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EPIDEMIOLOGY

A BRCA2 mutation incorrectly mapped in the original BRCA2 reference sequence, is a common West Danish founder mutation disrupting mRNA splicing

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Abstract Inherited mutations in the tumor suppressor genes BRCA1 and BRCA2 predispose carriers to breast and ovarian cancer. The authors have identified a mutation in BRCA2, 7845+1G>A (c.7617+1G>A), not previously regarded as deleterious because of incorrect mapping of the splice junction in the originally published genomic reference sequence. This reference sequence is generally used in many laboratories and it maps the mutation 16 base pairs inside intron 15. However, according to the recent reference sequences the mutation is located in the consensus donor splice sequence. By reverse transcriptase analysis, loss of exon 15 in the final transcript interrupting the open reading frame was demonstrated. Furthermore, the mutation segregates with a cancer phenotype in 18 Danish families. By genetic analysis of more than 3,500 Danish breast/ovarian cancer risk families, the mutation was identified as the most common BRCA2 mutation in West Denmark, while it is rare in Central and East Denmark and not identified in South Sweden. Haplotype analysis using dense SNP arrays indicated a common founder of the mutation approximately 1,500 years ago.

Keywords Hereditary breast cancer · BRCA2 · 7845+1G>A · Founder mutation · Mutation age · SNP array · RT-PCR

Introduction

Breast cancer is the most common female cancer in the western world affecting more than 10% of women. Approximately 5–10% of cases are believed to be inherited [1]. Germline mutations in the BRCA1 and BRCA2 genes predispose to breast and ovarian cancer and to a lesser

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extend to other cancers. The two genes have been used increasingly for clinical diagnostics and prognosis during the last 10–15 years. In Denmark, mutations in the two genes are identified in 28% of high risk families which is comparable to other western populations [2]. The relatively low frequency of mutations in the high risk families indicates the influence of other genes or insufficient analysis of BRCA1 and BRCA2. For example promoters and the majority of intron sequences are usually not analyzed and interpretation of variants in these regions is difficult. Even for variations located near the consensus AG and GT acceptor and donor splice sites, functional analyses are necessary to reveal splice defects. Furthermore, the applied techniques do not have 100% sensitivity, resulting in missed mutations. High quality cDNA and genomic reference sequences are essential to ensure correct interpretation of gene structure and clinical significance of identified variations.

The mutational spectrum for BRCA1 and BRCA2 is wide and analysis of entire coding regions and flanking sequences is necessary in most populations. However, founder mutations are evident in isolated genetic populations like the inhabitants in Iceland [3], Norway [4], Greenland [5], and among Ashkenazi Jews [6]. The authors have previously reported the mutational spectrum in BRCA1 and BRCA2 and only found weak indications of local founder mutations in Denmark [7]. However, at that time a mutation in BRCA2 intron 15, was interpreted as likely non deleterious in most Danish laboratories because of an error in the mapping of the splice junction in the reference sequence. Here, functional analysis of the mutation was performed, and the authors have analyzed the prevalence and the geographical distribution of the mutation in Denmark and Southern Sweden in samples forwarded for mutation screening from departments of clinical genetics. Moreover, the genetic origin and the age of the mutation were addressed by haplotype analysis using dense SNP arrays.

Materials and methods

Mutation analysis

BRCA1 and BRCA2 testings were requested after genetic risk assessment and counseling at the departments of clinical genetics. Blood samples were preferably collected from affected index persons. In some families no affected persons were alive and indirect testing was performed on a number of healthy relatives [8]. Mutation analysis of Danish samples was performed in four separate laboratories (Aalborg, Odense, Copenhagen, and Lund). Analysis of Swedish samples was performed in Lund. Mutation screening was performed by slightly different techniques, but most laboratories performed prescreening by DHPLC (denaturing high-performance liquid chromatography) and in some laboratories TGCE (Temperature gradient capillary electrophoresis [9]) and PTT (Protein truncation test [10]) followed by sequencing of relevant regions. MLPA (Multiplex ligation dependent probe amplification) was used to identify large genomic rearrangements. As part of these screenings, the exon 15 region of BRCA2 was analyzed by MLPA, TGCE, DHPLC, and/or sequencing.

