ORIGINAL ARTICLE

Takashi Yokota · Tomoko Nakata · Shiro Minami
Jyoji Inazawa · Mitsuru Emi

Genomic organization and chromosomal mapping of *ELKS*, a gene rearranged in a papillary thyroid carcinoma

Received: August 18, 1999 / Accepted: September 22, 1999

Abstract We recently isolated a novel cDNA, designated *ELKS*, that was fused to *RET* cDNA in a papillary thyroid carcinoma. Its encoded polypeptide sequence was rich in glutamic acid (E), leucine (L), lysine (K), and serine (S), and was characterized by the presence of nine alpha-helical coiled-coil domains consisting of periodic heptad repeats. We have now cloned the entire structure of the human *ELKS* gene from within a 700-kb genomic region represented by overlapping bacteriophage P1-derived artificial chromosome (PAC) and bacterial artificial chromosome (BAC) clones, and localized it to chromosomal band 12p13.3 by fluorescence in situ hybridization. The gene is approximately 500kb long, with 19 exons and 18 introns; the transcription initiation site within exon 1 is separate from the initiation codon (in exon 2). Analysis of the exon/intron structure revealed that introns interrupt the coding sequence in such a way that many functional segments of the protein are encoded by distinct exons. Exon 1 encodes the 5' non-coding region; exons 2, 3, 6, 7, 8, 9, 11, 14, and 15 encode the nine coiled-coil domains. Exons 17–19 constitute the 3' non-coding region. Analysis of the region immediately upstream of exon 1 showed that it was extremely rich in G/C nucleotides and contained multiple Sp-1 and AP2 binding sequences. The *ELKS-RET* gene fusion rearrangement we had observed in a papillary thyroid carcinoma occurred between intron 10 of the *ELKS* gene and intron 11 of *RET*.

Key words *ELKS* · Fusion gene · Break point · Papillary thyroid carcinoma

Introduction

Proto-oncogenes *TRKA* and *RET*, both of which encode receptor-tyrosine kinases, are occasionally rearranged in primary papillary thyroid carcinomas. *TRKA* is a receptor for nerve growth factor (Klein et al. 1991); *RET* forms receptor complexes with other molecules that transmit signals of its ligands, which include glial cell line-derived neurotrophic factor (GDNF; Jing et al. 1996), neurturin (NTN; Klein et al. 1998), persephin (PSP; Milbrandt et al. 1998), and artemin (Balon et al. 1998). In affected tumors, these two genes form chimeric oncogenes by fusing their tyrosine kinase domains with 5' portions of different activating genes (Jhiang and Mazzaferri 1994). Four kinds of chimeric oncogenes involving *RET* have been reported in papillary thyroid carcinomas: *RET/PTC1* (Grieco et al. 1990), *RET/PTC2* (Bongarzone et al. 1993), *RET/PTC3* (Bongarzone et al. 1994; Jhiang and Mazzaferri 1994; Santoro et al. 1994), and *RET/PTC5* (Klugbauer et al. 1998). Their 5'-end sequences consist of genes encoding, respectively, H4, regulatory subunit $Ri\alpha$ of protein kinase A, RFG/ELE1, and RFG5.

Recently, we identified a novel cDNA (*ELKS*), the 5' portion of which was fused to the *RET* gene in a papillary thyroid carcinoma as a consequence of a translocation, t(10;12)(q11;p13) (Nakata et al. 1999). The *ELKS* cDNA encodes a novel 948-amino-acid peptide that is expressed ubiquitously in human tissues. The presence of multiple coiled-coil domains in the *ELKS* product suggests that this protein is likely to form dimers. To investigate the molecular basis of the rearrangement we observed in the thyroid tumor, we determined the genomic structure and chromosomal localization of the human *ELKS* gene.

T. Yokota · M. Emi (✉)
Department of Molecular Biology, Institute of Gerontology, Nippon
Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211-8533,
Japan
Tel. +81-44-733-5230; Fax +81-44-733-5192
e-mail: memi@nms.ac.jp.

