1q21.1 deletion and a rare functional polymorphism in siblings with thrombocytopenia-absent radius–like phenotypes

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Abstract Thrombocytopenia-absent radii (TAR) syndrome, characterized by neonatal thrombocytopenia and bilateral radial aplasia with thumbs present, is typically caused by the inheritance of a 1q21.1 deletion and a single-nucleotide polymorphism in *RBM8A* on the nondeleted allele. We evaluated two siblings with TAR-like dysmorphology but lacking thrombocytopenia in infancy. Family NCI-107 participated in an IRB-approved cohort study and underwent comprehensive clinical and genomic evaluations, including aCGH, whole-exome, whole-genome, and targeted sequencing. Gene expression assays and electromobility shift assays (EMSAs) were performed to evaluate the variant of interest. The previously identified TAR-associated 1q21.1 deletion was present in the affected siblings and one healthy parent. Multiple sequencing approaches did not identify previously described TAR-associated SNPs or mutations in relevant genes. We discovered rs61746197 A > G heterozygosity in the parent without the deletion and apparent hemizygosity in both siblings. rs61746197 A > G overlaps a RelA–p65 binding motif, and EMSAs indicate the A allele has higher transcription factor binding efficiency than the G allele. Stimulation of K562 cells to induce megakaryocyte differentiation abrogated the shift of both reference and alternative probes. The 1q21.1 TAR-associated deletion in combination with the G variant of rs61746197 on the nondeleted allele is associated with a TAR-like phenotype. rs61746197 G could be a functional enhancer/repressor element, but more studies are required to identify the specific factor(s) responsible. Overall, our findings suggest a role of rs61746197 A > G and human disease in the setting of a 1q21.1 deletion on the other chromosome.

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INTRODUCTION

Thrombocytopenia-absent radius (TAR) syndrome (MIM#274000) is a rare inherited disorder characterized by neonatal thrombocytopenia and bilateral radial aplasia with thumbs present. The arm and hand anomalies in TAR are distinctly different than Fanconi anemia in which thumbs are absent or malformed (Hall et al. 1969; Toriello 1993). Congenital heart

disease has been reported in ~10% of individuals with TAR and structural renal anomalies in ~7% (Greenhalgh et al. 2002; Ahmad and Pope 2008). Additionally, bloody diarrhea or enteritis associated with cow's milk intolerance has been reported in ~20% of cases (Whitfield and Barr 1976; Toriello 1993). The thrombocytopenia in infancy typically improves within the first 1–2 years of life, although platelet counts may not reach normal levels (Manukjan et al. 2017). Notably, the degree of thrombocytopenia in TAR varies, and some patients have normal or near-normal platelet counts throughout life (Manukjan et al. 2017).

TAR is caused by biallelic autosomal recessive inheritance of a deletion at Chromosome 1q21.1 from one healthy parent and a single-nucleotide polymorphism (SNP) in the 5' untranslated region (UTR) (rs139428292 G > A) or intron 1 (rs201779890 G > C) of the RNA binding motif protein 8A gene (*RBM8A*) encoding the Y14 protein on the nondeleted 1q21.1 allele from the other parent (Klopocki et al. 2007; Houeijeh et al. 2011; Albers et al. 2012). Inheritance of two pathogenic variants in *RBM8A* has also been reported to cause TAR (Albers et al. 2012). The combination of the 1q21.1 deletion and the 5' UTR SNP (rs139428292) is associated with *RBM8A* haploinsufficiency and a classic TAR phenotype, including neonatal thrombocytopenia (Albers et al. 2012; Manukjan et al. 2017). In contrast, patients with the intron 1 SNP (rs201779890) and the 1q21.1 deletion were reported to have had normal or nearly normal platelet counts (Manukjan et al. 2017).