Reverse transcriptase PCR

Total RNA was extracted from blood from a carrier of 7845+1G>A using TRI reagent (Sigma) according to manufacturer's instructions. Random hexamer primed reverse transcription was carried out using SuperScriptIII (Invitrogen) at 50°C for 60 min, according to manufacturer's instructions. Primers (c2EX14F: 5′ GGA GGA AAA CAG ACA AAA GC 3′ and c2EX16R: 5′ CAT CCA CCA TCA GCC AAC 3′) encompassing exon 15 of *BRCA2* were designed. PCR was carried out using AmpliTaq DNA polymerase with GeneAmp10XPCR buffer II (Applied Biosystems) in the presence of 2 mM $MgCl_2$ under the following conditions: 95°C for 2 min followed by 38 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 60 s, and finally 72°C for 2 min. The PCR products were sequenced in both directions using a 3130XL Genetic Analyzer (Applied Biosystems).

To estimate the frequency of exon 15 skipping from the mutated allele, peak heights in positions in the sequence with dual base calls corresponding to exon 15 and 16 in the forward sequence and exon 14 and 15 in the reverse sequence were used. The fraction of loss was calculated as $F = h2/(h2 + (h1-h2)/2)$ were h1 and h2 are the heights of the two peaks in one position; h1 representing the base of the wild type allele and h2 representing the base of the mutated allele. This formula assumes equal expression of both alleles. The percentage of loss was calculated as the mean value of F for 10 dual base positions in each sequence multiplied with 100%.

SNP array analysis

Genome-Wide Human SNP 5.0 Arrays (Affymetrix) were used according to the manufacturer's instructions. In brief, two aliquots of 250 ng DNA were digested with Sty1 and Nsp1, respectively. Adaptors containing universal primer sites were ligated to the fragment ends and fragments were amplified in multiplex PCR. Purified PCR products were hybridized to 5.0 chips that were subsequently washed and scanned and cel files were generated. Genotypes were called from cel files using BRLMM-P algorithm. All chips

had present call rates above the QC limit of 86%, except one chip with 83%, which is allowed according to Affymetrix analysis guidelines. One chip was excluded because of lower present call rate resulting in 18 informative arrays representing 18 families. A common haplotype was estimated as the most common allele for each SNP on chromosome 13. The length of the haplotype was estimated in each individual as the longest fragment including BRCA2 without a homozygous SNP discordant with the common haplotype. The genetic lengths of the haplotypes measured in cM were calculated using recombination rates from UCSC (http://genome.ucsc.edu). The age of the mutation was calculated by assuming 100 generations to generate haplotypes of 1 cM and an average generation time of 25 years.

Pedigree analysis

Pedigrees from all families were constructed by the clinical genetic departments. Genetic testing of affected and healthy family members allowed co-segregation analysis of the mutation with the breast and ovarian cancer phenotypes. The authors calculated a likelihood ratio as proposed by Mohammadi et al. [11]. This method use penetrance as a function of age of onset, thereby avoiding the use of discrete liability classes used in LOD score methods. Furthermore, their method to calculate the combined likelihood score across families as the product of individual likelihood ratios was applied.

Results

As part of the routine screening for mutations in BRCA1 and BRCA2 the authors identified a G > A substitution in intron 15 of BRCA2. According to the genomic sequence originally reported [12], X95165, the identified variation was mapped 16 bases within intron 15. This reference sequence was also recommended by BIC (Breast Cancer Information Core) at that time. Furthermore, the entries in BIC still refer to exon/intron boundary at position 7829 in the cDNA in agreement with X95165. There are four entries for 7829+1G>A, 3 from Myriad and one of Danish origin reported by Bergthorsson et al. [13]. However, Bergthorsson named it 7845+1G>A in their publication. Furthermore, the mutation has been reported in one Spanish family [14]. Disagreement with X95165 was observed when sequencing the region. The authors, therefore, consulted more recent reference sequences that concurrently mapped the exon/intron boundary to position 7845, resulting in reclassification of the mutation as interrupting the consensus G in first position of the splice donor site in intron 15.