T. Nakata · S. Minami
Department of Bioregulation, Institute of Gerontology, Nippon
Medical School, Kawasaki, Japan

J. Inazawa
Medical Research Institute, Tokyo Medical and Dental University,
Tokyo, Japan

Material and methods

Cloning of full-length ELKS cDNA by 5' rapid amplification of cDNA ends (RACE)

5' RACE experiments were performed with a Marathon cDNA amplification kit (Clontech, Palo Alto, CA, USA), using human brain poly(A) RNA as starting material, according to procedures described previously (Nakajima et al. 1999b). The adapter-ligated cDNA was subjected to two rounds of polymerase chain reactions (PCR), using a first gene-specific primer, (5'-CCCTCCCGTACTGTTGGTTC-GACGGTG-3') that corresponds to nucleotides 488–515 of the ELKS cDNA (Nakata et al. 1999) and a nested gene-specific primer (5'-GTAGGAAAGGCGAAAGCCTCA-GCACTA-3') that corresponds to nucleotides 142–170 of ELKS cDNA. Sequencing was performed by the BigDye Terminator cycle-sequencing method, using a 377 ABI Prism automated DNA sequencer (ABI), as described previously (Nakajima et al. 1999a)

Isolation of the human ELKS gene

A bacteriophage P1-derived artificial chromosome (PAC) library and bacterial artificial chromosome (BAC) library containing human genomic DNA pooled in a three-dimensional structure (Genome Systems, St. Louis, MO, USA, and Roswell Research Center, Buffalo, NY, USA) was used for the isolation of genomic clones, as described previously (Tsukamoto et al. 1998), using oligonucleotides designed from the ELKS cDNA sequence.

Sequence analysis of exon/intron junctions and the 5'-flanking region

The nucleotide sequences of exons and their boundaries and the 5'-flanking region were determined by directly sequencing the isolated PAC and BAC clones, using oligonucleotide primers with sequence identity to ELKS cDNA (Nakata et al. 1999). The size of each intron was determined by direct sequencing of the overlapping PAC and BAC genomic clones or estimated by PCR amplification, using LA Taq and two oppositely oriented primers located in the respective flanking exons (Takara, Tokyo, Japan) according to procedures described previously (Nakajima et al. 1999b).

Chromosomal mapping of the human ELKS gene by fluorescence in situ hybridization (FISH)

To assign the human *ELKS* gene location on human chromosomes, a PAC clone carrying the human PAC chromosomal gene was used as a probe. Fluorescence in situ hybridization using PAC DNA was carried out as described previously (Mukae et al. 1998). The slides were examined through a Nikon epifluorescent microscope equipped with a

change-coupled device (CCD) camera (Photometrics, Tucson, AZ, USA). Images were captured with Quips (Vysis) software and processed with Adobe Photoshop 3.0 software.

Results and discussion

Genomic characterization of the ELKS gene

A 700-kb genomic region containing the entire human *ELKS* gene was obtained from seven overlapping clones. Five of these clones (232n23, 136j23, 927j12, 60g23, and 90j10) were isolated by screening a human genomic PAC library; the other two (366a16 and 359b12) were from a human genomic BAC library. The *ELKS* gene spanned more than 500kb and consisted of 19 exons interrupted by 18 introns (Fig. 1). Intron-exon boundary sequences and exon sizes are given in Table 1, along with intron sizes that were either determined by direct sequencing of genomic clones or estimated by PCR amplification using primers located in adjacent exons. Sequences at all exon-intron boundaries were compatible with the consensus sequence for splicing junctions, including AG-GT (Mount 1982). Comparison of the genomic and cDNA sequences revealed that exon 1 and the 5' half of exon 2 encode the 5' untranslated region (UTR), while the 3' portions of exon 2 through exon 16 are coding regions. Exon 17 encodes both the carboxyl-terminal coding region and 3' UTR; exons 18 and 19 encode only the 3' UTR.

Sequencing and characterization of the 5' portion of ELKS cDNA

The nucleotide sequence of the partial human ELKS cDNA that we described earlier (Nakata et al. 1999) contained the whole open reading frame (ORF) but lacked a complete sequence of its 5' UTR. We therefore carried out 5' RACE experiments and determined the full-length sequence that included an additional 12bp of cDNA sequence on the 5' end (Fig. 2). In this manner we determined that the full-length human ELKS cDNA consists of 406bp of 5' UTR, 2,844bp of coding sequence, and 1,705bp of 3' UTR.

