Mutations in PCYT1A Cause Spondylometaphyseal Dysplasia with Cone-Rod Dystrophy

Guilherme L. Yamamoto,1,2,* Wagner A.R. Baratela,1 Tatiana F. Almeida,1 Monize Lazar,2 Clara L. Afonso,3 Maria K. Oyamada,3 Lisa Suzuki,4 Luiz A.N. Oliveira,4 Ester S. Ramos,5 Chong A. Kim,1 Maria Rita Passos-Bueno,2 and Débora R. Bertola1,2

Spondylometaphyseal dysplasia with cone-rod dystrophy is a rare autosomal-recessive disorder characterized by severe short stature, progressive lower-limb bowing, flattened vertebral bodies, metaphyseal involvement, and visual impairment caused by cone-rod dystrophy. Whole-exome sequencing of four individuals affected by this disorder from two Brazilian families identified two previously unreported homozygous mutations in PCYT1A. This gene encodes the alpha isoform of the phosphate cytidylyltransferase 1 choline enzyme, which is responsible for converting phosphocholine into cytidine diphosphate-choline, a key intermediate step in the phosphatidylcholine biosynthesis pathway. A different enzymatic defect in this pathway has been previously associated with a muscular dystrophy with mitochondrial structural abnormalities that does not have cartilage and/or bone or retinal involvement. Thus, the deregulation of the phosphatidylcholine pathway may play a role in multiple genetic diseases in humans, and further studies are necessary to uncover its precise pathogenic mechanisms and the entirety of its phenotypic spectrum.

Spondylometaphyseal dysplasia with cone-rod dystrophy (SMD-CRD [MIM 608940]) is a rare autosomal-recessive condition that has been assigned to group 12 of the 2011 nosology and classification of skeletal diseases.1 It has been reported in nine families and is characterized by normal cognition, severe short stature, progressive bowing of the lower limbs, platyspondyly, metaphyseal irregularity, and cone-rod dystrophy causing visual impairment (Table S1 available online).2-4 The genetic defect that causes SMD-CRD is unknown.

We evaluated four Brazilian individuals with SMD-CRD from two families by whole-exome sequencing. The study was approved by the Ethics Committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo and informed consent was obtained prior to the collection of the samples. Family 1 comprises a pair of siblings whose parents are first-degree cousins: a 17-year-old boy (F1.1, IV-4 in Figure 1), who was born at term with a birth weight (BW) of 2,900 g and an unrecorded birth length (BL) and a 2-year-old girl (F1.2, IV-5 in Figure 1) who was born at term with a BW of 2,645 g and a BL of 44 cm. Both presented with visual impairment beginning in infancy that progressed to dramatically reduced visual acuity (hand movements only) along with short stature and bowing of the legs (Figure 2). A radiographic evaluation of these cases at our service at the ages of 2 and 17 years disclosed platyspondyly, with scoliosis in the older sibling, shortened long bones, generalized metaphyseal flaring, cupping and presence of spurs in the lateral and medial distal femur, and mild involvement of the tubular bones in the hands (Figure 2). An ophthalmologic evaluation in the older sibling revealed microphthalmia, a lack of fixed gaze, strabismus, nystagmus, corneal opacity in the right eye, corectopia and subluxation of the lens in the left eye, bilateral coloboma of the optic nerve, severe macular atrophy, and areas of choroidal and retinal atrophy (Figure 3A2). No electrical activity was detectable by a full-field electroretinogram (ERG) (Figure 3C2). His younger sister presented with very similar findings: pronounced microphthalmia, nystagmus, strabismus, inferonasal corectopia with subluxated lenses in both eyes, coloboma of optic nerve, severe macular atrophy, and areas of choroidal and retinal atrophy (Table S1). The cognitive development of both siblings was adequate, and they do not have facial dysmorphism.

The lipid profiles of the siblings showed that only the older brother had mildly reduced levels: triglycerides (TG) of 70 and 149 mg/dl in F1.1 and F1.2, respectively, and total cholesterol of 107 mg/dl (HDL 36 mg/dl) and 156 mg/dl (HDL 27 mg/dl). Abdominal ultrasonograms were normal in both cases.

