An incompletely penetrant novel MAFB (p.Ser56Phe) variant in autosomal dominant multicentric carpotarsal osteolysis syndrome

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Abstract. Multicentric carpotarsal osteolysis syndrome (MCTO) is a rare autosomal dominant skeletal dysplasia usually presenting in early childhood with variable phenotypic features and course. Clinical manifestations comprise aggressive osteolysis of the carpal and tarsal bones in particular, an often progressive nephropathy leading to end-stage renal disease, craniofacial anomalies and mental impairment. Recently, heterozygous missense mutations in the V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian) (MAFB) gene have been causally related to MCTO patients in 13 unrelated families investigated. Contrary to these findings suggesting complete penetrance, in the present study, we identified a novel missense MAFB variant present not only in the patient, but also in his unaffected mother, sister and maternal grandmother. This observation demonstrates an incomplete penetrance for some MAFB mutations, thereby suggesting that modifier genes, epigenetic mechanisms or environmental factors may modulate the MCTO phenotype. This should be considered in diagnosis and genetic counseling.

Introduction

Since the first description of a ‘disappearing bone disease’ (1) several rare congenital osteolysis syndromes have been described with their nosology being classified by Tyler and Rosenbaum (2) in 1976 and by the International Classification of Constitutional Disorders of Bone (3). Autosomal dominant multicentric carpotarsal osteolysis syndrome (MCTO; OMIM 166300) usually presents in early childhood, often with a clinical appearance mimicking juvenile idiopathic arthritis (JIA) (4). However, JIA can easily be differentiated from MCTO by clinical, laboratory and radiological findings (5). MCTO is characterized by progressive and most often bilateral destruction of the carpal and tarsal bones; however, osteolysis may also affect other bones. Affected individuals may develop proteinuria and progressive nephropathy leading to end-stage renal disease. Craniofacial anomalies and mental impairment have also been observed (5,6). Recently, Zankl et al (7) reported an association of MCTO with de novo missense mutations in the V-maf musculoaponeurotic fibrosarcoma oncogene homolog B avian) (MAFB) gene in 11 simplex cases. In two further multiplex families, MAFB missense mutations segregated in an autosomal dominant fashion. The protein encoded (MafB) by this gene is a basic domain leucine zipper transcription factor with an anti-osteoclastogenic function also involved in renal tubule survival, tissue differentiation within the pancreas and segmentation of the hindbrain (8-13). The disturbance of these different functions may explain the phenotypic features observed in association with MCTO.

In the present study, we identified a novel p.Ser56Phe MAFB missense variant in a German individual with MCTO. Surprisingly, an analysis of the family revealed presence of this variant in three unaffected female family members investigated.

Patients and methods

Patient description. The index case in this study is the second child of unrelated parents. He has a three years older healthy sister. At his first visit to the Genetics Department, University Hospital of Bonn, Bonn, Germany, the index case was 17 years of age. His early development was completely uneventful. By the age of four, he first visited the Children's Hospital for recurrent pain in his right hand with swelling of his wrist, raising the suspicion of the onset of juvenile rheumatoid arthritis. At the age of six he presented with dysesthesia in his right leg with swelling of his ankle. Subsequent diagnostic radiographic workup revealed several carpotarsal osteolytic spots. Extensive diagnostic testing did not reveal any immunological or infectious origin for his symptoms. During the course of his extensive diagnostic workup to explain his carpotarsal osteolysis, an electroencephalography revealed slow waves...
on his right temporoparieto-occipital recordings. Subsequent brain magnetic resonance imaging revealed an Arnold-Chiari malformation type I. During late puberty he developed androgenetic alopecia and slight non-immunological bilateral exophthalmos. At 19 years of age, an X-ray revealed irregular and dysplastic, partially missing carpalia and dysplastic changes of the tarsalia (Fig. 1). Renal functional studies and urine analysis showed normal renal function, without any sign of proteinuria or nephropathy.

The mother did not report any history of swelling of her wrists or ankles nor any radiographical signs of carpotarsal osteolysis. In addition she did not present with any ulnar deviation of her hands or wrist arthralgia. X-ray images, renal functional analyses and urine analysis were normal. The sister and maternal grandmother also showed no features characteristic of MCTO.

Sample collection and DNA analysis. After informed consent was obtained, EDTA blood samples were obtained from the family members. Genomic DNA was isolated using the QIAamp DNA Blood kit and amplification of the single MAFB exon was performed by polymerase chain reaction (PCR) using HotStarTaq DNA polymerase (Qiagen, Hilden, Germany). PCR primers (1F, 5'-GCTTGGCTCGCCGCGCTCC-3'; 1F2, 5'-CCGTGCGCGAGCTGAACCG-3'; 1R, 5'-GCCGCCAGGGACAGGTTCCG-3'; 1R2, 5'-TCGCGAGGGGATGAGCGTTC-3') also served as sequencing primers. For mutational analysis, PCR-amplified DNA products were subjected to direct automated sequencing using a 3130xl Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA) and both strands from the amplicons of the parents and patients were analyzed.

