Am. J. Hum. Genet. 68:81–91, 2001

Genotypic and Phenotypic Spectrum in Tricho-Rhino-Phalangeal Syndrome Types I and III

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Tricho-rhino-phalangeal syndrome (TRPS) is characterized by craniofacial and skeletal abnormalities. Three subtypes have been described: TRPS I, caused by mutations in the *TRPS1* gene on chromosome 8; TRPS II, a microdeletion syndrome affecting the *TRPS1* and *EXT1* genes; and TRPS III, a form with severe brachydactyly, due to short metacarpals, and severe short stature, but without exostoses. To investigate whether TRPS III is caused by *TRPS1* mutations and to establish a genotype-phenotype correlation in TRPS, we performed extensive mutation analysis and evaluated the height and degree of brachydactyly in patients with TRPS I or TRPS III. We found 35 different mutations in 44 of 51 unrelated patients. The detection rate (86%) indicates that *TRPS1* is the major locus for TRPS I and TRPS III. We did not find any mutation in the parents of sporadic patients or in apparently healthy relatives of familial patients, indicating complete penetrance of *TRPS1* mutations. Evaluation of skeletal abnormalities of patients with *TRPS1* mutations revealed a wide clinical spectrum. The phenotype was variable in unrelated, age- and sex-matched patients with identical mutations, as well as in families. Four of the five missense mutations alter the GATA DNA-binding zinc finger, and six of the seven unrelated patients with these mutations may be classified as having TRPS III. Our data indicate that TRPS III is at the severe end of the TRPS spectrum and that it is most often caused by a specific class of mutations in the *TRPS1* gene.

Introduction

Three types of tricho-rhino-phalangeal syndrome (TRPS I [MIM 190350], TRPS II [MIM 150230], and TRPS III [MIM 190351]) have been described in the literature. Features common to all three types are sparse, slowly growing scalp hair, laterally sparse eyebrows, a bulbous tip of the nose, and protruding ears. Highly characteristic are the long flat philtrum and the thin upper vermillion border. The most typical radiographic findings

Received August 16, 2000; accepted for publication October 20, 2000; electronically published December 7, 2000.

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0002-9297/2001/6801-0008$02.00

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in TRPS are cone-shaped epiphyses (CSEs), predominantly at the middle phalanges. Often, they are not detectable before 2 years of age. However, as a first manifestation of future CSEs, mild metaphyseal convexity can be detected during the first year of life in some cases (Giedion 1998). CSEs and premature closure of the growth plates can also be found at other tubular bones, however, with a much lower incidence (Giedion 1968). The skeletal age always lags behind the chronological age until puberty and then typically accelerates. Hip malformations such as coxa plana, coxa magna, or coxa vara are present in >70% of patients. In older patients, the hip abnormalities resemble degenerative arthrosis. Felman and Frias (1977) suspected that TRPS represents a condition that results from abnormal maturation of the epiphyses and growth plates, leading to mild to moderate growth retardation.

We have recently identified the *TRPS1* gene [MIM 604386, AF178030, AF183810], which maps to 8q24.1 and appears to encode a zinc-finger transcription factor (Momeni et al. 2000). The deletion of both *TRPS1* and *EXT1* leads to TRPS II. Patients have multiple cartilaginous exostoses in addition to the findings in TRPS I. Mental retardation has been described in many patients with TRPS II as well as in two patients with TRPS I and a cytogenetically visible deletion in 8q24 (Yamamoto et al. 1989; Hamers et al. 1990). In contrast, patients with TRPS I and a submicroscopic deletion or a *TRPS1* point mutation usually have normal intelligence (Brandt et al. 1997; Nardmann et al. 1997; Sasaki et al. 1997; Lüdecke et al. 1999).

Sugio and Kajii (1984) and Niikawa and Kamei (1986) described non–mentally retarded patients with the typical facial appearance of TRPS I but with more-severe brachydactyly and growth retardation. None of the patients had multiple cartilaginous exostoses. Niikawa and Kamei (1986) proposed the diagnosis of TRPS III for these patients. Since then, a few more patients with proposed TRPS III have been described (Nagai et al. 1994; Itin et al. 1996; Vilain et al. 1999). Kajii et al. (1994) used metacarpophalangeal pattern profile (MCPP) analyses in the family described by Sugio and Kajii (1984) and found that the more severe brachydactyly as compared with individuals with TRPS I was due to a generalized shortness of all phalanges and metacarpals. Furthermore, they found that the shortening of the tubular bones of the hands was a progressive process. The skeletal abnormalities such as CSEs or hip changes in TRPS I are rather variable, which is supported by the finding that they vary considerably within families and even between MZ twins with TRPS (Giedion et al. 1973; Naselli et al. 1998; authors' unpublished observations), whereas the facial appearance of all patients with TRPSs is rather similar. Thus, it has been speculated that TRPS III might represent only one extreme of the clinical spectrum of TRPS I. Here we describe the genotype-phenotype correlation in a large number of patients with TRPS I and TRPS III.

Patients and Methods

Patients

This study was approved by the local ethics committee at the Universitätsklinikum Essen. We searched for *TRPS1* mutations in 51 unrelated probands with TRPS, including 8 with proposed TRPS III. Thirty probands represent isolated cases and 20 represent familial cases (fig. 1). No pedigree information was available for patient 10569, who is an adopted child. In total, we analyzed 79 affected persons. Both parents of 15 isolated patients and the father of 1 additional isolated patient were available for analysis. In five familial cases (patients 12875, 6492, 13365, 13876, and 13916; table 1), only one affected individual could be studied. The patients are from Germany (28), Belgium (4), Turkey (4), The Netherlands (3), Norway (3), Great Britain (2), Japan (2), Austria (1), Canada (1), France (1), Israel (1), and Poland (1).

