EXTENDED REPORT

A gain of function mutation in TNFRSF11B encoding osteoprotegerin causes osteoarthritis with chondrocalcinosis

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ABSTRACT

Objective To identify pathogenic mutations that reveal underlying biological mechanisms driving osteoarthritis (OA).

Methods Exome sequencing was applied to two distant family members with dominantly inherited early onset primary OA at multiple joint sites with chondrocalcinosis (familial generalised osteoarthritis, FOA). Confirmation of mutations occurred by genotyping and linkage analyses across the extended family. The functional effect of the mutation was investigated by means of a cell-based assay. To explore generalisability, mRNA expression analysis of the relevant genes in the discovered pathway was explored in preserved and osteoarthritic articular cartilage of independent patients undergoing joint replacement surgery.

Results We identified a heterozygous, probably damaging, read-through mutation (c.1205A=>T; p.Stop402Leu) in TNFRSF11B encoding osteoprotegerin that is likely causal to the OA phenotype in the extended family. In a bone resorption assay, the mutant form of osteoprotegerin showed enhanced capacity to inhibit osteoclastogenesis and bone resorption. Expression analyses in preserved and affected articular cartilage of independent OA patients showed that upregulation of TNFRSF11B is a general phenomenon in the pathophysiological process.

Conclusions Albeit that the role of the molecular pathway of osteoprotegerin has been studied in OA, we are the first to demonstrate that enhanced osteoprotegerin function could be a directly underlying cause. We advocate that agents counteracting the function of osteoprotegerin could comply with new therapeutic interventions of OA.

INTRODUCTION

Osteoarthritis (OA) is a prevalent, complex, disabling disease of the articular joints, characterised by degradation of the hyaline articular cartilage and remodelling of the subchondrial bone with sclerosis. As the population ages and obesity rates rise, OA is becoming more prevalent and current estimates of symptomatic OA are 9.6% and 18.0%, respectively.1 Yet, there is no effective therapy to reverse or slow down the disease; analgesia, physiotherapy and, at end stage, joint replacement surgery are the main treatment options. As a result, OA has a large detrimental impact on the quality of life of the elderly and causes a major burden on health and social care systems, with indirect costs being predominant.1,2 To allow the development of new therapies, there is an ongoing need for insight into the underlying biological mechanisms driving OA. Despite the detection of compelling OA susceptibility loci,3–4 such insights, until now, remain limited. To detect rare mutations with large to moderate effect sizes, recently, focus has been to apply next-generation sequencing of exomes of severely affected family based patients. The value of identified rare variants lies in the characterisation of causal gene functions and underlying novel pathways in complex disease processes.5–6 Notably, in this respect, among the identified genes with mutations causing early onset families with OA associated syndromic skeletal phenotypes such as collagen type II (COL2A1),7 the growth differentiating factor 5 (GDF5),8 collagen type 11-α1 (COL11A1),9,10 cartilage oligomeric protein (COMP)11 and extracellular signalling molecule and member of the TGF-β superfamily (SMAD3)12,13 were three genes that appeared to additionally confer risk to common OA phenotypes, namely SMAD3,14 COL11A1,15,16 and the GDF5 gene which reached genome-wide significance.8,17

Previously, we reported on a single early onset family with familial generalised osteoarthritis (FOA) without any dysplasia, with radiographic chondrocalcinosis as reflected by calcifications within the joint and fibro-cartilage.18 Candidate gene screening, showed that none of the genes encoding the extracellular matrix structural proteins were involved in the pathogenesis of this osteoarthritic phenotype.18 Furthermore, a genetic linkage search in this and 6 other FOA families revealed significant linkage on chromosome 2q32 albeit that the attribution of this particular family to the linkage was moderate, and Sanger sequencing of positional exons failed to definitively indicate the causal mutation.19 Recently, a relevant extension was established in the ascertainment of the family under study; additional affected distant family members with the phenotype were detected, whereas, third-generation family members definitively developed the FOA phenotype. Such extensions increase the power of successful identification of genes considerably. To identify pathogenic OA mutations, we applied exome sequencing to two distant family members.
MATERIALS AND METHODS

