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Further Evidence That Chondrocalcinosis 1 (CCAL1) is a Confirmed Mendelian Phenotype With a Known Molecular Basis

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ABSTRACT

Chondrocalcinosis (CCAL), also known as calcium pyrophosphate dihydrate deposition disease (CPPDD), is a frequent multifactorial condition in the elderly, but there are two rare autosomal dominant Mendelian forms, CCAL1 (OMIM %600668) and CCAL2. Only three families with molecularly proven CCAL1 have been reported. Here, we describe an additional family from Germany (12 individuals, nine living) with CPPDD manifesting in the third decade of life, presenting with severe spinal problems and variable levels of disability, only two of them with hip problems. The mildly impaired growth in this family (median height at age 20: 10th percentile) may represent an as yet undescribed sign of CCAL1, or alternatively familial short stature. In all documented families and in this family, the disorder resulted from the recurrent heterozygous stop-loss variant NM_002546.4:c.1205A>T; p.(Ter402Leuext*19) in *TNFRSB11B* on chromosome 8q24, which predicts an extended osteoprotegerin protein with 19 additional amino acid residues. The pathomechanism of CCAL1 is not known. Biallelic *TNFRSF11B* loss-of-function variants cause autosomal recessive juvenile Paget's disease of bone (PDB5); shared features between CCAL1 and PDB5 include demineralization, osteoporosis, increased fractures, and a progressive height loss with age, but PDB5 and CCAL1 have very different phenotypes and heterozygous carriers of PDB5-associated variants are asymptomatic. Summing up, NM_002546.4:c.1205A>T represents a rare type of stop-lost variant, likely a gain-of-function variant, and to date no other variant is known to cause CCAL1. Our findings expand the phenotypic spectrum of CCAL1 and underscore that CCAL1 is a distinct rare Mendelian disorder for which the underlying molecular basis is now known.

1 | Introduction

Calcium pyrophosphate crystal deposition disease (CPPDD) is a frequent crystal-induced arthropathy characterized by calcium pyrophosphate crystal deposition (CPPD) in joints and soft tissues, leading to inflammation and joint destruction (Rosenthal and Ryan 2016). CPPDD phenotypes include asymptomatic CPPD, CPPD with osteoarthritis, chronic inflammatory CPP crystal arthritis, and acute CPP crystal arthritis (also known as pseudogout) (Zhang et al. 2011). Diagnosis is based

on clinical and/or radiological findings and the definitive diagnosis of CPPDD should include the microscopic detection of CPP crystals in the synovial fluid (Hollander et al. 1961; Zhang et al. 2011).

CPPDD is extremely common in older age and is idiopathic in most cases; it is associated with aging, osteoarthritis, gout, endocrine disorders, for example, hyperparathyroidism, genetic disorders such as haemochromatosis, and multifactorial familial predisposition (Doherty and Dieppe 1986; Ryan and McCarty 1997). In contrast, autosomal dominant CPPDD manifesting already in the third or fourth decade of life is very rare. Genetic studies have linked two chromosomal regions to the rare autosomal dominant familial forms of early-onset CPPDD, CCAL1 (OMIM %600668) on chromosome 8 and CCAL2 (OMIM #118600) on chromosome 5 (Baldwin et al. 1995; Hughes et al. 1995; Andrew et al. 1999). CCAL2 was found to be caused by various autosomal dominant mutations of the ANKH gene on chromosome 5p15, which increase pyrophosphate levels in the extracellular matrix and thus CPP crystal formation in the joints (Pendleton et al. 2002; Williams et al. 2002). The molecular basis of CCAL1 is less well understood. Only three molecularly proven CCAL1 families have been reported to date: an Israeli family first described in 1990 (Eshel et al. 1990) and revisited in 2018 (Williams et al. 2018), a family from the Netherlands (Ramos et al. 2015), and a US family of German-Italian ancestry from Long Island, NY (Williams et al. 2018). The families all presented a heterozygous c.1205A>T stop-loss (read-through) variant in TNFRSF11B (the gene encoding osteoprotegerin, OPG), predicting an abnormal OPG protein extended by 19 amino acid residues; p.(Ter402Leuext*19). The mutant protein was named OPG-XL (termination codon "X" replaced by leucine, "L"), and murine in vitro studies showed that in osteoclast formation assays, synthetic OPG-XL behaves as a loss of function protein (Mitton-Fitzgerald et al. 2021). The pathomechanism of CCAL1 in man is still unknown.