Chromosome 1q21.1 deletions and duplications are associated with phenotypes ranging from intellectual disability, autism, congenital heart defects, renal and urinary abnormalities, and dysmorphic faces in addition to TAR (Rosenfeld et al. 2012). The minimally deleted region of 1q21.1 associated with TAR is ~200 kilobases (kb) and not reported to overlap with deleted regions in other disorders. Mapping of 1q21.1–q21.2 has been challenging because of its highly repetitive nature, and the sequence was only considered "solved" in 2014 (O'Bleness et al. 2014).

In this study, we evaluated siblings with bony abnormalities and other clinical features consistent with TAR but without the typical TAR-associated thrombocytopenia of infancy. A series of genomic evaluations resulted in the fine mapping of the 1q21.1 deletion in these individuals and discovered a novel functional SNP (rs61746197) on 1q21.1 associated with their phenotypes.

RESULTS

Clinical Presentations

The clinical features of the patients are shown in Table 1 and Figure 1. The proband, NCI-107-1, had bilaterally absent radii, other bony abnormalities, and congenital heart disease but was not noted to have thrombocytopenia until about age 12 yr, despite having had several surgeries. TAR was not suspected in his older sister (NCI-107-2) until it was considered in the differential diagnosis for the proband. The sister's extremity abnormalities, congenital bilateral radial head dislocation, a slightly hypoplastic right radial head, and proximal ulnar hypoplasia were initially thought to be a result of in utero oligohydramnios. She had no history of thrombocytopenia. Both parents were evaluated and were healthy with no dysmorphism or medical problems. There was no other significant family history, and the family was of Caucasian ancestry.

Genomic Characterization

Both siblings and one unaffected parent carry a deletion at 1q21.1 (Chr 1:145,601,946–148,597,425, hg38) identified by research and confirmed by clinical aCGH testing. Targeted sequencing of the deleted region identified the common alleles of the previously

Table 1. Hematologic and nonhematological characteristics of study participants

Participant, sex	Age at study	Hematology				Nonhematological features
		Age (years) at test	WBC (/mCL)	Hb (g/dL)	Plt ($\times 10^9/L$)	
NCI-107-1, male	14 yr	0.2	19,200	10.8	222	Born at term with bilateral absent radii and hypoplastic ulnae, right thumb and right thenar eminence hypoplasia; bilateral proximal tibial deformities (Blount's disease); bilateral hip dysplasia; craniofacial dysmorphism with high prominent forehead and receding hairline, flat nasal bridge with bulbous tip, low-set ears, micrognathia, hypertelorism, and down-slanting eyes; atrial septal defect and cleft mitral valve. Height was 15th percentile for age.
		12	5730	12.8	116	
		14	5890	14.7	147	
NCI-107-2, female	22 yr	Birth	11,000	14	NR	Born preterm at 31-wk gestation with oligohydramnios and single umbilical artery. Flexion deformities of both elbows and bilateral radial head dislocation, hypoplastic left radius with bony fusion of radial head with distal humerus; duplicated collecting system in the right kidney; numerous small hemangiomas; hydrocephalus; craniofacial dysmorphism with high forehead and receding hairline, synophris, hypertelorism, high-arched palate, microsomia. No history of thrombocytopenia. Height was ninth percentile for age.
		3.8	9100	13.6	172	
		13	5100	14.2	169	
		22	4560	13.7	183	

See also Figure 1.

(WBC) White blood cell count, (Hb) hemoglobin, (Plt) platelets, (L) liter, (NR) not reported.

identified TAR-associated SNPs (rs139428292 and rs201779890) in all four family members and did not identify additional variants of interest. Whole-exome sequencing did not identify variants in genes associated with similar phenotypes such as *MECOM* (Niihori et al. 2015), *HOXA11* (Thompson and Nguyen 2000), *TBX5* (McDermott et al. 1993), or Fanconi anemia (Savage and Walsh 2018). There were three homozygous variants shared by both siblings, Chr 1:145515394 A > G (rs61746197, hemizygous because of 1q21.1 deletion), Chr 6:130504476 C > T, and Chr 12:52695897 G > A (rs35018791) aligned to hg19 (Table 2). rs35018791 is a missense variant in keratin 86 and is in a highly polymorphic region of the genome with many pseudogenes. The Chr 6:130504476 C > T variant has not been previously reported but is located in an intron of *SAMD3* (sterile α motif domain containing 3) and not bioinformatically predicted to be deleterious.

