UNCLASSIFIED SEQUENCE VARIANTS (UVs) AND GENETIC PREDISPOSITION TO CANCER

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Keywords : *BRCA* genes, hereditary predisposition, breast/ovarian cancer, unclassified sequence variants, pathogenicity

Abstract : Hereditary breast and ovarian cancers are mainly attributable to predisposition genes whose germinal mutations are responsible for the disease. The most common genes associated with breast/ovarian cancer are *BRCA1* and *BRCA2* but at least 20 other genes of medium of high penetrance have been associated with these types of cancer. Lifetime risk of breast cancer for *BRCA* mutations carriers approaches 90%. Appropriate medical follow-up is therefore essential for women carrying mutations in these genes. *BRCA* mutational spectrum has not been entirely characterized but not all sequence variants are pathogenic. These are classified as benign polymorphisms or unclassified variants (UV) with unknown pathological potential. To date, 43,5% of over 3500 genetic variants *BRCA1* and *BRCA2* are reported as having uncertain clinical significance. Whether one sequence variant has or not a pathogenicity implication is often a hard decision to take, involving important consequences for diagnosis and medical follow-up. Here we present several cases of unclassified sequence variants detection and interpretation by *in-silico* analysis.

INTRODUCTION

Breast cancer is one of the most common diseases in Europe. It is estimated that the annual incidence in Romania is 14,000 [Ferlay et al., 2001]. Breast cancer can be divided into sporadic, familial and hereditary. In 30% of all cases there is a familial segregation while in at least 10% a clear Mendelian inheritance is usually shown in different genetic backgrounds/populations. In familial and hereditary breast cancer cases, other types of cancer may be present and there is a strong link with ovarian cancer. According to the literature [Easton et al., 2004; Ford et al, 1995; Antoniou et al, 2003], in cases where breast/ovarian cancer is present in a very young age (17-40yrs) or in cases where multiple breast and other types of cancer (prostate, colorectal, stomach etc) are present, a germ line mutation is most often the cause of the disease. Today, in most European countries genetic counselling is offered to most cancer patients (<60 years old) in order to define if there is a defect in a cancer predisposing gene (or cancer syndrome) underlying the presence of the disease. In cases of early onset breast cancer (<45yrs) or when an important family history is present, genetic testing is offered in order to determine the molecular basis of the disease. Appropriate medical follow-up, including early and more frequent mammography and pelvic examinations for the early detection of ovarian cancer, is therefore essential for women carrying mutations in these genes. At the moment, the emphasis is on early detection; preventive measures are mostly limited to prophylactic surgery, most notably annexectomy in post-reproductive women to reduce the risk of both ovarian and breast cancer.

The most common genes associated with breast/ovarian cancer are *BRCA1* and *BRCA2* but at least 20 other genes (*CHEK2, PALB2, ATM, PTEN, RAD51C, CDH1, STK11* and others) of medium of high penetrance have been associated with these types of cancer, genes that modify the genetic risk in carriers of mutations (modifier genes) and possibly other yet to be discovered genetic factors [McClellan et al., 2010; Walsh et al., 2007]. The contribution of these different genes in breast cancer in different populations remains unknown. Important steps have been made for familial/hereditary breast cancer after the initial identification of the major susceptibility genes (*BRCA1* in 1994 and *BRCA2* in 1995) [Narod et al., 2004]. During the last years, many contributions have been brought to knowledge of structure, functions and roles of the proteins coded by BRCA genes [Bertwistle et al., 2000 ; Eccles et al., 2006 ; Feunteun et al., 2001 ; Honrado et al., 2005 ; Mullan et al., 2006 ; Shivji et al., 2004 ; Sowter et al., 2005 ; Rosen et al., 2003]. Intense research showed that BRCA proteins were involved in crucially important cellular process as DNA repair, transcription and cell cycle regulation in response to DNA damage [Yoshida et al., 2004].

