Missense Mutations and Gene Interruption in *PROSIT240*, a Novel *TRAP240*-Like Gene, in Patients With Congenital Heart Defect (Transposition of the Great Arteries)

Nadja Muncke, PhD; Christine Jung, MD; Heinz Rüdiger, MD; Herbert Ulmer, MD; Ralph Roeth; Annette Hubert; Elizabeth Goldmuntz, MD; Deborah Driscoll, MD; Judith Goodship, MD; Karin Schön; Gudrun Rappold, PhD

Background—Congenital heart disease represents the most common severe birth defect, affecting 0.7% to 1% of all neonates, among whom 5% to 7% display transposition of the great arteries (TGA). TGA represents a septation defect of the common outflow tract of the heart, manifesting around the fifth week during embryonic development. Despite its high prevalence, very little is known about the pathogenesis of this disease.

Methods and Results—Using a positional cloning approach, we isolated a novel gene, *PROSIT240* (also termed *THRAP2*), that is interrupted in a patient with a chromosomal translocation and who displays TGA and mental retardation. High expression of *PROSIT240* within the heart (aorta) and brain (cerebellum) was well correlated with the malformations observed in the patient and prompted further analyses. *PROSIT240* shows significant homology to the nuclear receptor coactivator *TRAP240*, suggesting it to be a new component of the thyroid hormone receptor–associated protein (TRAP) complex. Interestingly, several TRAP components have been previously shown to be important in early embryonic development in various organisms, making *PROSIT240* an excellent candidate gene to be correlated to the patient's phenotype. Subsequent mutational screening of 97 patients with isolated dextro-looped TGA revealed 3 missense mutations in *PROSIT240*, which were not detected in 400 control chromosomes.

Conclusions—Together, these genetic data suggest that *PROSIT240* is involved in early heart and brain development. **(*Circulation*. 2003;108:2843-2850.)**

Key Words: transposition of great vessels ■ heart defects, congenital ■ heart diseases

With a prevalence rate of 0.7% to 1% of live births,[1–4] congenital heart disease represents the most common severe birth defect. Despite this high prevalence, very little is known about the underlying mechanisms. Transposition of the great arteries (TGA) accounts for 5% to 7% of all congenital heart disease, affecting 0.2 per 1000 live births,[1,2,4,5] thereby representing the most frequent cyanotic heart defect diagnosed in the neonatal period. TGA occurs as a defect of the partition of the common outflow tract into the aorta and pulmonary arteries. The majority of patients show dextro-looped TGA (dTGA), which is characterized by atrioventricular concordance and ventriculoarterial discordance (Figure 1). As a result, the systemic and pulmonary circulations are completely separated, which is not compatible with life. The less common levo-looped TGA presents with both atrioventricular and ventriculoarterial discordance.

The pathogenesis of TGA is largely unknown. The genetic contribution to the pathogenesis of TGA versus environmental factors has been discussed controversially.[6,7] However, because historically only a few TGA patients survived to reproductive age, very little is known about the recurrence risk in offspring. Until now, mutations in 2 genes were thought to be associated with the pathogenesis of TGA in humans: *ZIC3*[8] and *CFC1* (human *CRYPTIC* gene).[9] Both genes were originally characterized in patients with heterotaxic phenotypes (randomized organ positioning)[10,11] and subsequently screened for mutations in patients with TGA. A contribution of the chromosomal region 22q11 to the pathogenesis of TGA, as suggested by Melchionda et al,[12] could not be verified in subsequent studies.[13,14] The low mutational frequency of *ZIC3* and *CFC1* in TGA patients cannot explain the high incidence of the disease, underlining the strong heterogeneity that we expect for dTGA.

In this study, we used positional cloning as a direct strategy to isolate genes involved in the pathogenesis of TGA. We report on a novel gene on 12q24 similar to the human *t*hyroid

Received August 8, 2003; de novo received September 17, 2003; accepted October 9, 2003.

From the Institut für Humangenetik (N.M., C.G., R.R., A.H., K.S., G.R.), Universität Heidelberg, and the Abteilung für Kardiologie (H.R., H.U.), Kinderklinik Heidelberg, Heidelberg, Germany; the Division of Cardiology (E.G., D.D.), Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pa; and the Institute of Human Genetics (J.G.), International Center for Life, Newcastle upon Tyne, Great Britain.

This article originally appeared Online on November 24, 2003 (*Circulation*. 2003;108:r136–r143).

Correspondence to Gudrun Rappold, PhD, Institute of Human Genetics, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany. E-mail gudrun_rappold@med.uni-heidelberg.de

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000103684.77636.CD**

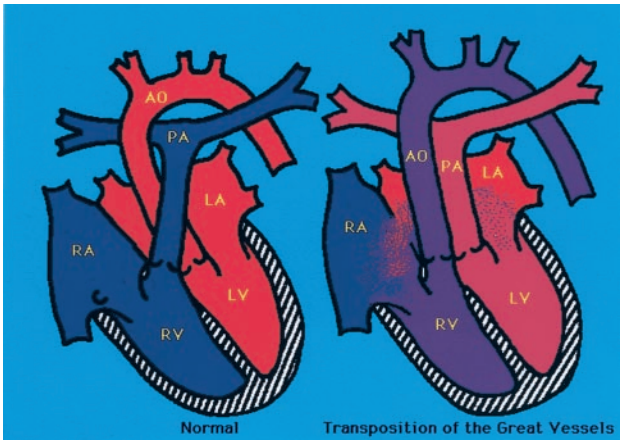

Figure 1. Scheme of normal heart (left) and dTGA (right). Atrial septal defect allows restricted mixing of oxygenated (red) and nonoxygenated (blue) blood. AO indicates aorta; LA, left atrium; LV, left ventricle; PA, pulmonary artery; RA, right atrium; and RV, right ventricle. Reprinted with permission from Randy Attwood, Director of University Relations, University of Kansas Medical Center, Kansas City.

