ARTICLE

Complex Inheritance Pattern Resembling Autosomal Recessive Inheritance Involving a Microdeletion in Thrombocytopenia–Absent Radius Syndrome

Eva Klopocki,* Harald Schulze,* Gabriele Strauß, Claus-Eric Ott, Judith Hall, Fabienne Trotier, Silke Fleischhauer, Lynn Greenhalgh, Ruth A. Newbury-Ecob, Luitgard M. Neumann, Rolf Habenicht, Rainer König, Eva Seemanova, André Megarbane, Hans-Hilger Ropers, Reinhard Ullmann, Denise Horn, and Stefan Mundlos

Thrombocytopenia–absent radius (TAR) syndrome is characterized by hypomegakaryocytic thrombocytopenia and bilateral radial aplasia in the presence of both thumbs. Other frequent associations are congenital heart disease and a high incidence of cow's milk intolerance. Evidence for autosomal recessive inheritance comes from families with several affected individuals born to unaffected parents, but several other observations argue for a more complex pattern of inheritance. In this study, we describe a common interstitial microdeletion of 200 kb on chromosome 1q21.1 in all 30 investigated patients with TAR syndrome, detected by microarray-based comparative genomic hybridization. Analysis of the parents revealed that this deletion occurred de novo in 25% of affected individuals. Intriguingly, inheritance of the deletion along the maternal line as well as the paternal line was observed. The absence of this deletion in a cohort of control individuals argues for a specific role played by the microdeletion in the pathogenesis of TAR syndrome. We hypothesize that TAR syndrome is associated with a deletion on chromosome 1q21.1 but that the phenotype develops only in the presence of an additional as-yet-unknown modifier (mTAR).

Thrombocytopenia–absent radius (TAR) syndrome (MIM 274000) is a clinically well-characterized malformation syndrome. According to the diagnostic criteria defined by J. Hall in 1969, typical clinical features are hypomegakaryocytic thrombocytopenia and bilateral absence of the radius in the presence of both thumbs.[1] These characteristic patterns differentiate TAR syndrome from other conditions with involvement of the radius—that is, Holt-Oram syndrome (MIM 142900), Roberts syndrome (MIM 268300), and Fanconi anemia (MIM 227650)—in which the thumb is usually absent or severely hypoplastic. Additional skeletal features associated with TAR syndrome include shortening and, less commonly, aplasia of the ulna and/or humerus. In the latter situation, the five-fingered hand arises from the shoulder. The hands may show limited extension of the fingers, radial deviation, and hypoplasia of the carpal and phalangeal bones. The lower limbs are frequently involved, but usually to a lesser extent than are the upper limbs. Dislocation of the hips and subluxation of the knees resulting in coxa vara are common. Extraskeletal manifestations comprise cardiac abnormalities, such as tetralogy of Fallot and atrial septal defects, and abnormalities of the genitourinary tract. Cow's milk allergy or intolerance appears to be relatively common among patients with TAR syndrome and may provoke eosinophilia.[2] Patients with TAR syndrome typically present with petechiae and severe bleeding during the first years of life. Platelet counts are often <50 platelets/nl (normal range 150–400 platelets/nl) due to impaired presence or maturation of megakaryocytic progenitors in the bone marrow.[3,4] Although platelet counts ameliorate over time, patients remain thrombocytopenic with continued risk of bleeding. In addition, young patients with TAR syndrome have been reported to have leukemoid reactions with white blood counts exceeding 35,000 cells/mm^3. Similar to the thrombocytopenia, they are transient in nature and not associated with true leukemia.[5]

The genetic basis of TAR syndrome remains unclear. Different modes of inheritance—that is, autosomal recessive, autosomal dominant, or autosomal dominant with reduced penetrance—have been discussed in the literature.[1,2,6] Generally, the pattern of inheritance is most consistent with autosomal recessive inheritance.[7] However, there does not appear to be an increased incidence of consanguinity in families with TAR syndrome, as would be expected for a rare autosomal recessive disorder. Several

From the Institut für Medizinische Genetik (E.K.; C.-E.O.; F.T.; D.H.; S.M.), Klinik für Allgemeine Pädiatrie (H.S.; G.S.; S.F.), and Institut für Humangenetik (L.M.N.), Charité Universitätsmedizin Berlin, and Max Planck Institut für Molekulare Genetik (H.-H.R.; R.U.; S.M.), Berlin; University of British Columbia, Vancouver (J.H.); Clinical Genetics, Royal Liverpool Children's Hospital, Liverpool, United Kingdom (L.G.); Clinical Genetics, Bristol Royal Hospital for Children, Bristol, United Kingdom (R.A.N.-E.); Kinderkrankenhaus Wilhelmstift, Hamburg, Germany (R.H.); Institut für Humangenetik, Universitätsklinikum Frankfurt, Frankfurt, Germany (R.K.); Institute for Biology and Medical Genetics, Charles University, Prague, Czech Republic (E.S.); and Service de Génétique Médicale, Université Saint-Joseph, Beirut, Lebanon (A.M.)

Received September 7, 2006; accepted for publication November 14, 2006; electronically published December 21, 2006.

Address for correspondence and reprints: Dr. Stefan Mundlos, Institut für Medizinische Genetik, Charité Universitätsmedizin Berlin, Campus Virchow Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail: stefan.mundlos@charite.de

* These two authors contributed equally to this work.

Am. J. Hum. Genet. 2007;80:232–240. 0002-9297/2007/8002-0004$15.00

Table 1. Clinical Description of Patients with TAR Syndrome

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

families have been reported with affected individuals spread across 2 or even 3 generations,[6] an unexpected finding in nonconsanguineous pedigrees. In addition, parent-to-child transmission has been reported.[8] Several explanations have been put forward to explain these unusual observations. Hall et al.[7] suggested that patients with TAR syndrome may be genetic compounds. In this case, TAR syndrome would follow a digenic inheritance similar to the situation described for Bardet-Biedl syndrome.[9] Also, the possibility that Roberts syndrome and TAR syndrome are allelic conditions has been discussed. However, Roberts syndrome was shown to be caused by mutations in the *ESCO2* gene, the protein product of which is required for the establishment of sister chromatid cohesion during S phase,[10] but *ESCO2* mutations are not observed in TAR syndrome.

