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ARTICLE

Worldwide mutation spectrum in cartilage-hair hypoplasia: ancient founder origin of the major 70A→G mutation of the untranslated *RMRP*

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Pleiotropic, recessively inherited cartilage-hair hypoplasia (CHH) is due to mutations in the untranslated *RMRP* gene on chromosome 9p13-p12 encoding the RNA component of RNase MRP endoribonuclease. We describe 36 different mutations in this gene in 91 Finnish and 44 non-Finnish CHH families. Based on their nature and localisation, these mutations can be classified into three categories: mutations affecting the promoter region, small changes of conserved nucleotides in the transcript, and insertions and duplications in the 5′ end of the transcript. The only known functional region that seemed to avoid mutations was a nucleolar localisation signal region between nucleotides 23–62. The most common mutation in CHH patients was a base substitution G for A at nucleotide 70. This mutation contributed 92% of the mutations in the Finnish CHH patients. Our results using linkage disequilibrium based maximum likelihood estimates with close markers, genealogical studies, and haplotype data suggested that the mutation was introduced to Finland some 3900–4800 years ago, and before the expansion of the population. The same major mutation accounted for 48% of the mutations among CHH patients from other parts of Europe, North and South America, the Near East, and Australia. In the non-Finnish CHH families, the A70G mutation segregated with the same major haplotype, although shorter, as in most of the Finnish families. In 23 out of these 27 chromosomes, the common region extended over 60 kb, and, therefore, all the chromosomes most likely arose from a solitary event many thousands of years ago.
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Introduction

Cartilage-hair hypoplasia (CHH) or McKusick-type metaphyseal chondrodysplasia (MIM 250250) is a pleiotropic disease, characterised by disproportionate short stature. Other common features include hypoplastic hair, defective immunity and risk for malignancies, Hirschsprung disease, hypoplastic anaemia, impaired spermatogenesis, and increased mortality.[1–6] The variation in the clinical severity is remarkable, even within affected siblings. CHH is especially common among the Old Order Amish in North America and Finns.[1,2] The carrier frequencies among the Amish and Finns were calculated to be as high as 1 : 19

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and 1 : 76, respectively.[2,7] There is no precise measure of worldwide incidence, although cases have been observed in numerous populations (International Skeletal Dysplasia Registry; personal observations).

After initial mapping of the *CHH* gene to 9p13,[8] linkage disequilibrium was detected among Amish and Finns.[7,9] Based on the Amish population history and haplotype data, only one mutation was suggested to account for the CHH cases,[1,7] but recently, multiple allelic mutations have been accepted as being possible.[10] Among Finns, 85–95% of the CHH chromosomes were estimated to originate from a common ancestor and contain an identical mutation.[9] As a result of several years of genetic and physical mapping,[11] the first disease-causing mutations in *RMRP* were described.[12] The untranslated *RMRP* gene encodes the RNA component of the RNase MRP complex. Normally, the RNase MRP complex is involved in multiple cellular and mitochondrial functions though the disease-causing functional impairment of the *RMRP* gene product remains to be characterised.[12,13]

Cartilage-hair hypoplasia is counted as being part of the so-called Finnish disease heritage, which consists of some thirty genetic diseases enriched in Finland[14] and has its origin in the special population history of Finland. The current population is thought to have originated from a relatively small founding population and very little immigration has occurred during the last 80–100 generations of expansion.[15–17] The early settlement up to the 1500s covered only the southwest and southeast regions and the coastal areas of the country. The northern and eastern area, or the late settlement region, was inhabited only 15–25 generations ago. The archaeological findings confirm that there has been sparse, although continuous, inhabitation in the southern coastal areas for about 10 000 years (http://virtual.finland.fi/finfo/english/prehistory.html).

In this study, we describe the results of genetic and mutation analyses of *RMRP* in Finnish cartilage-hair hypoplasia patients and 44 CHH families of other nationalities. The major mutation was introduced to the Finnish population 3900–4800 years ago and obviously well before the expansion of the population. Currently, this mutation contributes 92% of the *RMRP* mutations in Finland and 48% of the mutations in a collection of CHH patients from several other populations. We have demonstrated that the same haplotype is segregating with this mutation in Finland and abroad, thus, suggesting a single ancient occurrence and worldwide distribution. In total, eight different mutations in the promoter region and 28 mutations in the RNA coding region of 267 nucleotides have now been reported (this study).[12]

