

Case 6: Ambiguous test results and variants

Mary is 33 years old and has just been diagnosed with breast cancer. Her mother has a *BRCA1* mutation, so she assumes she will also test positive for this mutation. To Mary's surprise, she tests negative for the *BRCA1* mutation that her mother carries.

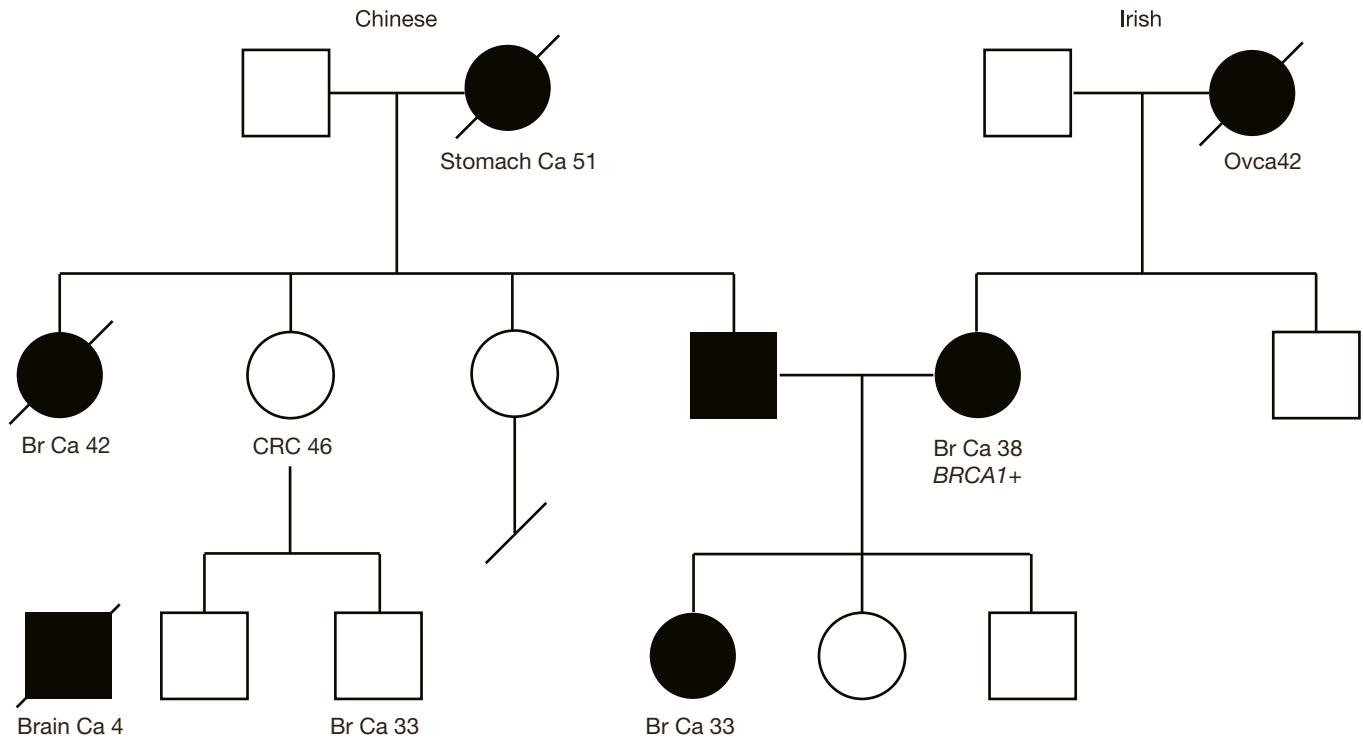
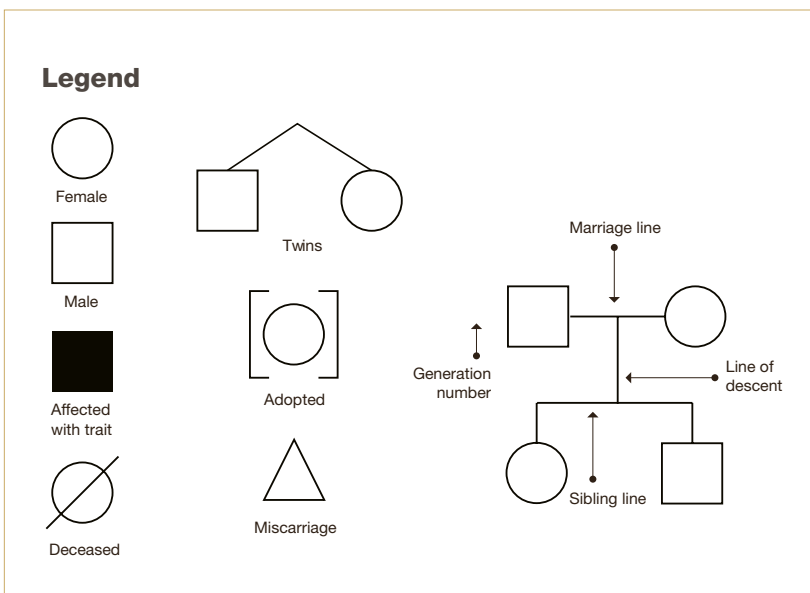