Here, we describe a four-generation family totaling 12 individuals (nine alive, three deceased) from Rhineland-Palatinate, Germany, with early-onset CPPDD and the heterozygous c.1205A>T variant *in TNFRSF11B*, indicating a proven diagnosis of CCAL1. We provide detailed clinical, radiological, and microscopic findings and show that the variant occurred on an allele with genetic polymorphisms identical to the Long Island family and different from the Israeli family. We propose that CCAL1 is a distinct, rare, and likely underdiagnosed Mendelian disorder for which the underlying molecular basis is now known.

2 | Subjects and Methods

2.1 | Ethical Considerations

All procedures were conducted according to the guidelines of the Declaration of Helsinki, and all living subjects in this study (II.2, III.1–3, III.4–6, and IV.1–3) provided written consent to participation according to the ethical approval of the University Medical Centre of the Johannes Gutenberg University Mainz. Furthermore, III.2 provided consent for her deceased mother (II.2) and grandfather (I.1).

2.2 | Subjects, Data Sources, and Methods

The four-generation family (pedigree, Figure 1A) originated from the Westerwald region of Rhineland-Palatinate, Germany, and has been known to us since 1999. Over the course of 22 years, we studied 12 affected individuals (eight females, four males; age at diagnosis 18–82 years, median age at diagnosis 49 years excluding the deceased subjects I.1 and II.1). In addition to these individuals, the pedigree included 11 family members who had not been available for study (Figure 1A: unnumbered diamond-shaped symbols); of these, four were allegedly affected (striped diamonds) and seven were allegedly unaffected (white diamonds) based on reports from studied subjects. These 11 family members were no part of this study, they were not seen by us, no genetic testing was done, and no data were available.

All subjects studied by us were diagnosed in the medical practice of M.W. The deceased great-grandfather (I.1) was diagnosed based on narratives of III.2 and III.5; his daughters II.1 and II.2 were diagnosed based on medical records and radiographs provided by their respective daughters (III.2 and III.5), and III.3 lived elsewhere and had requested diagnosis based on telephone interviews, medical records, and x-rays. The remaining subjects (III.1–2, III.4–6, and IV.1–3) were patients in the medical practice of M.W. Medical reports obtained in the medical practice included clinical, sonographical, radiological, and microscopic findings, and the subjects provided additional reports and data. Joint range of motion was measured using the neutral position method. Joint radiographs were obtained in two or three planes (knee: anterior–posterior, lateral, and patella tangential) and osteoarthritis (OA) was evaluated using the Kellgren-Lawrence (K/L) score (0: no OA, 1: doubtful OA, 2: minimal OA, 3: moderate OA, and 4: severe OA).

2.3 | Molecular Genetic Analysis and SNP Genotyping

Eight subjects (III.1–2, III.4–6, and IV.1–3) were available for molecular studies. The c.1205A>T variant in exon 5 of the *TNFRSF11B* gene (NM_002546.4, hg19) was first identified by one of us (M.A.B.) at the Australian Translational Genomics Centre in Queensland, Australia, in a genome-wide scientific study of blood DNA from subjects III.2, III.5, IV.2, and IV.3. Thereafter, we confirmed the findings at the Medical Care Center of the Johannes Gutenberg University Mainz, Germany, using genomic DNA from peripheral blood of subjects III.1–2, III.4–6, and IV.1–3 and Sanger sequencing with a CEQ Genetic Analysis System (Beckmann Coulter, Brea, CA).

To study the genetic background of the affected alleles in the family, we performed SNP genotyping using polymorphic markers rs2073618, rs1564858, rs10554146, and rs2228568 as described elsewhere for two other CCAL1 families (Williams et al. 2018).

2.4 | Statistical Analysis

Medians and percentages of the outcomes were calculated, but means, standard deviations, and comparative statistical tests were not used due to the small sample size.

