Table S1.

Summary of clinical manifestations in MPS VI patients. All patients except P7 had short stature, coarse facies, dolichocephaly, corneal clouding, hirsutism, pectus carinatum and brachydactyly.

Particulars	**Patient ID**								
	P1	**P2**	**P3**	**P5**	**P6**	**P7[1]**	**P8**	**P9**	**P10**
Family ID	F1	F2	F3	F5	F6	F6	F7	F8	F9
Mutation[2]	p.W450C/?	**p.L98R**	p.W450C	p.D53N	**p.R315P**	**p.R315P**	p.R160Q	**p.C91R**	**p.G38_G40del3**
Present age (years)	18	7	19	6	4	2	15	Expired at 10 years of age	7
Extent of severity	Moderate	Attenuated	Attenuated	Moderate	Moderate	NA	Moderate	Severe	Attenuated
Parental consanguinity	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Other affected members	None	None	None	None	Younger sister (P7)	Elder Sister (P6)	None	Younger brother	Cousin
Gender	Male	Female	Female	Male	Female	Female	Male	Male	Female
Age of onset	3 years	2 years	5 years	5 months	9 months	NA	3 years	9 months	18 months
Presenting feature(s)	Pectus carinatum, dolicho-cephaly	Short stature	Coarse facies, skeletal deformity	Delayed milestones	Kypho-scoliosis	Extensive Mongolian spots. Not yet developed clinical symptoms.	Large head, poor growth, corneal clouding, noisy breathing	Gibbus, inguinal hernia	Pectus carinatum, macrocephaly
Age at diagnosis (years)	6	3	6	1	3	1	14	7	4
Age at last clinical evaluation (years)	16	5	18	5	3	NA	15	7	7

Particulars	Patient ID								
	P1	**P2**	**P3**	**P5**	**P6**	**P7[1]**	**P8**	**P9**	**P10**
Short stature	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes
Coarse facies	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes
Ambulation	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes
Abnormal gait	Yes	No	Yes	No	No	NA	No	Yes	Yes
Dolichocephaly	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes
Frontal bossing	No	Yes	No	No	No	NA	No	Yes	No
Brain (MRI findings)	NE	No	Mild diffuse cerebral atrophy	NE	NE	NA	NE	NE	Diffuse cerebral atrophy, Left temporal arachnoid cyst, white matter disease
Intelligence	Normal	Normal	Normal	Normal	Normal	NA	Normal	Normal	Normal
Cognition	Normal	Normal	Normal	Normal	Normal	NA	Normal	Normal	Normal

Particulars	Patient ID								
	P1	**P2**	**P3**	**P5**	**P6**	**P7[1]**	**P8**	**P9**	**P10**
Surgery undergone	None	None	Tonsillo-adenoidectomy, Ventriculo-stomy, Bilateral supra-condylar osteotomy, Atlanto axial decompression, Carpel tunnel release	Bilateral inguinal hernia	None	NA	None	Inguinal hernia, tracheo-stomy	None
Hirsutism	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Corneal clouding	Yes	Yes	Yes	Yes	Yes. Bilateral disc edema was also present.	NA	Yes (Dense)	Yes	Yes
Hearing loss	No	No	No	Yes	NE	NA	Yes Significant in both ears, no hearing aids	Yes. (60% hearing loss. Used hearing aids	NE
Macroglossia	Yes	No	No	No	No	NA	Yes. Causes speech impairment	No	No
Respiratory function	NE	Normal	Normal	NE	Affected. Noisy breathing	NA	Affected. Noisy breathing and snoring	Affected	Normal
Sleep Disturbance	NE	NE	Mild	NE	Yes. Has snoring	NA	No	Yes. Had snoring	Mild obstructive sleep apnoea

