Human Molecular Genetics, 2003, Vol. 12, No. 21 ***2837–2844***
DOI: 10.1093/hmg/ddg306

Molecular defect of RAPADILINO syndrome expands the phenotype spectrum of *RECQL* diseases

H. Annika Siitonen[1], Outi Kopra[1], Helena Kääriäinen[2,3,4], Henna Haravuori[1], Robin M. Winter[5], Anna-Marja Säämänen[6], Leena Peltonen[1,7] and Marjo Kestilä[1,*]

[1]Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland, [2]Department of Medical Genetics, The Family Federation of Finland, Helsinki, Finland, [3]Department of Medical Genetics, University of Turku, Turku, Finland, [4]Department of Paediatrics, Turku University Central Hospital, Turku, Finland, [5]Department of Clinical and Molecular Genetics, Institute of Child Health, London, UK, [6]Department of Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland and [7]Department of Medical Genetics, University of Helsinki, Helsinki, Finland

Received July 7, 2003; Revised and Accepted August 27, 2003

The *RECQL4* helicase gene is a member of the *RECQL* gene family, mutated in some Rothmund–Thomson syndrome (RTS) patients. Other members of this gene family are *BLM* mutated in Bloom syndrome, *WRN* mutated in Werner syndrome and *RECQL* and *RECQL5*. All polypeptides encoded by *RECQL* genes share a central region of seven helicase domains. The function of RECQL4 remains unknown, but based on the domain homology it possesses ATP-dependent DNA helicase activity such as BLM and WRN. Rothmund–Thomson, Bloom and Werner syndromes have overlapping clinical features, of which high predisposition to malignancies is the most remarkable feature. Here we report a fourth syndrome resulting in mutations in the *RECQL* genes. RAPADILINO syndrome is an autosomal recessive disorder characterized by short stature, radial ray defects and other malformations, as well as infantile diarrhoea, but not by a significant cancer risk. Four mutations in the *RECQL4* gene were found in the Finnish patients, the most common mutation representing exon 7 in-frame deletion saving the helicase domain and showing dominant effect over other three nonsense mutations. The tissue expression of *Recql4* in mouse well agrees with the tissue symptoms of RAPADILINO. The skeletal malformations in RAPADILINO and RTS patients as well as the high osteosarcoma risk in RTS propose a special role for *RECQL4* in bone development.

INTRODUCTION

RAPADILINO syndrome (www.ncbi.nlm.nih.gov/Omim, MIM 266280) (1) is an autosomal recessive disorder and the acronym stands for hallmark features: RAdial hypo-/aplasia, PAtellae hypo-/aplasia and cleft or highly arched PAlate, DIarrhoea and DIslocated joints, LIttle size (height—2 SD or smaller) and LImb malformation, NOse slender and NOrmal intelligence. RAPADILINO belongs to the Finnish disease heritage, being more prevalent in Finland than in any other part of the world (2–4). We are aware of 14 Finnish RAPADILINO patients and three non-Finnish cases have been reported (5–7) as well. One of the non-Finnish cases (7) later developed poikiloderma, a typical feature for Rothmund–Thomson syndrome (RTS, MIM 268400) and was re-diagnosed as having a severe form of RTS or an entirely new syndrome (8). Clinical findings from RAPADILINO and RTS are shown in Table 1. Certain overlap between the symptoms of RAPADILINO and RTS prompted us to study whether the *RECQL4* gene, mutated in RTS, would also have a role in the etiology of RAPADILINO.

The gene mutated in some RTS-patients belongs to the *RECQL* gene family (9–13). Furthermore, it is becoming increasingly evident that RTS patients are a genetically heterogenous group and that patients with mutations in *RECQL4* belong to a osteosarcoma prone subgroup (13). Other members of the *RECQL* gene family are *BLM*, mutated in Bloom syndrome (BLM, MIM 210900), *WRN* mutated in Werner syndrome (WRN, MIM 277700) and *RECQL* and

*To whom correspondence should be addressed at: Department of Molecular Medicine, National Public Health Institute, Haartmaninkatu 8, 00290 Helsinki, Finland. Tel: +358 947448723; Fax: +358 947448480; Email: marjo.kestila@ktl.fi

Table 1. Comparison of the clinical picture of RAPADILINO and RTS-patients

Clinical findings	Finnish RAPADILINO patients; $n = 14$		RTS-patients;[a] $n = 41$		RTS-patients with known *RECQL4* mutations;[b] $n = 25$	
	Number	%	Number	%	Number	%
Sex	4 males/10 females	29/71	28 males/13 females	68/32	17 males/8 females	68/32
Small stature	13/14	93	25/38	66	6/6	100
Poikiloderma (± photosensitivity)	0/14	0	41/41	100	25/25	100
Sparse scalp hair	0/14	0	15/30	50	3/5	50
Sparse brows or lashes	0/13	0	19/26	73	2/2	100
Radial ray defects[c]	14/14	100	8/40	20	3/5	67
Cataracts	0/13	0	2/32	6	n.m.	
Osteosarcoma	1/14	7	13/41	32	15/25[d]	60
Patellar hypo/aplasia	12/14	86	n.m.		n.m.	
Joint dislocations	8/14	57	in some		n.m.	
Diarrhoea/vomiting/feeding problems in infancy	12/14	86	7/41	17	n.m.	
Normal intelligence	11/13[e]	85	39/41	95	5/5	100

[a]Wang *et al.* (22).
[b](9–13,24,25,28).
[c]Radial hypo/aplasia and/or aplastic/hypoplastic thumbs.
[d]None of the patients without OS were adults and five of them were only 1–3 years of age. Thus some of them may still have a risk of developing osteosarcoma in the future.
[e]Mildly delayed development in two; one of them had severe infantile meningitis.
n.m., not mentioned.