RNA analysis. RNA analyses were performed to investigate the expression of both alleles in the patient and his mother. Blood samples were collected in PAXgene tubes (PreAnalytiX, Hombrechtikon, Switzerland) and RNA for reverse transcription (RT)-PCR was prepared using the RNeasy Plus Micro kit (Qiagen) in accordance with the manufacturer’s instructions. Allele-specific amplification (ASA)-PCR and allele-specific quantitative PCR (AS-qPCR) were carried out as previously described (14). ASA-PCR may lack reliability since the mutated primer may still allow amplification of the normal allele as shown by Hezard et al (15). These authors showed that allele discrimination can be improved by incorporating an additional mismatch in the antepenultimate position by substituting the respective wild-type (WT) pyrimidine (purine) nucleotide by the respective pyrimidine (purine) base. Therefore, to adjust for similar annealing temperatures, ASA forward (F) primers and a universal reverse (UR) and forward (UF) primer were designed as follows (normal WT sequence, 5'-TGGAACTGAAAACCAGCGACCA-3'): WT-F, 5'-GAACTGAAAACCAGCGATCA-3'; mutant-G-F, 5'-GAACTGAAAACCAGGATCG; UR, 5'-GGCACTAACCTCTCAATGCTG-3'; and UF, 5'-TGGAACTGAAAACCAGCGACCA-3'. Reactions for AS-qPCR were performed on an ABI PRISM 7900HT Fast Real-Time PCR System (Applied Biosystems, Inc.) using SYBR-Green for detection. Each assay was performed as previously described (14) and included DNA from four controls (two male and two female samples) and the subject’s DNA.

Figure 1. X-ray of the right hand and foot at the age of 19 years shows (A) irregular and dysplastic, partially missing carpalia and (B) a bayonet-like wrist deformity with palmar subluxation. (C) Dysplastic changes of the tarsalia with consequent malposition of the metatarsal bones are less pronounced.
Results

The screening of MAFB PCR products obtained from the index case (Fig. 2A, III.2) revealed a single heterozygous c.167C>T nucleotide transition (Fig. 2B), predicting a p.Ser56Phe substitution (GenBank accession no. NM_005461.3, with the A of the start ATG as nucleotide 1). Gene analysis in the unaffected mother of the patient (II.2) provided evidence that she is a carrier of the mutant allele transmitted to her son. Moreover, the healthy sister and the healthy maternal grandmother also showed the heterozygous c.167C>T transition. These findings led us to perform functional analyses. To exclude the sole or predominant expression of the WT allele in the mother, the RT-PCR products were first analyzed by sequence analysis. Fluorescence intensity indicated the presence of the WT and variant transcript in similar amounts in the samples of the patient and his mother. To verify this finding we also performed AS-qPCR.

Prior to qPCR it had to be validated that the ASA primers only direct amplification on their complementary allele and that the conditions chosen avoid any other unspecific amplification. The ASA primers designed allowed the sole amplification of the respective allele without any additional unspecific priming, hence, qPCR was performed. This method involves PCR amplification of the product of interest and, in a separate reaction, amplification of an invariantly expressed internal standard (housekeeping gene) to be used as a denominator to normalize the amount of the target gene. Allele-specific qPCR also showed similar amounts of both transcribed alleles in the patient and his mother (data not shown).

Several clues point to a critical role for serine 56 located in the amino-terminal transcriptional activation domain of MAFB: First, the c.167C>T transition has not been deposited in the single nucleotide polymorphism (SNP, Build137) database and in the Deep Catalog of Human Genetic Variation (www.1000genomes.org), which argues against the possibility that this substitution is just a rare benign variant. In addition, the amino acid exchange substitutes a polar residue for a non-polar one and pathogenicity prediction with several algorithms (PolyPhen-2, SNPs&GO, PMut, SIFT) predicted this variant to be disease-causing or at least possibly damaging. Finally, Ser56 is absolutely conserved at its corresponding position in species orthologues as part of the transactivation motif at least as far down as X. laevis (Fig. 2C).

Discussion

The patient presented in this study showed the typical features of MCTO with wrist and ankle swelling, severe osteolysis of almost all carpal and tarsal bones (Fig. 1) and bilateral exoph-
thalamos. He showed no proteinuria or nephropathy at the
time of writing, but it is as yet unclear, whether his renal function
will remain normal. However, complete absence of renal
involvement has been frequently observed (6) and was not
reported in 50% of the patients reported by Zankl et al (7). We
further noted Arnold-Chiari malformation type I and andro-
genetic alopecia but are not aware of any other case showing
the coincidence of MCTO with these features.

