it is also clear that more studies concerning treatment in heavily pretreated patients are needed.

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LETTER TO THE EDITOR

A Danish national effort of *BRCA1/2* variant classification

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Introduction

With the technological development for sequencing and automation of sample handling, interpretation of data and classification of variants are becoming the more labor intensive part of genetic screening. By 2008, 107 unique pathogenic *BRCA1/2* variants had been identified in Danish hereditary breast and/or ovarian cancer families [1]. Identification of pathogenic *BRCA* variants affects not only choice of preventive measures but also affects the effect of treatment in cancer patients. The latter has most recently been shown in a Danish cohort of breast cancer *BRCA* carriers [2]. Since then, the number of identified pathogenic variants has almost tripled. All though the methods for variant classification have improved, the number of variants of unknown clinical significance has increased even more rapidly.

The five tier International Agency for Research on Cancer (IARC) classification system [3] is the classification system generally used in Denmark. All three participating laboratories (Rigshospitalet, Odense and Aalborg University Hospital) are longstanding members of Evidenced-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) [4] actively working towards classification of *BRCA* variants using a multifactorial likelihood model first described in 2004 [5] and subsequently revised and refined by incorporation of additional data [6–11]. Current ENIGMA classification rules can be found at https://enigmaconsortium.org/library/general-documents/.

For counseling and clinical decision-making, individuals with C5 (definitely pathogenic) and C4 (likely pathogenic) variants are treated equally and offered full high-risk surveillance programs. Likewise individuals with C1 (not pathogenic/low clinical significance) and C2 (likely not pathogenic/little clinical significance) variants are counseled based on family history and other risk factors and treated as 'no pathogenic *BRCA* variant detected'. Hence, misclassification between groups C1/C2 or C4/C5 will not have any clinical consequences, whereas misclassification between the group with no/little clinical significance (C1/C2) and the group of likely/definitely pathogenic variants (C4/C5) obviously would be severe. C3 is the group in between, representing variants of uncertain significance. This is a large group of variants with a probability of pathogenicity of 5–95%. However, an overly conservative and cautious approach leading to an overuse of C3 classifications could also be problematic and cause an unclear risk prediction. Thereby, leading to subjectivity in conveying and perceiving cancer risk.

Variant classification is not static. Obviously, reclassification of C3 variants is a natural consequence of growing information from new variant carriers, segregation and/or functional analyses. However, with the continuous gain of knowledge of protein function and particularly importance of naturally occurring isoforms there are examples of variant reclassification from C4/C5 to C1/C2. The most well-known example is *BRCA1*, LRG292t1:c.594-2A > C originally considered pathogenic due to exon10 skipping. However, further analysis showed when this variant occurs in *cis* with LRG292t1:c.641A > G it also produces 20–30% in-frame naturally occurring isoform Δ9,10 which retains the tumor suppressive function of BRCA1 [12]. In addition, there are reports of synonymous variants and deep-intronic variants originally deemed benign or likely benign subsequently showing an effect on splicing [13].

Here we present the concerted effort of our national Danish breast cancer variant classification group (DBKG) on streamlining *BRCA* variant classification.

Material and methods

Mutation screening of the *BRCA1* and *BRCA2* genes and variant classification were performed in three different laboratories (Rigshospitalet, Odense and Aalborg University Hospital). Variant lists from the three laboratories were collated from the uptake of *BRCA* screening (1999 Rigshospitalet, 2000 Odense and 2003 Aalborg) until the end of 2016. Nomenclature was revised according to current HGVS guidelines [14] and checked for consistency using https://mutalyzer.nl/.

Classification was updated using a batch search for ENIGMA approved classifications in ClinVar [15] on the 16 August 2017. Remaining variants were classified according to current ENIGMA rules by the representatives from the three laboratories. In addition, information on mRNA splicing analyses and functional studies previously published or carried out in the participating laboratories were taken into account.

Design of splicing assays is based on recommendations from ENIGMA [16] and when possible allele-specific in nature.