Mutation Analysis

We isolated high-molecular-weight DNA from the probands, either from peripheral leukocytes or lymphoblastoid cell lines, using the NUCLEON II kit (Amersham), and we screened for *TRPS1* gene mutations by direct sequencing of PCR products from genomic DNA, as described elsewhere (Momeni et al. 2000; Tricho-Rhino-Phalangeal Syndrome Project). For the detection of mutations in the patient's RNA, we isolated total RNA from either peripheral leukocytes or lymphoblastoid cell lines, using the QIAamp RNA Blood or the RNeasy Mini Kits (Qiagen). For first-strand DNA synthesis, we used random hexamer priming and Avian Myeloblastosis Virus reverse transcriptase (Gibco BRL; 10 min at room temperature, 15 min at 42°C, 5 min at 99°C, and 5 min at 5°C). We amplified the DNA, using amplimer primers PM101/PM73 (PM101: 5′-GAAGCTCGCGAGTCAAACAT-3′; PM73: 5′-GAGCTGCTCAGCCTGAAGT-3′; annealing temperature 56°C), which are located in exon 4 and exon 7 of *TRPS1* and are ~190 kb apart in genomic DNA. They amplify a 1,067-bp fragment from normal *TRPS1* RNA. RT-PCR products were sequenced with primer PM85 (PM85: 5′-GGGATAGTCCCAATGTGGAG-3′; annealing temperature 56°C), which is located in exon 5 of *TRPS1* and allows reading through exon 6 into exon 7.

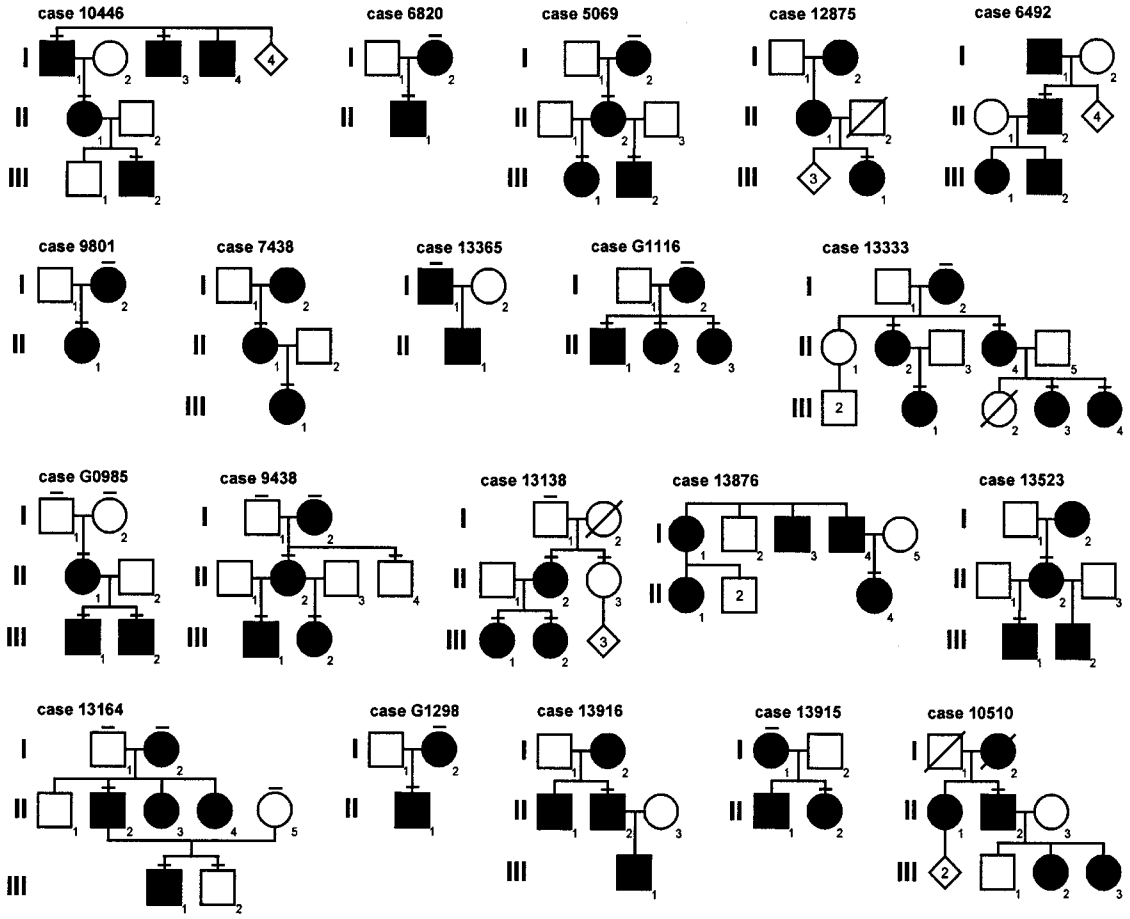

Figure 1 Pedigrees of familial cases of TRPS. Cases are shown in the order in which they appear in table 1. Affected individuals are indicated by blackened symbols. Individuals available for sequence analysis are denoted with bars above the symbol.

Anthropometric Analysis

The patients were heterogeneous for sex, age, and ethnicity. To compare the heights of the non-Japanese patients, we calculated the SDs for height with the mean values for the Western European population (van Wieringen et al. 1971) or the Turkish population (Neyzi et al. 1973); for the Japanese patients, we took data from the respective publications (table 1).

MCPP analysis is a graphic method used to depict the length of the tubular bones of the hand in relation to one another. It has been used to aid in establishing the diagnosis of many congenital malformations and bone dysplasias, including the TRPSs (Poznanski et al. 1974; Felman and Frias 1977; Kajii et al. 1994; Poznanski and Gartman 1997). For MCPP analysis, we measured the lengths of all 19 tubular bones of the hands from the original hand radiographs of 45 patients, and we calculated the deviation of each bone from the mean for corresponding age and sex, according to the technique of Poznanski et al. (1972). The deviations (so-called $\bar{z}$ scores) are expressed as multiples of SDs. The mean values and SDs had been established for American-born individuals of European ancestry by Garn et al. (1972). The calculations and plots of profiles, the comparison of the MCPPs from different individuals, and the calculation of Pearson's correlation coefficient (r) were done with the computer program ANTRO (release 4.83E; Hosenfeld et al. 1991). In some cases we encountered problems with the length measurements because of bowing of metacarpals and axial deviations at the interphalangeal joints, which lead to a distorted projection of the bones on the film.

