Syndromic Thrombocytopenia and Predisposition to Acute Myelogenous Leukemia Caused by Constitutional Microdeletions on Chromosome 21q

Marwan Shinawi*1, Ayelet Erez*1, Deborah L. Shardy2,3, Brendan Lee1,2, Rizwan Naeem2,3, George Weissenberger1,4, A. Craig Chinault1,4, Sau Wai Cheung1,4, Sharon E. Plon1

Departments of 1Molecular and Human Genetics and 2Pediatrics, 3Texas Children’s Cancer Center, Baylor College of Medicine and Texas Children’s Hospital; 4Medical Genetics Laboratories, Baylor College of Medicine

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* M.S. and A.E. contributed equally to this study.

Correspondence to:

Sharon E. Plon, M.D., Ph.D.
MC3-3320; 6621 Fannin Street
Houston, TX 77030
E-mail: splon@bcm.edu
Tel. 832-824-4251; Fax 832-825-4276
Abstract

Several lines of evidence support the presence of dosage-sensitive genes on chromosome 21 that regulate leukemogenesis and hematopoiesis. We report a detailed clinical and molecular characterization of three patients with chronic thrombocytopenia caused by distinct constitutional microdeletions involving chromosomal region 21q22.12. The patients exhibited growth restriction, dysmorphic features, and developmental delays. One patient developed acute myelogenous leukemia (AML) at 6 years of age. All three deletions included the RUNX1, CLIC6, DSCR, and KCNE1 genes. Our data provide additional support for the role of RUNX1 haploinsufficiency in megakaryopoiesis and predisposition to AML. The leukemic clone had trisomy 21 resulting from duplication of chromosome 21 containing the RUNX1 deletion. This demonstrates that genes other than RUNX1 must also play a role in AML associated with trisomy 21. We recommend that children with syndromic thrombocytopenia have clinical array-comparative genomic hybridization analysis and appropriate cytogenetic studies to facilitate our ability to provide a definitive diagnosis.
Introduction

Acquired and constitutional structural genomic aberrations leading to the activation of oncogenes or haploinsufficiency of tumor suppressor genes are well-known pathogenic mechanisms in cancer. The predisposition for leukemia and myelodysplasia (MDS) \(^1\)\(^2\) and the common hematological abnormalities\(^3\) seen in children with trisomy 21 suggest that dosage-sensitive genes on chromosome 21 are involved in leukemogenesis and hematopoiesis. The “leukemogenic” role of an additional chromosome 21 was further supported by showing the selective involvement of trisomic cells with leukemia in patients with trisomy 21 mosaicism.\(^4\) In addition, trisomy/polysomy 21and translocations that disrupt the $RUNX1$ gene on chromosome 21q22 are nonrandom and are among the most frequent acquired chromosomal abnormalities in acute lymphoblastic leukemia (ALL) and acute myelocytic leukemia (AML).\(^5\)-\(^7\) This dosage effect is also seen in a subtype of ALL patients with intrachromosomal amplification of chromosome 21 encompassing the RUNX1 gene (iAMP21) who have poor outcome.\(^8\)

The significant role of RUNX1 in megakaryopoiesis and leukemogenesis was further supported by the finding that haploinsufficiency of the $RUNX1$ gene is the genetic basis of the autosomal dominant familial platelet disorder with predisposition to acute myelogenous leukaemia (FPD/AML, MIM 601399).\(^9\) Similar to patients with the FPD/AML syndrome, there was an approximately 15% reduction in the number of platelets in the $Runx1^{+/−}$ mice,\(^10\) but the development of AML could not be recapitulated in these mice.\(^10\),\(^11\) In addition, somatic mutations in $RUNXI$ and its cofactor $CBFB$ are frequently found in acute leukemias and MDS.\(^12\),\(^13\)
Here we present the molecular analysis and fine mapping of constitutional microdeletions on 21q encompassing the \textit{RUNX1} gene in three patients with chronic thrombocytopenia. One of the three patients developed AML at the age of 6 years. In addition, the patients had growth restriction, dysmorphic features, and variable degree of developmental delay.

