Supplemental Material

Methods – Whole exome sequencing

DNA was extracted from peripheral blood leukocytes using Puregene reagents (Gentra Systems Inc., Minnesota, USA). WES was performed in collaboration with the Regeneron Genetics Center (RGC) or the PAH Biobank at Cincinnati Children's Hospital Medical Center. In brief, genomic DNA was prepared with a customized reagent kit from Kapa Biosystems and captured using the NimbleGen SeqCap VCRome 2 exome capture reagent or xGen lockdown probes. Samples were sequenced on the Illumina HiSeq 2500 platform with v4 chemistry, generating 75bp or 76bp pair-end reads. Patient DNA samples sequenced at the PAH Biobank/Cincinnati Children's Hospital Medical Center were prepared with the Clontech Advantage II kit and enriched using the NimbleGen SeqCap EZ Exome V2 capture reagent. Samples were sequenced using 125 bp paired-end sequencing on the Illumina HiSeq 2500 platform. We aimed to reach a coverage of ≥15x in ≥90% of targeted regions for all WES samples.

Supplemental Table 1. Mutations in known PAH risk genes identified in 155 pediatric-onset patients. Patients are heterozygous for the indicated variants.

ID	Gender	Age, dx or enrollment (y)	WHO class	Associated medical conditions	Primary method of detection	Gene	Mutation	Variant type	Inheritance	Previously-reported?	Allele frequency (ExAC)	CADD	MetaSVM	ACMG class (deleterious prediction)
FPPH060	F	9	1.2.2	HHT	Sanger	*ACVRL1*	1450 C>T, 1450-1451 ins G	LGD	unknown	Jones et al. 2014	---†	35	.	Likely Pathogenic
FPPH100	F	18	1.2.2	HHT	Sanger	*ACVRL1*	c.C1385G:p.S462X	LGD	unknown	Abdalla et al. 2004	---	42	.	Pathogenic
FPPH4606*	F	unknown	1.2.1		WES	*ACVRL1*	c.C1199T:p.A400V	D-Mis	paternal	No	---	35	D‡	Likely Pathogenic
JM0057	M	5	1.2.2	HHT	WES	*ACVRL1*	c.G955C:p.G319R	D-Mis	*de novo*	Machado et al. 2015	---	22.5	D	Likely Pathogenic
JM0035	F	13	1.1		WES	*BMPR1B*	c.T1328C:p.I443T	Mis	paternal	rs752045710	1.63E-05	23.8	T	VUS (medium) §
15-059	F	16	1.2.1		WES	*BMPR2*	c.1128+1G>A	LGD	unknown	rs863223420	---	26.5	.	Pathogenic
FPPH065	F	11	1.2.1		Sanger	*BMPR2*	c.2410-2413delGTCA: p.V804Pfsx1	LGD	paternal	Machado et al. 2009	---	35	.	Pathogenic
FPPH0709	F	12	1.2.1		Sanger	*BMPR2*	247+1delCAAGTG	LGD	unknown	Machado et al. 2015	---	24	.	Pathogenic
FPPH083	F	12	1.2.1		Sanger	*BMPR2*	del exon 2-3	LGD	unknown	Pfarr et al. 2011	---	.	.	Pathogenic
FPPH089	F	17	1.2.1		Sanger	*BMPR2*	c.189-209del21: p.delSTCYGLW	LGD	maternal	Machado et al. 2009	---	21.9	.	Pathogenic
FPPH1001	F	18	1.2.1		Sanger	*BMPR2*	c.1099-1103 delGGGGA: p.E368fsx1	LGD	unknown	Machado et al. 2009	---	35	.	Pathogenic
FPPH131-01	M	1.5	1.2.1		Sanger	*BMPR2*	del exon 2-3	LGD	maternal	Pfarr et al. 2011	---	.	.	Pathogenic
FPPH137-01	M	18	1.2.1		Sanger	*BMPR2*	c.C631T:p.R211X	LGD	unknown	Thomson et al. 2000	---	42	.	Pathogenic
FPPH3407	F	16 (postmortem)	1.2.1		Sanger	*BMPR2*	c.637C>T:p.R213X	LGD	unknown	Morisaki et al. 2004	---	35	.	Pathogenic
FPPH3711	F	1.5	1.2.1		Sanger	*BMPR2*	c.1214delA:p.D405fsx6	LGD	paternal	Machado et al. 2009	---	.	.	Likely Pathogenic
FPPH4206	M	35	1.2.1		Sanger	*BMPR2*	c.2441-2442delAC: p.H814fsx2	LGD	unknown	Machado et al. 2009	---	35	.	Likely Pathogenic
FPPH5403	F	9	1.2.1		Sanger	*BMPR2*	c.248-5delTATAGGinsAC	LGD	maternal	No	---	.	.	Likely Pathogenic
JM1101	F	12	1.2.1		WES	*BMPR2*	c.C961T:p.R321X	LGD	unknown	Koehler et al. 2004	---	40	.	Likely Pathogenic
JM860	M	16	1.2.1		WES	*BMPR2*	c.622-2A>C	LGD	unknown	No	---	24.6	.	Likely Pathogenic
15-051*	F	4	1.2.1/ 1.2.2		WES	*BMPR2*	c.T295C:p.C99R	D-Mis	maternal	Machado et al. 2006	---	25.8	D	Likely Pathogenic