Study design
To identify pathogenic mutations underlying development of OAm exome sequencing was performed for two distantly related FOA family members. After applying an eligible prioritisation scheme, five candidates were selected for de novo genotyping across the extended family (n=16). Linkage analysis was performed for the mutation that was shared among all affected members, and the mutation was validated by de novo genotyping of independent OA cases (n=1467) and healthy controls (n=744). Functionality of the mutation was investigated in an in vitro cell-based model, and generalisability was investigated by comparing gene expression in OA-affected cartilage with preserved cartilage (n=33).

An overview of the workflow for the analyses, identification of the causal mutant, and follow-up on the mutation is provided in online supplementary figure S1.

Study populations

Cases

FOA family members: Previously, we reported on an early onset family with FOA (MIM 165720-1) without any dysplasia, and with radiographic chondrodysplasia as reflected by calcifications within the joint and fibro-cartilage (figure 1A, and see online supplementary table S1).18 Family members were collected through rheumatology clinics from different parts of The Netherlands and France. The age of onset of OA in the family varies between 30 years and 50 years, and OA arises in the absence of mild or severe chondrodysplasia. The phenotype within these families is characterised by distinct progressive OA with symptoms and radiographic characteristics of OA at multiple joint sites simultaneously, including involvement of the hands with noduli, knees, hips and spine. More recently, relevant additional family members were ascertained (figure 1B, subjects 4, 5 and 16) with the unique FOA phenotype of this family. The study was approved by the medical ethic committee, and written informed consent was obtained from all participants.

Controls

Occurrence of the osteoprotegerin (OPG) read-through mutation in the population was tested by de novo genotyping in healthy subjects and OA cases. Middle-aged partners (n=744) of the offspring of nonagenarian siblings from the Leiden Longevity study (LLS)20 were considered as controls for OA cases and are further named ‘random controls’. OA cases included were selected from the Genetics osteoarthritis and Progression (GARP) study (n=177), 21 the Patients Prospectively Recruited in Knee and hip Arthroplasty (PAPRIKA) study performed at the Leiden University Medical Center (Dept. Orthopaedics; n=1137), 22 and the ongoing Research Arthritis and Articular Cartilage (RAAK) study (n=153) aimed at the banking of joint materials (cartilage, bone and where available, ligaments) and bone marrow-derived mesenchymal stem cells (hip joints only) of OA patients and controls in the Leiden University Medical Center and collaborating outpatient clinics in the Leiden area. More detailed description of the studies included can be found in the online supplementary methods.

Ethical permission for all studies described was obtained from the appropriate medical ethical committees. Written informed consent was obtained from all participants following detailed explanation of the study.

Exome sequencing

Exome sequencing of the FOA family members was performed by Illumina HiSeq2000 technology (Beijing Genome Institute). The sequences of each individual were generated as 100 bp paired-end reads, after enrichment of 44 MB exonic sequences by NimbleGen EZ (Roche NimbleGen, Madison, Wisconsin, USA). Raw image files were processed by Illumina base calling Software V1.7 with default parameters. SOAPaligner/SOAP2.21 was used to map reads onto the hg19 reference genome (GRCh37) at the UCSC Genome Browser website (http://genome.ucsc.edu/). Approximately, 71% of bases originated from the targeted exome, resulting in a mean coverage of 55.93-fold and 55.81-fold for subjects 1 and 4, respectively. More than 83% of the targeted exons were covered more than 10 times. Single-nucleotide variants were subsequently called by the SOAPsnp. The final variant-filtering scheme, which focused on sharing of novel23 pathogenic mutations between the distant related family members, is detailed in online supplementary table S2.

