Letters to the Editor

J Med Genet 1999;**36**:571–572

Biochemical and genetic characterisation shows that the BRCA1 IVS20 insertion is a polymorphism

EDITOR—Two breast cancer susceptibility genes, BRCA1 and BRCA2, have been identified.[1–3] Combined, these large and complex genes have over 800 reported genetic variants. More than 50 variants occurring within the introns of these genes are known. The clinical significance of these intronic variants, which could potentially impact RNA splicing, is largely undetermined.[4–7]

A variant within intron 20 of BRCA1 occurs through the duplication of 12 base pairs (bp) (GTATTCCACTCC) 48 bp from the donor junction. This variant has been observed in patients from cancer families.[8–10] Furthermore, biochemical analysis showed the loss of contribution by one chromosome to the normal RNA in a breast cancer patient with IVS20ins12.[11] This was shown by sequencing cDNA which showed the loss of a heterozygous base at the silent polymorphism at codon 1436 (serine TCT or TCC). These authors concluded that IVS20ins12 could represent a clinically significant regulatory mutation, although this variant was also present in a control specimen. More recently, IVS20ins12 was reported in four controls (with varying family histories of cancer, but otherwise healthy) and one early onset breast/ovarian cancer patient in a Polish BRCA1 study.[12] No aberrant splicing products were detected in these samples, but mRNA abundance and possible loss of transcript have not been assayed. These authors concluded that the issue merits a more extensive study.

Here, a patient diagnosed with breast cancer at the age of 40 and with cancer reported on the maternal side of her family is described. Clinical full sequence analysis of BRCA1 and BRCA2 (BRACAnalysis, Myriad Genetic Laboratories, Salt Lake City, UT) identified IVS20ins12, as well as common single nucleotide polymorphisms (SNPs), particularly S1613G (AGT for serine and GGT for glycine). The patient was counselled regarding the uncertain clinical significance of this variant and elected to participate in this research analysis.

cDNA was synthesised from RNA isolated from peripheral blood mononuclear cells from both the patient and a control sample that also contained S1613G. PCR reactions generated fragments spanning S1613G to exon 21. These products were separated by agarose gel electrophoresis and fragments corresponding in size to the normal transcript were isolated. No notable bands that might represent alternative/aberrant splice products were observed in either sample. The isolated fragments served as targets in subsequent PCR reactions designed to evaluate the sequence at either the polymorphism or the exon junctions. DNA sequencing clearly shows a heterozygous base at codon 1613 (fig 1A) and normal RNA splice junctions for exons 18-21 (fig 1B), indicating that both chromosomes contribute to normally spliced transcripts.

In seeming contrast to our analysis, Robledo *et al*[11] observed loss of transcript in an IVS20ins12 carrier. In patient RNA, only the sequence TCC was detected at