**Materials and Methods**

The parents of all three subjects gave informed consent to participate in human subjects protocols approved by the Institutional Review Board (IRB) of Baylor College of Medicine. Patient 1 was enrolled on IRB-approved protocol entitled: "The Molecular Basis of Familial Cancer Predisposition Syndromes". Patients 2 and 3 were enrolled on IRB-approved protocol entitled: "Use of microarrays (DNA chips) for global detection of cytogenetic abnormalities by comparative genomic hybridization". Both studies include forms that specifically allow parents to provide consent for the use of photographs for publication. The informed consents were provided according to the Declaration of Helsinki.

**Cytogenetic and fluorescence in situ hybridization (FISH) analyses**

Chromosome analysis of phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (for all patients) and of bone marrow (for patient #1) was performed by GTG- banding analysis using standard cytogenetic laboratory procedures. FISH analysis using probes specific for \textit{ETO} and \textit{RUNX1} genes and other specific probes for chromosome 21 were performed in PHA stimulated peripheral blood sample obtained from the patients. DNA for home brewed FISH probes was extracted and directly labeled according to the manufacturers’ instructions (Vysis).
Clinical chromosome microarray analysis

The Baylor College of Medicine (BCM) Chromosome Microarray Analysis (CMA) (http://www.bcm.edu/geneticlabs/cma/tables/detectionratesoligo.pdf) Version 6.3 (CMA V6.3) and CMA V5 were used to study patient #2 and patient #3, respectively.\textsuperscript{14, 15}

Deletion mapping by high-resolution oligonucleotide array

Further characterization of the deletions was performed utilizing the Agilent 244K Whole Human Genome CGH arrays (Agilent Technologies, Inc, CA, USA) containing 236,000 probes. The procedures for DNA digestion, labeling, hybridization, and data analysis were performed according to the manufacturer’s protocol (Agilent Technologies, Santa Clara, CA).

Results

Clinical Reports

In this report, we describe the clinical and molecular characterization of three patients with chronic thrombocytopenia, dysmorphic features (see Supplementary Figure), congenital anomalies and variable developmental delay. Table 1 provides a summary of the clinical features of all three patients. The average platelet counts were similar in all three patients; 78,000, 60,000, 74,000 in patients #1, #2, and #3, respectively. Prior to the cytogenetic analyses described below all three patients had extensive clinical work-ups, e.g. DEB breakage, which failed to reveal the cause of their thrombocytopenia. In patient #3, the platelet count was only requested after the karyotype and microarray analysis revealed that the deletion encompassed the $RUNXI$ gene. More detailed description of the clinical features and diagnostic work-up of each patient are provided in Supplementary material.
Clinical Cytogenetic, FISH and array-CGH analyses

When patient #1 was six years old, he presented to our center with chronic thrombocytopenia and newly diagnosed myelodysplastic syndrome and acute myelogenous leukemia (MDS/AML). Cytogenetic evaluation of unstimulated bone marrow revealed an abnormal cell line with duplication of chromosome 7p14-p15 along with 3 copies of chromosome 21 in 13 out of 20 cells examined (Figure 1A). Cytogenetic evaluation of PHA-stimulated peripheral blood revealed the same abnormal cell line in 7 out of 20 cells examined. Given chronic thrombocytopenia, AML, multiple congenital anomalies, and dysmorphic features the clinical diagnosis of a constitutional deletion involving the RUNX1 locus was made. The clinically available array comparative genomic hybridization (array-CGH) analysis at that time did not adequately cover the RUNX1 locus and, therefore, direct FISH analysis of the RUNX1 locus was requested. FISH evaluation of PHA-stimulated peripheral blood using probes specific for ETO and RUNX1 genes revealed one strong RUNX1 signal with one "dim" RUNX1 signal in 20% interphase cells examined and one strong RUNX1 signal and 2 "dim" RUNX1 signals in the remaining 80% of cells (Figure 1B). A bone marrow aspirate at the same time revealed similar findings with the same RUNX1 patterns described above (data not shown). FISH analysis of peripheral blood from both parents demonstrated two strong RUNX1 signals (data not shown) consistent with a de novo deletion in patient #1. The patient was successfully transplanted and follow-up studies revealed engraftment with female 46 XX donor cells.

