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Numerical chromosomal changes and risk of development of myelodysplastic syndrome–acute myeloid leukemia in patients with Fanconi anemia

Parinda A. Mehta[a,*], Richard E. Harris[a], Stella M. Davies[a], Mi-Ok Kim[b], Robin Mueller[a], Beatrice Lampkin[a], Jun Mo[c], Kasiani Myers[a], Teresa A. Smolarek[d]

[a]*Division of Bone Marrow Transplantation and Immune Deficiency, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229*

[b]*Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229*

[c]*Division of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229*

[d]*Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229*

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Abstract Fanconi Anemia (FA) is an inherited bone marrow failure syndrome characterized by congenital abnormalities, progressive marrow failure and predisposition to myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and solid tumors. The most common acquired chromosomal aberrations in FA patients are trisomy of 1q and monosomy of chromosome 7; the latter is known to be associated with poor prognosis. A few reports also suggest that gains of 3q are associated with progression to MDS–AML and overall poor prognosis. It is not uncommon for patients with Fanconi anemia to have easily detectable (oligoclonal) chromosomal alterations in their still normal (nonmalignant) marrow, which makes it even more challenging to determine the import of such alterations. We conducted a retrospective longitudinal analysis of fluorescent in situ hybridization (FISH) analysis for gains in 1q and 3q and for monosomy 7 and 7q deletions on 212 bone marrow samples from 77 children with FA treated at our institution between 1987 and 2007. Given the baseline increased chromosomal instability and defective DNA repair in patients with FA, which leads to unbalanced chromosomal aberrations such as deletions, insertions, and translocations, for the purpose of this analysis an abnormal clone was defined as ≥10% abnormal cells. Chromosome 3 and 7 aberrations were associated with increased risk of developing MDS–AML ($P = 0.019$ and $P < 0.001$ respectively), although the significance of chromosome 3 aberrations disappeared when different observation times were accounted for. Gain of 1q alone did not predict development of MDS–AML. In conclusion, children with FA should be followed closely with FISH analyses, because some of the clonal chromosomal abnormalities may be early indicators of progression toward MDS–AML and thus also of the need for hematopoietic stem cell transplantation.

1. Introduction

Fanconi anemia (FA) is a genetic disorder characterized by congenital abnormalities, progressive bone marrow failure, and predisposition to malignancies, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and squamous cell carcinoma of the head, neck, vulva, and uterine cervix [1–3]. The cellular phenotype of FA is characterized by an abnormally high level of baseline chromosomal breakage [4], along with an increased sensitivity to DNA cross-linking or alkylating agents that block DNA replication and RNA transcription [5]. Fanconi anemia is a complex genetic disorder, with 13 genes involved; these genes are reflected in the 13 FA complementation groups: A, B, C, D1 *(BRCA2)*, D2, E, F, G, I, J, L, M, and N [6]. Multiple FA gene products form a nuclear complex believed to function in the DNA damage response and repair pathway [7–11]. The biological steps leading to marrow failure or malignancy are poorly understood.

The most frequent malignancy in FA is AML, with a cumulative incidence approaching 33% by 40 years of

* Corresponding author. Tel.: (513) 636-5917; fax: (513) 636-3549.
E-mail address: parinda.mehta@cchmc.org (P.A. Mehta).

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age; the leukemic cells commonly show clonal chromosomal aberrations [12–14]. The most commonly acquired chromosomal clonal aberrations in patients with FA are gains in a portion of the long arm of chromosome 1 and monosomy of chromosome 7 or loss of a portion of the long arm of chromosome 7. Monosomy 7 and del(7q) have been associated with poor prognosis and progression to leukemia, but otherwise the clinical import and predictive value of transient or even persistent abnormal clones detected by conventional cytogenetic testing remain controversial [15–21].

Here we report a retrospective longitudinal analysis of fluorescence in situ hybridization (FISH) studies looking specifically for gains in chromosomes 1q and 3q and for monosomy 7 and 7q deletions in 212 evaluable marrow samples from 77 children with FA treated at Cincinnati Children's Hospital Medical Center (CCHMC) between 1987 and 2007.