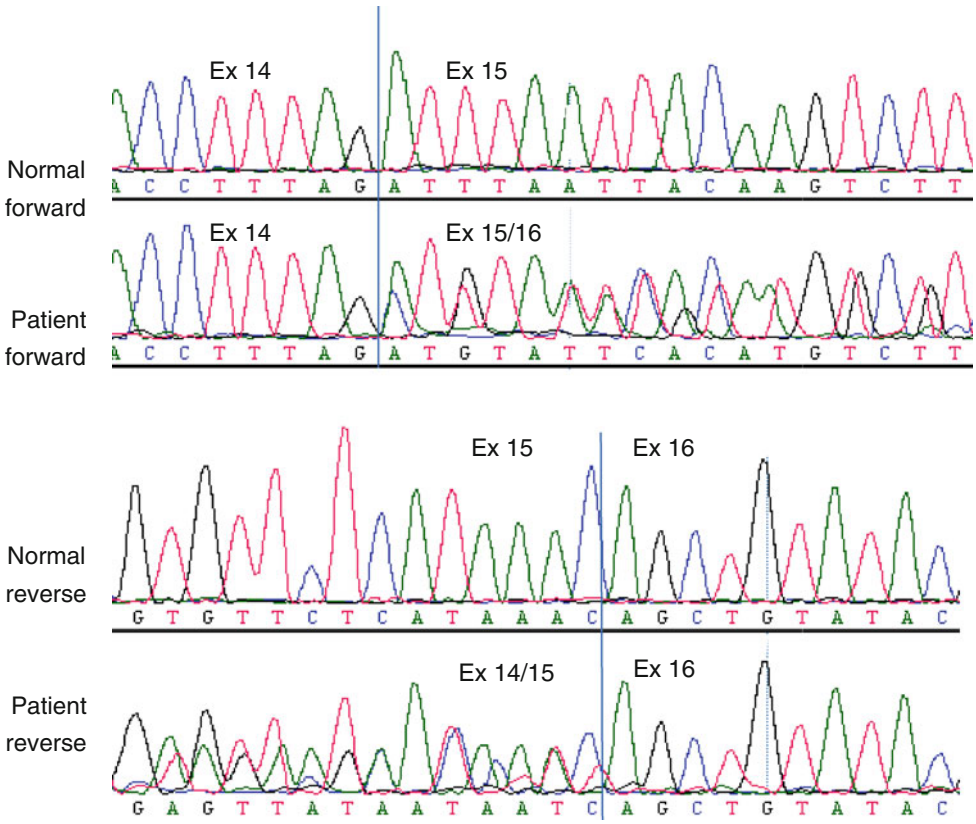

Fig. 1 Sequence of the exon 15 region *Upper* panel: electropherograms for forward sequences of cDNA obtained from a control sample and a patient carrying the 7845+1G>A mutation. *Lower* panel: Reverse sequences for control and patient sample

In general the authors interpret a mutation in this position as deleterious. However, disruption of the consensus donor site may lead to an in-frame transcript that may not be deleterious. Furthermore, because of the disagreement of the reference sequences, it was decided to perform functional splice analysis of the mutation. The authors isolated RNA from lymphocytes sampled from a carrier of the mutation for RT-PCR analysis. By using PCR primers located in exon 14 and 16 and sequencing the product, a mixed sequence in agreement with loss of exon 15 on one allele was obtained(Fig. 1). To estimate the fraction of loss of the mutated allele the authors calculated the percentage of mRNA missing exon 15 compared to the expected total amount of mutated allele. The values were 94% for exon 15 compared to exon 16 in the forward sequence and 90% for exon 15 compared to exon 16 in the reverse sequence, respectively. In conclusion the mutation causes almost complete loss (approximately 92%) of exon 15 in RNA from the mutated allele. Loss of the 192 bases will result in frame shift and stop codon 7 amino acids after exon 14.