hormone *r*eceptor–*a*ssociated *p*rotein (*TRAP*) 240,[15] which is disrupted by a translocation breakpoint in a patient with dTGA and mental retardation. The gene was therefore termed *PROSIT240* (*pro*tein *si*milar to *T*RAP240). Defects in TRAP function have been previously shown to affect nuclear receptor signaling, resulting in severe defects during embryonic development. Ablation of murine *TRAP220*, for example, has been shown to result in impaired heart and nervous system development.[16] *Drosophila* homologues of *TRAP240* and *TRAP230* are required for proper eye-antennal disc development.[17] Thus, TRAP family members do interfere with important processes in early embryonic patterning, making the *PROSIT240* gene a good candidate to be involved in the phenotype of the patient. Mutational screening of 97 patients with dTGA revealed several sequence variants, including 3 missense mutations, which were not detected in controls.

Methods

Case Report

The proband was a 7-year-old girl born as the first child to a healthy, 30-year-old mother and a healthy, 31-year-old father. The parents were not related, and the family history was unremarkable. The patient had a healthy, 4-year-old brother. The uneventful pregnancy ended in a spontaneous delivery in the 38th week of pregnancy (Apgar score 9/10/10). Birth length was 52 cm (75th percentile), birth weight was 2650 g (10th percentile), and head circumference was 32 cm (10th percentile). Echocardiography revealed dTGA, a perimembranous ventricular septal defect, and an open foramen ovale, as well as mild coarctation of the aorta. The TGA was operated on at the age of 14 months, and the remaining ventricular septal defect was corrected at the age of 4 years.

Postnatal microcephaly developed, and at the age of 2 months, a magnetic resonance imaging scan of the brain was performed. It showed no structural abnormalities, and myelinization was rather advanced. Motor development was mildly delayed. Discrete ataxia was present, and the sense of balance was impaired. Mental retardation became more obvious with age. Speech is nearly absent. The result of routine blood and urine examinations did not indicate metabolic disturbances. A deletion of chromosomal region 22q11 (CATCH22) was excluded by fluorescence in situ hybridization (FISH).

Human Subjects and Genomic DNA

Peripheral blood samples were taken from individuals after informed consent was obtained, after approval by the review board of ethics of the respective institutions (Medical Department, University of Heidelberg, Germany; Newcastle and North Tyneside Health Authority Joint Ethics Committee, England; and Children's Hospital of Philadelphia, Pa).

Genomic Clones and Breakpoint Mapping

Yeast artificial chromosome (YAC) clones were purchased from the German Resource Center (RZPD), Berlin. P1-derived artificial chromosome (PAC) and bacterial artificial chromosome (BAC) clones were obtained from BACPAC Resources, Oakland, Calif (RPCI-1,4 and RPCI-11) or from Research Genetics, Huntsville, Ala (HCIT, CTD). Isolation of metaphase chromosomes and FISH were performed as described elsewhere.[18]

Expression Studies

Human multiple-tissue Northern blots were purchased from Clontech Laboratories. Fragment Ex1/2, covering exons 1 and 2 (bp 91 to 283; FG33289 reverse, gacatcacgacgccatacac; FG493822 forward, gagcctggaggattgtcact), was used as a gene-specific probe, and a β-actin or a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe was used as a control. Labeling was performed with α-[^{32}P]dCTP (Amersham Bioscience). Probes were purified by using a purification kit (Qiaquick, Qiagen) and hybridized to the filter overnight at 65°C, as recommended by the manufacturer. The membranes were washed with 2× sodium chloride sodium citrate at room temperature and with 0.2× sodium chloride sodium citrate at 50°C and exposed at −80°C for 2 hours to 4 days, depending on signal intensity. Fetal cDNA panels were purchased from Clontech Laboratories.

Mutation Screening

Mutation screening was performed by denaturing high-performance liquid chromatography (DHPLC). A WAVE DNA fragment analysis system (Transgenomic Inc) was used. Untranslated regions were not analyzed; exons 10 and 17 had to be subdivided because the fragments were too large. (Primer sequences and polymerase chain reaction [PCR] conditions are available on request.)

Sequencing

Sequencing was performed on a MegaBACE sequencer (Amersham Bioscience) and with use of a DYEnamic ET terminator cycle sequencing kit, following the manufacturer's protocol. Sequencing reactions were performed on both DNA strands. Sequences were analyzed with the CLUSTAL program (German Cancer Research Center, Biocomputing Facility HUSAR).

Accession Numbers

During preparation of this manuscript, our sequence (accession No. AF515599) was confirmed on April 28, 2003, by an update of clone KIAA1025 (XM_034056), now also including exons 4 and 3 and part of exon 2.

Results

Physical Mapping and Characterization of Translocation Breakpoints

Routine cytogenetic analysis had revealed a de novo balanced chromosomal translocation 46,XX,t(12,17) (q24.1;q21). To map the breakpoints, FISH of YACs and PACs was used for gross mapping (data not shown). By using BAC and cosmid clones, we could isolate clones spanning the breakpoints, thus narrowing the critical interval (Figure 2A and 2B). Subsequent Southern blot analysis showed aberrant products in the