Neither height data nor hand radiographs were available for five patients with *TRPS1* mutations. Only one original hand radiograph and one photograph of a hand X-ray were available from patients in whom we could not identify a *TRPS1* mutation. Skeletal age was determined according to the tables of Greulich and Pyle (1959).

Table 1

Summary of *TRPS1* Gene Mutations, Height of Patients, and Radiographic Findings

Mutation[a]								Height		Hand Radiography		
Type	Location	Amino Acid Change	Case ID[b]	Individual	Sex	MR[c]	Age	in cm	SD	$\bar{z}$[d] (Left/Right)	Chronological Age	Skeletal Age
Nonsense:												
523C→T	Exon 3	Q175X	10446*	II-1	F	−	39 y	149.0	−2.8	−2.5/−2.6	32 y	Adult
				III-2	M	−	12 y 3 mo	126.0	−3.6	−2.9/−3.0	12 y 4 mo	9 y 3 mo
838delC	Exon 3	Stop at codon 322	G1097		F	−	13 y	157.5	0	NA		
1014C→A	Exon 4	C338X	6820[1,*]	I-2	F	−	28 y	167.0	0	−2.9/−3.3	32 y	Adult
				II-1	M	−	1 y 1 mo	73.0	−1.5	0/NA	1 y 1 mo	6 mo
1031delG	Exon 4	Stop at codon 351	5069*			?		NA		NA		
1137–1138insT	Exon 4	Stop at codon 380	12875*	III-1	F	−	14 y 9 mo	156.0	−1.2	−3.6/NA	14 y 9 mo	15 y 1 mo
			6492*	II-2	M	−	44 y	167.0	−1.6	−2.7/−2.6	39 y	Adult
1413–1426delA-T	Exon 4	Stop at codon 477	9801*	I-2	F	+	28 y	161.8	−0.7	NA		
				II-1	F	+	1 y 8 mo	82.0	0	NA		
1469C→G	Exon 4	S490X	13927		F	+/−	5 y 6 mo	111.5	−0.5	−0.4/−0.5	5 y 9 mo	3 y
1546G→T	Exon 4	E516X	10067		M	−	11 y	142.0	−0.4	NA		
1576C→T	Exon 4	Q526X	7438*		F	−		NA		NA		
1591C→T	Exon 4	R531X	10569		F	+/−	13 y 9 mo	152.0	−1.4	−3.0/−3.0	11 y 8 mo	9 y 2 mo
			13365*	I-1	M	−	25 y	161.0	−1.9	−3.8/−4.4	25 y	Adult
			14337		M	−	32 y	169.5	−1.2	−2.3/−1.7	32 y 4 mo	Adult
1595–1596delAT	Exon 4	Stop at codon 539	9273		F	?	2 y 6 mo	82.3	−2.7	NA		
1602–1603insA	Exon 4	Stop at codon 540	8413		F	−	8 y	121.0	−1.5	−1.4/NA	7 y 7 mo	6 y
1831C→T	Exon 4	R611X	G1116[2,*]	I-2	F	−	42 y	161.0	−0.8	NA		
				II-1	M	−	18 y 3 mo	169.5/−1.2		−0.3/NA	7 y 11 mo	6 y 10 mo
				II-2	F	−	15 y 7 mo	159.5	−0.8	−0.3/NA	5 y 3 mo	2 y 3 mo
				II-3	F	−	13 y 6 mo	162.0	0	−1.5/NA	3 y 2 mo	1 y 3 mo
			13333*	I-2	F	−	63 y	165.0	0	−0.9/−1.4	62 y 7 mo	Adult
				II-2	F	−	38 y	168.0	0	−2.6/−2.5	38 y 1 mo	Adult
				II-4	F	−	29 y	168.0	0	−1.8/−1.9	28 y 6 mo	Adult
				III-1	F	−	16 y 3 mo	169.0	0	−0.8/−0.9	16 y 3 mo	17 y
				III-3	F	−	7 y	122.0	0	−0.9/− 0.8	7 y	3 y 10 mo
			G1353		F	−		NA		NA		
			13738		F	−	21 y	146.0	−3.3	−2.5/−2.5	21 y	Adult
2047C→T	Exon 4	R683X	G0985*	II-1	F	−		NA		NA		
				III-1	M	−	9 y 7 mo	127.0	−1.9	NA		
				III-2	M	−	7 y 7 mo	126.0	0	NA		
2110delA	Exon 5	Stop at codon 766	12564		F	−	26 y	168.0	0	NA		
2113–2114delCA	Exon 5	Stop at codon 743	13854		F	+/−	5 y 7 mo	104.5	−2.0	−2.0/−2.8	6 y 8 mo	4 y 6 mo
2155C→T	Exon 5	Q719X	12736		F	?	3 y 8 mo	88.5	−2.4	−2.8/NA	3 y 8 mo	1 y 6 mo
2291–2292delTG	Exon 5	Stop at codon 788	431		F	?	6 y 6 mo	120.0	0	−0.9/−0.9	6 y 6 mo	3 y 9 mo
2301–2304delAGAG	Exon 5	Stop at codon 791	8230		M	−	12 y 6 mo	150.0	0	−2.2/−2.0	12 y 5 mo	9 y 9 mo
2406delG	Exon 5	Stop at codon 814	10794		F	−	10 y 3 mo	131.0	−1.6	−1.7/NA	10 y 4 mo	8 y 6 mo
2406–2407insG	Exon 5	Stop at codon 831	9438[3,*]	I-2	F	−	52 y	155.0	−1.8	NA		
				II-2	F	−	27 y	157.0	−1.5	−1.4/NA	23 y	Adult
				III-1	M	−	2 y 10 mo	90.0	−2.0	−1.8/NA	2 y 3 mo	1 y 1 mo
				III-2	F	−	2 y 3 mo	86.5	−1.5	−2.2/NA	2 y 2 mo	10 mo
2441–2442insT	Exon 5	Stop at codon 830	5650[4]		F	−	16 y	154.0	−1.8	NA		
2480–2481insT	Exon 5	Stop at codon 830	13138*	II-2	F	−	52 y	152.0	−2.3	−2.7/−2.4	51 y 10 mo	Adult
				III-1	F	−	26 y	146.0	−3.3	NA		
				III-2	F	−	22 y	148.0	−3.0	−2.0/−1.9	22 y 2 mo	Adult