The peripheral blood karyotype of patient #2 at age 19 months showed normal 46XX. Clinical array-CGH with CMA V6.3 revealed a loss in copy number on 21q,
detected with oligonucleotides emulating a single BAC clone (Figure 2A), and confirmed by FISH analysis with BAC clone [RP11-17O20] that includes the RUNX1 gene (Figure 2B). Parental FISH analysis with the same BAC revealed a normal pattern in the mother.

In the father, the BAC hybridized to one chromosome 21 and a second hybridization signal on the long arm of one chromosome 11(Figure 2B). The same insertional rearrangement was found in one of patient #2’s four siblings.

Karyotype analysis in patient #3 at birth showed a deletion at 21q22.1. The mother’s karyotype was normal but the father was not available for analysis. Based on the molecular results described below, FISH analysis was performed using two BAC clones from 21q: RP11-17O20 more distal and RP11-92D3 more proximal. In 15% of interphase nuclei there was one signal for both BACs and in 85% there were two signals for the proximal BAC and a single signal for the distal (more telomeric) BAC (data not shown).

The clinical CMA result for patient #3 also revealed a loss of copy number with 11 clones (from RP11-17O20 to clone RP4-639D23) that encompass at least 11 megabases on 21q22.1 (data not shown). The T-statistic values for centromeric clones were higher than for the more telomeric clones consistent with the finding from FISH analysis that there is a mixed population of cells with different size microdeletions.

**High-resolution genomic analyses by 244K array-CGH**

Patient #1 had high resolution analysis using the Affymetrix 50K SNPchip (data not shown) which was consistent with the Agilent array data described below. Subsequently, all three patients had research analysis using the Agilent 244K Whole Human Genome CGH Microarray in order to better define the extent of deletion. The
results are compared to genome sequence data (hg18; NCBI Build 36.1) (figure 3). The analysis was done using genomic DNA isolated from a blood sample for all three patients. This was performed at the time of diagnosis of leukemia in patient #1 and the deletion was found to be approximately 0.7 Mb in size on band 21q22.12 (position 34,796 to 35,507 Kb). This small microdeletion is embedded on a microarray profile that demonstrates gain of all the other oligonucleotides from chromosome 21 again reflecting trisomy 21 in the leukemic clone. In addition, the microarray analysis showed gain of approximately 24.1 Mb on the short arm of chromosome 7 between positions 19,767 and 44,578 Kb (data not shown).

The size of the deletion in patient #2 was ~1.81 Mb extending from 33,833 to 35,647 Kb on 21q22.12. The deletion in patient #3 is much larger and is approximately 19.8 Mb encompassing the region between 27,198 to 46,915 Kb (terminal part of 21q). Consistent with the CMA and FISH analyses there was evidence for a mixed population (mosaicism) with nonuniform deflection of the log2 ratio; about half with a 19.7 Mb deletion and approximately half with deletion of only the distal 9.1 Mb of the same region.

**Discussion**

We report three patients with chronic thrombocytopenia associated with multiple congenital anomalies, dysmorphic features, growth restriction, and developmental delay. One of the three patients developed AML at the age of 6 years. All three patients demonstrated constitutional microdeletions encompassing band 21q22.12. The deletions were variable in size and therefore were detected clinically by different techniques including routine karyotype for the largest deletion, clinical array-CGH, and targeted
FISH for the \textit{RUNX1} locus. Research studies using the Agilent 244K oligonucleotide array more finely delineated the deletions. There is a common deleted region of approximately 0.7 Mb among the three patients which encompasses 4 known genes: \textit{RUNX1}, \textit{CLIC6}, \textit{DSCR1}, and a portion of \textit{KCNE1} (Figure 4A).