RECQL5, not so far known to be mutated in any human diseases (14). The overlapping clinical features of Bloom, Werner and Rothmund–Thomson syndromes are chromosomal instability, growth retardation, dermatological changes and predisposition to malignancies (15). All polypeptides encoded by the *RECQL* genes share a central region of seven helicase domains (16). WRN and BLM are known to unwind certain DNA structures (17–19). The function of RECQL4 remains unknown, but based on the domain homology it too possesses ATP-dependent DNA helicase activity.

RESULTS

Identification of RECQL4 mutations

Three markers (www.gdb.org, D8S1836, D8S373 and D8S1925) flanking the *RECQL4* gene on 8q24.3 were analysed in six informative Finnish RAPADILINO families with a total of eight affected patients. The observed two-point LOD scores for these markers were 1.98, 2.28 and 2.12 (at the recombination fraction 0), TDT-LRT values being 0.0628, 0.0033 and 0.0002, respectively. Based on the linkage to the flanking markers it was reasonable to start analysing the 3.6 kb *RECQL4* gene (www.ncbi.nlm.nih.gov, GenBank accession no. NM_004260) as a candidate gene for RAPADILINO syndrome.

Four different *RECQL4* mutations were identified in the RAPADILINO families, a splice site mutation in intron 7 and three nonsense mutations in exons 5, 18 and 19. The splice site mutation, IVS7 + 2delT, is the Fin-major mutation, enriched in this isolated population. Nine out of 13 patients were homozygotes for the IVS7 + 2delT mutation, the remaining four being heterozygotes for this mutation. Detailed information about all the identified RAPADILINO mutations is presented in Table 2 and Figures 1 and 2. All the parents were found to be heterozygous for the *RECQL4* mutations and none of the healthy siblings were homozygous for any of the mutations. DNA analysis of 274 controls (Finns) for the IVS7 + 2delT mutation revealed two heterozygotes, implying a carrier frequency of 1 : 137, which is in good agreement with the incidence of RAPADILINO in Finland (1 : 75 000). A total of 22 *RECQL4* mutations have been previously reported in the RTS patients (9–13), all of them being different from any of our mutations (Fig. 1).

The IVS7 + 2delT mutation destructs the 5′ splice site of intron 7 and is predicted to result in an in-frame skipping of exon 7 (Fig. 2). To confirm this, PCRs of cDNA from numerous normal tissues were sequenced [Clontech fetal cDNA panel (catalogue no K1425-1), fetal cartilage and bone, adult intestine and fibroblast] and verified to contain exon 7. Contrary to this finding in control tissues, a smaller PCR product was obtained from fibroblast cDNA of a RAPADILINO patient homozygous for the IVS7 + 2delT mutation, and sequence analysis confirmed the deletion of exon 7. Elimination of exon 7 removes 44 amino acids of the 1208 amino acids RECQL4 polypeptide (GenBank accession no. NP_004251.1; www.ncbi.nlm.nih.gov/BLAST). Computer-assisted predictions of the deleted peptide sequence failed to identify any known domains or recognition sequences, e.g. binding sites (http://us.expasy.org/tools). However, this particular peptide region is very hydrophobic containing, for example, 10 proline residues and protein pI changes from 8.45 to 8.78 (http://us.expasy.org/tools/pi_tool.html), which most probably has an effect on the protein function. Interestingly, the corresponding region in the mouse polypeptide consists of 69 amino acids, whereas the domains encoded by other exons are more similar in size between human and mouse.

Table 2. RAPADILINO mutations

Family	Patient	Sex	Mutation
1	r104	F	Homozygote IVS7 + 2delT
2	r203	F	Homozygote IVS7 + 2delT
3	r303	F	Compound heterozygote IVS7 + 2delT & c.3271C > T (Q1091X)
3	r304	M	Compound heterozygote IVS7 + 2delT & c.3271C > T (Q1091X)
4	r405	F	Compound heterozygote IVS7 + 2delT & c.3214A > T (R1072X)
5	r504[a]	F	Homozygote IVS7 + 2delT
6	r605	F	Homozygote IVS7 + 2delT
6	r606	M	Homozygote IVS7 + 2delT
7	r704[b]	F	Compound heterozygote IVS7 + 2delT & c.806G > A (W269X)
8	r805	F	Homozygote IVS7 + 2delT
9	r903[c]	F	Homozygote IVS7 + 2delT
9	r904	M	Homozygote IVS7 + 2delT
10	r1003	M	Homozygote IVS7 + 2delT

[a]Osteosarcoma at the age of 15.
[b]Mutations of r704 were studied from her parents, because patient's DNA was not available.
[c]Lymphoma, deceased at the age of 25.

