Multicentric Carpotarsal Osteolysis Is Caused by Mutations Clustering in the Amino-Terminal Transcriptional Activation Domain of MAFB

Andreas Zankl,1,2,3,17 Emma L. Duncan,1,2,4,17,* Paul J. Leo,1 Graeme R. Clark,1 Evgeny A. Glazov,1 Marie-Claude Addor,5 Troels Herlin,6 Chong Ae Kim,7 Bruno P. Leheup,8 Jim McGill,9 Steven McTaggart,10 Stephan Mittas,11 Anna L. Mitchell,12 Geert R. Mortier,13 Stephen P. Robertson,14 Marie Schroeder,15 Paulien Terhal,16 and Matthew A. Brown1,*

Multicentric carpotarsal osteolysis (MCTO) is a rare skeletal dysplasia characterized by aggressive osteolysis, particularly affecting the carpal and tarsal bones, and is frequently associated with progressive renal failure. Using exome capture and next-generation sequencing in five unrelated simplex cases of MCTO, we identified previously unreported missense mutations clustering within a 51 base pair region of the single exon of MAFB, validated by Sanger sequencing. A further six unrelated simplex cases with MCTO were also heterozygous for previously unreported mutations within this same region, as were affected members of two families with autosomal-dominant MCTO. MAFB encodes a transcription factor that negatively regulates RANKL-induced osteoclastogenesis and is essential for normal renal development. Identification of this gene paves the way for development of novel therapeutic approaches for this crippling disease and provides insight into normal bone and kidney development.

Next-generation sequencing presents a new approach for disease-gene mapping in either small pedigrees or in small numbers of affected individuals and has been used to uncover the underlying cause for several skeletal dysplasias.1–4 Multicentric carpotarsal osteolysis (MCTO [MIM 166300]) is a rare skeletal dysplasia characterized by progressive bone resorption (osteolysis), predominantly (although not exclusively) of the carpal and tarsal bones. The early clinical appearance can mimic polyarticular juvenile idiopathic arthritis; however, subsequent clinical and radiographic appearances are characteristic (see Figure 1). Affected individuals can also have subtle craniofacial abnormalities (triangular faces, micrognathia, and exophthalmos), and many develop a progressive nephropathy leading to end-stage renal failure. Both simplex cases and families with autosomal-dominant inheritance have been reported.5–16 However, the responsible genetic defect has remained elusive. Mutations in MMP2 [MIM 120360], responsible for the autosomal-recessive Torg-Winchester syndrome [MIM 259600]17–19 that is also characterized by excessive bone resorption, have not been identified in patients with MCTO.20 Here, we report identification of the underlying genetic cause of MCTO.

We recruited simplex cases of MCTO and families with autosomal-dominant MCTO through the Queensland Bone Dysplasia Registry at the University of Queensland Centre for Clinical Research. Each individual had classical radiographic features of MCTO (complete destruction of carpal bones but relatively well-preserved metacarpals and phalanges) (Figure 1). Five of the 11 simplex cases had undergone renal transplantation. Of the remaining six, three had markedly impaired renal function but did not yet require dialysis. Three individuals all of whom were very young (7 years old or younger), had no evidence of renal dysfunction. The affected individuals in the two families with autosomal-dominant MCTO had no evidence of renal dysfunction. The study was approved by the University of Queensland Ethics Committee, and all subjects or their respective guardians gave informed written consent.

We performed whole-exome sequencing in five unrelated individuals with MCTO. Genomic DNA was extracted from peripheral blood samples via standard protocols. We used 5 μg DNA to construct DNA sequencing libraries with Illumina's DNA sample preparation kit for paired-end sequencing per the manufacturer's protocol. We used liquid phase hybridization for exome capture with...
Roche/Nimblegen SeqCap EZ Exome Library v1.0 kits (Roche/Nimblegen) according to the manufacturer’s protocol. Efficiency of sequence capture was assessed via quantitative real-time PCR with standard control primers as recommended by the manufacturer. Each DNA sequencing library was quality checked and quantified with Agilent 2100 Bioanalyser and DNA 1000 chip kits. We standardized the DNA concentration for sequencing to 10 nM. Each DNA library was individually sequenced via a 56 paired-end read sequencing protocol with a single flow cell lane on an Illumina Genome Analyzer II and with the manufacturer’s reagents.

