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

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ABSTRACT

Background Mucopolysaccharidoses (MPS) are monogenic metabolic disorders that significantly affect the skeleton. Eleven enzyme defects in the lysosomal degradation of glycosaminoglycans (GAGs) have been assigned to the known MPS subtypes (I–IX). Arylsulfatase K (ARSK) is a recently characterised lysosomal hydrolase involved in GAG degradation that removes the 2-0-sulfate group from 2-sulfoglucuronate. Knockout of Arsk in mice was consistent with mild storage pathology, but no human phenotype has yet been described.

Methods In this study, we report four affected individuals of two unrelated consanguineous families with homozygous variants c.250C>T, p.(Arg84Cys) and c.560T>A, p.(Leu187Ter) in ARSK, respectively. Functional consequences of the two ARSK variants were assessed by mutation-specific ARSK constructs derived by site-directed mutagenesis, which were ectopically expressed in HT1080 cells. Urinary GAG excretion was analysed by dimethylene blue and electrophoresis, as well as liquid chromatography/mass spectrometry (LC-MS)/MS analysis.

Results The phenotypes of the affected individuals include MPS features, such as short stature, coarse facial features and dysostosis multiplex. Reverse phenotyping in two of the four individuals revealed additional cardiac and ophthalmological abnormalities. Mild elevation of dermatan sulfate was detected in the two subjects investigated by LC-MS/MS. Human HT1080 cells expressing the ARSK-Leu187Ter construct exhibited absent protein levels by western blot, and cells with the ARSK-Arg84Cys construct showed markedly reduced enzyme activity in an ARSK-specific enzymatic assay against 2-O-sulfoglucuronate-containing disaccharides as analysed by C18-reversed-phase chromatography followed by MS.

Conclusion Our work provides a detailed clinical and molecular characterisation of a novel subtype of mucopolysaccharidosis, which we suggest to designate subtype X.

INTRODUCTION

Glycosaminoglycans (GAGs) are essential components of the connective tissue. They consist of disaccharide units, each composed of a uronic acid (glucuronate or iduronate) and an N-acetylhexosamine (glucosamine or galactosamine), which are further modified by N-sulfation and O-sulfation. Mucopolysaccharidoses (MPS) result from enzymatic defects in the stepwise degradation of sulfated GAGs, leading to lysosomal storage of heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), or their degradation intermediates.1 This typically results in multisystem disorders with variable manifestations including skeletal dysplasia (earlier termed ‘dysostosis multiplex’), short trunk disproportionate short stature, coarse facial features, corneal and lens opacity, retinopathy, hearing loss, valvular heart disease, cardiomyopathy, hepatosplenomegaly and, in some cases also neurodegeneration (OMIM: PS607014). Up to now, eleven different enzymes have been linked to MPS. Classification into different MPS subtypes was made clinically, depending on the phenotype and affected organ systems, followed by the assignment to specific enzyme defects. There is considerable overlap of the skeletal MPS phenotype with other disorders affecting the skeletal system, 461 of which have been summarised in the Nosology and Classification of Genetic Skeletal Disorders in 2019.2 As elevated urinary GAG excretion is a diagnostic hallmark of MPS, total GAG analysis using dimethylmethane blue (DMB) or uronic acid-based spectrophotometry and quantification of DS, HS and KS by liquid chromatography (LC) are commonly used methods for MPS screening in urine. Leucocyte enzyme activity assays and genetic testing can be used for specification and confirmation of respective MPS subtypes.13

Among other enzymes, several sulfatases are involved in the degradation of GAGs.4 The human lysosomal arylsulfatase K (ARSK (OMIM:*610011)) was identified by computational analysis of the human genome, based on its sulfatase-specific amino acid sequence at the catalytic site (sulfatase signature CXPSR).5 As in all human sulfatases, the conserved cysteine within this sequence undergoes post-translational modification and is converted into an α-formylglycine residue, which is essential for the enzyme activity of all human sulfatases.6 ARSK was found to remove the 2-O-sulfate group from 2-sulfoglucuronate, which led to its designation ‘glucuronate-2-sulfatase’ (GDS). Glucuronate-2-O-sulfation occurs in HS, DS and KS and, during
degradation, is selectively removed by ARSK (online supplemental figure S1). As sulfatas show no functional overlap and high substrate specificity, ARSK deficiency was discussed as a possible cause of a yet unidentified MPS subtype.

