Letter to the Editor

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Ambiguous pharmacogenetic genotyping results in a patient with bone marrow transplantation

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To the Editor,

This report describes a case of ambiguous pharmacogenetic test results from an oral swab specimen in a patient with previous bone marrow transplantation (BMT). These results indicate chimerism of donor and recipient genotypes in the DNA isolated from the patient's oral samples. Many laboratories do not accept oral samples for genetic testing of BMT recipients, as chimerism has been shown to be present in these samples [1]. In order to identify the recipient genotype, practice guidelines have suggested the use of fibroblast cultures, which are time-consuming to obtain and require specialized equipment that may not be available in a routine genetic testing laboratory [2]. Hair follicles have recently been used as a source of recipient DNA in one study but chimerism with donor-derived DNA has been observed in another study [2, 3]. In our report, we used trio analysis and donor genotyping of oral samples to infer the recipient genotype in pharmacogenes.

In this case, the patient (male, aged 8) was diagnosed with leukemia at the age of 2 and was treated by an allogeneic bone marrow transplant with his father as the donor. Millennium Health received an oral swab sample from the patient for its polymerase chain reaction (PCR)-based pharmacogenetics test (14 genes: *CYP2B6* (*1, *4, *6, *9, *18), *CYP2C9* (*1, *2, *3, *5, *8, *11), *CYP2C19* (*1, *2, *3, *4, *8, *17), *CYP2D6* (*1, *2, *2A, *3, *4, *4N, *5, *6, *9, *10, *17, *29, *35, *36, *41, allele-specific gene duplication), *CYP3A4* (*1, *22), *CYP3A5* (*1, *3), *DRD2* (rs1799732 Ins/Del), *HLA-B*15:02*, *HTR2C* (rs3813929C/T), *VKORC1* (rs9923231A/G), *COMT* (rs4680A/G), *OPRM1* (rs1799971A/G), *MTHFR* (rs1801131A/C, rs1801133C/T) and *UGT2B15* (*1, *2). The patient's lab test report indicated that results for nine pharmacogenes were unable to be determined. After the initial test results were inconclusive, the patient was tested twice again. A total of three separate oral samples were collected (the first two were collected using the DNA Genotek (Ottawa, Canada) ORAcollect OC-100 oral swab device and the third sample was saliva collected using the DNA Genotek OrageneDx OGD-510 device). Genotyping was performed using real-time PCR in a multiplex assay. The patient's test results failed laboratory quality control standards in all three attempts, prompting further investigation upon request from the treating physician. At this time, it was discovered that the patient was a BMT recipient, and a study protocol was developed and received Institutional Review Board (IRB) approval (Aspire IRB, Santee, CA) as per the Declaration of Helsinki. Samples from the mother and father (who was also the BMT donor) were also obtained.

Genotyping of the patient's (proband) three samples revealed a mix of interpretable and uninterpretable results, consistent with chimerism. Some results clearly aligned with genotype clusters and thus were interpretable, and other results were found outside of genotype clusters, which were uninterpretable (Figure 1A, B). The uninterpretable results from each of the three samples had slightly different positions compared to the genotype clusters, ranging from slightly out-of-cluster to substantially out-of-cluster. Comparison with the father's genotype revealed that for markers where the genotype of the proband matched that of the father, the proband results had high genotype confidence and were interpretable. For markers where the genotype of the proband mismatched that of the father, the results were shifted outside of the clusters and the genotype confidence was low (Figure 1C). As such, genotyping results were obtained for the proband at *COMT*, *CYP3A4*, *DRD2*, *HLA-B*15:02* and *MTHFR* genes (Table 1). Of these, abnormal predicted phenotypes were found for *COMT* and *MTHFR* [4–7].