Results

A total of 945 unique variants have been detected by the three Danish laboratories carrying out *BRCA1* and *BRCA2* screening in a diagnostic setting (Supplementary Table 1). Searching ClinVar for ENIGMA validated variant classification 164, 61, and 199 variants classified as C1, C2, and C5, respectively, resulting in 521 unclassified variants (Table 1). After classifying the remaining variants according to ENIGMA

Table 1. Proportion of variant classes.

Variant classification	ClinVar (ENIGMA)		DBKG	
Number	Percentage	Number	Percentage	
C1-benign	164	17%	167	18%
C2-likely benign	61	6.5%	211	22%
C3-uncertain significance	–	–	268	28%
C4-likely pathogenic	–	–	17	1.8%
C5-likely pathogenic	199	21%	282	30%
Not Classified by ENIGMA by 29 June 2017.	521	55%	–	–

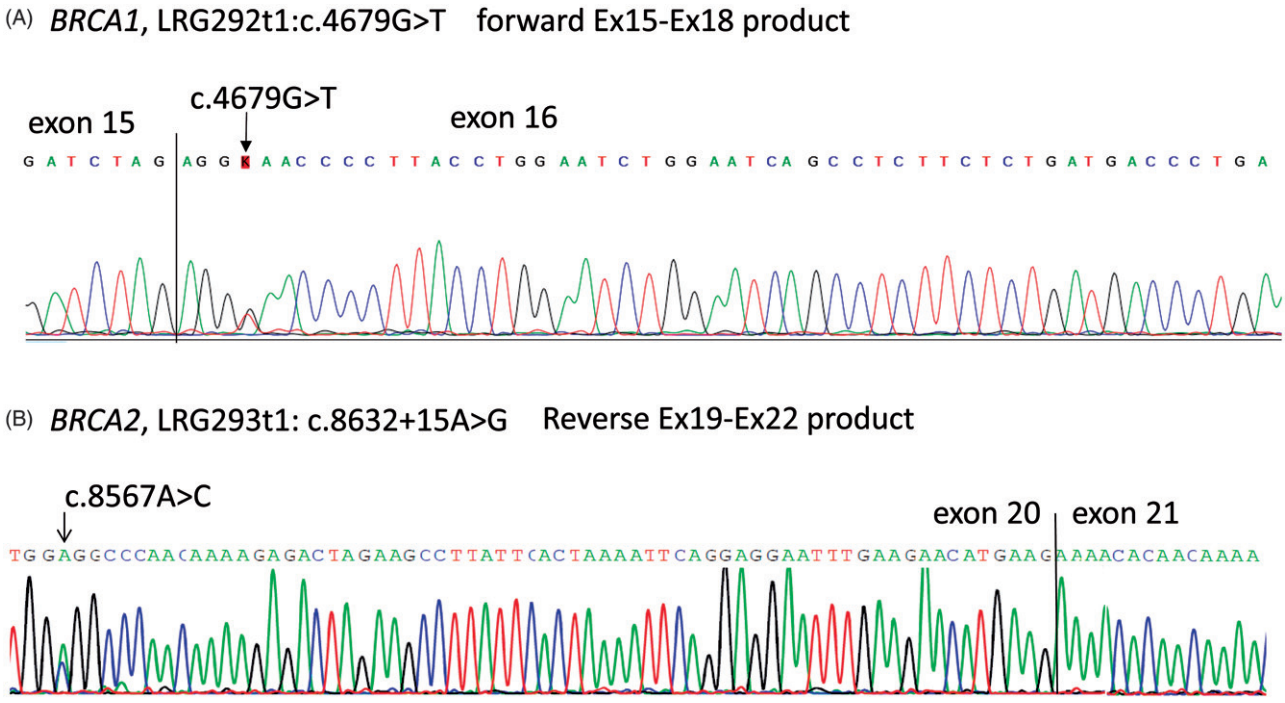

Figure 1. Allele specific mRNA splicing assays. (A) Direct Sanger sequencing electropherogram of RNA from a *BRCA1*, LRG292t1: c.4679G > T carrier showing equal representation of wildtype and variant alleles and no cryptic splicing in the RT-PCR products generated with primers located in exon 15 and 18. Albeit, the variant does not affect splicing, it is a missense variant and the effect on protein function has not been established. Hence it is still classified as C3. (B) Direct Sanger sequencing electropherogram of RNA from a *BRCA2*: LRG293t1: c.8633 + 15A > G carrier showing equal representation of wildtype and variant alleles as evident from exonic variant c.8567A > C in the RT-PCR products generated with primers located in exon 19 and 22. Furthermore, no cryptic splicing is observed at the exon 20–21 junction. Based on this c.8633 + 15A > G is classified as C2.

recommendations and based on additional available data 167, 211, 17, and 282 variants were classified as C1, C2, C4, and C5, respectively (Table 1), reducing the number of C3 to 268.