2518C→T	Exon 5	R840X	1277[5]		F	−	15 y	159.0	−0.8	NA		
2534T→A	Exon 5	L845X	13876*	II-4	F	−	40 y	152.0	−2.3	−2.1/−2.3	40 y	Adult
2816–2817insA	Exon 7	Stop at codon 942	13523*	II-2	F	−	39 y	146.0	−3.3	−1.8/−1.5	38 y	Adult
				III-1	M	−	17 y 2 mo	166.0	−1.5	−2.9/−2.9	17 y 1 mo	19 y
				III-2	M	−	11 y 3 mo	144.5	−0.4	NA		
2875C→T	Exon 7	Q959X	14338		M	−	3 y 11 mo	96.5	−2.0	−2.6/−2.4	3 y 10 mo	1 y
3012C→G	Exon 7	Y1004X	13164*	I-2	F	−	65 y	162.0	−0.7	NA		
				II-2	M	−	42 y	168.0	−1.4	NA		
				III-1	M	−	11 y 7 mo	135.0	−1.8	NA		
3360–3361insGGAG	Exon 7	Stop at codon 1162	11049[6]		M	−	33 y	176.0	0	NA/−3.8	33 y 4 mo	Adult
Splice site:												
IVS6+1G→T	IVS6	In-frame skipping, exon 6	13568		F	−	35 y	156.0	−1.7	−2.0/−2.3	35 y 1 mo	Adult
Missense:												
2681T→A	Exon 6	V894D	G1298*	I-2	F	+	31 y	156.0	−1.7	Originals NA		
				II-1	M	++	12 y 6 mo	101.0	−7.0	−4.8/NA	10 y 11 mo	5 y
2701A→C	Exon 6	T901P	13242[7]		M	−	29 y	147.0	−4.6	−7.1/NA	17 y 6 mo	14 y 6 mo
2723G→A	Exon 6	R908Q	5874		M	?	14 y 6 mo	159.0	−0.7	NA		
			7795		F	−	15 y 11 mo	150.8	−2.4	−4.5/−4.5	15 y 11 mo	18 y
2723G→C	Exon 6	R908P	13916[8,*]	II-2	M	−	35 y	150.0	−3.6	Originals NA		
2755G→A	Exon 6	A919T	13287		F	−	15 y 10 mo	153.0	−2.0	−3.6/−3.6	16 y 2 mo	16 y 6 mo
			13307		F	−	15 y	152.0	−1.1	−5.4/−5.6	14 y 5 mo	14 y
			13915[9,*]	I-1	F	−	28 y	142.5	−2.4	See Kajii et al. 1994		
				II-2	F	−	8 mo	69.0	0	See Kajii et al. 1994		
None identified:												
−/−			1778		M	−	17 y 3 mo	151.2	−3.7	−4.75/NA	17 y 3 mo	17 y
−/−			8827		F	−	11 y 6 mo	146.0	0	Originals NA		
−/−			10344		F	+	6 y 6 mo	114.0	−1.3	NA		
−/−			9595		F	−	13 y 11 mo	152.0	−1.4	NA		
−/−			13243		M	−	11 y 11 mo	139.0	−1.5	NA		
−/−			8198		F	−	4 y	101.5	−0.7	NA		
−/−			10510*			−		NA		NA		
TRPS1 deletions or disruptions:												
del *TRPS1*			G1386[10]		M	++	19 y	166.0	−0.7	−2.89/−2.68	37 y	Adult
del *TRPS1*			5182[11]		F	+/−	10 y 9 mo	129.0	−2.1	Originals NA	10 y 3 mo	6 y 3 mo
del *TRPS1*			10378[12]		F	−	11 y 9 mo	155.0	+0.5	+0.5/+0.7	10 y 1 mo	10 y 6 mo
t(7;8;13), del *TRPS1*			4314[13]		F	−	14 y 11 mo	162.0	−0.3	−2.4/−2.5	14 y 11 mo	14 y 11 mo
t(8;18)(q24.1;q23)	IVS 5		1729[14]	II-1	F	−	33 y	149.0	−2.8	Originals NA		
				II-2	M	−	27 y	163.0	−2.2	Originals NA		
inv(8)(pq)	IVS 5		8448[15]		F	−	4 y 1 mo	91.0	−3.2	Originals NA	4 y 1 mo	1 y 6 mo

NOTE. NA = not available; y = years.

[a] Nucleotide numbers refer to the *TRPS1* cDNA sequence; amino acid numbers refer to the deduced peptide sequence. The five recurrent mutations are underlined.

[b] Patients with designators followed by superscript numbers 1–15 have been described elsewhere, as follows: [1–6]Momeni et al. 2000; [7]Vilain et al. 1999; [8]Nagai et al. 1994; [9]Sugio and Kajii 1984; Kajii et al. 1994; [10]Hamers et al. 1990; Lüdecke et al. 1995; [11]Nardmann et al. 1997; [12]Lüdecke et al. 1999; [13]Brandt et al. 1997; [14]Marchau et al. 1993; Lüdecke et al. 1995; [15]Lüdecke et al. 1999. Asterisks (*) identify familial cases (see fig.1).

[c] MR = mental retardation. A minus sign (−) indicates normal intelligence, two plus signs (++) indicate severe retardation, and a question mark (?) indicates unknown.

[d] $\bar{z}$ = mean length deviation of the 19 tubular bones of the left or right hand.

The statistical significance of differences of heights of patients was tested by a one-way analysis of variance and subsequent two-tailed *t* test for single groups. Type 1 error was defined as $2\alpha = .05$ without adjustment for multiple tests.

Results

Spectrum of TRPS1 Mutations

We screened for mutations by sequencing the *TRPS1* coding region (exons 3–7) and the flanking intronic sequences until a mutation was identified in a particular patient. In cases of missense mutations, we sequenced the entire coding region as well as the presumptive noncoding exon 2 to exclude a further mutation.