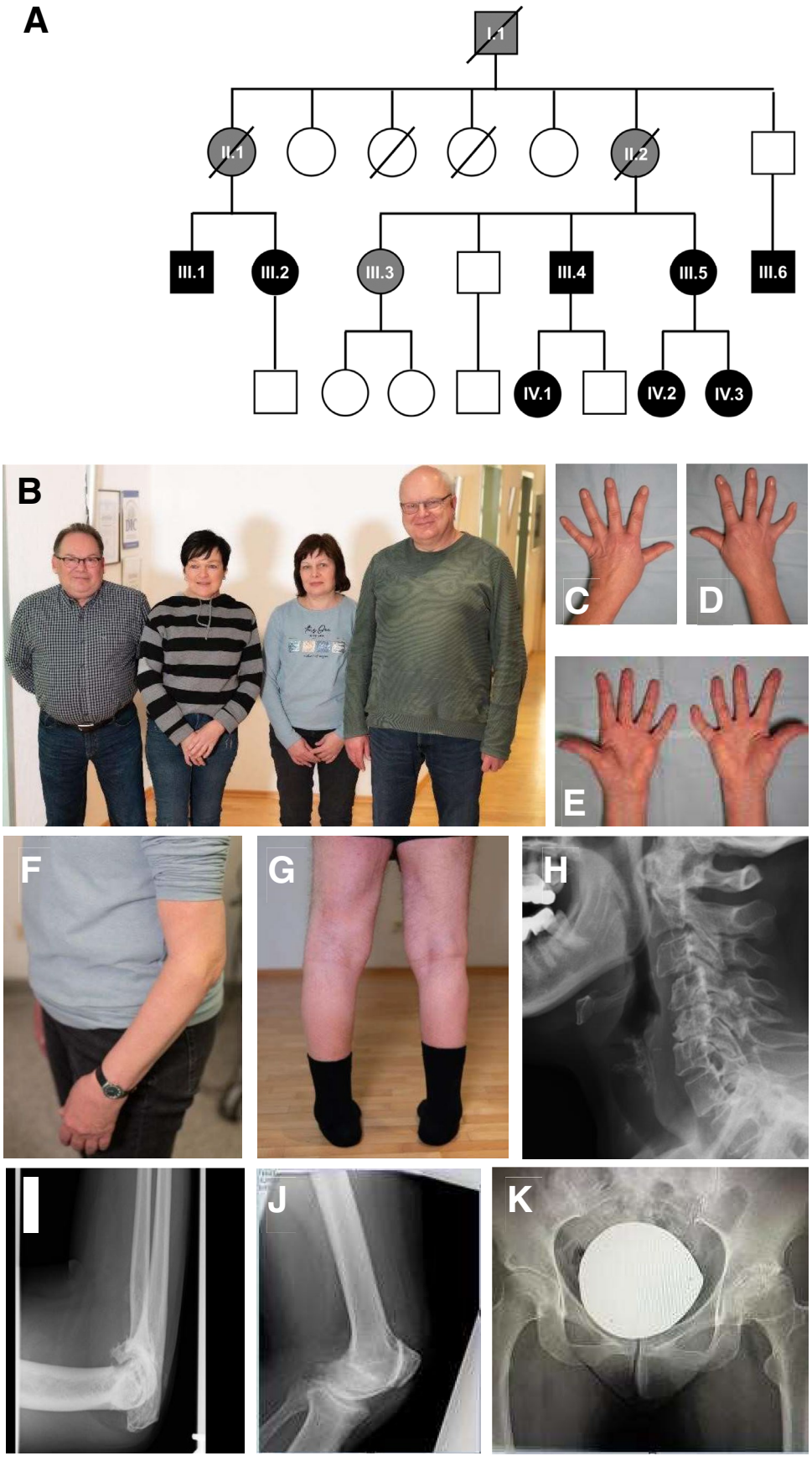


FIGURE 1 | Legend on next page.

FIGURE 1 | Family with early onset osteoarthritis and chondrocalcinosis. (A) Pedigree of the family: Black symbols ($n=8$) represent individuals with a clinical and molecular diagnosis of CCAL1 (genotype: *TNFRSF11B* c.1205A>T wt/mut) and gray symbols ($n=4$) represent individuals with a clinical diagnosis of CCAL1 only. In addition, the pedigree shows 11 family members who had been not available for study (blank circles and squares); the phenotype was not evaluated for these individuals. (B) Group photograph of family members: III.1 aged 61 years, III.2 aged 54 years, III.5 aged 54 years, and III.4 aged 56 years. Note the relatively short stature in almost all affected individuals, except for III.4 (C, D, E). Subject III.2 at the age of 53, dorsal and palmar views of the hands show doughy swellings over MCP joints II and III and fingers 2–5, and hyperextension and swelling of the metacarpophalangeal joint of the thumb, which is a clinical sign of rhizarthrosis. (F) Subject III.5, lateral view of the left elbow at the age of 54, indicating limitation of motion and extension deficit. (G) Subject III.1, dorsal view of the legs at the age of 60, note genu varum and massive instability of the left ankle joint. (H) Radiograph of III.5, lateral view of the cervical spine at the age of 56, note multisegmental osteochondrosis of C3–6, loss of cervical lordosis, degenerative kyphosis with anterolisthesis, C3/4 instability, and facet joint arthrosis of C4/5 and C5/6. (I) Radiograph of III.1, lateral view of the right elbow at the age of 54, note severe arthrosis of the humeroulnar and humeroradial joints. (J) Radiograph of III.1, lateral view of the left knee at the age of 56, note severe gonarthrosis, with marked calcification of the articular cartilage and suprapatellar recess (indicated by arrow). (K) X-ray of the pelvis of IV.3 showing severe coxarthrosis on the left side.