Particulars	Patient ID								
	P1	**P2**	**P3**	**P5**	**P6**	**P7[1]**	**P8**	**P9**	**P10**
Skeletal Involvement									
Pectus carinatum	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes
Cervical spine defects	NE	No	Cervical canal narrowing, prominent at cervico-medullary junction	Atlanto-axial dislocation, mild compression of spinal cord at C1/C2 level	NE	NA	NE	No	No
Thoraco-lumbar spine	Mild scoliosis	Mild gibbus	Mild scoliosis	Kyphosis	Kypho-scoliosis	NA	Lumbar lordosis	Kyphosis, lumbar lordosis	Normal
Stiff upper limbs	Yes	No	Yes	No	Yes	NA	Yes	No	No
Claw hands	Yes	Yes	Yes	Yes	No	NA	No	Yes	Yes
Carpal tunnel syndrome	No	No	Yes	No	No	NA	No	Yes	No
Brachydactyly	Yes	Yes	Yes	Yes (Mild)	Yes	NA	Yes	Yes	Yes
Bent knee	Yes	No	No	Yes	Mild	NA	Yes	Yes	No
Restricted joint mobility	Yes	Mild	Yes	Yes	Yes	NA	No	Yes	Mild

Particulars	Patient ID								
	P1	**P2**	**P3**	**P5**	**P6**	**P7[1]**	**P8**	**P9**	**P10**
Cardiovascular system									
Valvular defects									
Thickening of valve(s)	NE	Mitral and tricuspid valves	Aortic, mitral and tricuspid valves	Aortic valve	No	NA	No	Mitral valve	No
Mitral regurgitation	NE	Yes	Yes	Yes	No	NA	Yes	Yes	No
Tricuspid regurgitation	NE	Yes	Yes	No	No	NA	No	No	No
Mitral stenosis	NE	No	No	No	No	NA	No	No	No
Mitral valve prolapse	No	No	No	Yes	No	NA	Yes	No	No
Left ventricular hypertrophy	NE	Mild	Yes	No	No	NA	No	Yes	No
Other features	None	None	Pulmonary arterial hypertension	None	None	NA	None	None	None
Abdomen									
Umbilical hernia	Yes	Yes	Yes	Yes	No	NA	Yes	No	Yes
Inguinal hernia	No	No	No	Yes	No	NA	No	Yes	No
Organomegaly	No	No	No	Mild Hepatomegaly	Mild Hepato-megaly	NA	Hepato-spleno-megaly	Hepato-spleno-megaly	Mild hepato-megaly

Particulars	Patient ID								
	P1	**P2**	**P3**	**P5**	**P6**	**P7[1]**	**P8**	**P9**	**P10**
General									
Independence in daily activities	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes
Schooling	Normal	Normal	Normal	Normal	Normal	NA	Never sent	Normal	Normal

[1] Preclinical individual.

[2] Mutations were homozygous in all patients except P1, in whom mutation was found only in one allele.

Novel mutations are shown in bold.

NE – Not evaluated. NA – Not applicable. Patient P4 (from Family F4) is not a subject of this study.

Table S2.
Non-synonymous polymorphisms in patients.

Family ID	Patient ID	Mutation[1]	Non-synonymous polymorphisms	
			p.V358M	p.V376M
F1	P1	p.W450C	Heterozygous	Heterozygous
F2	P2	p.L98R	-	-
F3	P3	p.W450C	Homozygous	-
F5	P5	p.D53N	Homozygous	-
F6	P6	p.R315P	-	-
	P7	p.R315P	-	-
F7	P8	p.R160Q	-	-
F8	P9	p.C91R	Sample from patient not available	
F9	P10	p.G38_G40del3	-	Homozygous

[1] Mutations were homozygous in all patients except P1. In P1, one heterozygous mutation was found, and the second mutation was not found.
In P9, the mutation stated was inferred from genotype of parents. "-" indicates absence of polymorphism.

Table S3.
Variations of unknown significance (VUS) found in patients and control subjects.