We identified mutations in 44 (86%) of 51 patients with apparently normal chromosomes, in 24 (80%) of 30 patients representing sporadic cases, in 19 (95%) of 20 patients representing familial cases, and in the patient of unknown origin. We identified 35 different mutations (table 1) that are evenly distributed throughout the *TRPS1* gene (fig. 2). Five mutations are recurrent, and four of them were identified in patients of different ethnicities. For example, 1591C→T was found in patients 10569 (Norway), 13365 (Turkey), and 14337 (Belgium), and 2755G→A was found in patients 13287 (Belgium) and 13307 (Turkey) and in family 13915 (Japan). The 1137-1138insT mutation was found in two families from Germany. Careful examination of their pedigrees did not reveal any evidence for a relationship. Six of the mutations have been described elsewhere (Momeni et al. 2000). We found nine out-of-frame deletions (1–14 bp) and seven out-of-frame insertions (1–4 bp) in *TRPS1*. Although the frameshift mutations may add up to 61 aberrant amino acids (e.g., 2110delA) to the mutant protein, if the mutant transcripts are not degraded by nonsense-mediated decay (Frischmeyer and Dietz 1999), we consider them to be loss-of-function mutations, because the mutant proteins lack the C-terminal IKAROS-like zinc-finger motif (Momeni et al. 2000; fig. 2), which is considered essential for protein-protein interaction. Of the 19 single-base substitutions, 9 are transversions and 10 are transitions. Six transitions occur in CpG dinucleotides, and four of them are recurrent mutations. Thirteen single-base substitutions create premature stop codons.

One base substitution, IVS6+1G→T (patient 13568), alters the splice-donor site of intron 6. RT-PCR on RNA from the patient's leukocytes with primers PM101 and PM73 revealed two products: the 1,067-bp (normal) product and the 944-bp (mutant) product (fig. 3*A*). Sequencing of the RT-PCR product derived from the mutant allele showed that the mutation leads to an in-frame skipping of the 123-bp exon 6 (fig. 3*B*).

Five base substitutions cause missense mutations. All were found in exon 6, which encodes a presumptive GATA DNA-binding zinc finger. Of interest, we found only a mutant (2681T→A) but no normal *TRPS1* allele in individual II-1 of family G1298. This was confirmed by sequencing of the RT-PCR product from the patient's leukocyte RNA. This finding is unusual for a dominantly inherited disease. The patient is also homozygous for three intragenic single-nucleotide polymorphisms (SNPs; see below). By dosage-blot analysis with the *TRPS1* exon 6 amplimer probe PM65/PM64 and the control probe YL48E (15q12 [provided by Karin Buiting]), we determined that the patient has two copies of the mutant *TRPS1* gene (data not shown). We could not clarify whether the homozygosity is due to a maternal isodisomy at 8q24, because the patient's father is unknown and no material from his mother was left.

We did not find any mutation in the parents in sporadic cases or in apparently healthy relatives in familial cases. We want to emphasize that the parents of four of the five sporadic patients with missense mutations (patients 13242, 7795, 13287, and 13307) were available

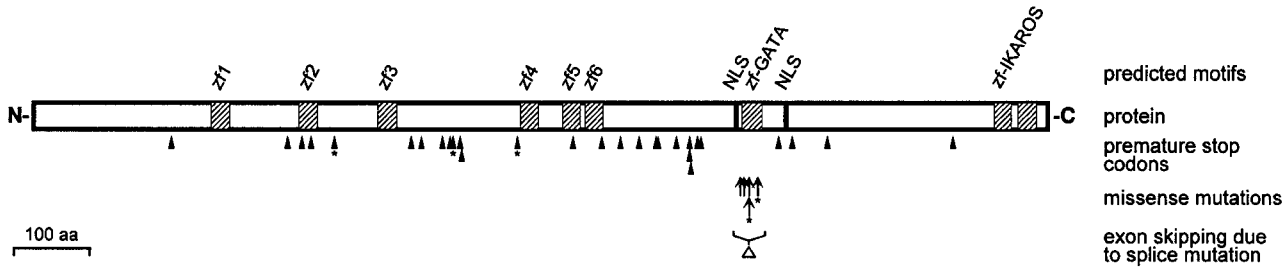

Figure 2 Structure of the TRPS1 protein and location of mutations. Hatched boxes, zinc-finger motifs (zf). Vertical bars, putative nuclear localization signals (NLSs). The location of the GATA and IKAROS-like zinc fingers are given. Blackened triangles indicate the localization of the premature stop codons, and missense mutations are indicated by arrows. The effect of the splice site mutation, skipping of exon 6, is denoted by an unblackened triangle. Recurrent mutations are identified by an asterisk (*). Note that two different insertion mutations lead to a premature stop codon at amino acid (aa) 830 and that we found two different missense mutation at aa 908.

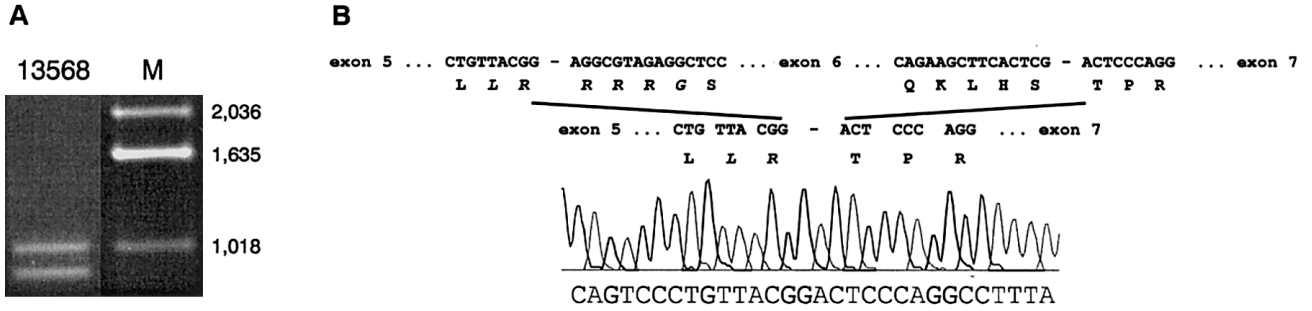

Figure 3 Splice-site mutation in patient 13568. *A,* Agarose gel with products of RT-PCR of RNA from the patient's leukocytes. The upper band (1,067 bp) is derived from the normal transcript and the lower band (944 bp) from the mutant transcript. M = length standard (1 Kb DNA ladder, Gibco BRL). *B,* Sequence analysis of the RT-PCR product from the mutant transcript. Exon 5 is spliced to exon 7. One putative NLS (LRRRRG, in italics) is truncated, and the GATA zinc finger is completely absent in the mutant protein.

for the analysis, and none of them has the respective base substitutions. This indicates that the substitutions are true de novo mutations and not polymorphisms.

