Supplementary file

Novel subtype of mucopolysaccharidosis caused by arylsulfatase K (ARSK) deficiency

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Figure S1: The role of ARSK in heparan sulfate degradation as an example for GAG degradation

ARSK removes the 2-O-sulfate group from 2-sulfoglucuronate. This figure, showing GAG degradation with the example of heparan sulfate, is adapted with permission from Dhamale et al. (2017)[1], © 2017, American Chemical Society.

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ARSA     SLCTPSRAALLTGRLPV    83
ARSB     PLCTPSRSQLLTGRYQI    105
ARSC     PLCTPSRAAFMTGRYPV    89
ARSD     PLCTPSRAAFLTGRHSF    103
ARSE     SLCTPSRAAFLTGRYPV    100
ARSF     SLCSPSRSAFLTGRYPI    93
ARSG     STCSPSRASLLTGRLGL    98
ARSH     SMCTPSRAAFLTGRYPI    69
ARSI     PICTPSRSQLLTGRYQI    107
ARSJ     PICTPSRSQFITGKYQI    136
GALNS    PLCSPSRAALLTGRLPI    93
GNS      ALCCPSRASILTGKYPH    105
SGSH     SSCSPSRASLLTGLPQR    84
SULF1    PMCCPSRSSMLTGKYVH    101
SULF2    PMCCPSRSSILTGKYVH    102
IDS      AVCAPSRVSFLTGRRPD    98
ARSK     PICCPSRAAMWSGLFTH    94
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Figure S2: Conserved sulfatase signature

The arginine in the CxPxR-motif is unexceptionally conserved in all human sulfatases indicating its relevance in formylglycine modification and catalytic activity.