BRCA mutational spectrum has not been entirely characterized. Over one thousand small sequence variations have been reported in the Breast Cancer Information Core database [BIC]. More than half of these mutations (over 300 in BRCA1 and 200 in BRCA2) cause the lost of function by premature protein synthesis termination [46], and around 60% are unique to a family [BIC]. Other variations include mis-sense alterations and intronic variants with unknown disease relevance. These are classified as benign polymorphisms or unclassified variants (UV) with unknown pathological potential. To date, 43,5% of over 3500 genetic variants BRCA1 and BRCA2 are reported as having uncertain clinical significance [BIC].

The distribution of BRCA mutations and other sequence variants has been studied in many populations, though recently in Romania. The overall data obtained by now from 19 HBOC families allowed in 2010 the first description of

genetic factors in Romanian population [L. Negura et al., 2010a]. We observed a variety of *BRCA* mutations, which may place the North-Eastern Romanian population somewhere between western populations (few recurrent, many rare or unique mutations) and eastern ones (recurrent mutations responsible for the majority of predisposed families, very few novel or unique mutations). The overall mutation frequency was 41%. Meantime, complete BRCA1 and BRCA2 sequencing permitted the description of several unclassified sequence variants, with uncertain pathogenicity, as well as common SNPs which defined local BRCA1 haplotypes [L. Negura et al., 2010b; L. Negura et al., 2010c].

Whether one sequence variant has or not a pathogenicity implication, is often a hard decision to take, involving important consequences for diagnosis and medical follow-up. Variants with unknown clinical significance (unclassified variants) always need further examination to define their possible pathogenic role Here we present several cases of unclassified sequence variants detection and interpretation by *in-silico* analysis.

PATIENTS AND METHODS

The main recruitment criterion for HBOC families was three or more breast or ovarian cancer cases within the same family line. Situations with less then three cases were considered for cancer cases were diagnosed before age 40, for breast and ovarian cases in the same family, for breast cancers in men, for bilateral breast cancer cases and for any medullar breast cancer. We also compared these criteria with INSERM family scores [Eisinger et al., 2004]. All patients agreed by written informed consent. We analyzed 26 patients from 17 unrelated HBOC families..

Genomic DNA was extracted from 10 ml peripheral blood by optimization of the Wizard® Genomic DNA purification kit (Promega Inc, Madison, WI, USA). DNA amount was estimated by spectrophotometry. Multiplex-PCR, allele-specific PCR and PCR-RFLP were performed for detection of known *BRCA1* mutations, as shown elsewhere [Negura et al., 2010b; Negura et al., 2009a; Negura et al., 2008]. *BRCA1* was also screened for large deletions and duplications by MLPA [Negura et al, 2009b].

The entire coding sequence of both genes, including exon/intron boundaries, was analysed using amplification and Sanger sequencing. Polymerase chain reaction amplifying BRCA exons were performed in a final volume of 20 µl containing 0.4mM each dNTP, 0.8 µM of each primer (sequence available on demand), 100 ng genomic DNA, and one unit of either ApliTaq® or AmpliTaq®Gold Polymerase with appropriate 1X Buffer (Applied Biosystems Inc, Foster City, CA, USA). PCR cycling comprised an initial denaturation step at 94ºC for 5 min followed by 30 cycles of 94ºC for 20 sec, 54ºC for 20 sec and 72ºC for 30 sec, and a final extension of 7 min at 72ºC. Amplicons were verified by electrophoresis on a 1,3% agarose gel, then purified by ExoSap® enzymatic digestion (Affymetrix Inc, USA), following producer's instructions. The product was sequenced in forward and reverse reactions, using the BidDye® Terminator Cycle Sequencing Kit (Applied Biosystems™), according to the manufacturer's instructions. Cycle sequencing consisted of an initial denaturation step at 94ºC for 11 min, followed by 30 cycles of 94ºC for 10 sec, 52ºC for 5 sec and 30ºC for 3 min. Sequence analysis was performed using the Seqman (DNA Star Inc, Madison, WI, USA) and the CEQ8000 Investigator (Beckman Coulter™) softwares.