Type of VUS	Total No.	Location	VUS	NCBI SNP ID	Patients	Controls
Synonymous VUS and those in non-coding regions	17	5'UTR portion of Exon 1	g.5109A>G	rs163126	+	NA
			g.5329A>G	rs57586329	+	NA
			g.5381C>G	rs62377914	+	NA
			g.5448insCA	rs3075605	+	NA
			g.5723A>G	rs59558132	+	NA
			g.5985A>G	rs163127	+	NA
		Intron 1	g.6761G>T	rs55953431	+	+
			g.6765G>A	rs163128	+	+
			g.22317T>C	rs3733895	+	-
		Intron 3	g.36011T>C	rs6870443	+	NA
		Intron 5	g.106089C>T	rs79555942	+	+
			g.152082A>C	rs25415	+	-
		Exon 6	g.152157G>A*	rs25413	+	NA
		3'UTR portion of Exon 8	g.211198G>A	rs2173012	+	NA
			g.213160T>G	rs7704939	+	NA
			g.214113G>T	rs3088247	+	NA
			g.214319T>G	rs11750774	+	NA
Non-synonymous	2	Exon 5	g.105881G>A (p.V358M)	rs1065757	+	+
			g.105935G>A (p.V376M)	rs1071598	+	+
Total	19					

No novel VUS were found.
*Synonymous polymorphism.
"+" indicates presence and "-" indicates absence of the VUS. NA – Not analyzed.

Table S4.
Missense mutations known to occur at L98 and R315 of human ARSB.

Amino acid residue	Mutations known	Codon change	Reference
L98	p.L98Q	C<u>T</u>G>C<u>A</u>G	[15]
	p.L98P	C<u>T</u>G>C<u>C</u>G	[46] (Cited from 47)
	p.L98R	C<u>T</u>G>C<u>G</u>G	This study
R315	p.R315Q	C<u>G</u>A>C<u>A</u>A	[48]
	p.R315P	C<u>G</u>A>C<u>C</u>A	This study

The nucleotides mutated are underlined. Mutations found through this study are shaded.

Table S5.

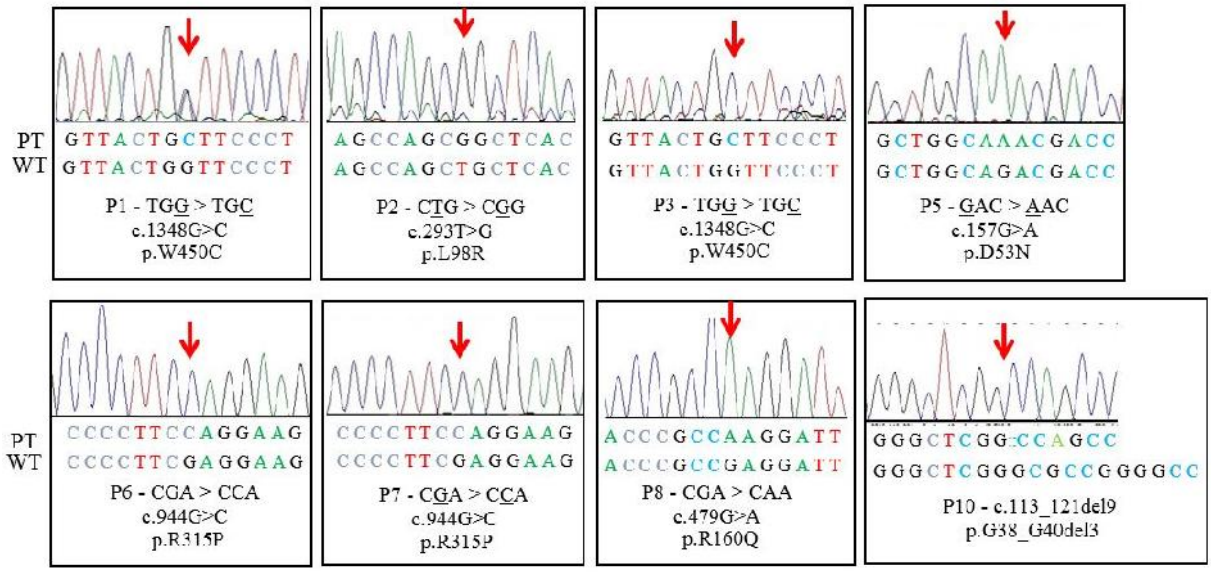
Active site mutations known to date in human ARSB.