ID	Gender	Age, dx or enrollment (y)	WHO class	Associated medical conditions	Primary method of detection	Gene	Mutation	Variant type	Inheritance	Previously-reported?	Allele frequency (ExAC)	CADD	MetaSVM	ACMG class (deleterious prediction)
FPPH0111	F	11	1.2.1		Sanger	*BMPR2*	c.C1471T:p.R491W	D-Mis	paternal	Deng et al. 2000	---	24.8	D	Pathogenic
FPPH070	F	15	1.2.1		Sanger	*BMPR2*	c.G932A:p.G311E	D-Mis	maternal	Machado et al. 2009	---	33	D	Pathogenic
FPPH0801	F	9	1.2.1		Sanger	*BMPR2*	c.C1471T:p.R491W	D-Mis	paternal	Deng et al. 2000	---	24.8	D	Pathogenic
JM0047	M	8	1.2.1		WES	*BMPR2*	c.G995C:p.R332P	D-Mis	unknown	No	---	34	D	Likely Pathogenic
JM1088	F	13	1.2.1		WES	*BMPR2*	c.C1585T:p.R529C	D-Mis	maternal	Machado et al. 2015	4.94E-05	34	D	Pathogenic
JM1334	F	12	1.2.1		WES	*BMPR2*	c.T295C:p.C99R	D-Mis	unknown	Machado et al. 2006	---	19.31	D	Likely Pathogenic
JM1342	M	8	1.2.1		WES	*BMPR2*	c.T297G:p.C99W	D-Mis	maternal	Machado et al. 2015	---	26.4	D	Likely Pathogenic
JM160	M	11	1.2.1		WES	*BMPR2*	c.G1472A:p.R491Q	D-Mis	unknown	Deng et al. 2000	---	35	D	Likely Pathogenic
JM365	F	5	1.2.1		WES	*BMPR2*	c.A200G:p.Y67C	D-Mis	maternal	Morisaki et al. 2004	---	26	D	Likely Pathogenic
JM625	M	3	1.2.1		WES	*BMPR2*	c.C1471T:p.R491W	D-Mis	*de novo*	Deng et al. 2000	---	24.8	D	Pathogenic
JM733	F	6	1.2.1	long QT syndrome	WES	*BMPR2*	c.C1154G:p.P385R	D-Mis	unknown	No	---	28.3	D	Likely Pathogenic
FPPH4606*	F	unknown	1.2.1		WES	*BMPR2*	c.A1509C:p.E503D	Mis	paternal	No	---	15.3	T	VUS (medium)
15-043	F	5	1.2.2		WES	*KCNK3*	c.641_642insGCAGAC: p.214insQT	In-frame	paternal	No	---	13.25	.	VUS (low)
15-033	M	13	1.2.2		WES	*KCNK3*	c.C675A:p.F225L	Mis	unknown	No	---	22.4	T	VUS (medium)
FPPH138-01	F	6	1.2.2		WES	*KCNK3*	c.G544A:E182K	Mis	paternal	Ma et al. 2013	---	32	T	Pathogenic
15-051*	F	4	1.2.1/ 1.2.2		WES	*SMAD9*	c.C880T:p.R294X	LGD	maternal	Drake et al. 2011	8.24E-06	40	.	Pathogenic
15-046	F	7	1.1	small patella syndrome	WES	*TBX4*	c.702+1G>A	LGD	unknown	No	---	27.3	.	Likely Pathogenic
15-062	F	10	1.1		WES	*TBX4*	c.842dupC:p.S281fs	LGD	unknown	No	---	34	.	Likely Pathogenic
FPPH4004	M	10	1.2		WES	*TBX4*	c.498_500del:p.166_167delS	In-frame	paternal	No	8.31E-06	23.2	.	VUS (medium)
FPPH9002	M	2	1.1		WES	*TBX4*	c.C1054T:p.R352X	LGD	*de novo*	No	---	35	.	VUS (high)
JM0064	F	3	1.1		WES	*TBX4*	c.664delA:p.T222fs	LGD	maternal	No	---	34	.	VUS (high)

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JM358	F	1.5	1.1		WES	*TBX4*	c.C577T:p.Q193X	LGD	maternal	No	---	38	.	Likely Pathogenic
JM587	M	1.5	1.1		WES	*TBX4*	chr17:59,534,897-59,560,878 25.9kb (exon 3-9) deletion	LGD	unknown	No	---	.	.	Likely Pathogenic
JM655	F	5	1.1		WES	*TBX4*	c.507_512del:p.169_171del	In-frame	paternal	No	---	20.8	.	VUS (medium)
JM921	M	0.6	1.1		WES	*TBX4*	c.148delC:p.P50fs	LGD	maternal	No	---	25.5	.	Likely Pathogenic
JM0034	F	14	1.1		WES	*TBX4*	c.C292G:p.P98A	D-Mis	maternal	No	---	27.8	D	VUS (high)
JM1341	F	1	1.1		WES	*TBX4*	c.A569C:p.H190P	D-Mis	unknown	No	---	24.1	D	Likely Pathogenic
JM1441	F	14	1.1		WES	*TBX4*	c.A380C:p.Y127S	D-Mis	paternal	No	---	27.6	D	Likely Pathogenic

Abbreviations: Age at dx, age at diagnosis; HHT, hereditary hemorrhagic telangiectasia; ACMG, American College of Medical Genetics; LGD, likely gene damaging; Mis, missense; D-Mis, damaging missense predicted by MetaSVM; VUS, variant of unknown significance.

*patient carries variants in both *BMPR2* and *ACVRL*.

†---, mutation not present in ExAC

‡Annotation toolkit predictions: B, benign; T, tolerant; P, possibly damaging; D, damaging; ., mutation class not included in toolkit.

§Deleteriousness prediction criteria if not ACMG classification "pathogenic" or "likely pathogenic": high= LGD or D-Mis plus CADD score≥15; medium= D-Mis or CADD≥15.

Supplemental Table 2. Mutations in known PAH risk genes identified in 257 adult-onset patients. Patients are heterozygous for the indicated variants unless noted.