Family 2 comprises two distant cousins, both products of consanguineous marriages: an 11-year-old girl (F2.1, V-5 in Figure 1), born at term with BW of 2,800 g and an unrecorded BL and a 14-year-old girl (F2.2, V-2 in Figure 1) born at term with BW of 2,250 g and a BL of 45 cm. These individuals presented with progressive lower limb bowing since the age of 1 year (Figure 2), severe short stature, normal cognitive development, no facial dysmorphism, and low myopia with no complaint of visual impairment. Both had initially normal fundoscopies. They were evaluated in our service at the ages of 11 and 14 years and a

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1Unidade de Genética, Instituto da Criança, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo 05403-000, Brazil; 2Centro de Estudos do Genoma Humano, Instituto de Biocéncias da Universidade de São Paulo, São Paulo 05508-090, Brazil; 3Departamento de Ofalmologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo 05403-000, Brazil; 4Departamento de Radiologia, Instituto da Criança, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo 05403-000, Brazil; 5Departamento de Genética, Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto 14049-900, Brazil

*Correspondence: guilherme.yamamoto@usp.br
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skeletal survey showed similar abnormalities to those observed in family 1 (Figure 2). Because these skeletal findings were highly suggestive of SMD-CRD, an ophthalmologic reevaluation was performed and provided the following results: retinography with reduced foveal brightness without atrophy (Figure 3A1) and visual acuities of 20/20 in both eyes in F2.1 and 20/30 OD, 20/40 OS in F2.2. An examination of optical coherence tomography (OCT) showed thinning of the retina in the macular region of both of individual F2.2’s eyes (Figure 3B). ERG was possible only in F2.2; in this person, we observed that the scotopic response was at the lower limit of normality and that the waves in the photopic phase were strongly reduced, featuring a cone dysfunction (Figure 3C1). The plasma lipid profile in these cases showed mildly reduced levels: TG of 80 and 33 mg/dl in F2.1 and F2.2 and total cholesterol of 141 mg/dl (HDL 21 mg/dl) and 121 mg/dl (HDL 31 mg/dl), respectively. An abdominal ultrasonogram revealed a diffuse hyperechogenic liver texture, compatible with increased lipid content in F2.1 and the presence of gallbladder stones in F2.2.

Exome sequencing of genomic DNA obtained from the peripheral blood of the four affected individuals was performed with Illumina’s TrueSeq kits for library preparation and exome capture and the Illumina HiScan sequencer. Alignment was made with the Burrows-Wheeler Aligner (BWA), and the Genome Analysis Tool Kit (GATK) was used for data processing and variant calling. Variant annotation was performed with ANNOVAR. Variants were filtered for a frequency of less than 1% in the control populations (1000 Genomes and 6500 Exome Sequencing Project from Washington University) and for an allele frequency of less than 5% among 62 Brazilians with different diseases sequenced in our service.

Because SMD-CRD is known to have a recessive mode of inheritance and both families show consanguinity, we hypothesized that homozygous mutations were the most likely genetic cause. There were initially 20,160 and 17,341 variants in families 1 and 2, respectively; after filtering, we were left with 18 variants in 15 genes in family 1 and 17 variants in 7 genes in family 2 (Table S2). The only gene with filtered variants in homozygosity shared by both families was PCYT1A (MIM 123695; RefSeq accession number NM_005017.2). Surrounding PCYT1A, at 3q39, all the affected individuals also had overlapping large blocks of homozygosity (chr3: 193,210,768–197,880,134 in family 1 and ch3: 192,053,274–197,236,838 in family 2).
Subjects F1.1 and F1.2 were homozygous for a c.385G>A (p.Glu129Lys) mutation, and subjects F2.1 and F2.2 were homozygous for a c.968dupG (p.Ser323Argfs*38) mutation. These mutations were confirmed by Sanger sequencing in the affected individuals and were identified in heterozygosity in their respective parents (Figure 1). Both are predicted to be deleterious by in silico analysis by Mutation Taster, SIFT, LRT, and PolyPhen2.

*PCYT1A* encodes for the enzyme choline-phosphate cytidylyltransferase A (CCT A), which is responsible for transforming phosphate-choline (P-choline) into cytidine-diphosphate-choline (CDP-choline) in glycerophospholipid metabolism. This enzyme exerts a key rate-limiting step in the CDP-choline pathway, the major pathway in the phosphatidylcholine (PC) biosynthesis, comprised of three steps (Figure S1). The ubiquitous and best-studied isoform of mammalian CCT (CCT A, 367 residues), which functions as a homodimer, has been described as having four domains: an N-terminal domain (75 residues) that contains its nuclear localization signal (NLS) sequence followed by a 150-residue catalytic domain, a 60-residue membrane binding domain (domain M), and a 50-residue unstructured phosphorylated tail that is known to house up to 16 phosphoserine sites, with unknown function (domain P) (Figure 1C). The missense mutation in family 1 (c.385G>A) causes a substitution (p.Glu129Lys) in a highly conserved amino acid in the catalytic domain (domain C), most probably altering the conformation of the region that is important for binding the nucleotide for phosphorylation. Site-directed mutagenesis in conserved motifs of the catalytic domain has been demonstrated to result in highly destructive effects on enzymatic activity. However, it is unclear how the c.968dupG mutation (family 2), which is predicted to result in a slightly truncated protein (p.Ser323Argfs*38), might exert effects, because nonsense-mediated RNA decay is unlikely. In addition, constructs containing deletions of the phosphorylation domain and substitutions of the serine amino acids were shown to have no effect on enzymatic activity or cell proliferation in vitro.