Mutation presence was systematically confirmed by forward and reverse sequencing on a second independent blood sample. All mutations and sequence variants are described according to HUGO approved systematic nomenclature [HGVS]. The nomenclature for BIC traditional mutations is also indicated. In-silico analysis, including Grantham scores, was performed using Alamut® (Interactive Biosoftware™), as well as freely available softwares as ESEfinder, GVGD alignment, SIFT (*Sorting Intolerant From Tolerant*) or PolyPhen (*Poly*morphism *Phen*otyping). BIC and NCBI (Entrez SNP) databases were used. NCBI reference sequences were U14680 and NP_009227.1 for *BRCA1*, respectively NM_000059.3 and NP_000050.2 for *BRCA2*.

RESULTS AND DISCUSSION

When completely sequencing *BRCA1* and *BRCA2* genes, beside deleterious mutations and known benign polymorphisms, a distinct sequence category can be identified, This include heterozygous nucleotide substitutions, very rare or unique within the population, with predictable effects on proteins, but with much less obvious involvement in hereditary predisposition to cancer. The incertitude of direct correlations with protein functions make us call these sequences variants with unknown clinical significance or "Unclassified variants" (UVs). In international databases, UVs are localized somewhere between deleterious mutations and benign SNPs, waiting for further research or bioinformatics analysis to confer them rather a pathogenic or SNP status. It is a very rude task to assign a consequence and to interpret identified UVs, and the

majority of explications often have just a speculative level. However, some clues could possibly allow an assignment within categories like “rather dangerous UVs” or “rather neutral UVs”.

Table I. Problematic sequence variants identified in BRCA genes

FAMILY	GENE	exon	BIC Nomenclature	HGVS nomenclature	Efect	BIC ?	TYPE	CLASS	AA conservation	grantham	domain	splice	ESE finder	GVGD alignment	SIFT	polyphen	NCBI
00101AN	B1	15	4763G>A	**c.4644G>A**	**p.Thr1548Thr**	no	silent	P		n/a		NO effect	no effect				
01601VH	B1	7	546G>C	**c.427G>C**	**p.Glu143Gln**	no	missens	UV	9 of 11 are E	29	brca1	NO effect	no effect	C0	tolerated	1.35 benign	
00701AC	B2	13	4486G>T	**c.4258G>T**	**p.Asp1420Tyr**	yes	missens	P	10 of 11 are E or D	160		NO effect	loss of 1	C15	not tolerated	2.17 probably	rs28897727
01301DT	B2	1109	4817A>G	**c.4589A>G**	**p.Lys1530Arg**	no	missens	UV	100%	26	brca2	NO effect	no effect		not tolerated	1.25 probably	
01902LM	B1	16	5075G>A	**c.4956G>A**	**p.Met1652Ile**	yes	missens	UV	100% hydrophobic	10	brct	NO effect	adds 1	C0	tolerated	1.11 benign	rs1799967

We identified five sequence variants other than mutations or common SNPs [Negura et al., 2010(a)]. In table I one can identify 3 variants for *BRCA1* and 2 for *BRCA2* gene. Two of these variants are already known and integrated in BIC database [BIC], while three others seem to be novel. All variants are mononucleotide substitutions. One of them has no consequence on amino acid sequence in the protein, due to the genetic code degenerescence, so it can be considered as silent. The other 3 variants generate amino acid substitutions, so we have to take in account Grantham substitution score and the amino acid position within the protein (conserved/not). We present below the 5 variants and their interpretation.

UV-1 (c.427G>C) – BRCA1

In figure 1 is presented (up), at a sequence level, the first sequence variant that we will call UV-1, in comparison with a wild-type sequence (down). The patient bearing this BRCA1 UV is also carrying the *BRCA2* c.8249_8251delAGA mutation within exon 18. We can see UV-1 is a heterozygous substitution of a G, within *BRCA1* exon 7, with a C nucleotide on modified allele.

