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A prospective study of brachytelephalangic chondrodysplasia punctata: identification of arylsulfatase E mutations, functional analysis of novel missense alleles, and determination of potential phenocopies

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Purpose: The only known genetic cause of brachytelephalangic chondrodysplasia punctata is X-linked chondrodysplasia punctata 1 (CDPX1), which results from a deficiency of arylsulfatase E (*ARSE*). Historically, *ARSE* mutations have been identified in only 50% of male patients, and it was proposed that the remainder might represent phenocopies due to maternal–fetal vitamin K deficiency and maternal autoimmune diseases.

Methods: To further evaluate causes of brachytelephalangic chondrodysplasia punctata, we established a Collaboration Education and Test Translation program for CDPX1 from 2008 to 2010. Of the 29 male probands identified, 17 had *ARSE* mutations that included 10 novel missense alleles and one single-codon deletion. To determine pathogenicity of these and additional missense alleles, we transiently expressed them in COS cells and measured arylsulfatase E activity using the artificial substrate, 4-methylumbelliferyl sulfate. In addition, clinical data were collected to investigate maternal effects and genotype–phenotype correlations.

Results: In this study, 58% of males had *ARSE* mutations. All mutant alleles had negligible arylsulfatase E activity. There were no obvious genotype–phenotype correlations. Maternal etiologies were not reported in most patients.

Conclusion: CDPX1 is caused by loss of arylsulfatase E activity. Around 40% of male patients with brachytelephalangic chondrodysplasia punctata do not have detectable *ARSE* mutations or known maternal etiological factors. Improved understanding of arylsulfatase E function is predicted to illuminate other etiologies for brachytelephalangic chondrodysplasia punctata.

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Key Words: arylsulfatase E; brachytelephalangic chondrodysplasia punctata; CDPX1; maternal autoimmune disease; vitamin K

Brachytelephalangic chondrodysplasia punctata (BCP) describes a group of heterogeneous conditions with overlapping phenotypes. X-linked recessive BCP (CDPX1) (OMIM no. 302950), originally recognized by Sheffield et al.,[1] is a panethnic congenital rare disorder that affects males. The most characteristic clinical features are as follows: [2,3] (i) Chondrodysplasia punctata, or stippled epiphyses, observed on X-ray. These minimally involve the ankle and distal phalanges but can also include long bones, vertebrae, hips, costochondral junctions, hyoid bone, and tracheal and bronchial cartilage. Chondrodysplasia punctata can be observed initially in the second trimester of pregnancy by ultrasound but tends to improve or disappear by age 2–3 years. (ii) Brachytelephalangy, or shortening of the distal phalanges. A characteristic finding on X-ray is an inverted triangle appearance of the distal phalanges, with punctata on either side. (iii) Nasomaxillary hypoplasia, referring to absence or hypoplasia of the anterior nasal spine, causing a flat nasal base, reduced nasal tip protrusion, shortened columella, and vertical grooves within the alae nasi. (iv) Proportionate short stature. Another commonly observed characteristic is mixed conductive and sensorineural hearing loss. Most children have normal intellect and life span, but comorbidities can be present, including compression of the cervical spinal cord associated with cervical vertebral abnormalities or stenosis of the upper and lower airways that result from extensive calcifications within the tracheal and bronchial cartilage.[3,4] Intrafamilial differences in disease severity have also been documented.[3]

The only known genetic cause of CDPX1 is defects in arylsulfatase E (ARSE). The *ARSE* gene is located at Xp22.3, within a cluster of contiguous arylsulfatase genes that share high sequence homology, escape X inactivation, and have pseudogenes on the Y chromosome.[5] There are 17 sulfatases in the human genome; all share extensive sequence homology and contain a highly conserved cysteine that undergoes a unique posttranslational modification essential for their catalytic activity.[6–8] ARSE is localized to the Golgi membranes,[9] and its transcript has been identified in multiple tissues.[5] Its protein product is a 589-amino-acid and 60 kD precursor, which is subject

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to N-glycosylation at four potential sites (asparagine residues 58, 125, 258, and 344) to give a mature 68 kD form. Aside from the conserved active site domain, there are two predicted transmembrane domains, through which it is likely to be anchored to the Golgi cisternae. Its physiological substrate is unknown. Enzymatic assays to determine endogenous ARSE activity in fibroblasts have not been successful thus far because of interference with other highly homologous arylsulfatases.[9] The ability of ARSE to hydrolyze the fluorogenic 4-methylumbelliferyl (4MU) sulfate permits the use of this artificial substrate to measure its activity in an *in vitro* system.[5] Studies showed that the enzyme activity is optimum at neutral pH.[5,9]