ID	Gender	Age, dx or enrollment (y)	WHO class	Associated medical conditions	Primary method of detection	Gene	Mutation	Variant type	Inheritance	Previously-reported?	Allele frequency (ExAC)	CADD	MetaSVM	ACMG class (deleterious prediction)
FPPH082	M	29	1.2.2	HHT	Sanger	*ACVRL1*	c.C1435T: p.R479X	LGD	paternal	Machado et al. 2009	---[†]	43	.	Likely Pathogenic
FPPH110	F	42	1.2.2	HHT	Sanger	*ACVRL1*	c.1031G>A: p.C344Y	D-Mis	unknown	Abdalla et al. 2000	---	31	D[‡]	Likely Pathogenic
FPPH139-01	F	20	1.2.2		Sanger	*ACVRL1*	c.1031G>A: p.C344Y	D-Mis	unknown	Abdalla et al. 2000	---	31	D	Pathogenic
FPPH091	F	44	1.2.2	HHT	Sanger	*ACVRL1*	c.C1120T: p.R374W	D-Mis	unknown	Berg et al. 1997	---	29.4	D	Pathogenic
JM120	M	29	1.2.2	PVOD	WES	*ACVRL1*	c.C139T: p.R47W	D-Mis	maternal	No	4.50E-05	24.1	D	Pathogenic
JM1348	F	32	1.2.2	HHT	WES	*ACVRL1*	c.G1451A: p.R484Q	D-Mis	unknown	Trembath et al. 2001	---	23	D	Pathogenic
FPPH172-1	F	33	1.2.2	HHT	Sanger	*ACVRL1*	c.1231C>T:p.R411W	D-Mis	unknown	Trembath et al. 2001	8.00E-06	35	D	Pathogenic
FPPH160-01*	F	48	1.2.2		WES	*ACVRL1*	c.C1376T: p.P459L	Mis	unknown	No	1.65E-05	24.7	T	Pathogenic
FPPH111	F	34	1.2.1		Sanger	*BMPR2*	c.248-2A>G	LGD	maternal	Machado et al. 2009	---	23.6	.	Pathogenic
FPPH114	M	unknown	1.2.1		Sanger	*BMPR2*	c.418+3A>T	LGD	unknown	Machado et al. 2006	---	14.14	.	Pathogenic
FPPH4413	F	24	1.2.1		Sanger	*BMPR2*	c.418+5G>A	LGD	unknown	Rosenzweig et al. 2008	---	17.27	.	Pathogenic
FPPH4905	F	unknown	1.2.1		Sanger	*BMPR2*	c.418+5G>A	LGD	unknown	Rosenzweig et al. 2008	---	17.27	.	Pathogenic
FPPH059	F	50	1.2.1		Sanger	*BMPR2*	c.968-5A>G	LGD	maternal	Machado et al. 2009	---	13.39	.	Pathogenic
FPPH3208	M	43	1.2.1		Sanger	*BMPR2*	c. G727T: p.E243X	LGD	paternal	Machado et al. 2001	---	43	.	Likely Pathogenic
FPPH2803	F	41	1.2.1		Sanger	*BMPR2*	c.1095delC: p.R365fsx8	LGD	maternal	Machado et al. 2009	---	29.4.	.	Pathogenic
JM629	M	26	1.2.1	kyphoscoliotic heart disease, sleep apnea	WES	*BMPR2*	c.1129-1G>A	LGD	unknown	rs863223420	---	26.5	.	Likely Pathogenic
FPPH2301	F	33	1.2.1		Sanger	*BMPR2*	c.1129-3C>G	LGD	unknown	Machado et al. 2001	---	27	.	Pathogenic
FPPH156-01	F	28	1.2.1		Sanger	*BMPR2*	c.1142insA: p.R381Kfsx17	LGD	unknown	No	---	35	.	Pathogenic
JM96	F	32	1.2.1	asthma	WES	*BMPR2*	c.115delC: p.P39fs	LGD	unknown	No	---	33	.	Likely Pathogenic
FPPH5301	F	37	1.2.1		Sanger	*BMPR2*	c.1189-1190delTG: p.C347X	LGD	maternal or *de novo*	Machado et al. 2009	---	35	.	Likely Pathogenic