Of the 19 identified families, pedigrees were available from 18. This information is summarized in Table 1.

Table 1 Clinical characteristics of families carrying *BRCA2* 7845+1G>A

Family	Screened individual (index person)	Family history of breast and ovarian cancer	Other cancers	LR
1	BC 61(+)	M BC 51; **S BC 54 + 60(−)**; MA BC; MA BC	B PC 63	0.0095
2	BC 54 71(+)		F PC + VEN 64; B PC + BOC 66	1.13
3	BC 54(+)	M BC 49; S BC 58 (+)		4.38
4	OC(+)	MA BC 41 + 53(+); MA BC 39(+); MA OC 64; MA BC; FC BC39(+); FC OC 51; FC BC 62(+); **FC BC 39(−)**	M AB(+); MU KI 78 + BL 81; MU TES 20	57.35
5	BC 36(+)	PA BC 45	F PC 77; M LU 53; MGF PC 83; PGM VEN 54	1.00
6	BC 58 + OC 58(+)	MC MBC 62(+)	F LEU(+); MC CER + VEN	11.39
7	BC 44(+)	M OC 50(+); FC OC 36 (+)	MU AST + GLI 51	11.10
8	BC 38(+)	M OC 59; FC BC	F PC; S LEU 6; PU LU 48; PU VEN 84	1.00
9	BC 44(+)	S BC 49(+); PA BC 45; PA BC 36 +37(+); PA BC 38; PA BC 42 + 50; PA BC 47 + OC 60(+); PA BC 35 FC BC 49; FC BC 30(+);		31.39
10	BC 46(+)	MA OC 64 + BC 64	M HN 77(+); MU VEN 53; MU VEN	1.08
11	BC 50(+)	M BC 43	MU PC; MU LU	0.87
12	BC 41 + OC 59(+)	M OC?; S BC 45(+); S BC 48(+); N BC 37(+); D BC 45; MGM BC 40(+)	B SKIN; ♀	12.04
13	BC 37(+)	M BC 49; MGM OC 72	FC HN; MU PAN 54	1.00
14	♂HN(+)	M BC 70; S OC 68; S OC 65		1.97
15	BC 56(+)	S BC 51; S BC 29 + 42(+)		1.19
16	BC 48 + 53(+)	D BC 25	F SKIN 75; S RC	1.00
17	BC 27(+)	PGM OC 50; PGMS BC 70; FFC BC 50; FFC BC		4.82
18	BC 47 + OC 63	S BC 56(+)		1.21
			Over all LR	1,662,078

BC female breast cancer, *MBC* male breast cancer, *OC* ovarian cancer, *PC* prostate cancer, *TES* testicular cancer, *VEN* gastric cancer; *AB* abdominal cancer; *LEU* leukemia, *CER* cervical cancer; *AST* astrocytoma, *GLI* glioblastoma; *HN* head and neck cancer; *KI* kidney cancer; *BL* bladder cancer; *RC* rectum cancer; *BOC* bone cancer

F father, *M* mother, *N* niece, *PU* paternal uncle, *MU* maternal uncle, *PA* paternal aunt, *MA* maternal aunt, *PGM* paternal grandmother, *MGM* maternal grandmother, *MGF* maternal grandfather, *FFC* Father's female cousin, *PGMS* paternal grandmothers sister (+): mutation carriers and obligate carriers, (−): individuals tested negative for the mutation

Individuals highlighted in bold are potentially in conflict with 7845+1G>A A being deleterious. *LR* likelihood ratio

To confirm that the mutation is deleterious, likelihood ratios were calculated for co-segregation of the mutation and breast and ovarian cancer. A significant exception from the segregation pattern was observed in family 1, where a female non-carrier developed bilateral breast cancer (54 and 60 years), resulting in a likelihood ratio of 0.0095. However, the likelihood ratios were near one or higher in the remaining families and the overall ratio across all families was 1,662,078 (Table 1).

