calculated using the GenePix Pro3.0 software (Axon, USA). In quantitative analysis, the wild-type–to–mutant signal ratios (W/M) is the spot of wild type probe (denoted as W) to mutant probe (denoted as M), can be employed to detect the genotype of point mutations. We employed an intensity ratio of 5:1 as the threshold for categorizing the positive and negative signals. Used to detect point mutations, the W/M produced unequivocal assignment of samples geno- type, with W/M > 5 for normal subjects, ~1 for heterozy- gotes, and < 0.2 for homozygous mutations. The Q-probe group was used to detect three deletions (α, α, α). Q1 probe was complementary to a 378-base-long fragment of normal α2 gene produced by 5‘-Cy5-cctcaggggacagagctac. Q2 probe was complementary to a 253-base-long fragment of the mutation (α) produced by 5‘-Cy6-cctcaggggacagagctac. Q3 probe was complementary to a 253-base-long fragment produced by linear amplification of PCR product (−50) by 5‘-Cy5-gccatctgggctctgtgttct. Q4 probe was complementary to a 155-base-long fragment produced by linear amplification of PCR product (−α) by 5‘-Cy5-gcaggttcgaggacagctga. When a signal of a deletion (−α, α, α) allele was present, the Q2 probe was used to provide a reliable identification of heterozygous, compound heterozygous and homozygous α thalassemia geno- types. Again, we employed an intensity ratio of 5:1 as the threshold to categorize the positive and negative signals in the Q probe group. The hybridization result with a sample (241-42) heterozygote, αα (−αα) is shown in Figure 1. It is apparent from these results that the discrimination of single-base mismatches is excellent. In addition, the results demonstrate the microarray's ability to detect and discrim- inate both homozygous and heterozygous mutations in human genomic DNA.

To demonstrate the accuracy of the method in genotyp- ing thalassemia, we investigated 1,880 unrelated individu- als whose thalassemia alleles had been typed previously. There was 100% agreement in all 1,880 samples. Based on these results we conclude that the method described here is a valuable technique, suitable for population screening.

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References

Stem Cell Disorders

Lack of mutations in the human telomerase RNA component (hTERC) gene in Fanconi’s anemia

As some patients with Fanconi’s anemia (FA) present excessive telomere shortening correlating with poor out- come, we investigated whether human telomerase RNA component (hTERC) mutations also play a role in telomere shortening in 115 FA patients. Only one patient was heterozygous for the G58A polymorphism. No other mutation or deletion was found. We conclude that hTERC gene mutations do not contribute to telomere shortening in FA.

Fanconi’s anemia (FA) is an autosomal recessive disorder clinically characterized by progressive pancytopenia due to bone marrow (BM) failure frequently evolving to acute leukemia, a variety of physical abnormalities, and increased predisposition to cancer. The onset of BM failure may be vari- able, presenting at older ages in some patients due, at least in part, to a myriad of genetic abnormalities. Eleven comple- mentation groups (A to J) of FA cells have been described. Increased telomere shortening also correlates with BM fail- ure in FA. Telomeres are responsible for the integrity and sta- bility of chromosome ends, and telomere length is maintained by telomerase-mediated addition of telomeric repeats. Exces- sive telomere shortening is observed in other types of BM failure syndromes, such as dyskeratosis congenita (DC) and acquired aplastic anemia. Mutations in genes encoding telom- erase complex components cause the X-linked type of DC (DKC1 gene) and the autosomal dominant type of DC (hTERC gene). Mutations in the hTERC gene are also found in a small subgroup of patients with acquired aplastic anemia.

In this study, we investigated whether hTERC mutations may also contribute to telomere shortening in FA. Peripheral blood samples were collected from 115 patients with FA before bone marrow transplantation and screened for hTERC mutations by direct sequencing, as previously described. Seventy-one were Brazilian patients treated at the Federal University of Paraná, Curitiba, PR, Brazil (37 males and 34 females; 42 were White, 25 Mulatto, seven Black and one Asian); the diagnosis was confirmed by diepoxybutane- induced chromosome breakage analysis. Complementation group analysis was available for 39 Brazilian patients (30 belonged to the FANC A group, four to the FANC C group, and five to the FANC G group). The other 44 cases studied were European patients treated at Hôpital Saint-Louis, Paris, France, and had a Caucasian ethnic background. Fifty-seven percent of these patients were male and 43% were females. Their diagnoses were confirmed by cytogenetics and nitrogen mustard-induced chromosome breakage analysis. Complement- ation group analysis was available for 17 patients (16 were FANC A and one was FANC C). Telomere length was determined in peripheral blood leukocytes by Southern blot in all European patients, as previously reported. Nineteen patients had a telomere shortening below 200 bp/year, whereas in 25 patients telomere shortening was greater than 200 bp/year. Blood samples for genetic analysis were collected after informed consent had been obtained for this study which was approved by the respective local Ethics Committees.