A recently published Arsk-deficient mouse model showed a mild lysosomal storage phenotype. Glycan reductive isotope labelling-LC/mass spectrometry (MS) revealed HS and CS accumulation in various tissues. Otherwise, the mouse model was characterised by a slight but non-significant elevation of urinary GAG excretion in the absence of skeletal or neurological abnormalities, however, a mild behavioural phenotype was observed.

Hence, although experimental data suggested an association of biallelic Arsk-variants with an MPS phenotype in mice, no association has yet been established in humans. This is the first report with a comprehensive phenotypical evaluation of four individuals from two unrelated families with ARSK (GDS) deficiency.

**MATERIAL AND METHODS**

**Subjects and enrolment**

In both families, parents were consanguineous and had two affected children with skeletal dysplasia, resembling spondyloepiphyseal dysplasia. The first, female child of family 1 was designated as subject 1 (S1), the second, male child as subject 2 (S2). In family 2, the first two children were unaffected. The third and the fourth male children were indicated as subjects 3 (S3) and 4 (S4), respectively.

Family 1 was referred for genetic analysis from a specialised orthopaedic centre in Austria. Following the identification of ARSK as a candidate gene in family 1, a search for additional affected individuals was carried out using GeneMatcher, which identified the second family in India.

Written informed consent for publication was obtained from both families. Explicit permission to publish photographs was obtained for S1 and S2.

**Clinical investigations**

Medical history and family history were taken during an onsite visit in all 4 subjects (S1–S4).

Reverse phenotyping was performed in subjects 1 and 2 after identifying the ARSK variant to search for further symptoms of MPS, including slit-lamp examination and fundoscopy, audiometry, cardiac and abdominal ultrasound, neurological examination, and dermatological assessment. Subjects 3 and 4 have had a thorough assessment of their skeletal phenotype, but no complementary investigations were possible as the patients were not available for follow-up examinations.

**Laboratory investigations**

A peripheral blood smear was performed with Giemsa staining and was investigated using light microscopy.

GAG excretion in urine (24-hour collection and random sample) was analysed quantitatively with uronic acid and DMB in S1, respectively, and DMB in S2. High-resolution electrophoresis of urinary GAG excretion was performed using standard methods.

Urine and plasma GAGs were measured using a multiplex assay with enzymatic digestion of HS, DS and KS followed by quantification of specific disaccharides by LC-MS/MS as described previously.

**Genetic analysis**

In family 1 exome sequencing was performed in S1, using DNA from leucocytes in a diagnostic setting. DNA was extracted using the QIASymphony DSP DNA Midi Kit on a QIASymphony SP instrument (QIAGEN, Hilden, Germany). Nextera DNA Flex Library Prep Kit was used for library preparation and sequencing was performed on a NextSeq 550 (Illumina, San Diego, California, USA). Sequence alignment of raw fastq files to the human reference sequence (GRCh37/hg19 assembly) and variant calling was performed with the DRAGEN Germline Pipeline V3.2.8 on Illumina BaseSpace (https://basespace.illumina.com/). Variant annotation, filtering and prioritisation using human phenotype ontology (HPO) terms (spondylo- or heterophyseal dysplasia HP:0002657 and disproportionate short-trunk short stature HP:0003521) was performed using VarSeq V2.2 (Golden Helix, Bozeman, Montana, USA, www.goldenhelix.com). MPS-associated genes (GALNS [OMIM:*612 222], GLB1 [OMIM:*611 458], GNS [OMIM:* 607 664], GUSB [OMIM:*611 499], HYAL1 [OMIM:*607 071], IDUA [OMIM:* 252 800], ARSB [OMIM:*611 542], HGSNAT [OMIM:* 405 653], NAGLU [OMIM:*609 701], SGSH [OMIM:* 605 270], IDS [OMIM:*300 823]) were analysed separately in S1 to exclude a known MPS form. Sanger sequencing was performed for segregation analysis of the ARSK variant in S2 and on the patients’ parents.