The patients included in the study were from five regions. The peninsula Jutland in West Denmark contains Aalborg in the north, Aarhus in the upper middle, Vejle in the lower middle and South Jutland. The island Funen is in the southern middle part of Denmark and East Denmark containing half of the Danish population is composed of Zealand and smaller islands (Fig. 2). The analysis revealed a skewed distribution of the mutation: more than half of the 7845+1G>A families were located in Vejle area; whereas only 13% of families undergoing genetic screening of BRCA1 and BRCA2 were from this area (466/3690, Table 2).

Compared to East Denmark where the 7845+1G>A mutation was identified only once in 1,300 mutation screenings, higher frequencies were observed in Jutland. When these frequencies were compared to the number of mutation screenings, significantly higher frequencies were

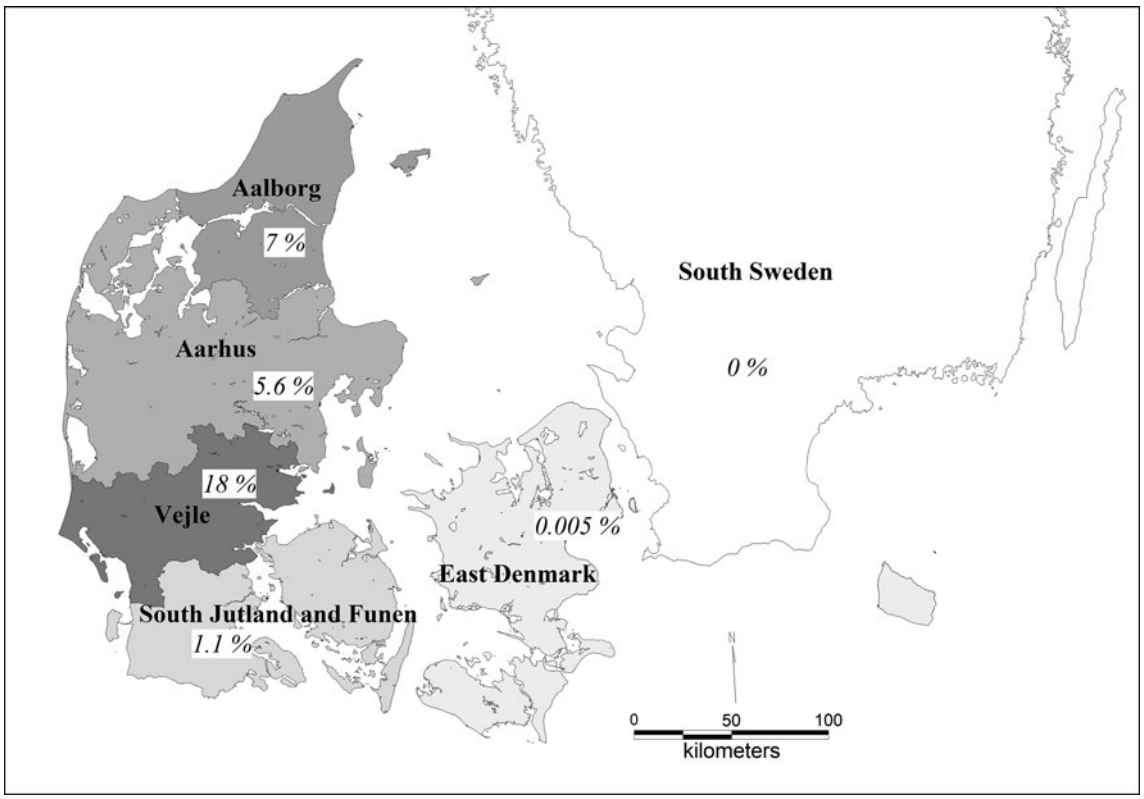

Fig. 2 Map of Denmark and South Sweden The fraction of 7845+1G>A mutation compared to the total number of pathogenic BRCA1 and BRCA2 mutations are indicated. The intensity of the *gray* coloring corresponds to the mutation frequency