In situ hybridization

It has been suggested that the expression of *RECQL4* is tissue-specific, with the highest expression level in thymus and testis (16). Since we found expression in all tested human tissues, we wanted to obtain more detailed information of the expression pattern, and performed *in situ* hybridization studies in fetal mouse tissues. E12.5 embryos did not show any hybridization signal, whereas at E15.5 and E18.5 a significant *Recql4* mRNA expression was detected in several tissues. Most intense signals were found in chondrocytes of developing bone and cartilage as well as in immature proliferating enterocytes of intestine (Fig. 3). Weaker signals were seen in multiple tissues, e.g. in the ventricular zone of the developing brain.

Clinical data

Detailed clinical data for this study were obtained from 14 RAPADILINO patients, 10 females and four males, while the RTS data was gathered from the literature (Table 1). When comparing clinical findings from the RAPADILINO patients with those from the RTS patients, the most distinct difference is poikiloderma, which is the hallmark symptom of RTS, but never present in the RAPADILINO patients. Both sensitivity and unsensitivity to light has been reported in the RTS patients (5,7,9), probably reflecting genetic heterogenity, but the RAPADILINO patients do not have photosensitivity. The only frequent dermatological sign in RAPADILINO is irregular areas of pigmentation resembling café-au-lait spots. A constant feature in the RAPADILINO patients is systematically identified radial ray defects, less frequent in RTS. Both the RAPADILINO and RTS patients are short in stature. In some RAPADILINO cases the other symptoms are so mild that it will be interesting to study whether *RECQL4* mutations could lead to a proportionate short stature without other clinical manifestations.

Surprisingly, RAPADILINO syndrome seems to be more common in females, in contrast with RTS, which seems to be more common in males. The severity of the disease has a rather wide range of manifestations, also within families. Of the three different sex sib-pairs, the affected male systematically presents distinctly milder clinical findings than his affected sister (Kääriäinen *et al.*, manuscript in preparation). In some of the boys, the features are so mild (i.e. small stature, small thumbs and hypoplastic patellae) that the diagnosis would have been missed without the more severely affected sister, which might explain the deviation in the sex ratio. However, the observed phenotypic variation implies some sex-influenced differences in the manifestation of the *RECQL4* mutations.

Feeding, vomiting and diarrhoea problems were observed in almost all of the RAPADILINO patients. Interestingly, these problems have been reported only for a few RTS patients. Maybe this feature has not been considered as a true symptom of RTS and has thereby been left unreported.

DISCUSSION

The expression pattern of *Recql4* in the fetal tissues of mouse is in good agreement with the tissue symptoms of RAPADILINO, including bone and cartilage malformations as well as infantile diarrhoea, implying dysfunction of cartilage and intestinal cells. It will be important to solve the significant role of *RECQL4* in the bone development and in the absorption of nutrients in the intestine.

Overlaps and differences have been observed in the clinical picture between RTS and RAPADILINO. In the future, careful characterization of symptoms, especially dermatological changes, is needed to evaluate whether these diseases are nevertheless a single disorder with multiple clinical features. Furthermore, the identification of *RECQL4* mutations and their effects on the synthesized polypeptide might provide an answer to this question.

Most *RECQL4* mutations found in the RTS patients represent nonsense or frameshift mutations, resulting in a truncated polypeptide (9–13) and in severely impaired or entirely missing helicase activity. The nonsense RAPADILINO mutations 2, 3 and 4 (Fig. 1) also lead to non-functional truncated polypeptides. Opposite to this, the IVS7 + 2delT mutation removes a hydrophobic stretch of amino acids, but leaves the helicase domain intact, although potentially affecting the normal folding of the polypeptide. Importantly, the all Finnish RAPADILINO patients, sharing similar clinical features distinctly different from RTS, are either homozygotes or heterozygotes for the IVS7 + 2delT mutation.

Since it is assumed that the premature stop mutations in the other allele of the patients result in an early degradation of mutant mRNA (9,20), the outcome is the functional predominance of the polypeptides carrying the IVS7 + 2delT deletion. This dominant effect of the in-frame deletion is sufficient to result in the RAPADILINO phenotype, although the nonsense mutations 2, 3 and 4 would predict the phenotype of RTS. In the literature there are only three RTS mutations (g.2626G > A, g.3684G > A and c.1704G > A) predicted to cause the skipping of an exon without frameshift. All these mutations are located in the helicase domain and they severely impair the

