Genetic changes in the RNA components of RNase MRP and RNase P in Schmid metaphyseal chondrodysplasia


METHODS
Subjects and families
As part of an ongoing research program on molecular mechanisms in skeletal dysplasias, which was approved by the Research Ethics Board of The Hospital for Sick Children, Toronto, Canada, DNA samples of 32 patients with the diagnosis of MCDS were studied for COL10A1 mutations. The diagnosis of MCDS was based clinically on short limbed short stature and absence of evident extra-skeletal manifestations (sparse hair, increased rate of infections, anaemia), and radiographically on compatible metaphyseal changes on skeletal radiographs. Causative mutations were identified in 12 patients (Susic & Cole, unpublished data); the 20 patients with MCDS but no COL10A1 mutations were included in the present study. None of these patients had a positive family history of metaphyseal chondrodysplasia. Probands SMC3 and SMC4 carrying mutations in RMRP are described here in more detail.

SMC3
This male patient was born to healthy, consanguineous parents (father is mother’s uncle) of white Canadian descent at 37 weeks of gestation with a birth length of 44 cm (< third percentile). The limbs were short, and skeletal radiographs at 10 months were consistent with a form of metaphyseal chondrodysplasia. Serum calcium, phosphate, and alkaline phosphatase levels were normal. The patient did not have anaemia, and a thorough immunological evaluation showed no evidence of cellular or humoral immune deficiency. At the age of 5 years, his height was 94 cm (< third percentile) and he remained mildly disproportionate with relatively short limbs. There was no history of recurrent infections. His hair and nails were normal. There was no excessive ligamental laxity but there was mildly limited extension at the elbows and mild varus deformity of the legs. Radiographs at the age of 4 years showed generalised metaphyseal changes with flaring and irregularities; these changes were most marked in the knees and hips (fig 1). The fibula was excessively long distally relative to the tibia. The hand radiographs showed short metacarpals and phalanges, and the phalangeal
epiphyses were small, but normal in shape. The vertebral bodies showed mild, non-specific changes.

**SMC44**

This 5 year old male patient was the second child born to healthy, non-consanguineous French-Canadian parents. A skeletal dysplasia was suspected at 32 weeks of gestation when an ultrasound revealed foreshortening of the extremities. He was born at 39 weeks of gestation with a birth weight of 3040 g (25th percentile) and birth length 44 cm (<third percentile). Metaphyseal chondrodysplasia was diagnosed soon after birth based on radiographic findings. The patient thrived in the post-natal period, although he developed pneumonia at 1 month of age, which required hospitalisation and treatment with intravenous antibiotics, to which he responded well. He had two further episodes of pneumonia (at 9 months and 5 years) as well as occasional bouts of otitis media, all of which necessitated treatment with oral antibiotics. At 2 years of age he contracted varicella, which followed a typical, uncomplicated course. He did not have difficulty tolerating standard vaccines. There was no history of constipation. The most recent evaluation of the patient at 5.6 years of age revealed a developmentally normal child in a good health without functional limitations (fig 2). The height was 90 cm and weight 14.5 kg (both well below the third percentile). The hair, eyelashes, and eyebrows appeared normal. The hands and feet were short and wide. There was a pectus carinatum, incomplete extension at the elbows,

![Figure 1](https://www.jmedgenet.com/)

**Figure 1** Radiographic findings in the two patients SMC3 (A, C) and SMC44 (B, D) with homozygous 70A→G mutations in the RMRP gene. Anteroposterior views of the knees (A, B) and hips (C, D) show metaphyseal flaring and irregularity. The changes are more marked in the knees than in the hips.

![Figure 2](https://www.jmedgenet.com/)

**Figure 2** Patient SMC44 at 5 years of age. He has short limbed short stature with short fingers and no obvious hair hypoplasia.
and mild hyperlaxity at the wrists and fingers. The spine was straight and skull shape was normal. A comprehensive haematological evaluation revealed a normal complete blood count and normal IgA, IgG, IgM, and IgE levels. Lymphocyte sub-populations were within the reference ranges, and mitogen induced lymphocyte stimulation tests were normal. Biochemical parameters of bone and mineral metabolism were unremarkable. A skeletal survey revealed multiple abnormalities, including shortening of the tubular bones, and widening and irregularity of the upper and lower extremity metaphyses (fig 1). The metaphyseal changes were most marked in the lower extremities. The fibula was excessively long distally relative to the tibia. The hand radiographs showed short metacarpals and phalanges, and multiple trapezoid shaped phalangeal epiphyses. The spine and skull were normal.