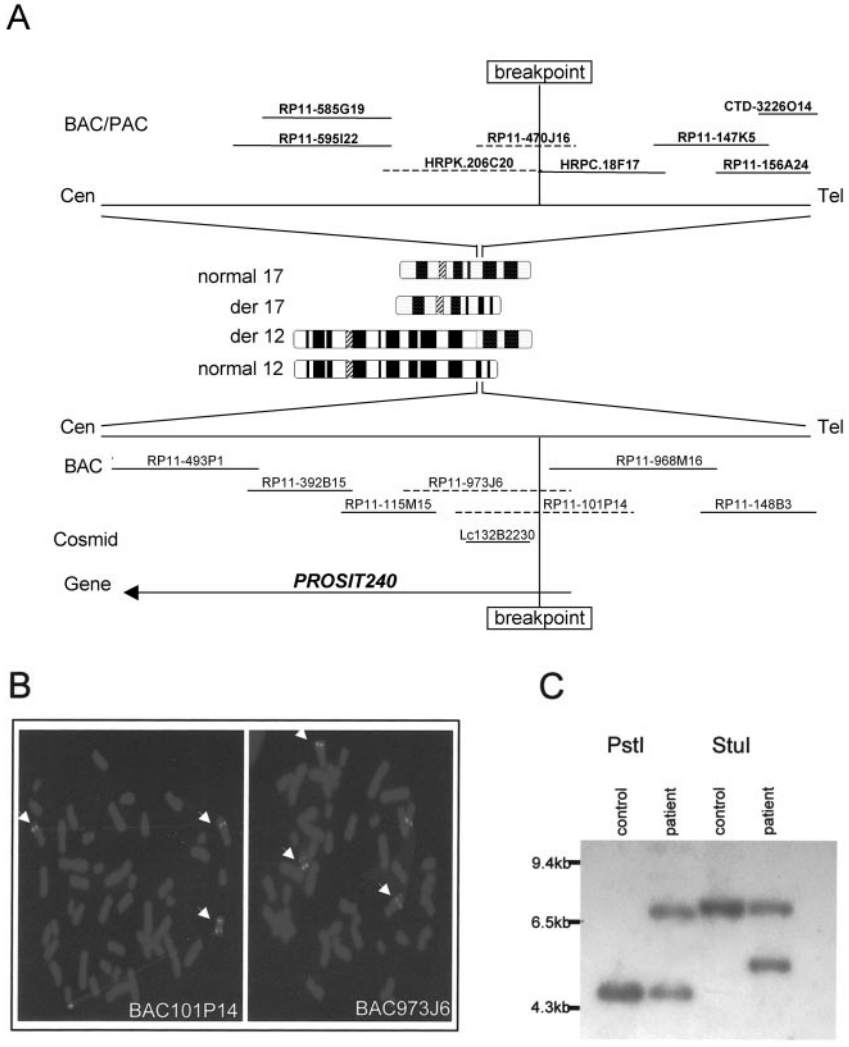

Figure 2. Physical mapping of chromosomal breakpoint region. A, BAC/cosmid map spanning 12q24 and 17q21 breakpoint region. Horizontal dotted lines indicate clones spanning breakpoint. Overlap of clones was verified by PCR or was based on sequence comparison. *PROSIT240* is shown; arrowhead indicates gene orientation. B, FISH analysis of metaphase chromosomes of affected individual. Biotinylated BACs spanning breakpoint were hybridized on patient's metaphase chromosomes, together with reference probe mapping to 17p. Arrows indicate split signal of BACs. C, Genomic Southern blot analysis of patient's and control DNAs derived from whole blood and digested with *Pst*I and *Stu*I. Only in patient's DNA was additional shifted band detected. Cen indicates centromere; Tel, telomere; and der, derivative.

patient's DNA but not in controls (Figure 2C). Cloning and sequencing of the junction fragments confirmed the location of the breakpoint and revealed a microdeletion of 16 nucleotides (data not shown).

On chromosome 17, no known gene was interrupted by the breakpoint, and no novel transcript could be isolated within this interval. The breakpoint on chromosome 12 resided in the overlapping region of RP11-101P14 and RP11-973J6. Using computer-based gene prediction (Nix at http://www.hgmp.mrc.ac.uk), a novel partial transcript of 192 bp could be amplified by reverse transcription (RT)–PCR in multiple tissues.

Isolation of a Novel Gene in 12q24 Interrupted by the Breakpoint

By RT-PCR (of heart, brain, and kidney mRNA; Clontech), subsequent cloning, and sequencing, we could show that the newly identified fragment forms a transcriptional unit with the partial cDNA clone KIAA1025, isolating 5 new exons 5′ to the already known sequence. In total, the novel gene encodes 31 exons with a transcript size of 9377 bp and spans a genomic locus of ≈317 kb. Exon 1, harboring the start codon at position 56, is embedded into a CpG island. A stop codon in exon 31 defines an open reading frame of 6633 bp, which encodes a putative protein of 2210 amino acids. A polyadenylation signal is predicted at position 9357. Figure 3 shows the genomic structure of the gene and gives the exon and intron sizes. The breakpoint resides between exon 1 (harboring the start codon ATG) and exon 2, therefore interrupting the genomic sequence of the gene.

Sequence Comparison

Alignment of the cDNA sequence with the databases revealed a significant homology with *TRAP240*.[15] The 2 large genes showed 55% homology on the nucleotide level over the total gene length according to the CLUSTAL program and 63% according to the BESTFIT program (German Cancer Research Center, Biocomputing Facility HUSAR). On the protein level, a total of 1138 amino acids are identical (51%) according to the CLUSTAL program, with 5 subregions showing identity of >70% (Figure 4). TRAP240 is evolutionarily conserved up to yeast[19] and represents a component of the human TRAP complex, a large multisubunit coactivator.[20–22] Approximately 20 different TRAP subunits have been identified so far, among which are several that have been shown to be involved in embryonic development.[16] TRAP240, 230, 170, and 100 were shown to possess at least 2 copies of the