By 2008, more than 140 male patients with a CDPX1 phenotype had been reported, and of these, around one-fourth were associated with Xp deletions or chromosomal rearrangements. Only 67 male probands had undergone *ARSE* mutation analysis, with mutations identified in 31.[3] Functional analysis was performed in 8 of 15 reported missense alleles (**Supplementary Table S1** online) by expressing the mutant *ARSE* complementary DNA in mammalian COS1 cells and measuring ARSE activity using 4MU sulfate.[5] In these experiments, all missense alleles had negligible activity.[9,10]

Considering the phenotypic resemblance of warfarin embryopathy in early gestation to BCP, Franco et al.[5] (1995) demonstrated that ARSE activity is inhibited in the presence of warfarin, an anticoagulant drug that decreases amounts of active vitamin K. These findings suggested that cases of BCP without *ARSE* mutations could be caused by inhibition of a normal ARSE enzyme in fetal development by reducing levels of vitamin K. Because placental transport of vitamin K is normally reduced,[11] vitamin K deficiency in the mother would be expected to result in greater vitamin K deficiency in the fetus. This hypothesis is attractive given that there have been several reports of BCP in offspring of both sexes in which gestational vitamin K deficiency was suspected, including mothers with severe hyperemesis gravidarum,[12] small-intestinal obstruction,[13] small-bowel syndrome,[14] pancreatitis,[15] or biliary lithiasis.[16] In those cases in which *ARSE* mutations were evaluated in their affected offspring, none were found.[3,13] Another group of children with BCP are born to mothers with autoimmune diseases.[17–19] It was proposed that maternal autoantibodies might perturb fetal vitamin K metabolism,[20] thereby reducing the etiology of all phenocopies to disturbances in vitamin K metabolism at a critical fetal time period. Although this hypothesis remains to be proven, it was not clear how many patients with BCP might be phenocopies due to maternal vitamin K deficiency states.

In 2008, through the Collaboration Education and Test Translation program (CETT),[21] sponsored by the National Institutes of Health Office of Rare Diseases Research, we established a pilot project to identify patients with CDPX1 and its phenocopies. The collaborative group included the clinical laboratory, which performed *ARSE* gene sequencing in patients referred with CDPX1 phenotypes and collected clinical information from the referring physician using a clinical data sheet designed for the CETT program for CDPX1 (**Supplementary Table S2** online). The research laboratory reviewed the clinical data sheets to evaluate genotype–phenotype correlations and performed functional analysis of the novel missense alleles identified. Because the majority of ARSE mutations identified thus far have been unique, functional analysis was important to demonstrate pathogenicity. This was also a unique opportunity to prospectively determine the incidence of CDPX1 phenocopies.

MATERIALS AND METHODS

CDPX1 phenotype

CDPX1 phenotype was defined as male sex, nasomaxillary hypoplasia, brachytelephalangy, and punctate calcifications on X-ray in children younger than 3 years.

Clinical data collection

The data sheet was designed to sample all the major features described in patients with BCP reported in the literature since 1978. In addition, it contained broad questions to identify other etiologies, including maternal vitamin K deficiency and autoimmune disease, that have been associated with BCP. The final data sheet was approved by the program staff and review board of the CETT program. Informed consent was obtained for the collection of clinical data, along with the consent for *ARSE* gene sequencing, according to the CETT program requirements. Referring physicians were asked to complete this form (shown in **Supplementary Table S2** online) at the time of sample submission. The clinical data sheets were deidentified and then

Table 1 Breakdown of patients and data sheets collected in this project

		NIH-CETT program (2008–2010)	Clinical data sheets obtained	Other referrals (2008–2010)	Clinical data sheets obtained
Male	*ARSE* mutation identified–CDPX1	17	13	3	2
	No *ARSE* mutation identified	12	6		
	Brother with CDPX1	2		1	
Female	No *ARSE* mutation identified	5			
	Carrier mother	6		3	
	Carrier relative	2			
	Noncarrier relative	1			

ARSE, arylsulfatase E; NIH CETT, National Institutes of Health Collaboration Education and Test Translation.

sent to the research laboratory for review. See **Table 1** for the breakdown of patients involved in this study.

Literature review

We searched PubMed from 1995 to 2010 to identify *ARSE* mutations in which functional analysis had not been done. Search terms used were CDPX1, ARSE, X-linked recessive CDP, and BCP.