Materials and methods

Subjects and families

The molecular genetic study on CHH was approved by the ethical committee of the Department of Medical Genetics at the University of Helsinki. The Finnish CHH patients were referred for diagnostic workup and genetic counselling from all over the country to the Helsinki University Central Hospital, where the CHH registry has been maintained from two epidemiological surveys in 1974 and in 1986.[2] Since the surveys, additional patients have been studied and evaluated, the current number of patients being 158 from 127 families. The clinical diagnostic features include disproportionate short-limbed, short stature, increased lumbar lordosis, joint laxity, extension limitation at the elbows, and bowed legs. Usually the patients presented with hair hypoplasia, but for inclusion, hair hypoplasia was accepted as a confirmatory feature only. Radiological findings included generalised metaphyseal dysplasia, and normal skull and spine in childhood films. A total of 115 patients from 91 families agreed to participate in this study, and the patients or their custodians signed the informed consent. The birthplaces of the Finnish patients' great-grandparents were inspected from the church parish registers.

The non-Finnish patients from a number of countries were collected through diagnostic consultations with clinicians and genetic counsellors named in the acknowledgments. The essential diagnostic and demographic data were obtained through clinical descriptions, patients' photographs, laboratory results, and copies of the skeletal radiographs of most patients. In addition, the consultants filled in a CHH data form. The ethnic background of the patients from America and Australia was often European. Data and samples were obtained from 63 patients from 53 families. Seven of these samples were obtained through the International Skeletal Dysplasia Registry maintained at the Cedars-Sinai Medical Center, Los Angeles, USA.

DNA samples from 845 regular blood donors of the Finnish Red Cross Blood Transfusion Service were studied for the Finnish major mutation (A70G). Smaller sets of control samples were studied for the other mutations. The control sample registry consists of a total of 971 samples from 35–55 year-old male donors, all of whose grandparents were born in the same geographic area in Finland, who were not aware of any of their relatives participating in the study, and had no knowledge that any of their more distant ancestors were from abroad. A sample was accepted after obtaining a written informed consent and fulfilling the criteria above. The samples were collected in eight regional centres, and the sampling density is representative of the Finnish population at the start of 1900. A sample consisted of the buffy-coat of the whole donation (about 450 ml) generated as a side product in the routine preparation of a leukocyte depleted red cell unit. DNA was extracted using a modified salting out protocol.[18] The ethical committee of the Finnish Red Cross Blood Transfusion Service approved the sample registry.

Mutation detection in RMRP

DNA samples were screened for insertions and deletions in the promoter region using PCR primers RM3IF 5′-CTTAGAAAGTTATGCCCGAAAAC-3′ and RM3IR 5′-GAAAGGGGAGGAACAGAGTC-3′ for amplification and 5% Sequagel (National Diagnostics) gels for separation. In case of changes in the length, this region was amplified using RM3F 5′-GGCCAGACCATATTTGCATAAG-3′ and RM3R 5′-CGGACTTTGGAGTGGGAAG-3′ primers and sequenced. The Finnish major mutation (A70G) was amplified using PCR primers RM70F 5′-GTGCTGAAGGCCTGTATCCT-3′ and RM70R 5′-CTAGGGGAGCTGACGGATG-3′. SSCP analyses were performed in 0.7xMDE (FMC BioProducts) gels at room temperature using 5W overnight. For sequencing of the whole RNA coding region of *RMRP,* PCR primers RMF 5′-CCAACTTTCTCACCCTAACCA-3′ and RMR 5′-AAGGCCAAGAACAGCGTAAA-3′ were used. In some samples, the 3′ region was separately amplified for sequencing using RM4F 5′-AGAGAGTGCCACGTGCATAC-3′ and RM4R 5′-CTTCATAGCAAGGCCAAGAAC-3′. All PAGE and SSCP gels were detected using silver staining.[19]

Analysis of polymorphic markers and reconstruction of haplotypes

Primers and separation conditions for D9S163, 99AC, TESK1 A/G, MN/CA9 C/A, D9S1804, 89AC, 47K16T7 SSCP,[11] 16P1, AB12GS, Del13, SIT45S, TP2A, TA13S, D4S, D6S, AC1, XL1S, CCS, and TG2S[12] have been described previously. Marker delG was amplified using primers 5′-TGCATGCTGCTTACTGGTTC-3′ and 5′-CCAATATCATGGGGGATGAC-3′ followed by separation in 0.7xMDE (FMC BioProducts) SSCP gels at 5W overnight. Primers for D9S165 and D9S1878 were as described (http://www.cephb.fr) and fragments were separated in 6% Sequagel (National Diagnostics) gels at 50°C. The four frequently polymorphic SNPs at nucleotides −149T/A, −58T/C, −48C/A, and at 274T/C were found during sequencing of the promoter and the RNA coding region of the *RMRP* gene (fragments RM3, RM4 and RM as described above). The distance between the transcription initiation site of *RMRP* and each marker was determined using our genomic contigs[12] and sequences AL133410 and AL135841 from the NCBI database at http://www.ncbi.nlm.nih.gov/entrez. In the case of AB12GS and 16P1, the length of BAC clone 341J2 (160 kb) determined by the pulsed-field gel electrophoresis was also needed.[11]