The FISH analysis using a \textit{RUNX1} probe on patient #1 demonstrated that the microdeletion was constitutional and not secondary to leukemia development. Both FISH and array-CGH demonstrate that the trisomic chromosome in the leukemia cells contains the \textit{RUNX1} deletion. This finding provides strong evidence that although deletion of the \textit{RUNX1} locus is responsible for the thrombocytopenia and predisposition to AML, other dosage sensitive genes on chromosome 21 also play a role in development of leukemia. In addition, the leukemic clone contains duplication of 7p that has not been previously reported in AML (http://www.cancerindex.org). Interestingly, the duplicated region contains the cluster of \textit{HOX} genes which have been implicated in AML pathogenesis.\textsuperscript{16,17}

In all three cases, the subjects had undergone extensive evaluations including a variety of different molecular and biochemical analyses, chromosome fragility testing, and muscle biopsy (see Supplemental Data). Clinical use of array-CGH analysis earlier in the diagnostic work-up for syndromic thrombocytopenia will allow for rapid identification of the causative deletion.

The molecular basis of the deletion was different in all three patients with patient #1 resulting from a \textit{de novo} deletion, #2 from a paternal insertional translocation, and #3 with likely postzygotic deletions resulting in mosaicism for two different sized deletions. Patient #3 had thrombocytopenia despite the fact that only 15\% of the cells were deleted
for the RUNX1 gene. Of note, the father and sibling of patient #2 are cytogenetically balanced and would appear normal on array-CGH analyses due to the ~1.8 Mb insertional rearrangement. This illustrates the need for the appropriate cytogenetic analyses of the parents of affected patients to correctly define the recurrence risk and to offer prenatal testing to the family if so desired.

Comparing these findings with those from published cases is challenging because hematologic parameters are not always reported, deletions are heterogeneous in nature and can occur with additional chromosomal rearrangements. Descriptions from the literature reveal that patients with partial monosomy 21 exhibit variable phenotype dominated by prenatal and postnatal growth restriction, severe developmental delay, abnormal muscle tone, a variety of dysmorphic facial features, and heart defects.18,19 Hematological findings and thrombocytopenia were reported in 4 cases with isolated 21q deletion only but may have been more commonly present if assayed.20, 21 All three patients described here share the following features: thrombocytopenia, variable developmental delay, hypertelorism and inverted nipples (Figure 4B). Phenotypic findings that were found in two of our patients included broad forehead, epicanthal folds, and abnormally shaped eyes and nose. Our data indicate that one or more of the genes within the minimal overlap region (RUNX1, CLIC6, DSCR1, and KCNE1) are involved in the dysmorphic phenotype and possibly the developmental delay of 21q microdeletion patients.

The KCNE1 gene encodes the potassium voltage-gated channel involved in the long QT syndrome (LQTS). Heterozygous loss-of-function mutations of KCNE1 cause the LQTS (MIM 176261)22 and homozygous or compound heterozygous mutations of
*KCNE* are responsible for prolonged QT and sensorineural hearing loss (Jervell and Lange-Neilsen syndrome; JLNS1; MIM 220400). Interestingly, although these abnormalities (including measurement of the QT interval) were not found in our patients, a cardiology evaluation is indicated in this microdeletion syndrome given that not all patients with LQTS show an increased QT interval at rest.

The molecular data on our patients provide additional support for the significant role of *RUNX1* haploinsufficiency in the development of thrombocytopenia as it results from a complete gene deletion as opposed to any potential dominant negative effect from point mutations as previously described in FPD/AML.9

The hematological abnormalities, clinical history, physical findings, and molecular defects in our patients add to our understanding of the heterogeneous group of hereditary thrombocytopenias.24 In particular, we demonstrate the clinical utility of array-CGH technology in the assessment of patients with thrombocytopenia and congenital anomalies and/or developmental delay.