Molecular analysis

All 11 exons and intronic flanking sequences of the *ARSE* gene were Sanger sequenced bidirectionally. In females, targeted array comparative genomic hybridization with exon-level resolution was performed concurrently with sequencing. Targeted mutation analysis was performed in affected male relatives of probands with *ARSE* mutations and in mothers and other female relatives to evaluate carrier status.

Mutant complementary DNA constructs

Wild-type *ARSE* complementary DNA (wt*ARSE*) was engineered in the mammalian expression vector, pALTER-MAX (Promega, Madison, WI) by inserting ARSE exon 2, obtained by RT-PCR from a liver RNA library, into the *ARSE* complementary DNA clone (GenBank: AA887688.1). It contained

Table 2 *ARSE* mutations detected in this study

	Exon	Allele	Predicted protein	Maternal heterozygote	Previously identified allele
P1	3	c. 126_128delTCT	Leu43del		Novel
P2	3	c. 139 G→A	D47N		Novel
P3	4	c. 217G→A	G73S	+	Novel
P4	4	c. 217G→A	G73S	+	
P5	4	c. 235 C→T	H79Y	+	Novel
P6	4	c. 268A→G	R90G		Novel
P7	4	c. 284 C→T	T95M	+	Novel
P8	5	c. 359 G→A	G120E		Novel
P9	6	c. 445G→T	G149C		Novel
P10	7	c. 916 A→G	T306A		Novel
P11	7	c. 949 G→A	G317R		Ref. 25[f]
P12[a]	9	c. 1130 G→A	G377E	+	Novel
P13	9	c. 1171G→A	G391R	+	Novel
P14[b]	10	c. 1300G→A	G434S	+	Novel
P15	10	c. 1387G→A	A463T		Novel
P16	11	c. 1442 C→T	T481M		Ref. 10
P17[c]	1–11	c. (?_67)_(1770_?)del	Null[e]	+	
P18[d]	1–11	c. (?_67)_(1770_?)del	Null[e]	+	
P19	1–11	c. (?_67)_(1770_?)del	Null[e]		
P20	1–11	c. (?_67)_(1770_?)del	Null[e]		
P21-32	1–11	No mutations found			
Previously reported alleles evaluated for ARSE activity in this study					
	3	c. 119 T→G	I40S	+	Ref. 3
	3	c. 169 G→A	G57S		Ref. 3
	5	c. 349 G→A	G117R		Ref. 5
	5	c. 410 G→C	G137A	+	Ref. 25
	7	c. 949 G→A	G317R	+	Ref. 25
	8	c. 1063 G→A	G355S		Ref. 26
	9	c. 1226 C→T	T409M	+	Ref. 3

ARSE, arylsulfatase E.

[a]This patient has a brother with similar features who was also found to have c. 1130 G→A. [b]This patient has a brother with similar features who was also found to have c. 1300G→A. [c]This patient has two sisters found to be heterozygous for complete deletion of *ARSE*. [d]This patient has a sister found to be heterozygous for complete deletion of *ARSE*. [e]Complete deletions of *ARSE* are reported in CDPX1; because the break points have not been determined, it is not known if these are identical. [f]This mutation was identified by Sheffield et al. (ref. 25) but there is no functional analysis information on it, indicated by a "?".