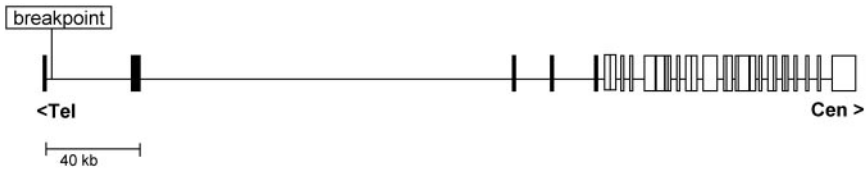

Figure 3. Scheme of genomic structure of *PROSIT240*, with black and white boxes representing exons. Exon 1 contains start, and exon 31 contains stop codon. Black portions indicate newly defined exons, and white portions indicate cDNA designated by KIAA1025. Breakpoint, residing between exons 1 and 2, is indicated. Exon and intron sizes and 5′ and 3′ borders within coding region of *PROSIT240* are given. Cen indicates centromere; Tel, telomere.

exon	5` border	3´ border	exon size in bp	intron size in bp
1	1	72	72	39343
2	73	310	238	173373
3	311	395	85	14479
4	396	479	84	26060
5	480	625	146	2486
6	626	820	195	366
7	821	1009	189	3950
8	1010	1175	166	2208
9	1176	1280	105	3666
10	1281	2012	732	765
11	2013	2238	226	1000
12	2239	2344	106	313
13	2345	2469	125	2767
14	2470	2569	100	5773
15	2570	2790	221	329
16	2791	2996	206	4519

exon	5` border	3´ border	exon size in bp	intron size in bp
17	2997	3934	938	3732
18	3935	4114	180	620
19	4115	4338	224	1894
20	4339	4531	193	640
21	4532	4955	424	514
22	4956	5175	220	1446
23	5176	5364	189	5013
24	5365	5588	224	202
25	5589	5731	143	2935
26	5732	5890	159	1308
27	5891	6067	177	1497
28	6068	6225	158	2698
29	6226	6387	162	2563
30	6388	6500	113	2009
31	6501	6633	133	

LXXLL domain, responsible for nuclear hormone receptor binding.[23] Two LXXLL motifs are also present in the novel gene at amino acids 668 and 1224 (Figure 4). Because of the structural (and possible functional) homology to *TRAP240*, we termed the novel gene *PROSIT240*. Alternatively, the name *THRAP2* also has been assigned by the nomenclature committee.

Expression Analysis

To examine the expression pattern of *PROSIT240*, we performed Northern blot analysis on human multiple tissues. Figure 5A shows that *PROSIT240* is expressed in multiple tissues, with high expression in the brain, heart, skeletal muscle, kidney, placenta, and peripheral blood leukocytes. Among the fetal tissues tested, the transcript showed strongest expression in fetal brain, but it was also expressed in all other tissues tested (Figure 5B). In addition, expression in fetal skeletal muscle, fetal spleen, and fetal thymus was shown by RT-PCR on a normalized cDNA panel (data not shown).

Because *PROSIT240* was identified in a patient with a severe heart defect and mental retardation, we also investigated the expression pattern in different subregions of the heart and brain. Figure 5C shows that within heart structures, the highest expression was detectable in the aorta. Among the tested brain regions, the cerebellum showed the strongest expression; however, the novel gene is expressed in all brain subregions (Figure 5D). Hence, *PROSIT240* is expressed at the right place during embryonic development to be involved in the pathogenesis of the patient's condition.

Using zoo blot analysis, we could show that *PROSIT240* is conserved within humans, mice, cattle, and chickens (data not shown). Database searches across species revealed a possible partially cloned mouse orthologue (XM_132318) residing in the syntenic region, with 88% homology on the nucleotide level and 92% homology on the protein level.

Mutational Analysis of Patients With dTGA

On the basis of the fact that the novel gene is interrupted in a patient with heart and neuronal defects, the correlating expression patterns of *PROSIT240*, and the high impact of related TRAP components in early embryonic development, we consider it an excellent candidate gene to be associated with the pathogenesis of the patient's phenotype. To prove the involvement of *PROSIT240*, additional patients with similar phenotypes were screened for mutations. Because the patient with the chromosomal translocation is also suffering from mental retardation, a mutation screen in patients with mental retardation would be formally possible. However, because such a patient pool is both clinically and genetically highly heterogeneous, this approach is not very promising. To test its involvement in the clinically more specifically defined defect of heart formation, we screened 97 patients with dTGA for mutations in *PROSIT240* by means of DHPLC and sequencing. In cases for which metaphase chromosomes were available (22 cases), FISH analysis was also performed to exclude large deletions. With the use of BACs RP11-973J6, RP11-392B15, and RP11-493P1 as hybridization probes, no gross deletion could be detected. The Table summarizes the results of mutation screening by DHPLC. In total, 6 intronic polymorphisms, 6 silent mutations, and 4 missense mutations were found, which were named according to the scheme of Antonarakis.[24] In cases for which a polymorphism or mutation was not found in 6 control individuals, the number of controls was increased. In the case of 2 intronic polymorphisms (IVS5+41C→T and IVS19+22T→C) and 1 silent mutation (1563C→T), the variation could not be detected in the control cohort of 100 and 68 individuals,