In family 2 exome sequencing of the probands and two unaffected siblings was performed. Genomic DNA was extracted using the standard phenol-chloroform method. Exome was captured using Illumina’s Nextera Rapid Capture Kit followed by massively parallel sequencing using the NextSeq500 Sequencer (Illumina, San Diego, California, USA) with a targeted average coverage depth of 100×. An inhouse pipeline with integrated burrows-wheeler aligner 1 and genome analysis toolkit V3.6 best practices2 was used for data analysis. Variants were annotated using ANNOVAR3 and allele frequencies derived from 983 in-house exomes of Indian ethnicity, OMIM phenotypes and HPO terms were integrated in the annotation with the help of inhouse Perl scripts. Region of homozygosity (ROH) analysis was done with the help of FILTER tool4 using exome data of affected and unaffected siblings. As the family has two affected siblings and reported consanguinity, a pathogenic variant was assumed in the shared homozygous region. The ARSK variant was validated by Sanger sequencing.

**Generation of ARSK and ARSG constructs**

Different ARSK constructs were generated to prove that the detected variant in family 1, NM_198150.2:c.250C>T, p.(Arg84Cys), results in reduced enzymatic function with a specific enzymic assay.

Wild-type-ARSK (ARSK-WT) and ARSK-Cys80Ala constructs were used for comparison of enzymatic function, as normal function was defined by the WT-construct and loss of sulfatase activity had previously been shown for the Cys80Ala construct. An additional construct with the enzyme arylsulfatase G (ARSG [OMIM:* 610 008], another human sulfatase, was used to show substrate specificity of ARSK. All constructs used in this study include a C-terminal histidine-tag (RGSHHHHHH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-HH-H
**Transfection and immunoblotting**

ARSK-WT, ARSK-Arg84Cys, ARSK-Leu187Ter, ARSK-Cys80Ala and ARSG (N-sulfoglucosamine 3-O-sulfatase) were transiently expressed in HT1080 cells by polyethylenimine (PEI) transfection protocol. Cells were harvested 48 hours after transfection and lysed in phosphate-buffered saline (PBS)/0.5% TX100, sonication was performed on ice (3×10 s), and homogenates were obtained by centrifugation (15000 g, 4°C). Protein determination was performed by detergent compatible (DC) assay (BioRad). Homogenates (50 µg of total protein) were analysed by immuno-noblotting on polyvinylidene difluoride (PVDF) membrane and antibodies directed against ARSK (biorbyt 160024), ARSG (biorbyt 318995) and GAPDH (Santa Cruz sc-25778, FL-335) as loading control.

**Glucuronate-2-sulfatase activity assay**

The disaccharide 2-sulfoglucuronate-N-acetyl-glucosamine (G2A0) was prelabelled with the fluorescent dye 2-aminoacridone (AMAC).10 AMAC-labelled G2A0 (12,5 nmol) was incubated with 100 µg of protein of the appropriate homogenates in a final volume of 52,5 µl in 250 mM ammonium-acetate buffer pH 4.6 for 24 hours at 37°C. After centrifugation (15000 g, 4°C), the samples were analysed by C18-reversed-phase (RP)-chromatography in ammonium acetate buffer (60 mM, pH 5.6) with a flow rate of 1 mL/min with the Ettan LC system (GE Healthcare). The saccharides were eluted and fractionated with an acetoneitrile gradient, in which AMAC-labelled disaccharides were detected by ultraviolet (UV) absorbance at 255 nm. The fractions containing AMAC-mediated fluorescence peaks were vacuum dried, resolved in 25 µl acetoniitride and analysed by nano-electrospray ionization mass spectrometry (ESI-MS) in a negative ion mode.