Initial base calling was performed with the Illumina Data Analysis Pipeline software (CASAVA 1.7). Sequence data were aligned to the current build of the human genome (hg19, released February 2009) with the Novoalign alignment tool (V2.07.09 1); sequence alignment files were converted with SAMtools (v0.1.14) and Picard tools (v1.42). SNPs and indels were called with the Genome Analysis Toolkit (GATK v5506) and were annotated with ANNOVAR. An average of 1.96 Gb of sequence per individual (range 1.48–2.38 Gb) was generated, with mean on-target (exons ± 200 bp) sequence of 87.0% (range 85.5%–88.1%). Mean depth of exomic coverage was 50-fold (range 37.0–60.5) with 84.9% and 75.9% achieving 10-fold and 15-fold coverage, respectively (mapping and coverage statistics and detailed base calling information is presented in Tables S1 and S2, available online).

Custom scripts with R and Bioconductor were used to analyze and filter the sequencing data. SNPs and indels with good quality reads were retained (minimum depth of coverage for SNP calling, greater than 10-fold for homozygous SNPs and greater than 15-fold for heterozygous SNPs). Additionally, we used the GATK-provided quality parameters: we included SNPs/indels with values QUAL > 50, QD > 0.5, HRUN < 5, and SB < 0.1 (defined as: QUAL, combined recalibrated quality score at the SNP position; DP, sequencing depth at the SNP position; QD, QUAL/DP ratio at the SNP position; HRUN, maximal length of the homopolymer run; SB, strand bias at the SNP position). We retained SNPs and indels with potentially damaging consequence (non-synonymous SNV, splicing, frameshift substitution, stop-gain SNV, and stoploss SNV based on either RefSeq or UCSC transcripts). We excluded SNPs observed in the National Center for Biotechnology Information (NCBI) dbSNP (release 132), 1000 Genomes, 1000 Genomes small indels (called with the DINDEL program), the SNPs of 46 genomes released by Complete Genomics and 16 other whole exomes from control samples run internally with the same capture technology that was used in this project.

We assessed the five sequenced individuals for heterozygous carriage of either a single previously unreported nonsynonymous SNP shared by all individuals or a previously unreported nonsynonymous SNP in each individual within a common gene. After quality filtering (detailed in Table 1), a single gene was identified, MAFB (v-maf musculoaponeurotic fibrosarcoma oncogene ortholog B [avian]...
region of unreported heterozygous missense mutations in the same simplex cases with MCTO also demonstrated previously sequencing in the five probands. Further, the six additional confirmed the mutations identified through exomic sequencing, we also sequenced the same region and segregated in the five probands. We also sequenced 164 unre-lated white European controls. Sanger sequencing itance of MCTO (Figure 3). We also sequenced 164 unre-

Table 1. Summary of Identified Variants from Exome Sequencing

| Variants in three genes remained. Two of these were dinucleotide repeats in splice sites and did not fit appropriate autosomal-dominant inheritance pattern. Thus variants in only one remaining gene (MAFB) fitted all the criteria. |