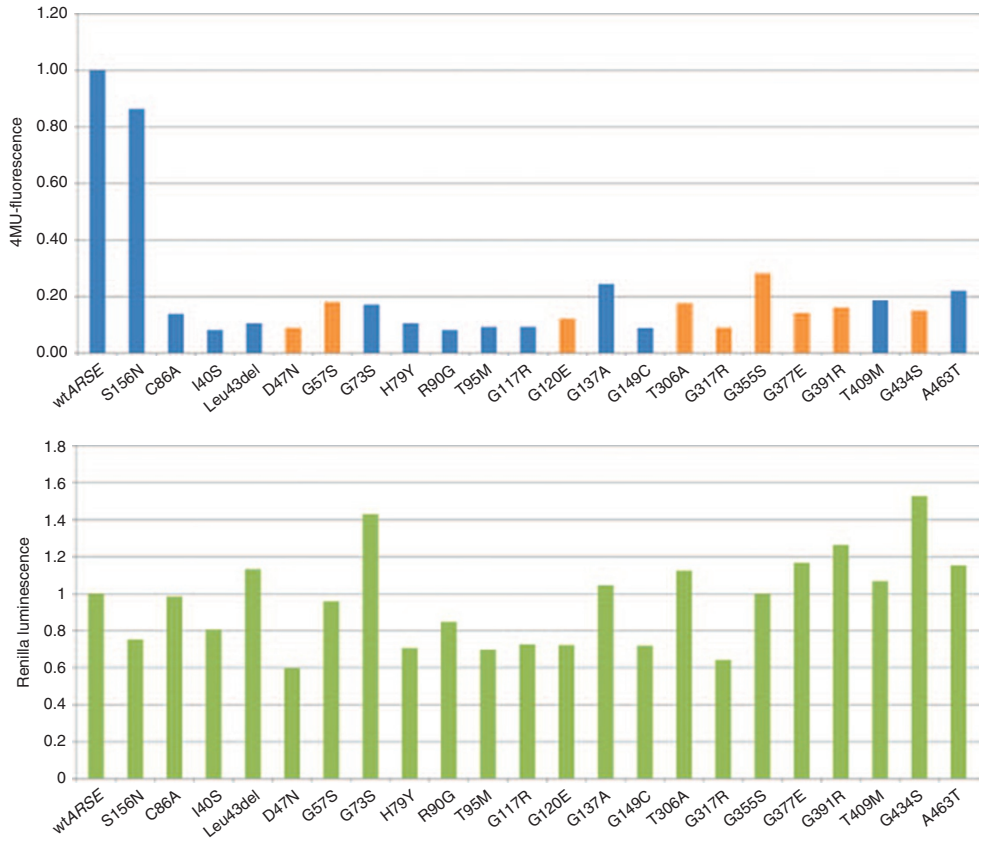

Figure 1 Activity of ARSE missense proteins. Top, mutations are listed on the X axis from N to C terminus of ARSE. C86A alters the putative active site of ARSE and was used as a negative control. S156N is a reported *ARSE* polymorphism. Missense proteins in orange were subsequently tested for residual activity. The Y axis represents the ratio of averaged fluorescence of the mutant proteins to wt*ARSE*. Bottom, expression of Renilla plasmid cotransfected with each ARSE mutant protein. Y axis shows measured Renilla luminescence units. Experiments represent three independent replications. ARSE, arylsulfatase E; wt*ARSE*, wild-type ARSE.

the open reading frame, bps 1–1856, including 86 nucleotides of the 3′ untranslated region. wt*ARSE* diverges from the reference sequence (NM_000047.2) by having the synonymous substitutions c.4T→C and c.1771A→G, and the polymorphisms reported in dbSNP: c.495 T→C, c.1270 G→A, and c.1692 C→T. Site-directed mutagenesis of wt*ARSE* was performed using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and resequenced after mutagenesis for confirmation. Primers used to generate *ARSE* mutations are listed in **Supplementary Table S3** online.

COS cell transfections

COS1 cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum at 37 °C and 5% CO_2. Overnight cultures were seeded at 10×10^5 cells per 21 cm^2 culture plate and cotransfected with 5 µg of *ARSE* and 2.5 µg of *Renilla reniformis* luciferase (Promega) plasmids using 15 µl of FuGENE HD (Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions. Transfections were performed in triplicate. Cells were harvested 48 h after transfection, washed with phosphate-buffered saline, trypsinized, and resuspended in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum, centrifuged at 1,000 rpm for 4 min and resuspended in 200 µl of lysis buffer: 0.1 mmol/l Tris HCl, 0.15 mol/l NaCl, and 1% Triton X-100 at pH 7.5. The lysate was incubated on ice for 20 min, then centrifuged at 20,800 rcf × 20 min at 4 °C. Supernatant was collected and protein concentration was measured by Bradford assay. For time course experiments, 0.5 µg of Renilla plasmid was used and lysis buffer was 0.05 mol/l Tris HCI at pH 7.5.

ARSE assay

The reported assay[5,12] was modified as follows: incubation mixture (100 µl) contained 20 µg of lysate and 0.2 mmol/l 4MU sulfate (Sigma-Aldrich, Saint Louis, MO) in 0.05 mol/l Tris HCI buffer (pH 7.5). Incubation was performed for 3 h at 37 °C in a 96-well plate; the reaction was stopped with 1.8 ml of glycine–carbonate buffer (pH 10.7). Fluorescence was determined (Wallac1420 Multilabel Counter, software version 3.00; PerkinElmer, Waltham, MA) at 365 nm (excitation) and 460 nm (emission). Each sample was run in triplicate and the experiment was repeated three times. Time course experiments on selected mutations were stopped at 0 h, 30 min, 1 h, 2 h, and 2 h 30 min. Renilla luminescence was measured by the Dual-Luciferase Assay and a GloMax 96 Microplate Luminometer (Promega).