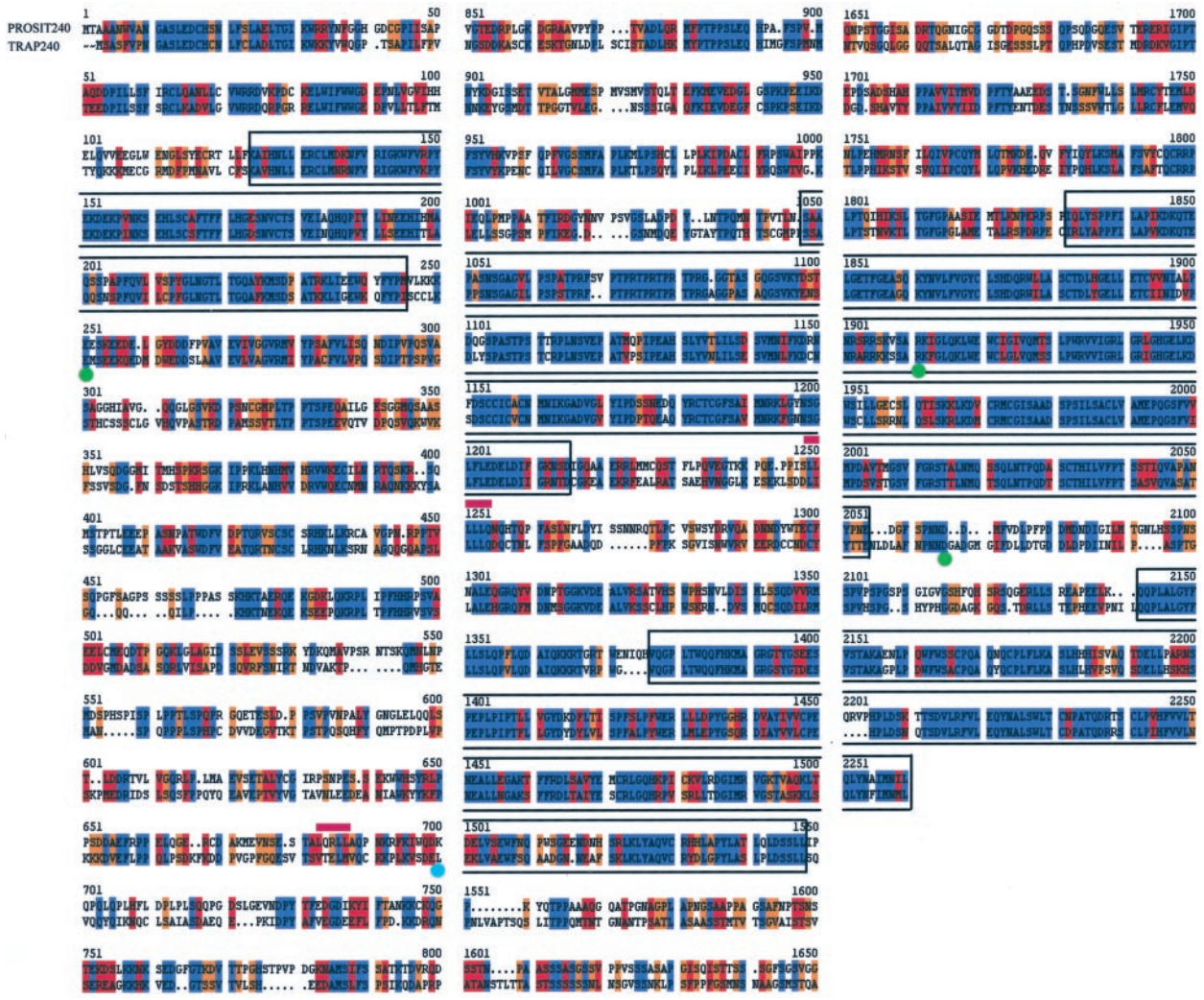

Figure 4. Sequence comparison between PROSIT240 (top) and TRAP240 (lower) based on CLUSTAL program (HUSAR). Blue color indicates identical amino acids, red color indicates amino acids with strong similarity, and orange indicates amino acids with weak similarities. Boxed areas represent regions with identities >70%. Two LXXLL domains are indicated by horizontal pink lines at positions 683 and 1249. Green circles below amino acid indicate missense mutations found in conserved amino acids; turquoise circle indicates missense mutation in nonconserved amino acid.

respectively. Therefore, these cases cannot be excluded to be functionally important without further analysis. One of the missense mutations (2056A→C, Lys686Gln) was found once in a control cohort of 200 individuals, representing an allelic frequency of 0.25%. Because the mutation did not affect a conserved amino acid and we could not obtain further information about the person showing the mutation, we did not pursue further analysis on this case at the moment, despite the fact that it also could not be excluded to be of functional significance.

Most notably, we found 3 missense mutations (752A→G [Glu251Gly], 5615G→A [Arg1872His], and 6068A→G [Asp2023Gly]) that could be detected only in patients and not in any of the 200 ethnically matched control persons. Parental DNA was available for 3 of the 6 respective parents. The mutation Glu251Gly could also be detected in the patient's mother, who does not have a clinically defined TGA. All 3 mutations affect amino acids, which are conserved between *PROSIT240* and *TRAP240* (Figure 4), and 2 mutations (Arg1872His and Asp2023Gly), which reside within the available mouse sequence, affect amino acids conserved between the human and murine *PROSIT240* sequence. Two of the variations (Glu251Gly and Asp2023Gly) furthermore significantly change the biochemical properties of the amino acids.

Discussion

Early embryonic development and organogenesis require spatially and temporally tight coordination of gene expression. Transcriptional regulation in such complex processes therefore involves not only basic activators or repressors but also additional modifiers. The TRAP complex, composed of several TRAP components, represents such a global coactivator, influencing transcriptional regulation of nuclear hormone receptors[20–22] and other activators like p53 or VP16.[25] TRAPs have been shown to be essential for early embryonic development. TRAP220$^{-/-}$ mouse embryos, for example, die at ≈11 days after conception because of severe heart prob-