3 | Results

3.1 | Clinical Findings

Clinical data of all symptomatic and pre-symptomatic (IV.2–3) family members are summarized in Table 1. Subjects III.1–2, III.4–6, and IV.1 presented with severe early-onset chronic and acute arthritis manifesting between age 20 and 30 years and mostly affecting the knees, ankles, elbows, cervical spine, and wrists. All showed early-onset OA and joint calcifications (CPPD) by physical examination, sonography, and radiography. Subjects III.1, III.2, III.5, III.6, and IV.1 were additionally examined by magnetic resonance imaging (MRI), and III.2 and III.5 also by computed tomography (CT). The diagnosis of CPPDD was proven by microscopic detection of CPP crystals in synovial fluid obtained during synovectomy for inflammatory arthritis of III.5 (right elbow) and III.1 (right knee). The other subjects had multiple acute episodes of acute joint inflammation but no synovectomy. Figure 1B depicts four subjects with severe joint destructions (from left to right: siblings III.1 and III.2, and siblings III.5 and III.4; aged 61, 54, 53, and 55 years, respectively); subject III.3 was similarly affected but lived elsewhere and was not available for Figure 1B, and III.6 had not yet been diagnosed. Figure 1C–K shows clinical and radiographic findings.

Previous reports have associated CCAL1 also with arthrosis of the hips (Eshel et al. 1990; Baldwin et al. 1995; Ramos et al. 2015). In the five most seriously affected subjects of this family, evaluation of the hip joints using the Kellgreen scale showed severe OA of the hips (K/L grade 4) in only two subjects (III.3 and IV.1), requiring artificial joint replacement at ages 52 and 30 years, respectively. Subjects III.1, III.2, and III.5 (aged 56, 52, and 48 years at radiography) demonstrated no or only doubtful OA of the hip joints (K/L grade 0–1).

The degree of disability (Grad der Behinderung, GdB, by German social welfare law) reflects the deterioration in a person's health and the loss of independence in daily activities. GdB scores range from 20 (mildly disabled) to 100 (most severely disabled), and GdB ≥ 50 signifies severe disability by law. In this family, severe disability was acknowledged at a median age of 47 years (range: 30–53 years). Subjects II.2, III.2, and III.5 retired at ages 50, 51, and 52 years, respectively, while III.1 and III.3 could work beyond age 60 in office jobs.

None of the affecteds had additional rheumatic or endocrine disorders. Comorbidity included cancer at old age in two subjects: In II.1, a melanoma of the right eye was diagnosed at age 61 years and treated by surgery; she died 2 years later of liver metastases. II.2 was diagnosed at age 68 years with a hormone receptor-positive breast cancer, was successfully treated with mastectomy and tamoxifen, and died aged 81 years of Covid-19.

The available subjects over the age of 50 were approximately 6–7 cm shorter than they had been as young adults, showing a marked decline in height (Figure 1, Table 1). Interestingly, with the exception of III.4, all subjects with recorded height data (generations II–IV) had always been of relatively short stature (below the 50th percentile for age); the median height in the family at age 20 years was at the 10th percentile (Table 1). Nine out of 11 subjects had a height between the 1st and 21st percentile at age 20, and III.3 and III.4 had heights at the 43rd and 69th percentiles, respectively. We do not know whether the short height is due to the disorder or reflects familial short stature.

3.2 | DNA Sequencing, gnomAD Data, and SNP Genotyping of TNFRSF11B

Molecular analysis of subjects III.1, III.2, III.4–6, and IV.1–3 was performed in 2016–2021 and revealed a heterozygous variant NM_002546.4:c.1205A>T p.(Ter402Leuext*19) in *TNFRSF11B* (Figure 2A), which had been previously reported in CCAL1 (Ramos et al. 2015; Williams et al. 2018). In the relevant databases, the alteration was initially classified as a "variant of uncertain significance" (ACMG classification, American College of Medical Genetics and Genomics), but meanwhile this was changed to "pathogenic." At the protein level, the variant predicts a read-through mutation of the termination codon (Ter402Leu), with an exchange at position 402 of the stop codon to a leucine amino acid (aa) residue and a protein elongation of 19 aa residues. The wild-type OPG comprises 401 aa, and the mutant OPG-XL molecule spans 420 aa.