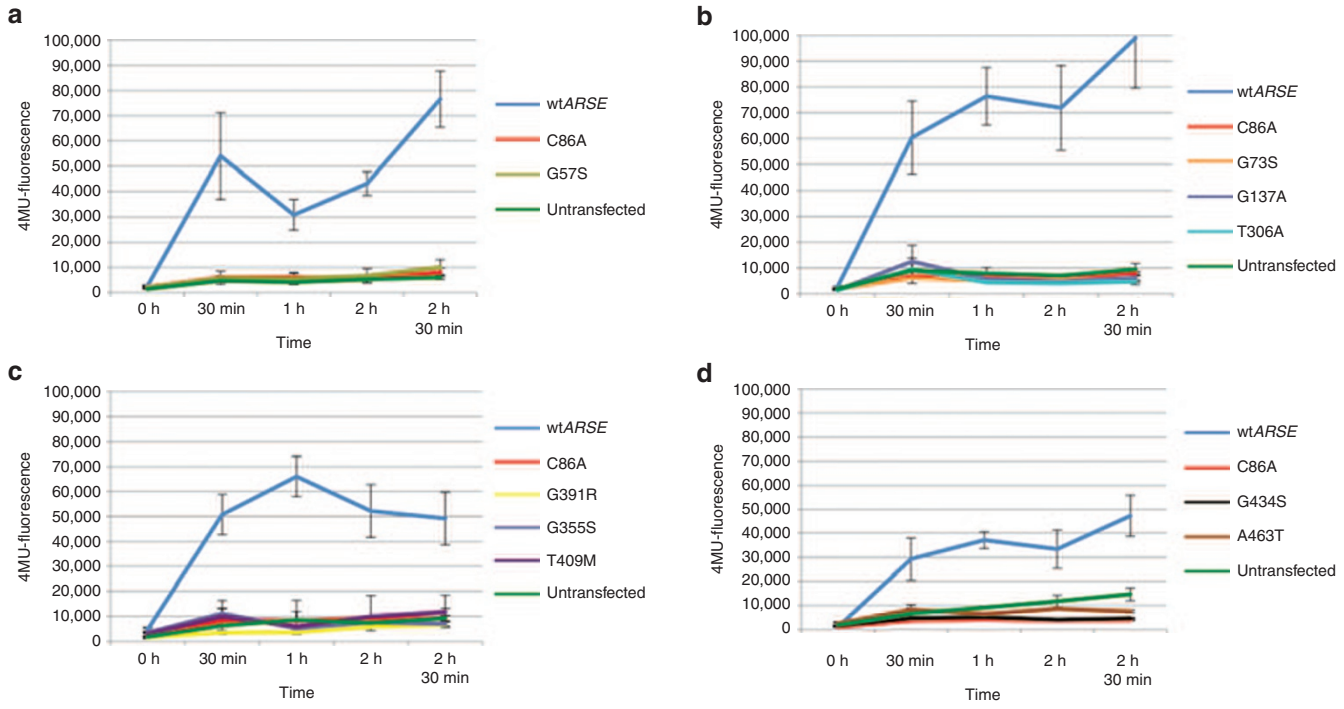

Figure 2 Time course experiments for selected alleles. (**a**) G57S; (**b**) G73S, G137A, T306A; (**c**) G391R, G355S, T409M; (**d**) G434S and A463T show negligible activity over time compared with wt*ARSE*. The Y axis represents 4-methylumbelliferyl-fluorescence (1.0s) (counts). Error bars represent the SD of the measurements. Experiments represent three independent replications. ARSE, arylsulfatase E; wt*ARSE*, wild-type ARSE.

RESULTS

Clinical and molecular analysis

Twenty nine male probands were referred prospectively through the CETT program, and clinical data sheets were collected on 19. Three additional male probands with *ARSE* missense alleles were contributed by the Molecular Genetics Laboratory of the Royal Devon and Exeter National Health Service Foundation Trust, and clinical data sheets were collected on two of them. From these 21 data sheets, the frequency of relevant clinical features in *ARSE* mutation-positive and mutation-negative probands was collated in **Supplementary Table S4a,b** online, respectively.