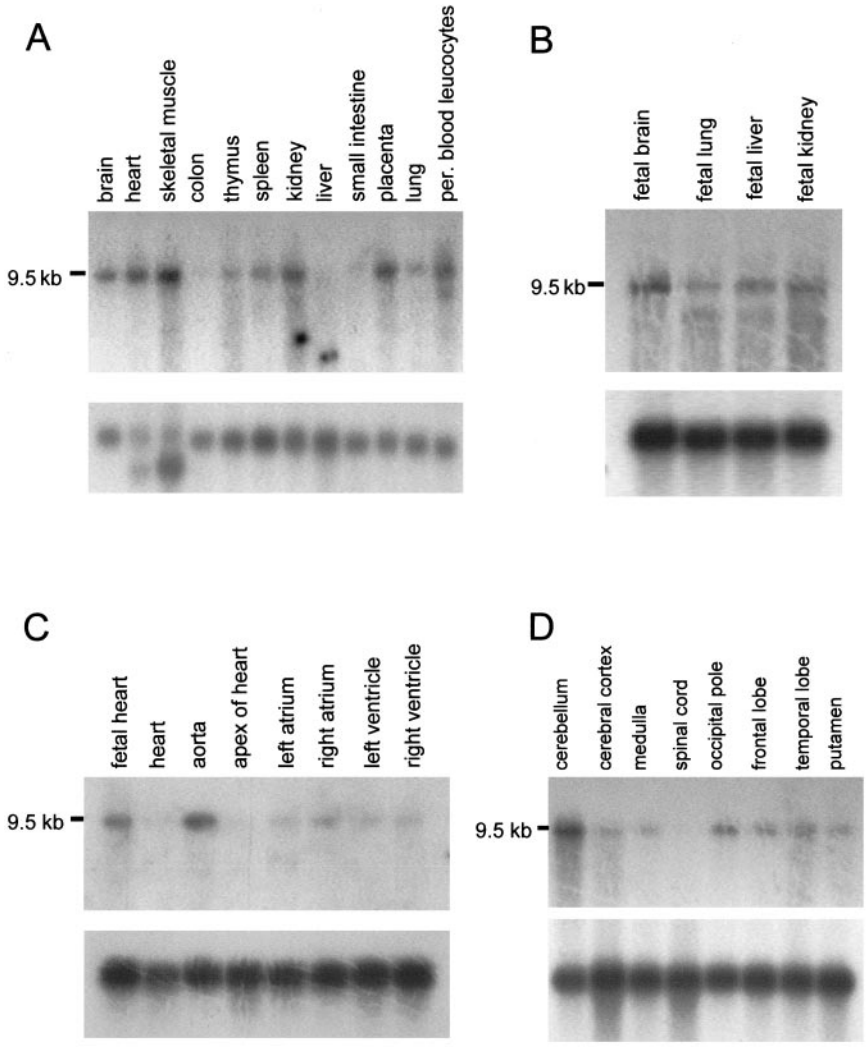

Figure 5. Expression studies of *PROSIT240* by Northern blot analysis. Multiple-tissue Northern blots (Clontech) were hybridized with probe spanning part of exon 1 and exon 2 (bp 33 to bp 228 of coding region). Expression was checked in human adult tissues (A), human fetal tissues (B), and various subregions of heart (C) and brain (D). β-Actin cDNA (A, B, and D) or GAPDH probe (C) was used as control. Per indicates peripheral.

lems and impaired neuronal development.[16] Also, some of the interacting partners of TRAPs have long been known to be involved in developmental processes. Thyroid hormone and its receptor TR, for example, play key roles in the development of the central nervous system.[26,27] Patients with point mutations in the TRβ gene, for example, show mental retardation and emotional disturbance.[27] The influence of nuclear receptors in heart formation has been strikingly demonstrated for RXRα and RARα. RXR/RAR double mutants display outflow tract malformations and abnormalities of the great arteries.[26,28] Using a positional cloning approach, we could show that *PROSIT240/THRAP2*, a novel *TRAP240*-like gene, is interrupted in a patient with a severe heart defect (dTGA) and mental retardation.

The *PROSIT240*/*THRAP2* gene comprises 31 exons encoding a putative protein of 2210 amino acids. On the protein level, 1138 amino acids (51%) are identical between PROSIT240 and TRAP240, with 5 subregions showing identity of >70%. PROSIT240 contains 2 LXXLL domains, which have been shown to be responsible for receptor binding. Both *TRAP240* and *PROSIT240* are ubiquitously expressed, with the highest expression in skeletal muscle. In contrast to *TRAP240*, however, *PROSIT240* is also strongly expressed in the heart (aorta) and brain (cerebellum), where it could possibly substitute for TRAP240 function. The expression pattern correlates well with a possible role for PROSIT240 in the pathogenesis of the patient's phenotype. Because of the high sequence homology to *TRAP240* and the overlapping expression pattern, we suggest that the novel gene is functionally related to *TRAP240*. Future studies will have to show whether *PROSIT240* is indeed a functional relative of *TRAP240*. It will be interesting to see whether and with what kind of activators (or repressors) the putative protein will interact, defining its biologic role during development. Considering the phenotype, RAR/RXR and TR would be particularly interesting candidates to check for interaction with PROSIT240.

For the pathogenesis of dTGA, the involvement of 2 genes has already been discussed. A nonsense mutation in the gene *ZIC3*, originally characterized in patients with laterality defects,[10] was shown to segregate with dTGA in a Lebanese family. Surprisingly, a healthy, maternal uncle also showed the same mutation, pointing toward incomplete penetrance.[8] Besides *ZIC3*, the EGF-CFC gene *CFC1* represents an interesting candidate because of gene-targeting studies in mice. *CFC1*$^{-/-}$ mice develop laterality defects and complex