S. No.	Mutation	Homozygosity/ Heterozygosity	Severity of phenotype[1]	Ethnicity of patient[1]	Original reference for mutation
1	p.D53N	Homozygous	Not stated[2]	Indian	[1]
2	p.D54N	Homozygous	Not stated. Patient was expected to have early onset and rapidly progressing disease based on high level of urinary GAG and no detectable activity of ARSB	Portugal	[15]
3	p.C91Y	Compound heterozygous	Intermediate phenotype	Polish	[45]
4	p.C91R	Homozygous	Severe	Indian	This study
5	p.R95Q	Compound heterozygous	Severe in one patient, mild in two patients	Not known	Litjens et al. (1996) *Am. J. Hum. Genet.* **58**:1127-1134
6	p.D300E	Compound heterozygous	Not stated	UK	[15]
7	p.N301K	Homozygous	Severe	Turkish	Brands et al. (2013) *Orphanet J. Rare Dis.* **8**:51

[1] Severity and ethnicity mentioned here are as reported in the original publication of the mutation.

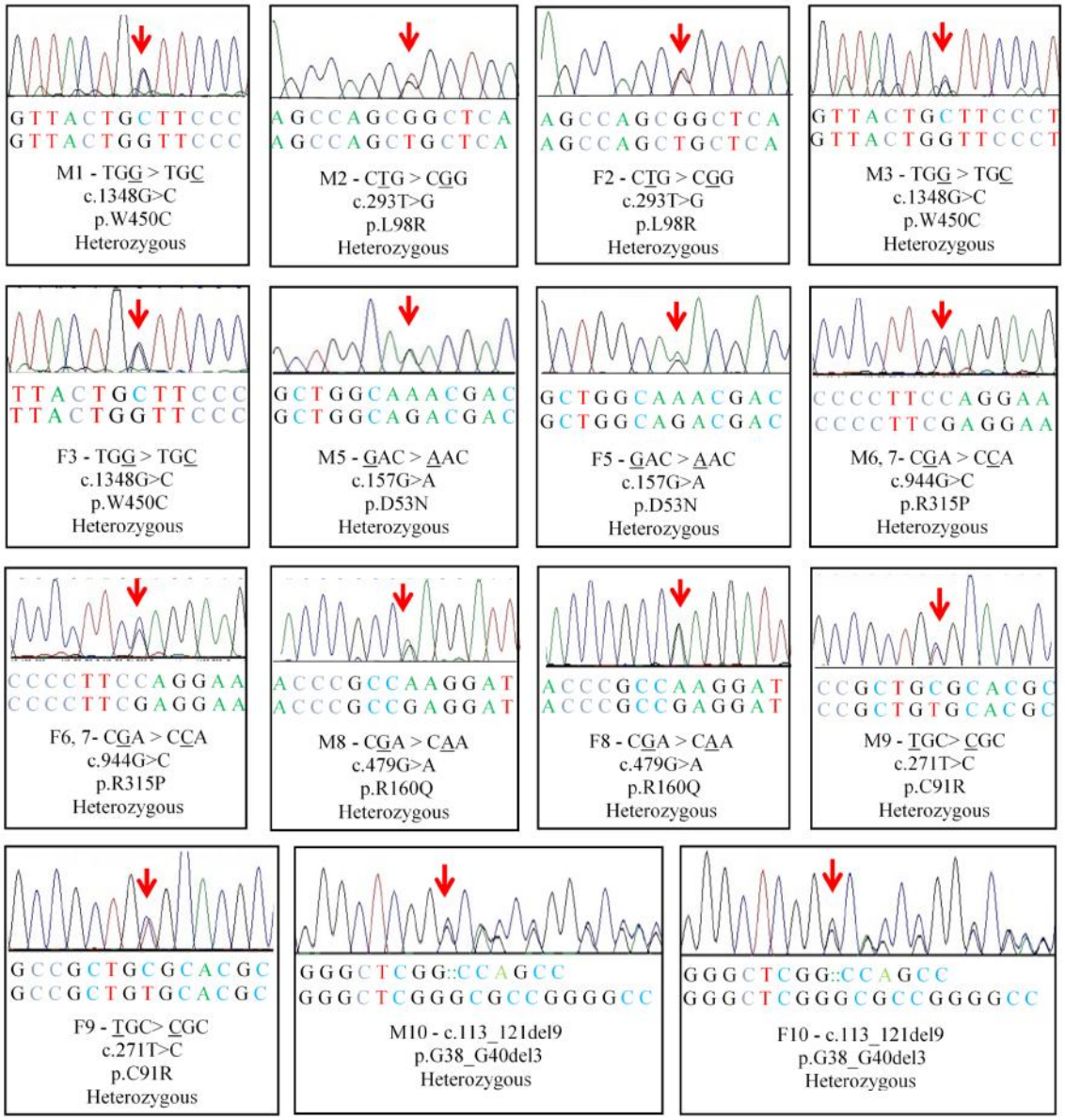
[2] Disease severity for the patient with p.D53N mutation was not reported in original publication. In this study, the patient with this mutation had “moderate” disease severity.