ARSE mutations were identified in 17/29 male probands from the CETT project and included four complete gene deletions, 1 single-codon deletion, and 12 missense mutations. The mutations identified are shown in **Table 2**. All missense alleles were predicted to be damaging by either PolyPhen[22] or SIFT[23] analysis, except for T306A, which was predicted to be benign. None of these mutations were polymorphisms identified in dbSNP (V132).[24] All residues affected by the mutations were evolutionarily conserved among all ARSE proteins and human arylsulfatate C (ARSC).

In addition, four females with clinical features of BCP were referred for *ARSE* mutation analysis. No mutations were found, and clinical data were obtained in only one patient. She had nasomaxillary hypoplasia, flattened nose, anteverted nostrils, brachytelephalangy, ichthyosis, delayed motor, and cognitive development. At the age of 11 months, there was no evidence of chondrodysplasia punctata, and her karyotype was normal. The distribution of all patients included in this project is shown in **Table 1**.

ARSE functional analysis

We performed functional analysis on the 12 novel missense alleles, the single codon deletion identified in our cohort, and 7 novel missense alleles reported previously in which functional analysis had not been done[3,5,25,26] (**Table 2**). These 20 *ARSE* alleles were engineered by site-directed mutagenesis and coexpressed in COS1 cells with Renilla luciferase to control for transfection efficiency. Whole-cell lysates were incubated with 4MU sulfate for 2 h, and ARSE activity was measured by detection of 4MU product. We found that all the mutant alleles had negligible ARSE activity, suggesting that they were pathological (**Figure 1**). However, we observed minimally increased fluorescence in some alleles, especially those located in the C-terminal region, suggesting potential residual activity. To determine if residual activity was present, time course experiments were performed for nine selected alleles. We found that none of these alleles showed an increase of ARSE activity over time as compared with wt*ARSE* (**Figure 2a–d**).

DISCUSSION

Spectrum of *ARSE* mutations

Twenty nine male probands with CDPX1 phenotype were referred through the CETT program. Molecular analysis identified *ARSE* mutations in 17, providing a mutation identification rate ~58%, similar to previous reports.[3,5,10]