Summary of *PROSIT240* Sequence Variations in dTGA Study Cohort

Type of Variation/Specific Variation		Patients (n=97) Variant Frequency, n (%)	Controls No. of Controls	Controls Frequency, n (%)
Intronic variations				
IVS5+41C>T	Intron 5	1 (1.03)	100	0 (0)
IVS5–27A>C	Intron 5	19 (19.59)	100	22 (22.00)
IVS19+22T>C	Intron 19	1 (1.03)	100	0 (0)
IVS21–40A>G	Intron 21	24 (24.74)	6	2 (33.3)
IVS26–42G>C	Intron 26	1 (1.03)	68	3 (4.41)
IVS27–8C>T	Intron 27	1 (1.03)	100	6 (6.00)
Silent mutations				
948G>A (Lys316Lys)	Exon 7	1 (1.03)	6	1 (16.66)
1563C>T (Ser521Ser)	Exon 10-1	1 (1.03)	68	0 (0)
1773G>A (Gln591Gln)	Exon 10-2	25 (25.77)	6	2 (33.33)
3070T>C (Leu1024Leu)	Exon 17-1	25 (25.77)	6	2 (33.33)
5928T>C (Thr1976Thr)	Exon 27	23 (23.71)	68	20 (28.99)
6354C>T (Pro2118Pro)	Exon 29	10 (10.31)	6	1 (16.66)
Missense mutations				
2056A>C (Lys686Gln)	Exon 11	1 (1.03)	200	1 (0.50)
752A>G (Glu251Gly)	Exon 6	1 (1.03)	200	0 (0)
5615G>A (Arg1872His)	Exon 25	1 (1.03)	200	0 (0)
6068A>G (Asp2023Gly)	Exon 28	1 (1.03)	200	0 (0)

cardiac malformations reminiscent of human heterotaxy syndrome.[29,30] Most interestingly, 82% of the homozygous mutant mice have malconnection of the great arteries, including TGA, as well as other cardiac malformations.[29] Bamford et al[11] identified 2 distinct mutations within the gene *CFC1* in 3 independent patients with laterality defects and dTGA. Both the deletion and the missense mutation, however, were also found in normal controls or in a healthy parent of the patient.[11] An additional splice-donor mutation was detected in the same gene, creating an alternative splice site, which is predicted to cause a frameshift.[9] However, functional studies could not be performed. The influence of the chromosomal region 22q11 (CATCH22) in dTGA was also discussed controversially. Whereas 2 studies provided evidence that a deletion of 22q11 is causative for dTGA in 12% of patients,[12,31] 2 other studies found no correlation between the deletion and the disease.[13,14] These controversial data and the low mutation frequency of *ZIC3* and *CFC1* in dTGA patients underline the heterogeneity of this disease and demonstrate that only initial steps have been made so far toward an understanding of the pathogenesis of dTGA.

To clarify the involvement of *PROSIT240* in heart formation, we screened 97 patients with dTGA for mutations. In total, we found 6 intronic polymorphisms, 6 silent mutations, and 4 missense mutations. Although intronic and silent mutations generally cannot be excluded to be functionally relevant,[32,33] especially when not found in controls, we focused on the missense mutations at this stage. Three missense mutations (Glu251Gly, Arg1872His, and Asp2023Gly) found in the patient cohort could not be identified in 400 control chromosomes. These mutations all affect amino acids that are conserved between PROSIT240 and TRAP240 (Figure 4) and could therefore be important for protein function. Glu251Gly and Asp2023Gly represent exchanges that significantly change biochemical properties of the respective amino acid. One of the mutations (Glu251Gly) is also carried by the patient's mother, who did not present with dTGA. Unfortunately, this mother was not available for further clinical testing to check for previously undiagnosed (subtle) heart defects. The mutation could represent a rare polymorphism, yet finding the mutation in a healthy parent can also be explained by incomplete penetrance, which has been previously reported in congenital heart disease (eg, in ZIC3; see above).

Detecting 3 missense mutations and 1 gene interruption (leading to haploinsufficiency) in dTGA patients strongly suggests a contribution of *PROSIT240* to heart development. The putative relatedness of *PROSIT240* to *TRAP240* could point to an involvement of the TRAP complex. The *Drosophila* homologues of *TRAP240* and *TRAP230* were shown to act together to control cell affinity to establish cell boundaries, a process that might also be relevant to our observation relating *PROSIT240* malfunction to dTGA.[34] Amino acid exchanges caused by missense mutations could alter the ability of PROSIT240 to interact with target activators or repressors. Understanding more about the mechanisms leading to dTGA might also help to determine whether dTGA could be considered a manifestation of a left-right laterality defect

concerning the heart, a point that has been raised based on data from animal models[35,36] and the fact that *ZIC3* and *CFC1* mutations are detected both in patients with dTGA and in heterotaxy problems.[8,11] In animal models, most interestingly, Pitx2$^{-/-}$ and Dvl$^{-/-}$ mice present with outflow tract abnormalities, including TGA and laterality defects. Both genes are part of the Wnt/Dvl/β-catenin $\rightarrow$Pitx2 pathway, which was shown to recruit TRAP components.[36] It will be interesting to see whether there will be a common pathogenetic mechanism involved in causing dTGA and laterality defects. With *PROSIT240*, we were able to bring a novel, exciting player into the game, leading the way toward new questions that can be raised concerning the complexity of heart formation.

Acknowledgments

N.M. was supported by the Landesgraduiertenförderung Baden-Württemberg, Germany. We thank Dr Rüdiger Blaschke for critically reading the manuscript and Maike Boerger, Dr Beate Niesler, and Dr Bernd Frank for helpful support.