The putative promoter sequence of the *ELKS* gene was examined with the TESS program to identify any binding sites for transcription factors. The G/C-rich 5'-flanking region was found to contain several Sp1 sites and AP2 sites. No CAAT or TATA box was present in the 5'-flanking region of the *ELKS* gene. These features are characteristic of the promoters of housekeeping genes (Dyran 1986).

Chromosomal mapping

PAC genomic clones served as probes to localize the *ELKS* gene by fluorescence in situ hybridization on metaphase chromosomes. Clear fluorescent signals were present on chromosomal band 12p13.3 (Fig. 3). We had already linked

Fig. 1. Upper panel, Genomic structure and map of the human *ELKS* gene. Overlapping PAC and BAC genomic clones that cover the entire region are shown above the map. Lower panel, Relationship between exonic organization and functional domains of *ELKS*. A translation initiation codon (ATG) is present in exon 2, and a translation stop

codon (TGA) in exon 17. Exons are represented by *black-outlined boxes*, and the breakpoint of the *ELKS* gene rearrangement observed in a papillary thyroid carcinoma is indicated by the *vertical arrow*. Coiled-coil domains are indicated by *hatched boxes*

Table 1. Exon/intron organization of human *ELKS* gene

Exon Number	Exon size (bp)	Sequence at exon/intron junction ^a				Intron size ^b (kbp)
		3' Splice acceptor	5' End of exon	3' End of exon	5' Splice donor	
1	250			GCGGAGCTGTAG	gtgaggtcttcgcgacccct	ND
2	825	gtgtgatctttcattacag	ATATGGTGTAAG	GAGGAAAACCAG	gtaagttctacgtgtgtta	ND
3	417	tctttttgtcttggtctga	CACATGCAGATG	ATGTTGAGAGAG	gtatgtgactacttttttag	ND
4	75	tttctgcctcttttctacag	GAGATCGATCGA	ATTGAGATGAAG	gtaagtgagaatctagtaaa	3.8
5	156	aaatcttcattgtcttcag	GATTCAAAAATT	ATGAAAAATAAG	gtaatggcatgtgagacttt	ND
6	168	gtgttttactcttttctag	ATTGGCCAGGTG	CTGCAGACTGAG	gtagaacaattctgggatt	ND
7	169	taaaactctcatctcttag	GTGGATGCTCTC	CTTCAGAAGAAG	gtaaggtacaggtgtttcga	39
8	137	aaatattatgtacatttag	ATTGAAAATCTT	CTTCAGAGAGAAA	gtgagtggctggcccaact	1.3
9	141	ttgatattctctcatcag	GAGCGGACAATT	TCAGAGAAAGAG	gttaagctcccaaaatgga	1.2
10	141	agatgtgtgttctctttt	AGGCTTCACTTT	CAATTGAAAAAG	gttaaagaaaaaattcaca	6.4
11	194	tgaatgtctttctttgtag	GCACATGAGGCA	TGAGTTGGAAAAG	gtaagaaagtgaagctgatt	14.6
12	12	ggatttctgtttctctcag	TCTCACCTCAAG	TCTCACCTCAAG	gtcagtagctgtgactaaa	32
13	136	tttctctctctgtcatttag	GCAAGTGAAAAGA	CAGCAGCTACAG	gttagaacacaaaggagaatc	53
14	161	cttcccatatttatgatag	GTGGAGGAGTTA	TCTGGAGATGAA	gtaagtgtttgtagtcttat	81.9
15	145	gcatttgcctttctttgcag	GCAAGAAGCTCT	CTTAAGCAGCAG	gtattattaaatgccagcag	36
16	99	tatgaataatttctcttag	ACGCAAAATCGA	TCCCCAGACCAG	gtaagatggctctggaatgt	2.2
17	65	gtcatctctctccgtgtag	GATGAGGAGGAG	TTGTTCAGAACG	gtgagaacacagcttcccta	34.3
18	189	attttattttttgcttag	ATCATCCAGCCC	ACCCGGGGGGCAG	gtgagcctctactcaaaact	45
19	1475	agaagatgacccggggcag	CTTCAGGATGAG			