With genotypes from both the mother and father, we were able to predict additional proband genotypes at *HTR2C* and *VKORC1* using Mendelian inheritance (Table 1). Finally, using the parent genotypes along with

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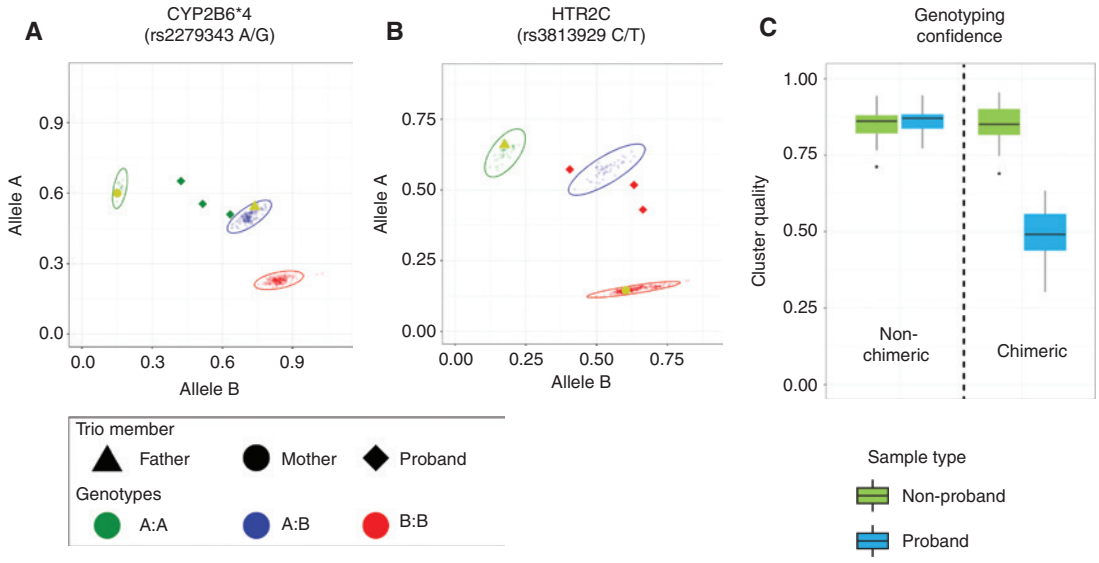

Figure 1: (A, B) Scatter plots for SNP genotyping results with mismatched patient and donor genotypes. Results are shown for each of the two representative markers from five genotyping runs which contained the mother, father (BMT Donor) and three separate patient (proband) samples. All other samples included in the runs are displayed as small semi-transparent circles. Genotypes were assigned based on cluster profiles. The ellipses represent the genotype cluster, within which a genotyping call would be made (points outside the cluster have low confidence). The patient's signal (diamond) is a consistent outlier from the genotyping clusters, and as such would not be interpreted. The patient's genotypes at these markers were presumptively assigned by trio analysis as shown in Table 1. (C) Box-and-whisker plot of genotyping quality scores for proband and non-proband samples. Genotyping result quality is shown for markers in which the genotype matched (non-chimeric) or did not match (chimeric) the donor genotype. Proband genotypes are evaluated separately from non-proband genotypes to show the relationship of cluster confidence for patient results when similar or different to parent/donor results. Cluster quality is computed by subtracting the average distance of a point to members of its own cluster from the average distance of a point to members of the next closest cluster and dividing this difference by the larger of the two distances. A score of 1 identifies a correct unambiguous assignment and a score of 0 is interpreted as an arbitrary assignment.

the patient's raw genotyping results relative to other samples, we were able to infer presumptive genotypes at *CYP2B6*, *CYP2C19*, *CYP2C9*, *CYP2D6*, *CYP3A4/CYP3A5*, *OPRM1* and *UGT2B15*. Notably, these included genotypes that are associated with increased (ultrarapid) metabolism for *CYP2C19* and poor metabolism for *CYP2B6* [8]. While these assignments are presumptive, other phenotypes can be ruled out; for example, poor metabolizer phenotype for *CYP2C19* can be ruled out based on the parent genotypes.