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FPPH1101	F	25	1.2.1		Sanger	*BMPR2*	c.1248-1251del ATTT:p.F417fs	LGD	paternal	Machado et al. 2009	---	35	.	Pathogenic
SPH586NW2874	F	64	1.2.1		WES	*BMPR2*	c.1277-1G>-	LGD	unknown	No	---	24.4	.	Likely Pathogenic
JM883	F	35	1.2.1		WES	*BMPR2*	c.1604delG: p.R535fs	LGD	unknown	No	---	34	.	Likely Pathogenic
FPPH081	M	28	1.2.1		Sanger	*BMPR2*	c.186insTACC: p.G63fsx1	LGD	maternal	Machado et al. 2009	---	14.98	.	Pathogenic
JM100	F	22	1.2.1	deaf/mute	WES	*BMPR2*	c.2305delC: p.R770Gfs	LGD	unknown	No	---	35	.	Likely Pathogenic
FPPH068	F	32	1.2.1		Sanger	*BMPR2*	c.261insA: p.87fsx9	LGD	unknown	Machado et al. 2009	---	34	.	Pathogenic
FPPH132-01	M	36	1.2.1		Sanger	*BMPR2*	c.354-355TA>AG: p.C118X	LGD	paternal	Machado et al. 2009	---	27.9	.	Likely Pathogenic
FPPH1703	F	48	1.2.1		Sanger	*BMPR2*	c.507-510delCTTTinsAAA: p.C169X	LGD	maternal	Deng et al. 2000	---	.	.	Pathogenic
FPPH2201	M	31	1.2.1		Sanger	*BMPR2*	c.690-691delAGinsT: p.K239fsx21	LGD	unknown	Machado et al. 2009	---	33	.	Pathogenic
SPH604HK2894	F	40	1.2.1		WES	*BMPR2*	c.918_921del: p.H306fs	LGD	unknown	No	---	.	.	Pathogenic
JM118	F	39	1.2.1	sleep apnea	WES	*BMPR2*	c.C1750T: p.R584X	LGD	unknown	Machado et al. 2001	---	40	.	Pathogenic
FPPH1804	F	56	1.2.1		Sanger	*BMPR2*	c.C2617T: p.R873X	LGD	unknown	Deng et al. 2000	---	0.44	.	Likely Pathogenic
FPPH4701	M	34	1.2.1		Sanger	*BMPR2*	c.C2695T: p.R899X	LGD	unknown	Lane et al. 2000	---	39	.	Pathogenic
SPH966EM5361	F	43	1.2.1		WES	*BMPR2*	c.C2695T: p.R899X	LGD	unknown	Austin et al. 1993	---	43	.	Likely Pathogenic
FPPH0401	F	unknown	1.2.1	teratoma	Sanger	*BMPR2*	c.C439T: p.R147X	LGD	paternal	Machado et al. 2001	---	38	.	Pathogenic
JM863	M	49	1.2.1		WES	*BMPR2*	c.C470A: p.S157X	LGD	unknown	No	---	35	.	Likely Pathogenic
JM1427	M	30	1.2.1		WES	*BMPR2*	c.C541T: p.Q181X	LGD	unknown	Machado et al. 2015	---	35	.	Likely Pathogenic
FPPH093	F	unknown	1.2.1	appetite suppressant	Sanger	*BMPR2*	c.C631T: p.R211X	LGD	unknown	Thomson et al. 2000	---	42	.	Pathogenic
JM766	F	28	1.2.1		WES	*BMPR2*	c.C631T: p.R211X	LGD	unknown	Thomson et al. 2000	---	36	.	Likely Pathogenic
FPPH2003	F	35	1.2.1		Sanger	*BMPR2*	c.C961T: p.R321X	LGD	maternal	Koehler et al. 2004	---	40	.	Likely Pathogenic
FPPH4303	M	47	1.2.1		Sanger	*BMPR2*	c.C961T: p.R321X	LGD	paternal or *de novo*	Koehler et al. 2004	---	40	.	Likely Pathogenic

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FPPH116	F	23	1.2.1		Sanger	*BMPR2*	c.C994T: p.R332X	LGD	unknown	Thomson et al. 2000	---	38	.	Pathogenic
FPPH1201	M	29	1.2.1		Sanger	*BMPR2*	c.C994T: p.R332X	LGD	unknown	Thomson et al. 2000	---	38	.	Likely Pathogenic
SPH833MS5175	F	44	1.2.1		WES	*BMPR2*	c.C994T: p.R332X	LGD	unknown	Thomson et al. 2000	---	36	.	Pathogenic
FPPH096	F	20	1.2.1		Sanger	*BMPR2*	c.G1398A: p.W466X	LGD	paternal or *de novo*	Koehler et al. 2004	---	.	.	Pathogenic
JM675	F	37	1.2.1		WES	*BMPR2*	c.G210A: p.W70X	LGD	unknown	No	---	37	.	Likely Pathogenic
JM87	M	50	1.2.1	hypothyroidism	WES	*BMPR2*	c.T1146G: p.Y382X	LGD	unknown	Rosenzweig et al. 2008	---	27.8	.	Pathogenic
FPPH125	F	39	1.2.1		Sanger	*BMPR2*	c.T201G: p.Y67X	LGD	unknown	Rosenzweig et al. 2008	---	35	.	Pathogenic
FPPH134-01	F	48	1.2.1		Sanger	*BMPR2*	chr2:203,142,934-203,424,670 281.7kb (exon 1-12) deletion	LGD	unknown	No	---	.	.	Likely Pathogenic
FPPH095	F	37	1.2.1		Sanger	*BMPR2*	del exon 2, 3	LGD	paternal	Pfarr et al. 2011	---	.	.	Pathogenic
FPPH2501	M	42	1.2.1		Sanger	*BMPR2*	del exon 3	LGD	unknown	Pfarr et al. 2011	---	.	.	Pathogenic
FPPH0312	F	57	1.2.1		Sanger	*BMPR2*	deletion exons 4 & 5	LGD	unknown	Cogan et al. 2005	---	.	.	Pathogenic
FPPH0901	F	30	1.2.1		Sanger	*BMPR2*	deletion exons 4-10	LGD	paternal	No	---	.	.	Pathogenic
FPPH4504	F	23	1.2.1		Sanger	*BMPR2*	c.G296A: p.C99Y	D-Mis	paternal	Machado et al. 2009	---	27.7	D	Pathogenic
FPPH136-03	F	22	1.2.1		Sanger	*BMPR2*	c.A200G: p.Y67C	D-Mis	paternal	Morisaki et al. 2004	---	17.2	D	VUS (high) [§]
FPPH077	M	21	1.2.1		Sanger	*BMPR2*	c.A200G: p.Y67C	D-Mis	paternal	Morisaki et al. 2004	---	17.2	D	Pathogenic
FPPH2104	F	29-39	1.2.1		Sanger	*BMPR2*	c.C1471T: p.R491W	D-Mis	paternal	Deng et al. 2000	---	24.8	D	Pathogenic
FPPH5201	F	46	1.2.1		Sanger	*BMPR2*	c.C250T: p.C84R	D-Mis	paternal	Machado et al. 2009	---	27.8	D	Pathogenic
FPPH067	F	unknown	1.2.1		Sanger	*BMPR2*	c.G1040A: p.C347Y	D-Mis	unknown	Lane et al. 2000	---	28.5	D	VUS (high)
JM093	F	43	1.2.1		WES	*BMPR2*	c.G1040A: p.C347Y	D-Mis	paternal or *de novo*	Lane et al. 2000	---	28.5	D	Pathogenic
FPPH108	F	30	1.2.1		Sanger	*BMPR2*	c.G1042A: p.V348E	D-Mis	unknown	Machado et al. 2009	4.53E-04	25.3	D	Pathogenic
SPH534UH2819	F	23	1.2.1		WES	*BMPR2*	c.G1156C: p.E386Q	D-Mis	paternal or *de novo*	Austin et al. 1993	---	32	D	Likely Pathogenic