The lowercase letters refer to intron sequences and the uppercase letters to exon sequences

^aThe sequences of exon/intron boundaries were determined as described under Materials and Methods

^bThe intron sizes were estimated by size-fractionating polymerase chain reaction-amplified fragments, using agarose gel electrophoresis, or by direct sequencing of PAC and BAC genomic clones, as described under Material and Methods

ND; Not determined

Fig. 2. Nucleotide sequence of the 5'-region of human *ELKS*. The putative transcriptional start site predicted by the 5' rapid amplification of cDNA ends (RACE) experiment is indicated by an *asterisk*. The initiation codon (ATG) is indicated by *bold type* and *underlined*. Potential SP-1 and AP-2 binding sequences are indicated

5'- AGCAGCCAGG	GTCGATCCCG	GCGCTGCTCC	CGCGGCGCTT	GGCTGCCCCG
		SP-1		
GGCGTAACGG	GCCACGAGAA	GTAGGGGGTG	GGGCGCCGCT	TAGGTTTGGG
AAGACACGAA	GCGGCTCCTC	GGGCTTCCAG	CGTCCACTTC	CTACGGCAAG
CAGGAGGAGA	CAAGCGCCAC	CCTGCGCTCG	CGGAGCCGAC	CCCGGCTCTC
CCCGAACCGT	GGCCGCAGGG	GTCGTGTGTG	TGTGTGTGTG	AGTGTGTGTG
TGTGTGTGCA	GGGACGCTCC	GCGCCCATCA	CTCTGTTTCG	GTGCTAATCG
			AP-2	
ACCGCCGTGG	CGCCGATTTC	CCCCGCTTCC	CAGTTCCTCA	CCCGCGTGCC
	AP-2			
CCGGAAGGCG	CCCGGAGGCC	CCCGGGCCGC	CGCGTCACGC	TCCCGGGAGG
CTGAGGGAAA	GGGAGGCAGC	CGAGCCGGGA	GCCCCGGGAG	GCGCGCTGCG
		SP-1		
CCCCGCGGCG	GGGCTGGGGG	CGGGGCTAGG	TGTCTCGCGC	AGGGGGCGGG
GCGCGGTGAC	GTCGCGGGCG	CCTGGGCCGT	GCTGTGGCGG	CGGCGGCGGC
GGTAGTGGCG	GCGGCGGGCG	TGCCCCGGCG	GCAGCGGCAG	CAGTAGCGGC
AGCCCTGAGG	ACGGTGAGAC	GGCCGCGGCG	GCGGCGACAG	CGCGGAAGTC
GCGCGGGACC	CAGAGCTTCC	CCCCTGTCGC	TAGTGCTGAG	GCTTTCGCCT
TTCCTACCCA	GCTTCTTGCT	CACGGCCCCG	GCTGGGCTGG	GCAGGGCAAC
GCGCCCCCGG	GCAGGGGCGG	AGCTGTAGAT	ATGGTGTAAG	ATACTTCTTC
AAGATTGGAC	AGCTGGGGAC	CTTCTTCTGA	TTAACCCTAA	ACCAACTTGT
AGCCATAGAG	ACACCTCACA	AGGTTCCCAT	TTTTGTTGTT	GTTGTTGTTG
			-1+1	
ATTTTCTGCT	CACACCTTTC	CTGACCTTGC	AACC <u>ATG</u> -3'	

the *ELKS* sequence to DNA markers on 12p13 by radiation hybrid mapping, using the Stanford G3 hybrid panels (Nakata et al. 1999).

Exon organization and protein domains

The lower panel of Fig. 1 confirms the relationship between exon organization and protein domains that we had predicted previously (Nakata et al. 1999). The secondary structure of the *ELKS* sequence is likely to contain alpha-helical coiled-coil domains, because of the presence of heptad repeats in each of the helical-domain regions. The Multi Coil program (version 1-0, window size 2.1) predicted that *ELKS* protein would contain nine coiled-coil domains. The transcription-initiation site within exon 1 is separate from the initiation codon in exon 2. Exons 2–17 are coding elements;

exons 17–19 encode the 3' UTR. Introns interrupt the protein-coding sequence in such a way that many protein segments are encoded by distinct exons; thus, each of seven coiled-coil domains are encoded by exons 2, 3, 6, 7, 11, 14, and 15, respectively. The other two coiled-coil domains are encoded by exons 7–8 and exons 8–9. An exon/intron structure of this nature indicates that the gene consists of a mosaic of exons that individually encode functional domains.

