,Title :

Phenotype characterization of *TBX4* mutation and deletion carriers with neonatal and pediatric pulmonary hypertension.

Supplementary material.

Table S1.

Case #	Origin of testing	NGS panel	CNV array	Sanger sequencing	Other findings	Family testing
1, 7, 13, 15, 19	Bambino Gesù Children's Hospital, Rome	*ABCA3, SFTPB, SFTPC, SFTPA1, SFTPA2, SFTPD, NKX2.1, ENG, CAV1, BMPR2, SMAD1, SMAD5, SMAD9, ACVRL1, TBX4, FOXF1, MEOX2, CSF2RB, CBLN2, CRHR1, CRHBP, PPARG* *	GCH array	#7,13,15,19 : TBX4	no	#1,7: CGH trio analysis #15 : TBX4 Sanger in parents
4,5,11	Boston Children's Hospital	no	#4, 5 : CGH array	#11	#4 : Xp22.31 duplication*	no
12,14,18	Munich University	*ACVRL1, BMPR1B, BMPR2, CAV1, EIF2AK4, ENG, GDF2, KCNA5, KCNK3, SMAD1, SMAD4, SMAD9, TBX4***	no	#12, 14, 18	n/a	#12, 18 : confirmatory TBX4 Sanger sequencing in mother and maternal grandmother (SPS affected)
2, 8, 9, 16, 17	Children's Hospital Colorado	#8,16,17: *BMPR2, ALK1, ENG, CAV1, KCNK3, EIF2AK4, SMAD 9* #9: *BMPR2, ALK1, ENG, CAV1, KCNK3, EIF2AK4, SMAD 9, ABCC8, KCNA5, SNAD4, GDF2, TBX4* ***	#2 : SNP array	#8, 16, 17	#9 : Del(3)(q26.2-q26.32) (inheritance not confirmed)	#2 : Trio FISH analysis
#3, 10	Vanderbilt University Hospital	#10: *BMPR2, ALK1, ENG* ****	#3 : CGH array	#10	no	no
#6	University of Iowa Hospital	Not performed	ChiP array	no	no	no

Cases were selected from the following registries and centers : the Pediatric Pulmonary Hypertension Network (PPHNet) [s1] (cases #2,8,9,16,17), Childhood Interstitial Lung Disease – Europe consortium (chILD-EU) [s2] (cases #12,13,14,18), Bambino Gesù Children's Hospital, Rome, Italy (1,7,15,19), Boston Children's Hospital, Boston, MA (cases #4,5,11), Vanderbilt University Children's Hospital, Nashville, TN (cases # 3,10) and Iowa Children's Hospital, Iowa city, IA (case #6). Genetic testing: * Custom research panel target; ** Center for Human Genetic, Munich University diagnostic panel; *** National Institute of Health Pulmonary Arterial Hypertension Biobank reserach panel, former and current versions ; ****Commercial pulmonary hypertension diagnostic panel.

Table S2: initial and subsequent chest imaging.

	Chest radiogram	Chest high-resolution computed tomography	
		Early (first year of life)	Late (1-18 years)
1	1 day : decreased expansion, decreased vascular markings.		
2			1 year : mild interlobular septal thickening and ground glass opacities in the lower lobes, juxtapleural nodules in upper and lower lobes.
3	3 years : mild, generalized hyperinflation ; moderate, parahilar, peribronchial wall thickening.		9 years : normal
7	1 day : decreased expansion, diffuse groud glass opacity, right tension pneumothorax,	20 days : diffuse areas of hyperinflation and dysventilation, ground-glass opacities.	
8		1 month : nonspecific lower lobes septal thickening, patchy areas of atelectasis ands hyperinflation.	
9	.		3 years : RV and pulmonary artery enlargement, Multiple small nodules in right upper lobe with patchy ground glass opacities, peripheral septal thickening
10			18 years : multifocal densities with cystic changes. Central emphysema and fibrosis ; right ventricular hypertrophy, enlarged pulmonary artery and veins ; mediastinal/hilar lymphadenopathy.
12	1 day : decreased expansion, left tension pneumothorax.		9 years : Patchy ground glass opacities, septal thickening, consolidation, mosaic perfusion, regional hyperinflation and air trapping
13	1 day : bilateral pneumothorax, diffuse ground glass opacities, interlobular thickening, regional hyperinflation.	4 weeks : mild diffuse ground glass opacities	2.5 years : severe diffuse patchy ground glass and consolidations
14	1 day : right pneumothorax, diffuse ground-glass opacities	12 days : prominent PA, very discrete diffuse ground glass	
15	2 years : Minimal perilar bronchial thickening		9 years : mild bronchial collapse, expiratory air trapping; no significant interstitial lung disease.
16			9 years : small diffuse opacities (possible PCH), mild peribronchial thickening, enlarged pulmonary arteries.
17			13 years : mild central bronchial wall thickening, patchy air trapping and lower lobe atelectasis, lobar nodular opacities.
18	1 month : diffuse ground glass opacities, interlobular thickening, consolidations, hyperinflated areals .	5 months: bilateral diffuse ground glass opacities, interlobular thickening, consolidations, hyperinflated areals	7 years : irregular emphysema (mosaic like pattern) septal thickenings, consolidations
19	3 years : bilateral diffuse thickening of bronchial walls prevalent in basal lungs (3 yrs)		10 years : interstitial thickening, centrilobular nodules (« tree in bud » pattern), diffuse bronchiectasis, initial honeycombing.