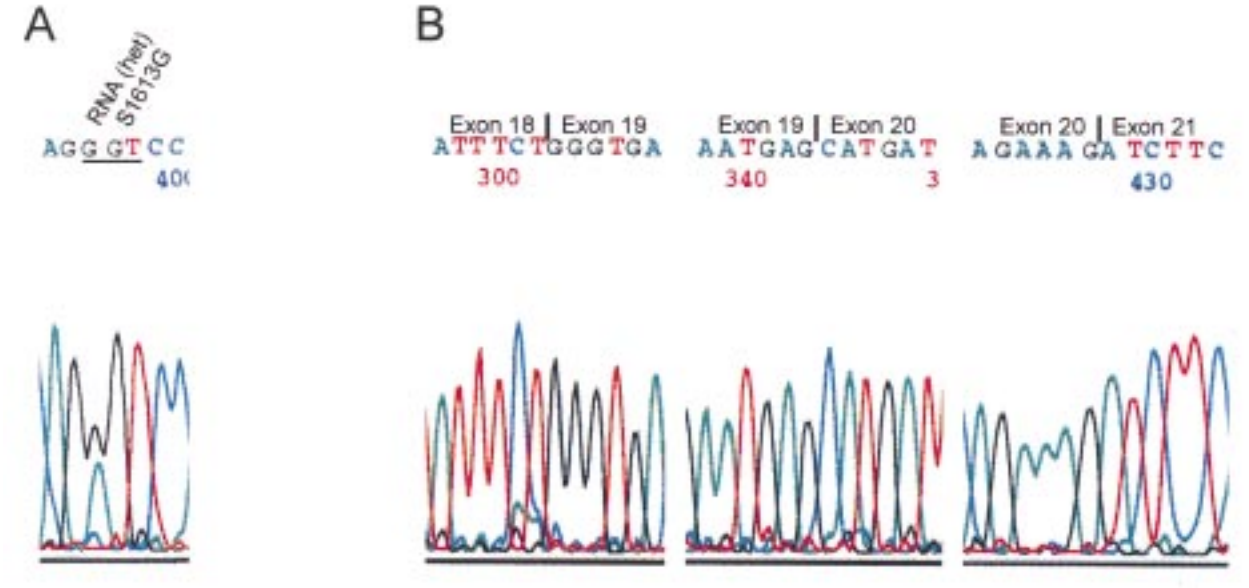

Figure 1 Sequence analysis for RNA transcripts from a patient with IVS20ins12. (A) Heterozygous base pair at codon 1613 in the patient RNA sample. A fragment derived from a primary amplification reaction using the primer pair 14F and 21R was gel purified and used as a target in a subsequent reaction with the primer pair 14F and 16R. The product from the second reaction was sequenced (the data were extracted with ABI Prism V2.1.1 sequencing analysis software). (B) Normal RNA splice junctions at exons 18 to 21 on transcripts contributed by both chromosomes of a patient with IVS20ins12. This sequence was derived from the same primary fragment described in (A) except that secondary amplification used the primer pair 16F and 21R. The exon junctions are normally spliced. The primers used in these experiments contained the following designations and gene specific sequences (all numerical base pair designations conform to GenBank submission U14680[1]): 14F, 5'-GTGTCTGCAGATAGTTCTACCAGTA (bases 4554-4578), 16F, 5'-CAGCTGCTGCTCATACTACTGATA (bases 4960-4983), 16R, 5'-TGACCCTTTCTGTTGAAGCTGTCAA (bases 5058-5034), 21R, 5'-TGAAGGGCCCATAGCAACAGA (bases 5516-5496). To facilitate nucleotide sequencing M13 tails with the sequences 5'-GTTTTCCCAGTCACGACG for forward or 5'- AGGAAACAGCTATGACCAT for reverse were incorporated on all primers. Double stranded dye primer thermocycle sequence products were detected on automated instruments (Model 377, Applied Biosystems, Foster City, CA).

A

Haplotype	Exon4-49C/T	Q356R	D693N	S694S	L771L	P871L	E1038G	S1040N	K1183R	S1436S	S1613G	Haplotype designation[13]
												1
				■	■	■	■		■	■	■	2
		■										4
								■				5
	■		■	■	■	■	■		■	■	■	7
						■						8

B

Anon ID	Exon4-49C/T	Q356R	D693N	S694S	L771L	P871L	E1038G	S1040N	K1183R	S1436S	S1613G	Haplotype Pairs
816	0	0	0	1	1	1	1	0	1	1	1	2 + 1
917	0	0	0	2	2	2	2	0	2	2	2	2 + 2
1319	0	0	0	1	1	1	1	1	1	1	1	2 + 5
2033	0	0	0	1	1	1	1	0	1	1	1	2 + 1
2047	0	0	0	2	2	2	2	0	2	2	2	2 + 2
2687	0	1	0	1	1	1	1	0	1	1	1	2 + 4
3886	0	0	0	1	1	1	1	0	1	1	1	2 + 1
4024	1	0	1	2	2	2	2	0	2	2	2	2 + 7
5147	0	0	0	1	1	1	1	0	1	1	1	2 + 1
5185	0	0	0	2	2	2	2	0	2	2	2	2 + 2
5435	0	0	0	1	1	1	1	0	1	1	1	2 + 1
5695	0	0	0	2	2	2	2	0	2	2	2	2 + 2
6131	0	0	0	1	1	1	1	0	1	1	1	2 + 1
6167	0	0	0	1	1	1	1	0	1	1	1	2 + 1
7397	0	0	0	1	1	1	1	0	1	1	1	2 + 1
8664	0	0	0	1	1	1	1	0	1	1	1	2 + 1
8984	0	0	0	1	1	2	1	0	1	1	1	2 + 8
9141	0	0	0	2	2	2	2	0	2	2	2	2 + 2
9709	0	0	0	2	2	2	2	0	2	2	2	2 + 2