Fig. 1.

A family pedigree that shows multi-generational cancers occurring at young ages, on both the maternal and paternal sides. This patient could have inherited a genetic mutation placing her at increased risk of cancer from either side.



Should Mary consider further genetic testing?

Yes, there are two main reasons that Mary should consider additional genetic testing. One reason is her young age at diagnosis of breast cancer. National Comprehensive Cancer Network (NCCN) guidelines recommend consideration of genetic testing for women diagnosed with breast cancer ≤ 45 years old. A second reason is her strong paternal family history of cancer. Most inherited cancer risks follow an autosomal dominant inheritance pattern, meaning there is a 50-50 chance that an affected parent will pass the mutation to a child of either sex. Therefore, the paternal family history is equally as important as the maternal history for hereditary cancer risk assessment. Mary's paternal family history is suggestive of an inherited risk for cancer.

What type of additional genetic testing should Mary pursue?

Historically, genetic testing has been offered in a sequential manner, gene by gene. This approach can be timely and expensive if there are multiple genes being considered for testing. Recent advances in genetic testing with next generation sequencing make it possible to test multiple genes simultaneously, with lower cost and faster turnaround time for results.

Mary's personal and paternal family history is suggestive of more than one inherited cancer syndrome. At a minimum, complete testing of *BRCA1* and *BRCA2* should be offered. Testing for *TP53* (Li Fraumeni syndrome) should also be considered in women diagnosed with breast cancer under age 31, even in the absence of family history of cancer(1). While it remains unclear whether breast cancer is associated with Lynch syndrome, her father's diagnosis of colon cancer at 46 years of age and her paternal grandmother's diagnosis with stomach cancer raise suspicion for Lynch syndrome. There are also many other genes that have been implicated in hereditary cancer risk with overlapping cancer types.

Current NCCN guidelines recommend considering a multi-gene panel when more than one gene could explain an inherited cancer syndrome, or when someone tests negative for a specific inherited cancer syndrome but their history remains strongly suggestive of an inherited cancer syndrome. A multi-gene panel would be a reasonable approach to testing for Mary, so that multiple hereditary cancer genes could be assessed at once. Genes and their variants can have variable penetrance, meaning that a low, moderate or high proportion of people can be affected by the trait associated with the gene or variant, which in this

context is increased risk for cancer. While most cancer susceptibility genes are rare and highly penetrant, our understanding of genes with low and moderate penetrance is increasing, with *ATM*, *BARD1*, *BRIP1*, and *CHEK2* as examples in HBOC. Due to the complexities of genetic testing options, medical and psychosocial implications, and results interpretation, genetic testing should be pursued in the context of genetic risk evaluation by a genetics professional.

Mary pursues a multi-gene cancer panel that tests for mutations in 24 genes associated with hereditary cancer risk. She is found to have a variant of uncertain significance (VUS) in the *CHEK2* gene.

Since Mary was found to carry a variant of uncertain significance (VUS), what does this mean to her and her family members?

Variants of uncertain significance are changes in the sequence of the DNA where there is too little information known about the specific DNA change to classify it as disease-causing (pathogenic or likely pathogenic) or normal variation (benign polymorphism). VUS are more commonly reclassified as benign changes when more information becomes available, but some are eventually considered pathogenic mutations. Various commercial labs report VUS rates that range from 9 to 41% in multi-gene panels.