The precise mechanism of how these mutations have an impact on bone and/or cartilage and retina is currently unknown, and future functional studies are required to uncover its exact role. Nevertheless, some data from experiments in tissues and animal models available in the literature provide some speculative ideas of how this could be occurring. Pathogenicity could derive either from lack of PC in specific cells or from increased levels of acetylcholine (ACh) and ACh receptor activation.

Phospholipids play an important role in maintaining membrane stability. PC is the major phospholipid component of the eukaryotic plasma membrane external leaflet, which includes the matrix vesicles, and is responsible for initiating extracellular mineral formation. Previously, loss-of-function mutations in *CHKB* (MIM 612395), one of the two genes that encode choline kinase (CK), along with *CHKA* (MIM 118491), were identified as the cause of muscular dystrophy with mitochondrial structural abnormalities (MIM 602541), the only genetic disorder in humans that has been associated with deregulation of the PC pathway. The same mechanisms that were proposed...
by the authors to be responsible for muscular dystrophy may be applied to SMD-CRD, i.e., decreased levels of PC and altered phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratios, as well as a different pattern of fatty acid composition in the PC. Moreover, tissue specificity was also demonstrated in the study of Mitsuhashi et al.\(^1\) because in muscles, only the CKB isoform was present; CKA was not detectable.\(^19\) In our cases, a tissue-specific pattern may also be operating because a second enzyme, CCT B, encoded by the \textit{PCYT1B} gene (MIM 604926) and expressed as two isoforms (CCT B1 and CCT B2), seems to have the same function as CCT A. A lack of complete redundancy between CCT A and CCT B was demonstrated by the discordant phenotypes of \textit{Pcyt1a} knockout mice, which showed early embryonic lethality,\(^20\) and the \textit{Pcyt1b} knockout mice, which had only gonadal dysfunction and impaired axonal branching.\(^16\)

Although no skeletal or retinal abnormalities were observed in the affected individuals with muscular dystrophy, the \textit{md} spontaneous mutant mice, which present a deletion in \textit{Chkb} and the same muscle phenotype as humans, were also visibly smaller than their nonaffected littermates and showed an outward rotation of the forelimbs caused by severe long bone bowing; this suggests that the deregulation of the CDP-choline pathway can have an impact on bone formation.\(^19\)

**Figure 3. Ophthalmological Evaluation of F2.2 and F1.1**

Shown are retinography (A), OCT (B), and full-field ERG (C). In the retinography, F2.2 presents only decreased macular brightness and mild pigmented changes in the fovea bilaterally (A1). Despite this mild phenotype, decreased macular thickness is evident in the OCT (B), and there is a severely reduced photopic response in ERG with the scotopic response being at the lower limit of normality (C1). A severe phenotype with vascular narrowing, and RPE and macular atrophy (A2, top) is associated with eye malformations (coloboma of the optic nerve) in F1.1 (A2, bottom). No electrical activities were detectable in the full-field ERG (C2).
Decreased levels of PC may also be associated with the mildly reduced levels of TG and total cholesterol that were observed in our adolescent individuals and the increased lipid content in the liver of F2.1. This has been demonstrated in rats fed a choline-deficient diet that resulted in decreased levels of PC, the accumulation of TG in the liver, and the impaired secretion of VLDL and LDL, but not HDL cholesterol, with a reduction of their plasmatic levels.21