In addition to the five probands who had undergone exomic sequencing, we also sequenced the same region in their (clinically unaffected) parents; a further six unrelated simplex cases with MCTO and their parents (with a single exception, individual 81.3, for whom no parental DNA sample was available); and all available family members of two families with autosomal-dominant inheritance of MCTO (Figure 3). We also sequenced 164 unrelated white European controls. Sanger sequencing confirmed the mutations identified through exomic sequencing in the five probands. Further, the six additional simplex cases with MCTO also demonstrated previously unreported heterozygous missense mutations in the same region of MAFB (Figure 2). None of the parents of the affected probands carried the mutations present in their child, demonstrating that these are previously unreported autosomal-dominant mutations in the affected individuals. In the two families with autosomal-dominant inheritance of MCTO, heterozygous missense mutations were also demonstrated in the same region and segregated with disease status (Figure 3). None of the 164 control samples carried any mutations in this region (328 alleles). The location of the mutated amino acids within the protein domain is shown in Figure 4. We used three SNP function prediction algorithms (Polyphen [Poly-

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inhibited osteoclastogenesis by interfering with the key transcription factors normally activated by RANKL, whereas reduced MafB expression enhanced osteoclastogenesis. Conversely, RANKL downregulated expression of MafB. Thus, MafB might regulate the development of pluripotent myeloid precursors by enhancing macrophage differentiation while repressing osteoclastogenesis. MafB is itself regulated by the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ); and osteoclastic bone resorption is increased in mice with C/EBPβ deficiency and in mice expressing only the short isoform of C/EBPβ in both of which MafB expression levels are attenuated.

The observed mutations in MAFB all lie within a short region of the amino-terminal transcriptional activation domain (amino acids 54-71; see Table 2 and Figure 4). Of note, amino-terminal truncation of MAFB (residual amino-acids 195-311 fused to the N terminus) exerted a dominant negative effect upon myeloid colony formation and macrophage differentiation. The observed heterozygous missense mutations in MAFB in individuals with MCTO have a dominant effect; however, whether the mechanism for this is haploinsufficiency or a dominant negative effect requires further study.

In common with other large Maf proteins, MafB is phosphorylated by glycogen synthase kinase-3. The conserved phosphorylation sites shared by Maf proteins lie in the same region as the observed mutations; there are four mutations predicted to affect putative phosphorylation sites (amino acids 54, 62, 66, and 70). Mutations of the corresponding amino acids in MafA prevented phosphorylation and decreased its transcriptional activity. It is possible that the mutations we observed might have a similar effect upon MafB phosphorylation and subsequent function. However, functional studies are required to confirm such speculation.

It is not clear why the bony changes in MCTO are seen predominantly in the carpal and tarsal bones. Systemic bone mineral density (BMD) has not been studied comprehensively in patients with MCTO; however, one case report noted osteopaenia in a child before significant renal dysfunction had developed. In adult patients with advanced renal failure, it is not possible to partition the effects of renal osteodystrophy from direct systemic bone effects from MAFB mutations. Whether polymorphisms in MAFB are associated with variation in BMD in the normal population is unclear. In our GWAS of 2,055 postmenopausal women with either extremely high or low BMD, three SNPs in MAFB were associated with lumbar spine BMD with p value < 10^{-3} (not significant at a genome-wide level), a finding sufficiently suggestive to warrant further investigation in larger data sets.

Patients with MCTO commonly develop renal impairment and frequently progress to end-stage kidney disease. Renal biopsy is often only obtained at a late stage of disease, by which time nonspecific findings of glomerulosclerosis and severe tubulointerstitial fibrosis dominate.
the biopsy appearance. However, two studies report renal biopsies obtained from children with MCTO and early renal dysfunction. One child, with isolated proteinuria, had focal and segmental glomerulosclerosis with distortion, fusion, and effacement of the podocyte foot processes. Another child, only 2 years old but with hypertension, proteinuria, and mildly elevated serum creatinine, had a normal biopsy with light microscopy, but electron microscopy also showed abnormal fusion of the podocyte foot processes and discrete abnormalities of the glomerular basement membrane. MafB is essential for normal development of podocyte foot processes and is expressed in both the podocyte late in renal development and in the mature glomerulus. MafB−/− homozygous knockout mice have renal dysgenesis, including loss of normal foot processes of podocytes, abnormal glomerular differentiation with decreased mature glomeruli, tubular dysgenesis due to abnormal tubular survival, and renal cysts. The mice have decreased renal expression of nephrin, podocin, and CD2AP, critical proteins for normal podocyte development, and glomerular function. Although abnormalities in these basement membrane proteins have not been studied in patients with MCTO, the histopathological features that have been demonstrated in homozygous mice are consistent with the renal biopsy findings observed in patients with MCTO and renal impairment.