**RESULTS**

**Biallelic ARSK variants cause a novel MPS phenotype**

Genetic testing was performed in S1–S4 between the ages of 14 years and 20 years. Until then, their skeletal dysplasia has not been assigned to a specific genetic disorder. The main radiological abnormalities in all four subjects were thickened calvaria, platyspondyly, anterior inferior beaking of the thoracolumbar vertebrae, broadening of clavicles and ribs, narrowing of lower parts of iliac bones, small epiphyses, metaphysial striae, and hypoplastic carpal bones (online supplemental table S3, figures 1 and 2). Cranial MRI performed due to headache in S1 at 12 years (I). S1–S4, subjects 1–4.

![Figure 1: Phenotype of skull, thorax and spine. Lateral skull radiographs show mildly thickened calvarias of S1 (A, B) and S2 (C–D). S1 and S2 show an open bite (A, C). Radiographs of chest and spine show broad (oar-shaped) ribs (S1 at 17 years (E, F) and S2 at 14 years (H)), broad vertebral bodies, platyspondyly and irregular endplates (S1 at 17 years (G), S3 at 18 years (J)). S1 shows a bell-shaped thorax (E). Radiographs of lateral spine additionally illustrate anterior beaking and irregular vertebral end plates (J). Lateral spine radiograph of the lumbar area shows platyspondyly with scalloping of the posterior end plates (S1 at 12 years (G)). Anterior inferior beaking of the cervical spine of S3 at 20 years (l). S1–S4, subjects 1–4.](http://jmg.bmj.com/)

regurgitation. S1 also showed mild ventricular hypertrophy suggesting mild storage cardiomyopathy, a reduced distensibility of the aorta and an elevated stiffness, as additional known cardiac manifestations of MPS.

All four subjects of this study had normal behaviour and attended a regular school, S1 and S2 at A level. S3 and S4 were not available for follow-up examinations, therefore their behaviour and cognitive function in adulthood remains unknown.

Phenotypical features of S1 and S2 are shown in figure 3 and are summarised in table 1.

Reverse phenotyping in S1 and S2 clearly revealed an MPS phenotype with multisystem involvement. In these two subjects, coarse facial features, short trunk disproportionate short stature and short neck, genu valga, and hip pain were noticed at the end of the first decade. Furthermore, reverse phenotyping revealed mild opacity of the lens and vitreous body. The optical coherence tomography image of the retina of S1 showed loss of interdigitation zone temporal of the macula. Mild aortic valve stenosis and mild left ventricular hypertrophy were noted in cardiac ultrasound in S1 and S2.

Auditory tests revealed normal results in S1 and S2 at the age of 16 years and 14 years, respectively.

Early MPS-related symptoms like macrocephaly at birth (S1 and S2), recurrent ear infections (S2) and sleeping disorder (S2) were initially not attributed to an underlying genetic condition (see timeline figure 3J). Of note, head circumference in S3 and

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S4 was below the third percentile, proportionate to height at the ages of 18 years and 17 years, respectively.

Absence of vacuolisation in peripheral leucocytes
A peripheral blood smear in S1 and S2 did not reveal abnormal vacuolisation in leucocytes.

Urinary GAG excretion pattern and plasma GAG concentration
Urine analysis in S1 at 11 years of age revealed normal urinary GAG excretion but elevated CS and KS excretion (online supplemental table S1).

Measurement of urinary GAG excretion by LC-MS/MS analysis in S1 and S2 at the ages of 15 years and 17 years, respectively, showed a threefold and fourfold elevation of specific DS-derived disaccharides in a morning urine sample, respectively (online supplemental table S2), while all other specific GAG-derived disaccharides were in the normal range.

Enzyme activity of β-hexosaminidase, β-galaktosidase, arylsulfatase A, α-iduronidase, iduronat-2-S sulfatase, sulfamidase, acetylglucosaminidase, GlcNAc-transferase, GlcNAc-6-S-sulfatase, GalNAC-6-S-sulfatase, arylsulfatase B and β-glucuronidase was measured in fibroblasts of S2 and were all within normal range.