rs61746197 A > G is in the 3' UTR of *RBM8A* but was not located in the 1q21.1 deleted region in human genome build hg19. Using the UCSC Genome Browser liftover tool, we found that rs61746197A > G is located in the deletion in build hg38. Whole-genome sequencing of NCI-107-2 with alignment to hg38 confirmed that rs61746197 A > G is in the deleted region of 1q21.1 (Table 2). Sanger sequencing confirmed that both siblings carried only the variant (G) allele, and the parent without deleted 1q21.1 was heterozygous A/G at this location. The minor allele frequency (MAF) of rs61746197 A > G in the gnomAD database is 0.5% (997/183,070). Table 3 compares the MAF of this 3' UTR *RBM8A* SNP with that of the 5' UTR and intron 1 SNPs.

The rs61746197 Variant Allele Disrupts Transcription Factor Binding

rs61746197 A > G is a noncoding variant located 1861 base pairs 3' of *RBM8A* and predicted to overlap with three transcripts of the *GNRHR2* pseudogene and the promoter of *PEX11B* (Fig. 2). We first evaluated whether rs61746197 A > G was associated with changes in expression of nearby genes *LIX1L*, *PEX11B*, and/or *RBM8A* by performing qPCR of RNA from patient-derived primary fibroblast cultures and/or EBV-transformed lymphoblastoid cell lines.

Figure 1. Skeletal features of study participants. (A–C) Proband, NCI-107-1. (A) Absent left radius and hypoplastic left ulna; (B) absent right radius and short right ulna; status post-multiple corrective surgeries for right thumb hypoplasia including tendon transfer and centralization; (C) normal right humerus. (D,E) Sister, NCI-107-2. (D) Wide left radius with mild hypoplasia and bony fusion of the proximal radial head to the distal humerus at the left elbow; (E) the right radius and ulna are normal; (D,E) both hands are normal with complete skeletal maturation.

There were no significant differences in the expression of *RMB8A*, *PEX11B*, or *LIX1L* between the individuals with both the 1q21.1 deletion and rs61746197 G compared with those carrying one or the other (Supplemental Fig. 1).

We hypothesized that the rs61746197 variant allele (G) could possibly alter TFBS and used TRANSFAC to query the region surrounding the SNP (Matys et al. 2006). According to the TRANSFAC database, rs61746197 A > G overlaps a putative RelA–p65 binding motif, a component of the NF- κ B transcription factor complex (Fig. 2). We also examined the ENSEMBL database to determine additional TFBSs overlapping with the SNP. TFBS motifs in Ensembl with high information content under the SNP include PBX4::HOXA10, CUX1, SOX9, and others (Table 4). Given the known roles of several of these factors in hematological development (Stein and Baldwin 2013) and/or their importance in bone remodeling and development (Wu et al. 2007), we proceeded to test whether the rs61746197 variant allele (G) disrupts transcription factor binding in vitro by EMSA.

EMSA probes containing the rs61746197 reference (A) or the alternative allele (G) were incubated with nuclear extracts of K562 cells (Fig. 3A). Unstimulated K562 extract produced a bandshift on both the reference (A) and alternative (G) probe. This shift was competed away

Table 2. Coordinates by genome build of 1q21.1 deletion and SNPs of interest

	Chr	hg18 start	hg18 stop	hg19 start	hg19 stop	hg38 start	hg38 stop
Brother aCGH	1	144,003,055	144,967,596	146,950,065	147,253,662	145,601,946	148,597,425
Sister aCGH	1	144,003,055	144,967,596	146,919,485	147,253,662	145,601,946	148,597,425
rs61746197	1	144,227,001	144,227,001	145,919,695	145,919,695	145,919,695	145,919,695
RelA-p65	1			145,515,380	145,515,409	145,919,688	145,919,698
Sister whole-genome seq	1					145,370,001	146,190,000
TAR common deleted region ^a	1	144,215,938	144,320,529	145,504,581	145,609,172	145,825,941	145,930,512
<i>RBM8A</i>	1	144,218,995	144,422,801	145,507,557	145,513,535	145,921,556	145,927,536
rs139428292 ^b	1	Not in hg18	Not in hg18	145,927,447	145,927,447	145,927,447	145,927,447
rs201779890 ^b	1	Not in hg18	Not in hg18	145,927,328	145,927,328	145,927,328	145,927,328