Sequencing of candidate genes like *HoxA10, HoxA11, HoxD11,* and *c-mpl,* the thrombopoietin receptor gene, was performed, but no mutations were identified.[11,12] Given the unclear inheritance and the frequently sporadic nature of TAR syndrome, we searched for genomic aberrations in 30 patients with TAR syndrome and their families, using high-resolution microarray-based comparative genomic hybridization (array CGH[13,14]). We identified a common 200-kb microdeletion on the long arm of chromosome 1 in all the affected and in 25 (32%) of the 78 unaffected family members. Our results indicate that TAR syndrome is associated with a microdeletion on 1q21.1 that is necessary but not sufficient to cause the phenotype.

Material and Methods

Patients

We examined samples from 30 unrelated patients with TAR syndrome. All patients fulfill the diagnostic criteria for TAR syndrome: bilateral radial aplasia in the presence of both thumbs and thrombocytopenia.

Figure 1. Pedigrees of TAR-affected families. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Patients were clinically assessed by at least one of us. Blood samples were collected from the patients, their parents, and additional family members if available. Informed consent was obtained from all patients, their parents, and family members from whom a blood sample was collected. One patient (patient 16), described elsewhere, was suggested to have an overlapping phenotype of Roberts syndrome and TAR syndrome.[15] Clinical data are summarized in table 1. Pedigrees of all families are shown in figure 1. The investigated patients with TAR syndrome originate from different regions of the world, including Germany, the United Kingdom, the United States, Lebanon, and India.

Cytogenetics and Array CGH

Karyotyping of GTG-banded chromosomes from lymphocytes at 450-band resolution was performed according to standard procedures. Four patients were analyzed by array CGH by use of a submegabase-resolution whole-genome tiling path BAC array consisting of the human 32K Re-Array set (DNA and clones kindly provided by Pieter de Jong, BACPAC Resources Center[17–19]), the 1-Mb Sanger Clone set (clones kindly provided by Nigel Carter, Wellcome Trust Sanger Institute[20]), and a set of 390 subtelomeric clones (generated in the course of the European Union initiative COSTB19: Molecular Cytogenetics of Solid Tumors). Array CGH hybridization and analysis by CGHPRO was performed as described elsewhere.[21–23] Detailed protocols are also available at the Max Planck Institute for Molecular Genetics: Molecular Cytogenetics Group Web site. Copy-number gains and losses were determined by use of a conservative threshold of 0.3 and −0.3, respectively. Aberrant signals including three or more neighboring BAC clones were considered genomic aberrations and were further evaluated by FISH, unless they coincided with a published

Table 2. Primer Sequences for qPCR Analysis

Primer	Name	Primer Sequence (5′→3′)		Amplicon Position	
		Forward	Reverse	Start	Stop
1	Chr1-No3	TTGGGTAAGAACGGAGATGG	CACCTTTCATCAAGGGAGGA	144014365	144014445
2	Chr1-No5	AATCTAGGTGGGGCTGTGTG	CCTTTCTCATCCAGCAGCTC	144041972	144042069
3	NK	GCTTCAATGTCTCCCGTGTTCT	TGGCCGTAGATAATCTCATATGTTTG	144096959	144097039
4	NK2	TGAGTGGTCTTCGGGTGATAGA	CCCATCCCACTGAAAACTGAA	144110432	144110519
5	hChr1-A	CAGATGCTTGAAATGGAGTAAATGC	TCCCTGAGATAGCAACAGTATCTGA	144128930	144129015
6	PIAS3-I9-10	CTTCCCACTGAGCATTGCAA	GAGGTTAAGACTAGCAAGATCAAAAGATT	144294022	144294099
7	POLR3C I13-14	GGAAAATGGTAAAGGGTGGTG	CATCTTCCCTAACCTGGGAGA	144305571	144305643
8	POLR3C I11-12	GTAACCAACCAAGGGCGTAA	GTTTGCTTGGGGGTTCAGTA	144308167	144308260
9	ZNF364 I1-2	TCCTGAGTGTGATTGATTCTGGAT	AAATTCGAAGTGCTGTACTTCACAA	144339188	144339268
10	hChr1-C (ZNF364 I2-3)	TGGTATCCAGCAGTTCCTGTGA	TGTCCCCATGAAATTTGCAA	144360856	144360946
11	hChr1-D (ZNF364 I4-5)	GAGAAGAAACAGCCAGGGAAGTAC	TGCTAAAACCAGGTAAGAGTCTTCAC	144387303	144387389

Table 3. Primer Sequences for Sequencing Analysis

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

DNA copy-number variant as listed in the Database of Genomic Variants (version June 2006).

FISH

BAC clones RP11-698N18 and RP11-258G05 (obtained from the RZPD, Deutsches Resourcenzentrum für Genomforschung, Berlin, Germany) were fluorescently labeled using nick translation and were hybridized to metaphase spreads of the patient's lymphocytes by use of standard procedures. BAC clones were labeled with Spectrum Orange. Cep1 (Vysis) labeled with Spectrum Green was used as a control probe. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole. Image analysis was performed using the ISIS analysis system (Metasystems).