GnomAD (v4.1.0, searched March 18, 2025) indicated a germline classification of the c.1205A>T variant as pathogenic (single submitter) and an allele frequency of 4 in 1,614,162 (2.48e−6), with zero homozygotes, which would indicate a frequency in the population of 1:201,770 individuals. GnomAD (2025) lists two neighboring variants altering the *TNFRSF11B* stop codon,

TABLE 1 | Clinical findings in the probands.

Proband	Gender and age (years) when last seen	OA with CPPDD	Acute CPP crystal arthritis	Chronic inflammatory arthritis	Number of affected joints[a]	Age (years) at first joint replacement	Number of artificial joints	Height (cm) and percentile at age 18–20, taken from identity card	Age, height (cm) and percentile when last measured	Comments
I.1	M 65	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	Born in Molzhain, Westerwald, Rhineland-Palatinate, Germany, in 1899. Died at the age of 65.
II.1	F 63	+	+	+	10	55	1	160 (10th)	n.k.	Bilateral rhizarthrosis and arthrosis of MCP II + III and DIP IV, died aged 63 from metastatic melanoma of the right eye.
II.2	F 82	+	+	+	7	61	1	158 (6th)	n.k.	Died aged 82 from COVID-19.
III.1	M 62	+	+	+	10	58	1	164 (1st)	61 years 158 (1st)	Ventral spondylodesis C3-Th1 at age 57, right knee replacement at age 58.
III.2	F 55	+	+	+	10	44	3	160 (10th)	54years 153 (1st)	Arthrosis of DIP II-V on the left, surgery by dorsal spondylodesis of L4-L5 at 41 and of C2-Th3 at 51.
III.3	F 61	+	+	+	7	51	1	167 (43th)	n.k.	Bilateral arthrosis of DIP II + III, left hip replacement at age 52.

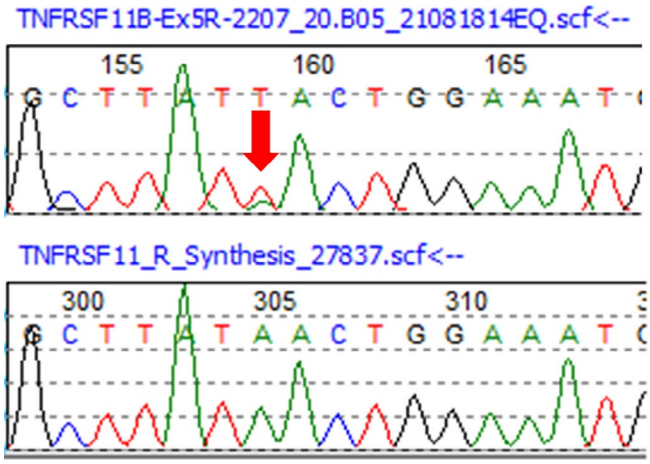
(Continues)

TABLE 1 | (Continued)

Proband	Gender and age (years) when last seen	OA with CPPDD	Acute CPP crystal arthritis	Chronic inflammatory arthritis	Number of affected joints[a]	Age (years) at first joint replacement	Number of artificial joints	Height (cm) and percentile at age 18–20, taken from identity card	Age, height (cm) and percentile when last measured	Comments
III.4	M 58	+	+	+	9	44	2	184 (69th)	56 years 177 (69th)	Left ankle arthrodesis at age 47 and right ankle arthrodesis at age 53.
III.5	F 55	+	+	+	9	45	4	162 (17th)	54 years 155 (2nd)	Spinal canal stenosis C3–C6 awaiting surgery. Condition after bilateral ankle arthroplasty aged 44 and 47 and partial right knee arthroplasty aged 48.
III.6	M 53	+	+	+	5	52	1	160 (< 1st)	n.k.	Massive degenerative deformity C1–C7, left knee replacement aged 53. Incidental finding: Congenital clubfoot.
IV.1	F 32	+	+	+	5	30	1	160 (10th)	n.k.	First symptoms at age 20, bilateral severe coxarthrosis and gonarthrosis, hip replacement at age 30.
IV.2	F 26	−	−	−	1	0	0	163 (21st)	n.k.	Knee problems from the age of 20.
IV.3	F 21	−	−	−	0	0	0	162 (17th)	n.k.	Diagnosed by presymptomatic genetic testing, no symptoms yet.

Abbreviations: CPP, calcium pyrophosphate; CPPDD, calcium pyrophosphate deposition disease; DIP, distal interphalangeal joint; F, female; M, male; MCP, metacarpophalangeal joint; n.k., not known; OA, osteoarthritis.
[a]Kellgreen-Lawrence score ≥ 3.