Genotyping

Heterozygous deleterious gene variants shared among the two family members were chosen to fit efficiently in a Sequenom multiplex assay designed by the Assay Designer software V3.1 (Sequenom, San Diego, California, USA). Single nucleotide polymorphisms (SNP) were genotyped by mass spectrometry (the homogeneous MassARRAY system; Sequenom, San Diego, California, USA).
California, USA) using standard conditions and as described elsewhere. In short, PCR reactions were carried out in a final volume of 5 μL and contained 2.5 ng of genomic DNA. Genotypes were assigned by using GenoTyper V3.0 software (Sequenom, San Diego, California, USA). Variants were genotyped across available FOA members (see online supplementary table S1 and supplementary table S3), OA case studies (GARP), additional mutations by PCR artefacts was confirmed by sequencing from both strands.

**In vitro mutagenesis**

A mammalian expression vector (pORF9) containing the human TNFRSF11B open-reading frame was obtained from InvivoGen. The point mutation causing read-through was introduced using the QuickChange Site-Directed Mutagenesis kit (Agilent Technologies) according to the manufacturer’s protocol with the following primers: Forward 5'-TAAGCTGTTATCATCTGGAAATGGCATTGAC-3' and Reverse 5'-GCTCAATGGGCATTTCCTGTAAGCAGCTTATA-3'.

Introduction of the aimed mutation and exclusion of additional mutations by PCR artefacts was confirmed by sequencing from both strands.

**Production of recombinant osteoprotegerin and Western blotting analysis**

For the production of recombinant OPG, HEK293T cells were cultured in OPTI-MEM I-Reduced Serum Medium (Invitrogen). Cells were transfected either with empty vector or vector with wild-type or mutant TNFRSF11B using FuGENE 6 reagent (Roche). Tissue culture supernatants were collected after 3 days, cleared of debris by centrifugation, and stored frozen. To confirm expression and quality of the recombinant proteins in the conditioned media, Western blotting was performed on 45 μL tissue culture supernatant as described previously using the rabbit monoclonal antibody EPR3592 (Epitomics; 1:5000 diluted) to detect OPG. Subsequently, concentration of OPG in the tissue culture supernatant was determined by ELISA (R&D Systems) following the manufacturer’s instructions.

**In vitro osteoclastogenesis**

Putative differences in the activity of wild-type and mutant OPG was investigated by applying an in vitro model of osteoclastogenesis on 96-wells plates with pieces of human bone. Poietics human osteoclast precursors (Lonza) were seeded onto the OsteoAssay Human Bone Plate (Lonza; 10^4 cells per well) in the absence (negative control) or in the presence (positive control) of receptor activator of the nuclear factor-KB ligand (RANKL). Either nothing, or equal volumes of tissue culture supernatant from the HEK293T cells was added to osteoclast growth and differentiation medium (Lonza) to a final concentration of 100 ng/mL OPG. Osteoclast precursors were cultured for 6 days before adding fresh osteoclast growth and differentiation medium. Subsequently, cells were grown for 16–18 h, and media were harvested for analysis.

To analyse osteoclastogenesis, tartrate-resistant acid phosphatase staining (TRAP; Takara Bio) at day 7 of poietics human pre-osteoclast cultures was carried out following the manufacturer’s protocol. Concentration of CTX-I in the media was determined by applying CTX-I CrossLaps ELISA (IDS) according to the manufacturer’s protocol. The amount of CTX-I released in the tissue culture supernatant reflects the amount of bone resorption by mature osteoclasts. The in vitro osteoclastogenesis assays were carried out twice, in triplicate.

**Gene expression analysis**

To explore the generalisability of the discovered pathway towards common forms of OA, mRNA expression was analysed in an available dataset (Illumina HT-12 v3 microarray) of 33 paired preserved and osteoarthritic cartilage samples of the RAAK study. Following RNA isolation of the collected cartilage, samples were prepared for gene expression by microarray analysis as described in detail in the online supplementary methods. Subsequently, data were exported for analyses using Limma. As implemented in Limma, a paired t test was applied to all sample pairs. Overall mean normalised probe expression levels of the measured genes in cartilage ranged from 6.58 to 14.91 with a mean of 7.4 and a median of 7.1 with a strong right tailing.