2. Materials and methods

2.1. Patient selection

All patients with FA seen at the FA Comprehensive Care Center at CCHMC who had a bone marrow aspiration performed at least once were eligible for inclusion. Institutional Review Board approval was obtained for the study. The database of all bone marrow samples performed at CCHMC from 1987 to May 2007 was queried for marrow sampling performed for patients with FA as a diagnosis. Bone marrow aspirate and biopsy reports (as well as FISH reports for studies of specific regions of chromosomes 1, 3, and 7) were abstracted, along with clinical data, including the development of MDS or leukemia, or the performance of a hematopoietic stem cell transplantation (HSCT).

FISH was not routinely performed on bone marrow samples from FA patients before 1987. We located additional unstained marrow smears in storage on the marrow samples obtained prior to this date and performed FISH on these slides for this study. Prior to initiating this phase of the study, we refined the technique for this procedure by practicing on stored bone marrow smears using samples that had FISH studies done for clinical care at the time of the original bone marrow study. We demonstrated that performing FISH studies on stored samples (stored for as long as 10 years) was feasible, and that the results were in agreement with the results obtained on the fresh marrow samples. To account for positive correlation among multiple samples taken on the same patients, we conducted generalized estimating equations (GEE) analysis with compound symmetry within patient dependency structure. Diagnosis of MDS–AML was included as a covariate. No significant differences were found. *P*-values from the GEE analysis were 0.945 for chromosome 1 aberration, 0.288 for chromosome 3 aberration, and 0.073 for chromosome 7 aberration, suggesting that FISH studies can be reliable when performed on old stored bone marrow smears.

2.2. FISH methodology

Fluorescent in situ hybridization was performed on fresh bone marrow samples using Vysis probes (Abbott Molecular, Des Plaines, IL) for 1q25, 1p36, 3q27 (*BCL6* locus, B-cell CLL/lymphoma 6), CEP 7 (the centromere of chromosome 7), and 7q31. These probes were used to evaluate the marrow samples for gains in the short or long arm of chromosome 1, gains in the long arm of chromosome 3, and either deletion of the long arm of chromosome 7 or monosomy 7. Each sample was evaluated using fluorescence microscopy, with at least 200 cells evaluated for most patients. Stored marrow smears were evaluated in the same way, with 200 cells examined for each of the probes.

A FISH result was considered abnormal if the number of cells with an abnormal finding exceeded that found in normal marrow samples by at least two standard deviations. In general, the finding of $\geq$4% abnormal cells by this method is considered to be abnormal. However, given the baseline increased chromosomal instability and defective DNA repair in patients with FA, which leads to unbalanced chromosomal aberrations such as deletions, insertions, and translocations, for the purpose of this analysis, the results were categorized conservatively, as representing an abnormal clone if the percent of abnormal cells was $\geq$10%.

2.3. Marrow aspirate and biopsy studies

The 500 cell differential counts performed on Giemsa-stained marrow smears were examined closely for evidence of myelodysplasia or leukemia by standardized methods. Each marrow sample was coded by an experienced hematopathologist at CCHMC from 0 to 4 (none, minimal, mild, moderate, and severe, respectively) for evidence of dysplasia in the erythroid, myeloid, and megakaryocytic cell lines. The hematopathologist was unaware of the clinical picture or the results of the cytogenetics or FISH studies.

2.4. Statistics

Patients were grouped into two categories, those who did and those who did not develop MDS–AML, and compared for frequency of FISH abnormalities. Fisher's exact test was used to determine whether the presence or absence of chromosome 1, 3, and 7 aberration is associated with risk of MDS–AML. Cumulative risk of MDS–AML over time was estimated using Kaplan–Meier survival curves. The log-rank test was used to test the association between the presence or absence of chromosome 1, 3, and 7 aberration and the risk of MDS–AML, accounting for different follow-up times.