hg38 coordinates are based on alignments of patient whole-genome sequence data to the hg38 (GRCh38.p7) reference sequence. aCGH was performed with probes for hg18. Liftover of Chromosome 1q21.1 coordinates was performed with default settings of the UCSC genome browser liftover tool with the exception of the aCGH liftover from hg18 to hg38 in which there was limited alignment because of repetitive sequence. The RelA-p65 binding site, rs139428292, and rs201779890 were not present in hg18. *Italics* indicates build in which the region or variant was first identified. SNP locations in hg38 are based upon dbSNP build 151.

(Chr) Chromosome, (SNP) single-nucleotide polymorphism, (cCGH) array comparative genomic hybridization, (seq) sequencing, (TAR) thrombocytopenia-absent radii syndrome.

^aRegion reported by Klopocki et al. (2007).

^bSNPs previously associated with TAR.

with equimolar quantities of cold reference (A) probe but required threefold excess cold alternative (G) probe to perturb the shift characteristics. These differences indicate that transcription factors bind to the reference allele (A) with a higher binding efficiency than the alternative (G) allele. Interestingly, stimulation of the K562 cells with TPA to induce the megakaryocyte differentiation pathway abrogated the observed shift of both the reference and alternative probes. Because RelA-p65 is a component of the NF- κ B transcription factor complex, we evaluated whether the addition of anti-NF- κ B-p65 antibody changed binding between the probe and nuclear factor(s). Addition of anti-NF- κ B-p65 antibody disrupted the bandshift but did not result in a super-shift (Fig. 3B), indicating that RelA is likely binding to the locus, but the antibody blocks this interaction.

DISCUSSION

Our data show that the 1q21.1 TAR-associated deletion in combination with the G variant of rs61746197 on the nondeleted chromosome results in a TAR-like phenotype notable for lack of thrombocytopenia but with other clinical features of TAR. This SNP appears to be

Table 3. Frequency of TAR-associated SNPs in the literature, ClinVar, and gnomAD

SNP	SNP location in relation to <i>RBM8A</i>	Number of reported cases	Number of ClinVar submissions	Allele count/allele number, MAF in all of gnomAD
rs61746197 A > G	3' UTR	2 (the siblings in this report)	None	997/183,070, 0.005446
rs139428292 G > A	5' UTR	24/38 cases reported and summarized by Manukjan et al. (2017)	9	4831/269,236, 0.01794
rs201779890 G > C	Intron 1	14/38 cases reported and summarized by Manukjan et al. (2017)	8	1515/265,508, 0.005706

References: ClinVar (Landrum and Kattman 2018), <https://www.ncbi.nlm.nih.gov/clinvar/>; gnomAD (Karczewski et al. 2019), <https://gnomad.broadinstitute.org>.

associated with a milder hematologic presentation as the proband developed very mild thrombocytopenia at age 12 yr and thrombocytopenia has not occurred in his sister. The upper limb bony abnormalities in these siblings are consistent with other reported TAR cases (Toriello 1993). About one-third of TAR cases have been noted to also have hypoplasia of the ulnae and/or humerus (Houeijeh et al. 2011). Similar to as noted in the sister, unilateral radial aplasia or hypoplasia has been reported in six literature cases (Houeijeh et al. 2011). Both siblings had other congenital anomalies sometimes reported in TAR, including heart and renal abnormalities.