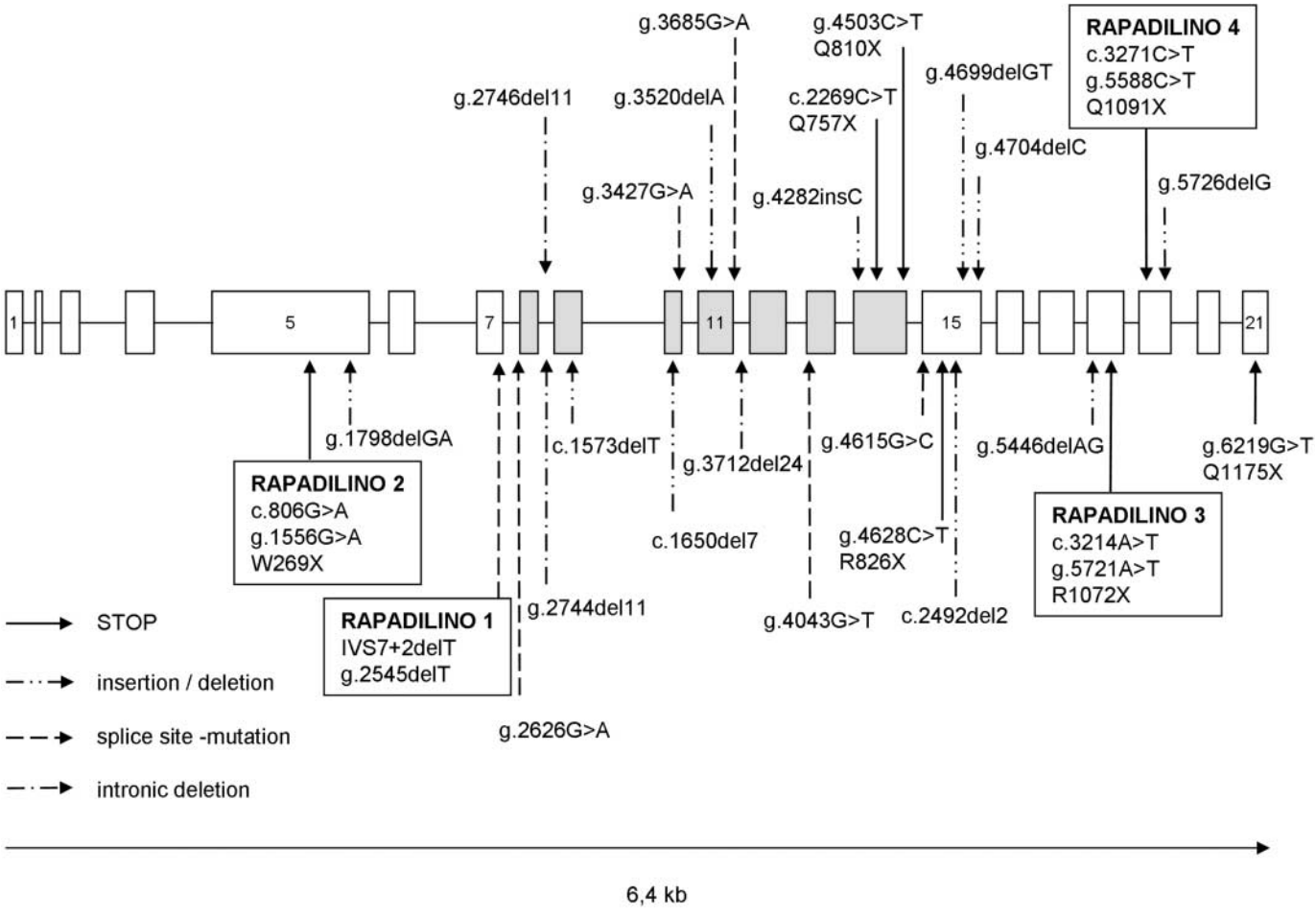

Figure 1. Gene structure of *RECQL4* and known mutations in RAPADILINO and RTS. The genomic structure of the *RECQL4* helicase gene contains 21 exons and it has a typical house-keeping promoter. The whole genomic sequence of *RECQL4* (AB026546) is 6462 bp long, mRNA (NM_004260) being 3627 bp. This sequence encodes protein of 1208 amino acids (NP_004251.1), resulting in a 133 kDa molecule. The RTS mutations have been published previously (9–13). The sites of the mutations have been presented in the genomic sequence or in the cDNA sequence according to the numbering in the original article. In the picture the helicase domain is marked as grey.

helicase activity, unlike IVS7 + 2delT mutation, which is located outside the helicase domain.

An analogous example showing a different effect of mutations of the helicase gene on the clinical phenotype is the xeroderma pigmentosum group D (*XPD*) gene. Mutations in the *XPD* gene directly affecting the repair activity of the ATP-dependent DNA helicase result in the classical XP-D clinical phenotype (XPD, MIM 278730), whereas the effect of other types of mutations is distinctly different, leading to Cockayne Syndrome (CS, MIM 216400) or Trichothiodystrophy (TTD, MIM 601675) (21).

Another interesting feature of the genotype–phenotype correlation of *RECQL* gene defects merges from the well-established tendency of malignancies in Werner, Bloom and Rothmund–Thomson syndromes. Patients with Bloom syndrome are very cancer-prone, in Werner syndrome sarcomas are a common feature, and osteosarcomas are especially frequent among RTS patients (13,15,22). This shared tendency for malignancies could be due to the crucial role of the RECQL helicases in retaining the stability of chromosomal DNA. Fifteen of the 25 RTS patients with mutations in *RECQL4* were reported to have osteosarcoma and no information was given from one patient (9–11,13,23–25). In addition, Wang *et al.* (13) found *RECQL4* mutations in five infantile RTS-patients, who may develop osteosarcoma in the future. Among RTS patients the median age at the time of the osteosarcoma diagnosis has been nine years (13), while the literature reviewed showed that only one RTS patient with the *RECQL4* mutations was over 20 years old when osteosarcoma was diagnosed (9,24). At the moment nine of the RAPADILINO patients are over 20 years old and only one has osteosarcoma. It seems that the osteosarcoma incidence is higher in RTS than in RAPADILINO. A hypothesis could be proposed that the defective helicase activity in the Werner, Bloom and RTS patients makes them prone for cancer and sarcomas, whereas in the RAPADILINO patients, with prevailed helicase activity, the cancer risk is lower.

