Misinterpretation of \textit{TPMT} by a DTC Genetic Testing Company

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23andme has suspended marketing of health-related reports due to US Food and Drug Administration approval violations. This has fostered discussions on the actual risks associated with consumer use of these reports. In the case described below, rare genotypes for the gene encoding thiopurine methyltransferase (\textit{TPMT}) were misinterpreted by a direct-to-consumer (DTC) company, and risk calculations for breast cancer were offered when accuracy was not possible from the available information. Politics aside, these examples illustrate risks associated with DTC genetic testing without professional interpretation.

Dr J. is a half-Ashkenazi, half-Irish man with a family history of Crohn’s disease. He decided to obtain a direct-to-consumer (DTC) genetic test. The test performed was one of two versions of an Illumina HumanHap550+ BeadChip platform used by the company at the time, which included single-nucleotide polymorphisms (SNPs) from the standard HumanHap550 panel, augmented with a custom set of ~25,000 additional SNPs selected by company staff.\textsuperscript{1} The report stated that for \textit{TPMT} he has “one *3B mutation and one *3C mutation, a homozygous nonfunctional alleles pattern resulting in significant enzyme deficiency. A person with these mutations has an increased risk of toxicity when treated with thiopurine drugs at standard doses.”

The report went on to state “See the technical report for more details. May have other mutations in the \textit{TPMT} gene (not reported here).” However, the top of the report reads: “Only a medical professional can determine whether a thiopurine drug is right for a particular patient. The information contained in this report should not be referred to as the “*3B mutation” or the “*3C mutation”—both of these SNPs are also in the *3A allele, and the C>T SNP at rs1800460 and T>C SNP at rs1142345 can rarely occur in other \textit{TPMT} alleles as well.

The Clinical Pharmacogenomics Service (CPS) at Boston Children’s Hospital also became suspicious that the \textit{3B/3C} genotype was very unlikely for Dr J. Because \textit{TPMT*3A} alleles contain both the SNPs found in the \textit{TPMT*3B} and \textit{TPMT*3C} alleles, most assays (including the DTC test discussed here) cannot distinguish between the \textit{TPMT*1/TPMT*3A} (intermediate enzyme activity) and the \textit{TPMT*3B/TPMT*3C} genotype (no or low enzyme activity).\textsuperscript{2} In addition to Dr J’s result, the CPS learned of two other results with the exact same \textit{3B/3C} call around the same time period from the same DTC company. The first was a member of the Boston Children’s Hospital CPS service and the second was her spouse, who was adopted as a child. It was statistically improbable that three people who knew each other but were unrelated, with vastly different phenotypes and ethnic backgrounds, were all \textit{3B/3C}. With an allele frequency of 0.000461 for *3B (in Caucasians and 0.00562 in Middle Eastern populations),\textsuperscript{3} it was much more likely that the three *3B/3C calls were actually *1/3A (the *3A allele frequency is 0.0356 for Caucasian and 0.0114 for Middle Eastern populations). However, that was not mentioned on the front page of the DTC report, and although Dr J. has a PhD, he did not read the “technical report,” which discusses the fact that *3B and *3C often exist on the same gene copy, defined as the *3A allele. This lack of clear, concise, and understandable front-page information led Dr J. (and others) to assume that the *3B and *3C variants were on different chromosomes, resulting in him having a homozygous variant phenotype.

The CPS genotyped Dr J’s parents, wife, and children to confirm the *3B/*3C finding using single-gene Sanger sequencing in a Clinical Laboratory Improvement Amendments (CLIA)–approved laboratory. DNA was collected by the Manton Center for Orphan Disease Research, Gene Discovery Core, under informed consent governed by the institutional review

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board of Boston Children's Hospital. TPMT sequencing was performed by Claritas Genomics (Cambridge, MA). Dr J.’s parents were *1/*1 and *1/*3A, his wife was *1/*1, and his children were also *1/*1 and *1/*3A. Thus, it would be virtually impossible for Dr J. to be *3B/*3C (Figure 1b). This testing confirmed his *1/*3A status, showing that the DTC summary is misleading.

If it has not been determined if an individual’s TPMT SNPs are on the same or opposite alleles, caution should always be applied when interpreting this genotype, should there be discordance between the inferred diplotype and the phenotype. If Dr J. required treatment for his Crohn’s disease with 6-mercaptopurine and was able to convince the physician of his *3B/*3C status with the report he held in his hands, it would not be catastrophic. Per the current Clinical Pharmacogenetics Implementation Consortium guideline,3 Dr J. would be prescribed a dose that is only 10% of the standard dose. This is in comparison with the recommended dose reduction of 30–70% for patients with the *1/*3A genotype. There could be disease progression or worsening of symptoms, in addition to unnecessary discomfort, if he was started on the significantly lower dose, and valuable time would be lost in adjusting his dose.

However, consider a patient in a similar situation with a form of leukemia that is standardly treated with a thiopurine. It is not likely that a DTC result would be used in leukemia treatment dosing, but it is possible. A wary doctor might use this reported genotype to prescribe a dramatic 90% reduction in dose, which could result in less effective (and therefore more expensive) treatment, with serious consequences including progression of disease. The DTC TPMT report contains the same information as reports issued from hospital-utilized CLIA–approved laboratories, in which results have been analytically and clinically validated. This particular report contains information that serves no other purpose than to give information about thiopurine dosing. It is both a burden and a real risk to clinicians to simultaneously consider and discount the pharmacogenomics content. In medicine, there are decision points that make just-in-time verification much more difficult (for the Boston Children’s Hospital CPS, the clinical turnaround time in a CLIA–approved laboratory currently averages 7 days for most tests), and the provider is left to make decisions with all available information.

Even though the DTC company offered consultations with a genetic counselor (at a premium), the most reasonable interpretation of the genotype was not presented clearly, and patients may not realize that they need interpretative support. Just as the Personal Genomes Project requires that potential participants pass a test on the risks of reidentification, perhaps truly informed consent on the risks of incomplete or misinterpreted information is necessary here.4

Mrs J. has a family history of breast cancer. The DTC company provided false reassurance when the breast cancer summary classified her as having “typical risk,” with a four-star confidence rating (Figure 1c, screenshot taken in November 2013. This appears to be clarified as of February 2014). Although this company tested for the three specific predominant BRCA mutations in Ashkenazi Jews (with appropriate disclaimers), there are hundreds of different mutations in non-Ashkenazis (Mrs J. is not of Ashkenazi descent), and an overall “breast cancer risk” score was presented on a summary page without information on most of those known causative mutations. Even Ashkenazi women with a strong family history need to be sequenced comprehensively for nonfounder mutations and for mutations in the other genes that cause or are associated with the disease.5

Figure 1 DTC interpretation and pedigree. (a) Screenshot of TPMT interpretation given by the DTC company. (b) Screenshot of TPMT alleles for pedigree. (c) “Four-star” confidence rating for “breast cancer risk,” although most known causative mutations for breast cancer are not included in the equation. DTC, direct-to-consumer; TPMT, thiopurine methyltransferase.
positive BRCA mutation finding from this DTC company may be actionable, but any “negative” result may lead the individual to falsely interpret her breast cancer risk from the summary and would be actively, and dangerously, misleading.

We are not advocating the end of DTC testing, nor are we universally denouncing DTC genetic testing companies. However, we feel that the interpretations must be accurate and reasonable, with adequate and freely available interpretation support for consumers and physicians.

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CONFLICT OF INTEREST
The authors declared no conflict of interest.