To functionally characterize the potential role of rs61746197 A > G in the clinical manifestations in these siblings, we first evaluated the expression of genes in the region near the

Table 4. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect (substitution, deletion, etc.)	dbSNP/dbVar ID	Genotype (heterozygous/homozygous)
PEX11B: 2KB upstream variant; GNRHR2: noncoding transcript variant; RBM8A (3' UTR)	1	NC_000001.11: g.145919695T > C GRCh38.p12 Chr 1	N/A	SNV	Substitution	rs61746197	A > G heterozygous
Deletion; Chr 1: 145,601,946–148,597,425	1	Chr 1:145,601,946–148,597,425 GRCh38.p12 Chr 1	N/A	Deletion	Deletion	N/A	Heterozygous deletion

Figure 3. Electromobility shift assays (EMSAs) suggest that rs61746197 A > G is a functional SNP. (A) Ten micrograms of unstimulated nuclear extract from K562 cells was incubated with reference (Ref, R) or alternative (Alt, A) probe as indicated. Cold Ref probe titrates signal away from Alt at a lower stoichiometric ratio than the converse (arrow and compare lanes 11–13 with 6–8). (B) Unstimulated extract (left panel, U) exhibits a markedly stronger baseline shift than TPA stimulated extract (right panel, S). Unstimulated nuclear extract (U) when incubated with labeled alternative probe (A) produces a novel secondary shift not observed with either the reference probe (R) or the TPA stimulated extract (S). Addition of RelA–p65 antibody abrogates this secondary shift (arrow).

SNP and in the deleted locus. However, we detected no differences in the expression of *RMB8A*, *PEX11B*, or *LIX1L* genes in cell lines derived from the siblings reported here or their parents. Notably, we also did not detect significant differences in *LIX1L*, *PEX11B*, and/or *RMB8A* expression for 5' UTR rs139428292G > A carriers with versus those without the 1q21.1 deletion. The 5' UTR SNP (rs139428292G > A) is a predicted expression quantitative trait locus (eQTL) in many tissues (Supplemental Fig. 1), and reports of TAR patients with this SNP and a 1q21.1 deletion suggest reduced *RMB8A* expression, in addition to classic TAR clinical phenotypes. In contrast, the intron 1TAR-associated SNP (rs201779890 G > C) and this new 3' UTR SNP (rs61746197 A > G) are not predicted eQTLs in GTEx (GTEx Consortium 2013), have similar minor allele frequencies (Table 3), and are reported

in patients with physical manifestations of TAR but lacking the typical neonatal thrombocytopenia.

We hypothesized that the role of the regulatory element(s) containing rs61746197 A > G is important in other tissue types, in specific stages of development, and/or in earlier stages of hematopoiesis. To further explore this hypothesis, we performed EMSAs and found that the shift observed on the alternative (G) allele could be disrupted by incubation with NF- κ B-p65(RelA) antibody. NF- κ B-p65(RelA) is one of the factors predicted to bind to the locus, and its core binding site lies over rs61746197 A > G. We also observed that only unstimulated and not TPA-stimulated nuclear extracts of K562 cells contained sufficient levels of the factor that induce the mobility shift. Previous reports indicate that RelA translocates into the nucleus upon TPA treatment in HeLa cells, but not in K562 cell lines (Hansen et al. 1994). Taken together, these results suggest the G variant of rs61746197 A > G could be a functional enhancer/repressor element in certain cell types and at particular stages of development.

In summary, the clinical phenotypes of these patients and EMSA data suggest an association between hemizygosity for rs61746197 A > G and absent radius syndrome, a variant of thrombocytopenia-absent radius syndrome. The 1q21 chromosomal locus remains challenging to annotate and multiple sequencing approaches may be required to identify novel disease-associated variants. Additional studies are warranted to identify the specific factor(s) responsible and their timing in relation to hematopoiesis and development.

