TITLE: Predictive genetic testing of a bone marrow recipient – ethical issues involving unexpected results, gender issues, test accuracy, and implications for the donor.

Authors: A. Sexton[1*], L. Rawlings[2*], M. Jenkins[3], and I. Winship[1]

[1]Familial Cancer Centre, Royal Melbourne Hospital, 2-Centre, Grattan St, Parkville VIC 3052 Australia

[2]Institute for Medical and Veterinary Science, Genetics & Molecular Pathology, Frome Rd, Adelaide, SA, Australia

[3] MEGA, School of Population Health, University of Melbourne, Parkville, Melbourne 3052, Australia

* AS and LR are both primary authors of this work.

*Corresponding author: Dr Adrienne Sexton, Royal Melbourne Familial Cancer Centre, Level 2-Centre, Royal Melbourne Hospital, Grattan St, Parkville VIC 3050, Australia

T: +613 9342 7151

F: +613 9342 4267

E-mail: adrienne.sexton@mh.org.au

Suggested running head: Case report on practical and ethical issues in genetic testing post-bone marrow transplant

Abstract

We present a case where an apparently straightforward Lynch syndrome predictive genetic test of DNA from a blood sample from a woman yielded an unexpected result of X/Y chromosome imbalance. Furthermore, it demonstrates the complexities of genetic testing in people who have had bone marrow transplants. This highlights the potential for multiple ethical and counselling challenges, including the inadvertent testing of the donor. Good communication between clinics and laboratories is essential to overcome such challenges and to minimise the provision of false results.

Key words: genetic counselling, ethical, donor, recipient, genetic test, Lynch syndrome, stem cell transplant, incidental findings

Introduction

Predictive genetic testing for cancer susceptibility is usually technically straightforward, as the test does not require extensive analysis of an entire gene, but simply determines whether a specific mutation known to be segregating in a family is present or absent, by comparing with test results of a familial positive control.

Lynch syndrome is caused by a germline mutation in one of four mismatch repair genes: *MLH1*, *MSH2*, *MSH6* and the most recently discovered gene *PMS2*. People with Lynch syndrome have a high risk of colorectal and endometrial cancer (and also an increased risk of some other cancers including stomach, ovary, small intestine, biliary tract, and urinary tract cancers) with the risks varying depending on which gene is involved (Win et al. 2012). Here we discuss a family with a *PMS2* mutation c.989-?_1144+?del (p.?) leading to a deletion of exon 10. This mutation has been previously reported in other families by Senter et al. (2008).

In the past eight years, more data have become available on *PMS2* as an autosomal dominant cause of Lynch syndrome (Herkert et al. 2011; Senter et al. 2008; Worthley 2005). Biallelic *PMS2* mutations cause a distinct phenotype involving a high risk cancer from early childhood and a predisposition to gastrointestinal adenomas (Herkert et al. 2011). Mutation detection in *PMS2* is challenging due to the presence of multiple highly homologous pseudogenes. Current methods seek to enhance the detection of the real *PMS2* gene and reduce the selection of the pseudogenes by targeting the few differences in nucleotide sequence between them (Vaughan et al. 2010; Vaughan et al. 2011). Phenotypic testing for signs of mismatch repair deficiency of bowel and endometrial tumours is useful in identifying patients with *PMS2* mutations, as *PMS2* mutations are associated with high microsatellite instability and loss of *PMS2* expression as detected by immunohistochemistry (Hendriks et al. 2006). There

is some evidence that the cancer risks for *PMS2* Lynch syndrome are lower than for the other three genes, with colorectal cancer lifetime risk to age 70 years estimated at 15-20%, and at 15% for endometrial cancer (Senter et al. 2008).

The literature regarding genetic testing in bone marrow recipients has focused on HLA testing for donor matching. The extent of chimerism for donor cells in blood post-transplant (Lion et al. 2012) will clearly be a potential confounding factor in subsequent genetic testing. The case we present here highlights some of the ethical, practical and counselling issues.