Controls

Fragment H1A was sequenced in DNA samples from 25 unrelated controls from The Centre d’Etude du Polymorphisme Humain (CEPH). In addition, 64 unrelated CEPH samples and samples from 34 unrelated controls from Europe, the Near East, and North and South America were studied using single strand conformation polymorphism (SSCP) analysis for the base pair change G → A at nucleotide (nt) 129.

Mutation detection in RMRP

Insertions and deletions in the promoter region were screened using PCR primers RM3IF and RM3IR as described previously. The major mutation (A70G) in cartilage–hair hypoplasia was detected on SSCP gels following PCR with primers RM70F and RM70R. In order to confirm SSCP findings and to detect other possible sequence changes, all DNA samples were sequenced. The promoter region was amplified using RMF and RMR. PCR products were purified using QiaGen Gel Extraction Kit (Qiagen GmbH, Hilden, Germany) prior to automated sequencing (ABI3100 Sequencer; Applied Biosystems, Foster City, CA, USA).

Mutation detection in H1RNA

The promoter and the transcript (341 nts) of H1RNA (GenBank accession no. AL355075, X16612) were amplified in two overlapping fragments using primers H1AR 5'-CCTGCAATATTGATAGT-3'; H1BF 5'-CCGGAGCTTGGAACAGACT-3'; H1AF 5'-CTCTGTTCCCAAAGGGTTT-3'; H1BR 5'-CCCTGTTTCCAAAAGGGTTT-3'. The PCR products were 446 bp and 407 bp (corresponding to nts –105 to +341 and +234 to +743, respectively). SSCP was performed on all PCR products at room temperature and 5 W for 20 h. A large number of band patterns were detected and thus, 25 samples still available were re-amplified using the same primers. The PCR products were purified and sequenced as described above.

RESULTS

In the search for RMRP mutations among 20 patients with diagnosis of MCDS, we found two probands, SMC3 and SMC44, to be homozygous for a base change A → G at nt 70. This mutation is the worldwide major mutation causing CHH. DNA samples from parents of SMC3 were available and both were found to be heterozygous for 70A → G, thus showing recessive inheritance. Clinically, SMC3 had short limbed short stature with no extra-skeletal features suggestive of CHH. This latter diagnosis had been clinically excluded on the basis of normal hair, normal ligamental laxity and normal history of infections with normal immunological and haematological findings, even though the family history of consanguinity was suggestive of an autosomal recessive rather than dominant condition. The radiographic findings were consistent with metaphyseal chondrodysplasia. The only extra-skeletal manifestation of CHH demonstrated by patient SMC44 was mild joint laxity. All other CHH features, aside from the radiographic changes, were absent. He had a history of recurrent respiratory infections and otitis media, but their frequency was in keeping with the normal rate of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Status</th>
<th>All changes in H1RNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nt –18</td>
</tr>
<tr>
<td>SMC1</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC3</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC4</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td></td>
<td>Mother of SMC4</td>
<td>G/G</td>
</tr>
<tr>
<td>SMC11</td>
<td>Patient</td>
<td>G/G</td>
</tr>
<tr>
<td>SMC12</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td></td>
<td>Mother of SMC12</td>
<td>–</td>
</tr>
<tr>
<td>SMC15</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC20</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC23</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC29</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC32</td>
<td>Patient</td>
<td>G/G</td>
</tr>
<tr>
<td>SMC33</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC34</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC40</td>
<td>Patient</td>
<td>–</td>
</tr>
<tr>
<td>SMC41</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC42</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC44</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC45</td>
<td>Patient</td>
<td>T/C</td>
</tr>
<tr>
<td>SMC46</td>
<td>Patient</td>
<td>A/G</td>
</tr>
<tr>
<td>SMC47</td>
<td>Patient</td>
<td>–</td>
</tr>
<tr>
<td>SMC48</td>
<td>Patient</td>
<td>G/G</td>
</tr>
<tr>
<td>Controls</td>
<td>–</td>
<td>39/11</td>
</tr>
</tbody>
</table>

†H1RNA sequence in X1.6612 was used as a reference.
infections in the first few years of life. Similar to patient SMC3, a comprehensive immunological and haematological investigation was normal. Furthermore, additional co-morbid conditions, such as Hirschprung’s disease and gastrointestinal malabsorption, were not present in either patient.

All genomic changes found in the transcript and the promoter region up to nt –47 of H1RNA are listed in table 1. A heterozygous base change (G→A) at nt129 resides in the transcript and was found in five patients and in one healthy mother. One of these patients (SMC44) was homozygous for G/G at nt70 in RMRP. A base change A→G at nt –34 was detected as homozygosity in one patient and as heterozygosity in two patients. This nucleotide resides only four nucleotides upstream from the TATA box of H1RNA. The other changes were all base substitutions in the region between the TATA box and transcription initiation site (nts –7, –8, –9, and –18; table 1).