Haplotypes of the Finnish CHH families were reconstructed assuming a minimum number of recombinations in each family and grouped according to the mutation in *RMRP*. Haplotypes segregating with the Finnish major mutation and the healthy haplotypes were used to calculate the age of this mutation in the Finnish population. The two patients showing UPD[20] were excluded from this analysis.

In order to find out if the A70G mutation had arisen once or several times in the non-Finnish CHH patients homozygous or heterozygous for the A70G mutation, 17 CHH families and two Amish CEPH (Centre d'Etude Polymorphisme Humain) controls were genotyped. These controls were previously found to be nucleotide 70G carriers.[12] Haplotypes were reconstructed using 15 markers and assuming a minimum number of recombinations in each family.

Methods of computing the age of the major mutation in the Finnish population

To estimate the age of the major A70G mutation, we used the DMLE programme[21] which, in its original form was designed to estimate the recombination distance between a biallelic marker and a disease gene based on observed (or supposed) historical recombinations in the data rather than the age estimation. The possibility of estimating the age of a mutation was implemented in a more recent version (beta 0.25, released April, 1999) of the programme given the parameters of 'known' recombination rate, population growth rate, frequencies of the disease associated marker alleles, (ie identical by state, IBS)[21] in sampled disease chromosomes and in the general population, and the proportion of the disease chromosomes sampled from the population under study. We chose the following parameters for the estimate; the recombination rate was based on the estimated physical distance of 630 kb between D9S163 and *RMRP,* as calculated using the genomic sequence and the BAC contig[11] and the genetic distance of 0.3 cM in the same interval.[9] These numbers led us to assume that 2.1 Mb correspond to 1 cM in this chromosomal region. The general approximation 1 Mb corresponding to 1 cM was used as well. Population growth rate (exponential) was estimated to be 0.070 by actual later historical census data from the 18th century and assuming that the age reaches far beyond the start of the Common Era. The disease chromosome associated allele frequencies were directly calculated from the data, and their population frequencies from the healthy parental chromosomes. The proportion of sampled disease chromosomes was calculated from the frequency study in the blood donors' samples (10/845), taking the Finnish population size as 5 million. Two million Monte Carlo replicates using different seeds were computed for the 2.1 Mb correspondence, and the average likelihoods taken to represent the final values because of the poorer convergence to a definite maximum as compared to the 1 Mb correspondence to genetic length of 1 cM, where only one million replicates were used. We also computed the means-of-moments estimator for the most recent ancestor (TMRCA)[22] to evaluate the agreement with DMLE age estimates. We did not apply the correction as suggested by Labuda *et al.*[23] Markers TESK1 A/G SSCP, delG, D4S, AC1, and XL1S were selected for the estimation, since they had more than one instance of presumably non-ancestral allele presented in the haplotype in disease chromosomes. However, the two most centromeric markers

(CCS and TG2S) were not included in any of the calculations because of an obvious recombination hotspot separating them from the rest of the haplotype.

Results

RMRP mutations segregate with specific haplotypes in the Finnish population

Approximately 160 patients with cartilage-hair hypoplasia have been diagnosed in Finland (The Finnish CHH patient registry).[2] After finding the first CHH-causing mutations in *RMRP,* we continued the mutation search in the rest of the 115 Finnish patient samples from 19 multiplex and 72 uniplex families. We found the Finnish major mutation G at nucleotide 70 (A70G) to be homozygous in 74 families (Table 1). All patients in the remaining families were compound heterozygotes for this mutation and represented one of the four minor mutations for the other allele. The most common minor mutation was also a base substitution (G262T) and was found in 13 families. The other minor mutations were detected only in one or two Finnish families, as shown in Table 1. All mutations found in the Finnish CHH patients resided either in the evolutionally conserved nucleotides of the *RMRP* transcript[24,25] or disrupted the function of the promoter region.[12] The G262T mutation was not found among 173 anonymous Finnish blood donors, nor were the other minor mutations found among 120 of these controls and 160 non-Finnish controls. Ten heterozygous carriers for the mutation G at nucleotide 70 were found among 845 anonymous blood donors of the Finnish Red Cross Blood Transfusion Service, representing the geographic population distribution in the beginning of the 20th century.

