

Brief report

Incidence and clinical implications of *GATA1* mutations in newborns with Down syndromeSharon R. Pine,¹ Qianxu Guo,¹ Changhong Yin,¹ Somasundaram Jayabose,¹ Charlotte M. Druschel,² and Claudio Sandoval¹¹Department of Pediatrics, Division of Hematology/Oncology, Maria Fareri Children's Hospital and Westchester Medical Center, New York Medical College, Valhalla, NY; ²Congenital Malformations Registry New York State Department of Health, Troy

Somatic mutations in the *GATA1* gene are present in almost all cases of Down syndrome (DS)–associated acute megakaryoblastic leukemia (AMKL) and transient leukemia (TL). An in utero origin of the *GATA1* mutation suggests it is an early leukemogenic event. To determine the detectable incidence and clinical relevance of *GATA1* mutations in DS newborns, we screened Guthrie cards from

590 DS infants for mutations in the *GATA1* gene. Twenty-two (3.8%) of 585 evaluable infants harbored a predicted functional *GATA1* mutation; 2 were identified exclusively within intron 1. Hispanic newborns were 2.6 times more likely to have a mutated *GATA1* gene than non-Hispanics ($P = .02$). Two newborns with a *GATA1* mutation subsequently developed AMKL, and none of the infants without a func-

tional *GATA1* mutation were reported to have developed leukemia. In addition to screening for TL, a *GATA1* mutation at birth might serve as a biomarker for an increased risk of DS-related AMKL. (Blood. 2007;110:2128-2131)

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Introduction

Newborns with Down syndrome (DS) are at an increased risk of being diagnosed with transient leukemia (TL), or transient myeloproliferative disorder, which is characterized by an accumulation of immature clonal megakaryoblasts in the peripheral blood and liver. The disease usually spontaneously resolves within 3 months, although in rare cases it is fatal due to hepatic fibrosis or liver failure.¹⁻³ Children with DS have a 1 in 500 chance of developing acute megakaryoblastic leukemia (AMKL),⁴⁻⁶ and 19% of DS (or trisomy 21 mosaic) neonates who are diagnosed with TL develop AMKL within 4 years.^{3,7}

Somatic mutations of *GATA1* are found in almost all cases of DS or mosaic trisomy 21–associated TL and AMKL.⁸ *GATA1* mutations have not been detected in healthy children without DS, in DS children with other types of leukemia, or in AMKL cells of non-DS/non-trisomy 21 mosaic children, with the exception of a single case of adult AMKL without DS or acquired trisomy 21.^{8,9} This suggests that *GATA1* mutations play an important role in TL and AMKL development in the DS/mosaic trisomy 21 setting. We and others have shown that the *GATA1* mutation is cleared after resolution of TL or AMKL,¹⁰⁻¹³ and therefore the *GATA1* mutation appears to be restricted to the blast cells. All reported mutations occur in the 5' end of the gene, mostly within exon 2. Mutations either introduce a premature stop codon, a frameshift, or the loss of exon 2 from altered splicing, resulting in the expression of a shortened *GATA1* protein, termed *GATA1s*, that lacks exon 2 and its N-terminal transactivation domain.¹⁴

The overall incidence of *GATA1* mutations in DS infants and their clinical relevance for predicting development of AMKL is

uncertain. *GATA1* mutations were detected in 2 of 21 neonatal blood-spot samples from DS children.¹⁰ *GATA1* mutations were also found at birth in DS infants who later developed AMKL, including cases that did not have diagnosed TL,¹⁰ suggesting that either the TL was subclinical or that *GATA1* mutations in the absence of TL still participate in leukemia development. To determine the incidence of *GATA1* mutations in a large cohort of DS newborns, we screened 590 blood spots from DS neonates. We then examined the association between the *GATA1* mutations and risk of developing AMKL in the study population.

Patients, materials, and methods

Guthrie cards from 590 DS infants born in New York State between January 1997 and December 1999 were obtained from the New York Congenital Malformation Registry. Infants were matched to the New York State Cancer Registry (Albany, New York State Department of Health). The institutional review boards of New York Medical College, Westchester Medical Center, and the New York State Department of Health approved this project. Informed consent was obtained in accordance with the Declaration of Helsinki.