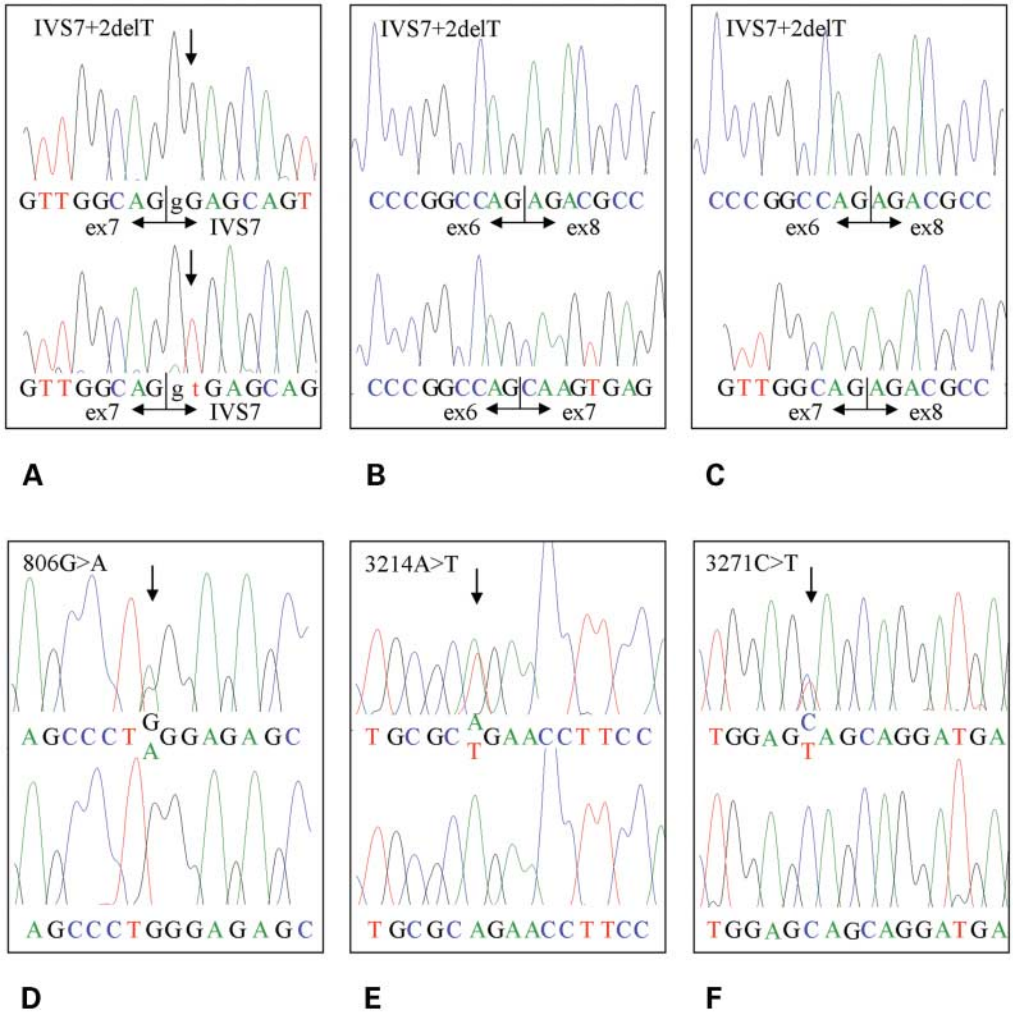

Figure 2. DNA sequence chromatograms of four known RAPADILINO mutations and corresponding controls (upper row; patient, lower row; wild-type control). (**A**) Genomic sequence from patient (r605) homozygous for IVS7 + 2delT and healthy control. The consensus sequence of the donor splice site has been marked with lower case letters. (**B**) cDNA sequence from patient (r605) shows skipping of exon 7. In this case sequence from the end of exon 6 continues to the beginning of exon 8. The splice error causes no frameshift. In the control sequence (cDNA from bone of human embryo) sequence contains exon 7 as expected. The exon boundary of exons 6 and 7 is shown in (B) and the exon boundary of exons 7 and 8 is shown in (**C**). (**D**) Nonsense mutation c.806G > A (W269X) in exon 5. Sequence is from patient r704's mother. (**E**) Nonsense mutation c.3214A > T (R1072X) in exon 18 and (**F**) Nonsense mutation c.3271C > T (Q1091X) in exon 19.

MATERIALS AND METHODS

Patients

Since the rare RAPADILINO syndrome was first described (1), we have conducted a systematic search of RAPADILINO cases in Finland among children born during the years 1970–1990 (M. Mentula, unpublished data). After that, new patients have come to our knowledge only accidentally. In each family, at least one affected child has fulfilled the criteria shown in Kääriäinen *et al.* (1).

Based on this nationwide search, we identified 11 Finnish RAPADILINO families with 14 affected individuals. All these families were contacted and asked to participate. One family refused to participate [patient 1 in Kääriäinen *et al.* (1)], and from one family only the mother and father could be contacted (patient 2 in the same paper). All the other families agreed to participate.

