SHORT REPORT

Mutations in WNT1 are a cause of osteogenesis imperfecta

Somayeh Fahiminiya,1 Jacek Majewski,1 John Mort,2 Pierre Moffatt,2 Francis H Glorieux,2 Frank Rauch2

ABSTRACT

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder that is mainly characterised by bone fragility and often short stature. Extraskeletal findings, such as tooth abnormalities (dentinogenesis imperfecta), can also be observed. OI is usually transmitted in an autosomal dominant fashion and is mostly caused by mutations in COL1A1 and COL1A2, the genes encoding the collagen type I α1 and α2 chains. Other dominantly and recessively inherited forms of OI are rare and can be caused by mutations in at least 10 different genes (CRTAP, LEPRE1, PPBP, SERPINH1, FKBP10, SP7, SERPINF1, BMP1, TMEM38B, IFTM5). The classification of OI is a topic of ongoing debate, and there is no consensus as to how new genetic findings should be integrated. According to the clinical criteria of the expanded Silence classification, OI type I represents the least severe part of the OI bone fragility spectrum, OI type II is the lethal form, and OI type III comprises the most severely affected individuals surviving the neonatal period. OI type IV is intermediate with regard to the severity of bone fragility, between OI type I and III.

Over the past 15 years, 148 individuals have been diagnosed with OI type IV in our centre at the Shriners Hospital for Children in Montreal. We first sequenced the coding regions and splicing sites of the two main OI genes (COL1A1/COL1A2) in all of these patients, which resulted in the identification of disease-related mutations in 134 subjects (67 in COL1A1; 67 in COL1A2). Further Sanger sequencing in an additional six patients revealed mutations in other genes that are known to cause bone fragility (LEPRE1, N=3; PPBP, N=1; LRP5, N=1; FKBP10, N=1). In the remaining eight individuals with OI type IV (from seven families) who were negative in initial molecular testing, whole exome sequencing was performed and the results are described here. The Institutional Review Board of McGill University approved the study. Clinical data were extracted by retrospective chart review and appropriate informed parental consent was obtained.

A total of 3 μg of genomic DNA was used for library preparation and exome capturing with Agilent SureSelect v3 (three samples) or v4 kits (five samples) (Agilent Technologies, Santa Clara, California, USA). The captured DNA samples were subsequently sequenced with 100 nucleotide paired-end reads on Illumina HiSeq2000. Bioinformatics analysis of generated exome sequencing data was performed with our pipeline as previously described. Briefly, the human genome assembly hg19 was used as the reference genome and the high-quality trimmed paired-end sequences were mapped against it using a Burrows-Wheeler algorithm (V0.5.9). Variant calling and annotation were performed by SAMtools V0.1.17 and ANNOVAR, respectively. The final list of variants composed of those with an allele frequency <0.0005 in the 1000 Genomes database, not previously seen in >5 individuals in our exome database (containing >550 samples) at Genome Quebec Innovation Center and predicted to be protein altering substitutions. Finally, we manually examined all of the potential candidate variants using IGV (Integrative Genomics Viewer).

Because of the presence of affected siblings in family 1 and the unaffected status of parents in all families, we carried out the analysis under the hypothesis of recessive mode of inheritance. In four of the eight patients, the analysis uncovered novel variants in the gene WNT1, which have not

To cite: Fahiminiya S, Majewski J, Mort J, et al. J Med Genet Published Online First: [please include Day Month Year] doi:10.1136/jmedgenet-2013-101567
Genotype-phenotype correlations

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of genes</th>
<th>Gene names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>19</td>
<td>WNT1; CFHR4; NEB; AKAP9; TTF1; LRP4; FOLH1; OR4A15; HNRRNPUL2; PCNX13; PC; PDZRN4; DIRP2; COQ10A; TPPP3; NUDT7; ADAD2; SPIRE2; ZNF347 †</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>WNT1; FRMD4A; KDM5A; CSRNP2; HSD17B6; LOR; KIAA0404; SMPD1 †</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>WNT1; PREP; FSP2; MTUS2; WDR66 †</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>INPP5B; WDR65; NBPF16; GIMAP8; TOPORS; NDUFA1; SLC12A1; LDHAL6B; UNC45A; TRCN18 †</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>FBXO2; SPARC; AH11; UBR2; ZAR1; FER116; ASPN; TNC; ANKR2D6; OR51G2; SPTBN5 †</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>ZMIZ2; KIR2DL3; EXO3; CD209; PMML1 †</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>HSFBAP1; ORK2; FAT1; GLE1; GLB112; CARD10; FKBP10 †</td>
</tr>
</tbody>
</table>