Rearrangement of *ELKS* and *RET* genes in a papillary thyroid carcinoma

We previously showed that amino acid 691 of *ELKS* was fused to amino acid 713 of *RET* in a papillary thyroid carcinoma; ie, the kinase domain of *RET* starting from exon 12

Fig. 3. Chromosomal localization of human *ELKS* gene to 12p13.3 (arrows) by fluorescence in situ hybridization in metaphase chromosomes

of the *RET* gene was juxtaposed to a coiled-coil structure of the 5' portion of the *ELKS* cDNA sequence (Nakata et al. 1999). The fusion occurred at the end of exon 10 of the *ELKS* gene, indicating that rearrangement in the tumor genome involved intron 10 of *ELKS* and intron 11 of *RET*.

Four other kinds of chimeric oncogenes involving *RET* have been reported by others in papillary thyroid carcinomas: *RET/PTC1* (Grieco et al. 1990), *RET/PTC2* (Bongarzone et al. 1993), *RET/PTC3* (Bongarzone et al. 1994; Jhiang et al. 1994; Santoro et al. 1994), and *RET/PTC5* (Klugbauer et al. 1998). Their 5'-end sequences consist of genes encoding, respectively, H4, regulatory subunit R α of protein kinase A, RFG/ELE1, and RFG5. The breakpoints of *RET/PTC1* and *RET/PTC3* occur within intron 1 of the *H4* gene and intron 5 of the *ELE1* gene, respectively (Smanik et al. 1995). The genomic breakpoints of the *RET* proto-oncogene in all four previously described forms of *RET* rearrangement had occurred within intron 11 of the *RET* structure, a location which separates the transmembrane domain from the tyrosine kinase domain of *RET* protein. As the *ELKS-RET* gene fusion also disrupted intron 11 of *RET*, our results support the idea that all *RET*-related fusion genes encode proteins with a *RET* kinase domain at the carboxyl terminus. We previously synthesized a ^{35}S -Met-labeled *ELKS-RET* fusion protein in vitro and showed that it actually did form dimers (Nakata et al. 1999). The combined results appear to indicate that constitutive dimerization of rearranged *RET*, mediated by events within intron 11 that fuse the kinase domain to genes that

have coiled-coil structures, may be a general mechanism for activating *RET*-receptor tyrosine kinases and promoting oncogenesis in papillary thyroid carcinomas.

Acknowledgments This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan and the Ministry of Health and Welfare of Japan; and by a Novartis Foundation Research Grant.

References

- Balon RH, Tansey MG, Lampe PA, Fahrner TJ, Enomoto H, Simburger KS, Leitner ML, Araki T, Johnson EM, Milbrandt J Jr (1998) Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFR α 3-*RET* receptor complex. *Neuron* 21:1291–1302
- Bongarzone I, Monzini N, Borrello MG, Carcano C, Ferraresi G, Arighi E, Mondellini P, Della Porta G, Pierotti MA (1993) Molecular characterization of a thyroid tumor-specific transforming sequence formed by the fusion of ret tyrosine kinase and the regulatory subunit RI α of cyclic AMP-dependent protein kinase A. *Mol Cell Biol* 13:358–366
- Bongarzone I, Butti MG, Coronelli S, Borrello MG, Santoro M, Mondellini P, Pilotti S, Fusco A, Della Porta G, Pierotti MA (1994). Frequent activation of ret protooncogene by fusion with a new activating gene in papillary thyroid carcinomas. *Cancer Res* 54:2979–2985
- Dynan WS (1986) Promoters for house-keeping genes. *Trends Genet* 2:196–197
- Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della Porta G, Fusco A, Vecchio G (1990) PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. *Cell* 60:557–563

