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LETTER

Unclassified variants in disease-causing genes: nonuniformity of genetic testing and counselling, a proposal for guidelines

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With the introduction of high-throughput technologies in the DNA diagnostic laboratories for the mutation scanning of the major cancer-predisposing genes, an increasing number of missense and intronic variants are detected. The consequence of many less common variants in terms of cancer risk is often unknown. These changes often do not lead to truncated proteins upon translation like most disease-causing pathogenic mutations. For this reason, these variants are named unclassified variants (UVs). In the database of the Breast cancer Information Core (BIC, http://research.nhgri.nih.gov/bic/), 32% of all reported BRCA1 sequence changes and 53% of the BRCA2 variants fall into this category. Hence, a BRCA-UV is detected in a significant proportion of tested individuals.

There are two important steps in the clinical management of a DNA test result. One is the interpretation of the detected sequence change by the laboratory in terms of functional consequence on the protein structure and disease outcome (a disease-causing 'pathogenic mutation' *versus* a not with disease-associated 'neutral polymorphism'). Second is the communication of the test result by the clinical geneticist to the counselee. At the moment, there are no commonly accepted guidelines for either of these steps in the case of a UV test result. Therefore, we investigated by means of a questionnaire how many BRCA-UVs all Dutch DNA laboratories detected in the samples they received for mutation scanning of the BRCA genes in 2001, and if and how they reported them. A second questionnaire asked the clinical geneticist, responsible for the policy of hereditary breast cancer in his centre, how he informed his counselees about the UV test result in terms of risk for breast and ovarian cancer and what his policy was regarding presymptomatic testing. A reply from representatives of the 10 departments for genetic counselling and from the eight laboratories for DNA diagnostics was obtained (100% response rate).

The eight DNA diagnostic laboratories in the Netherlands, which test the BRCA genes, apply different combinations of mutation scanning technologies to scan the total coding region of the *BRCA1* and *BRCA2* genes. Table 1 shows that the four laboratories for which the data are complete differed considerably in the reported percentages of BRCA-UV's detected in the samples received in 2001 ($\chi^2 = 10.23$, df = 3; $P = 0.017$), in contrast to the reported percentages of detected mutations ($\chi^2 = 20832$, df = 3; $P = 0.972$). Most laboratories report the detected UV to the requesting counsellor and offer cosegregation analysis as an additional test to investigate the segregation of a variant with the disease. Nonetheless, there is heterogeneity towards offering other types of information to the clinical geneticist (Table 2). The observed lack of consistency in interpretation of the pathogenicity of a variant among the various laboratories, which is most probably the reason for the reported differences in the amount of detected BRCA-UVs by the different laboratories, is internationally also evident in the BIC database, in which some of the multiple entered variants are reported both as polymorphism and as UV.

All clinical geneticists indicated that they have received reports in which a UV was mentioned (Table 3). Five of them discuss the possibility of the detection of a UV with the counselee during the first consultation. Nine of the 10 inform the counselee when a UV is detected, of whom eight have the feeling that the counselee understands the meaning and implications of the detection of the UV. Seven of the 10 clinical geneticists offer cosegregation analysis to try to clarify the clinical meaning of the detected variant. None of the clinical geneticists offer presymptomatic testing routinely to a family in which a UV is detected, but all base their advice with regard to breast surveillance on the family history, sometimes in combination with information about the segregation analysis.

Hence, there is little consistency in the information provided by the Dutch clinical geneticists to probands with a BRCA-UV. This has also been observed in the United States.[1] Petrucelli *et al* conclude that most but not all counsellors mention a BRCA-UV as a possible test result and that the clinical interpretation of a BRCA-UV differed significantly between respondents.