We found three SNPs in the *TRPS1* gene. Two SNPs are located in the noncoding exon 2. SNP1 is a G→A transition at position −117, and SNP2 is a T insertion between nucleotides −47 and −48. SNP3 is located in intron 5 (IVS5+29A→C). Five of the seven patients, in whom we could not find a mutation, are heterozygous for at least one SNP.

Anthropometric Analysis

Short stature and brachydactyly are common features of TRPS and are determined by means of simple measurements of height and MCPP analysis. To establish a genotype-phenotype correlation, we collected data on height and analyzed the hand radiographs of patients with TRPS I or TRPS III. The genetic status and the anthropometric data are summarized in table 1.

Height measurements were available for 76 patients. We observed great variability in the individual relative heights, even in unrelated patients with identical mutations, if the height of unaffected relatives was taken into consideration as well. The relative heights ranged from 0.5 SD above the mean to 7.0 SD below the mean. The lowest value was found in individual II-1, case G1298. The patient is very severely affected, with nearly absent scalp and body hair and low-set, large, protruding, and dysmorphic ears. Skeletal anomalies include scoliosis and pectus carinatum, coxa retrosa, and genu recurvatum. An X-ray of his right hand at age 10 years 11 mo shows CSEs of all metacarpals; of all proximal, middle, and distal phalanges; and of the distal radius, with severe shortness of all tubular bones of this hand. Skeletal age was severely retarded (~5 years). Because the individual is homozygous for the missense mutation V894D, we excluded him from further calculations. The average height ($\bar{z}$) of the remaining 75 patients was 1.41 SD below the average height of the respective population (with the standard deviation $s = 1.15$ for single values). Although the average values for the seven patients with a chromosomal abnormality ($\bar{z} = -1.54$ SD; $s = 1.38$) and the six patients in whom we could not identify a mutation ($\bar{z} = -1.43$ SD; $s = 1.25$) are also in this range, we did not include them in our calculations, because effects of other genes cannot be excluded.

Growth retardation in TRPS is considered to occur postnatally and as a progressive process. This is supported by our finding that the lengths at birth, available for 16 patients, were in the normal range (49–54 cm) even if patients were born to affected parents ($n = 5$). Furthermore, the average relative height of the 28 adult patients with a *TRPS1* mutation was smaller ($\bar{z} = -1.68$ SD; $s = 1.29$) than that of the 34 children ($\bar{z} = -1.16$ SD; $s = 0.96$).

Because we had found that most patients suspected of having TRPS III had missense mutations in the *TRPS1* gene, we compared the heights of these patients with the heights of the patients with nonsense mutations. On average, the nine patients with heterozygous missense mutations are smaller ($\bar{z} = -2.06$ SD; $s = 1.42$) than the 53 patients with nonsense mutations ($\bar{z} = -1.28$ SD; $s = 1.06$). The heights of children with missense mutations ($\bar{z} = -1.24$ SD; $s = 0.96$; $n = 5$) are comparable to those with nonsense mutations ($\bar{z} = -1.15$ SD; $s = 0.98$; $n = 29$). However, when only the adult patients with missense ($\bar{z} = -3.01$ SD; $s = 1.28$; $n = 4$) or nonsense ($\bar{z} = -1.45$ SD; $s = 1.15$; $n = 24$) mutations are compared, a statistically significant ($P < .05$) difference can be seen, although the ranges for patients with missense mutations (−1.7 to −4.6 SD) and patients with nonsense mutations (0 to −3.3 SD) overlap. Finally, comparison of the average height of adult patients with missense mutations ($\bar{z} = -3.01$ SD; $s = 1.28$; $n = 4$) with the average height of children with missense mutations ($\bar{z} = -1.24$ SD; $s = 0.97$; $n = 5$) proves ($P <$

.05) that growth retardation in TRPS is a progressive process.

The predominant locations of the TRPS-characteristic CSEs of the Giedion type 12 or the characteristic cone-shaped deformity at the base of the affected phalanx in adults are the middle phalanges (Giedion 1968). This deformity is the result of a premature closure of the growth plate, which leads to growth retardation. Poznanski et al. (1974) used MCPP analyses in 11 patients with TRPS I to establish a characteristic pattern profile, with shortness of the middle phalanges and ulnar metacarpals ("mean Poznanski"; Poznanski et al. 1974). Kajii et al. (1994) found a difference between this TRPS I–pattern profile and the profiles of patients with proposed TRPS III (family 13915 of the present study), but they concluded that MCPP analysis is only supportive but not diagnostic of TRPS III. Because these analyses were done before the *TRPS1* gene had been identified, we have set out to correlate the pattern profiles with the particular genetic defect in our patients. The original hand radiographs were available from 45 patients, and photographs of hand X-rays were available from 9 further patients. For MCPP analysis, we used only the original hand X-rays of the 44 patients in whom we had found a *TRPS1* mutation, because we wanted to calculate the mean length deviation (mean SD, $\bar{z}$) of all 19 tubular bones of a hand as a measure of the severity of brachydactyly (table 1). The X-rays of the youngest patient, individual II-1 of family 6820, did not show CSEs, and MCPP analysis could not disclose brachydactyly. The MCPPs of the remaining 43 patients disclosed a spectrum of affected bones, ranging from only two middle phalanges bilaterally in patient 10378 ($\bar{z} = +0.05/+0.7$), with hemizygosity for *TRPS1,* to nearly uniform shortness of all 19 bones of both hands, as in patient 13242 ($\bar{z} = -7.1$ at age 17 years), in whom TRPS III was diagnosed (Vilain et al. 1999). Figure 4*A* shows examples of individual pattern profiles. With one exception (case 13287), we found that patients with a missense mutation had, on average, shorter metacarpals than did those with a nonsense or the splice-site mutation. Therefore, we calculated mean pattern profiles for either the 35 patients with a heterozygous nonsense (and the splice-site) mutation ("mean TRPS I"; fig. 4*B*) or the four patients with a heterozygous missense mutation ("mean TRPS III"). Mean pattern profiles were calculated from the left-hand MCPPs, except for patient 11049, for whom only the right-hand X-ray was available. For reasons explained above, we did not include patient G1298 (II-1), patients with chromosomal abnormalities, or patients with an unknown genetic defect in this analysis. We compared the mean MCPPs by calculating the product moment correlation (Pearson's *r*; Poznanski et al. 1972) with the mean Poznanski profile and with each other. The mean TRPS I profile correlates