Identification of biallelic ARSK variants in four individuals with a skeletal disorder resembling spondyloepiphysial dysplasia
Exome analysis in S1 revealed a homozygous variant in ARSK, NM_198150.2:c.250C>T, p.(Arg84Cys), located within a 38.4 Mb sized ROH (GRCh37/hg19 Chr5:82940273–121330223). The variant was confirmed in a homozygous state in S2 and a heterozygous state in the patients’ parents (figure 3A).
Table 1 Genetic variants and phenotype of patients with ARSK deficiency

| Subject 1 (family 1) | Subject 2 (family 1) | Subject 3 (family 2) | Subject 4 (family 2) |
|----------------------|----------------------|----------------------|----------------------|
| **Variant in ARSK gene (NM_198150.2)** | c.250C>T, p.(Arg84Cys), homozygous | c.250C>T, p.(Arg84Cys), homozygous | c.560T>A, p.(Leu187Ter), homozygous |
| **Ethnicity** | Turkish | Turkish | Indian |
| **Last examination (age in years, sex)** | 16 y, female | 14 y, male | 18 y, male |
| **Previous suspected diagnoses** | MPS, spondyloepiphysial dysplasia, Turner syndrome | MPS, spondyloepiphysial dysplasia | Brachymelia, MPS, spondyloepiphysial dysplasia |
| **Height** | 146.5 cm, –3.25 SD | 150 cm, –1.94 SD | 157 cm, –3.22 SD |
| **Weight** | 68 kg | 49 kg | n.a. |
| **Head circumference** | 59 cm, 2.52 SD | 58.5 cm, 1.95 SD | 53 cm, –2.21 SD |
| **Arm span** | 150.5 cm | 155 cm | 164 cm |
| **Length upper/lower body segment** | 71.5 cm / 75 cm | 70 cm / 80 cm | 73 cm / 84 cm |
| **Facial phenotype** | Coarse facial features | Coarse facial features | Coarse facial features, long philtrum, broad nasal root |
| **Eyes** | Mild myopia since 14th year of life, mild lens and vitreous opacity, mild retinal pigmentation temporal of the fovea | Mild lens and vitreous opacity, mild retinal pigmentation temporal of the fovea | Normal |
| **Auditory system** | Normal audiogram and tympanogram | Normal audiogram and tympanogram | Normal |
| **Jaw and teeth** | Open bite, wide spaced teeth, diastemata, canine-like appearance of lateral incisors | Open bite, wide spaced teeth, canine-like appearance of lateral incisors | Normal |
| **Hands/wrist** | Hands normal | Hands normal, intermittent paresthesia | Normal |
| **Skeletal features** | Disproportionate short-trunk short stature, genu valgus, mild scoliosis | Disproportionate short-trunk short stature, mild genu valgus | Disproportionate short-trunk short stature, genu valgus |
| **Liver, spleen** | Normal in size and structure (ultrasound examination) | Normal in size and structure (ultrasound examination) | Normal on clinical examination |
| **Kidneys** | Normal in size and structure (ultrasound examination) | Normal in size and structure (ultrasound examination) | n.a. |
| **Heart** | Systolic murmur, mild aortic valve stenosis and regurgitation, thickened ends of aortic cusps, mild left ventricular hypertrophy | Systolic and diastolic murmur, mild aortic valve stenosis and regurgitation, thickened ends of aortic cusps | Normal on clinical examination |
| **Neurological examination, cognition** | Normal | Normal | Normal |

ARSK, arylsulfatase K; MPS, mucopolysaccharidosis; n.a., not available; y, years.

Targeted variant analysis of known MPS-associated genes in the exome data set of S1 did not reveal pathogenic or likely pathogenic variants in GALNS, GLB1, GNS, GUSB, HYAL1, IDUA, ARSB, HGSNAT, NAGLU, SGSH and IDS.