A



B

SNP	Position in *TNFRSF11B*	Change	Average Heterozygosity	Israeli Family	Long Island Family	German Family
rs2073618	Exon 1	c.9C>G	0.522	+	+	+
rs1564858	Intron 2	c.400+4C>T	0.105	+	-	-
rs10554146	Intron 3	c.592 +55_56del	0.215	+	-	-
rs2228568	Exon 4	c.768A>G	0.105	+	-	-

FIGURE 2 | (A) Electropherograms showing (top) the heterozygous *TNFRSF11B* c.1205A>T p.(Ter402Leuext*19) variant in patient III.2 (base substitution indicated by red arrow) and (bottom) the wild-type variant. (B) Table comparing the results of SNP analyses of four polymorphic variants located within the *TNFRSF11B* gene in the US family from Long Island and the Israeli family (Williams et al. 2018) with the present family. Note that the German family and the Long Island family showed similar markers, whereas the Israeli family is clearly unrelated.

c.1204T>C and c.1206A>C, that predict very similar stop lost variants of, respectively, p.Ter402GlnextTer19 and p.Ter402TyrextTer19. GnomAD reports these variants with respective allele frequencies of 8 in 1,614,016 (4.96e−6) and 1 in 1,614,142 (6.20e−7), with zero homozygotes, indicating population frequencies of 1:100,876 and 1:807,071 individuals, respectively. In gnomAD, these variants are listed without germline classification, but prediction tools such as the VarSome and Franklin databases classify them as variants of uncertain significance.

In subjects III.1, III.2, III.4–6, and IV.1–3, genotyping of SNPs within and adjacent to TNFRSF11B showed SNPs identical to the US Long Island family and different from the Israeli family (Williams et al. 2018), indicating that the variant originated on at least two different genetic backgrounds (Figure 2B).

4 | Discussion

We describe a German family with autosomal dominant CCAL1. The family showed the c.1205A>T p.(Ter402Leuext*19) stopLoss variant in the *TNFRSF11B* gene at chromosome 8q24.12 (the OPG-XL variant) that had been previously reported in all three molecularly confirmed CCAL1 families, originating from the Netherlands (Ramos et al. 2015), from Israel, and from Long Island, USA (Eshel et al. 1990; Williams et al. 2018). Only one other family (from Maine, USA) with likely molecularly confirmed CCAL1 is known, which showed genetic linkage to a 32-cM region on chromosome 8q (Baldwin et al. 1995). Findings from SNP genotyping show that the c.1205A>T variant in this family and the Long Island family could have originated on the same allele.

Clinical signs and age at onset in this family were similar as in the previously described families. All families had early-onset radiographic chondrocalcinosis. In this family, we demonstrated the presence of CPP crystals by microscopy of synovial fluid from knee and elbow joints obtained during synovectomy. Recurrent acute inflammatory episodes and early joint destruction led to severe pain and to functional limitations beginning in the 3rd decade of life (range 20–40 years), and to early joint replacement from the 4th to 6th decade (range 30–58 years). Significant hip arthritis was observed in only two out of 9 informative subjects (22%) from this family, and had been reported previously in only three out of 10 members (30%) of the Maine family (Baldwin et al. 1995). In contrast, in the families from Israel, the Netherlands, and Long Island, all major weight-bearing joints of the lower extremities, including the hips, were described as typically affected (Eshel et al. 1990; Ramos et al. 2015; Williams et al. 2018). The reasons for these differences remain unknown.

Body height was not documented in the previously described families. From official documents issued around age 20 years, we

collected body height data for 11 individuals in this family and found a median height at the 10th percentile. Possibly, this could represent familial short stature, or alternatively a mild growth impairment caused by the OPG variant representing a limited phenotypic overlap with juvenile-onset Paget's disease 5 (PDB5). Short stature is a typical symptom in PDB5, which is caused by biallelic *TNFRSF11B* loss-of-function variants. Further body height data from CCAL1 families, including affected and unaffected family members, are needed to determine whether the OPG-XL protein causes defects of cartilage and bone metabolism leading not only to changes in bone density, but also to a mildly defective bone development and thus to a tendency towards short stature. A recent study (including human induced pluripotent stem cells expressing OPG-XL) demonstrated increased cartilage mineralization with concurrent lower subchondral bone mineralization as a result of the TNFRSF11B readthrough variant, which could possibly affect endochondral ossification during skeletal development towards reduced stature (Rodríguez Ruiz et al. 2022). Findings highlight the importance of studying multiple tissues/cells/phenotypes to determine the underlying pathways of disease caused by mutations such as the *TNFRSF11B* c.1205A>T stopLoss variant.