Case Report

This family had participated in the Australasian Colorectal Cancer Family Study (ACCFS) (Winship and Win 2012). The participation in the ACCFS (prior to the scope of this case report) was approved by the Human Research Ethics Committee of the University of Melbourne (IRB), according to the National Statement on Ethical Conduct in Human Research (2007). Research testing of blood samples from participants for mutations in DNA mismatch repair genes had been ongoing over the past ten years. Approximately ten years after enrolment, participating members of this family were notified by the study that their family's DNA had been examined and this had identified information relevant to them and their relatives. As the results were obtained under research conditions (i.e. not clinical conditions), they were also informed that if they wanted this information, the ACCFS would organise a referral to a clinical genetics service. The clinical service would offer genetic counselling and genetic testing in an accredited diagnostic laboratory. The research protocol does not allow identifying information about any participants to be released without written consent of the participant. In this case, the clinic was informed that a *PMS2* deletion mutation was identified in at least one family member. The pedigree (Fig. 1) was provided

by the client to the clinic. The client, a 58 year old woman who had a history of melanoma and leukaemia in her early 50s and a benign parotid tumour in childhood, wanted testing for the benefit of her three children. She reported that one of her brothers had bowel cancer at 46 years of age, her mother had bowel cancer at 70 years of age and two of her mother's cousins had bowel cancer. Breast and ovarian cancer history (not shown, for de-identification purposes) on the maternal side was accounted for by another gene, for which our client's parent tested negative. Her father had gastric cancer in his late 50s, but there was no known bowel cancer history reported on the paternal side. Her unaffected brother had not requested clinical testing at that stage, and therefore clinicians in our service offered testing to our client and her elderly parents, who chose to attend together.

The normal protocol for predictive testing for Lynch syndrome is to collect two blood samples, separated by time and analysed independently, so that a final result can be delivered. Accordingly, the request for predictive *PMS2* testing by MLPA comprised two 10ml peripheral blood samples in EDTA which were received and manually extracted using PureGene® kit and robotically using QIAGEN QIAcube®. The extracted DNA samples were tested for the known *PMS2* exon 10 deletion using MRC Holland MLPA SALSA® kit P008-B1 and analysed by fragment analysis on an Applied Biosystems 3730 DNA analyser. Upon testing, a negative test result was determined, but in addition, both X and Y chromosome control markers were detected.

The clinical authors' (AS and IW) first contact with this family was to review the test results of the predictive test for the *PMS2* gene mutation and provide these results to the client. Prior to the results appointment, the diagnostic laboratory requested repeat blood samples to clarify a potential sample mix-up. The second blood collection yielded the same unexpected result: the Y chromosome control marker used in the deletion kits was detected in our female client's sample. This was the first time that the laboratory had found such a result. After

discussion among clinical and laboratory staff, both the first and second samples were tested with human identity markers using 17 microsatellite loci, including X and Y markers, which confirmed that all samples were from the same patient. The family-specific mutation was not present in any of the samples. On review of the pedigree, the client was recorded as having three natural children.

When providing the results to the client, the need to explain the uncertainty about the Y chromosome result raised issues of individual privacy and sensitive information, particularly since she had chosen to receive her results along with her parents. Therefore we spoke to her alone first, framing this as routine so as not to cause alarm, before asking her parents to join us. We explained that there was uncertainty about the accuracy of her *PMS2* result due to the presence of the Y marker, and discussed a range of explanations, such as the possibility of a Y marker chromosome or other chromosome rearrangement which may account for the result. She was surprised by this result but not distressed, and summarised it in her own words as showing that she was "a little bit man." On questioning, she did not have any signs of gender imbalance nor any fertility problems, having had three uncomplicated pregnancies. Our counselling approach was to explain that the presence of a complete Y chromosome was unlikely. We discussed that a molecular karyotype could provide some clarity about any chromosome abnormalities and raised the possibility of chromosomal implications for her children.