Quantitative Real-Time PCR (qPCR)

To evaluate genomic DNA for the microdeletion 1q21.1 and to map the breakpoint at a higher resolution, we developed a qPCR test, using a set of 11 primer pairs located within the deletion. Genomic DNA was extracted from blood or buccal smears by use of standard methods. qPCR was performed in a total volume of 24 μl in each well containing 12 μl of SYBR Green PCR Master Mix (Applied Biosystems), 10 μl of genomic DNA (1 ng/μl), and 2 μl of primers (0.2 μmol each). Samples were run in triplicate in separate tubes to permit the quantification of the target sequences normalized to *Albumin*. PCR conditions were as follows: initial denaturation step at 95°C followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. By use of calibrator samples of normal control DNA, the gene copy number was estimated on the basis of the comparative $\Delta\Delta C_t$ method. In addition, we performed a sex determination for the individuals, calculating the *Factor VIII* exon 3 relative to our endogenous control *Albumin*, to assure its reliability. Primer sequences are given in table 2.

Candidate-Gene Sequencing

To test for possible point mutations or small deletions in genes within the deleted region, we sequenced the coding region of the genes *HFE2, LIX1L, PIAS3, ANKRD35, ITGA10, RBM8A, PEX11B, POLR3GL, TXNIP,* and *GNRR2* on the nondeleted allele in three patients with TAR syndrome. Primer sequences are given in table 3.

Results

Clinical Features of Patients with TAR Syndrome

The patients with TAR syndrome examined in this study included 19 females and 11 males, with ages ranging from 6 months to 45 years. The study includes one case of vertical transmission (an affected mother had an affected fetus; patients 29 and 30). Six probands have unaffected siblings, and, in one family, two brothers are affected (patients 25 and 26) (for pedigrees, see fig. 1).

All patients studied here had a documented thrombocytopenia defined as a platelet count <150 platelets/nl and bilateral radial aplasia (for clinical details, see table 1). We observed a considerable clinical variability with some individuals presenting with minor additional upper-limb abnormalities, whereas others showed severe phocomelia (fig. 2). Lower-limb involvement, including hip dysplasia, genua vara, and bowing of long bones, was present in 16 patients, and short stature (height ⩽3rd percentile) was seen in 9 patients. Cardiac anomalies, such as ventricular septal defect, occurred in three patients, and genitourinary anomalies, including horseshoe kidney, hypoplasia of the uterus and vagina, and renal pelvis dilation, were observed in four patients. Eight patients were described as having

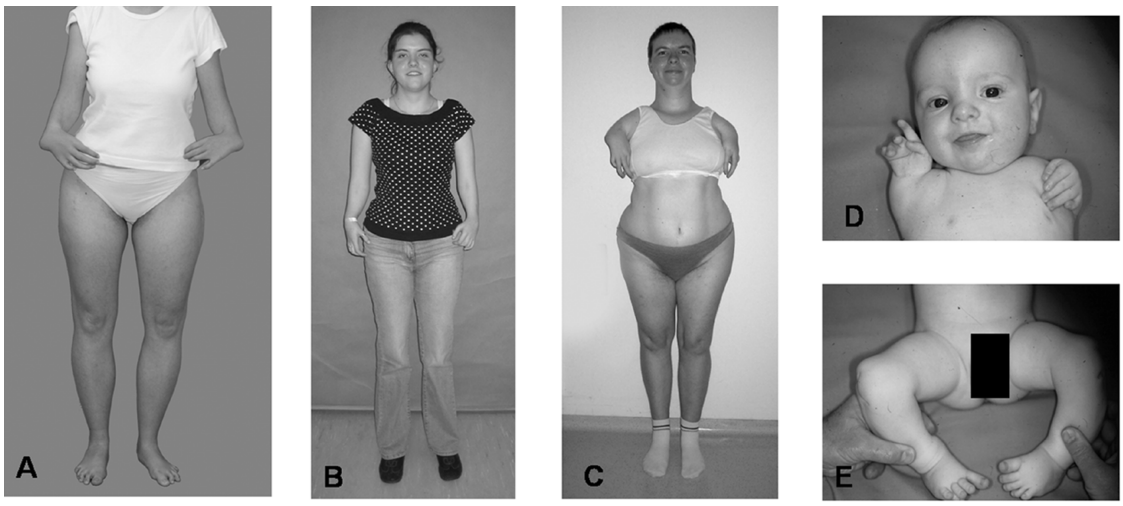

Figure 2. *A,* Adult patient (patient 1) with moderate upper-limb involvement. Note the radial deviation of both hands. *B,* Patient 9, showing mild upper-limb involvement with slightly reduced lengths of the arms. *C,* Severe phenotype with phocomelia (patient 20). *D,* Phocomelia in a severely affected patient (patient 8), and bowing of the long bones (*E*).

cow's milk intolerance and increased susceptibility to gastroenteritis. Two patients had sensorineural hearing loss and one had a cleft lip and palate.

Microdeletion Associated with TAR Syndrome

No cytogenetic abnormalities were detected by standard karyotyping of GTG-banded metaphase chromosomes from patients' lymphocytes. To investigate the genomic DNA of patients with TAR syndrome for submicroscopic aberrations, we performed array CGH analysis, using a whole-genome tiling path BAC array.[18,19,23,24] Initially, we analyzed four patients with TAR syndrome and detected an interstitial deletion on chromosome 1q21.1 (fig. 3*A*). Array CGH data were submitted to the Gene Expression Omnibus (GEO) database (provisional series number GSE5781). According to the array CGH data, the two breakpoints were located between BAC clones RP11-458D21 and RP11-698N18 (proximal breakpoint) and RP11-258G05 and RP11-399E17 (distal breakpoint). The deletion was determined to be localized between positions 144.1 Mb and 144.6 Mb on chromosome 1 (Ensembl, v39, June 2006). The deletion encompasses 19 annotated genes and transcripts (fig. 4). The flanking BACs RP11-458D21 (proximal to deletion) and RP13-553C06 (distal to deletion) showed normal $\log_2$ ratios. Both breakpoints were found to be enriched for low-copy repeats, and, according to the Segmental Duplication Database, the deleted segment is flanked by duplication clusters with 93%–99% sequence identity. The proximal breakpoint coincided with published DNA copy-number polymorphisms.[25] The detected deletion was not present in 700 control samples with different phenotypes examined by one of us (R.U.) using the same array platform. Furthermore, a similar deletion is not described in the Database of Genomic Variants (version June 2006).