**Conclusion**

Deletions encompassing the *RUNX1* gene on 21q22.12 are responsible for syndromic thrombocytopenia and predisposition to AML. Patients with this contiguous gene syndrome also exhibit dysmorphic features, developmental delay, and growth restriction (Figure 4B). Molecular analysis of leukemia samples from one patient deleted for *RUNX1* confirm that haploinsufficiency of *RUNX1* is associated with AML development but that other genes on chromosome 21 also play a role in trisomy 21 associated with AML. We predict that a wider clinical application of array-CGH will significantly improve our ability to provide a definitive diagnosis for patients with
syndromic chronic thrombocytopenia and/or AML, and will limit the extensive diagnostic work up that is usually performed for these patients.
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Contribution: M.S. wrote the paper and performed the clinical evaluation of patient #3; A.E. performed the 244K microarray experiments; D.L.S participated in the molecular analysis of patient #1; B.L. participated in the clinical evaluation of patient #2; R.N. performed the FISH analysis on patient #1; G.W. participated in the 244K microarray experiments C.C. participated in the 244K microarray experiments and data analysis; SWC reviewed and provided the cytogenetic and clinical microarray data. S.E.P., the P.I. of this study, performed the clinical evaluation of patient #1 and edited the paper.

Conflict-of-interest disclosure: The Department of Molecular and Human Genetics at Baylor College of Medicine (BCM) offers extensive genetic laboratory testing and SWC, ACC, and GW derive revenue from this activity.

Correspondence: Sharon E. Plon, MC3-3320; 6621 Fannin Street, Houston, TX 77030; E-mail: splon@bcm.edu
References


10. Sun C, Downing JR. Haploinsufficiency of AML1 results in a decrease in the number of LTR-HSCs while simultaneously inducing an increase in more mature progenitors. Blood 2004; 104: 3565–3572


Table 1 – Clinical features of the three patients described with syndromic thrombocytopenia

<table>
<thead>
<tr>
<th>Feature</th>
<th>Patient #1 (male)</th>
<th>Patient #2 (female)</th>
<th>Patient #3 (female)</th>
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<td>Race/Ethnicity</td>
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<td>Caucasian-European/Filipino</td>
<td>Caucasian-Hispanic</td>
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<td>Age thrombocytopenia first detected</td>
<td>18 months</td>
<td>19 months</td>
<td>9 months</td>
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<td>Average platelet count (platelets/mm$^3$)</td>
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<td>74,000</td>
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<td>Other hematologic findings</td>
<td>MDS/AML – age 6 years</td>
<td>Macrocytosis</td>
<td>-</td>
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<td>Birth weight (gms) - (percentile)</td>
<td>2920 (25$^{th}$)</td>
<td>2041 (&lt;3$^{rd}$)</td>
<td>1955 (&lt;3$^{rd}$)</td>
</tr>
<tr>
<td>Growth delay</td>
<td>+</td>
<td>+(G-tube)</td>
<td>+</td>
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<tr>
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<td>Fine motor</td>
<td>Global</td>
<td>Global</td>
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<td>&lt;3$^{rd}$ percentile</td>
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<tr>
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<td>5$^{th}$ percentile</td>
<td>&lt;3$^{rd}$ percentile</td>
<td>&lt;3$^{rd}$ percentile</td>
</tr>
<tr>
<td>Height*</td>
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<td>&lt;3$^{rd}$ percentile</td>
<td>&lt;3$^{rd}$ percentile</td>
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<td>Eyes</td>
<td>Hypertelorism, almond-shaped</td>
<td>Hypertelorism, epicanthal folds, sparse eyebrows</td>
<td>Hypertelorism, epicanthal folds, small/deep-set, strabismus,</td>
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<td>Intact/ smooth philtrum</td>
<td>Intact</td>
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<td>Short/anteverted</td>
<td>Flat nasal bridge</td>
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<td>Inverted nipples</td>
<td>Inverted nipples</td>
<td>Inverted nipples</td>
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<td>Skull</td>
<td>Prominent forehead</td>
<td>Normocephalic</td>
<td>High broad forehead, flat occiput</td>
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<td>Other anomalies</td>
<td>Transposition of great arteries, absence of left testis</td>
<td>Toenail hypoplasia, delayed myelination and dysgeneic corpus callosum</td>
<td>Neonatal seizures, alopecia</td>
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</tbody>
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(*) Measurements at last clinical evaluation; FOC, fronto-occipital circumference;