During this discussion and review of her medical history, we elicited a previously unrecorded fact that she had undergone a bone marrow transplant four years previously, with her brother (unaffected by cancer) as the donor. This explained the presence of the Y chromosome, but cast doubt on the accuracy of her *PMS2* result because other (donor) DNA was present. Our client was happy to proceed with *PMS2* testing of buccal samples and with the microarray analysis.

We later invited her parents to join the consultation, and we explained that her father had the *PMS2* deletion and the implications for his side of the family. Her mother was surprised at her own negative result, as her side of the family had been extensively investigated for cancer predisposition, and she had had bowel cancer herself. She took on the role of communicating the information to her husband's relatives. Our client chose to share her own uncertain result with her parents without hesitation.

Buccal sample results were equivocal, as some donor DNA contamination was still detected, so the laboratory requested skin biopsy samples. Knowing that the referral to our clinic had been generated as a result of research participation, we investigated whether any DNA remained in storage with the researchers of the Australasian Colorectal Cancer Registry, since her participation had been several years prior to the bone marrow transplant. With her consent, the DNA stored by the research laboratory was transferred to the diagnostic laboratory for the *PMS2* test. Definitive testing on this sample confirmed that she did not carry the mutation in the *PMS2* gene. The microarray result showed complete chimerism for donor (male) DNA, suggesting that the predictive testing on all four clinical samples provided prediction and a risk assessment for the donor rather than the female patient.

The clinical authors (AS and IW) were then able to explain the negative *PMS2* result from the pre-transplant DNA, with counselling to acknowledge and address the previous uncertainties and multiple sample types and to allay any doubts and confusion. Our client reached her own accurate conclusion that in doing the original *PMS2* test on her blood, we had actually inadvertently tested her brother. While acknowledging that the test result was a likely reflection of her brother's genetic status, we emphasised the need for him to have a predictive test to confirm this, and he subsequently contacted the clinic regarding predictive testing.

Discussion

The initial question raised in the laboratory was whether the laboratory has a responsibility to report this incidental finding to the clinician. An incidental finding is defined as: "a finding concerning an individual research participant that has potential health or reproductive importance and is discovered in the course of conducting research but is beyond the aims of the study" (Wolf et al. 2008, p. 219; Wolf 2008).

There are limited ethical guidelines regarding the reporting of incidental findings. In a study involving the professional opinions of 16 genetic specialists, there was >80% concordance to report the incidental finding in a whole exome/genome sequencing assay of a variant of known pathogenicity, but only 29-33% consensus to report the incidental detection of a missense variant (Green et al. 2012). Similar discussions have been reported in the field of imaging (The Royal College of Radiologists 2011). Given the recent release of The American College of Medical Genetics and Genomics recommendations on incidental findings in exome/genome sequencing, this case report is a timely example of the complex ethical decisions and communication between patient, clinician and laboratory (Green et al. 2013). It was the diagnostic laboratory scientist's (LR) decision in this case to make contact with the clinical team, because ignoring a factor that indicated a potential inaccuracy could have led to a false test result, and therefore had significant clinical implications.

If medical history about bone marrow transplantation has not been elicited, this can lead to the provision of inaccurate genetic results. This is especially problematic if the recipient is the same sex as the donor, because the genetic chimerism would remain undetected (for predictive tests involving DNA sequencing, the patient's gender would not be detected at all). Furthermore, it raises other major ethical quandaries, including inadvertent testing of the donor, and potential distress for the recipient due to a result showing an opposite gender

marker. In this case, the predictive test result was negative in the donor blood cells, but a positive result would have caused an ethical dilemma of knowing about a serious potential risk for the donor without their consent. The consultand would receive equivocal personal data, but might feel beholden to inform the donor. We were unable to find data on whether these situations have previously occurred in the familial cancer field.

Confounding of genetic results due to bone marrow transplantation has been reported in a few other cases, including a false negative genetic result for Factor V Leiden (Crookston et al. 1998). In that case, the false negative result occurred because the genetic test was done on peripheral blood cells, but the patient had received a stem cell transplant and the normal donor cell DNA masked the patient's own Factor V genetic status.