Sequencing

Sequencing of genomic DNA was done by ABI 373A sequencer (Applied Biosystems) according to the manufacturer's instructions. Primers for the mutation detection and RT–PCR were selected by using the human *RECQL4* genomic sequence (AB026546.1). For the detection of mutation 1 (Fin-major) in intron 7 the forward primer 5′-GTGGCCAGTGGTTGTCTTG-3′ and the reverse primer 5′-TTAGGGGA-CAAGCAGCAGTT-3′ were used. The forward primer for RAPADILINO mutation 2 in exon 5 was 5′-TACAATTGA-GCGTGGGGACT-3′ and

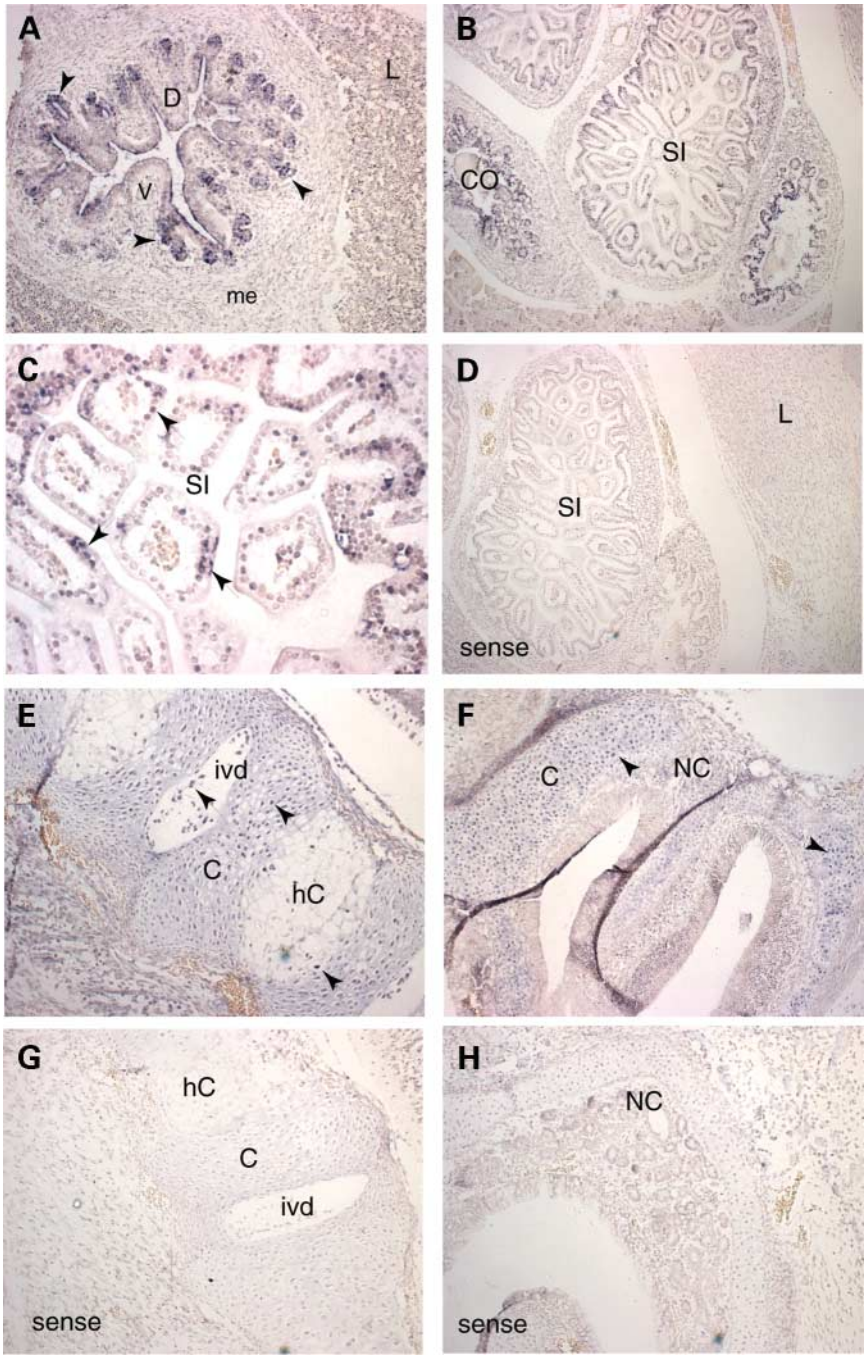

Figure 3. *Recql4* mRNA expression in fetal mouse (E18.5) is strongest in the organs most affected in RAPADILINO syndrome. Most signal was detected in the intestine (**A**–**C**) and the developing bone (**E**) and cartilage (**F**). In the intestine the most prominent expression is seen in epithelial cells (arrows in A and C). A strong specific signal is also found in vertebral (E) and forelimb cartilage (data not shown) as well as in the nasal cavity (F). In the developing bone and cartilage the *Recql4* mRNA is localized in the proliferating chondrocytes (arrows in E and F). mRNA expression was also found in some other organs including liver (A), ventricular zone of the brain and lung (data not shown). Hybridization with the sense probe gives a negative result (**D**, **G**, **H**). D, duodenum; V, villus; L, liver; me, muscularis externa; CO, colon; SI, small intestine; ivd, invertebral disc; C, cartilage; hC, hypertrophic cartilage; NC, nasal cavity. Magnifications: A, B and D, ×10; C, ×40; E, F, G and H, ×20.

the reverse primer was 5′-CCCA-CATAGGAGGGTCACTG-3′. For RAPADILINO mutations 3 and 4 in exons 18 and 19 respectively the forward primer was 5′-GTTGGAGACGAGG-TTGGAGA-3′ and the reverse primer was 5′-CACTGCATCC-ACAGAGCAAG-3′.