Exome analysis in family 2 revealed a homozygous nonsense-mutation in ARSK, NM_198150.2:c.560T>A, p.(Leu187Ter), in S3 and S4, located within a ROH of 14 Mb (GRCh37/hg19 Chr5:81721372–95865500). The parents and the unaffected brother were found to be heterozygotes. The unaffected sister did not carry this variant (figure 3B). Both variants have not been reported in variant databases such as ClinVar and Human Gene Mutation Database (HGMD). The detected missense variant c.250C>T, p.(Arg84Cys), results from a C to T substitution, replacing the highly conserved arginine at codon 250 with cysteine, an amino acid with highly different physicochemical properties. The variant affects the sulfatase signature (CXPSR) within the N-terminal sulfatase domain of the protein, which is essential for forming the catalytic site of sulfatases. In gnomAD, this variant is listed with a total allele frequency of 0.002129% (6/281810 alleles). The variant c.560T>A, p.(Leu187Ter), is listed with a total allele frequency of 0.0003980% (1/251248 alleles). Of note, no individuals with homozygous loss-of-function variants are represented in gnomAD.

Affected individuals’ ARSK variants cause ARSK deficiency

The arginine at amino acid position 84 in ARSK is part of the highly conserved sulfatase signature 80-CCPSR (6/281810 alleles). The variant c.560T>A, p.(Leu187Ter), is listed with a total allele frequency of 0.0003980% (1/251248 alleles). Of note, no individuals with homozygous loss-of-function variants are represented in gnomAD.

Ectopic expression of the constructs in HT1080 cell lysates revealed comparable expression levels for ARSK-WT, ARSK-Arg84Cys, slightly lower amounts of ARSK-Cys80Ala and lack of protein formation for ARSK-Leu187Ter in western blot analyses, using an ARSK-specific antibody (figure 4D, online supplemental figure S3D). This indicated comparable stability.
of ARSK-WT and ARSK-Arg84Cys, probable lower stability of ARSK-Cys80Ala, and was compatible with nonsense-mediated mRNA decay as a consequence of the Leu187Ter variant. ARSK-WT-HT1080, ARSK-Arg84Cys-HT1080 and ARSK-Cys80Ala-HT1080 cell lysates only revealed moderate differences in total acid sulfatase activity using the artificial pseudo-substrate p-nitrophenyl sulfate at acidic pH, indicating a high background of the entire set of endogenous lysosomal and non-lysosomal sulfatases (data not shown).

Therefore, we used the specific ARSK disaccharide substrate G2A0 prelabelled with the fluorescent dye AMAC at the reducing end of the N-acetylglucosamine residue, so that the non-reducing end of the disaccharide was still accessible for enzymatic degradation. After expressing the different ARSK constructs and the ARSG construct, respectively, HT1080 cell lysates were incubated with AMAC-labelled G2A0 and were subsequently analysed by RP HPLC fractionation. Incubation of the AMAC-labelled G2A0 substrate (26 mL retention volume, buffer control) with ARSK-WT homogenates resulted in a main AMAC-mediated detection peak with a retention volume of 28 mL indicating an efficient 2-O-desulfation of the disaccharide leading to the desulfated G0A0 product (figure 4A).

However, most other samples (except the buffer sample) including the Cys80Ala variant and the ARSG (N-sulfoglucosamine 3-O-sulfatase) also showed a minor peak at 28 mL (online supplemental figure S3B,C), which was most likely due to endogenous ARSK activity in HT1080 cells rather than residual ARSK activity of ARSK-Arg84Cys and ARSK-Cys80Ala, respectively. MS analysis of indicated peak fractions (figure 4C, online supplemental figure S3; m/z values) containing AMAC-labelled disaccharides revealed expected masses of m/z 670.15 for the 2-O-desulfated disaccharide educt peaks and m/z values of 590.19 for the desulfated product within the peaks shifted towards a higher retention volume (figure 4C, online supplemental figure S3).

These data clearly demonstrate that the human ARSK-Arg84Cys variant, similar to the previously published ARSK-Cys80Ala construct, is not able to efficiently desulfate the glucuronate-2-O-sulfated disaccharide. Exchange of cysteine and arginine at positions 80 and 84, respectively, leads to strongly reduced sulfatase activity.