To confirm the array CGH data and to examine additional patients with TAR syndrome, we performed FISH analysis, using two BAC clones mapping to the deleted region at 1q21.1 (fig. 4). The FISH results confirmed an interstitial deletion of ~500 kb on chromosome 1q21.1 in all investigated patients with TAR syndrome. A single signal was detected on index patients' metaphases with BAC clone RP11-698N18 (located at the proximal deletion breakpoint) (fig. 3*B*), whereas the more distal BAC clone RP11-258G05 located at the distal deletion breakpoint showed two signals. However, one signal was always weaker, indicating that BAC clone RP11-258G05 spans the distal breakpoint (fig. 3C). Thus, the FISH results confirmed an interstitial deletion of ~500 kb on chromosome 1q21.1. In two families, deviating FISH signals were detected: patient 7 showed complete deletion of both BAC clones on one chromosome 1 (fig. 3*D*), whereas deletion of RP11-698N18 and two signals of equal intensity for the distal probe RP11-258G05 were observed in patient 20 (fig. 3*E*). These results demonstrate that the distal breakpoint is variable and that the common deletion is smaller in

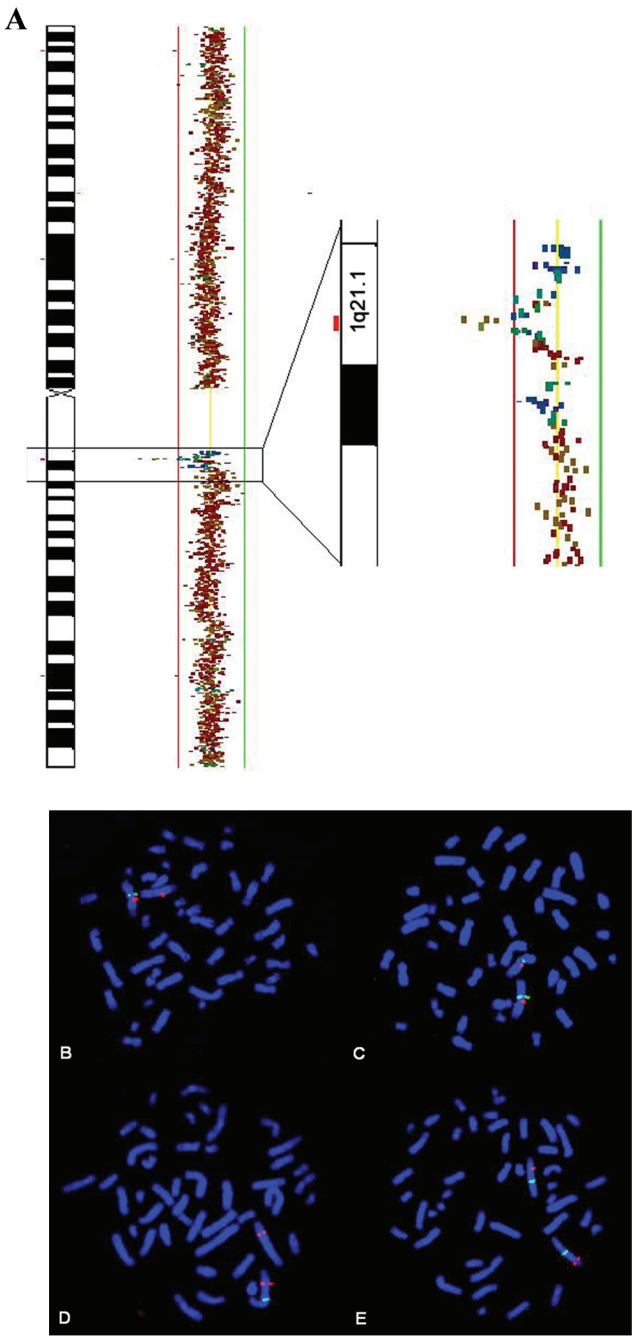

Figure 3. *A,* Array CGH profile of chromosome 1 (patient 1). Note the microdeletion in 1q21.1. *B–E,* Confirmation of the microdeletion by FISH. *B,* Deletion of RP11-698N18 (labeled with Spectrum green) and two normal signals of control probe CEP1 (labeled with Spectrum orange) (patient 4). *C,* Partial deletion of RP11-258G05 (labeled with Spectrum green) and two normal signals of control probe CEP1 (labeled with Spectrum orange) (patient 4). *D,* Deletion of RP11-258G05 (labeled with Spectrum green) and two normal signals of control probe RP11-5P4 located in chromosomal band 1p31.3 (labeled with Spectrum orange) (patient 7). *E,* Two normal signals of RP11-258G05 (labeled with Spectrum green) and of control probe RP11-5P4 (labeled with Spectrum orange) (patient 20).