A previous study reported osteopenia with CCAL1 (Williams et al. 2018) and likewise we found osteopenia and additionally osteoporosis in this family. The OPG-XL osteoprotegerin was initially described as a gain-of-function mutation (Ramos et al. 2015), but results of murine in vitro studies with synthetic OPG-XL were considered to indicate a loss-of-function (Mitton-Fitzgerald et al. 2021). In vitro, the OPG-XL protein resulted in a loss-of-function in the RANKL-mediated osteoclastogenesis and in increased osteolytic activity, offering a possible explanation for osteopenia and arthritis in CCAL1. In contrast, a gain-of-function would have promoted inadequate bone resorption and osteopetrosis. Nevertheless, clinical findings support a genetic gain-of-function (Ramos et al. 2015). In humans, the c.1205A>T stopLoss variant represents the only known heterozygous, genetically dominant disease-causing variant, whereas all null mutations of *TNFRSF11B* function as PDB5-causing recessives with apparently healthy heterozygous carriers.

Biallelic inactivating *TNFRSF11B* variants reduce OPG function and result in the (severe) autosomal recessive, juvenile-onset phenotype of PDB5, while the heterozygous OPG-XL mutation causes the (relatively milder) adult-onset phenotype of CCAL1. PDB5 is a rapidly disabling disorder beginning in the first decade of life and characterized by increased bone resorption and deposition. Clinical features include skeletal demineralization, osteoporosis, osteosclerosis, cortical and trabecular thickening, progressive skeletal deformities, fractures, vertebral collapse, and skull enlargement, and occasionally extra-skeletal manifestations such as hearing loss and retinopathy (Cundy et al. 2002). However, despite their differences, PDB5 and CCAL1 share a small subset of features. The current CCAL1 family presented demineralization, osteoporosis, vertebral collapse, and progressive height loss, which are typical features of PDB5 as well. Possibly, short stature could represent another shared sign of PDB5 and CCAL1.

All familial forms of CPPDD are rare, but the literature completely lacks population-based studies to date. Given that in all molecularly proven CCAL1 families, all affected individuals presented rather uniformly physically severely disabled at an age of 40–60 years, we would hypothesize, based on the gnomAD data (see results) that the paucity of reported families does not reflect reduced penetrance or highly variable expressivity. In gnomAD, the c.1205A>T variant was present with an allele frequency of 4 in 1,614,162 (2.48e−6) and zero homozygotes, which would equate to a frequency of the heterozygous variant in the population of 1:201,770 individuals and would suggest that there should be dozens of affected families in the US as well as in Europe, and many more worldwide. Furthermore, if other stop lost alterations of *TNFRSF11B* (e.g., c.1204T>C and c.1206A>C, see results) would be associated with the clinical signs and symptoms of CCAL1 as well, this would predict an even higher prevalence of the disorder. Hence, the molecular data suggest that CCAL1 is a rare and underdiagnosed disorder.

In conclusion, we describe the fourth molecularly proven CCAL1 family with a total of 12 individuals with CPPDD manifesting in the third decade of life. In all families, including this one, the disorder resulted from a recurrent heterozygous stop-loss variant c.1205A>T in the *TNFRSB11B* gene on chromosome 8q, which predicts an extended osteoprotegerin protein with 19 additional amino acid residues; p.(Ter402Leuext*19). The clinical signs and symptoms of CCAL1 (caused by the recurrent dominant stop-loss variant in *TNFRSF11B*) and the corresponding disorder of PDB5 (caused by various recessive loss-of-function variants in *TNFRSF11B*) show only limited phenotypic overlap, including osteoporosis, osteopenia, joint degeneration, vertebral collapse, and possibly growth impairment/short stature, suggesting different underlying pathomechanisms. The rare type of mutation suggests that CCAL1 is a rare disorder; but we anticipate that there could be a few further *TNFRSF11B* stop lost variants causing CCAL1. The findings underscore that CCAL1 is a distinct rare Mendelian disorder for which the underlying molecular basis is now known.

Author Contributions

The study was carried out as part of the medical thesis of Anna-Christina Pansa. Anna-Christina Pansa suggested the subject of the thesis, and Oliver Bartsch was her doctoral supervisor. Anna-Christina Pansa and Oliver Bartsch drafted the manuscript and assembled Figure 1 and Table 1, and Mareike Selig contributed Figure 2. Mareike Selig and Matthew A. Brown critically revised the manuscript. All authors collected and/or interpreted clinical, radiological, and/or molecular data of the patients, and all authors gave final approval of the version to be published. Oliver Bartsch agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Andrew, L. J., V. Brancolini, L. S. de la Pena, et al. 1999. "Refinement of the Chromosome 5p Locus for Familial Calcium Pyrophosphate Dihydrate Deposition Disease." *American Journal of Human Genetics* 64: 136–145.