One patient (a 12-year old, African-Brazilian male with FANC A complementation group, homozygous for the 3788-
3790 FANC A mutation) was heterozygous for the G58A transition. No other mutation or deletion was found among these patients. It is important to note, however, that other large deletions or promoter region mutations could not be detected if beyond the limits of our primers. The G58A substitution was previously described in one patient with constitutional aplastic anemia and in two with acquired aplastic anemia by Vuilliamy et al.9 Yamaguchi et al.9 also found two Black patients with myelodysplastic syndrome and one patient with acquired aplastic anemia who were heterozygous for this substitution. More recently, Wilson et al.8 described a 2-year-old Black myelodysplastic patient heterozygous for the G58A transition. Four of eight relatives of this patient carry this gene variant, but none presents any hematologic abnormality. The prevalence of the G58A substitution varies from 4 to 20% in African-Americans and is not a disease-causing mutation, but a common polymorphism, especially among normal Black subjects.9 In the present study, the only patient carrying this substitution was an African-Brazilian. BM failure was diagnosed at the age of 3 years, and the patient had a short stature, low weight, narrow eyelid gap, tenar hypotrophy, and café-au-lait spots. No HLA-matched donor was available, but the BM showed relative response to treatment with oxymetholone and the boy became transfusion independent.

Nucleotide G58, located immediately 3’ of the template, is not phylogenetically conserved in vertebrates, does not influence the telomerase RNA tertiary structure, and does not influence telomerase activity in vitro.8,9 Taken together, these data strongly suggest that this polymorphism is unlikely to play a role in the pathogenesis of BM failure in FA or in other BM failure syndromes.

In conclusion, although excessive telomere length shortening is observed in some patients with FA and seems to predict evolution to aplastic anemia, mutations in the hTERC gene do not contribute to this phenomenon in the setting of FA. However, mutations in other genes encoding other components of the telomerase complex might play a role. Alternatively, increased hematopoietic proliferative demands and/or the oxidative stress in response to BM failure may ultimately lead to increased telomere shortening in FA.

**References**


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**Acute Lymphoblastic Leukemia**

**Molecular diagnosis of leukemic cerebrospinal fluid cells in children with newly diagnosed acute lymphoblastic leukemia**

Cytomorphology and IgH/T-cell receptor γ clonal gene rearrangements detected by polymerase chain reaction (PCR) homo/heteroduplex analysis and direct sequencing were evaluated in cerebrospinal fluid (CSF) free of red blood cells at diagnosis of 37 children with acute lymphoblastic leukemia. Molecular CSF involvement was greater as detected by molecular analysis than observed by morphologic criteria (45.9% vs 5.4%). The 4-year event−free survival was lower in the group with molecularly detected CSF involvement (p = 0.01).

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**Letters to the Editor**

In the majority of treatment centers the diagnosis of central nervous system (CNS) involvement is defined by the presence of pleocytosis of more than 5 cells per cubic millimeters in the cerebrospinal fluid (CSF), with blast cells or the presence of cranial nerve palsies. The detection of CNS involvement has been associated with a high risk of relapse.12 Some studies have also demonstrated that patients with any identifiable blast cells in CSF, as well CSF contamination by blastic cells during traumatic lumbar puncture, have an increased risk of CNS relapse.13 Others, however, did not find this association.5 Conventional cyto- logical analysis has proven useful, but the analysis of cells in CSF, especially when the cell count is low, is more difficult than is widely admitted and nor is it always conclusive.

We analyzed 37 of 66 children with acute lymphoblastic leukemia (ALL) admitted to our institution (18 were classified as having standard risk and 19 as high risk). Twenty-nine patients were excluded, 13 because of the presence of red blood cells in the CSF, suggesting puncture accident, 10 because of the absence of stored CSF or bone marrow at