ID	Gender	Age, dx or enrollment (y)	WHO class	Associated medical conditions	Primary method of detection	Gene	Mutation	Variant type	Inheritance	Previously-reported?	Allele frequency (ExAC)	CADD	MetaSVM	ACMG class (deleterious prediction)
FPPH1901	F	32	1.2.1		Sanger	*BMPR2*	c.G1472A: p.R491Q	D-Mis	unknown	Deng et al. 2000	---	35	D	Pathogenic
FPPH1401	F	36	1.2.1		Sanger	*BMPR2*	c.G248A: p.G83E	D-Mis	maternal	Machado et al. 2009	---	27.7	D	Pathogenic
FPPH092	F	30	1.2.1		Sanger	*BMPR2*	c.G350A: p.C117Y	D-Mis	paternal	Thomson et al. 2000	---	27.9	D	Pathogenic
FPPH1301	F	23	1.2.1		Sanger	*BMPR2*	c.T295C: p.C99R	D-Mis	paternal or *de novo*	Machado et al. 2006	---	19.3	D	VUS (high)
JM586	M	72	1.2.1	hypothyroidism prostate cancer	WES	*BMPR2*	c.G674A: p.R225H	Mis	unknown	rs148682262	0.0003	24.8	T	VUS (medium)
FPPH6401	F	unknown	1.2.2	breast cancer	WES	*CAV1*	c.471delC: p.D157fs	LGD	unknown	No	---	33	.	Pathogenic
FPPH066	M	50	1.2		WES	*EIF2AK4*	c.C1387T: p.R463X (homozygous)	D-Mis	unknown	Eyries et al. 2014	8.28E-06	38	.	Pathogenic
FPPH170-1	M	35	1.6	PCH	WES	*EIF2AK4*	c.C3766T:p.R1256X; c.1150dupG:p.S383fs	LGD; LGD	unknown	Best et al. 2014	0.0001; 3.31E-05	49; 32	.	Pathogenic
FPPH135-01	F	37	1.2.2	HHT	WES	*ENG*	c.714dupG: p.E239Gfs	LGD	maternal or *de novo*	Bossler et al. 2006	---	27.9	.	Pathogenic
SPH863DA5217	F	39	1.2.2		WES	*SMAD9*	c.G218A: p.R73H	D-Mis	unknown	No	---	20.3	D	VUS (high)
FPPH1601	M	33	1.2		WES	*TBX4*	c.1115dupC: p.Pro372fs	LGD	paternal	No	---	34	.	Likely Pathogenic
FPPH160-01*	F	48	1.2.2		WES	*TBX4*	c.G1055T: p.R352L	Mis	unknown	rs747237133	8.40E-06	32	T	VUS (medium)
SPH627PH2920	M	47	1.1		WES	*TBX4*	c.G1207A: p.G403R	Mis	unknown	rs146829316	0.0001	27.1	T	VUS (medium)

Abbreviations: Age at dx, age at diagnosis; HHT, hereditary hemorrhagic telangiectasia; PCH, pulmonary capillary hemangiomatosis; CF, cystic fibrosis; PVOD, pulmonary veno-oclusive disease; LGD, likely gene damaging; D-Mis, damaging missense predicted by MetaSVM; ACMG, American College of Medical Genetics.

*patient carries variants in both *ACVRL* and *TBX4*.

†---, mutation not present in ExAC.

‡Annotation toolkit predictions: B, benign; T, tolerant; P, possibly damaging; D, damaging; ., mutation class not included in toolkit.

§Deleteriousness prediction criteria if not ACMG classification "pathogenic" or "likely pathogenic": high= likely gene damaging or D-Mis plus CADD score≥15; medium= D-Mis or CADD≥15.

Supplemental Table 3. Lack of enrichment of rare genetic variants in known PAH risk genes*, excluding *BMPR2 and TBX4*, in patients of European ancestry.

	Mutation type†	Observed in cases	Observed in controls (n=1319)	Enrichment rate	p-value
Pediatric-onset (n=88)	SYN	2	22	1.36	0.66
	LGD	0	2	0	1
	MIS	4	53	1.13	0.78
	D-Mis	1	21	0.71	1
Adult-onset (n=160)	SYN	5	22	1.87	0.21
	LGD	1	2	4.12	0.29
	MIS	4	53	0.62	0.52
	D-Mis	0	21	0	0.16

**ACVRL1*, *BMPPR1A*, *BMPR1B*, *CAV1*, *EIF2AK4*, *ENG*, *KCNK3*, *SMAD9 and SMAD4*

†SYN, synonymous; LGD, likely gene damaging; MIS, all missense; D-Mis, damaging missense predicted by MetaSVM.

Supplemental Table 4. Enrichment of *de novo* LGD variants in very early-onset (≤5 years of age) pediatric patients.

Variant type*	Age ≤5y (n=16)	Age >5-18y (n=18)	Enrichment	p-value
SYN	2	8	3.6	0.12
MIS	10	16	1.4	0.44
D-Mis	3	6	1.8	0.51
LGD	6	0	NA	0.01

*SYN, synonymous; LGD, likely gene damaging; MIS, all missense; D-Mis, damaging missense predicted by MetaSVM.

Supplemental Table 5. List of *de novo* LGD and missense variants in pediatric-onset IPAH trios (n=36).