- Jhiang SM, Mazzaferri EL (1994) The *ret*/PTC oncogene in papillary thyroid carcinoma. *J Lab Clin Med* 123:331–337
- Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M, Tamir R, Antonio L, Hu Z, Cupples R, Louis JC, Hu S, Altmann BW, Fox GM (1996) GDNF-induced activation of the *ret* protein tyrosine kinase is mediated by GDNFR- α , a novel receptor for GDNF. *Cell* 85:1113–1124
- Klein R, Jing SQ, Nanduri V, O'Rourke E, Barbacid M (1991) The *trk* proto-oncogene encodes a receptor for nerve growth factor. *Cell* 65:189–197
- Klein RD, Sherman D, Ho WH, Stone D, Bennett GL, Moffat B, Vandlen R, Simmons L, Gu Q, Hongo JA, Devaux B, Poulsen K, Armanini M, Nozaki C, Asai N, Goddard A, Phillips H, Henderson CE, Klugbauer S, Demidchik EP, Lengfelder E, Rabes HM (1998) Detection of a novel type of *ret* rearrangement (PTC5) in thyroid carcinomas after Chernobyl and analysis of the involved *ret*-fused gene RFG5. *Cancer Res* 58:198–203
- Klugbauer S, Demidchik EP, Lengfelder E, Rabes HM (1998) Detection of a novel type of RET rearrangement (PTC5) in thyroid carcinomas after chernobyl and analysis of the involved RET-fused gene RFG5. 58:198–203
- Milbrandt J, Desauvage FJ, Fahrner TJ, Baloh RH, Leitner ML, Tansey MG, Lampe PA, Heuckeroth RO, Kotzbauer PT, Simburger KS, Golden JP, Davies JA, Vejsada R, Kato AC, Hynes M, Sherman D, Nishimura M, Wang LC, Vandlen R, Moffat B, Klein RD, Poulsen K, Gray C, Garces A, Henderson CE, et al. (1998) Persephin, a novel neurotrophic factor related to GDNF and Neurturin. *Neuron* 20:245–253
- Mount SM (1982) A catalogue of splice junction sequences. *Nucleic Acids Res* 10:459–472
- Mukae N, Enari M, Sakahira H, Fukuda Y, Inazawa J, Toh H, Nagata S (1998) Molecular cloning and characterization of human caspase-activated DNase. *Proc Natl Acad Sci USA* 95:9123–9128
- Nakajima T, Ota N, Kodama T, Emi M (1999a) Isolation and radiation hybrid mapping of a highly polymorphic CA repeat sequence at the SREBP cleavage-activating protein (SCAP) locus. *J Hum Genet* 44:350–351
- Nakajima T, Hamakubo T, Kodama T, Inazawa J, Emi M (1999b) Genomic structure and chromosomal mapping of the human sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) gene. *J Hum Genet* 44:402–407
- Nakata T, Kitamura Y, Shimizu K, Tanaka S, Fujimori M, Yokoyama S, Ito K, Emi M (1999) Fusion of novel gene, ELKS, to RET due to translocation t(10;12)(q11;p13) in a papillary thyroid carcinoma. *Genes Chromosom Cancer* 25:97–103
- Pierotti MA, Santoro M, Jenkins R, Sozzi G, Bongarzone I, Grieco M, Monzini N, Miozzo M, Herrmann M, Fusco A, Hay I, Della Porta G, Vecchio G (1992) Characterization of an inversion on the long arm of chromosome 10 juxtaposing D10S170 and *ret* and creating the oncogenic sequence *ret/ptc*. *Proc Natl Acad Sci USA* 89:1616–1620
- Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, Pierotti MA, Vecchio G, Fusco A (1994) Molecular characterization of RET/PTC3; a novel rearranged version of the RET proto-oncogene in a human thyroid papillary carcinoma. *Oncogene* 9:509–516
- Smanik P, Furminger T, Mazzaferri E, Jhiang S (1995) Breakpoint characterization of the *ret*/PTC oncogene in human papillary thyroid carcinoma. *Hum Mol Genet* 4:2313–2318
- Tsukamoto K, Ohta N, Shirai Y, Emi M (1998) A highly polymorphic CA repeat marker at the human interleukin 6 receptor (IL6R) locus. *J Hum Genet* 43:289–290