Fig. S1. Chromatograms of mutations identified in MPS VI patients (P1–P3, P5–P8, and P10).



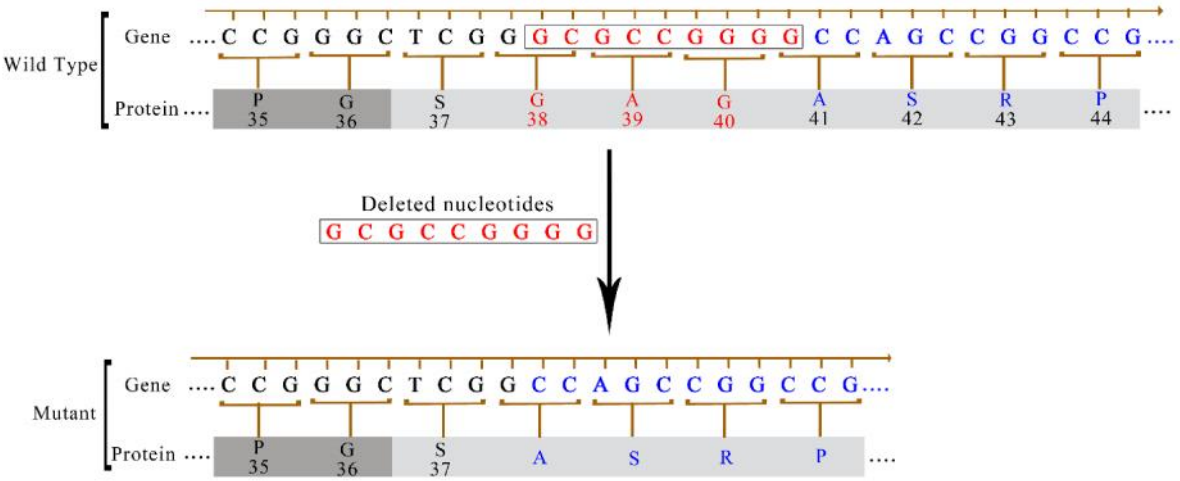
PT – Nucleotide sequence of *ARSB* from patients, WT – Nucleotide sequence of wild type *ARSB*. Red arrows indicate differences in nucleotides.

Fig. S2. Chromatograms of samples from parents of MPS VI patients.



M indicates sample from mother, F, sample from father.

Fig. S3. A representation of the p.G38_G40del3 mutation.



Deleted nucleotides and corresponding amino acid residues are shown in red. The region before the deletion is shown in black and that after the deletion in blue. Amino acid residues that correspond to the signal peptide are shaded in dark gray in the precursor. Amino acid residues that correspond to the mature protein are shaded in light gray. The position of each amino acid residue in the precursor is indicated below the residue.