Table 2 Geographical distribution of *BRCA2* 7845+1G>A mutation

	# mutation screenings	# mutations BRCA1/2	7845+1G>A	*P*-value (analyses)	*P*-value (mutations)
Aalborg	259	28 (10.8%)	2 (7%)	0.07	0.049
Aarhus	806	89 (11.0%)	5 (5.6%)	0.03	0.016
Vejle	466	55 (11.8%)	10 (18%)	0.00001	0.000005
South Jutland/Funen	859	85 (9.9%)	1 (1.1%)	1	0.52
East Denmark	1,300	190 (14.6%)	1 (0.005%)		
Total	3,690	447	19		

P-values were calculated by comparing the geographical regions to East Denmark

found for Aarhus ($P = 0.03$) and Vejle ($P = 0.00001$) whereas it was borderline significant for Aalborg ($P = 0.07$). Similarly, when the number of families with 7845+1G>A mutations was compared to the number of families with identified pathogenic mutations in BRCA1 and BRCA2, increased frequencies for Vejle ($P = 0.000005$), Aarhus ($P = 0.016$) and Aalborg ($P = 0.049$) were found compared to East Denmark (Fig. 2). An insignificantly higher frequency of 7845+1G>A was found in South Jutland and Funen compared with East Denmark. The mutation was not identified in South Sweden supporting its skewed distribution.

To clarify if the 7845+1G>A mutation had one common founder, the authors analyzed DNA from index persons from 18 of the 19 families using SNP arrays. Of the 500 K SNPs, approximately 17 K was located on chromosome 13. The longest shared haplotype was estimated from the most common allele for each SNP. The ends of the haplotypes were limited by SNPs that were homozygous and different from the common type (bold in Fig. 3). The results are in agreement with one common founder haplotype. The length of the shared haplotype ranged from 0.2 to 3.3 Mb corresponding to 415 and 23 generations, respectively. The mean number of generations across all chips was 63.8 and assuming 25 years for each generation the mutation was founded 1,500 years ago in the Danish Iron Age.

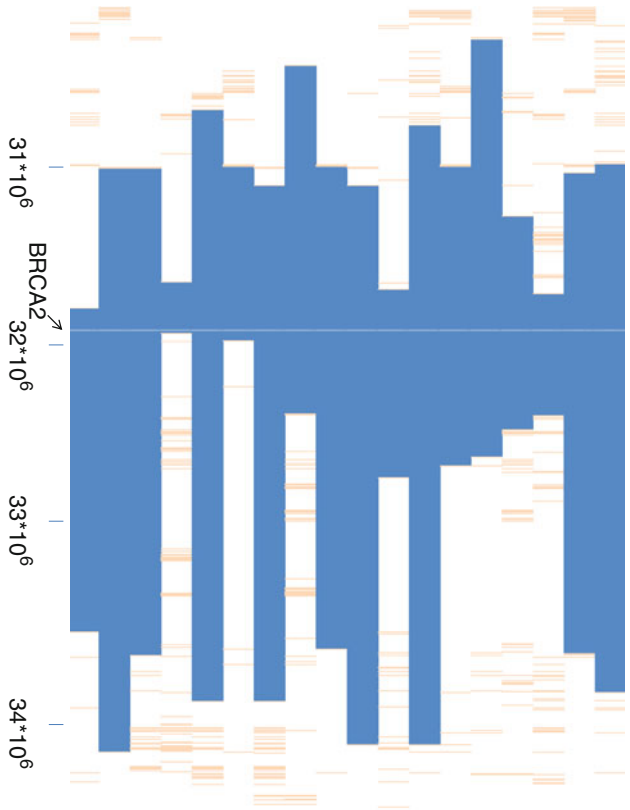

Fig. 3 Haplotypes in the BRCA2 region among carriers of the 7845+1G>A mutation The *dark* regions indicate the longest fragment of the common haplotype in 18 index cases. *Light* lines indicate SNPs where the genotype breaks the haplotype. The *y-axis* indicates position on chromosome 13 in base pairs