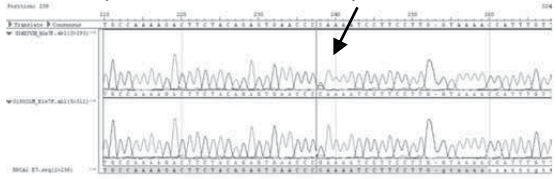
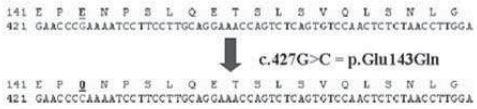


Figure 1. Identification of UV-1 (c.427G>C) in *BRCA1*-exon7

The substitued nucleotide has the position 427 in the coding *BRCA1* sequence (ref. U14680), so the variant will be called c.427G>C in HUGO nomenclature and 546G>C in BIC nomenclature. Affecting the first nucleotide of the codon 143 (GAA – glutamic acid), the substitution generates a CAA codon 143, coding for glutamine, so we will have a mismatch substitution causing p.143E>Q, as we can see below.



The Alamut software is considering that no splicing or ESE (*exonic splicing enhancer*) site is affected and is estimating that E143Q is rather a tolerated substitution (confirmed by SIFT). Polyphen is according a rather benign score of 1,35, while Grantham score for Glu>Gln is very low, 29. However, there is a 82% conservation of the Glu143 when comparing several species, and we should take this in account when assigning UV-1 as not a silent variant, even if tolerated. Still, since *BRCA1* c.427G>C occurred in a family with a deleterious *BRCA2* mutation, we believe it is unlikely to cause disease.

UV-2 (c.4644G>A) – BRCA1

Figure 2 presents, in forward and reverse sequencing, a sequence variant identified in two patients from the same HBOC family. Both are carrier of the deleterious *BRCA1* c.342_344delTC mutation. This UV-2 variants consists in a mismatch substitution of a G, within exon 15 of *BRCA1* gene, with an A on the modified allele, which appears heterozygous G/A for this site in the image below.

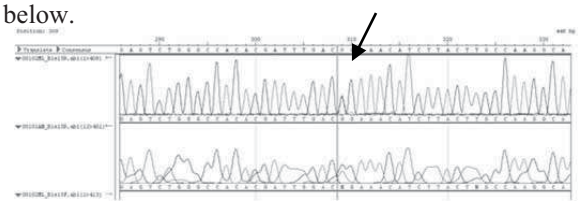


Figure 2. Identification of UV-2 (c.4644G>A) in *BRCA1*-exon15

The affected nucleotide is the 4644 in the coding *BRCA1* sequence (ref. U14680), so the variant will be called c.4644G>A in HUGO nomenclature and 4763G>A in BIC nomenclature. For the protein level, this nucleotide is the third of a ACG codon, coding for threonine 1584. The A for G substitution will have no consequence, since all codons starting with AC are coding for threonine. As we can see in the image below, the variation is translating in the protein as Thr1548Thr, so the UV-2 variant (c.4644G>A), although unidentified before, can clearly be considered as silent. Alamut software proved that neither splicing, nore ESE sites were affected by this sequence variation.

UV-3 (c.4956G>A) – BRCA1

A third sequence variation on *BRCA1* was observed within exon 16. As one can observe in figure 3, heterozygous substitution of a G nucleotide with an A appears in the upper sequence, in one patient's DNA (up), while the mother is wild-type (down)

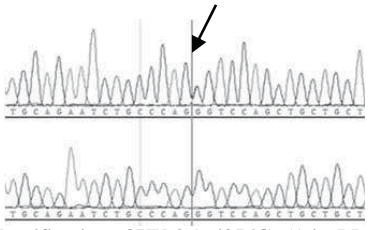
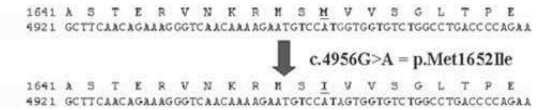


Figure 3. Identification of UV-3 (c.4956G>A) in *BRCA1*-exon16

The substituted G is situated in the position 4956 in coding *BRCA1* sequence (ref. U14680), so the variant will be called c. 4956G>A in HUGO nomenclature and 5057G>A in BIC nomenclature. It is the last nucleotide of the 1652 codon (ATG – coding for Methionine). Its replacement with an A will generate an ATA 1625 codon, coding for Isoleucine, so UV-3 is a mis-sense genetic variation with a p.Met1652Ile consequence, as we can see below.



