Clinical Investigation and Reports

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 TRAP2), 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
by guest on April 12, 2017 http://circ.ahajournals.org/ Downloaded from

PROSIT240
dTGA and mental retardation. The gene was therefore termed

important processes in early embryonic patterning, making

TRAP230

A deletion of chromosomal region 22q11

retardation became more obvious with age. Speech is nearly absent.

showed no structural abnormalities, and myelinization was rather

magnetic resonance imaging scan of the brain was performed. It

septal defect was corrected at the age of 4 years.

operated on at the age of 14 months, and the remaining ventricular

perimembranous ventricular septal defect, and an open foramen

birth weight was 2650 g (10th percentile), and head circumference

ended in a spontaneous delivery in the 38th week of pregnancy

uneventful pregnancy

30-year-old mother and a healthy, 31-year-old father. The parents

The proband was a 7-year-old girl born as the first child to a healthy,

Case Report

hormone receptor–associated protein (TRAP) 240,15 which is

disrupted by a translocation breakpoint in a patient with
dTGA and mental retardation. The gene was therefore termed

PROSIT240 (protein similar to TRAP240). Defects in TRAP

function have been previously shown to affect nuclear recep-
tor 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 develop-

ment.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

(Appgar 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 Hei-
delberg, Germany; Newcastle and North Tyneside Health Authority

Joint Ethics Committee, England; and Children’s Hospital of Phila-
delphia, Pa).

Genomic Clones and Breakpoint Mapping

Yeast artificial chromosome (YAC) clones were purchased from the

German Resource Center (RZPD), Berlin. PI-derived artificial

chromosome (PAC) and bacterial artificial chromosome (BAC)

clones were obtained from BACPAC Resources, Oakland, Calif

(RPCI-14 andRPCI-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, gacatcacgacgccatac; FG493822 forward, gagc-
tgagaggtcact), was used as a gene-specific probe, and a β-actin or

glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe was

used as a control. Labeling was performed with α-32PdCTP

(Amersham Bioscience). Probes were purified by using a purifica-
tion kit (QiapQuick, 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 Laborato-
ries.

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. Sequencing reactions were performed on both DNA

strands. Sequences were analyzed with the CLUSTAL program (Ger-
man 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 cosm
did clones, we could isolate clones spanning the breakpoints, thus

narrowing the critical interval (Figure 2A and 2B). Subse-
quent Southern blot analysis showed aberrant products in the

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.
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. 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 and represents a component of the human TRAP complex, a large multisubunit coactivator. Approximately 20 different TRAP subunits have been identified so far, among which are several that have been shown to be involved in embryonic development. TRAP240, 230, 170, and 100 were shown to possess at least 2 copies of the
LXXLL domain, responsible for nuclear hormone receptor binding. 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. 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,
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 and other activators like p53 or VP16. 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-
lems and impaired neuronal development. 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. Patients with point mutations in the TRβ gene, for example, show mental retardation and emotional disturbance. 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. Using a positional cloning approach, we could show that PROSIT240, 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, was shown to segregate with dTGA in a Lebanese family. Surprisingly, a healthy, maternal uncle also showed the same mutation, pointing toward incomplete penetrance. 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
cardiac malformations reminiscent of human heterotaxy syndrome. Most interestingly, 82% of the homozygous mutant mice have malconnection of the great arteries, including TGA, as well as other cardiac malformations. Bamford et al. identified 2 distinct mutations within the gene \textit{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. An additional splice-donor mutation was detected in the same gene, creating an alternative splice site, which is predicted to cause a frameshift. 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, other studies found no correlation between the deletion and the disease. These controversial data and the low mutation frequency of \textit{ZIC3} and \textit{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 \textit{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, 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 \textit{PROSIT240} and \textit{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 \textit{ZIC3}; see above).

Detecting 3 missense mutations and 1 gene interruption (leading to haploinsufficiency) in dTGA patients strongly suggests a contribution of \textit{PROSIT240} to heart development. The putative relatedness of \textit{PROSIT240} to \textit{TRAP240} could point to an involvement of the TRAP complex. The \textit{Drosophila} homologues of \textit{TRAP240} and \textit{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 \textit{PROSIT240} malfunction to dTGA. Amino acid exchanges caused by missense mutations could alter the ability of \textit{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 and the fact that ZIC3 and CFC1 mutations are detected both in patients with dTGA and in heterotaxy problems. In animal models, most interestingly, Pitx2c−/− and Dvl1−/− mice present with outflow tract abnormalities, including TGA and laterality defects. Both genes are part of the Wnt/Dvl/β-catenin→Pitx2 pathway, which was shown to recruit TRAP components. 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

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

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

\textit{Circulation}. 2003;108:2843-2850; originally published online November 24, 2003; doi: 10.1161/01.CIR.0000103684.77636.CD

\textit{Circulation} is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/108/23/2843

\textbf{Permissions}: Requests for permissions to reproduce figures, tables, or portions of articles originally published in \textit{Circulation} can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

\textbf{Reprints}: Information about reprints can be found online at:
http://www.lww.com/reprints

\textbf{Subscriptions}: Information about subscribing to \textit{Circulation} is online at:
http://circ.ahajournals.org//subscriptions/