*Family 1 is a consanguineous family with two affected children, sharing 19 rare homozygous and multiple variants.
†The variants within 7TN, MUC4, MUC12, MUC5B, and MUC16 genes are not counted because these genes are known to accumulate mutations at a high rate, and thus are unlikely to be causative genes.
MAF, minor allele frequency.

previously been reported in dbSNP, 1000 Genome, EVS (Exome Variant Server) or our exome database (table 1). In those four patients, we did not identify any variants within the 12 known genes associated with autosomal dominant and recessive OI. The two of the patients were siblings from a consanguineous family (family 1, figure 1). In both siblings we detected a non-synonymous homozygous variant (c.428G>T (p.Cys143Phe)) in exon 3 of WNT1, located within an overlapping region of homozygosity of approximately 30 Mb length (see online supplementary figure S1). Both children were born at term without apparent abnormalities and had normal cognitive development. Muscle hypotonia was first noted between 3–6 months of age, but motor milestones were reached at the appropriate ages. The girl sustained the first long bone fracture at 17 months of age after a fall from standing height. In the following 2 years, she suffered 15 long bone fractures involving both the upper and lower extremities. When first evaluated at our institution at the age of 3 years, multiple vertebral compression fractures were noted (figure 1). She was short (height Z score −3.5), there was no joint or skin hyperlaxity, sclera were white, and teeth appeared normal on inspection. Lumbar spine areal bone mineral density (after two pamidronate infusion cycles) was very low (Z score of −3.8). The younger brother had the first fracture (clavicle) in the second week of life, sustained 12 long bone fractures in the following 4 years, and also had multiple vertebral compression fractures (see online supplementary figure S2). His height Z score was −2.6 at 4 years of age.

In subject II-1 of family 2, a 14 bp homozygous frameshift deletion (NM_005430: c.287_300del (p.Gln96Profs)) was detected. Sanger sequencing confirmed the homozygous deletion in this subject and showed that her parents were heterozygous for this deletion (see online supplementary figure S3). This girl had short stature (height Z score −3.4 at 3 years of age) and a history of multiple long bone and vertebral compression fractures (see online supplementary figure S2).

Subject II-1 of family 3 was compound heterozygous for two WNT1 variants (NM_005430: c.946_949insAACA (p.Ser317Lysfs) and c.1063G>T (p.Val355Phel)). Although parental DNA was not available, because of the proximity of the two loci, we were able to use the exome sequencing data to determine that the mutant alleles were mutually exclusive in paired reads, and were thus present in trans. Both variants were subsequently confirmed using direct Sanger sequencing (see online supplementary figure S3). This girl had a history of 30 long bone fractures before the age of 3 years, had multiple vertebral compression fractures (see online supplementary figure S2) and was short (height Z score −3.5 at 3 years of age).

All of the children with WNT1 mutations received intravenous bisphosphonate treatment, starting between 8–32 months of age. Nevertheless, they continued to suffer between 2–5 new long bone fractures per year.

In the other four individuals with OI type IV, exome sequencing revealed that one patient was compound heterozygous for a deletion of the entire exon 1 and a stopgain mutation in FKBP10 (data not shown), a gene associated with recessive OI.12 No conclusive result emerged from the analysis in the other three patients (table 1).

In this study we found rare and most likely deleterious WNT1 mutations in four children—originating from three unrelated families—with a clinical diagnosis of OI type IV. This strongly suggests that disruption of WNT1 causes OI in our subjects. Wnt proteins are known to be key regulators of bone mass and the Wnt signalling pathway is an important target for novel bone anabolic treatments of osteoporosis.13 14 Wnt proteins interact with cell surface LRP5 and other targets to activate bone forming cells.14 Homozygous loss of function mutations in LRP5 lead to osteoporosis-pseudoglioma syndrome, which resembles OI type IV with regard to its skeletal features.15 16 WNT1 defects have not previously been linked to bone diseases. The skeletal phenotype of the Wnt1 knockout mouse apparently has not been reported, but this mouse model has severe abnormalities in brain development.17 18 Neurological problems were not conspicuous features in our patients with WNT1 mutations. All patients were reported to have reached motor milestones at the appropriate ages and cognitive development was normal. However, the two children from family 1 were noted to have muscle hypotonia. For this reason, the girl had undergone cranial CT, but no abnormalities were reported. At least one of our subjects (II-1 in family 2) has a mutation that is expected to result in the total loss of function of WNT1, similar to the Wnt1 knockout mouse model. Thus, we hypothesise that the phenotypic discrepancy is related to different functions and/or compensatory mechanisms of WNT1 across species. It should be noted that, even in mice, the expressivity of the neurological phenotype ranged from adult onset ataxia to neonatal death,19 which may help us to understand the different effects of the loss of function in the vastly different genetic backgrounds of mice and humans.