This variant is already known in BIC database, where numbers of cases have been reported. The amino-acid in the 1625 position has a 100% hydrophobic conservation, which may be tolerated (Alamut-SIFT) as both Met and Ile are hydrophobic. Since the 10 Grantham score is one of the lowest possible in substitutions, and Polyphen is also calculating a 1,11 very low score, the benign level of the substitution seems quite certain. No effect is either estimated on splicing sites, although an additional ESE (*exonic splicing enhancer*) is estimated to appear within exon 16. Still, there is not enough argument to consider Met1652Ile a UV. Moreover, there is a SNP code for this variant in NCBI EntrezSNP database (rs1799967) and it happened to be used in H5 haplotype assignment in Judkins haplotype characterization [Judkins et al., 2005]. Overall, UV-3 is not a UV at all, it is just a simple benign common polymorphism.

UV-4 (c.4258G>T) – BRCA2

The first sequence variant identified in BRCA2 gene is a T for G substitution localized within exon 11. The patient bearing this UV-4 is carrying the BRCA1 deleterious c.5266dupC mutation (same as the recurrent 5382 eastern founder mutation). In figure 4, the heterozygous variation (G and T) can be observed in the upper sequence, comparing with the downer wild-type patient (G only).

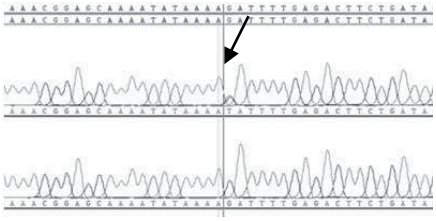
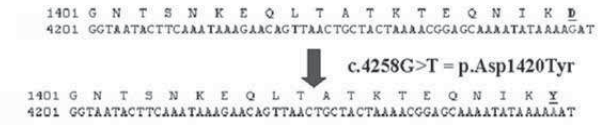


Figure 4. Identification of UV-4 (c.4258G>T) in *BRCA1*-exon11

In HUGO nomenclature, the variation is called c.4258G>T, while it is 4486G>T in the BIC nomenclature (ref. NM_000059.3). The affected nucleotide is the first G of the 1420 GAT codon coding for aspartic acid. Its substitution with a T brings a TAT Tyrosine coding 1420 codon, so the consequence will be p.Asp1420Tyr, as we can see below.



There is a lot to say about our UV-4, even if it already exists in BIC database and also in NCBI EntrezSNP as the rs28897727. Alamut software estimates a 90% conservation of the 1420 amino acid either as a aspartic (D) or a glutamic (E) acid form. SIFT doesn’t tolerate its substitution with a tyrosine, and the Grantham score of such substitution is huge (160). Polyphen is also estimating for Asp1420Tyr a probably damaging 2,17 score. An ESE site is generated

within exon 11 by the same substitution, even if splicing sites are not affected. GVGD gives a big C15 score of untolerance. All this should make c.4258G>T a damaging pathogenic substitution, but it is considered polymorphic by BIC, as common is certain populations and not affecting any known protein domain (why being so conserved then?). SNP frequency (0.26 calculated on 1000 Americans with European origin) makes homozygous context possible, although no such situation has ever been reported (here again, why?).

In our patient, this UV-4 has a special status, as it is making a genetic difference between two HBOC families bearing the same recurrent *BRCA1* 5382insC mutation [Negura et al., 2010a, Negura et al., 2010b]. Interestingly, the UV-4 bearing family shows an ovarian cancer history, whilst the non-bearing is rather a breast cancer family; this could open a discussion about wether *BRCA2* c.4258G>T could modifying the cancer risk and could influence cancer phenotype in *BRCA1* mutation carriers, even if not pathogenic by itself. The situation is far of being definitive and we believe *BRCA2* c.4258G>T is the typical situation of UV pending for a decision, with arguments for both being pathogenic or simply polymorphic.

UV-5 (c.4589A>G) – BRCA2

The last sequence variant we will discuss here is a G for A substitution within again BRCA2 exon 11. In figure 5, this variation clearly appears heterozygous in a breast cancer patient, with familial history and not carrying a BRCA mutation.

