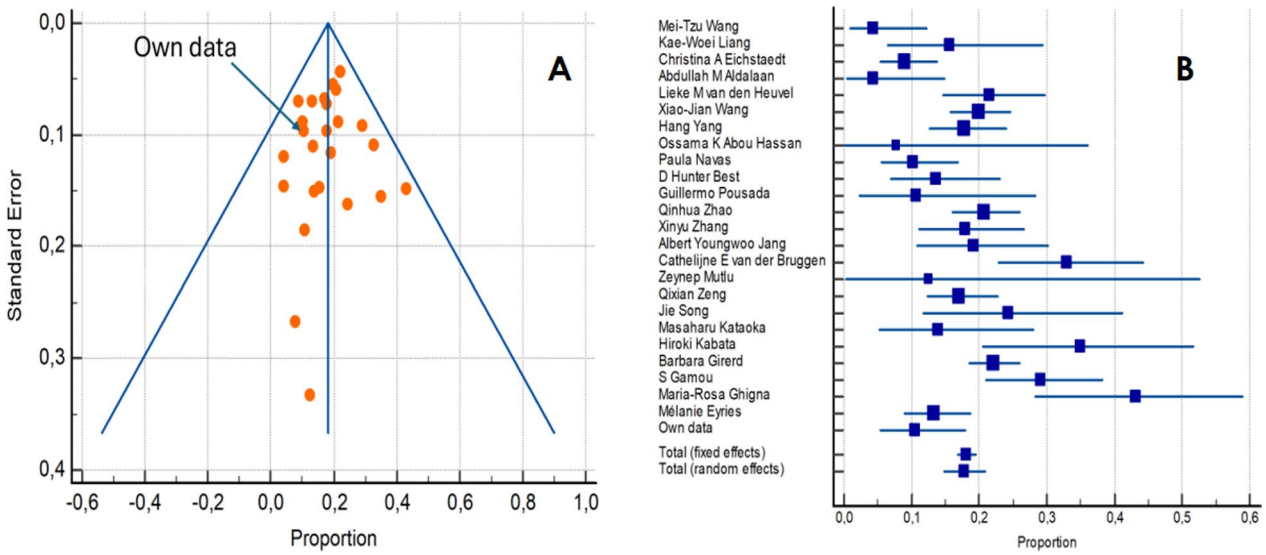

Fig. 4 Meta-analysis of the frequency of pathogenic *BMPR2* gene variants in patients with IPAH/HPAH. **A** Funnel plot of the meta-analysis of the frequency of pathogenic *BMPR2* gene variants in patients with IPAH/HPAH. **B** Meta-analysis data of the frequency of pathogenic *BMPR2* gene variants in patients with IPAH/HPAH

variant's impact on protein function, allele frequency within the population, functional studies, the variant's position in relation to other known pathogenic variants, and other relevant factors [49].

Currently, there is no universally accepted methodology for the use of specific bioinformatics tools to evaluate the pathogenicity of genetic variants. The challenge of calibrating analytical tools remains highly relevant [50]. One of the most cited and widely used programs for interpretation of variant pathogenicity is InterVar, which automates the classification process based on 18 out of 28 criteria defined by the ACMG. InterVar usage significantly reduces analysis time and minimizes variability in interpretation among different experts [51–58]. Nevertheless, it should be noted that the interpretation of sequencing data using InterVar in patients with pulmonary hypertension has limitations, the main one being the low penetrance of the disease. The authors of this software product suggest that InterVar is most effective for interpreting variants associated with severe congenital or early developmental disorders with nearly 100% penetrance, and its performance may be less reliable for late-onset diseases or recessive conditions [11].

A meta-analysis of the literature, with variants reassessed according to ACMG criteria, indicated a slightly higher average *BMPR2* mutation frequency of 17.75% (14.88–20.82). Despite the heterogeneity observed in the global data, our meta-analysis showed that the prevalence of *BMPR2* mutations in the Russian population does not significantly differ from the global average.

Conclusion

The prevalence of pathogenic variants in the BMPR2 gene within our group was found to be 10.48%, which does not statistically differ from the 17.75% carrier frequency reported in the meta-analysis. It is essential to consider the revised pathogenicity criteria established since 2015 and to utilize state-of-the-art tools for reevaluating the data.

Study limitations

This study had several limitations. Firstly, InterVar uses only part of the ACMG criteria for pathogenicity assessment. The authors of the studies included in the meta-analysis did not publish the complete set of identified variants, including benign and likely benign variants. As a result, we only reclassified P, LP, and VUS variants, as well as those for which the authors did not provide a classification (N/A). A limitation of this approach is that pathogenic variants may exist among the benign and likely benign variants.

Abbreviations

ACMG	American College of Medical Genetics and Genomics
AMP	Association for Molecular Pathology
BMPR2	Bone morphogenetic protein receptor type-2
CTEPH	Chronic thromboembolic pulmonary hypertension
HPAH	Hereditary pulmonary arterial hypertension
InDel	Insertion and deletion
IPAH	Idiopathic pulmonary arterial hypertension

LP variant	Likely pathogenic variant
mPAP	Mean pulmonary arterial pressure
MOOSE	Meta-analysis of observational studies in epidemiology
NT-proBNP	N-terminal prohormone of Brain Natriuretic Peptide
P variant	Pathogenic variant
PH	Pulmonary hypertension
PAH	Pulmonary arterial hypertension
Pts	Patients
PVR	Pulmonary vascular resistance
PWP	Pulmonary wedge pressure
RAP	Right atrial pressure
SNV	Single nucleotide variant
SV	Structural variant
TAPSE	Tricuspid annular plane systolic excursion
VUS	Variants of uncertain significance
WU	Wood units

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03214-9.

Supplementary Material 1.

Supplementary Material 2.

Author contributions

ED, EZ, AL, AD, NO performed patients selection and inclusion. GO, IB, VZ, MP performed bioinformatics analysis. IB, DN performed literature analysis. LP performed meta-analysis. DZ and MP supervised the study. GO, IB, VZ, DN, ED, LM, DZ and MP analysed the data and wrote the manusucript. All authors read and approved the manuscript.

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Data availability

The sequencing data of 105 IPAH patients that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at the Bioinformatics Lab at HSE University.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki, approved by the Ethics Committee of City Clinical Hospital Nu 51, Moscow, Russia (protocol 19/15, 22 Oct 2014), and confirmed for extension (protocol #03/23, 4 Sept 2023) by the local Ethics Committee of the City Clinical Hospital Nu 29, Moscow, Russia.

Consent for publication

Non-applicable.

Competing interests

The authors declare no competing interests.

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