Discussion

The authors have identified a mutation that may be misinterpreted because of an error in the previously used reference sequence. Many laboratories tend to use a fixed reference sequence to ensure consistency in nomenclature. However, the results highlight the importance of using updated reference sequences.

The authors have confirmed that the identified mutation is most likely to be deleterious by calculating likelihood ratios for co-segregation and by RT-PCR analysis. For one family a disagreement with co-segregation was observed, because a woman with bilateral breast cancer turned out to be a non-carrier. However, no deleterious BRCA1 and BRCA2 mutations were identified in 84.7% of the women with bilateral breast cancer diagnosed at a mean age of 46 years in the WECARE study [15] supporting that the case is a phenocopy. Another potential phenocopy, a non carrier from family four developed breast cancer 39 years old. However, five other affected women in that family were all mutation positive and the resulting LR was 57.35 supporting co-segregation of disease and breast and ovarian cancer. The overall likelihood ratio for all families was 1,662,078, strongly supporting co-segregation of the mutation with the disease.

RT-PCR analysis demonstrated skipping of exon 15. Quantification of the mutated and the wildtype alleles indicated that exon 15 is lost in approximately 92% of mRNAs produced by the mutated allele, suggesting slightly reduced penetrance of this mutation. However, correct splicing using a non-consensus donor site is highly unlikely and the result may reflect uncertainty in the measurement. The most likely explanation for the observation that the RNA lacking exon 15 did not constitute 50% of mRNA, is nonsense mediated decay of mRNA because of premature termination of transcripts. The results are in agreement with Gutierrez-Enriquez et al. [14], although they did not quantify the the fraction of exon 15 loss.

The age of the mutation may seem relatively high (1,500 years) taking its skewed location into account, i.e., the strong over-representation in central Jutland compared to the rest of Denmark. This may be surprising because the Danish population is regarded genetically homogeneous with no genetically isolated sub-regions. The most obvious explanation may be that the mutation was founded in the Vejle area, and that individuals carrying this mutation have not tended to move to Funen and East Denmark. Another

potential explanation could be that the mutation was missed in some laboratories. However, this is highly unlikely because all Danish laboratories identified the mutation at least once and the mutation was confirmed in the Swedish laboratory in a positive control sample from Denmark.

In conclusion, the 7845+1G>A mutation is perceived a deleterious founder mutation located mostly in central Jutland. The authors have not examined the presence of the mutation in other countries than Denmark and Sweden and draw the attention of other laboratories to the incorrect annotation in the X95165 sequence to avoid clinically important errors.

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Conflict of interest None.