When the DNA samples of the parents were available, we genotyped them and reconstructed haplotypes, segregating

Table 1 *RMRP* mutations in Finnish and foreign CHH patients

Mutations in RMRP	*Nationality*	*Number of families*	
		Uniplex	*Multiplex*
70G/G[a]	Finnish	59[d]	15
70G/262T[b]	Finnish	9	4
70G/211G	Finnish	1	
70G/154T	Finnish	1	
70G/dupTACTCTGTGA at −13[c]	Finnish	2	
70G/G	US, Canadian, Dutch, Turkish, English, US Amish	12	1
70G/63T	Australian	1	
70G/79A	US	1	
70G/180A	Mexican	1	1
70G/182C	Dutch, English	1	1
70G/211G	US	1	
70G/dup TCTGTGA at −13	US		1
70G/dup AAGCTGAG at −6	Mexican		1
70G/dup TGAAGCTGAG at −6	French		1
70G/ins CCTGAG at −6[c]	German	1	
70G/dup AAGCTGAGGACGTG at 1	US	1	
70G/dup GGACGTGGTT at 4	Brazilian	1	
70G/ins TTCCGCCT at 57	French	1	
70G/dup TG at 98[c]	Canadian	1	
118G/214T	German		
126T/T	Arabian		1
146A/A	Chinese	1	
152G/243T	Canadian	1	
193A/242G	Canadian	1	
195T/238T	Israelis	1	
195T/242G	Brazilian	1	
211G/230T	Polish		1
236G/238T	US	1	
261T/T	Israelis	1	
264A/A	Austrian	1	
4T/del AG at 94	English	1	
193A/dup TCTGTGAAGCTGAGGAC at −3[c]	English		
195T/ins GGACGTGGTT at −4	US	1	
dup TG at 98/dup TG at 98	Turkish	1	
dup TG at 98/2 times dup ACTACTCTGTGAAGC at −10[c]	Swiss	1	

Many of the US patients represent evidence of Caucasian family history. [a]including 42 previously reported families (30 uniplex and 12 multiplex); [b]including six previously reported families (five uniplex and one multiplex); [c]previously reported family/families;[12] [d]including two patients with uniparental disomy for chromosome 9 (patients A and B in[20]).

with the different *RMRP* mutations. The major mutation always segregated with the Finnish major haplotype (in this study),[12] the shortest haplotype being less than 29 kb in length between markers D13 and a base substitution polymorphism in the first exon of *MN/CA9* (data not shown). The minor mutation G262T and the 10 bp duplication at −13 segregated with their own haplotypes as depicted in Figure 1A. All minor haplotypes were more than 200 kb

A

D9S165	D9S1878	D9S163	99AC	16P1	AB12GS	TESK1 A/G	Del13	SIT45S	149T/A	58T/C	48C/A	*RMRP*	274T/C	MN/CA9 C/A	TP2A	TA13S	delG	D6S	AC1	XL1S	CCS	TG2S	D9S1804	89AC	47K16T7 SSCP
7	2	3	6	1	1	1	1	1	T	T	C	nt70G	T	1	1	1	1	1	1	1	1	1	3	9	3
5	3	4	6	2	2	2	2	2	A	C	A	nt262T	T	1	1	1	1	2	2	2	1	1	3	8	2
6	4	4	6	2	2	2	2	2	A	C	A	nt262T	T	1	1	1	1	2	2	2	1	1	3	8	1/2
7	4	4	6	2	2	2	2	2	A	C	A	nt262T	T	1	1	1	1	2	2	2	1	1	3	8	2
5	4	4	6	2	2	2	2	2	A	C	A	nt262T	T	2	1	1	1	2	2	2	1	1	3	8	2
5	4	4	6	2	2	2	2	2	A	C	A	nt262T	T	2	1	1	1	2	2	2	1	1	3	8	2
5	N	4	6	2	2	2	1	2	A	C	A	nt262T	T	1	1	1	1	2	2	2	1	1	3	8	2
5	3	4	6	2	2	2	2	2	A	C/T	A	nt262T	T	1	1	1	1	2	2	2	1	1	3	8	2
6	N	5	6	2	2	1	2	1	A	C/T	A	nt262T	T	1	1	1	1	2	2	2	1	1	3	8	3
5	3	4	6	2	2	2	2	2	A	C/T	A/C	nt262T	T	1	1	1	1	1/2	2	2	1	1	3	8	2
5/7	2/4	4	6	1/2	1/2	1	1/2	1/2	T/A	C/T	A/C	nt262T	T	1	1	1	1/2	1/2	2	2	1	1	3	8/9	2
5	N/4	4	6	2	2	1/2	1/2	1/2	A	C/T	A/C	nt262T	T	1/2	1	1	1	2	2	2	1	1	3	8	2
5/7	N/4	4	N/6	2	1/2	1/2	1/2	1/2	T/A	C/T	A/C	nt262T	T	1	1	1	1/2	1/2	2	1/2	1	1/2	3	8	1
3/4	1/3	3/4	6	1/2	1/2	1/2	1/2	1/2	T/A	C/T	A/C	nt262T	T	1	1	1	1	1/2	1/2	1/2	1	1	3	9	2/3
5	N	3	6	1	1	2	2	2	T	C	A	dup	C	2	2	2	1	1	1	1	1	1	2	4	2
7	N	3	6	1	1	2	1/2	2	T	C	A	dup	C	2	2	2	1	1	1	1	1	1	3	9	2
7	4	3	6	1	1	2	2	2	T	C	A	nt211G	C	2	2	1/2	2	1	2	1	1	1	3	8	1
7	4	3	6	1	1	2	2	2	T	C/T	C	nt154T	T/C	1	2	2	2	1	2	1	2	1	3	5	1

