Downloaded from jmg.bmj.com on February 23, 2013 - Published by group.bmj.com
JMG Online First, published on February 23, 2013 as 10.1136/jmedgenet-2013-101567

SHORT REPORT

Mutations in WNT1 are a cause of osteogenesis imperfecta

Somayyeh Fahiminiya,[1] Jacek Majewski,[1] John Mort,[2] Pierre Moffatt,[2] Francis H Glorieux,[2] Frank Rauch[2]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jmedgenet-2013-101567).

[1]Department of Human Genetics, McGill University and Genome Quebec Innovation Center, Montreal, Quebec, Canada
[2]Genetics Unit, Shriners Hospital for Children and McGill University, Montreal, Quebec, Canada

Correspondence to
Dr Frank Rauch,
Genetics Unit, Shriners Hospital for Children, 1529 Cedar Avenue, Montréal, Québec, Canada H3G 1A6;
frauch@shriners.mcgill.ca

Received 29 January 2013
Revised 5 February 2013
Accepted 6 February 2013

ABSTRACT

Background Osteogenesis imperfecta (OI) is a heritable bone fragility disorder that is usually due to dominant mutations in *COL1A1* or *COL1A2*. Rare recessive forms of OI, caused by mutations in genes involved in various aspects of bone formation, have been described as well.

Objective To identify the cause of OI in eight children with severe bone fragility and a clinical diagnosis of OI type IV who had had negative results on *COL1A1*/*COL1A2* Sanger sequencing.

Methods Whole exome sequencing was performed in genomic DNA samples from all eight individuals.

Results *WNT1* mutations were found in four children from three families. *WNT1* was the only gene where mutations were found in all of these four patients. Two siblings from a consanguineous family had a homozygous missense mutation affecting a highly conserved cysteine residue in WNT1 (c.428G>T (p.Cys143Phe)). One girl had a homozygous frameshift deletion (c.287_300del(p.Gln96Profs)). A girl from a third family was compound heterozygous for a frameshift insertion and a missense mutation affecting a conserved amino acid (c.946_949insAACA (p.Ser317Lysfs); c.1063G>T (p.Val355Phe)). All of these children had short stature, low bone density, and severe vertebral compression fractures in addition to multiple long bone fractures in the first years of life. The Wnt signalling pathway is one of the key regulators of osteoblast activity.

Conclusions Recessive inactivating mutations in *WNT1* are a new cause of OI type IV.

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), and blue or grey sclera can be associated.[1] 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 α chains.[2] Other dominantly and recessively inherited forms of OI are rare and can be caused by mutations in at least 10 different genes (*CRTAP*, *LEPRE1*, *PPIB*, *SERPINH1*, *FKBP10*, *SP7*, *SERPINF1*,[2] *BMP1*,[3] *TMEM38B*,[4] *IFITM5*[5]).

The classification of OI is a topic of ongoing debate, and there is no consensus as to how new genetic findings should be integrated.[2 6] According to the clinical criteria of the expanded Sillence 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.[1] OI type IV is intermediate with regard to the severity of bone fragility, between OI type I and III.[1]

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; *PPIB*, 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. Bioinformatic analysis of generated exome sequencing data was performed with our pipeline as previously described.[7] Briefly, the human genome assembly hg 19 was used as the reference genome and the high quality trimmed paired-end sequences were mapped against it using a Burroughs-Wheeler algorithm (V.0.5.9).[8] Variant calling and annotation were performed by SAMtools V.0.1.17[9] and ANNOVAR,[10] 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).[11]

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

Copyright Article author (or their employer) 2013. Produced by BMJ Publishing Group Ltd under licence.

Downloaded from jmg.bmj.com on February 23, 2013 - Published by group.bmj.com

Table 1 Genes with rare homozygous and compound heterozygous variants mutated in each of the families (MAF<0.0005)

Family	Number of genes	Gene names
1*	19	*WNT1*; *CFHR4*; *NEB*; *AKAP9*; *TTF1*; *LRP4*; *FOLH1*; *OR4A15*; *HNRNPUL2*; *PCNXL3*; *PC*; *PDZRN4*; *DIP2B*; *COQ10A*; *TPPP3*; *NUDT7*; *ADAD2*; *SPIRE2*; *ZNF347* †
2	8	*WNT1*; *FRMD4A*; *KDM5A*; *CSRNP2*; *HSD17B6*; *LOR*; *KIAA0040*; *SMPD1* †
3	5	*WNT1*; *PREPL*; *FSIP2*; *MTUS2*; *WDR66* †
4	10	*INPP5B*; *WDR65*; *NBPF16*; *GIMAP8* ; *TOPORS*; *NDUFAF1*; *SLC12A1*; *LDHAL6B*; *UNC45A*; *TNRC18* †
5	11	*FBXO2*; *SPARC*; *AHI1*; *URB2*; *ZAR1*; *FER1L6*; *ASPN*; *TNC*; *ANKRD26*; *OR51G2*; *SPTBN5* †
6	5	*ZMIZ2*; *KIR2DL3*; *EXD3*; *CD209*; *PNMAL1* †
7	7	*HSPBAP1*; *OR9K2*; *FAT1*; *GLE1*; *GLB1L2*; *CARD10*; *FKBP10* †

*Family 1 is a consanguineous family with two affected children, sharing 19 rare homozygous and multiple heterozygous variants.
†The variants within *TTN*, *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.