Proband ID	Variant type	Variant Class	Gene	Transcript ID	Nucleotide change	AA change	ExAC pLI	ExAC mis-z	MetaSVM	CADD	ExAC frequency	Deleterious prediction	Other medical conditions
15-002*	D-Mis[†]	nonsynonymous SNV	*TUBB6*	NM_001303527	c.C40T	p.R14W	5.11E-04	4.11	D[‡]	14.4	8.29E-06	low	
15-002*	D-Mis	nonsynonymous SNV	*ALDH9A1*	NM_000696	c.A545G	p.Y182C	1.29E-05	-0.19	D	19.3	1.65E-05	high	
15-002*	missense	nonsynonymous SNV	*TCN2*	NM_000355	c.C1163T	p.A388V	0.02	-2.67	T	1.62	4.12E-05	low	
15-006	D-Mis	nonsynonymous SNV	*KDM3B*	NM_016604	c.C3298T	p.P1100S	1.00	4.99	D	31.0	---[§|]	high	
15-028	missense	nonsynonymous SNV	*SEMA6D*	NM_153618	c.C1818G	p.I606M	1.00	0.82	T	11.8	---	low	
15-042	D-Mis	nonsynonymous SNV	*ITPR1*	NM_001168272	c.C3614T	p.A1205V	1.00	5.75	D	15.6	1.08E-05	high	
15-054*	D-Mis	nonsynonymous SNV	*EMID1*	NM_001267895	c.G1114A	p.G372R	0.04	-0.29	D	21.0	---	high	
15-054*	missense	nonsynonymous SNV	*TF*	NM_001063	c.C1376T	p.T459I	0.43	0.71	T	14.0	---	low	
JM0004*	LGD	stopgain	*ZNF620*	NM_175888	c.G74A	p.W25X	6.83E-06	-0.37	.	16.2	---	high	
JM0004*	LGD	frameshift_deletion	*AMOT*	NM_001113490	c.957delC	p.S319fs	0.21	-0.28	.	.	---	high	
JM0008	missense, LGD	nonsynonymous SNV, frameshift_deletion	*ALPPL2*	NM_031313	c.C1565A, c.1561delG	p.T522N, p.G521fs	6.22E-06	1.59	T	6.6	---	high	esophageal dysmotility
JM0013	D-Mis	nonsynonymous SNV	*RBL2*	NM_001323608	c.A1067C	p.E356A	0.96	0.58	D	17.5	0	high	
JM0023	missense	nonsynonymous SNV	*CARNS1*	NM_020811	c.C2305G	p.R769G	0.02	-0.65	T	16.5	---	medium	
JM0028*	D-Mis	nonsynonymous SNV	*MAPK6*	NM_002748	c.G1528A	p.A510T	0.53	0.47	D	19.4	---	high	
JM0028*	D-Mis	nonsynonymous SNV	*TRH*	NM_007117	c.C253A	p.H85N	9.18E-06	0.14	D	20.5	---	high	
JM0028*	missense	nonsynonymous SNV	*CSNK2A2*	NM_001896	c.A551G	p.H184R	1.00	3.49	T	15.0	---	medium	
JM0035*	missense	nonsynonymous SNV	*MYO16*	NM_001198950	c.T1420C	p.S474P	1.00	1.53	T	0.99	0	low	
JM0035*	missense	nonsynonymous SNV	*CNTN4*	NM_001206956	c.A722G	p.H241R	1.00	-0.64	T	26.1	0	medium	
JM0039	missense	nonsynonymous SNV	*MEFV*	NM_000243	c.C2281T	p.R761C	1.12E-08	-0.53	T	9.07	7.42E-05	low	
JM0042*	missense	nonsynonymous SNV	*CHID1*	NM_001142675	c.C217T	p.R73W	0.00	0.58	T	12.6	---	low	
JM0042*	missense	nonsynonymous SNV	*RARS2*	NM_020320	c.A770G	p.K257R	3.49E-06	-0.48	T	15.2	---	medium	
JM0065	missense	nonsynonymous SNV	*CCDC40*	NM_017950	c.G2926A	p.E976K	6.32E-11	0.14	T	10.9	---	low	
JM1307*	missense	nonsynonymous SNV	*NEU1*	NM_000434	c.C32G	p.P11R	1.93E-05	1.85	T	0.00	0	low	
JM1307*	LGD	stoploss	*EMC8*	NM_006067	c.G633C	p.X211Y	0.26	0.90	.	0.12	8.24E-06	low	
JM1321	missense	nonsynonymous SNV	*PRDM9*	NM_001310214	c.G2487T	p.E829D	1.95E-22	-0.42	T	8.5	---	low	
JM1344*	D-Mis	nonsynonymous SNV	*ATP6V0A2*	NM_012463	c.A1184G	p.N395S	4.74E-06	-0.01	D	15.3	---	high	autism
JM1344*	missense	nonsynonymous SNV	*DAB2*	NM_001244871	c.G1655T	p.W552L	0.98	-1.08	T	18.1	---	medium	autism
JM1416	missense	nonsynonymous SNV	*SPATC1*	NM_001134374	c.C794T	p.S265L	3.59E-08	-0.78	T	4.97	8.74E-06	low	
JM171	LGD	frameshift insertion	*NUCB1*	NM_006184	c.567dupT	p.R189fs	4.17E-05	0.84	.	.	---	high	
JM216	D-Mis	nonsynonymous SNV	*SLC25A24*	NM_013386	c.C649T	p.R217C	4.90E-09	0.21	D	24	---	high	
JM559*	D-Mis	nonsynonymous SNV	*ABCC8*	NM_000352	c.G2870A	p.R957H	3.94E-15	2.60	D	26.5	1.65E-05	high	
JM559*	missense	nonsynonymous SNV	*PREX1*	NM_020820	c.G4069A	p.E1357K	1.00	4.11	T	17.9	---	medium	