The complicated testing implications post-transplant also raised the question for our client that "If my brother [bone marrow donor] has the Lynch syndrome gene problem, and I now have his cells, is there an increased cancer risk for me?" We counselled that an increased risk of Lynch syndrome cancers due to circulating *PMS2* donor cells was very unlikely.

The problematic progression of genetic testing for *PMS2* raised the question for our client of the risk of transmission of a hereditary condition via a related donor. This has been noted in other conditions, for example leukaemia originating from cells of a donor with Bloom syndrome (Bielori et al. 2003), and deep-vein thrombosis resulting from a donor liver with a Factor V Leiden mutation (Willems et al. 2003). Regarding hereditary cancer, genetic risks acquired from bone marrow donors could be an important consideration in syndromes with increased risk of haematological cancers such as Li Fraumeni syndrome, or when organ transplantation is required and there is a possible hereditary cause, such as genetic kidney disease. This has the potential to occur in Lynch syndrome, and one case report documents testing of related kidney donors to exclude a transplant affected by the familial *MSH2*

mutation (Lynch et al. 2003). Lynch et al. (2003) discuss that a kidney transplant from a donor relative with an *MSH2* mutation may increase the risk of another kidney cancer in the recipient, as well as potentially endangering the donor who is left with only one kidney and is at increased risk of Lynch syndrome cancers.

The present case illustrates the potential benefit of planned storage of pre-transplant DNA of the recipient in families where hereditary cancer is suspected, to facilitate possible future genetic testing. Where pre-transplant DNA is not available, accurate genetic testing requires a skin biopsy or other non-blood, non-buccal sample to ensure no contamination from donor DNA. Alternatively, specific methods for recovering DNA enriched for recipient DNA rather than donor DNA from a post-transplant sample would be required (Khan et al. 2012). The microarray result in our patient suggests that this would not have been feasible.

Another issue raised by this case is that MLPA kits have control probes for other genes on the same chromosome [e.g. an *MLH1* probe is present in a Von Hippel Lindau MLPA kit (MRC Holland Salsa Kit P016)] or genes on other chromosomes which have the potential to yield unexpected results aside from the sex chromosome markers (Schouten et al. 2002). This leads to the question of whether unexpected results which are unrelated to the gene being tested should be reported.

Conclusion

This case demonstrates the complexities of genetic testing in bone marrow recipients, especially when the donor is a family member and therefore at risk of the same inherited condition. We suggest that a routine question about whether treatment by bone marrow transplant (no matter how long ago) should be asked prior to any genetic test for patients with

a history of any haematological malignancy or immune deficiency. Furthermore, all patients undergoing testing could be routinely asked whether they have had a blood transfusion in the previous two months, to avoid erroneous test results. This case also shows that predictive tests have potential to yield unexpected results due to the presence of control probes, and good communication between genetic clinic staff, patients, and laboratory scientists is essential in resolving problems, avoiding provision of incorrect test results and ascertaining whether unexpected results are relevant to patient care.

Acknowledgements

We are very grateful to the patient for her consent to publish this case study. We thank Jessica Taylor (Familial Cancer Centre Royal Melbourne Hospital) and Ivan Macciocca (Victorian Clinical Genetics Service) for helpful discussions, D Henry, G McKavanagh, J Rossini, A Tirimacco and M Zawitkoswki (Institute for Medical and Veterinary Science) for laboratory testing, and Dan Buchanan (Cancer and Population Studies Group, Queensland Institute of Medical Research).