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.Val355Phe)). 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 *Wnt* 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

Downloaded from jmg.bmj.com on February 23, 2013 - Published by group.bmj.com

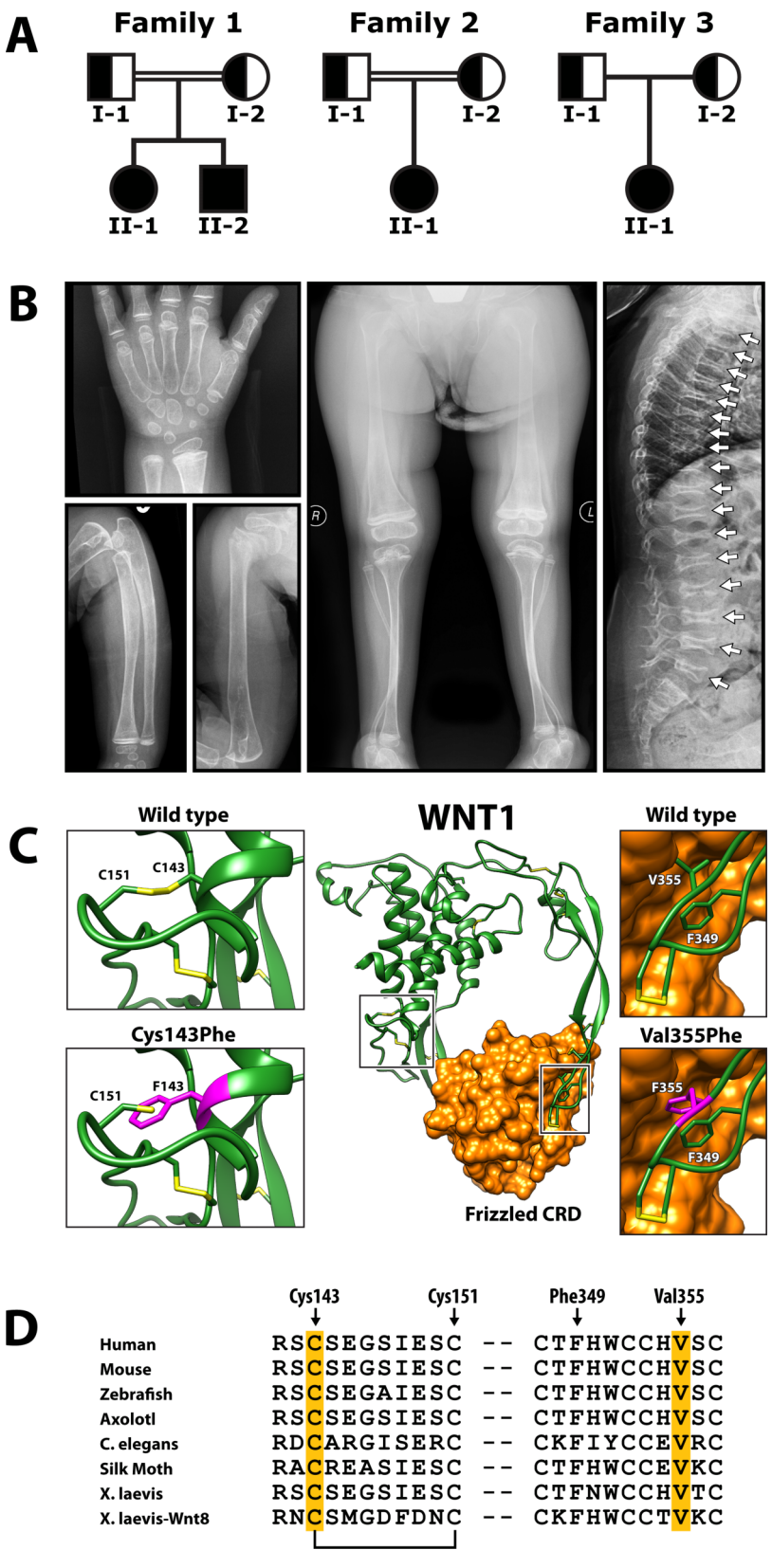

Figure 1 (A) Pedigrees of families with *WNT1* mutations. (B) Radiographic findings in a girl with a homozygous p.Cys143Phe mutation in *WNT1*. The images were obtained after two cycles of intravenous pamidronate treatment, which explains the sclerotic bands close to the growth plates on all long bones. 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 disulfide bridges that are the invariable hallmark of all Wnt proteins (figure 1, online supplementary table S1).[22 23] 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.[23] 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).