Proband ID	Variant type	Variant Class	Gene	Transcript ID	Nucleotide change	AA change	ExAC pLI	ExAC mis-z	MetaSVM	CADD	ExAC frequency	Deleterious prediction	Other medical conditions
JM630*	missense	nonsynonymous SNV	*HIRA*	NM_003325	c.C2173T	p.R725W	1.00	4.00	T	22.3	9.91E-05	medium	
JM630*	LGD	stopgain	*ZMYM2*	NM_001190965	c.C1618T	p.R540X	0.97	1.85	.	41.0	---	high	
JM758*	D-Mis	nonsynonymous SNV	*SAMHD1*	NM_015474	c.C25T	p.P9S	1.50E-05	2.20	D	7.92	0	high	
JM758*	missense	nonsynonymous SNV	*SPTA1*	NM_003126	c.T6297G	p.F2099L	5.85E-05	-3.70	T	9.61	0	low	
JM758*	missense	nonsynonymous SNV	*C19orf68*	NM_199341	c.C229T	p.R77W	.	.	T	33.0	0	medium	
JM852	LGD	stopgain	*KEAP1*	NM_012289	c.C1752A	p.Y584X	0.252	4.75	.	19.8	---	high	incontinentia pigmenti, neonatal seizures, spastic diplegia, developmental delay, sleep apnea
JM905	missense	nonsynonymous SNV	*ADAMTS18*	NM_001326358	c.T303A	p.F101L	3.74E-15	-3.13	T	11.3	0	low	

*ID, patient carries mutations in more than one candidate gene.

†Variant classifications: LGD, likely gene damaging; D-Mis, damaging missense predicated by MetaSVM; SNV, single nucleotide variant.

‡Annotation toolkit predictions: B, benign; T, tolerant; P, possibly damaging; D, damaging; ., mutation class not included in toolkit.

§|---, mutation not present in ExAC.

Supplemental Table 6. Lack of enrichment of genome-wide rare variants in pediatric-onset IPAH patients of European ethnicity.

Mutation type*	Observed in cases (n=66)	Observed in controls (n=1,319)	Enrichment	p-value
SYN	3,140	59,864	1.05	1
MIS	5,838	113,810	1.03	1
D-Mis	882	17,388	1.01	1
LGD	410	7,597	1.08	0.95

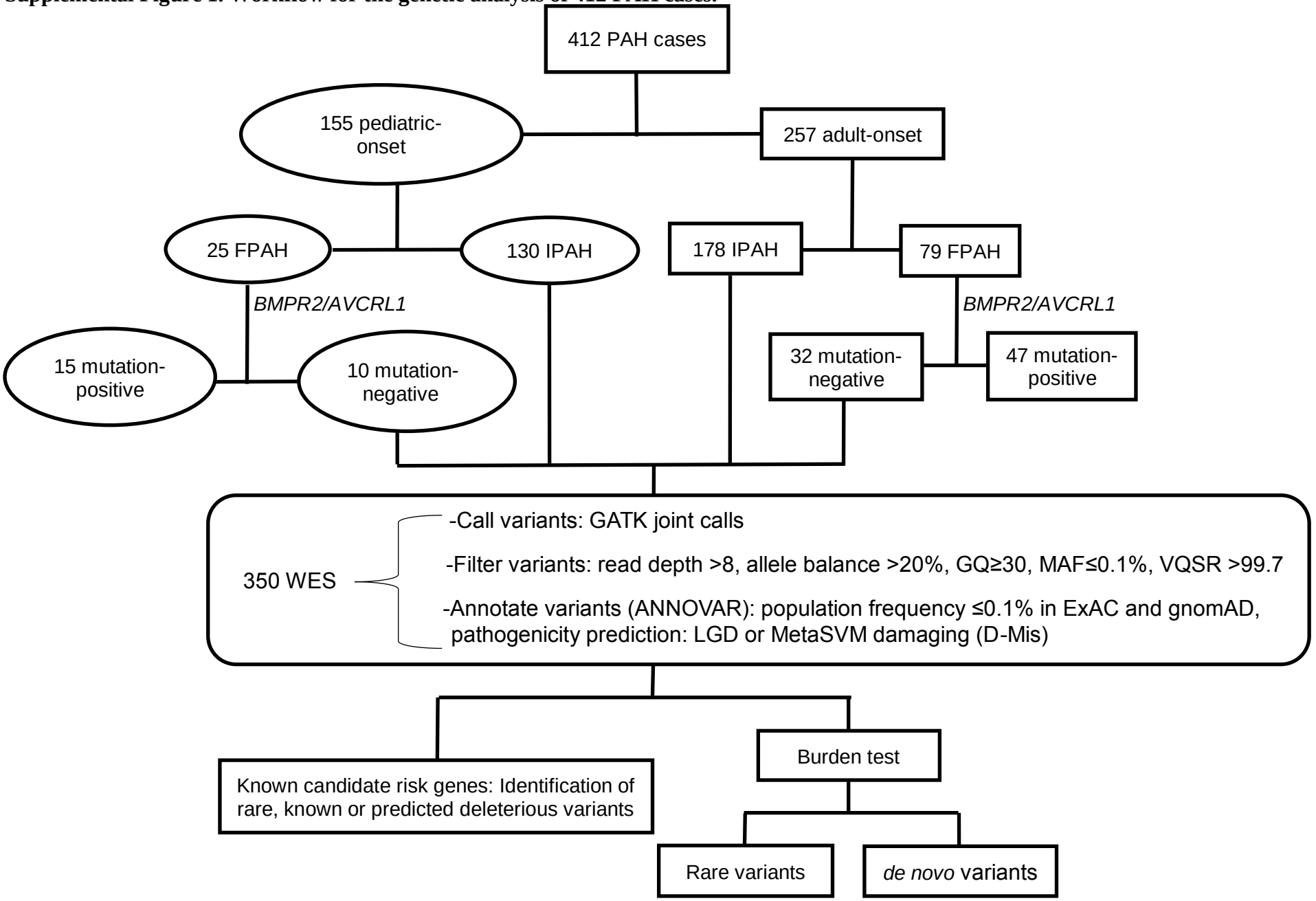
*SYN, synonymous; LGD, likely gene damaging; MIS, all missense; D-Mis, damaging missense predicted by MetaSVM

Supplemental Table 7. Lack of enrichment of genome-wide rare variants in adult-onset IPAH patients of European ethnicity.