Renal disease was present in most but not all patients in our cohort. Individuals 43.3, 81.3, and 22.3 have as yet no evidence of renal disease. However, these patients are only 3, 6 and 7 years old, respectively, and it is as yet unclear whether their renal function will remain normal. Of note, individual 81.3 has a unique mutation (c.176C>T [p.Pro59Leu]); and individuals 22.3 and 43.3 share the same mutation (c.211C>T [p.Pro71Ser]) although at the same location as another individual who has manifested renal disease. The affected individuals in family 82 and family 83 (who carry the same mutation, c.161C>T [p.Pro54Leu]), many of whom are adults, do not have renal disease, with the exception of individual 1.2 in Family 83 who at a very old age developed renal dysfunction of unknown aetiology. Thus it seems likely that MAFB

Figure 3. Sanger Sequencing and Family Trees for Families 82 and 83
(A) Family 82.
(B) Family 83.
Black indicates known affected and white indicates known unaffected; a question mark indicates unknown status.

Figure 4. Schematic picture of MafB, showing protein domains and positions of the observed mutations in individuals and families with MCTO
mutations are also responsible for MCTO in the absence of renal disease. Patients with MCTO also have a subtle craniofacial abnormalities that include micrognathia, a slim nose, maxillary hypoplasia, and consequent exophthalmos.8,12 A recent GWAS of cleft lip with and without cleft palate found significant association with multiple variants near MAFB and expression studies demonstrated MafB expression in palatal tissue.34 Cleft lip and/or palate have not been reported as a feature of patients with MCTO.

Studies in mice have suggested that MAFB plays important roles in pancreatic β cell development and in hindbrain development. MCTO is not known to be associated with pancreatic or central nervous system abnormalities, although these have not been investigated in detail.

Of the ten separate mutations observed, all were predicted to be probably or possibly damaging (Table 2). The protein structure of MafB is only established for the fragment of protein interacting with its DNA recognition motif, Cmare (amino acids 238–264).35 The mutations we observed cluster in a narrow region of the transitional activation domain where the protein structure is not yet established, although of note the protein domains in this regions are highly evolutionarily conserved (Figure 2). The function prediction algorithm PolyPhen predicts that all the variants are damaging or possibly damaging (Table 2).

Skeletal dysplasias highlight important pathways in bone of relevance to the general population and to common bone diseases such as osteoporosis. For example, the identification of SOST [MIM 605740] as the gene underlying sclerosteosis [MIM 269500]36 and van Buchem’s disease [MIM 239100]12 has lead to the development of anti-sclerostin antibodies as a treatment for osteoporosis.38 For patients with MCTO, bisphosphonates have not proven useful in treatment to date. Our data suggest that it might be worth targeting MafB signaling instead in these patients. Anti-RANKL antibody therapy is now available for the treatment of osteoporosis39 and could be considered for treatment of at least the bone disease that characterizes the MCTO phenotype.

In conclusion, we have identified previously unreported missense mutations clustering in a narrow region of MAFB in 11 unrelated simplex cases and in two families with MCTO. MAFB is known to play a critical role in regulation of osteoclastogenesis and in normal renal development, making this a highly plausible gene underlying this skeletal dysplasia. This study opens up the possibility of new therapeutic options in these patients and other patients with excessive osteoclastic activity.

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

Acknowledgments
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<tr>
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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, http://www.1000genomes.org/
sequence-data/download-data/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
PolyPhen, http://sift.jcvi.org/
SIFT, http://sift.jcvi.org/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
UniProt, http://www.uniprot.org/uniprot/

References