Baldwin, C. T., L. A. Farrer, R. Adair, R. Dharmavaram, S. Jimenez, and A. Anderson. 1995. "Linkage of Early-Onset Osteoarthritis and Chondrocalcinosis to Human Chromosome 8q." *American Journal of Human Genetics* 56: 692–700.

Cundy, T., M. Hegde, D. Naot, et al. 2002. "A Mutation in the Gene *TNFRSF11B* Encoding Osteoprotegerin Causes an Idiopathic Hyperphosphatasia Phenotype." *Human Molecular Genetics* 21: 19–27.

Doherty, M., and P. Dieppe. 1986. "Crystal Deposition Disease in the Elderly." *Clinical Rheumatology* 12: 97–116.

Eshel, G., A. Gulik, N. Halperin, et al. 1990. "Hereditary Chondrocalcinosis in an Ashkenazi Jewish Family." *Annals of the Rheumatic Diseases* 49: 529–530.

GnomAD. 2025. *NM_002546.4(TNFRSF11B):c.1205A>T*. GnomAD [v4.1.0]. https://gnomad.broadinstitute.org/gene/ENSG00000164761?dataset=gnomad_r4.

Hollander, J. L., R. A. Jessar, and D. J. McCarty. 1961. "Synovianalysis: An Aid in Arthritis Diagnosis." *Bulletin on the Rheumatic Diseases* 12: 263.

Hughes, A. E., D. McGibbon, E. Woodward, J. Dixey, and M. Doherty. 1995. "Localisation of a Gene for Chondrocalcinosis to Chromosome 5p." *Human Molecular Genetics* 4: 1225–1228.

Mitton-Fitzgerald, E., C. M. Gohr, C. J. Williams, A. Ortiz, G. Mbalaviele, and A. K. Rosenthal. 2021. "Effects of the *TNFRSF11B* Mutation Associated With Calcium Pyrophosphate Deposition Disease in Osteoclastogenesis in a Murine Model." *Arthritis and Rheumatology* 73: 1543–1549.

Pendleton, A., M. D. Johnson, A. Hughes, et al. 2002. "Mutations in *ANKH* Cause Chondrocalcinosis." *American Journal of Human Genetics* 71: 933–940.

Ramos, Y. F., S. D. Bos, R. van der Breggen, et al. 2015. "A Gain of Function Mutation in *TNFRSF11B* Encoding Osteoprotegerin Causes Osteoarthritis With Chondrocalcinosis." *Annals of the Rheumatic Diseases* 74: 1756–1762.

Rodríguez Ruiz, A., M. van Hoolwerff, S. Sprangers, et al. 2022. "Mutation in the CCAL1 Locus Accounts for Bidirectional Process of Human Subchondral Bone Turnover and Cartilage Mineralization." *Rheumatology (Oxford, England)* 62: 360–372.

Rosenthal, A. K., and L. M. Ryan. 2016. "Calcium Pyrophosphate Deposition Disease." *New England Journal of Medicine* 374: 2575–2584.

Ryan, L. M., and D. J. McCarty. 1997. "Arthritis and Allied Conditions: A Textbook of Rheumatology." In *Calcium Pyrophosphate Crystal Deposition Disease, Pseudogout, and Articular Chondrocalcinosis*, edited by W. J. Koopman, 13th ed., 2103–2126. Williams and Wilkins.

Williams, C. J., U. Qazi, M. Bernstein, et al. 2018. "Mutations in Osteoprotegerin Account for the CCAL1 Locus in Calcium Pyrophosphate Deposition Disease." *Osteoarthritis and Cartilage* 26: 797–806.

Williams, C. J., Y. Zhang, A. Timms, et al. 2002. "Autosomal Dominant Familial Calcium Pyrophosphate Dihydrate Deposition Disease Is Caused by Mutation in the Transmembrane Protein ANKH." *American Journal of Human Genetics* 71: 985–991.

Zhang, W., M. Doherty, T. Bardin, et al. 2011. "European League Against Rheumatism Recommendations for Calcium Pyrophosphate Deposition. Part I: Terminology and Diagnosis." *Annals of the Rheumatic Diseases* 70: 563–570.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1** Supporting Information.