Minisequencing

The identification of carriers for IVS7 + 2delT was performed by minisequencing (26) (with slight modifications) of DNA samples from 274 healthy Finns. The forward primer for PCR

was 5′-GCTCCCATTCTACCCTCTCC-3′ and the biotinylated reverse primer was 5′-CAGACAGGATCCGCATGACT-3′. The detection primer for the deletion was 5′-CCCTCAGGGCA GTTGGCAGG-3′.

In situ hybridization

The *in situ* hybridization studies comprised mice (C57BL/ National Public Health Institute, Helsinki, Finland) at embryonic days 12.5, 15.5 and 18.5 (E12.5-18.5). The embryos were fixed by overnight immersion in freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Samples were embedded in paraffin, cut into 5 µm thick sections and mounted onto SuperFrost/Plus microscope slides (Merck, Germany). Animal care and handling were at all times consistent with the guidelines set out in the National Research Council's guide for laboratory animal care and use.

Probes for *in situ* hybridization were prepared by amplifying the 510 bp fragment corresponding to nucleotides −27–483 from 17 days mouse embryo cDNA (Clontech/BD Biosciences). Primers were selected by using the mouse *Recql4* mRNA sequence (XM_128258.1). The forward primer was 5′-TTCCCCTAAACTCACGTTCG-3′ and the reverse primer was 5′-AGGAAGTGCATCGTCAGCTT-3′. The fragment was cloned into the pGEM-T Easy Vector (Promega) and the digoxigenin-labelled anti-sense and sense probes were generated using a DIG RNA labeling kit (Roche) according to the manufacturer's instructions. The anti-sense and sense probes were diluted in 1 : 100 in a hybridization mixture containing 50% formamide, 5× SSC, 10% dextran sulfate, 1× Denhardt's solution, 1 µl/ml RNAse inhibitor and 500 µg/ml tRNA. *In situ* hybridization was performed as described previously by Breitschopf *et al.* (27) with slight modifications. Samples were analysed and photographed using a Zeiss Axioplan 2 imaging microscope (Zeiss). The final figures were prepared using Adobe Photoshop 5.0 and Adobe Illustrator 8.0 software.

Cells

Establishment of fibroblast cell line was done from a skin biopsy of patient r605 homozygous for Fin-major mutation. PolyA mRNA was purified from fibroblast cell line using an Oligotex Direct mRNA midi/maxi kit (Qiagen). RT–PCR from polyA mRNA was performed by using an Advantage RT-for-PCR kit (BD Biosciences). Amplification of *RECQL4* exons 6–9 from cDNA was performed with the forward primer 5′-GAAGTGGCGGAAGAAAGGGGAGTG-3′ and the reverse primer 5′-TAGAGCAGCGCTGGGAGCTGGTAG-3′. Immortalization of lymphoblasts was attempted twice from male patient r904 and both attempts failed. Immortalization of male patient r1003 seemed to be unsuccessful but cells started to grow after about 2 months of incubation. We have not found any difficulties immortalizing lymphoblasts from our female patients (r405 and r504). Miozzo *et al.* (25) reported that they too had failed to immortalize lymphoblasts from RTS male patient.

ACKNOWLEDGEMENTS

We thank all the families for participating in this study. We thank Mirva Södeström, Eero Vuorio and Mikko Kuokkanen for RNA samples, Ritva Timonen for technical assistance and Jani Saarela for advice in protein analyses. This research was supported by the Academy of Finland. A.-M.S. was supported by the Academy of Finland grant number 52940 and L.P. by grant number 202887.