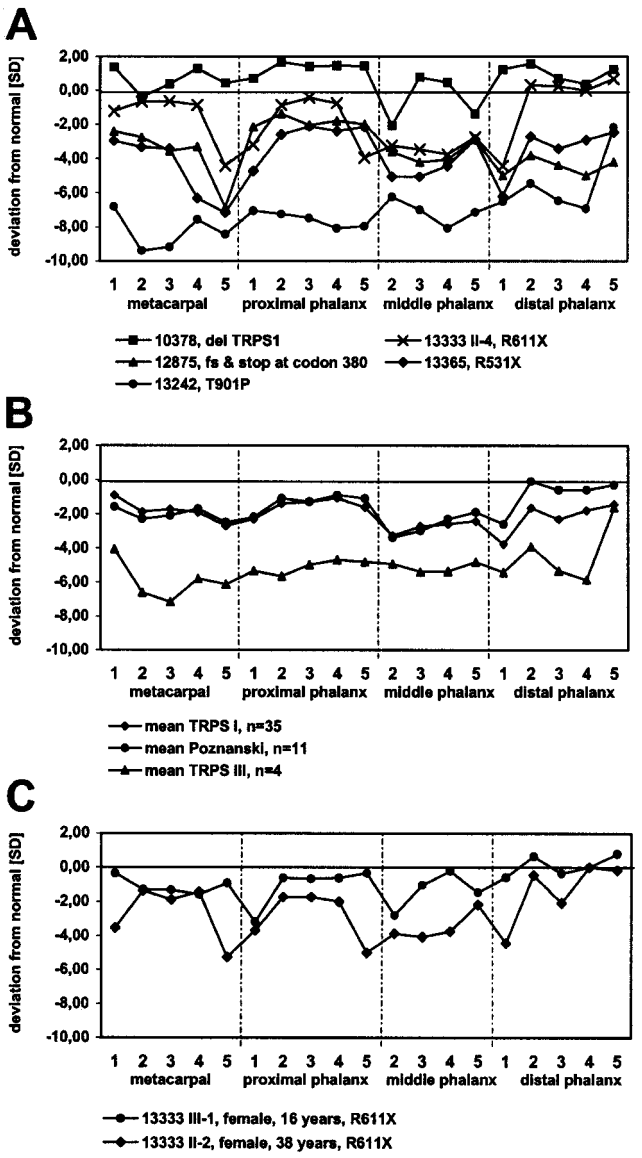

Figure 4 MCPP analyses in TRPS. *A,* Spectrum of MCPPs of patients with different *TRPS1* mutations. *B,* Comparison of mean pattern profiles (see text). *C,* MCPPs of mother (individual II-2) and daughter (III-1) of family 13333 show no significant correlation ($r = 0.38$).

better with mean Poznanski ($r = 0.68$; $P < .01$) than mean TRPS III does ($r = 0.47$; $P < .05$), but mean TRPS I is significantly different from mean TRPS III ($r = 0.27$). Like the difference between the mean heights of patients with TRPS I or TRPS III, the difference between the pattern profiles "mean TRPS I" and "mean TRPS III" proves that patients with a missense mutation tend to be more severely affected than patients with nonsense mutations. However, MCPP analysis cannot be recommended as an unequivocal diagnostic tool in TRPS. For example, we found nonsymmetrical changes in 3 of 30

patients (patients 6820 (I-1), 13365, and 13568), for whom X-rays of both hands were available. In patient 13365 (I-1), who has the recurrent R531X mutation, the discrepancy between hands was so extreme that the left-hand MCPP correlated with the mean TRPS I MCPP ($r = 0.73$ [$P < .001$] vs. $r = 0.37$; no correlation with the mean TRPS III), whereas the right-hand MCPP would suggest TRPS III ($r = 0.64$ [$P < .01$] vs. $r = 0.5$ [$P < .05$] for TRPS I). Furthermore, we found, for example, different MCPPs in a mother (individual II-2) and her daughter (individual III-1) in case 13333 ($r = 0.38$, no correlation, fig. 4*C*) and in unrelated female patients of the same age with identical missense mutations, patients 13287 and 13307 ($r = 0.19$; no correlation; not shown).

Hand X-rays at different chronological ages were available from six patients (patients 13927, 13738, 13242, 7795, 13287, and 4314). In all cases, MCPP analysis revealed that the growth retardation of the bones was a progressive process. This is in agreement with the comparison of the heights of children and adults with TRPS.

The X-rays of most children showed a retarded skeletal age compared with chronological age, until puberty. At puberty, an acceleration of the skeletal age in comparison with chronological age was noted. Of interest, the only exception was patient 13242, with TRPS III (Vilain et al. 1999). At age 17.5 years, the patient's skeletal age corresponded to 14.5 years.

Discussion

The present and previous studies reveal a disease-causing *TRPS1* mutation in 50 (88%) of 57 unrelated cases of either TRPS I or TRPS III. This indicates that *TRPS1* is the major if not the only locus for TRPS I and TRPS III. We found five molecular classes of patients with (i) a *TRPS1* gene deletion or disruption (6 cases), (ii) a nonsense mutation (35 cases), (iii) an in-frame splice mutation (1 case), (iv) a missense mutation (8 cases), or (v) no mutation identified (7 cases). However, evaluation of the clinical features revealed a broad spectrum rather than distinct clinical classes. The mild mental retardation of some of the patients cannot be correlated with a particular *TRPS1* mutation, and the severe mental retardation in case G1386 is most probably due to the large cytogenetically visible deletion (Hamers et al. 1990; Lüdecke et al. 1995).