**RESULTS**

**Identification of read-through mutation in TNFRSF11B**

Whole exome sequencing was applied to two distant FOA family members (figure 1B; subjects 1 and 4) and identified 57 018 and 60 652 variants, respectively, which fulfilled the quality criteria in each patient. To identify pathogenic mutations, a prioritisation scheme was applied in which we excluded variants present in dbSNPv132 (including 1000 Genomes Pilot project data), synonymous variants, tolerated missense variants as determined by SIFT (http://sift.jcvi.org/), intergenic variants and intron variants. Additionally, we excluded variants detected in in-house analysed human genomes (n=221), in-house analysed human exomes (n=43), and analysed independent human genomes by the Genome of The Netherlands project (GoNL n=473; http://www.nlgenome.nl). This reduced the number of variants to, respectively, 102 and 105 variants per subject that were predicted to affect gene function (see online supplementary table S2). Given that these two FOA patients were diametrically opposed (figure 1B subject 1 and 4; six meioses apart) they are expected to share the causal variant. We found, in total, five heterozygous deleterious gene variants that were shared among the two patients: 3 novel missense damaging, 1 splice site mutation, and 1 read-through mutation located in, respectively, the preferentially expressed antigen in melanoma family member 22 (PRAMEF22), dynemin axonemal heavy chain 10 (DNAH10), tetrarospin 8 (TSPAN8), mitotic control homologue gene (DIS3), and the tumour necrosis factor receptor superfamily member 11b (TNFRSF11B; table 1).

Genotyping of these variants in all members of the extended FOA family (n=16, figure 1) showed complete cosegregation with the phenotype across three generations only for the read-through mutation within the TNFRSF11B gene, resulting in a genome wide significant linkage analysis at this locus (LOD-score of 3.48; see online supplementary table S4). Furthermore, the
variant did not occur in 1467 other OA-affected subjects (the GARP, RAAK and PAPRIKA study) nor in 744 random controls (LLS) indicating that the mutation may be private for this family. These data indicate that the A>T nucleotide change (A1205T) at the STOP codon of the TNFRSF11B gene-encoding OPG is likely causal to the FOA phenotype in this family. The read-through mutation results in the generation of a Leucine amino acid (X402L), and subsequent 19 additional amino acids at the C-terminus of OPG (c.1205A=>T; p.Stop402Leu; see online supplementary figure S3).

**Effect of read-through mutation on OPG protein function**

OPG is a soluble decoy receptor which inhibits osteoclastogenesis by competing with the receptor activator of the nuclear factor-κB (RANK), expressed on the membrane of pre-osteoclasts, for the binding of the nuclear factor κB ligand (RANKL; see online supplementary figure S4). To investigate the putative effect of the (X402L) read-through mutation on OPG protein function, its activity was investigated in an in vitro osteoclastogenesis assay. Mutant OPG gene was generated by site-directed mutagenesis and recombinant wild-type and mutant OPG proteins were produced in transfected HEK293T cells. Protein integrity was confirmed by Western blotting (see online supplementary figure S5). Subsequently, recombinant proteins were added to differentiating poietics human preosteoclasts seeded onto OsteoAssay Human Bone Plates. After 1 week, efficiency of bone resorption upon osteoclastogenesis of the osteoclast precursors was quantified by determining the amount of CTX-I released in the medium of the differentiating osteoclasts (figure 2A). A clear difference could be observed between cells cultured in the presence or in the absence of OPG. Figure 2 shows that the addition of RANKL to pre-osteoclasts greatly enhanced osteoclastogenesis, as can be concluded from the higher concentrations of CTX-I (figure 2A, +). Adding medium from HEK293T cells transfected with empty vector did not have an effect (figure 2A, + sup). However, addition of recombinant wild-type OPG (figure 2A, + wt OPG) or mutant OPG (figure 2A, + mt OPG) significantly reduced bone resorption, respectively, 1.2-fold (p=0.049) and 1.6-fold (p=8.4×10⁻⁵), reflecting a statistical, significantly increased capacity of the mutant-OPG to inhibit osteoclastogenesis as compared to the wild type (p=4.7×10⁻⁵). To visually inspect the osteoclastogenesis in this experiment, a tartrate-resistant acid phosphatase (TRAP) staining was performed for the different conditions. Mature osteoclasts are distinguished as large multinucleated TRAP-positive cells (figure 2B, indicated with an arrow). Together, these data demonstrate that the identified read-through mutation in TNFRSF11B, causing the FOA phenotype, enhances OPG protein function.