MDS/AML, myelodysplastic syndrome and acute myelogenous leukemia
Legends

Figure 1. Karyotype and FISH analysis in patient #1. (A). Cytogenetic analysis of peripheral blood abnormal cell showing duplication of 7p14-p15 and three copies of chromosome 21 (arrows). (B). FISH on peripheral blood using probes specific for ETO (orange) and RUNX1 (green) genes. Note the presence of one strong and one dim green signal in the metaphase and lower left interphase nucleus confirming the constitutional RUNX1 deletion. In the lower right interphase nucleus there are one strong and 2 “dim” RUNX1 signals representing a trisomy 21 cell line with duplication of the deleted chromosome 21.

Figure 2. Array-CGH and FISH analyses of patient #2. (A). An output of the clinical array-CGH (CMA v. 6.3) of patient #2. This profile represents the averaged combined data of hybridizations performed using reference DNA. Oligonucleotides emulating the clone RP11-17O20 (arrow) shows down displacement indicating a loss of chromosome 21 material in the patient versus the reference DNA. (B). Representative data from metaphase FISH analysis in patient #2 and her father. The RP11-17O20 clone was used as probe (pink). The green signal is a subtelomeric probe used as a control. The patient has a single pink signal consistent with a deletion on one of the two copies of chromosome 21. The FISH analysis in the father shows one pink signal on chromosome 21 and the second signal inserted in the long arm of chromosome 11. Reverse banding (inset) confirmed the insertion to chromosome 11.

Figure 3. Agilent 244K Human Genome CGH Microarray hybridization profile of chromosome 21 for the three patients. The size of the deletions is indicated by the blue
bars. The small microdeletion in patient #1 is embedded within a profile that demonstrates copy number gain with all the other oligonucleotides from chromosome 21 (red bar) reflecting the trisomy 21. The profile of patient #3 shows that the deflection of the log2 ratio (the green deflection from 0) is not uniform indicating the presence of mosaicism in the cell population. Based on FISH data, only 15% of the cells in patient #3 have the large (~19.7 Mb) deletion and are deleted for the RUNX1 gene.

**Figure 4. Summary of the genetic and clinical findings in our patients.** (A)

Schematic representation of the deleted regions on 21q22.12 (not drawn to scale). The deletion map was constructed using the USCS human genome browser (http://genome.ucsc.edu/). The black lines show the extents of the deletions. The shaded area is the overlap between the deletions in the three patients. The genes located within this interval are at the bottom of the figure. **(B)** Diagram of the shared and unique clinical features of the three patients described in this report.
Figure 1

A

B

21 (AML1)

8 (ETO)

21 (AML1) dim
Figure 2

A

Chromosome

12 13 14 15 16 17 18 19 20 21 22  X

B

Patient #2

Father
Figure 3
Figure 4

A

Chromosome 21

q21.3  q22.11  q22.12  q22.13  q22.3

Patient #1
Patient #2
Patient #3

KCNE1  DSCR1  CLIC6  RUNX1

B

Patient #2
Feeding Problems

Patient #3
Seizures

Thrombocytopenia
Growth Restriction
Variable Developmental Delay
Dysmorphic Features
Inverted Nipples

Congenital Heart Disease
Absent Testis
AML

Patient #1
Syndromic thrombocytopenia and predisposition to acute myelogenous leukemia caused by constitutional microdeletions on chromosome 21q

Marwan Shinawi, Ayelet Erez, Deborah L. Shardy, Brendan Lee, Rizwan Naeem, George Weissenberger, A. Craig Chinault, Sau Wai Cheung and Sharon E. Plon