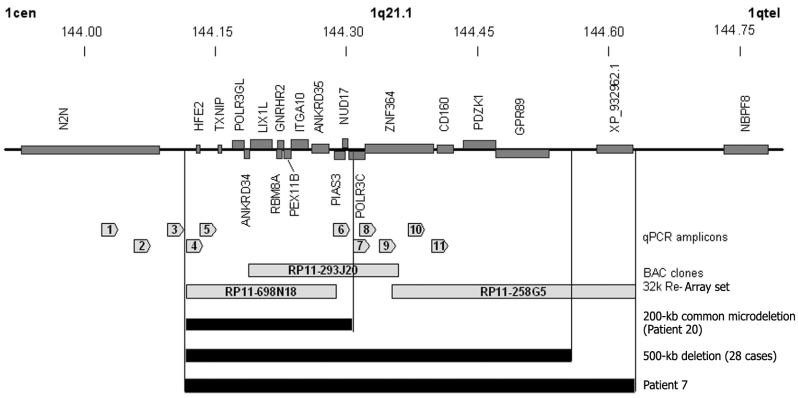

Figure 4. Schematic representation of the 1q21.1 genomic region. Positions of the genes, BAC clones, and qPCR amplicons are indicated. The extent of the microdeletions detected in patients with TAR syndrome are indicated by bars. The number of patients with the respective deletion is given on the right side.

patient 20 than in patient 7. Despite carrying the smallest deletion, patient 20 presents with a severe phenotype, including phocomelia and lower-limb involvement, indicating that the critical region for TAR syndrome is located within the region covered by the more proximally located BACs RP11-698N18 and RP11-293J20 (fig. 4). To further investigate the telomeric breakpoint, we performed a qPCR analysis of the deleted region in patient 20, which narrowed the breakpoint to a 2.5-kb region within the *POLR3C* gene between positions 144,305,643 and 144,308,167 bp on chromosome 1 (fig. 5*B*). In total, 20 patients with TAR syndrome and 59 unaffected family members were examined by FISH. Since metaphase preparations were not available for 31 individuals (10 patients with TAR syndrome and 21 unaffected relatives), we established a qPCR assay to analyze the 1q21.1 region. The deletion was detected by qPCR in an additional 10 of 10 patients with TAR syndrome and 6 of 21 relatives (fig. 5*A*). To rule out point mutations on the other nondeleted allele, we analyzed the coding sequences of 10 genes (*HFE2, LIX1L, PIAS3, ANKRD35, ITGA10, RBM8A, PEX11B, POLR3GL, TXNIP,* and *GNRR2*) located within the deletion in three patients with TAR syndrome. We detected no mutations but several polymorphisms that were also found in a control population and were not present in all the patients with TAR syndrome examined.

Inheritance of the Deletion

Taken together, the FISH and qPCR results revealed that the microdeletion was inherited from one of the unaffected parents in the majority of patients. We observed maternal inheritance for 12 patients, paternal inheritance for 5 patients, and de novo occurrence for 5 patients. In the remaining patients, the pattern of inheritance could not be investigated, because DNA samples from the parents were not available for examination. The deletion can be traced back to the generation of the grandparents in four families (families 2, 4, 7, and 8). In two instances, the microdeletion occurred de novo in one of the parents (one male and one female, in families 5 and 9, respectively). In several families, we were able to demonstrate that unaffected siblings and relatives had inherited the deletion. In the family with vertical transmission, the deletion was demonstrated in both the affected mother and the affected fetus.

Discussion

Here, we describe a common microdeletion in patients with TAR syndrome that encompasses 200 kb and 11 genes on chromosome 1q21.1. In 25% of the investigated patients, the deletion occurred de novo. In the other 75%, however, the deletion was inherited from either the unaffected mother or the unaffected father. The deletion is likely to be causative, because deletions and duplications in this genomic region were not observed in 700 other individuals and are also not described as DNA copy-number variants in the Database of Genomic Variants. Several genes with known functions are located within the deleted region (fig. 4). *PIAS3* is the most conspicuous candidate among the genes within the deleted region. It acts as a negative regulator of activated, phosphorylated Stat3, a transcription factor involved in hematopoietic growth-factor signaling.[26] Binding of thrombopoietin to its receptor, c-Mpl, results in activation of Jak2 kinase and distinct Stat family members, among which Stat3 is the most prominent.[27,28] Nevertheless, monoallelic expression of a negative regulator should result in stabilization of the activated Jak2/Stat3 pathway in response to thrombopoietin, which we did not observe in patients with TAR syndrome.[29] A simple model including *PIAS3* hemizygosity can therefore be excluded, although more-complex models including the PIAS3-binding protein Gfi-1 are feasible.[30] *Lix1L* is a second candidate gene, harboring com-