Mary and her family members should be counseled about future cancer risks based on assessment of the family history of cancer, not the presence of the VUS. The VUS cannot be used to define future cancer risks for Mary, nor for cancer risk management recommendations. If multiple family members affected with cancer carry the VUS, then it is more suggestive of causation, but 50% of first-degree relatives will carry the VUS just by chance, so it takes a large family or multiple families to prove the VUS segregates with cancer. Testing unaffected relatives for a VUS is not useful unless the commercial lab offers for the family to participate in a variant study.

Does Mary's multi-gene test result rule out an inherited cancer risk for her and her family members?

Mary's test result does not rule out an inherited risk for cancer for her or her family members. While Mary did not inherit the *BRCA1* mutation that her mother carries and her multi-gene panel test was inconclusive with a *CHEK2* VUS, the fact remains that Mary was diagnosed with breast cancer at a very young age. In addition, she has a paternal family history of cancers that is unexplained and

suggestive of inherited risk for cancer. Mary and her family members remain at increased risk for the cancers present in close family members.

It is possible that Mary has an inherited mutation in a different cancer risk gene or a mutation in a targeted gene that was not identified. There may be additional genes associated with hereditary cancer risk that are not yet incorporated into multi-gene panels. Additionally, genetic testing is not 100% sensitive, so mutations may be missed in the targeted genes.

It is also possible that Mary's cancer is due to multifactorial cancer risk, where multiple small genetic factors she inherited from one or both sides of her family have combined with environmental and/or lifestyle factors to increase her risk for cancer. There is currently no clinical genetic testing for these types of smaller inherited risk factors.

Finally, it cannot be ruled out that Mary has a sporadic breast cancer diagnosis. It may be that her father and other paternal family members have a mutation in an inherited cancer risk gene, but Mary did not inherit it and simply developed breast cancer sporadically at a young age.

What surveillance is recommended to Mary and her family members?

Mary and her family members should consider cancer risk management options based on the family history of cancer.

This history may not be explained by the *BRCA1* mutation on the maternal side. Management recommendations would include colon and breast cancer screening at an earlier age.

Mary should be encouraged to keep in touch with her genetics professional regarding the interpretation of the *CHEK2* VUS and advances in genetic testing. If the *CHEK2* VUS is reclassified in the future to either a benign polymorphism or a pathogenic mutation, that information will benefit both Mary and her family members. She should also update her genetics professional with any changes to her personal or family cancer history as this may alter her family history assessment. As knowledge of inherited cancer risk genes advances, there may be additional genetic testing for Mary to consider in the future.

Her father should seek genetic risk evaluation to consider appropriate genetic testing based on his diagnosis of cancer and family history of cancer. If he were found to have an inherited gene mutation, it would be important to verify whether Mary's panel test would have identified that specific mutation, and her siblings could also consider testing for the mutation.

Mary's siblings should seek genetic counseling and consider genetic testing for the known *BRCA1* mutation that their mother has, since they are each at 50% risk to inherit this mutation despite Mary's negative testing for it.

References

- NCCN Guidelines. https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf
- A.K. Win, N.L. Lindor, M.A. Jenkins. Risk of breast cancer in Lynch syndrome: a systematic review. *Breast Cancer Res.* 15 (2) (2013 Mar 19) R27.
- J. Scalia-Wilbur, B.L. Collins, R.T. Penson, D.S. Dizon. Breast cancer risk assessment: moving beyond *BRCA1* and 2. *Semin. Radiat. Oncol.* 26 (1) (2016) 3-8.
- J.L. Berliner, A.M. Fay, S.A. Cummings, B. Burnett, T. Tillmanns. NSGC Practice Guideline: Risk Assessment and Genetic Counseling for Hereditary Breast and Ovarian Cancer. *J. Genet. Counsel.* 22 (2) (2013) 155-163.
- C.G. Selkirk, K.J. Vogel, A.C. Newlin, S.M. Weissman, S.M. Weiss, C. Wang, P.J. Hulick. Cancer genetic testing panels for inherited cancer susceptibility: the clinical experience of a large adult genetics practice. *Fam. Cancer.* 13 (4) (2014) 527-36.
- N. Tung, C. Battelli, B. Allen, R. Kaldate, S. Bhatnagar, K. Bowles, et al. Frequency of mutations in individuals with breast cancer referred for *BRCA1* and *BRCA2* testing using next-generation sequencing with a 25-gene panel. *Cancer* 121 (1) (2015) 25-33.