B

Distance (nt)	630 000 D9S163	189 000 AB12GS	51 000 TESK1 A/G	47 500 Del13	7 500 SIT45S	219 149T/A	128 58T/C	118 48C/A	0 *RMRP* nt70	204 274T/C	23 240 MN/CA9 C/A	31 500 TP2A	55 000 TA13S	82 000 delG	95 000 D6S	198 000 AC1
English	6	1	2	1	1	T	T	C	G	T	1	2	1	1	1	2
English	3	1	2	1	1	T	T	C	G	T	1	1	1	2	1	2
French	3	1/2	2	1	1	T	T	C	G	T	1	2	1	2	1	2
German	6	1	2	1	1	T	T	C	G	A	1	2	1	2	1	2
Dutch	6	1	2	1	1	T	T	C	G	T	1	1/2	1	1	1	1/2
Dutch	6	1	2	1	1	T	T	C	G	T	1	1/2	1	2	1	1/2
Dutch	6	1	2	2	1	T	T	C	G	T	1	2	1	2	2	2
Turkish	6	2	2	1	1	T	T	C	G	T	1	2	1	2	1	2
Turkish	6	2	2	1	1	T	T	C	G	T	1	2	1	2	1	2
Canadian	6	2	2	1	1	T	T	C	G	T	1	1	1	1	1	1
Canadian	6	2	2	1	1	T	T	C	G	T	1	1	1	1	1	1
Canadian	2	2	2	1	1	T	T	C	G	T	1	2	1	2	1	2
Canadian	6	1	2	1	1	T	T	C	G	T	1	2	1	2	1	2
Canadian	6	1	2	1	1	T	T	C	G	T	1	2	1	2	1	2
US	4	1	2	2	1	T	T	C	G	T	1	2	1	2	1	1
US	6	1/2	2	1	1	T	T	C	G	T	1	2	1	2	1	2
US	4	1/2	1	2	1	T	T	C	G	T	2	2	2	2	1	2
US	3/6	1	2	1	1/2	T	T	C	G	T	1	2	1	2	1	2
US	6	1	2	1	1	T	T	C	G	T	1	2	1	2	1	2
US	6	1	1	1	1	T	T	C	G	T	1	2	1	2	1	2
Brazilian	3	1	2	1	1	T	T	C	G	T	1	1	1	1	1	1
Mexican	4	1/2	1/2	1/2	1	T	T	C	G	T	1	2	1	2	1	2
Mexican	6	2	1/2	1	1	T	T	C	G	T	1	2	1	2	1	2
US Amish	6	2	1	1	1	T	T	C	G	T	1	1	1	2	1	1
US Amish	6	2	1	1	1	T	T	C	G	T	1	1	1	2	1	1
Amish (CEPH)	4/6	2	1	1	1	T	T	C	G	T	1	1/2	1	1/2	1	1/2
Amish (CEPH)	3/6	1	1	1	1	T	T	C	G	T	1	1	1	1	1	1/2

Figure 1 Haplotype analyses in the Finnish and non-Finnish CHH families. (**A**) The haplotypes segregating with the Finnish minor mutations are different from that segregating with the Finnish major mutation G for A at nucleotide 70 of *RMRP*. The major haplotype determined in Finnish CHH families is depicted in blue. (**B**) The non-Finnish chromosomes segregating with the major mutation show the Finnish major haplotype. The four Amish CHH haplotypes are reconstructed from an Amish CHH family and from two Amish CEPH controls. On top are shown the distances between the markers and the nucleotide 70 of *RMRP*. The telomere is to the left. N, an undetermined allele.

in length and longer than a large proportion of the major haplotypes suggesting a more recent occurrence of these mutations.