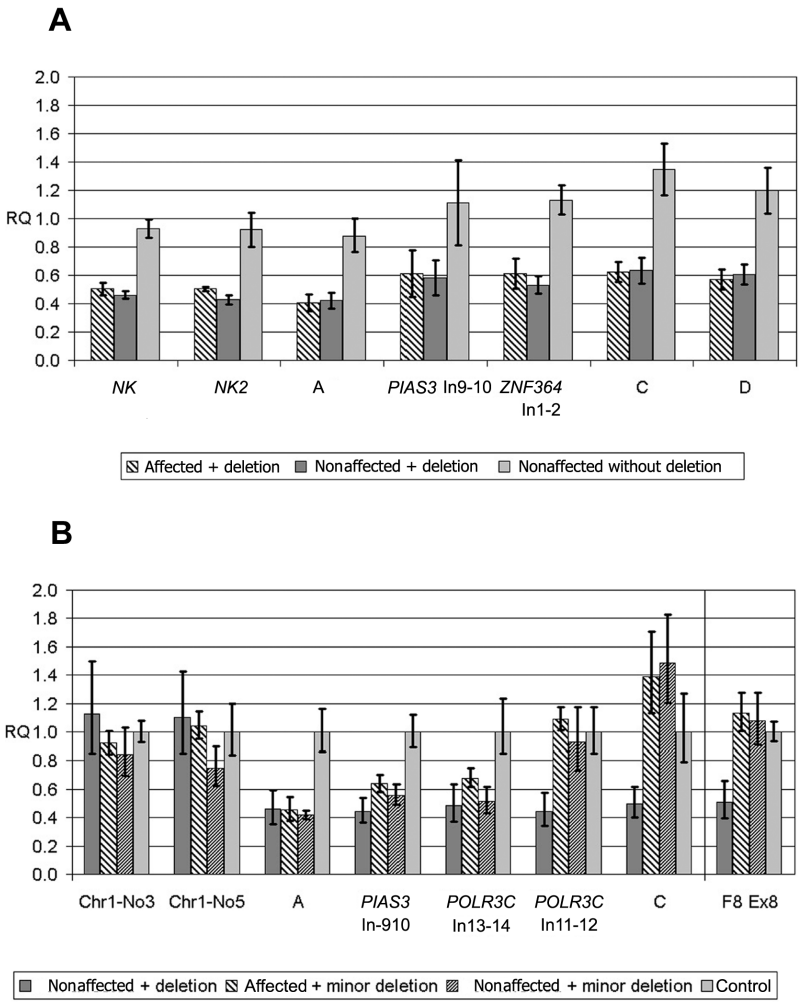

Figure 5. *A,* Copy-number analysis by qPCR. The mean values for relative quantification (RQ) were exported from the 7500 SDS software. For all three groups (affected with deletion, nonaffected with deletion, and nonaffected without deletion) mean values and SDs (*error bars*) for each target primer relative to *Albumin* as a two-copy reference gene were calculated. Results were calibrated to the mean value determined for a healthy female control. A, C, and D refer to primers shown in table 2 (hChr1-A, hChr1-C, and hChr1-D, respectively). *B,* For determination of the distal breakpoint, the two carriers of the small deletion (patient 20 and nonaffected mother) were compared with the same control as in panel A and one nonaffected male deletion carrier. The breakpoint of the smaller deletion maps to the region between introns 11–12 and introns 13–14 of *POLR3C* (~2.5 kb). Two target sequences located proximal to the deletion (Chr1-No3 and Chr1-No5) displayed normal copy numbers in all samples. The error bars indicate the 95% CI as calculated by the 7500 SDS software. F8 = factor VIII gene.

putational sequence homology to *Lix1,* a gene reported to be transiently expressed during chick hind-limb development.[31] None of the other genes within the deleted interval has a known function in limb development or hematopoiesis.

Our finding that carriers of the microdeletion (i.e., clinically inconspicuous parents and grandparents) are not affected implies that haploinsufficiency of the deleted region is not sufficient to cause TAR syndrome or, for that matter, any obvious signs or symptoms in the carrier. The possibility of a deletion on one allele and a point mutation or small deletion on the other allele cannot be excluded. However, the absence of any significant change in the genes located within the deletion on the nondeleted allele argues against this possibility. Furthermore, the occurrence of vertical transmission—one case described here and additional cases in the literature[8]—argues in favor of other models of inheritance. Thus, TAR syndrome does not appear to follow a standard autosomal dominant or autosomal recessive pattern of inheritance. The possibility of genomic imprinting as an underlying mechanism can be excluded, since we detected maternal (12/22) as well as paternal (5/22) transmission of the deletion among the unaffected carrier parents. In addition, reports of maternal

as well as paternal uniparental disomy of chromosome 1 describe no severe phenotypes, no malformations, and no hematopoietic abnormalities in affected individuals.[32–34]

Another explanation for the unusual pattern of inheritance observed is random monoallelic expression caused by methylation of one allele, as described elsewhere for association with Paris-Trousseau/Jacobsen thrombocytopenia.[35] To a certain degree, this mechanism can be regarded as an autosomal analogue to X inactivation resulting in a mosaic state. Mutations in one allele further reduce the gene dosage to a disease-causing level. Since the inactivation is random, the differences between affected and unaffected carriers of the mutation can be explained only by stochastic effects or by alterations in a second gene that controls the methylation in this region.

The idea of multiple allelism in TAR syndrome was raised by Hall.[1] Autosomal recessive inheritance on the basis of two nonidentical mutant alleles was discussed as one option. Digenic inheritance must also be considered, and it implies that mutations (or, in this case, at least one microdeletion) in each of two unlinked loci have to be present in an affected individual, and only this combination of the two genetic hits—that is, double heterozygosity—causes the disease phenotype or possibly affects the severity of the phenotype. Carriers with alterations at only one of the two loci do not show the disease phenotype. Recently described examples for digenic inheritance in human disease are retinitis pigmentosa[36] and Bardet-Biedl syndrome.[37] Two distinct categories have been specified[38]: (1) two mutations act in a synergistic fashion with multiplicative effect on the phenotype or (2) both mutations have additive effects, with the second mutation acting as a modifier to increase the severity of the phenotype.

In TAR syndrome, this would involve two genetic changes: one rare mutation (the microdeletion) and one relatively frequent change or polymorphism that functions in this situation as a modifier of the other. The modifier theory has been studied in detail in the context of the dactylaplasia mutation (*Dac*) in mice, the murine equivalent to human ectrodactyly. Affected patients and *Dac* mice have hypoplasia of the middle part of the distal limbs, a phenotype that is extremely variable, ranging from syndactyly to monodactyly. Because *Dac* mice exhibit the ectrodactyly phenotype only on certain genetic backgrounds, it was postulated that the manifestation of the mutant gene *Dac* is controlled by another locus, which was termed "*mdac*."[39] The mutation is expressed as an autosomal semidominant trait only if the *mdac* allele is homozygous. This hypothesis was verified by the identification of the *Dac* mutation[40] and the mapping of *mdac* to mouse chromosome 13.[41]

A similar situation may exist in TAR syndrome. The observed microdeletion is the prerequisite for the phenotype. This deletion mutation is rare and predisposes an individual to TAR syndrome, but the presence of an additional modifier is required to elicit the phenotype. The modifier (mTAR) is likely to be a relatively common polymorphism, as indicated by the ratio of affected to unaffected deletion carriers. mTAR could be inherited in a dominant or recessive fashion, with both possibilities resulting in the observed recurrence risk of ~25%. Sporadic cases arise if the deletion occurs de novo and if the modifier mTAR is inherited from one parent (dominant) or both parents (recessive). If one of the parents carries the deletion, it will be transmitted to 50% of the children, but only those who have also inherited mTAR will develop TAR syndrome. Likewise, a parent-to-child transmission is possible if the child inherits the deletion from the affected parent along with mTAR from either or both parents. Although one would expect TAR syndrome in 25% of the offspring in the case of a dominant modifier, parent-to-child transmission of TAR syndrome is rare. Thus, a model with one or more recessively acting modifiers appears more likely. We therefore propose that TAR syndrome be considered not a single-gene disease but a complex trait requiring at least two unlinked alleles—one rare, the other frequent—to manifest the phenotype.