The recent observations of Zankl et al (7) suggested a
heterozygous de novo MAFB mutation in our isolated MCTO
patient. As expected, we observed a single heterozygous
p.Ser56Phe amino acid substitution that surprisingly was also
present in three unaffected females in this family. MAFB
RNA analysis in the patient and his mother revealed similar
amounts of mutant and WT mRNAs providing no evidence,
that the phenotypic differences are a result of a preferential
transcription of the WT allele in the mother.

Nevertheless, several strands of evidence suggested that the
amino acid substitution observed represents a pathogenic muta-
tion. Aside from its absence in databases, comparative genomics
revealed apparent evolutionarily conservation of Ser56 at least
as far down as X. laevis (Fig. 2C). In addition, pathogenicity
prediction revealed this substitution to be disease-causing or at
least possibly damaging. Moreover, this novel variant resides in
the transactivation domain of MafB, affected by all the missense
mutations (residues 54-71) described to date (Fig. 2C) (7).

Variable phenotypes and even intra-familial variability
was observed in the probands investigated by Zankl et al (7).
This suggests a role for as yet unidentified modifier gene(s),
epigenetic mechanisms or environmental factors in disease
penetrance which could also explain the absence of the associ-
ated renal phenotype. Gender differences in penetrance due
to a protective modifier gene may also be an explanation for
the presence of three healthy females in our family. Actually,
Incomplete penetrance occurring only in female carriers
has been described in familial hypokalemie periodic paralysis
due to mutations in the skeletal muscle calcium channel α1
subunit gene (16). Also, asymptomatic females carrying the
same survival motor neuron 1 gene mutation as their affected
relatives with recessive spinal muscular atrophy have been
observed (17). On the other hand, such a protective effect in
females should result in an unequal gender ratio. However,
a review of the data of all MCTO patients reported to date
showed no male predominance of the disease.

Similar to the data presented, several other bone diseases
exhibit variable expressivity and incomplete penetrance.
For example, heterozygous mutations in the sequestosome
(SQSTM1) gene are now suggested not to cause Paget’s disease
of bone (OMIM 601530) but only predispose a carrier to the
disease (18). The fact that a single mutant allele can be insufficient
to cause the disorder has also been observed in osteoclasis 1
(OMIM 6166800) (19), characterized by alternating phases of
bone resorption and formation, as well as growth differentiation
factor 6-attributable oculo-skeletal phenotypes (20).

Finally, it would of interest to observe the effects of the
heterozygous p.Ser56Phe and other missense mutations in a
mouse model. Complete MafB deficiency has been shown
to cause defective respiratory rhythogenesis and fatal
central apnea at birth (21). However, all mouse models were
generated by mutations not comparable with the missense
mutations observed in MCTO patients. In the first model, MafB/
Kreisler (kr), MAFB has been shown to be the ortholog of the
gene affected by the mouse Kreisler mutation, that involves
a submicroscopic inversion, leaving 30 kb of 5’ sequence and
the coding region intact (8,22). Of note, in these homozygous
mice, the expression of the MafB/kr gene is still maintained
outside of the hindbrain indicating a selective, tissue-specific
gene inactivation (22). Using N-ethyl-N-nitrosourea (ENU),
Cordes and Barsh (8) introduced an A-to-G transition at nucleo-
tide 743 resulting in a p.Asp248Ser substitution. The mutation
is located in the putative DNA-binding domain and there seems
to be a similar effect of the kr<sup>ENU</sup> mutation which is suggested
to retain partial function in the absence of DNA binding,
possibly through interactions with other bZip transcription
(co)factors (13). A previous study demonstrated that kr<sup>ENU</sup>
was able to form dimers with WT MafB and transactivated a reporter
gene (23). Finally, a targeting vector (MafB<sub>green fluorescent protein</sub>)
was designed and RT-PCR failed to detect transcripts
in mutant renal cells and macrophages (13). Again, all MafB<sup>−/−
</sup> mice died within 24 h after birth. A phenotype strikingly
similar to that of the Kreisler mutant, was observed in these
MafB<sup>−/−</sup> neonates but not in their heterozygous siblings. Hence,
it remains elusive, whether a heterozygous missense mutation in
the amino-terminal transcriptional activation domain of mouse
MafB mirrors the phenotype observed in humans and whether
incomplete penetrance can also be observed in this model.

In conclusion, the novel MAFB variant observed is expected
to be responsible for the MCTO phenotype in our patient. The
effect of a modifier, incomplete penetrance, epigenetic mecha-
nisms or environmental factors may account for the variable
expressivity of the disease in the family presented in our study.
This not only increases the complexity of genetic counseling
but should also be considered in patient care, at least in renal
prognosis.

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