As outlined above, we consider all frameshift mutations as loss-of-function mutations. If synthesized at all, only the three mutant proteins in patients 14338, 13164, and 11409 may have the potential to enter the nucleus, because, in all others, at least one nuclear localization signal (NLS) is destroyed or absent. This will be clarified in ongoing experiments. None of the mutant proteins would contain the C-terminal IKAROS-like zinc fingers. The double–zinc finger motif of IKAROS is known to mediate the formation of homo- or heterodimers with other IKAROS isoforms (Sun et al. 1996). This dimerization appears to be necessary for normal function of the IKAROS transcription factor. The finding of a mutation that ablates only the 160 most–C-terminal amino acids of the TRPS1 protein (patient 11049) may indicate that homo- or heterodimerization is necessary for the normal function of TRPS1, as well. Experiments aiming at the identification of potential binding partners for TRPS1 are in progress. As shown by RT-PCR, the splice mutation in patient 13568 leads to a stable, truncated transcript. The predicted mutant protein lacks the GATA DNA-binding zinc finger and has a truncated N-terminal NLS (fig. 3*B*) and, thus, probably does not enter the nucleus. The phenotype of most patients with a nonsense mutation or the splice mutation fits into the observed spectrum of TRPS I and resembles the phenotype of patients with a *TRPS1* deletion or disruption. Because the *TRPS1* gene seems to be normally expressed biallelically, we assume that all nonsense mutations and the splice-site mutation, all found in a heterozygous state, reduce the dosis of TRPS1 protein by half, as do complete *TRPS1* deletions or *TRPS1* disruptions. This supports the hypothesis that haploinsufficiency at *TRPS1* is the major cause of TRPS I.

All missense mutations identified so far are located in *TRPS1* exon 6, in the GATA zinc-finger domain. The V894D mutation is located at the N-terminal side of this domain. It exchanges a neutral with an acidic amino acid. The phenotype of the index patient (G1298 II-1) resembles severe TRPS III; however, his mother (individual I-2), who is heterozygous for the missense mutation, is typical for TRPS I. Thus, this missense mutation seems to have a mild effect in the heterozygote, comparable to a nonsense mutation. This is compatible with the finding that the homozygote is viable. The four other missense mutations alter the GATA DNA-binding zinc finger. Patient 13242, with TRPS III (Vilain et al. 1999), is the most severely affected patient in our study, with a heterozygous missense mutation. He carries a T901P mutation, which is expected to disrupt the β-sheet structure of this portion of the zinc finger and to alter the shape of the entire zinc finger. Patient 13916 II-2, with TRPS III (fig. 1), has an arginine to proline substitution at position 908 (R908P), which is likely to disrupt the β-sheet structure at the tip of the zinc finger. Of interest, we found another missense mutation at position 908 in two unrelated patients from The Netherlands and Germany. This R908Q mutation changes a basic to a neutral, polar amino acid at the very tip of the zinc finger. Patient 5874 is a boy with moderate mental retardation and without a family history of

TRPS I. Unfortunately, the original X-rays of his hands were not available. However, photographs of his hands showed prominent interphalangeal joints, which is indicative for CSEs. Photographs of his feet showed short rays 4 and 5, bilaterally. This patient is of nearly normal height (−0.7 SD). Patient 7795 is a 16-year-old girl with normal intelligence and mild short stature (−2.4 SD). MCPP analysis revealed severe brachydactyly ($\bar{z} = -4.5$) with involvement of metacarpals II–V. The A919T missense mutation exchanges a nonpolar to a polar amino acid within the Zn^{2+}-chelating domain of the zinc finger, which probably destabilizes the finger structure by an impairment of Zn^{2+} binding. The A919T mutation was found in a family from Japan (case 13915, individuals I-1 and II-2) and in two sporadic patients from Belgium (case 13287) and Turkey (case 13307). For the Japanese patients, the diagnosis of Ruvalcaba syndrome had been proposed (Sugio and Kajii 1984) but was corrected to TRPS III 10 years later (Kajii et al. 1994). The three elder patients with the A919T mutation have moderate short stature but remarkably short hands. On average, adult patients with *TRPS1* missense mutations are smaller than those with nonsense mutations but are not as small as patients with the contiguous gene syndrome TRPS II, in whom the deletion of the *EXT1* gene, which is also involved in bone development, exerts an additive effect (average height $\bar{z} = -3.91$ SD; $n = 20$; Langer et al. 1984).

All proteins with missense mutations are expected to be able to enter the nucleus and form dimers or multimers through the IKAROS-like zinc-finger domains. These mutants probably have a decreased affinity to DNA, because of the altered GATA zinc fingers, and exert a dominant negative effect as a component of a multimeric protein complex, in contrast to haploinsufficiency supposed for the remaining mutations. Our data demonstrate that TRPS has a broad spectrum with regard to growth retardation and brachydactyly. However, patients with a missense mutation in the GATA zinc finger tend to be more severely affected, and patients with the T901P and R908P mutations, which are supposed to lead to dramatic conformational changes, define the severe end of the TRPS spectrum.

Acknowledgments

We thank the patients and their families for their cooperation; L. Brueton, A. Caliebe, M. Cohen, H. Enders, M. Frydman, J. A. Goodship, S. Kleier, R. König, P. Meyer, L. Neumann, S. C. Niemann-Seyde, A. Rackowitz, I. Schwarze, H. Seidel, and B. Spiegl for sending blood or DNA samples from patients; and E. Passarge for continuous support. This study was supported by grants Lu483/4-2 and Ho949/13-3 from the Deutsche Forschungsgemeinschaft. We want to dedicate this article to Prof. Eberhard Passarge on the occasion of his 65th birthday.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/index.html (for *TRPS1* genomic sequence [accession number AF178030] and for *TRPS1* cDNA sequence [accession number AF183810])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for TRPS I [190350], TRPS II [150230], TRPS III [190351], *TRPS1* [604386])

Tricho-Rhino-Phalangeal Syndrome Project, http://www.uni-essen.de/~thg020/TRPS1/Table3.html (for sequences of primers used for mutation analysis and generation of the *TRPS1* exon 6 hybridization probe)

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