Acknowledgments

We thank all patients and their families for participating in this study. The excellent technical assistance of Gundula Leschik is acknowledged. We thank Ines Krause-Plonka, Sigrid Tinschert, and Dietmar Müller for arranging contact with the patients with TAR syndrome and their families. Thanks to Pieter de Jong and the BACPAC Resources Center, for providing the DNA of the human 32K Re-Array set; the COSTB19 initiative, for the clones of the subtelomeric set; Nigel Carter and the Mapping Core and Map Finishing groups of the Wellcome Trust Sanger Institute, for initial clone supply and verification of the 1-Mb clone set; Claus Hultschig, for spotting the arrays; and Wei Chen, for bioinformatics support. H.S. and S.F. have been supported by a grant from the Deutsche Forschungsgemeinschaft (SCHU 1421/3-1).

Web Resources

The URLs for data presented herein are as follows:

BACPAC Resources, http://bacpac.chori.org/pHumanMinSet.htm
Database of Genomic Variants, http://projects.tcag.ca/variation/
Ensembl, http://www.ensembl.org/
Gene Expression Omnibus (GEO), http://www.ncbi.nlm.nih.gov/geo/
Max Planck Institute for Molecular Genetics: Molecular Cytogenetics Group, http://www.molgen.mpg.de/~abt_rop/molecular_cytogenetics/Protocols.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for TAR syndrome, Holt-Oram syndrome, Roberts syndrome, and Fanconi anemia)
Segmental Duplication Database, http://humanparalogy.gs.washington.edu/

References

1. Hall J, Levin J, Kuhn J, Ottenheimer E, Berkum Kv, McKusick V (1969) Thrombocytopenia with absent radius (TAR). Medicine 48:411–439