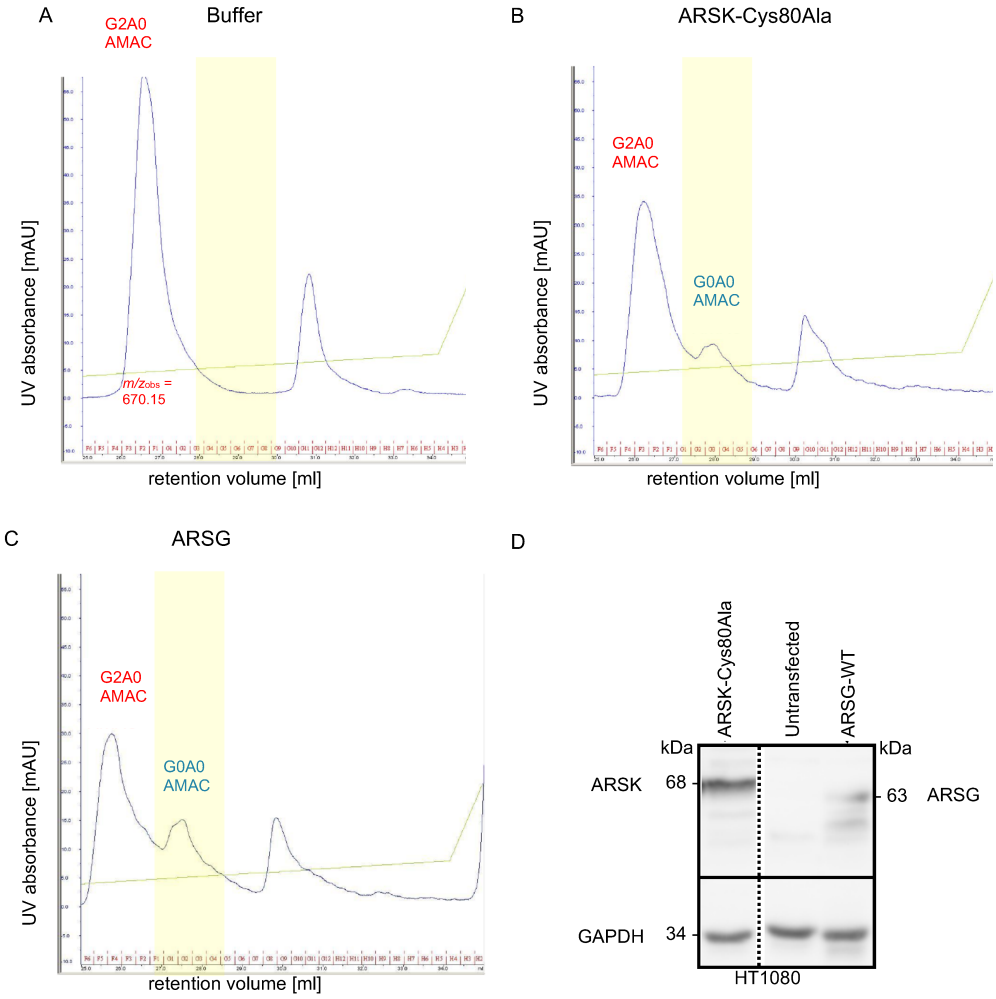

Figure S3: Functional consequences of ARSK variants - additional measurements

ARSK-Cys80Ala and ARSG did not desulfate the synthetic G2A0 disaccharides. Incubation of the AMAC-labeled G2A0 disaccharide with buffer only as control (a) resulted in a major peak of the unreacted educt (*m/z* 670.15) and ubiquitous peak at a retention volume > 30 ml). Incubation of AMAC-labeled G2A0 with ARSK-Cys80Ala (b) and ARSG (c) resulted in minor G0A0 product peaks, which might be attributed to the activity of endogenous HT1080 ARSK rather than to ectopic expression of ARSK-Cys80Ala or ARSG, respectively. (d) Western blot analysis: Expression of ARSK-Cys80Ala in HT1080 cell lysates. No expression in untransfected or ARSG-transfected cells. GAPDH was used as loading control.

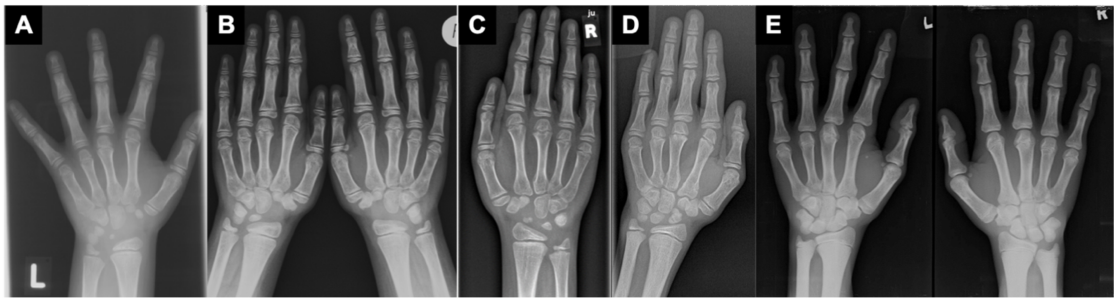

Figure S4: Progression of skeletal dysplasia – hands of S1-4 at different ages

Hand radiographs of affected individuals demonstrate short third, fourth and fifth metacarpals, small carpal bones and small epiphyses of lower ends of radius and ulna. S1 at 10 years (A) and 12 years (B), S2 at 14 years (C), S4 at 17 years (D), S3 at 18 years (E)

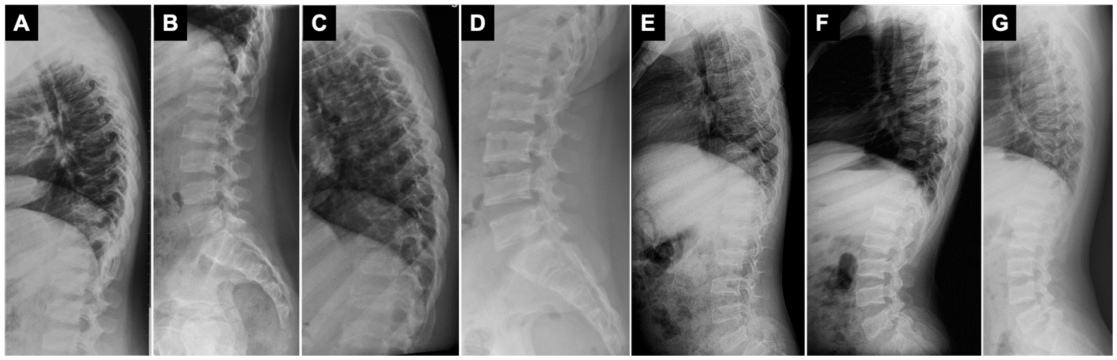

Figure S5: Progression of skeletal dysplasia – lateral spine of S1-4 at different ages

Radiographs of lateral spine illustrate platyspondyly, anterior beaking and irregular vertebral end plates. S1 at 12 years (A, B), S2 at 14 years (C), S1 at 17 years (D), S4 at 17 years (E), S3 at 18 years (F) and at 20 years (G)

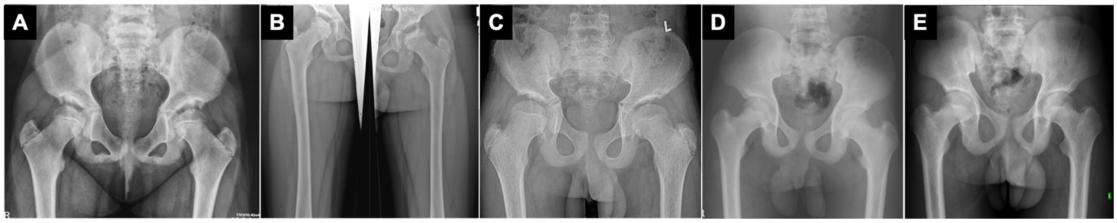

Figure S6: Progression of skeletal dysplasia – pelvis of S1-4 at different ages

Pelvic radiographs of affected individuals demonstrate irregular acetabular roofs, reduced hip joint space and small capital femoral epiphyses. S1 at 12 years (A), S2 at 14 years (B), S4 at 17 years (C), S3 at 18 years (D) and at 20 years (E)