The birthplaces of the great-grandparents of 107 CHH patients were reported[2] to reside relatively evenly both in the early and late settlement regions of Finland (Figure 2A). We separately collected the information about the great-grandparents of the patients with the minor mutations and plotted their birthplaces on the map of Finland. Grandparents of the minor mutation carriers were found more often in the late settlement region (Figure 2B), whereas the birthplaces of grandparents of the major mutation carriers were distributed more evenly in both the early and late settlement regions. The distribution of the birthplaces of the grandparents of the blood donors carrying the A70G mutation in the early and late settlement regions is shown in Figure 2C.

The A70G substitution is the most common mutation among CHH patients worldwide

We studied patients from 44 different families, representing nationalities from Europe, North and South America, the Near East, Australia, and China (Table 1). Patients from 13 families were homozygous for the G mutation at nucleotide 70, whereas patients from 15 families were heterozygous for this mutation and represented one of the numerous rare mutations in the pairing allele (Table 1). Patients from 16 families had only rare mutations. Patients from five of these families were homozygous for their private mutation, suggesting parental consanguinity (Table 1). In addition to the major mutation, the base substitution G at nucleotide 211 was the only mutation found both among the Finnish (one family) and non-Finnish patients (two families). Like the Finnish mutations, most of the rare mutations in the foreign samples were base substitutions in the transcribed region. In the 5′ end of the coding region, duplications or insertion of 8–14 nucleotides represent a new type of mutation in the CHH patients. All sites for the different types of mutation in the transcript are evolutionally conserved[24,25] and were not found among 120 Finnish and 160 non-Finnish controls. We did not find any *RMRP* mutations in nine patients who probably do not have CHH but another type of chondrodysplasia.

Since the mutation G at nucleotide 70 was common in several countries, we asked the question whether this mutation had a single origin or if this nucleotide was a hot spot for mutations. Eight homozygous and nine heterozygous patients and their family members, and two Amish CEPH (Centre d'Etude Polymorphisme Humain) controls were genotyped and haplotypes were reconstructed. Chromosomes from different nationalities and the Old Order Amish carrying G at the nucleotide 70 shared the same