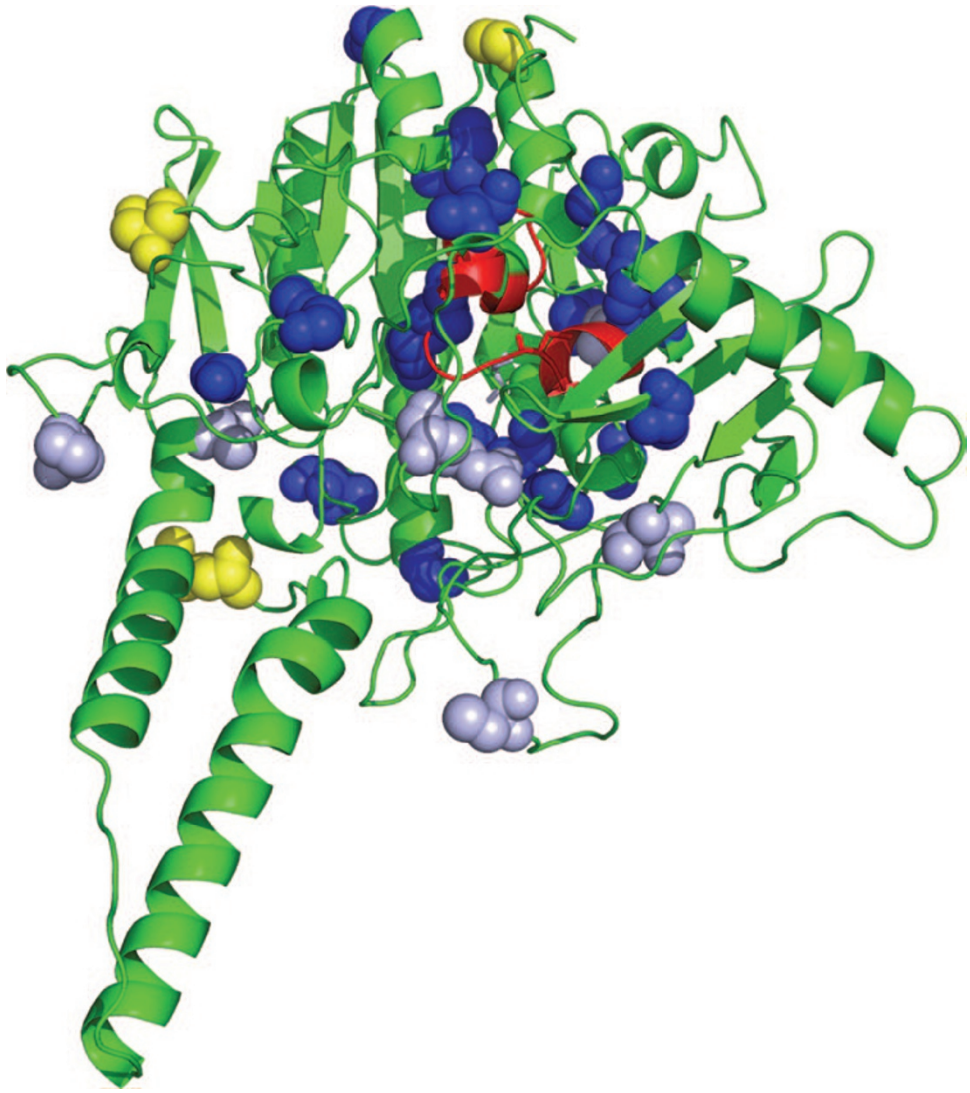

Figure 3 Model of ARSE. ARSE was superimposed on arylsulfatase C crystal structure and visualized by PyMOL. β Sheets, connecting loops, and α helices are green. The 18-amino-acid active site is red. ARSE residues involved in mutations are depicted as blue spheres, and polymorphisms are yellow spheres (see **Table 1**). *ARSC* mutation residues analyzed by Ghosh (2004) (ref. 28) are light blue spheres. The two transmembrane domains are shown as parallel α helices at the bottom of the molecule. ARSC, arylsulfatate C; ARSE, arylsulfatase E.

Although the majority of *ARSE* mutations identified overall have been unique, several reported *ARSE* mutations occurred more than once in unrelated families, including G73S, G137A, G317A, T481M, P578S, and W581X. Of these, only G73S and T481M represent transitions at CpG dinucleotides. We also noted that the maternal carrier frequency may be high. Although only nine mothers were tested in this study, all were found to be carriers of the *ARSE* mutations identified in their sons. Similarly, nine mothers of previously reported males with CDPX1 who had carrier testing were found to be carriers.[3,25,27] Maternal carriers were identified among both *ARSE* deletion and point mutation alleles (see **Table 2**). We suggest that the high frequency of maternal carriers, if proven, could be secondary to *de novo* mutations occurring more often in the germline of the maternal grandfather. Alternatively, given that patients with CDPX1 often survive and reproduce, the frequency of new mutations may be low.

Absence of genotype–phenotype association

The majority of missense substitutions were located in residues that were conserved in evolution for both ARSE and other arylsulfatases, ASA, ASB, ASC, ASD, ASH, and ASF. All missense alleles were distributed along most of the full protein and had negligible activity as compared with wt*ARSE*. Of note, there were no missense alleles identified in exon 2, which contains the start methionine codon, and none in the putative transmembrane domains. These results, taken together with indistinguishable phenotypes of patients with *ARSE* gene deletions and nonsense and missense alleles, indicate the absence of an association between mutation and disease severity. This is further supported by variation in

phenotype severity between affected males within the same family.[3]

It is possible that the missense alleles interfere with the proper localization of the protein or result in a catalytically inactive or misfolded and degraded protein. Daniele et al.[9] showed that the *ARSE* missense alleles, R11P, G137V, G245R, and G492Y, when overexpressed in COS cells had negligible activity but retained stability and Golgi location, suggesting that these residues may be important for the catalytic activity of ARSE.

ARSE protein modeling

ARSE is most closely related evolutionarily to ARSC and likely derives from this gene by duplication.[6] The amino acid identity between ARSE and ARSC is 51%. Recently, the crystal structure of ARSC, a transmembrane endoplasmic reticulum protein, was determined.[28] The crystal structure implies that the active site rests near the membrane surface, suggesting an intriguing role for the lipid bilayer in catalysis. Sequence analysis predicts that ARSE also contains two putative membrane-spanning domains, which likely anchor ARSE to Golgi membranes.[9] To further evaluate the effect of *ARSE* missense alleles on protein function, we modeled ARSE onto ARSC[29] using the automated alignment mode in SWISS-MODEL.[30] Structural 3D assessment for this model was created using the PyMOL Molecular Graphics System (Version 1.2r3pre; Schrödinger, LLC). Using this model, we visualized the location of the *ARSE* missense alleles identified in this article, as well as three known polymorphisms (**Figure 3**). Of note, we found that most mutations clustered around the active site (shown in red in **Figure 3**), similar to previously evaluated *ARSC* missense mutations suspected of impairing catalytic activity.[28] A smaller group of three mutations clustered around an *ARSC* mutation, also known to be inactive, that was positioned in a loop that might associate with the membrane (**Figure 3**). Of note, the known polymorphisms were all positioned toward the external surface. As previously predicted, there were no mutations located in the transmembrane domains. This structural comparison suggests that *ARSE* missense mutations inactivate ARSE by impairing its catalytic site.