Examples of mRNA splicing assays with information on allelic usage, as recommended by ENIGMA are shown in Figure 1. Variants with equal contribution to assumed full-length transcript from both alleles and no usage of cryptic splice sites are considered likely benign (C2) if they do not cause direct changes to the protein sequence. Therefore, the intronic mutation in *BRCA2*, LRG293t1:c.8632 + 15A > G (Figure 1(B)) is classified C2, whereas *BRCA1* LRG292t1:c.4679G > T, although not causing aberrant splicing, is classified C3 because of the resulting rare missense variant p.(Gly1560Val). Variants producing no full-length transcript or no naturally occurring isoforms from the variant allele are deemed likely pathogenic (C4).

Discussion

As evident from the collection of variants classified across Denmark, a large proportion of variants detected in a routine diagnostic setting have not been formally classified by ENIGMA in ClinVar. Additional variants may be classified by searching the literature or carrying out functional and splicing assays. However, to ensure consistency in variant classification the efforts must be concerted, which is a major priority of the national initiative DBKG.

The example in Figure 1 illustrates the results of splicing assays performed according to ENIGMA's rules. *BRCA2*, LRG293t1:c.8632 + 15A > G is classified as C2 by splice assay. In order to reach a final classification as C1, support from multifactorial analysis using co-segregation, pathology information etc. is necessary. Likewise, the C3 variant *BRCA1*, LRG292t1:c.4679G > T requires multifactorial analysis possibly supported by functional data to be classified further. This calls for a collaborative approach among clinicians and molecular genetic laboratories. This approach have already been applied for many variants in ENIGMA and a large number of additional variants, likely counting the variants presented here, will be included in coming analyses.

The classification of variants collected for this study is updated and presented in the Supplementary material. However, classifications are not static and therefore the listed results should not be used for clinical purposes in the current form. DBKG will ensure continuous revision of classifications kept in an updated national database and eventually upon formal ENIGMA validation will be posted in ClinVar.

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Disclosure statement

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LETTER TO THE EDITOR

The accuracy of preoperative staging of the axilla in primary breast cancer: a national register based study on behalf of Danish Breast Cancer Group (DBCG)

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Introduction

Staging of axillary lymph nodes in women with breast cancer is an important guide for treatment decisions. For decades, axillary lymph node dissection (ALND) was the standard procedure in staging the axilla, but today, sentinel lymph node biopsy (SLNB) is the standard of care in clinically node negative women.

In Denmark, patients suspicious of breast cancer are referred for a triple test assessment which consists of clinical examination, mammography, whole-breast ultrasonography (US) and needle biopsy of suspicious lesions. The preoperative examination also includes US of the axillary lymph nodes and fine needle aspiration cytology (FNAC) if enlarged or suspicious lymph nodes are present [1]. This examination is an important tool in the preoperative staging of patients with primary breast cancer [2,3].

Patients are classified as clinically node negative if no suspicious axillary lymph nodes are seen on US. These patients will be offered a SLNB, followed by ALND if macrometastases (tumor deposits >2 mm) are found in the sentinel lymph node(s). SLNB was completely implemented in Denmark by the end of 2004 [4].

Patients with preoperatively verified axillary metastases will have ALND performed immediately or receive neoadjuvant treatment. Accurate preoperative axillary lymph node status can reduce the numbers of patients having unnecessary SLNB performed. This reduces the time of the surgical procedure and it has been shown to lower healthcare costs [5,6].

The sensitivity of the preoperative staging of the axilla has been shown to vary between studies from [2,5]. In the meta-analysis by Diepstraten et al. [5] the sensitivity were found to be 50%.

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