All these aforementioned changes were found in the controls also. Among the 25 sequenced CEPH controls, base substitutions were found at sites –7, –8, –9, –18, and –34 in six, six, two, 11, and three samples, respectively (table 1). As new polymorphisms, changes T→A and T→C at nt –4 were detected (T/A/C: 48/1/1). The significance and frequency of such three nucleotide polymorphisms within the non-coding sequence, observed also at nt –7 in the patients (table 1), remains unclear. The 129G→A change in the transcript was found in seven of 123 control samples, and although nt129 is evolutionarily conserved,17 we consider all these changes as polymorphisms because of their frequent occurrence also in the controls. In addition, in the controls a new base substitution was found once in H1RNA at evolutionarily conserved nts 73, 125, and 239.17 Based on our data, it is not possible to determine if these changes were rare polymorphisms or pathogenic mutations.

DISCUSSION

Mutations in COL10A1 cause Schmid type metaphyseal chondrodysplasia, and although mutations in many collagen genes can cause more than one disease entity, mutations in COL10A1 have only been reported in MCDS.9 As no mutations have been identified in a large number of MCDS cases, we performed a study on the genes coding for the RNA components of RNase MRP and RNase P. These enzymes are structurally related, share the same protein components, show nuclear localisation, and function in similar processes.15 16

Mutations in RMRP, the gene coding for the RNA component of RNase MRP, cause CHH.15 In a set of 20 patients with a diagnosis of MCDS without COL10A1 mutations, we found two patients homozygous for the 70A→G change in RMRP, the worldwide major mutation in CHH.15 Parents’ samples were available for mutation determination in one case; both parents were found to be heterozygous carriers for 70A→G. Different from the autosomal dominant inheritance of MCDS, this is consistent with an autosomal recessive mode of inheritance as in CHH.

Phenotypic variability in CHH is remarkable, even within sibships.19 It has been suggested that patients with radiographic findings consistent with CHH but without apparent hair hypoplasia have a variant form of CHH.19 However, based on the results of the present study and some previous reports,19 it is more likely that such patients represent the mild end of the spectrum of clinical severity. Therefore, CHH cannot be excluded based on the absence of extra-skeletal manifestations. Other clinical features, such as age at onset of growth failure, can be used in differentiating CHH clinically from MCDS (table 2). In CHH the growth failure is usually already present at birth and always during the first year of life, as was seen also in both patients in this study, whereas in MCDS short stature is seldom the presenting feature and is not present during the first year of life.14 Although radiographic metaphyseal changes are present in both conditions, they differ in severity and distribution (figs 1 and 3). In CHH the metaphyseal changes are most prominent at the knees.
with significant flaring and irregularity of the metaphyses.13 The proximal femurs and other long bones are affected but to a lesser extent. Hand radiographs show short metacarpals and phalanges with metaphyseal irregularity and, especially in older children, cone shaped epiphyses.19 In MCDS, the radiographic changes are most severe at the hips with enlarged capital femoral epiphyses, short femoral neck with significant metaphyseal irregularity, and severe coxa vara deformity with vertically oriented growth plate (fig 3). The metaphyseal changes are marked also at the knees with irregular, thickened, and widened appearance of the growth plates. Hand radiographs are normal in patients with MCDS but no mutation in \textit{COL10A1}, screening for mutations in the \textit{RMRP} gene is recommended. Differentiating CHH from MCDS has important clinical implications. It is not known whether patients with \textit{RMRP} mutations but no extra-skeletal manifestations of CHH are at a similarly increased risk of malignancies as patients with phenotypically typically usual CHH.20 However, our previous studies have shown that the risk of malignancies cannot be predicted on the basis of immunological parameters in an individual patient.21 Therefore, life long follow up of all patients with metaphyseal chondrodysplasia and \textit{RMRP} mutations, irrespective of their phenotypic presentation, is recommended. In addition, appropriate genetic counselling is not possible without proper DNA diagnosis when the family history does not confirm dominant inheritance.

RNase \textit{P} is an endoribonuclease, the function of which is related to that of RNase MRP.18 Owing to the many similarities between RNase MRP and RNase \textit{P}, and the \textit{RMRP} mutations in two patients in this set of MCDS cases, we also sequenced \textit{HIRNA}, the gene coding for the RNA component of RNase \textit{P}. The structure of \textit{HIRNA} is very similar to that of \textit{RMRP}. As CHH results from mutations either in the \textit{RMRP} transcript or those that change the distance between the TATA box and transcription initiation site of \textit{RMRP},11 we sequenced the corresponding regions of \textit{HIRNA}. Altogether, base substitution polymorphisms were found at six sites in the patients and controls upstream from the transcription initiation site. We also detected a frequent base substitution polymorphism 129G→A in the transcript. Finally, three controls harboured a base substitution at one of the conserved nucleotides of \textit{HIRNA}; a rare polymorphism or mutation could not be excluded in these cases. Mutations in the RNA components and proteins of RNase MRP and RNase \textit{P} cause altered growth phenotypes in yeast.22–26 In humans, only mutations in \textit{RMRP} have been characterised and the possible role of the RNA \textit{P} RNA and the protein components of these endoribonucleases in diseases remain open for investigation.