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

Downloaded from jmg.bmj.com on February 23, 2013 - Published by group.bmj.com

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 Québec 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

1 Rauch F, Glorieux FH. Osteogenesis imperfecta. *Lancet* 2004;363:1377–85.
2 Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. *Nat Rev Endocrinol* 2011;7:540–57.
3 Martinez-Glez V, Valencia M, Caparros-Martin JA, Aglan M, Temtamy S, Tenorio J, Pulido V, Lindert U, Rohrbach M, Eyre D, Giunta C, Lapunzina P, Ruiz-Perez VL. Identification of a mutation causing deficient BMP1/mTLD proteolytic activity in autosomal recessive osteogenesis imperfecta. *Hum Mutat* 2012;33:343–50.
4 Shaheen R, Alazami AM, Alshammari MJ, Faqeih E, Alhashmi N, Mousa N, Alsinani A, Ansari S, Alzahrani F, Al-Owain M, Alzayed ZS, Alkuraya FS. Study of autosomal recessive osteogenesis imperfecta in Arabia reveals a novel locus defined by TMEM38B mutation. *J Med Genet* 2012;49:630–5.
5 Cho TJ, Lee KE, Lee SK, Song SJ, Kim KJ, Jeon D, Lee G, Kim HN, Lee HR, Eom HH, Lee ZH, Kim OH, Park WY, Park SS, Ikegawa S, Yoo WJ, Choi IH, Kim JW. A single recurrent mutation in the 5′-UTR of IFITM5 causes osteogenesis imperfecta type V. *Am J Hum Genet* 2012;91:343–8.
6 Van Dijk FS, Pals G, Van Rijn RR, Nikkels PG, Cobben JM. Classification of Osteogenesis Imperfecta revisited. *Eur J Med Genet* 2010;53:1–5.
7 McDonald-McGinn DM, Fahiminiya S, Revil T, Nowakowska BA, Suhl J, Bailey A, Mlynarski E, Lynch DR, Yan AC, Bilaniuk LT, Sullivan KE, Warren ST, Emanuel BS, Vermeesch JR, Zackai EH, Jerome-Majewska LA. Hemizygous mutations in SNAP29 unmask autosomal recessive conditions and contribute to atypical findings in patients with 22q11.2DS. *J Med Genet* 2012;50:80–90.
8 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754–60.
9 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009;25:2078–9.
10 Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164.
11 Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. Integrative genomics viewer. *Nat Biotechnol* 2011;29:24–6.
12 Alanay Y, Avaygan H, Camacho N, Utine GE, Boduroglu K, Aktas D, Alikasifoglu M, Tuncbilek E, Orhan D, Bakar FT, Zabel B, Superti-Furga A, Bruckner-Tuderman L, Curry CJ, Pyott S, Byers PH, Eyre DR, Baldridge D, Lee B, Merrill AE, Davis EC, Cohn DH, Akarsu N, Krakow D. Mutations in the gene encoding the RER protein FKBP65 cause autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet* 2010;86:551–9.
13 Baron R, Hesse E. Update on bone anabolics in osteoporosis treatment: rationale, current status, and perspectives. *J Clin Endocrinol Metab* 2012;97:311–25.
14 Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. *Gene* 2012;492:1–18.
15 Beighton P, Winship I, Behari D. The ocular form of osteogenesis imperfecta: a new autosomal recessive syndrome. *Clin Genet* 1985;28:69–75.
16 Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 2001;107:513–23.
17 McMahon AP, Bradley A. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. *Cell* 1990;62:1073–85.
18 Thomas KR, Capecchi MR. Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. *Nature* 1990;346:847–50.
19 Thomas KR, Musci TS, Neumann PE, Capecchi MR. Swaying is a mutant allele of the proto-oncogene Wnt-1. *Cell* 1991;67:969–76.
20 Witte F, Dokas J, Neuendorf F, Mundlos S, Stricker S. Comprehensive expression analysis of all Wnt genes and their major secreted antagonists during mouse limb development and cartilage differentiation. *Gene Expr Patterns* 2009;9:215–23.
21 Rauner M, Sipos W, Pietschmann P. Age-dependent Wnt gene expression in bone and during the course of osteoblast differentiation. *Age (Dordrecht, Netherlands)* 2008;30:273–82.
22 Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol* 1998;14:59–88.
23 Janda CY, Waghray D, Levin AM, Thomas C, Garcia KC. Structural basis of Wnt recognition by Frizzled. *Science* 2012;337:59–64.

Downloaded from jmg.bmj.com on February 23, 2013 - Published by group.bmj.com

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/