Reference List

1. Collaborativ group (2002) Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50,302 women with breast cancer and 96,973 women without the disease. Lancet 360: 187–195
2. Gerdes AM, Cruger DG, Thomassen M, Kruse TA (2006) Evaluation of two different models to predict BRCA1 and BRCA2 mutations in a cohort of Danish hereditary breast and/or ovarian cancer families. Clin Genet 69:171–178
3. Thorlacius S, Sigurdsson S, Bjarnadottir H, Olafsdottir G, Jonasson JG, Tryggvadottir L, Tulinius H, Eyfjord JE (1997) Study of a single BRCA2 mutation with high carrier frequency in a small population. Am J Hum Genet 60:1079–1084
4. Dorum A, Hovig E, Trope C, Inganas M, Moller P (1999) Three per cent of Norwegian ovarian cancers are caused by BRCA1 1675delA or 1135insA. Eur J Cancer 35:779–781. doi:S0959804999000507
5. Hansen TV, Ejlertsen B, Albrechtsen A, Bergsten E, Bjerregaard P, Hansen T, Myrhoj T, Nielsen PB, Timmermans-Wielenga V, Andersen MK, Jonson L, Nielsen FC (2009) A common Greenlandic Inuit BRCA1 RING domain founder mutation. Breast Cancer Res Treat 115:69–76. doi:10.1007/s10549-008-0060-z
6. Abeliovich D, Kaduri L, Lerer I, Weinberg N, Amir G, Sagi M, Zlotogora J, Heching N, Peretz T (1997) The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. Am J Hum Genet 60:505–514
7. Thomassen M, Hansen TV, Borg A, Lianee HT, Wikman F, Pedersen IS, Bisgaard ML, Nielsen FC, Kruse TA, Gerdes AM (2008) BRCA1 and BRCA2 mutations in Danish families with hereditary breast and/or ovarian cancer. Acta Oncol 47:772–777. doi:791892967[pii];10.1080/02841860802004974
8. Cruger DG, Kruse TA, Gerdes AM (2005) 'Indirect' BRCA1/2 testing: a useful approach in hereditary breast and ovarian cancer families without a living affected relative. Clin Genet 68:228–233. doi:CGE484[pii];10.1111/j.1399-0004.2005.00484.x
9. Li Q, Liu Z, Monroe H, Culiat CT (2002) Integrated platform for detection of DNA sequence variants using capillary array electrophoresis. Electrophoresis 23:1499–1511. doi:10.1002/1522-2683(200205)23:10<1499:AID-ELPS1499>3.0.CO;2-X
10. Hogervorst FB, Cornelis RS, Bout M, Van Vliet M, Oosterwijk JC, Olmer R, Bakker B, Klijn JG, Vasen HF, Meijers-Heijboer H (1995) Rapid detection of BRCA1 mutations by the protein truncation test. Nat Genet 10:208–212. doi:10.1038/ng0695-208
11. Mohammadi L, Vreeswijk MP, Oldenburg R, Van den Ouweland A, Oosterwijk JC, van der Hout AH, Hoogerbrugge N, Ligtenberg M, Ausems MG, van der Luijt RB, Dommering CJ, Gille JJ, Verhoef S, Hogervorst FB, van Os TA, Gomez GE, Blok MJ, Wijnen JT, Helmer Q, Devilee P, van Asperen CJ, van Houwelingen HC (2009) A simple method for co-segregation analysis to evaluate the pathogenicity of unclassified variants; BRCA1 and BRCA2 as an example. BMC Cancer 9:211. doi:10.1186/1471-2407-9-211
12. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378: 789–792
13. Bergthorsson JT, Ejlertsen B, Olsen JH, Borg A, Nielsen KV, Barkardottir RB, Klausen S, Mouridsen HT, Winther K, Fenger K, Niebuhr A, Harboe TL, Niebuhr E (2001) BRCA1 and BRCA2 mutation status and cancer family history of Danish women affected with multifocal or bilateral breast cancer at a young age. J Med Genet 38:361–368
14. Gutiérrez-Enríquez S, Coderch V, Masas M, Balmaña J, Diez O (2009) The variants *BRCA1* IVS6-1G>A and *BRCA2* IVS15+1G>A lead to aberrant splicing of the transcripts. Breast Cancer Res Treat 117:461–465. doi:10.1007/s10549-008-0154-7
15. Borg A, Haile RW, Malone KE, Capanu M, Diep A, Torngren T, Teraoka S, Begg CB, Thomas DC, Concannon P, Mellemkjaer L, Bernstein L, Tellhed L, Xue S, Olson ER, Liang X, Dolle J, Borresen-Dale AL, Bernstein JL (2010) Characterization of BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance in unilateral and bilateral breast cancer: the WECARE study. Hum Mutat 31:E1200–E1240. doi:10.1002/humu.21202