Table S3. Phenotypic spectrum in published 17q23 deletions including the current cohort.

Studies	Tech	n	Sex	TBX2/TBX4	PAH	CHD	HL	DD	facial	skel	Remarks
S3	Mic	1	F	-	(+)*	-	+	+	+	-	Hydrocephalus, * secondary PAH, comfort care
S4	Mic	1	M	-	-	-	-	+	-	-	Epilepsy, microcephaly
S5	Mic	1	M	-	-		+	+	+	+	TEF, microcephaly, hypothyroidism
S6	Mic	1	F	-	-	-	+	+	+	+	
S7	Kar	1	F	-	-	-	-	+	+	+	TEF
S8	Kar	1	F	-	-	-	-	+	+	+	Epilepsy
S9	Kar	1	M	+	-	-	-	+	+	+	Craniosynostosis
S10	Kar	1	M	+	-	-	unk	+	+	+	TEF, VSD, PS, death 3 mo
S11	Kar	1	F	+	+*	+	unk	unk	+	+	* death at 17 days ; PAH changes at autopsy
S12	Kar	1	M	+	-	-	+	+	+	+	
S13	Kar	1	M	+	+*	+	unk	unk	+	unk	TEF, VSD, * likely PAH, death 4 mo
S14	Mic	1	F	+	+	-	+	+	+	+	Microcephaly
S15	Mic	2	F	+	-	-	+	+	-	-	
			M	+	-	-	+	+	-	-	
S16	Mic	7	F	+	-	+	+	+	+	+	
			F	+	-	+	-	+	+	+	Esotropia
			M	+	+	-	-	-	+	+	PPHN
			F	+	+	+	+	-	-	+	
			F	+	-	+	-	unk	+	+	Cutis aplasia
			F	+	-	-	-	+	+	+	
			F	+	+	+	-	+	+	+	
This study	Mic	6	M	+	+	-	-	unk	-	-	Hypothyroidism, cortisol deficiency, death 5 mo
			M	+	+	+	-	-	-	-	Omphalocoele, seizures, nystagmus
			F	+	+	+	-	+	+	+	
			F	+	+	+	-	-	+	+	
			F	+	+	+	-	+	+	+	
			F	+	+	+	+	+	+	+	Microcephaly, esotropia
Statistics :											
Total (n)		27	17F (63%)	21/27 (78%)	13/27 (48%)	11/27 (40%)	10/24 (42%)	19/23 (83%)	21/27 (78%)	20/26 (77%)	
TBX2/TBX4 -		6			1/6 (17%)	0/6 (0%)	3/6 (50%)	6/6 (100%)	5/6 (83%)	4/6 (67%)	
TBX2/TBX4 +		21			12/21 (57%)	12/21 (57%)	7/18 (39%)	13/17 (76%)	16/21 (76%)	16/20 (80%)	
P value					0.16	0.02	0.66	0.28	1	0.59	

Legend : Tech : testing technique ; Mic : chromosomal microarray ; Kar : karyotype ; unk : unknown ; PAH pulmonary arterial hypertension ; CHD : congenital heart disease (patent ductus arteriosus, atrial septal defect) ; HL hearing loss ; DD developmental delay ; digits - abnormal toes and/or fingers ; facial – facial dysmorphisms ; skel – other skeletal anomalies ; TEF tracheo-esophageal fistula ; VSD ventricular septal defect. Statistics : n : number of positive subjects over total tested per category; TBX2/TBX4 - : deletions exclusive of the TBX2/TBX4 loci ; TBX2/TBX4 + : deletions inclusive of the TBX2/TBX4 loci. P values determined by Fisher exact test.