On the other hand, not only a decrease in PC product, but also an excess of substrates, ultimately leading to an increase in ACh, may be involved in the pathogenesis of SMD-CRD. In the past two decades, the characterization of a nonneuronal cholinergic system has been proposed. Several groups have demonstrated that not only neurons, but different types of cells in several tissues, including retina, cartilage, and bone, express the complete acetylcholine pathway, responsible for an autocrine or paracrine tissue-specific cholinergic signaling.23,24 Unpublished data from our laboratory confirm that all the genes in the acetylcholine pathway are expressed in preosteoblasts derived from mesenchymal cells. ACh acts as a signaling molecule, controlling basic cell functions such as proliferation, differentiation, and the establishment and maintenance of cell-cell contacts. ACh exerts its action by binding to muscarinic or nicotinic receptors (nAChRs). The latter are members of a superfamily of ligand-gated ion channels that, being activated by binding of agonists with high affinity, control cell function by ionic signals, including calcium.22,24–26 There is evidence for the presence of alpha7 nicotinic acetylcholine receptor (alpha7nAChR) in the retina pigmented epithelium (RPE) and in cartilage.28 Therefore, we can speculate that the SMD-CRD phenotype may also be a consequence of elevated P-choline leading to elevated choline and ultimately acetylcholine levels, which in turn activates acetylcholine receptors (AChRs) in an autocrine or paracrine manner (among them alpha7nAChR), resulting in calcium influx and downstream signaling.

Different animal models support the hypothesis of skeletal dysplasia resulting from excessive acetylcholine activation, at least in the embryonic stages. Nicotine is the major candidate compound that has been implicated in intrauterine growth retardation and skeletal growth retardation in newborns of tobacco-using mothers. Prenatal nicotine exposure in mice induces delayed chondrogenesis, specifically mediated via fetal alpha7nAChR, and the mechanism underlying this process may involve the downregulation of IGF-1 signaling and the inhibition of matrix synthesis by growth plate chondrocytes. The same matrix synthesis inhibition is observed in human growth plate chondrocytes in suspension cultures that are treated with nicotine.28,29 Moreover, long bone and vertebral abnormalities have been demonstrated in chick and rat embryos exposed to organophosphates (inhibitors of acetylcholinesterase, the enzyme that degrades Ach).30–33

In summary, the possible explanations of the effective mechanisms of the deregulation of PC pathway (decreased levels of PC and/or increased levels of ACh) leading to bone and/or cartilage and retinal disease may include: (1) an imbalance of calcium itself, because it is of major importance to bone development and signaling, both in bone and in the RPE; (2) a loss of cell adhesion caused by cytoskeletal disaggregation and induced by muscarinic and/or nicotinic AChR activation;22,34,35 (3) apoptosis secondary to decreased PC levels,8,36 and/or (4) a modulation of the inflammatory response in cartilage caused by the nonneuronal cholinergic system (Figure S1).

All persons that have been described so far with SMD-CRD present a phenotype comprising uniform skeletal findings, whose first signs (rhizomelia and bowed lower limbs) are observed mostly during the first year of life, and RPE dystrophy, which has a more variable age of onset. Although the majority of the affected individuals showed signs in infancy and early childhood, some of them, including the cousins from family 2, presented ophthalmologic abnormalities in late childhood and adolescence. During development, severe disproportionate short stature is observed, with a final height of less than 100 cm. Multiple orthopedic interventions were required to correct the bowed lower limbs in some individuals. The spine abnormalities do not require interventions because the scoliosis is usually mild. The visual loss is progressive, and although it evolves with stabilization during adolescence, this causes a significant burden for the affected individuals (Table S1).

Unlike the other cases described in the literature, family 1 in this study showed eye malformations. It remains to be determined whether these additional findings are part of the spectrum of PC deregulation or, alternatively, if they may be caused by mutations in a different gene with a recessive mode of inheritance, such as mutations in APOD (Table S2), which encodes for apolipoprotein D, a lipid transporter associated with retinol metabolism.38 Retinol insufficiency or depletion during early development is a well-established cause of congenital eye malformations.39,40

The identification of mutations in PCYT1A as the cause of SMD-CRD substantiates the hypothesis that, in humans, a deregulation of the choline pathway is responsible, not only for muscular dystrophy, but also for skeletal and retinal abnormalities. Although we speculated on how the deregulation of the pathway could interfere with bone and retinal development, further functional studies are essential for this confirmation. An increased understanding of this mechanism may lead to better management of the clinical findings of SMD-CRD, with ultimately prevention of the visual loss.

Supplemental Data

Supplemental Data include one figure and two tables and can be found with this article online at http://www.cell.com/AJHG/.

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Web Resources

The URLs for data presented herein are as follows:

- 1000 Genomes, http://browser.1000genomes.org
- HomozygosityMapper software, http://www.homozygositymapper.org/
- MutationTaster, http://www.mutationtaster.org/
- Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
- PolyPhen-2, http://www.genetics.bwh.harvard.edu/pph2/

References