2. Geddis A (2006) Inherited thrombocytopenia: congenital amegakaryocytic thrombocytopenia and thrombocytopenia with absent radii. Semin Hematol 43:196–203
3. Ballmaier M, Schulze H, Strauß G, Cherkaoui K, Wittner N, Lynen S, Wolters S, Bogenberger J, Welte K (1997) Thrombopoietin in patients with congenital thrombocytopenia and absent radii: elevated serum levels, normal receptor expression, but defective reactivity to thrombopoietin. Blood 90: 612–619
4. Letestu R, Vitrat N, Massé A, Couedic J-PL, Lazar V, Rameau P, Wndling F, Vuillier J, Boutard P, Plouvier E, et al (2000) Existence of a differentiation blockage at the stage of a megakaryocyte precursor in the thrombocytopenia and absent radii (TAR) syndrome. Blood 95:1633–1641
5. Greenhalgh K, Howell R, Bottani A, Ancliff P, Brunner H, Verschuuren-Bemelmans C, Vernon E, Brown K, Newbury-Ecob R (2002) Thrombocytopenia-absent radius syndrome: a clinical genetic study. J Med Genet 39:876–881
6. Schnur R, Eunpu D, Zackai E (1987) Thrombocytopenia with absent radius in a boy and his uncle. Am J Med Genet 28: 117–123
7. Hall J (1987) Thrombocytopenia and absent radius (TAR). J Med Genet 24:79–83
8. Ward R, Bixler D, Provisor A, Bader P (1986) Parent to child transmission of the thrombocytopenia absent radius (TAR) syndrome. Am J Med Genet Suppl 2:207–214
9. Beales P (2005) Lifting the lid on Pandora's box: the Bardet-Biedl syndrome. Curr Opin Genet Dev 15:315–353
10. Vega H, Waisfisz Q, Gordillo M, Sakai N, Yanagihara I, Yamada M, von Gosliga D, Kayserili H, Xu C, Ozono K, et al (2005) Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion. Nat Genet 37:468–470
11. Fleischman R, Letestu R, Mi X, Stevens D, Winters J, Debili N, Vainchenker W (2002) Absence of mutations in the HoxA10, HoxA11 and HoxD11 nucleotide coding sequences in thrombocytopenia with absent radius syndrome. Br J Haematol 116:367–375
12. Strippoli P, Saoia A, Iolascon A, Tonelli R, Savino M, Giordano P, D'Avanzo M, Massolo F, Locatelli F, Borgna C, et al (1998) Mutational screening of thrombopoietin receptor gene (c-mpl) in patients with congenital thrombocytopenia and absent radii. Br J Haematol 103:311–314
13. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, et al (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20: 207–211
14. Solinas-Toldo A, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Döhner H, Cremer T, Lichter P (1997) Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 20:399–407
15. Urban M, Opitz C, Bommer C, Enders H, Tinschert S, Witkowski R (1998) Bilaterally cleft lip, limb defects, and haematological manifestations: Roberts syndrome versus TAR syndrome. Am J Med Genet 79:155–160
16. Seemanova E, Spicakova V, Kulovany E, Zwinger A, Veprek P, Salichova J (1985) TAR syndrome in three unrelated children. Cesk Pediatr 40:511–514
17. Osoegawa K, Mammoser AG, Wu C, Frengen E, Zeng C, Catanese JJ, de Jong PJ (2001) A bacterial artificial chromosome library for sequencing the complete human genome. Genome Res 11:483–496
18. Krzywinski M, Bosdet I, Smailus D, Chiu R, Mathewson C, Wye N, Barber S, Brown-John M, Chan S, Chand S, et al (2004) A set of BAC clones spanning the human genome. Nucleic Acids Res 32:3651–3660
19. Ishkanian A, Malloff C, Watson S, DeLeeuw RJ, Chi B, Coe B, Snijders A, Albertson D, Pinkel D, Marra M, et al (2004) A tiling resolution DNA microarray with complete coverage of the human genome. Nat Genet 36:299–303
20. Fiegler H, Carr P, Douglas EJ, Burford DC, Hunt S, Scott CE, Smith J, Vetrie D, Gorman P, Tomlinson IP, et al (2003) DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. Genes Chromosomes Cancer 36:361–374
21. Chen W, Erdogan F, Ropers H, Lenzner S, Ullmann R (2005) CGHPRO—a comprehensive data analysis tool for array CGH. BMC Bioinformatics 6:85
22. Klopocki E, Fiebig B, Robinson P, Tönnies H, Erdogan F, Ropers H, Mundlos S, Ullmann R (2006) A novel 8 Mb interstitial deletion of chromosome 8p12-p21.2. Am J Med Genet A 140: 873–877
23. Erdogan F, Chen W, Kirchhoff M, Kalscheuer V, Hultschig C, Müller I, Schulz R, Menzel C, Bryndorf T, Ropers H, et al (2006) Impact of low copy repeats on the generation of balanced and unbalanced chromosomal aberrations in mental retardation. Cytogenet Genome Res 115:247–253
24. Fiegler H, Carr P, Douglas EJ, Burford DC, Hunt S, Scott CE, Smith J, Vetrie D, Gorman P, Tomlinson IP, et al (2003) DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. Genes Chromosomes Cancer 36:361–374
25. Tuzun E, Sharp A, Bailey J, Kaul R, Morrison V, Pertz L, Haugen E, Hayden H, Albertson D, Pinkel D, et al (2005) Fine-scale structural variation of the human genome. Nat Genet 37: 727–732
26. Ihle J (2001) The Stat family in cytokine signaling. Curr Opin Cell Biol 13:211–217
27. Ezumi Y, Takayama H, Okuma M (1995) Thrombopoietin, c-Mpl ligand, induces tyrosine phosphorylation of Tyk2, JAK2, and STAT3, and enhances agonists-induced aggregation in platelets in vitro. FEBS Lett 374:48–52
28. Schulze H, Ballmaier M, Welte K, Germeshausen M (2000) Thrombopoietin induces the generation of distinct Stat1, Stat3, Stat5a and Stat5b homo- and heterodimeric complexes with different kinetics in human platelets. Exp Hematol 28: 294–304
29. Ballmaier M, Schulze H, Cremer M, Folman C, Strauss G, Welte K (1998) Defective c-Mpl signaling in the syndrome of the thrombocytopenia with absent radii. Stem Cells 16:177–184
30. Rodel B, Tavassoli K, Karsunky H, Schmidt T, Bachmann M, Schaper F, Heinrich P, Shuai K, Elsasser HP, Moroy T (2000) The zinc finger protein Gfi-1 can enhance STAT3 signaling by interacting with the STAT3 inhibitor PIAS3. EMBO J 19: 5845–5855
31. Swindell EC, Moeller C, Thaller C, Eichele G (2001) Cloning and expression analysis of chicken Lix1, a founding member of a novel gene family. Mech Dev 109:405–408
32. Miura Y, Hiura M, Torigoe K, Numata O, Kuwahara A, Matsunaga M, Hasegawa S, Boku N, Ino H, Mardy S, et al (2000) Complete paternal uniparental isodisomy for chromosome 1

revealed by mutation analyses of the TRKA (NTRK1) gene encoding a receptor tyrosine kinase for nerve growth factor in a patient with congenital insensitivity to pain with anhidrosis. Hum Genet 107:205–209
33. Wassink T, Losh M, Frantz R, Vieland V, Goedken R, Piven J, Sheffield V (2005) A case of autism and uniparental disomy of chromosome 1. Hum Genet 117:200–206
34. Zeng W, Gao H, Brueton L, Hutchin T, Gray G, Chakrapani A, Olpin S, Shih V (2006) Fumarase deficiency caused by homozygous P131R mutation and paternal partial isodisomy of chromosome 1. Am J Med Genet A 140:1004–1009
35. Raslova H, Komura E, Couédic JL, Larbret F, Debili N, Feunteun J, Danos O, Albagli O, Vainchenker W, Favier R (2004) *FLI1* monoallelic expression combined with its hemizygous loss underlies Paris-Trousseau/Jacobsen thrombopenia. J Clin Invest 114:77–84
36. Kajiwara K, Berson E, Dryja T (1994) Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 264:1604–1608
37. Katsanis N, Ansley S, Badano J, Eichers E, Lewis R, Hoskins B, Scambler P, Davidson W, Beales P, Lupski J (2001) Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. Science 293:2213–2214
38. Ming J, Muenke M (2002) Multiple hits during early embryonic development: digenic diseases and holoprosencephaly. Am J Hum Genet 71:1017–1032
39. Chai C (1981) Dactylaplasia in mice. J Hered 72:234–237
40. Sidow A, Bulotsky M, Kerrebrock A, Birren B, Altshuler D, Jaenisch R, Johnson K, Lander E (1999) A novel member of the F-box/WD40 gene family, encoding dactylin, is disrupted in the mouse dactylaplasia mutant. Nat Genet 23:104–107
41. Johnson K, Lane P, Ward-Bailey P, Davisson M (1995) Mapping the mouse dactylaplasia mutation, Dac, and a gene that controls its expression mdac. Genomics 29:457–464