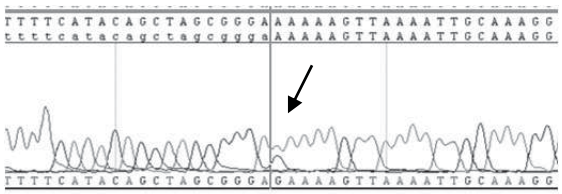


Figure 5. Identification of UV-5 (c.4589A>G) in *BRCA1*-exon11

As the substituted nucleotide is the 4589 within the coding sequence of BRCA2 gene (ref. NM_000059.3), the variation is called c.4589A>G in HUGO nomenclature and 4817A>Gin BIC nomenclature. It is the second nucleotide of a series of 6 adenines, ant it occupies the second position in a lysine coding AAA codon; when modified, the codon becomes AGA, coding for 1530 arginine, so the effect on the protein is p.Lys1530Arg, as shown below.

The conservation for the amino acid 1530 is estimated by Alamut at 100%, while SIFT does not tolerate substituting the concerning lysine with any other amino acid, although the low 26 Grantham score would allow it. No effect is estimated on splice sites or on ESE, and Polyphen is giving a low 1,25 score considering the substitution rather benign. Our UV-5 substitution was never identified before and doesn't exist in BIC database [Negura et al., 2010a]. The 100% conservation of the lysine and the lack of tolerance estimated for any substitution in this position make us consider c.4589A>G having an important pathogenic potential. In fact, is the most

probably pathogenic of all 5 UVs described here. Further investigations are necessary to determine whether this UV is responsible for the disease in the carrier patient.

CONCLUSIONS

BRCA sequence variants of unclear clinical significance (Table I) do not bring directly useful information. From five UVs described here, only one (*BRCA2* c.4589A>G) has a clear pathogenic potential, due to 100% conservation of the amino acid substituted. Two other UVs, *BRCA1* c.427G>C and less probably *BRCA1* c.4956G>A, need additional data to confirm a pathogenic potential. Two variants, *BRCA1* c.4644G>A and *BRCA2* c.4258G>T, are clearly defined as benign, either because being silent or quite common in the population. Three of four such UVs were found in patients also carrying a deleterious mutation, though this situation isn't much relevant since mutation and UV affect different genes in two of those cases. More interesting is rather the presence of the *BRCA2* c.4258G>T unclear UV in one patient harbouring the BRCA1 recurrent c.5266dupC, but not in other carrier patients from unrelated family. Still, its clinical involvement is not proven and whether c.4258G>T could be responsible for the difference of phenotypes between c.5266dupC carriers remains speculative.

Investigations continue in order to establish more clear involvement of those sequence variants into pathology.

REFERENCES

Ferlay J. et al., 2001, GLOBOCAN 2000 : Cancer incidence, mortality and prevalence worldwide. *International Agency for Research on Cancer Press*; IARC Cancer base no.5.

Easton D.F., Hopper J.L., Thomas D.C., Antoniou A., Pharoah P.D.P., Whittemore A. and Haile R.W., 2004, Breast cancer risks for BRCA1/2 carriers. *Science*, 306, 2187-2188.

Ford D., Easton D.F. and Peto J., 1995, Estimates of the gene frequency of BRCA1 mutations and its contribution to breast and ovarian cancer incidence. *Am J Hum Genet*, 57, 1457–1462.

Antoniou A. et al., 2003, Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*, 72(5), 1117-1130.

McClellan J. and King M.C., 2010, Genetic heterogeneity in human disease. *Cell*, 141(2), 210-217.

Walsh T. and King M.C., 2007, Ten genes for inherited breast cancer. *Cancer Cell*, 11(2), 103-105.

Narod S.A. and Foulkes W.D., 2004, BRCA1 and BRCA2: 1994 and beyond. *Nat Rev Cancer*, 4(9), 665-676.

Bertwistle D. and Ashworth A., 2000, BRCA1 and BRCA2. *Curr Biol*, 10(16), R582.