**Generalisability of the OPG/RANK/RANKL pathway in OA**

To investigate the response of TNFRSF11B and the tumour necrosis factor superfamily member 11 (TNFSF11) gene, encoding RANKL, to the general OA pathophysiological process in articular cartilage, we explored a microarray expression dataset of OA affected and preserved cartilage, taken from the same joints, of 33 independent OA patients that underwent joint replacement due to end-stage OA disease (RAAK study). TNFRSF11A encoding RANK appeared to be expressed at a very low level (in the lowest quartile of expression) and was therefore not detected well. By contrast, the expression levels of TNFRSF11B in osteoarthritic and preserved cartilage samples appeared in the highest expression quartile with little interindividual variation. A highly significant upregulation of TNFRSF11B (2.1-fold change, p value=1.9×10⁻⁸ and TNFSF11 (1.2-fold change, p value=3.1×10⁻⁴) was observed in osteoarthritic cartilage, as compared with preserved cartilage of the same joint, indicating that these genes are responsive to the OA process in articular cartilage (see online supplementary table S5).

It has been shown that bone homeostasis merely depends on the ratio between TNFRSF11B and TNFSF11.²⁹ Therefore, we compared this ratio in osteoarthritic and preserved cartilage. As shown in figure 3, we found a significant increase of the TNFRSF11B/TNFSF11 ratio (p=2.0×10⁻⁴) in osteoarthritic articular cartilage which may contribute to respective mineralisation of the cartilage and eventually formation of bone, a major hallmark of the ongoing OA disease process.³⁰

**DISCUSSION**

We have identified a heterozygous read-through mutation (c.1205A=>T; p.Stop402Leu) in TNFRSF11B that is likely causal to the severe OA phenotype in the here-studied, extended, FOA family. Functional analyses showed that this mutation establishes a gain of function of the encoding protein OPG. Given that the mutant OPG decoy receptor more efficiently antagonises osteoclastogenesis, and the FOA phenotype is characterised by calcifications within the joint and fibrocartilage, we hypothesise that the detected mutation acts via an unfavourable interplay between (subchondral) bone and cartilage towards enhanced matrix mineralisation. Gene expression analyses of preserved and osteoarthritic articular cartilage of independent OA patients showed that enhanced OPG-mediated antagonism is a general phenomenon of the pathophysiological process within osteoarthritic articular cartilage. This is in line with other reports showing increased expression of OPG in arthritic cartilage compared to healthy cartilage.³¹ ³² Also, during intervertebral disc degeneration, a process highly comparable to the pathological processes occurring in articular cartilage during OA, OPG appeared to be increased in correlation

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**Table 1** Detected novel variants overlapping in family members 1 and 4