METHODS

Study Participants

Individuals with TAR and their families were participants in the IRB-approved longitudinal cohort study at the National Cancer Institute (NCI) entitled “Etiologic Investigation of Cancer Susceptibility in Inherited Bone Marrow Failure Syndromes (IBMFS)” (ClinicalTrials.gov Identifier: NCT00027274, <https://marrowfailure.cancer.gov>) (Alter et al. 2018). Participants completed comprehensive family and individual history questionnaires. Medical records were reviewed in detail, and biospecimens were collected. The individuals reported here, family NCI-107, also underwent comprehensive clinical evaluations by the IBMFS study team at the National Institutes of Health Clinical Center, Bethesda, MD. DNA was extracted from whole blood by standard methods.

Genomic Characterization

The genomic characterization of family NCI-107 started in 2007 and proceeded over many years as methods for detecting variants in the 1q21.1 chromosomal region improved. Initially, array comparative genomic hybridization (aCGH) using Agilent 244K Human aCGH design 14693 and Agilent custom 4 × 44K designs 17773, 18388, and 19085 was performed on whole-blood DNA from the affected siblings and their parents (Agilent, Inc.). The presence of the 1q21.1 deletion in the siblings and one parent was confirmed in a CLIA-certified laboratory. A custom sequencing library was created with SureSelect probes (Agilent, Inc.) designed to target the deleted region (hg19 Chr 1:145341190-146164640) to evaluate the nondeleted region for novel variants.

The sequence alignment challenges at 1q21.1 led us to perform whole-exome sequencing (WES) on the siblings and their parents to assess coding regions for potential disease-associated variants as previously described (Ballew et al. 2013). Synonymous and low-quality variants were removed, and the remaining variants were filtered to identify variants shared between the siblings. We first focused on variants at 1q21.1 that were homozygous in the

children and present in the parent without the 1q21.1 deletion. We filtered variants in the exome using autosomal recessive inheritance models.

Whole-genome sequencing was also performed on NCI-107-2 in a pilot study utilizing 10× Genomics Gemcode technology initially aimed at testing the ability of this platform to detect heterozygous deletions. The WGS alignments were conducted on hg38.

We used the liftover tool in the UCSC Genome Browser to map all coordinates to hg38 (Table 2). TRANSFAC was used to identify putative TFBS at or around the SNP of interest, rs61746197 (QIAGEN) (Matys et al. 2006).

Functional Studies of rs61746197 A > G

Changes in expression of *RBM8A* (Hs04234933_g1), *PEX11B* (Hs00948294_m1), and *LIXL1* (Hs00542268_m1) were evaluated by qPCR using RNA isolated from EBV-immortalized lymphoblasts and/or fibroblast cells from all four family members. EBV-lymphoblasts were cultured at 5%O₂, 5%CO₂, 2 psi in an AVATAR incubator system (Xcell Biosciences) in RPMI supplemented with 10% FBS. Cells were harvested at >70% viability; $\sim 5 \times 10^6$ cells were harvested for RNA extraction. Fibroblast RNA was extracted directly from cryopreserved primary cell cultures. RNA was extracted using RNeasy plus mini kit (QIAGEN), and 50 ng of total RNA was reverse transcribed using Superscript III first-strand synthesis kit primed with random hexamers (Thermo Fisher). Changes in target levels were quantified using TaqMan probes (Thermo Fisher) by the $\Delta\Delta CT$ method after normalization to GAPDH (Hs99999905_m1) using a minimum of three technical replicates.

EMSA were performed using TPA-stimulated and unstimulated nuclear extracts of K562 cells (Active Motif). Ten micrograms of the nuclear extracts were incubated on ice for 30 min with 3.3 nM of IR700 labeled probes (Supplemental Table 1) (Integrated DNA Technologies), poly-dIdC, glycerol binding buffer, and increasing molar equivalents of unlabeled competitor probe as indicated (where 1× = 3.3 nM, 2× = 6.7 nM, 3× = 10 nM, final concentration of unlabeled competitor probe). In some reactions, anti-NF- κ B-p65 subunit antibody (MAB3026, Millipore Sigma) or volumetric equivalent of glycerol was added after the initial 30 min and incubated for an additional 15 min at room temperature. Reactions were halted with the addition of 5× loading dye and loaded quickly on 7.5% Criterion gels (Bio-Rad). The gels were run for 50 min at 150 V and imaged on a Chemidoc MP system (Bio-Rad).