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Table S1: Results of DMB urine electrophoresis in S1 (age 11 and 16 years) and S2 (age 14 years)

	DMB urine analysis		
	S1 (at 11 years)*1	**S1 (at 16 years)*2**	**S2 (at 14 years)*2**
GAG mg /creatinine mmol (DMB method)	8.9 mg/mmol	7 mg/mmol	18 mg/mmol
	expected <18 mg/mmol	expected ≤10 mg/mmol	expected ≤18 mg/mmol
Chondroitin sulfate	67%	78%	**93% ↑**
	expected 70-80%	expected 70-80%	expected 70-80%
Heparan sulfate	9%	22%	7%
	expected 20-30%	expected 20-30%	expected 20-30%
Keratan sulfate	**24%**	not detected	not detected
	expected undetectable	expected undetectable	expected undetectable

*1: analyses performed at the Center of Pathobiochemistry and Genetics, Medical University of Vienna, Austria
*2: analysis performed at the Laboratory of Metabolic Diseases, Department of Pediatrics and Adolescent Medicine, Medical University of Graz, Austria

Table S2: Results of LC-MS/MS analysis of urine and plasma in S1 (age 17 years) and S2 (age 15 years)

	LC-MS/MS urine analysis*3		**LC-MS/MS plasma analysis*3**	
	S1 (at 17 years)	**S2 (at 15 years)**	**S1 (at 17 years)**	**S2 (at 15 years)**
Dermatan sulfate	**165 µg/mmol creatinine ↑**	**234 µg/mmol creatinine ↑**	42 ng/ml	39 ng/ml
	expected 0-53 µg/mmol creatinine		average in reference samples: 34 ng/ml, range 8 - 131 ng/ml	
Heparan sulfate	230 µg/mmol creatinine	288 µg/mmol creatinine	131 ng/ml	146 ng/ml
	expected 0-323 µg/mmol creatinine		average in reference samples: 257.2 ng/ml, range 66 - 1346 ng/ml	
Keratan sulfate	60 µg/mmol creatinine	239 µg/mmol creatinine	839 ng/ml	2146 ng/ml
	expected 0-314 µg/mmol creatinine		average in reference samples: 1017.1 ng/ml, range 457 - 2442 ng/ml	
Total GAG content in plasma			1012 ng/ml	2330 ng/ml
			average in reference samples 1308.3 ng/ml, range 619 - 2691 ng/ml	

*3: analysis performed at the Laboratory Genetic Metabolic Diseases, Amsterdam UMC, University of Amsterdam, Departments of Clinical Chemistry and Pediatrics, Core Facility Metabolomics, Emma Children's Hospital, Amsterdam Gastroenterology Endocrinology Metabolism, Amsterdam, The Netherlands

Table S3: ARSK-associated skeletal phenotype

***ARSK*-associated skeletal phenotype in comparison to radiologic MPS-specific features**						
Radiologic MPS specific features[2-6]		**S1**	**S2**	**S3**	**S4**	**Subject specific details**
Skull	Thickened cortical bone of the calvaria	x	x	x	x	
Spine	Rounded or bullet-shape vertebral bodies	-	-	-	-	Irregular vertebral end plates
	Platyspondyly	x	x	x	x	
	Anterior inferior beaking of the thoracolumbar vertebrae	x	x	x	x	
	Posterior scalloping of the vertebral bodies.	x	x	x	x	
	Thoracolumbar junction kyphosis	-	-	-	-	
	Kyphosis with gibbus	-	-	-	-	
	Hyperlordosis	-	-	x	x	
	Scoliosis	-	x	-	x	
	Spinal cord compression	-	-	-	-	
Hip/pelvis	Poor development of acetabulum and medial proportion of proximal femoral epiphysis	x	x	x	x	Irregular acetabular roofs, reduced hip joint space
	Severe hip dysplasia	x	x	x	x	
Hands/feet	Carpal and tarsal bones are hypoplastic and irregularly shaped	x	x	x	x	
	Metacarpal bones are proximally pointed, shortened and thickened	x	x	x	x	Short third, fourth and fifth metacarpals
Long bones	Hypoplastic and thinned epiphyses cortically with osteoporosis	x	x	x	x	Small epiphyses of lower ends of radius, ulna and capital femur
		x	x	x	x	Metaphyseal striae
Chest/ribs	Abnormally shaped ribs, spatulate ribs, broadening of clavicles and ribs	x	x	x	x	

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