In conclusion, our study suggests that MCDS and CHH may be challenging to distinguish on clinical and radiological grounds. If there is a family history consistent with an autosomal dominant condition, CHH as a cause of the metaphyseal chondrodysplasia can usually be excluded. If no such family history is present and if no mutation in \textit{COL10A1} is found, a diagnosis of CHH should be considered for patients presenting with a metaphyseal chondrodysplasia, even in the absence of extra-skeletal manifestations of the disease. The importance of specific diagnosis is highlighted by the need for appropriate genetic counselling and for proper follow up of the patients. This study showed no conclusive evidence for causative mutations in \textit{HIRNA} in patients with metaphyseal chondrodysplasia, therefore, the role of this gene in skeletal dysplasias remains unclear.

\section*{Acknowledgements}
This study was financially supported by The March of Dimes Birth Defects Foundation (6-FY00-294), Helsinki University’s Research Funds, and the Helsinki University Central Hospital (to M Ridandpaa); by the Shriners of North America (to L Ward and F H Glorieux); by a grant from the Canadian Institutes of Health Research (to W G Cole); and by the Foundation for Paediatric Research, Helsinki, Finland and by a European Society for Paediatric Endocrinology Research Fellowship, sponsored by Novo Nordisk A/S (to O Makiitie).

\section*{Authors’ affiliations}
M Ridandpaa, S Rockas, M Sarkkoja, H Makiitie, Folkhalsan Institute of Genetics and Department of Medical Genetics, Biocoremid, Helsinki, Finland; L M Ward, Department of Pediatrics, University of Ottawa, Ottawa, Ontario, Canada; F H Glorieux, Genetics Unit, Shriners Hospital for Children, McGill University, Montreal, Quebec, Canada; M Susic, W G Cole, O Makiitie, Centre for the Study of Heritable Connective Tissue Diseases, Research Institute, University of Toronto, Toronto, Ontario, Canada; O Makiitie, Hospital for Children and Adolescents, FI-00029 Helsinki University Central Hospital, Finland.

\section*{References}
Transplanting CY282 heterozygous livers is risky

More safeguards may be needed in liver transplantation, after the discovery of a new mutation in a recipient sparking iron overload. This seemingly rare event questions the supposed suitability of donor livers heterozygous for the C282Y gene—the most common type transplanted.

The case centred on iron overload detected in a liver biopsy specimen taken from a recipient four years after transplantation during a hernia repair. Hepatic iron concentration (HIC) was way above normal and hepatic iron index (HII) indicated hereditary haemochromatosis. Yet the recipient did not have the condition before transplantation and was negative for HFE mutations C282Y and H63D. The donor was heterozygous for the C282Y gene and had only mild iron stores in the liver.

Sequencing of the HFE gene disclosed single base heterozygosity in exon 1 g.189G→R, causing serine substitution at codon 6 (R6S) in the recipient, and C282Y heterozygosity in the donor. Two thirds of the recipient’s living relatives were heterozygous for R6S but not C282Y or H63D mutation; none showed iron overload. Thirty five European controls without hereditary haemochromatosis tested negative for the R6G mutation. The authors propose an interaction between R6S and C282Y mutations to explain the events.

The donor was a 46 year old woman with HIC and HII values within the normal range and the recipient a 34 year old man with cirrhosis of the liver from alcohol misuse. Both were Caucasian.

Transplantation of livers heterozygous for C282Y is widespread, but this discovery casts doubt on safety.

Genetic changes in the RNA components of RNase MRP and RNase P in Schmid metaphyseal chondrodysplasia

M Ridanpää, L M Ward, S Rockas, M Särkioja, H Mäkelä, M Susic, F H Glorieux, W G Cole and O Mäkitie

doi: 10.1136/jmg.40.10.741

Updated information and services can be found at:
http://jmg.bmj.com/content/40/10/741

These include:

References
This article cites 28 articles, 10 of which you can access for free at:
http://jmg.bmj.com/content/40/10/741#BIBL

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.

Topic Collections
Articles on similar topics can be found in the following collections
Genetic screening / counselling (886)

Notes

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/