<table>
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<tr>
<th>CHR</th>
<th>POS</th>
<th>Base*</th>
<th>Codon*</th>
<th>AA subst</th>
<th>Gene name</th>
<th>Expri</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>13036596</td>
<td>T/C</td>
<td>ATA&gt;ACA</td>
<td>I223T</td>
<td>Preferentially expressed antigen in melanoma Family member 22 (PRAF22)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>124281300</td>
<td>C/A</td>
<td>TCT&gt;Tat</td>
<td>S577Y</td>
<td>Dynetin, axonemal, heavy chain 10 (DNAH10)</td>
<td>7.1</td>
<td>7.1×10⁻¹</td>
</tr>
<tr>
<td>7</td>
<td>71523146</td>
<td>C/A</td>
<td>GTT&gt;TTG</td>
<td>V209F</td>
<td>Tetraspan 8 (TSPAN8)</td>
<td>7.2</td>
<td>9.9×10⁻¹</td>
</tr>
<tr>
<td>13</td>
<td>73335781</td>
<td>C/T</td>
<td>splice site</td>
<td>splice site</td>
<td>Mitotic control homologue gene (DIS3)</td>
<td>7.3</td>
<td>9.8×10⁻¹</td>
</tr>
<tr>
<td>8</td>
<td>11993614</td>
<td>T/A</td>
<td>TAA=TIA</td>
<td>“&gt;”=L</td>
<td>Tumour necrosis factor receptor superfamily, member 11b (TNFRSF11B)</td>
<td>10.2</td>
<td>2.9×10⁻⁸</td>
</tr>
</tbody>
</table>

*Variants are novel, missense damaging, splice-sites or read-through, not present in dbSNP132 (including 1000 Genomes Pilot project data), and not detected in inhouse analysed human genomes (LLS, n=221), inhouse analysed human exomes (GARP, n=43) and analysed human genomes by means of the Genome of The Netherlands Project (GoNL, n=473). AA subst., amino acid substitution; Base, base change; CHR, chromosome; codon, codon change; Expri, relative expression level as determined in the microarray analyses of 33 osteoarthritic and preserved paired cartilage samples; GARP, Genetics osteoarthritis and Progression; LLS, Leiden Longevity study; ND, not determined; p Value, nominal p-value of the differential expression between paired osteoarthritic and preserved samples (n=33 pairs); POS, chromosomal position.
with degeneration grade and coinciding with the presence of microscopic calcifications.33 Albeit that the role of the molecular pathway of OPG has been studied in OA, a direct causal role towards matrix mineralisation has not been reported up until now, and it may provide new clues to the development of anti-osteoarthritic drugs.

Retrospectively, we observed in our family a positional linkage signal at the position of the TNFRSF11B gene (see online supplementary table S4).19 34 Additionally, of note is a report of Baldwin et al35 that indicated linkage to chromosome 8q in a family with early onset OA and chondrocalcinosis at a locus encompassing the TNFRSF11B gene. Given the results, it would be interesting to further investigate the status of the TNFRSF11B gene of the reported family by next-generation sequencing. Although TNFRSF11B was not previously detected as OA susceptibility gene by virtue of genome-wide association studies, consistent genetic associations of TNFRSF11B with knee OA were found in the general population,32 36 in particular, in association with OA progression as measured by changes in osteophyte grade. More investigations are necessary to explore whether genetic variations in genes of the OPG pathway confer risk to common OA.

Transgenic mice that overexpress TNFRSF11B had increased skeletal radiodensity, osteopetrosis and increased bone density due to decreased numbers of mature osteoclasts,37 however, no reference or investigation has been made to OA as being part of the phenotypes of these mice or, for that matter, radiographic chondrocalcinosis. Since long, epidemiological studies have shown that high bone mineral density is associated with development of OA38 39 and may also affect OA progression.40 Therefore, alternatively to the mentioned enhanced matrix mineralisation of the cartilage, expression of mutant OPG could result in systemic higher bone mass, thereby predisposing to OA due to a suboptimal cartilage-bone interplay. Since we have no bone density measurements available for our FOA family
members, the role of systemic high bone density in the OA onset requires further investigation. By contrast, loss of function mutations in the TNFRSF11B gene, that abolish the inhibitory action on osteoclastogenesis, have been found to cause Juvenile Pagets disease, characterised by accelerated bone turnover, fractures of the long bone and hyperphosphatasa. 41 Furthermore, SNPs within the TNFRSF11B gene have been found to be associated in genome-wide association studies to common osteoporosis or low bone mineral density. 42