Supplementary references :

s1 Abman SH, Raj U. Towards improving the care of children with pulmonary hypertension: The rationale for developing a Pediatric Pulmonary Hypertension Network. Prog Pediatr Cardiol 2009: 27(1-2): 3-6.

s2 Griese M, Seidl E, Hengst M, Reu S, Rock H, Anthony G, Kiper N, Emiralioglu N, Snijders D, Goldbeck L, Leidl R, Ley-Zaporozhan J, Kruger-Stollfuss I, Kammer B, Wesselak T, Eismann C, Schams A, Neuner D, MacLean M, Nicholson AG, Lauren M, Clement A, Epaud R, de Blic J, Ashworth M, Aurora P, Calder A, Wetzke M, Kappler M, Cunningham S, Schwerk N, Bush A, the other ch ILDEUc. International management platform for children's interstitial lung disease (chILD-EU). Thorax 2018: 73(3): 231-239.

s3 Khattab M, Xu F, Li P, Bhandari V. A de novo 3.54 Mb deletion of 17q22-q23.1 associated with hydrocephalus: a case report and review of literature. *Am J Med Genet A* 2011; 155A: 3082-3086.

s4 Coppola A, Tostevin A, McTague A, Pressler RM, Cross JH, Sisodiya SM. Myoclonic epilepsy in a child with 17q22 q23.1 deletion. Am J Med Genet A 2013; 161A: 2036-2039.

s5 Puusepp H, Zilina O, Teek R, Mannik K, Parkel S, Kruustuk K, Kuuse K, Kurg A, Ounap K. 5.9 Mb microdeletion in chromosome band 17q22-q23.2 associated with tracheo-esophageal fistula and conductive hearing loss. Eur J Med Genet 2009; 52: 71-74.

s6 Boutry-Kryza N, Labalme A, Till M, Schluth-Bolard C, Langue J, Turleau C, Edery P, Sanlaville D. An 800 kb deletion at 17q23.2 including the MED13 (THRAP1) gene, revealed by aCGH in a patient with a SMC 17p. Am J Med Genet A 2012; 158A: 400-405.

s7 Park JP, Moeschler JB, Berg SZ, Bauer RM, Wurster-Hill DH. A unique de novo interstitial deletion del(17)(q21.3q23) in a phenotypically abnormal infant. Clin Genet 1992; 41: 54-56.

s8 Mickelson EC, Robinson WP, Hrynchak MA, Lewis ME. Novel case of del(17)(q23.1q23.3) further highlights a recognizable phenotype involving deletions of chromosome (17)(q21q24). Am J Med Genet 1997; 71: 275-279.

s9 Thomas JA, Manchester DK, Prescott KE, Milner R, McGavran L, Cohen MM, Jr. Hunter-McAlpine craniosynostosis phenotype associated with skeletal anomalies and interstitial deletion of chromosome 17q. Am J Med Genet 1996; 62: 372-375.

s10 Marsh AJ, Wellesley D, Burge D, Ashton M, Browne C, Dennis NR, Temple K. Interstitial deletion of chromosome 17 (del(17)(q22q23.3)) confirms a link with oesophageal atresia. J Med Genet 2000; 37: 701-704.

s11 Levin ML, Shaffer LG, Lewis R, Gresik MV, Lupski JR. Unique de novo interstitial deletion of chromosome 17, del(17) (q23.2q24.3) in a female newborn with multiple congenital anomalies. Am J Med Genet 1995; 55: 30-32.

s12 Khalifa MM, MacLeod PM, Duncan AM. Additional case of de novo interstitial deletion del(17)(q21.3q23) and expansion of the phenotype. Clin Genet 1993; 44: 258-261.

s13 Dallapiccola B, Mingarelli R, Digilio C, Obregon MG, Giannotti A. Interstitial deletion del(17) (q21.3q23 or 24.2) syndrome. Clin Genet 1993; 43: 54-55.

s14 Nimmakayalu M, Major H, Sheffield V, Solomon DH, Smith RJ, Patil SR, Shchelochkov OA. Microdeletion of 17q22q23.2 encompassing TBX2 and TBX4 in a patient with congenital microcephaly, thyroid duct cyst, sensorineural hearing loss, and pulmonary hypertension. *Am J Med Genet A* 2011; 155A: 418-423.

s15 Schonewolf-Greulich B, Ronan A, Ravn K, Baekgaard P, Lodahl M, Nielsen K, Rendtorff ND, Tranebjaerg L, Brondum-Nielsen K, Tumer Z. Two new cases with microdeletion of 17q23.2 suggest presence of a candidate gene for sensorineural hearing loss within this region. *Am J Med Genet A* 2011; 155A: 2964-2969.

s16 Ballif BC, Theisen A, Rosenfeld JA, Traylor RN, Gastier-Foster J, Thrush DL, Astbury C, Bartholomew D, McBride KL, Pyatt RE, Shane K, Smith WE, Banks V, Gallentine WB, Brock P, Rudd MK, Adam MP,

Keene JA, Phillips JA, 3rd, Pfotenhauer JP, Gowans GC, Stankiewicz P, Bejjani BA, Shaffer LG. Identification of a recurrent microdeletion at 17q23.1q23.2 flanked by segmental duplications associated with heart defects and limb abnormalities. *Am J Hum Genet* 2010; 86: 454-461.