ADDITIONAL INFORMATION

Data Deposition and Access

Genomic data are available through controlled access in dbGaP per the National Institutes of Health (NIH) genomic data sharing policy (Study Accession: phs001481.v1.p1). The variants were submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and can be found under accession numbers SCV000995057 and SCV000995062.1.

Ethics Statement

This study was approved by the Institutional Review Board of the National Cancer Institute (study ID number 02-C-0052; ClinicalTrials.gov Identifier: NCT00027274). The participants, their parents, or legal guardians provided written, informed consent. Minors provided written, informed assent.

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Author Contributions

S.A.S., N.G., and B.P.A. evaluated patients and collected clinical data. S.A.B. and J.P.R.-A. performed experiments. S.L.A., Y.J., Y.W., Y.J.Z., and J.B. performed sequencing, genotyping, and aCGH. S.A.B., J.P.R.-A., J.D., M.S., J.W., D.R., B.J.B., W.Z., and P.S.M. performed the bioinformatics analysis. S.A.S. conceived of the project. S.A.S., J.P.R.-A., and S.A.B. wrote the manuscript. All authors edited and approved the final manuscript.

Competing Interest Statement

The authors have declared no competing interest.

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REFERENCES

- Ahmad R, Pope S. 2008. Association of Mayer–Rokitansky–Küster–Hauser syndrome with thrombocytopenia absent radii syndrome: a rare presentation. *Eur J Obstet Gynecol Reprod Biol* **139**: 257–258. doi:10.1016/j.ejogrb.2007.01.018
- Albers CA, Paul DS, Schulze H, Freson K, Stephens JC, Smethurst PA, Jolley JD, Cvejic A, Kostadima M, Bertone P, et al. 2012. Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit *RBM8A* causes TAR syndrome. *Nat Genet* **44**: 435–439. S431–432. doi:10.1038/ng.1083
- Alter BP, Giri N, Savage SA, Rosenberg PS. 2018. Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up. *Haematologica* **103**: 30–39. doi:10.3324/haematol.2017.178111
- Ballew BJ, Yeager M, Jacobs K, Giri N, Boland J, Burdett L, Alter BP, Savage SA. 2013. Germline mutations of regulator of telomere elongation helicase 1, *RTEL1*, in Dyskeratosis congenita. *Hum Genet* **132**: 473–480. doi:10.1007/s00439-013-1265-8
- Greenhalgh KL, Howell RT, Bottani A, Ancliff PJ, Brunner HG, Verschuuren-Bemelmans CC, Vernon E, Brown KW, Newbury-Ecob RA. 2002. Thrombocytopenia-absent radius syndrome: a clinical genetic study. *J Med Genet* **39**: 876–881. doi:10.1136/jmg.39.12.876
- GTEx Consortium. 2013. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* **45**: 580–585. doi:10.1038/ng.2653
- Hall JG, Levin J, Kuhn JP, Ottenheimer EJ, van Berkum KA, McKusick VA. 1969. Thrombocytopenia with absent radius (TAR). *Medicine (Baltimore)* **48**: 411–439. doi:10.1097/00005792-196948060-00001
- Hansen SK, Baeuerle PA, Blasi F. 1994. Purification, reconstitution, and I κ B association of the c-Rel-p65 (RelA) complex, a strong activator of transcription. *Mol Cell Biol* **14**: 2593–2603. doi:10.1128/MCB.14.4.2593
- Houeiijeh A, Andrieux J, Saugier-Verber P, David A, Goldenberg A, Bonneau D, Fouassier M, Journel H, Martinovic J, Escande F, et al. 2011. Thrombocytopenia-absent radius (TAR) syndrome: a clinical genetic series of 14 further cases. impact of the associated 1q21.1 deletion on the genetic counselling. *Eur J Med Genet* **54**: e471–e477. doi:10.1016/j.ejmg.2011.05.001
- Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, et al. 2019. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. *bioRxiv* doi:10.1101/531210
- Klopocki E, Schulze H, Strauß G, Ott CE, Hall J, Trotier F, Fleischhauer S, Greenhalgh L, Newbury-Ecob RA, Neumann LM, et al. 2007. Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. *Am J Hum Genet* **80**: 232–240. doi:10.1086/510919