Although in situ hybridisation studies have not detected Wnt1 expression during in utero mouse limb development,20 Wnt1 expression can be detected by real-time PCR in postnatal mouse long bones, including osteoblasts.21 Given the predominance of...
the brain phenotype in the global Wnt1 knockout mouse, this mouse model may not be ideal for elucidating the function of Wnt1 in bone cells. However, a mouse model of targeted Wnt1 inactivation in osteoblasts could be useful to assess whether WNT1 plays a direct role in regulating the activity of bone forming cells.

As to therapeutic considerations, our very limited experience suggests that intravenous bisphosphonate treatment, an antiresorptive approach, is less effective in children with WNT1 mutations than in other children with OI. It may be worthwhile exploring the effect of treatment approaches that stimulate the Wnt signalling pathway in bone forming cells. However, even

Figure 1  (A) Pedigrees of families with WNT1 mutations. (B) Radiographic findings in a girl with a homozygous p.Cys143Phe mutation in WNT1. The images were obtained after two cycles of intravenous pamidronate treatment, which explains the sclerotic bands close to the growth plates on all long bones. Cortices are very thin in extremity bones. The lateral view of the thoracic and lumbar spine shows severe compression fractures of all vertebral bodies. (C) Location of p.Cys143Phe and p.Val355Phe mutations in the WNT1 molecule. The WNT structure resembles a hand with the thumb and index finger grasping the cysteine-rich domain of the Frizzled receptor (shown as molecular surface view). Cys143 is involved in one of the 11 intramolecular disulfi de bridges that are the invariable hallmark of all Wnt proteins (figure 1, online supplementary table S1). Substitution of Cys143 by phenylalanine is likely to change the three dimensional structure of WNT1, destabilising the thumb region. Val355 is located in the 'index finger' of WNT1. This amino acid residue has been shown to interact directly with three amino acids on the surface of the Frizzled receptor. Substitution of Val355 by a phenylalanine is likely to disrupt the structure of the finger region due to the clash of the larger side chain with that of Phe349. This would undermine the interaction between WNT1 and Frizzled. (D) Amino acid conservation of the Cys143 and Val355 residues in WNT1 protein. Amino acid alignments were generated using Vector NTI. These residues are also conserved across the various Wnt proteins in humans (see online supplementary table S1).
though such treatments are under development,13 14 they are presently not available for use in children.

In summary, we detected WNT1 mutations in four children out of a group of 148 individuals with a clinical diagnosis of OI type IV. Disruption of WNT1 thus appears to be a rare cause of severe bone fragility in children.

Acknowledgements We thank Mark Lepik for the preparation of the figure and Patty Mason for technical assistance. We also wish to acknowledge the contribution of the high-throughput sequencing platform of the McGill University and Genome Quebec Innovation Centre (Montreal, Canada). FR received salary support from the Chercheur-Boursier Clinicien programme of the Fonds de la Recherche du Québec–Santé. JM is a recipient of a Canada Research Chair. This study was supported by the Shriners of North America.

Contributors All authors contributed to the concept, design, acquisition of data or analysis and interpretation of data. All authors contributed to drafting or revising the content and approved the final version.

Funding This study was supported by the Shriners of North America and the Fonds de recherche du Québec–Santé.

Competing interests None.

Patient consent Obtained.

Ethics approval The Institutional Review Board of McGill University approved the study.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES


Mutations in WNT1 are a cause of osteogenesis imperfecta

Somayyeh Fahiminiya, Jacek Majewski, John Mort, et al.

J Med Genet published online February 23, 2013
doi: 10.1136/jmedgenet-2013-101567

Updated information and services can be found at:
http://jmg.bmj.com/content/early/2013/02/22/jmedgenet-2013-101567.full.html

These include:

Data Supplement
“Supplementary Data”
http://jmg.bmj.com/content/suppl/2013/02/22/jmedgenet-2013-101567.DC1.html

References
This article cites 23 articles, 6 of which can be accessed free at:
http://jmg.bmj.com/content/early/2013/02/22/jmedgenet-2013-101567.full.html#ref-list-1

P<P
Published online February 23, 2013 in advance of the print journal.

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

Advance online articles have been peer reviewed, accepted for publication, edited and typeset, but have not not yet appeared in the paper journal. Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/