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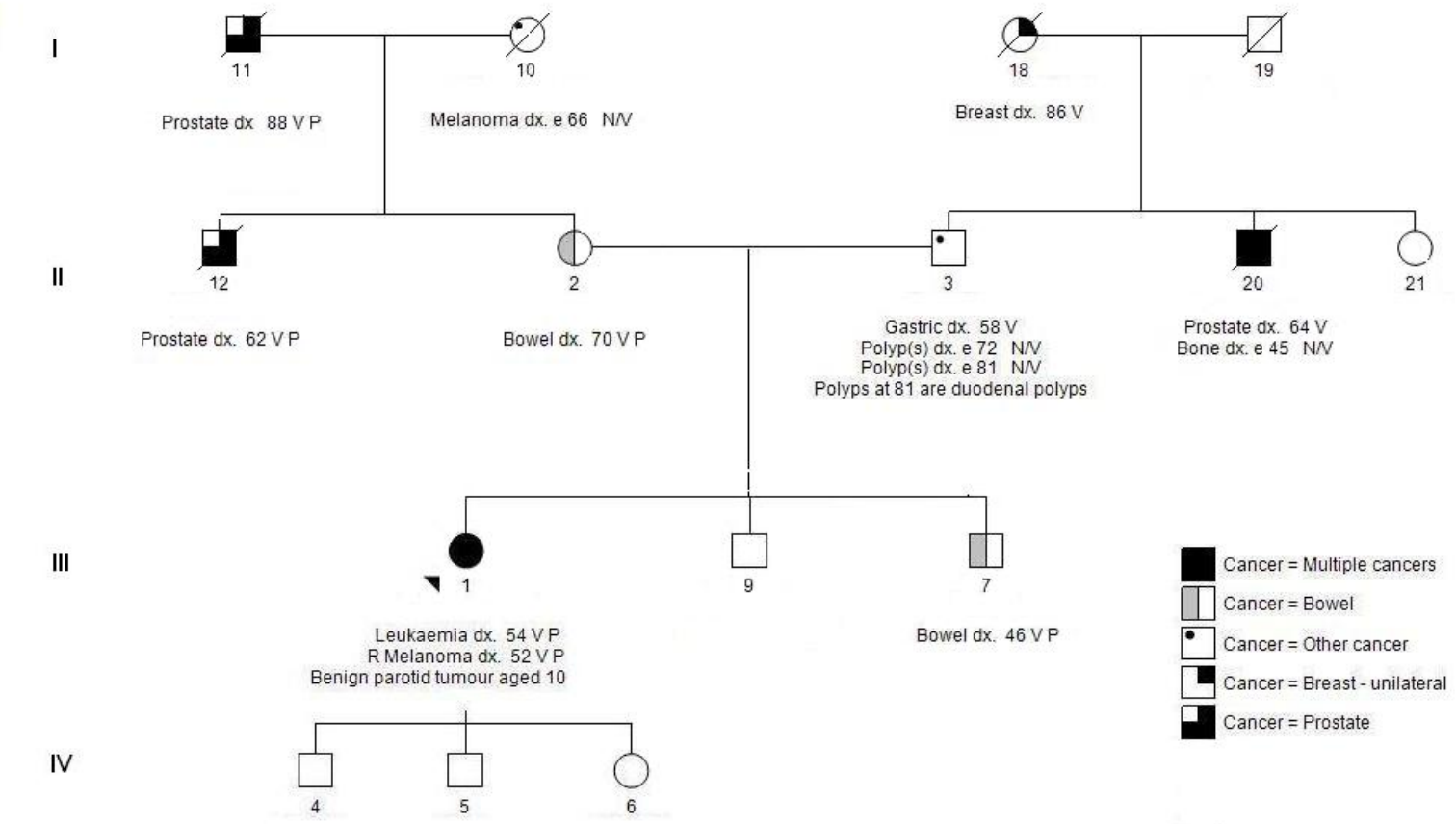
Figure 1. Pedigree of the family. V, verified; VP, verified plus pathology report; N/V, not verified; e, estimated; dx., diagnosed.



Author/s:
Sexton, A; Rawlings, L; Jenkins, M; Winship, I

Title:
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Date:
2014-02-01

Citation:
Sexton, A., Rawlings, L., Jenkins, M. & Winship, I. (2014). Predictive Genetic Testing of a Bone Marrow Recipient-Ethical Issues Involving Unexpected Results, Gender Issues, Test Accuracy, and Implications for the Donor. JOURNAL OF GENETIC COUNSELING, 23 (1), pp.33-37. https://doi.org/10.1007/s10897-013-9643-x.

Persistent Link:
http://hdl.handle.net/11343/220451