DISCUSSION

In this study, we report the identification of biallelic ARSK variants as the underlying cause of a novel subtype of MPS in four individuals of two unrelated families. Affected individuals had skeletal abnormalities and coarse facial features suggestive for MPS, while multisystem involvement with cardiac and ophthalmo-mological findings was only identified by targeted reverse phenotyping.

We demonstrate that the ARSK variant c.250C>T, p.(Arg84Cys) in family 1 leads to ARSK deficiency, proven by a functional GDS assay, whereas the variant c.560T>A, p.(Leu187Ter) in family 2 results in the absence of any ARSK-specific signal in western blot analysis, most probably due to nonsense-mediated mRNA decay.

The effect of ARSK enzymatic deficiency on GAG storage in vivo was recently shown in an Arsk knockout mouse model.20 2-O-sulfated HS was increased in isolated liver lysosomes of Arsk KO mice,20 while no evidence of storage pathology was noted in liver, spleen and kidney of S1–S2 by ultrasound examination. Arsk-deficient mice had a normal skeletal morphology, whereas skeletal abnormalities are the main feature in the human subjects of this study. Although the delineated phenotype in mice is milder and not directly comparable to the human phenotype assessed in this study, the mouse model provides additional evidence that ARSK deficiency leads to a novel subtype of MPS.20

Detailed history and reverse phenotyping of two of the affected individuals (S1 and S2) revealed recurrent bilateral ear infections and sleep disturbance, as noted in 80%–90% of children affected by MPS (type I, II, III, IV).17 Assessment for...
known ophthalmological features of MPS revealed mild lens and vitreous opacity and mild pigmentary changes of the retina in S1 and S2. The heart abnormalities which were found in S1 and S2 are in accordance with those typically observed in MPS, like cardiac murmur (aortic or mitral), valve disease, ventricular hypertrophy, thickened leaflets, and regurgitation, reduced distensibility of the aorta and elevated stiffness.  

In S1 total urinary GAG excretion was normal at 11 years of age, which could be due to a decline of urinary GAG excretion with age.  

Electrophoresis in S1 showed elevated excretion of CS and KS, pointing to a possible diagnosis of MPS IV, but enzyme analysis of galactosamine-6-sulfatase (Morquio A) and β-galactosidase (Morquio B) was normal. Repeated GAG analysis in S1 and S2 at age 16 years and 14 years, respectively, revealed normal results. In addition, the application of the recently developed LC-MS/MS method for the analysis of specific GAG-derived disaccharides revealed a sensitivity for the DMB method of only 90%, underpinning the risk of false-negative results for MPS screening by DMB.  

Urinary GAG analysis by LC-MS/MS in S1 and S2 at 17 years and 15 years of age, respectively, revealed a threefold and fourfold increase of specific DS-derived disaccharides. This is in line with the higher sensitivity of the LC-MS/MS method. The elevated proportion of CS (and KS) detected by electrophoresis in S1 at age 11 years and S2 at age 14 years, respectively, could probably be explained by the overlapping band of accumulated sulfated DS with CS and KS, while no unsulfated DS band was visible on either sample. We speculate that urinary GAG screening by DMB may fail to detect ARSK-associated MPS, which probably contributed to the late identification of this MPS type. Further studies with DMB and electrophoresis, as well as the detection of specific GAG-derived disaccharides by LC-MS/MS, are needed to characterise this novel MPS subtype at the level of accumulating compounds.

In conclusion, the results of this study establish a novel enzyme defect in the group of MPS. ARSK deficiency caused by biallelic loss-of-function mutations or missense mutations affecting the sulfatase signature is associated with childhood-onset disproportionate short-trunk short stature, and skeletal, cardiac and ophthalmological abnormalities. ARSK deficiency should be considered in patients with unclarified skeletal dysplasia or clinically suspected but unassigned MPS, even in the presence of normal urinary GaG excretion. We suggest designating ARSK deficiency as novel subtype X within the group of MPS.

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