- Landrum MJ, Kattman BL. 2018. ClinVar at five years: delivering on the promise. *Hum Mutat* **39**: 1623–1630. doi:10.1002/humu.23641
- Manukjan G, Bösing H, Schmugge M, Strauss G, Schulze H. 2017. Impact of genetic variants on haematopoiesis in patients with thrombocytopenia absent radii (TAR) syndrome. *Br J Haematol* **179**: 606–617. doi:10.1111/bjh.14913
- Matys V, Kel-Margoulis OV, Fricke E, Liebich I, Land S, Barre-Dirrie A, Reuter I, Chekmenev D, Krull M, Hornischer K, et al. 2006. TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucleic Acids Res* **34**: D108–D110. doi:10.1093/nar/gkj143
- McDermott DA, Fong JC, Basson CT. 1993. Holt–Oram syndrome. In *GeneReviews*[®] (ed. Adam MP, Ardinger HH, Pagon RA, et al.). University of Washington, Seattle.
- Niihori T, Ouchi-Uchiyama M, Sasahara Y, Kaneko T, Hashii Y, Irie M, Sato A, Saito-Nanjo Y, Funayama R, Nagashima T, et al. 2015. Mutations in *MECOM*, encoding oncoprotein EVI1, cause radioulnar synostosis with amegakaryocytic thrombocytopenia. *Am J Hum Genet* **97**: 848–854. doi:10.1016/j.ajhg.2015.10.010
- O’Bleness M, Searles VB, Dickens CM, Astling D, Albracht D, Mak AC, Lai YY, Lin C, Chu C, Graves T, et al. 2014. Finished sequence and assembly of the DUF1220-rich 1q21 region using a haploid human genome. *BMC Genomics* **15**: 387. doi:10.1186/1471-2164-15-387
- Rosenfeld JA, Traylor RN, Schaefer GB, McPherson EW, Ballif BC, Klopocki E, Mundlos S, Shaffer LG, Aylsworth AS, q21.1 Study Group. 2012. Proximal microdeletions and microduplications of 1q21.1 contribute to variable abnormal phenotypes. *Eur J Hum Genet* **20**: 754–761. doi:10.1038/ejhg.2012.6
- Savage SA, Walsh MF. 2018. Myelodysplastic syndrome, acute myeloid leukemia, and cancer surveillance in fanconi anemia. *Hematol Oncol Clin North Am* **32**: 657–668. doi:10.1016/j.hoc.2018.04.002
- Stein SJ, Baldwin AS. 2013. Deletion of the NF-κB subunit p65/RelA in the hematopoietic compartment leads to defects in hematopoietic stem cell function. *Blood* **121**: 5015–5024. doi:10.1182/blood-2013-02-486142
- Thompson AA, Nguyen LT. 2000. Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with *HOXA11* mutation. *Nat Genet* **26**: 397–398. doi:10.1038/82511
- Toriello HV. 1993. Thrombocytopenia absent radius syndrome. In *GeneReviews*[®] (ed. Adam MP, Ardinger HH, Pagon RA, et al.). University of Washington, Seattle.
- Whitfield MF, Barr DG. 1976. Cows’ milk allergy in the syndrome of thrombocytopenia with absent radius. *Arch Dis Child* **51**: 337–343. doi:10.1136/adc.51.5.337
- Wu S, Flint JK, Rezvani G, De Luca F. 2007. Nuclear factor-κB p65 facilitates longitudinal bone growth by inducing growth plate chondrocyte proliferation and differentiation and by preventing apoptosis. *J Biol Chem* **282**: 33698–33706. doi:10.1074/jbc.M702991200

1q21.1 deletion and a rare functional polymorphism in siblings with thrombocytopenia-absent radius –like phenotypes

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