DNA samples were screened for *GATA1* mutations by single-strand conformation polymorphism (SSCP) and sequencing of polymerase chain reaction (PCR) products, as described.¹¹ Sixty samples were randomly chosen and analyzed in an independent assay.

Potential single-nucleotide polymorphisms (SNPs) were compared with known SNPs.^{15,16} Potential splicing aberrations were analyzed by using GENSCAN^{17,18} and NNSPLICE version 0.9.¹⁹

Calculations were performed using STATA software v.9.0 (Stata, College Station, TX) using 2-tailed χ^2 or Fisher exact tests or analysis of variance (ANOVA).

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Table 1. Characteristics of Down syndrome infants and incidence of GATA1 mutations

	Wild-type GATA1	Mutated GATA1	P
n	563	22	—
Sex, no.			
Male	284	15	—
Female	279	7	.10†
Race, no.			
White	439	20	—
African American	97	1	.15‡
Asian	24	1	1.00‡
Unknown	3	0	—
Hispanic status, no.			
Hispanic, any race	110	9	—
Non-Hispanic, any race	409	12	.02†
Non-Hispanic white*	313	11	.06†
Non-Hispanic African American*	72	0	.02‡
Unknown	44	1	—
Mean age of mother, y	33.0	34.5	.31§
Developed AMKL, no.			
Yes	0	2	—
No	563	20	.001‡

— indicates not applicable.

*Compared with those with a Hispanic ethnicity with any race.

† χ^2 test.

‡Fisher exact test.

§ANOVA test.

||One patient developed leukemia of unspecified phenotype.

Results and discussion

Frequency and spectrum of GATA1 mutations in DS infants

Table 1 summarizes the selected demographic characteristics of the infants. There was a 99.2% completion rate (585 samples) and a 100% concordance rate (3/60 duplicated samples harbored a mutation). Twenty-eight (4.8%) of 585 DS infants had mutations within intron 1 or exon 2 (Table 2). The sensitivity of detection was 5% (data not shown).

Twenty-one GATA1 mutations from 20 patients were within exon 2 and predicted to express only the GATA1s protein starting at codon 84. Two distinct GATA1 mutations were identified in patient 7, confirming previous reports^{10,21} that multiple independent GATA1 mutations can be simultaneously present. Three additional DS infants (patients [pts] 26-28) harbored synonymous substitutions within exon 2 that did not match currently known polymorphisms.

Interestingly, 5 GATA1 mutations (pts 21-25) were detected within the 3' end of intron 1. One of the mutations (pt 23) matched a known SNP (rs12849226). The mutations in patients 21 and 22 were predicted to abolish the exon 2 splicing site and result in expression of only the GATA1s isoform (Table 2). Functional analysis is required to confirm that the splicing site was abolished. There was no change in the predicted splicing sites for the mutations identified in patients 23 to 25, and those mutations did not match any known polymorphisms. The mutations identified in pts 23 to 28 were likely germ line and nonfunctional, but future studies are necessary for confirmation. In total, a predicted functional GATA1 mutation was identified in 22 (3.8%) of 585 analyzed neonatal blood spots.

Characteristics of DS infants carrying a GATA1 mutation

Because the incidence and frequency of genetic abnormalities in childhood leukemia vary by race and ethnicity, we examined the

frequency of GATA1 mutations based on these variables. White individuals had an elevated, nonsignificant risk of having a GATA1 mutation compared with African Americans and Asians. Hispanic infants had a higher incidence of GATA1 mutations compared with non-Hispanics (7.6% and 2.9%, respectively; $P = .02$). The observation of a higher GATA1 mutation incidence among Hispanics in this study may have occurred by chance; thus, additional studies are required for confirmation. Males had an elevated, nonsignificant risk of having a GATA1 mutation compared with females. There was no difference in the mean age of the mothers of DS newborns with or without a mutated GATA1 sequence.