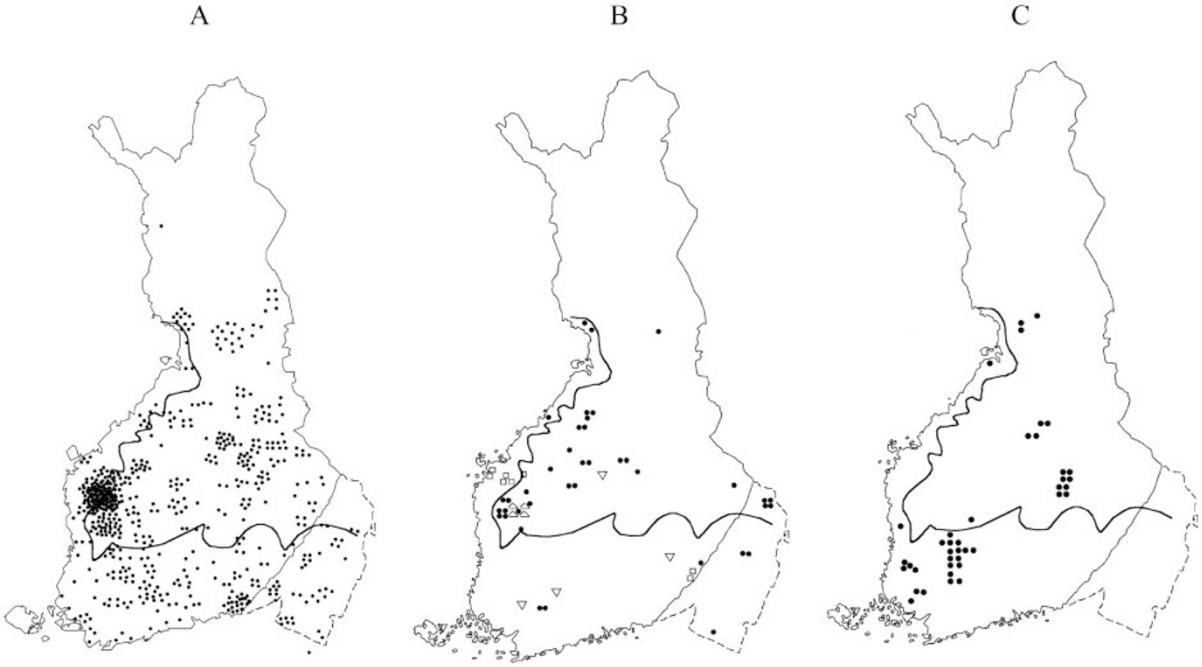

Figure 2 Geographical distribution of the *RMRP* mutations in Finland. (**A**) Birthplaces of the great-grandparents of 107 CHH patients (83 CHH families).[2] (**B**) Birthplaces of grandparents of the carriers (or CHH patients' parents) of the minor mutations in *RMRP*. ●, G262T; □, the Finnish duplication; △, G154T; ▽, C211G (**C**) Birthplaces of the grandparents of the blood donors carrying substitution G at nucleotide 70. In each map, one symbol depicts one great-grandparent/grandparent. The line denotes the approximate border between the old and new settlement in Finland. The area depicted by the dotted line belonged to Finland before the Second World War.

alleles with the Finnish major haplotype (Figure 1B). Twenty-three of the 27 haplotypes were similar, at least in a region of 60 kb around the *RMRP* mutation, clearly pointing to a common origin of this base substitution rather than due to frequent occurrence.

The Finnish major mutation is ancient

According to our maximum likelihood estimates with the DMLE program[21] the major *RMRP* mutation arrived in Finland some 130–160 generations ago corresponding to an age (given a generation time of 30 years)[26] of approximately 3900–4800 years (Figure 3). The estimates were computed using five of the markers (TESK1 A/G SSCP, delG, D4S, AC1 and XL1S) and averaging the likelihood points, each consisting of either two or one million Monte Carlo replicates (Figure 3). Using the same five markers and estimates for the genetic and physical correspondence, the method of Risch *et al*[22] also resulted in high age estimates, although with much wider limits of 150–314 generations. These high age estimates are in keeping with the distribution of the mutation, which also suggests an ancient introduction of this mutation into the Finnish population.

Discussion

Recently, we have reported that two types of mutation in *RMRP* can lead to cartilage-hair hypoplasia. Duplications and insertions between the TATA box and the transcription initiation site silence transcription from that allele, and changes involving at most two highly conserved nucleotides of the transcript lead to a homozygous or compound heterozygous mutant transcript.[12] Altogether, in a set of 91 Finnish and 44 CHH families from other countries, we have found 36 different mutations in the *RMRP* gene; 28

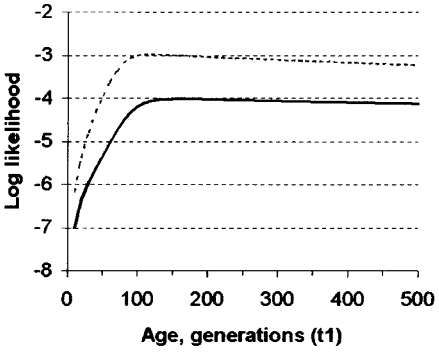

Figure 3 Age estimate average plots by the Disequilibrium Maximum Likelihood Estimate (DMLE) programme using five markers, TESK1 A/G SSCP, delG, D4S, AC1, and XL1S, around the A70G mutation in *RMRP* in Finns. A continuous line is for assuming a correspondence of 1 cM to 2.1 Mb and a broken line for a correspondence of 1 cM to 1 Mb. The maximum likelihoods are t1=160 generations and t1=130 generations, respectively. The lower 95 CI boundary is at around 30 generations for each age estimate, whereas the upper may not be plotted at all.

of them are reported for the first time in this paper. These can be classified into three groups. The first group of mutations consists of eight insertions and duplications that affect the promoter region. The second group of mutations consists of 24 base substitutions and two other changes in conserved nucleotides of the transcript. In addition, we have characterised herein three insertions and duplications in the 5′ end of the transcript, which form a third group of mutations in *RMRP*. These mutations are gathered around the nucleolar localisation signal region[27] and the regions reported to be important for binding the different proteins of the RNase MRP complex.[13] Only heterozygosity of insertion and further duplication mutations in the promoter region or 'null' alleles of *RMRP* were detected, further suggesting that expression of the RNase MRP RNA is indeed essential for life.[12,28]