With regard to clinical relevance, the patient's response to medications is likely to be impacted by both donor and recipient phenotypes. Many of the genes tested code for medication metabolism enzymes produced by liver hepatocytes. Mature hepatocytes can develop from bone marrow-derived donor cells [9–11], and the patient's liver is likely to be a chimeric mix of donor-derived and recipient hepatocytes. Donor hepatocytes have been shown to account for 5% of total hepatocytes as early as 13 days after transplantation [10], and in some cases have been found at higher levels (as much as 40% of total hepatocytes) months after transplantation [9]. When both donor and recipient phenotypes are the same, the clinical relevance is straight-forward. Normal phenotypes were predicted for *CYP2D6* for both donor and recipient, indicating that the patient may not experience a genetic influence on CYP2D6-metabolized medications such as codeine or risperidone [8]. When donor and recipient phenotypes are different, then the impact on medication response is less clear but can still be informative; e.g. for *CYP2C19*, the donor phenotype is normal while the recipient phenotype is ultrarapid metabolizer. As the extent of chimerism in the patient's liver is unknown, the patient's ability to metabolize CYP2C19 substrates, such as sertraline [8], cannot be predicted. However, the patient is unlikely to poorly metabolize such medications. With regard to pharmacodynamic genes, both the donor and recipient phenotypes were abnormal at *COMT*, *MTHFR* and *VKORC1*, and thus the patient may not respond to medications associated with those genes [7, 12, 13]. On the other hand, both the donor and recipient had normal responder phenotypes for *DRD2*, which may impact the patient's response to antipsychotics [14]. Similarly, the patient does not have the *HLA-B*15:02* allele in either

Table 1: Genotypes and predicted phenotypes.

Gene	Mother		Father/donor		Proband	
	Genotype	**Predicted phenotype**	**Genotype**	**Predicted phenotype**	**Genotype**	**Predicted phenotype**
COMT	A/A	Normal activity	A/G	Reduced activity	A/G	Reduced activity
CYP2B6	*6/*6	Poor metabolizer	*1/*4	Normal metabolizer	*4/*6	*Intermediate metabolizer*
CYP2C19	*1/*17	Ultrarapid metabolizer	*1/*1	Normal metabolizer	*1/*17	*Ultrarapid metabolizer*
CYP2C9	*1/*1	Normal metabolizer	*1/*3	Intermediate metabolizer	*1/*1	*Normal metabolizer*
CYP2D6	*1/*35	Normal metabolizer	*1/*5	Normal metabolizer	*5/*35	*Normal metabolizer*
CYP3A4; *CYP3A5*	*1/*1; *3/*3	Intermediate metabolizer	*1/*1; *1/*3	Extensive metabolizer	*1/*1; *3/*3	*Intermediate metabolizer*
DRD2	C/C	Normal responder	C/C	Normal responder	C/C	Normal responder
*HLA-B*15:02*	Negative	Typical risk of hypersensitivity	Negative	Typical risk of hypersensitivity	Negative	Typical Risk of hypersensitivity
HTR2C	C/C	Normal expressor	T/–	Variant expressor	C/–	Normal expressor
MTHFR	C/T (C677T); A/A (A1298C)	Reduced activity	T/T (C677T); A/A (A1298C)	Greatly reduced activity	T/T (C677T); A/A (A1298C)	Greatly reduced activity
OPRM1	A/G	Reduced expressor	A/G	Reduced expressor	*A/A*	*Normal expressor*
UGT2B15	*1/*1	Normal metabolizer	*1/*2	Intermediate metabolizer	*1/*1	*Normal metabolizer*
VKORC1	G/G	Normal activity	A/A	Reduced activity	A/G	Reduced activity

Genotypes and predicted phenotypes were assigned as per standard clinical genotyping based on cluster profiles. Additionally, proband genotypes at HTR2C and VKORC1 were predicted using Mendelian inheritance using parental genotypes. Shaded fields identify presumptive genotypes, which were inferred by the donor genotype as well as the location of the patient's raw genotyping results relative to other samples within each assay.

donor or recipient genotypes, and thus does not have increased risk of severe hypersensitivity in response to carbamazepine [15].

In conclusion, our results indicate that bone marrow transplants can confound genotyping even when oral samples are used for testing. Our data illustrate that donor cells can be obtained with oral specimens from both oral swab and saliva collections, and can interfere with the proper determination of a patient's genotype. Unlike the current practice of culturing fibroblasts to obtain patient genotypes, this method incorporates both recipient and donor genotypes. While many laboratories do not accept samples from BMT patients, we have shown that genotyping of the patient along with the donor and parents may allow for identification of test results than can be regarded as conclusive, as well as potentially useful presumptive information about other pharmacogenes. With this process, BMT patients and their doctors may be able to access genetic data that can be used to guide medication selection.

Author contributions: FE: sample processing, data collection and analysis; MD: data analysis and drafting the manuscript; AD: interpretation, drafting and critical revision of the manuscript; TM: interpretation, data analysis and critical revision of the manuscript; JV: conception, design, interpretation, drafting and critical revision of the manuscript. All authors read and approved the final manuscript.

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