References

1. Hoffman JI. Incidence of congenital heart disease, I: postnatal incidence. *Pediatr Cardiol*. 1995;16:103–113.
2. Hoffman JI. Incidence of congenital heart disease, II: prenatal incidence. *Pediatr Cardiol*. 1995;16:155–165.
3. Schoetzau A, van Santen F, Sauer U, et al. Kardiovaskuläre Fehlbildungen in Bayern 1984–1991. *Z Kardiol*. 1997;86:496–505.
4. Samanek M. Congenital heart malformations: prevalence, severity, survival, and quality of life. *Cardiol Young*. 2000;10:179–185.
5. Fyler DC, Buckley LP, Hellenbrand WE, et al. Report of the New England regional infant cardiac program. *Pediatrics*. 1980;65(suppl): 375–461.
6. Burn J, Brennan P, Little J, et al. Recurrence risks in offspring of adults with major heart defects: results from first cohort of British collaborative study. *Lancet*. 1998;351:311–316.
7. Digilio MC, Casey B, Toscano A, et al. Complete transposition of the great arteries: patterns of congenital heart disease in familial precurrence. *Circulation*. 2001;104:2809–2814.
8. Megarbane A, Salem N, Stephan E, et al. X-linked transposition of the great arteries and incomplete penetrance among males with a nonsense mutation in ZIC3. *Eur J Hum Genet*. 2000;8:704–708.
9. Goldmuntz E, Bamford R, Karkera JD, et al. CFC1 mutations in patients with transposition of the great arteries and double-outlet right ventricle. *Am J Hum Genet*. 2002;70:776–780.
10. Gebbia M, Ferrero GB, Pilia G, et al. X-linked situs abnormalities result from mutations in *ZIC3*. *Nat Genet*. 1997;17:305–308.
11. Bamford RN, Roessler E, Burdine RD, et al. Loss-of-function mutations in the EGF-CFC gene *CFC1* are associated with human left-right laterality defects. *Nat Genet*. 2000;26:365–369.
12. Melchionda S, Digilio MC, Mingarelli R, et al. Transposition of the great arteries associated with deletion of chromosome 22q11. *Am J Cardiol*. 1995;75:95–98.
13. Takahashi K, Kido S, Hoshino K, et al. Frequency of a 22q11 deletion in patients with conotruncal cardiac malformations: a prospective study. *Eur J Pediatr*. 1995;154:878–881.
14. Goldmuntz E, Clark BJ, Mitchell LE, et al. Frequency of 22q11 deletions in patients with conotruncal defects. *J Am Coll Cardiol*. 1998;32: 492–498.
15. Ito M, Yuan CX, Malik S, et al. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol Cell*. 1999;3:361–370.
16. Ito M, Yuan CX, Okano HJ, et al. Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. *Mol Cell*. 2000;5:683–693.
17. Treisman JE. *Drosophila* homologues of the transcriptional coactivation complex subunits TRAP240 and TRAP230 are required for identical processes in eye-antennal disc development. *Development*. 2001;128: 603–615.
18. Lichter P, Cremer T. *Human Cytogenetics: A Practical Approach*. Oxford/New York/Tokyo: IRL Press/Oxford University Press; 1992.
19. Samuelsen CO, Baraznenok V, Khorosjutina O, et al. TRAP230/ARC240 and TRAP240/ARC250 mediator subunits are functionally conserved through evolution. *Proc Natl Acad Sci U S A*. 2003;100:6422–6427.
20. Fondell JD, Ge H, Roeder RG. Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. *Proc Natl Acad Sci U S A*. 1996;93:8329–8333.
21. Fondell JD, Guermah M, Malik S, et al. Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID. *Proc Natl Acad Sci U S A*. 1999;96: 1959–1964.
22. Yuan CX, Ito M, Fondell JD, et al. The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. *Proc Natl Acad Sci U S A*. 1998;95:7939–7944.
23. Ren Y, Behre E, Ren Z, et al. Specific structural motifs determine TRAP220 interactions with nuclear hormone receptors. *Mol Cell Biol*. 2000;20:5433–5446.
24. Antonarakis SE. Recommendations for a nomenclature system for human gene mutations: Nomenclature Working Group. *Hum Mutat*. 1998; 11:1–3.
25. Malik S, Roeder RG. Transcriptional regulation through mediator-like coactivators in yeast and metazoan cells. *Trends Biochem Sci*. 2000;25: 277–283.
26. Kastner P, Mark M, Chambon P. Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell*. 1995;83: 859–869.
27. Forrest D, Reh RA, Rusch A. Neurodevelopmental control by thyroid hormone receptors. *Curr Opin Neurobiol*. 2002;12:49–56.
28. Kastner P, Grondona JM, Mark M, et al. Genetic analysis of RXRα developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. *Cell*. 1994;78:987–1003.
29. Gaio U, Schweickert A, Fischer A, et al. A role of the cryptic gene in the correct establishment of the left-right axis. *Curr Biol*. 1999;9:1339–1342.
30. Yan YT, Gritsman K, Ding J, et al. Conserved requirement for EGF-CFC genes in vertebrate left-right axis formation. *Genes Dev*. 1999;13: 2527–2537.
31. Marble M, Morava E, Lopez R, et al. Report of a new patient with transposition of the great arteries with deletion of 22q11.2. *Am J Med Genet*. 1998;78:317–318.
32. Krawczak M, Reiss J, Cooper DN. The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. *Hum Genet*. 1992;90:41–54.
33. Cartegni L, Chew SL, Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet*. 2002;3: 285–298.
34. Janody F, Martirosyan Z, Benlali A, et al. Two subunits of the *Drosophila* mediator complex act together to control cell affinity. *Development*. 2003;130:3691–3701.
35. Yasui H, Morishima M, Nakazawa M, et al. Anomalous looping, atrioventricular cushion dysplasia, and unilateral ventricular hypoplasia in the mouse embryos with right isomerism induced by retinoic acid. *Anat Rec*. 1998;250:210–219.
36. Kioussi C, Briata P, Baek SH, et al. Identification of a Wnt/Dvl/β-catenin$\rightarrow$Pitx2 pathway mediating cell-type-specific proliferation during development. *Cell*. 2002;111:673–685.