the majority of sickled cells. As expected, the HbF percentage correlated positively and negatively with the percentage of normal and abnormal cells, respectively (Figure 1B–C).

Fluorescent labeling of HbF allowed us to discriminate non-F-cells (Figure 1Di–ii) from F-cells (Figure 1Diii–iv), and analyze their shape as captured in the brightfield images. The images confirmed the prediction that some RBCs with detectable HbF content still sickle (Figure 1Div), and also identified RBCs that are resistant to sickling despite no detectable HbF (Figure 1Dii). The percentage of non-F-cells sickling on deoxygenation was significantly higher than among F-cells (20.08% [95% CI 15.56–24.60] vs 13.44% [95% CI 10.21–16.68], P < .0001). This difference was statistically significant both in patients not taking HU and in treated patients (Figure 1F). F-cells from patients on HU sickled significantly less than F-cells from patients off HU, and the same difference was borderline significant when comparing non-F-cells from patients on HU with those off HU.

Our observations support that the threshold used for detection of F-cells is not the same threshold that defines protection against sickling. Similar to previous investigators, we also have found that the very high concentration of intraerythrocytic hemoglobin poses a difficult challenge in achieving the antigen saturation with anti-γ-globin antibody that is necessary to measure accurately the amount of HbF per F-cell,2 which will be needed ultimately to test fully the mathematical modeling predictions of Steinberg et al. Differential RBC susceptibility of non-F-cells to sickling between patients on or off HU also supports the existence of additional beneficial mechanisms of HU other than HbF induction.3 The identification of factors besides HbF that modulate sickle hemoglobin polymerization may help in the design of novel therapies for HU-resistant SCA patients.

Kleiber Yotsumoto Fertrin
Sickle Cell Vascular Disease Section, Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD

Eduard J. van Beers
Sickle Cell Vascular Disease Section, Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD

Leigh Samsel
Flow Cytometry Core Facility, National Heart, Lung, and Blood Institute, Bethesda, MD

Laurel G. Mendelsohn
Sickle Cell Vascular Disease Section, Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD

Rehan Saiyed
Sickle Cell Vascular Disease Section, Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD

To the editor:

Misleading results from saliva samples of patients post-BMT in exome analyses

We routinely perform clinical exome sequencing on blood samples from patients without bone marrow transplants (BMTs). Moreover, we accept saliva samples due to the acceptable DNA quality, noninvasiveness, and improved percentage of participants.1 We assess the quality at the wet laboratory as well as the bioinformatics process which includes monitoring the number of variants after inheritance model filtering. Recently, a 4-member family (mother, father, female proband, and affected sister) was referred for exome analysis due to combined immune deficiency of undetermined genetic etiology in the proband. The samples that were submitted for analysis were blood for both parents and saliva for both affected sisters. All of the quality controls passed the set thresholds except for the inheritance...
model variants which deviated from their normal range. Specifically, the number of de novo variants was inflated, whereas the opposite was true for the autosomal-dominant variants (Figure 1). We hypothesized that a switch of DNA samples had occurred either during collection or processing in the laboratory. Short tandem repeat (STR) analysis confirmed that the relationship among family members was correct, however, unidentified peaks at all STR loci were present in both sisters, including the presence of a Y chromosome peak suggesting contaminating male DNA. This confirmed that the variant number discrepancy of the inheritance models can be attributed to the male DNA contaminating the proband’s sample. Upon further investigation with the referring physician, it was indicated that both sisters had undergone BMTs from 2 distinct unrelated male donors.

Several XY fluorescence in situ hybridization (FISH) studies showed that a small percentage of bone marrow donor-derived cells can repopulate every part of the gastrointestinal tract (0%-4.6%), liver (0%-7%), and epithelial cells (0.2%-7.3%) in humans.\(^2\)\(^4\) However, other XY FISH studies reported higher levels of donor cells in buccal (0.8%-12.7%), liver (4%-43%), mouthwash (25%), and fingernail (8.9%-72.9%) samples.\(^3\)\(^5\) An STR study reported 25% of donor leukocytes in mouthwash cell pellets from BMT patients.\(^5\) Similarly, DNA extracted from fingernail samples of BMT patients showed coexistence of the donor pattern of the STRs (8.9%-72.9%).\(^5\) Consistent with 3 studies that showed high levels (>25%) of donor, based on STR analysis, our saliva samples had 61% and 28% and cytobrushes had 37% and 12% of donor in the proband and sister, respectively. Additionally, blood XY FISH analysis of the proband and sister revealed an incidence of 99% and 62%, respectively.

Several points should be noted. Saliva samples may have a higher level of donor cells than anticipated that will interfere with genotyping assays including exome sequencing. Thus, the level of donor chimerism must be established by STR analysis prior to genetic testing. Second, the inheritance modeling used in the exome analysis pipelines is sensitive to sample switching and contaminating DNA. Therefore, the importance of quality-assurance measures at each step of a complex exome test is critical to ensure accuracy. Third, fibroblast cells should be the sample choice for genetic testing of BMT patients, otherwise exome sequencing results may be misleading if saliva samples or buccal samples are processed.

References

Misleading results from saliva samples of patients post-BMT in exome analyses

C. Alexander Valencia, Subba Rao Indugula, Abhinav Mathur, Chao Wei, Jenice Caitlin Brown, India Cole, Sarah Dell, Jessica Connor and Kejian Zhang