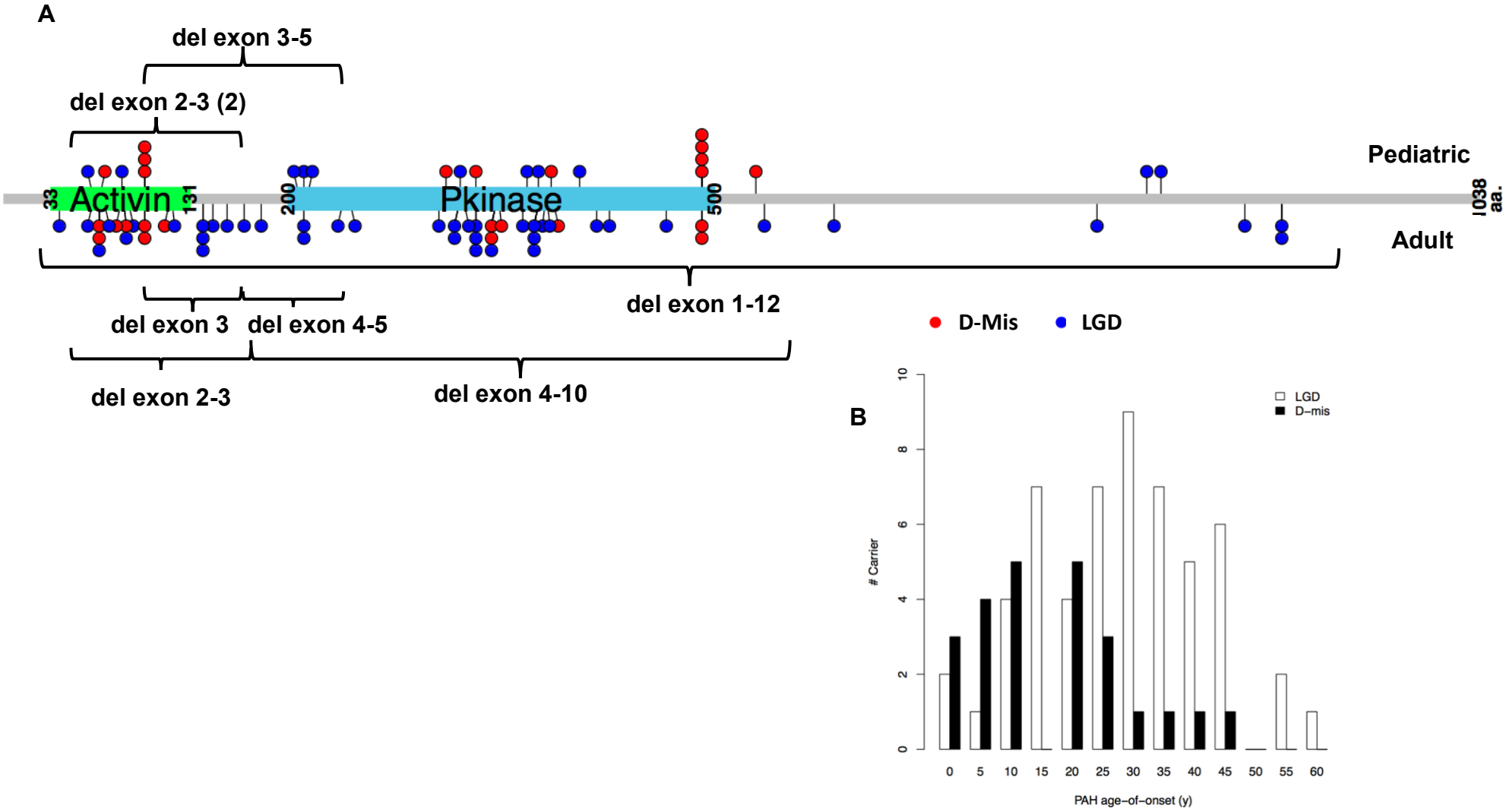
Mutation type*	Observed in cases (n=120)	Observed in controls (n=1319)	Enrichment	p-value
SYN	5,514	59864	1.01	1
MIS	10,538	113810	1.02	1
D-Mis	1,621	17,388	1.02	1
LGD	680	7,597	0.98	1

*SYN, synonymous; LGD, likely gene damaging; MIS, all missense; D-Mis, damaging missense predicted by MetaSVM

Supplemental Figure 1. Workflow for the genetic analysis of 412 PAH cases.



Supplemental Figure 2. ***BMPR2*** **mutations identified in 412 pediatric- and adult-onset PAH patients. A,** Rare LGD and D-Mis mutations identified in pediatric-onset patients are indicated above the protein schematic; mutations in adult-onset patients are below the schematic. D-Mis, damaging missense mutations predicted by MetaSVM (red), LGD, likely gene damaging (blue), Pkinase, conserved protein kinase domain. **B,** LGD variants occurred more frequently in patients with older age-of-onset compared to D-Mis variants. P =0.0006, Mann–Whitney U test.



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