Also other countries, where DNA diagnostic services are being offered, must be having the same problems with the

Table 1 DNA test results of mutation scanning of the BRCA1 and BRCA2 genes by five Dutch DNA diagnostic laboratories of the samples received in 2001

		BRCA1			BRCA2		
Laboratory[a]	*Number of analysed families*	*Total (%)*[b]	*Pathogenic (%)*[b]	*UV (%)*[b]	*Total (%)*[b]	*Pathogenic (%)*[b]	*UV (%)*[b]
1	144	19 (13)	10 (7)	9 (6)	30 (21)	6 (4)	24 (17)
2	117	23 (20)	10 (9)	13 (11)	18 (15)	5 (4)	13 (11)
3	104	9 (9)	6 (6)	3 (3)	16 (15)	6 (6)	10 (10)
4	161	15 (9)	12 (7)	3 (2)	22 (14)	6 (4)	16 (10)
5	180	16 (9)	14 (8)	2 (1)	2 (1)	2 (1)	0[c]

[a]Data of three laboratories are not included because the data were not complete or received after a second reminder, by which time, new information about eight UVs was published. [b]Relative to the total number of families. [c]At the time of the survey, the BRCA2 test-results on the samples of 2001 from this laboratory included only the Protein Truncation Test for the large central exons 10 and 11.

Table 2 Additional testing and information offered by the eight DNA diagnostic laboratories to the clinical geneticists

Additional information or tests offered	*Number of centres*
Report the detected UV to the requesting counselor	7
UV has a high probability of being pathogenic[a]	4
UV has been detected before[a]	4
Co-segregation analysis of variant and disease	7
Loss of heterozygosity analysis of tumour tissues	5
Investigating mRNA for splice-defects	3
Presymptomatic testing of UV	0[b]

[a]As published in the literature or in the BIC database. [b]Three consider it in special cases and one laboratory considers this to be the decision of the clinical geneticist.

Table 3 Policy of the clinical geneticists regarding the detection of a UV in BRCA1 or BRCA2

Clinical geneticist	*Answers received*	*Number of compliants*
Received UV reports	10	10
Discuss the possibility of finding a UV before testing	10	5
Inform the counselee when a UV is detected	10	9[a]
Feels that the counselee understands the UV-report	9	8
Always discuss cosegregation analysis	10	7[b]
Offer presymptomatic testing	10	0

[a]One does this sometimes, depending on the UV. [b]In addition to these seven, two other counselors do this sometimes depending on family structure, and one principally does not offer segregation analysis.

clinical handling of UVs, but to our knowledge, they have not surveyed this. Our department has thus formulated standards for the interpretation of sequence variations and guidelines for making clinical recommendations. We propose that:

- All variants for which the pathogenicity is not demonstrated or excluded in peer-reviewed published literature, in a mutation database, or on the basis of own findings, are called UVs.
- Patients are informed by the genetic counsellor at the initiation of a DNA test about the possibility of a UV as the result of the mutation scanning.
- The DNA diagnostic laboratory reports a detected UV to the requesting counsellor, who in turn communicates this to the counselee.
- The uncertainties surrounding the pathogenicity of the detected variant are discussed, as is the possibility of classification of the UV after further research. An explanation that further research might involve the cooperation of the counselee and his relatives should also be given.
- Presymptomatic testing of family members is not offered. Surveillance is offered on the basis of the family history. If a family history fits a hereditary breast cancer syndrome, surveillance is offered as in families with a BRCA1 or BRCA2 mutation.[2]
- Patients can request prophylactic surgery, but the decision to perform this surgery should be based on the family history and not be influenced by the detection of the UV.

We hope that these guidelines will be of assistance in those situations where mutation detection in disease-causing genes has become an integral part of clinical decision-making.

The clinical problem posed by UVs is not restricted to BRCA1 and BRCA2, but is also evident for many other disease-related genes. Understanding the clinical significance of these variants will require a multidisciplinary approach involving studies on protein function, evolutionary gene sequence conservation, linkage analysis, and

population genetics. Although some of these data are becoming available in public databases and in the literature,[3,4] other important information (eg of cosegregation analysis, RNA analysis and LOH analysis in tumour tissue) is in the private domain of the clinical genetic centres, who are recommended to perform these tests to clarify the pathogenicity of UVs. It is important that even the results of these tests are compiled in a publicly available resource and that guidelines are formulated according to which information the pathogenic status of a UV can be changed. With such a resource, guidelines and a tight collaboration between the genetics community and the family clinics, the consistency in interpretation of the pathogenicity of variants will increase, the associated cancer risk will be clarified and counselees will receive more balanced information about their risk.

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