Clinical comparison of BCP patients with and without *ARSE* mutations

Clinical data sheets were returned on 21/32 male probands (including those from the United Kingdom) but were not always fully completed. Nevertheless, the frequency of clinical findings (**Supplementary Table S4a,b** online) was similar to those reported before.[3] In exception, rhizomelia and ichthyosis, atypical findings for CDPX1, were reported once in patients with *ARSE* mutations R90G and D47N, respectively. Ganglioneuroblastoma was reported once in a patient without an *ARSE* mutation. Cervical spine abnormalities, frequently reported in patients with BCP, were not reported in the group without *ARSE* mutations in this study. Most surprising was the absence of information related to potential phenocopies in the *ARSE* mutation–negative group. The limitations of this data collection included its nonmandatory requirement to be completed. In addition, more specific questions on maternal vitamin K deficiency states should have been included. These features limit the interpretation of the data.

Etiologies for BCP in patients without *ARSE* mutations

From the 12 male probands identified without *ARSE* mutations, there were no mothers reported with conditions previously associated with gestational vitamin K deficiency. With the caveat that the clinical data sheets might be incomplete for maternal history, it seems likely that genetic heterogeneity or other environmental factors not considered also contribute to mutation-negative patients. Future studies could evaluate the X-linked ARSE paralogs ARSD, ARSF, and ARSH. ARSD has been sequenced in some patients with BCP previously without mutations reported.[5,25] Improved understanding of ARSE function would help to identify additional pathway proteins that, when defective, could be candidates for causing BCP.

One of these pathways may involve vitamin K, as fetal vitamin K deficiency in early gestation may be responsible for an unknown proportion of BCP phenotypes.[3,12,13,16] Supporting these observations, offspring of pregnant women receiving warfarin showed shortening and flattening of the nose, epiphyseal stippling, and cerebral hemorrhage.[31] Warfarin inhibits the VKORC1 complex, which recycles vitamin K. Offspring of pregnant rats treated with warfarin showed calcification and disruption of hypertrophic chondrocytes in long-bone epiphyses. These studies indicate that early gestational warfarin exposure results in bone dysplasia, whereas later exposure affects coagulation factors. Reduced bone γ-carboxyglutamate levels in the pups suggested that this phenotype was related to inhibited synthesis of vitamin K–dependent skeletal proteins.[32] Vitamin K–dependent proteins are activated in the endoplasmic reticulum through posttranslational modification by γ-glutamyl carboxylase. γ-Glutamyl carboxylase adds CO_2 to a bound glutamate residue to form γ-carboxyglutamate. Vitamin K is a cofactor in this reaction and is simultaneously oxidized.[33,34] γ-Carboxyglutamate or Gla residues bind calcium with high affinity. Vitamin K–dependent proteins involved in bone metabolism are bone Gla protein (osteocalcin), expressed in osteoblasts and odontoblasts and matrix Gla protein, i.e., expressed in vascular smooth muscle cells and chondrocytes.[35] Matrix Gla protein acts as an inhibitor of extracellular matrix mineralization and prevents vascular calcification.[36] Mutations in *MGP* cause Keutel syndrome, which features chondrodysplasia punctata, midfacial hypoplasia, peripheral pulmonary stenosis and progressive soft tissue, and vascular calcifications.[37,38] We speculate that ARSE might undergo γ-carboxylation and have functions similar to matrix Gla protein and osteocalcin, by catalyzing the desulfation of a critical growth plate component that inhibits calcification. Alternatively, ARSE may require vitamin K for normal growth plate mineralization in a pathway independent of γ-carboxylation.

In this project, the CETT program fostered collaboration between clinicians, researchers, and clinical laboratories. The

direct depositing of mutations from the clinical laboratory into a public database[39] allows access by the entire biomedical community for further investigation.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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