Despite the fact that it has been convincingly demonstrated that a strong interaction between subchondral bone and the articular cartilage exists, and that changes in mineralisation of bone and cartilage reflect pathophysiology of OA, several seemingly contradictory observations have been made, and the direction of the changes of the subchondral bone density and mineralisation in OA patients appeared unclear, and possibly dependent on the disease status. 43 44 More recently, OA patients were shown to benefit from treatment with strontium ranelate, which is a drug licensed for osteoporosis and acts by increasing bone formation while decreasing bone resorption. 45 These studies, however, have been the subject of debate, 46 47 and seem in contrast with our current findings, and with the extensive literature indicating that individuals with high systemic bone mass are at increased risk for the incidence of OA. 48 It could be speculated that, initially, strontium ranelate enhances stabilisation of the dynamically changing OPG/RANKL ratios as it was observed across different grades of OA, 49 resulting in OPG and RANKL levels in the joint balancing towards a net ratio of bone formation. This may temporarily strengthen the joint and decelerate OA progression. Although therapeutic treatment of rats with another bone-forming agent, OPG-Fc, did not directly improve the OA phenotype, it did decrease pain in particular when starting the treatment at early stages. 50 Increased bone thickness as a result of the treatment may prevent evanescence of the joint leading to a ‘desensitisation’. Careful investigation of the long-term effects of these bone-forming therapies in OA is, definitely, necessary 50 thereby taking the existence of different (sub)types of OA characterised by differences in bone mineral density 51 into account.

The molecular pathway of the OPG/RANKL in terms of its manipulation for therapeutic benefit has been studied extensively, especially to enhance the OPG-mediated RANK antagonism in osteoporosis, or, in the case of cancer, growth of bone metastasis. 52 These studies showed that all the C-terminal domains of OPG are necessary for high-affinity association with RANKL. 53 As a result, the design of short recombinant, monomeric OPG molecules that mimic the receptor binding and dimerisation functions of the multidomain, 100-kDa homodimeric OPG polypeptide has been indicated as challenging. 54

Given that the here-detected read-through mutation results in the addition of 19 amino acids at the C-terminus of the OPG protein, we hypothesise that the gain of function may be established through an enhanced stability of OPG dimers, thereby increasing the efficiency of RANKL binding. The use of the here-identified X402L mutant OPG protein could provide a putative new starting point to further establish such a stable molecule.

In summary, we have identified a read-through, gain of function mutation within the TNFRSF11B gene that is likely causal to the FOA phenotype in the here-studied family. With this result, we are the first to demonstrate that enhanced OPG-mediated RANK antagonism could be directly underlying the onset of OA. The exact mechanism on how this is brought about remains to be established. Given that enhanced OPG-mediated antagonism appears to be a more general phenomenon in the pathophysiological process of osteoarthritic cartilage, we advocate that, in contrast to the use of bone-forming therapies, agents counteracting OPG function could comply with the development of new disease-modifying treatments in OA.

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Contributors YFM, PES and IM contributed to the overall study design, data interpretation and writing of the manuscript. YFMR performed the functional studies. RvdB contributed to DNA sample preparations and confirmation of exome sequencing findings by de novo genotyping. SDB contributed the mRNA expression analyses. MK and RGHHN contributed to collection and interpretation of clinical data and cartilage samples. KY and E-WEMWL contributed to the bio-informatics of the exome sequencing data. All authors read and approved the manuscript.

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Competing interests None.

Patient consent Obtained.

Ethics approval Ethical permission for all studies described was obtained from the appropriate medical ethical committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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Basic and translational research
Basic and translational research


A gain of function mutation in TNFRSF11B encoding osteoprotegerin causes osteoarthritis with chondrocalcinosis

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