REFERENCES

1. Kääriäinen, H., Ryöppy, S. and Norio, R. (1989) RAPADILINO syndrome with radial and patellar aplasia/hypoplasia as main manifestations. *Am. J. Med. Genet.*, **33**, 346–351.
2. Norio, R. (2003) Finnish Disease Heritage I: characteristics, causes, background. *Hum. Genet.*, **112**, 441–456.
3. Norio, R. (2003) Finnish Disease Heritage II: population prehistory and genetic roots of Finns. *Hum. Genet.*, **112**, 457–469.
4. Norio, R. (2003) The Finnish Disease Heritage III: the individual diseases. *Hum. Genet.*, **112**, 470–526.
5. Vargas, F.R., de Almeida, J.C., Llerena Junior, J.C. and Reis, D.F. (1992) RAPADILINO syndrome. *Am. J. Med. Genet.*, **44**, 716–719.
6. Jam, K., Fox, M. and Crandall, B.F. (1999) RAPADILINO syndrome: a multiple malformation syndrome with radial and patellar aplasia. *Teratology*, **60**, 37–38.
7. Kant, S.G., Baraitser, M., Milla, P.J. and Winter, R.M. (1998) Rapadilino syndrome–a non-Finnish case. *Clin. Dysmorphol.*, **7**, 135–138.
8. Hilhorst-Hofstee, Y., Shah, N., Atherton, D., Harper, J.I., Milla, P. and Winter, R.M. (2000) Radial aplasia, poikiloderma and auto-immune enterocolitis–new syndrome or severe form of Rothmund–Thomson syndrome? *Clin. Dysmorphol.*, **9**, 79–85.
9. Kitao, S., Shimamoto, A., Goto, M., Miller, R.W., Smithson, W.A., Lindor, N.M. and Furuichi, Y. (1999) Mutations in RECQL4 cause a subset of cases of Rothmund–Thomson syndrome. *Nat. Genet.*, **22**, 82–84.
10. Lindor, N.M., Furuichi, Y., Kitao, S., Shimamoto, A., Arndt, C. and Jalal, S. (2000) Rothmund–Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome. *Am. J. Med. Genet.*, **90**, 223–228.
11. Wang, L.L., Worley, K., Gannavarapu, A., Chintagumpala, M.M., Levy, M.L. and Plon, S.E. (2002) Intron size constraint as a mutational mechanism in Rothmund–Thomson syndrome. *Am. J. Hum. Genet.*, **71**, 165–167.
12. Balraj, P., Concannon, P., Jamal, R., Beghini, A., Hoe, T.S., Khoo, A.S. and Volpi, L. (2002) An unusual mutation in RECQ4 gene leading to Rothmund–Thomson syndrome. *Mutat. Res.*, **508**, 99–105.
13. Wang, L.L., Gannavarapu, A., Kozinetz, C.A., Levy, M.L., Lewis, R.A., Chintagumpala, M.M., Ruiz-Maldanado, R., Contreras-Ruiz, J., Cunniff, C., Erickson, R.P. *et al.* (2003) Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund–Thomson syndrome. *J. Natl Cancer Inst.*, **95**, 669–674.
14. Kitao, S., Lindor, N.M., Shiratori, M., Furuichi, Y. and Shimamoto, A. (1999) Rothmund–Thomson syndrome responsible gene, RECQL4: genomic structure and products. *Genomics*, **61**, 268–276.
15. Mohaghegh, P. and Hickson, I.D. (2001) DNA helicase deficiencies associated with cancer predisposition and premature ageing disorders. *Hum. Mol. Genet.*, **10**, 741–746.
16. Kitao, S., Ohsugi, I., Ichikawa, K., Goto, M., Furuichi, Y. and Shimamoto, A. (1998) Cloning of two new human helicase genes of the RecQ family: biological significance of multiple species in higher eukaryotes. *Genomics*, **54**, 443–452.
17. Sun, H., Karow, J.K., Hickson, I.D. and Maizels, N. (1998) The Bloom's syndrome helicase unwinds G4 DNA. *J. Biol. Chem.*, **273**, 27587–27592.
18. Fry, M. and Loeb, L.A. (1999) Human Werner syndrome DNA helicase unwinds tetrahelical structures of the fragile X syndrome repeat sequence d(CGG)n. *J. Biol. Chem.*, **274**, 12797–12802.

19. Constantinou, A., Tarsounas, M., Karow, J.K., Brosh, R.M., Bohr, V.A., Hickson, I.D. and West, S.C. (2000) Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. *EMBO Rep.*, **1**, 80–84.
20. Yamabe, Y., Sugimoto, M., Satoh, M., Suzuki, N., Sugawara, M., Goto, M. and Furuichi, Y. (1997) Down-regulation of the defective transcripts of the Werner's syndrome gene in the cells of patients. *Biochem. Biophys. Res. Commun.*, **236**, 151–154.
21. Lehmann, A.R. (2001) The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, three diseases. *Genes Dev.*, **15**, 15–23.
22. Wang, L.L., Levy, M.L., Lewis, R.A., Chintagumpala, M.M., Lev, D., Rogers, M. and Plon, S.E. (2001) Clinical manifestations in a cohort of 41 Rothmund–Thomson syndrome patients. *Am. J. Med. Genet.*, **102**, 11–17.
23. Wang, L.L., Levy, M.L., Lewis, R.A., Gannavarapu, A., Stockton, D.W., Burks, L.M., Lev, D., Cunniff, C. and Plon, S.E. (2000) Evidence for genetic heterogeneity in Rothmund–Thomson syndrome. *Am. J. Hum. Genet.*, **67** (suppl.), 376.
24. Lindor, N.M., Devries, E.M., Michels, V.V., Schad, C.R., Jalal, S.M., Donovan, K.M., Smithson, W.A., Kvols, L.K., Thibodeau, S.N. and Dewald, G.W. (1996) Rothmund–Thomson syndrome in siblings: evidence for acquired *in vivo* mosaicism. *Clin. Genet.*, **49**, 124–129.
25. Miozzo, M., Castorina, P., Riva, P., Dalpra, L., Fuhrman Conti, A.M., Volpi, L., Hoe, T.S., Khoo, A., Wiegant, J., Rosenberg, C. *et al.* (1998) Chromosomal instability in fibroblasts and mesenchymal tumors from 2 sibs with Rothmund–Thomson syndrome. *Int. J. Cancer*, **77**, 504–510.
26. Syvänen, A.C., Aalto-Setälä, K., Harju, L., Kontula, K. and Söderlund, H. (1990) A primer-guided nucleotide incorporation assay in the genotyping of apolipoprotein E. *Genomics*, **8**, 684–692.
27. Breitschopf, H., Suchanek, G., Gould, R.M., Colman, D.R. and Lassmann, H. (1992) *In situ* hybridization with digoxigenin-labeled probes: sensitive and reliable detection method applied to myelinating rat brain. *Acta Neuropathol. (Berl.)*, **84**, 581–587.
28. Tong, M. (1995) Rothmund–Thomson syndrome in fraternal twins. *Pediatr. Dermatol.*, **12**, 134–137.