Mutations in *RMRP* seem to avoid nucleotides 23–62 which have been reported to be a nucleolar localisation signal region.[27] We found a compound heterozygous mutation at nucleotide 57 in one patient, but due to the sequence content of this duplication it does not change the sequence of the nucleolar localisation signal. On the other hand, the mitochondrial localisation signal region has been reported to reside between nucleotides 118–175 in the mouse[29] which corresponds to nucleotides 118–167 in the human *RMRP*. This stretch harbors four different substitution mutations; two patients were even homozygous for this kind of mutation. Functional studies are needed to further clarify the viability of mutations in the nucleolar localisation signal region and the significance of the mutations in the mitochondrial localisation signal region.

Cartilage-hair hypoplasia and five other diseases of the Finnish disease heritage, namely aspartylglucosaminuria (AGU), progressive myoclonus epilepsy (PME), infantile neuronal ceroid lipofuscinosis (INCL), juvenile neuronal ceroid lipofuscinosis (JNCL), and congenital nephrotic syndrome of the Finnish type (CNF), show similar distribution in Finland, and in each disease, the major mutation has been found in 78–98% of the Finnish disease chromosomes.[30–34] The age of these major mutations in Finland has not been calculated, but the distribution of the birthplaces of the great-grandparents supports a hypothesis that these mutations were present in the founding population. The most common mutation among the Finnish CHH patients is the base substitution G for A at nucleotide 70 of *RMRP* representing 92% (211/230) of the disease mutations (this study).[12] Our computation, using Monte Carlo sampling, results in an estimate that the major mutation is ancient in Finland and was most likely introduced before the hypothesised time of the population expansion. The most conspicuous single parameter in these estimates is the correspondence of the physical map and recombination probabilities at very short map lengths. We are able to estimate that 2.1 Mb corresponds to 1 cM on the telomeric side

of the *RMRP* locus where only one out of the five markers suitable for the age calculations resides. Due to the vicinity of the centromere it is possible that 1 cM corresponds to a longer physical distance, while at the same time, we have observed a possible recombination hotspot on the centromeric side. It was also obvious that the algorithm implemented in the DMLE programme does not converge very well when the age of the mutation increased. Our assumption that the introduction of the major mutation to the Finnish population has occurred only once may be wrong since the major mutation appears to be common worldwide (see below). On the other hand, solitary introduction of the mutation is strongly favoured by the length of the shared haplotype segregating among Finns. The means-of-moments estimator calculations suffer the same reservations and, furthermore, do not account for the population parameters and are even more sensitive to assumed recombination rate. As an independent observation, the birthplaces of the great-grandparents of CHH patients are distributed in the early and late settlement regions relatively evenly, and strengthen the assumption that the major mutation was present at the time of the beginning of the population expansion some 80–100 generations ago.[2,17] Similarly, our haplotype data, and the distribution of different mutations in Finland, allow us to assume that the four Finnish minor mutations are younger than the major mutation and that the local enrichment of CHH in the western Finland is, at least in part, due to the enrichment of the rare mutations in that region.

The Finnish major mutation is also the most common mutation (48%) among the CHH patients in 44 families from other countries. Patients from 13 families are homozygous, whereas patients from 15 families are heterozygous for the A70G substitution representing nationalities from Europe, North and South America, the Near East, and Australia. The ethnic background of the patients from America and Australia, in particular, was often European and mixed. In all non-Finnish CHH families, the major mutation segregates with the same major haplotype as in the Finnish families. In 23 out of the 27 chromosomes, the common region expands over 60 kb. Interestingly, one of the homozygous US patients is Amish. In this Amish patient and two Amish CEPH control chromosomes, which were available for genotyping, the haplotypes are the same as the Finnish major haplotype extending over 100 kb region around the *RMRP* locus. A more extensive collection of Amish samples is needed in order to find out if the A70G substitution accounts for all the frequent CHH cases among Amish or whether several different mutations are also involved in that population.

In conclusion, different insertions and duplications in the promoter region and a large number of mutations in the *RMRP* transcript can cause CHH. Our findings clearly support the hypothesis that a single ancient nucleotide 70 G mutation in *RMRP* causes most of the Finnish CHH cases and contributes to the majority of the non-Finnish cases, possibly also including the Amish enrichment. It is very likely that all of the nucleotide 70G mutations reported in the study represent a single occurrence of this base substitution. The ancient A70G mutation was introduced to Finland by the founder individuals and is highly enriched in the current population.

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