Concordance of GATA1 mutations in twins

Three sets of twins were revealed after the analyses were completed. Two sets were African-American females who carried wild-type GATA1. The third set, Hispanic males (pts 12 and 13), carried identical GATA1 exon 2 mutations. This finding is consistent with previous reports of the same GATA1 mutation being present in twins with AMKL^{12,22} and confirms the prenatal origin of GATA1 mutations.

Subsequent leukemia in DS infants

Two of the patients with a known functional GATA1 mutation were reported to subsequently develop leukemia. Patients 10 and 22 were both Hispanic males who developed AMKL at the ages of 11 and 17 months, respectively. The mutation in patient 22 was located within intron 1. Unfortunately, DNA was not available from the time of AMKL diagnosis, so it is unknown if this mutation was present in the AMKL blasts. Of the 563 DS infants who had only a wild-type GATA1 detected, there were no cases of leukemia reported. Based on estimates from migration data from the United States Census Bureau,²³ approximately 56 (9.6%) children in our study population moved outside New York State and thus were lost to follow-up of cancer. Because 1 in 500 DS children will develop AMKL, it is possible, but unlikely, that a child in our study population who moved out of New York State developed AMKL.

Technical factors could account for a low frequency of GATA1 mutations in this DS population, such as a sensitivity of 5%, low numbers of cells on the Guthrie cards, and our inability to detect deletions of the entire exon 2 or within exon 3.

The presence of a GATA1 mutation in our cohort of DS newborns conferred an elevated risk of developing AMKL. We propose that GATA1 mutations can serve as an early biomarker for a subgroup of DS children with an increased risk of developing AMKL. Because the samples analyzed in our study were blinded, we were unable to obtain clinical records regarding a TL diagnosis or blood counts. A large prospective DS screening study is needed to make a direct comparison between TL, GATA mutations, and subsequent AMKL development in DS newborns to determine the prognostic usefulness of GATA1 mutations in addition to TL screening.

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Table 2. Mutation characteristics and cancer outcome in DS neonates with GATA1-mutations

Patient	Mutation*	Protein	Cancer registry match
Mutations within exon 2 predicted to generate the GATA1s isoform			
1	c.-25_3del	No initiation codon	No
2	c.30_31dupG	Frameshift	No
3	c.38_39delAG	Frameshift	No
4	c38_39delAG	Frameshift	No
5	c.49C>T	Stop codon	No
6	c.90_91delAG	Frameshift	No
7	c.158_159insAGCTG	Frameshift	No
7	c.170_171insTGCGCAGC	Frameshift	No
8	[c.160_161insCCGTGCT; c.165T>C]	Frameshift	No
9	c.[162_172dup; 173_174insTGGC]	Frameshift	No
10	c.[163_174dup; 174_175insTG]	Frameshift	Unspecified leukemia
11	c.164_177dup	Frameshift	No
12	c.166_167insCAGCGCTG	Frameshift	No
13	c.166_167insCAGCGCTG	Frameshift	No
14	c.166_185dup	Stop codon	No
15	c.173_174insACTGTAGTA	Stop codon	No
16	c.177_178insCTGGGGCA	Frameshift	No
17	c.182_195dup	Frameshift	No
18	c.187_205delins18	Frameshift	No
19	c.212_220+41del	Altered splicing	No
20	c.216_220+2del	Altered splicing	No
Mutations exclusive to intron 1 predicted to alter splicing of exon 2 to generate the GATA1s isoform			
21	c.-20G>A	Altered splicing	No
22	c.-30T>A	Altered splicing	AMKL
Mutations not predicted to alter the GATA1 protein structure			
23	c.-68T>G	SNP: rs12849226	No
24	c.-8dupG	No altered splicing	No
25	c.-8_-6dup	No altered splicing	No
26	c.174G>A	Ala-Ala, not a known SNP	No
27	c.201G>A	Glu-Glu, not a known SNP	No
28	c.201G>A	Glu-Glu, not a known SNP	No

*Following the HGVS mutation nomenclature guidelines.²⁰

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Authorship

Contribution: S.R.P. designed and supervised research and wrote the manuscript; Q.G. performed research and analyzed data; C.Y. performed research and analyzed data; S.J. designed research and reviewed the manuscript; C.M.D. contributed patients and data;

and C.S. designed and supervised research and reviewed the manuscript.

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