Eccles D.M., 2006, Breast Cancer Treatment and Genetics. *Fam Cancer*, 5(2), 127-128.

Feunteun J., 2001, BRCA1 et BRCA2: des echafaudages de reparation des lesions de l'ADN. *Medecine/Sciences*, 17, 1070-1071.

Honrado E., Benitez J. and Palacios J., 2005, The molecular pathology of hereditary breast cancer: genetic testing and therapeutic implications. *Mod Pathol*, 18(10), 1305-1320.

Mullan P.B., Quinn J.E. and Harkin D.P., 2006, The role of BRCA1 in transcriptional regulation and cell cycle control. *Oncogene*, 25, 5854-5863.

Shivji M.K.K. and Venkitaraman A.R., 2004, DNA recombination, chromosomal stability and carcinogenesis: insights into the role of BRCA2. *DNA Repair*, 3, 835-843.

Sowter H.M. and Ashworth A., 2005, BRCA1 and BRCA2 as ovarian cancer susceptibility genes. *Carcinogenesis*, 26(10), 1651–1656.

Rosen E.M., Fan S., Pestell R.G. and Goldberg I.D., 2003, BRCA1 gene in breast cancer. *J Cell Physiol*, 196, 19-41.

Yoshida K. and Miki Y., 2004, Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci*, 95(11), 866-871.

Breast Information Core (BIC) database (http://research.nhgri.nih.gov/bic/).

Negură L., Uhrhammer N., Negură A., Artenie V., Caraseviciv E. and Bignon Y.-J., 2010(a), Complete BRCA mutation screening within breast and ovarian cancer predisposition families in North-Eastern Romanian population. *Familial Cancer,* 9(4), 519-523.
Negură L., Carasevici E., Negură A., Uhrhammer N. and Bignon Y.-J., 2010(b), Identification of a recurrent BRCA1 mutation in two breast/ovarian cancer predisposition families with distinct phenotypes, by allele-specific multiplex-PCR. *Romanian Review of Laboratory Medicine*, 18(2), 53-61.
Negură L., Uhrhammer N., Negură A., Artenie V., Caraseviciv E. and Bignon Y.-J., 2010(c), Haplotype characterization of BRCA1 gene in North-Eastern Romania. *European Journal of Human Genetics*, Volume 18, Supplement 1, 165-166.
Eisinger F. et al., 2004, Identification and management of hereditary predisposition to cancer of the breast and the ovary. *Bull Cancer*, 91(3), 219-237.
Negură L., Carasevici E., Negură A., Uhrhammer N. and Bignon Y.-J., 2009(a), Rapide PCR-RFLP screening for a novel BRCA2 mutation found in a north-eastern Romanian population. *FEBS Journal*, Volume 276, Supplement 1, p. 331.
Negură L., Carasevici E., Negură A., Uhrhammer N. and Bignon Y.-J., 2008, Molecular investigation of BRCA gene mutations by multiplex-PCR optimization within HBOC families in North-Eastern Romania. *FEBS Journal*, Volume 275, Supplement 1, p. 419.
Negură L., Carasevici E., Artenie V., Negură A., 2009(b), Using MLPA for large genomic rearrangements detection in breast cancer predisposition genes. *Rev. Med. Chir. Soc. Med. Nat. Iasi*, 113(4), 1182-1190.
Human Genome Variation Society (HGVS) Nomenclature for the description of sequence variations (http://www.genomic.unimelb.edu.au/mdi/mutnomen/).
Judkins T. et al., 2005, Application of embryonic lethal and other obvious phenotypes to characterize the clinical significance of genetic variants found in trans with known deleterious mutations. *Canc Res*, 65(21), 10096-10103.

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ACKNOWLEDGEMENTS
This study was possible with partial financial support from the Romanian Ministry for Education and Research, by the CNCSIS research grant PN-II-ID-PCE-2008, code 1990/2008, contract 1199/2009, financed by UEFISCSU (Unitatea Executivă pentru Finanţarea Învăţământului Superior şi a Cercetării Ştiinţifice Universitare).