Figure 2 Informative polymorphisms and haplotype pair assignment among the set of IVS20ins12 carriers. (A) SNP haplotype designations. The presence of a non-consensus allele in common haplotypes is depicted schematically with solid squares. (B) Polymorphisms among IVS20ins12 carriers. The figure contains numbers of non-consensus alleles in each informative locus (0, 1, or 2), as well as the pair of common haplotypes accounting for a particular allele combination. An anonymised BRCA1 sequence variation database used in the present study contained 5593 individual full BRCA1 sequences, encompassing all sequences of BRCA1 exons between exon 2 and the end of the coding sequence, as well as adjacent intronic sequences, as described by Shattuck-Eidens et al.[13] In addition, the database contained ethnic ancestry identifiers, whenever available. Note that the probands were not ascertained using a single set of criteria, and phenotype/family history information has been stripped on anonymisation. Therefore, essentially no genotype-phenotype correlation could be made solely on the basis of the information present in the database. The following frequent BRCA1 SNP loci were used for haplotype pair assignment[13]: exon 4-49C→T, Q356R, D693N, S694S, L771L, P871L, E1038G, S1040N, K1183R, S1436S, and S1613G.

codon 1436; RNA from the consensus (TCT) allele was lost. The question remained whether the lost allele came from the same chromosome that also contained IVS20ins12. A single nucleotide polymorphism haplotype analysis of patient samples addressed this question.

Our anonymised BRCA1 sequence variation database contained 19 observations of IVS20ins12 and they all shared SNP haplotype 2 (fig 2). There were six homozygous haplotype 2 carriers. The rest contained a haplotype 2 chromosome along with a chromosome with one of the five other previously described common haplotypes. Therefore, IVS20ins12 is found solely on a haplotype 2 background. This finding agrees with the previous observation that the vast majority of infrequent sequence variations within the BRCA1 gene are only found on a single SNP haplotype background.[13 14] Indeed, given that this population includes 3550 subjects who do not have a single copy of haplotype 2, and none of these carries an insertion in IVS20, there is 90% confidence that the frequency of IVS20ins12 on non-haplotype 2 backgrounds does not exceed 0.033%. In contrast, 0.77% of 2452 instances of haplotype 2 carry IVS20ins12. Since IVS20ins12 is present on the background of haplotype 2, for which codon 1436 is encoded by TCC, the RNA detected by Robledo *et al*[11] was transcribed from the chromosome carrying the insertion. It appears that the loss of transcript resulted from a hitherto unidentified BRCA1 mutation on the other chromosome.

These data and analyses strongly suggest that BRCA1 IVS20ins12 is a polymorphism with no obvious clinical significance. Biochemical evaluation of RNA encoding BRCA1 from a patient carrying the variant shows that both chromosomes contribute to the normally spliced transcript. Although these transcripts were isolated from leucocytes, it is highly unlikely that tissue specific splicing events would create mutant transcripts. This conclusion is augmented by the clarification of a seemingly conflicting result. Here, genetic analysis showed the presence of IVS20ins12 on the only functional copy of the BRCA1 gene in a previously described carrier of a loss of transcript mutation.[11]

THOMAS SCHOLL
MICHAEL T PYNE
BRIAN WARD
DMITRY PRUSS

Myriad Genetic Laboratories, 320 Wakara Way, Salt Lake City, UT 84108, USA

1 Miki Y, Swensen J, Shattuck-Eidens D, *et al*. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;**266**:66-71.
2 Tavtigian SV, Simard J, Rommens J, *et al*. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nat Genet* 1996;**12**:333-7.
3 Wooster R, Bignell G, Lancaster J, *et al*. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1994;**378**:789-92.
4 Friedman LS, Ostermeyer EA, Szabo CI, *et al*. Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in families. *Nat Genet* 1994;**8**:399-404.
5 Serova O, Montagna M, Torchard D, *et al*. A high incidence of BRCA1 mutations in 20 breast-ovarian cancer families. *Am J Hum Genet* 1996;**58**: 42-51.
6 Hoffman JD, Hallam SE, Venne VL, *et al*. Implications of a novel cryptic splice site in the BRCA1 gene. *Am J Med Genet* 1998;**80**:140-4.
7 Scholl T, Pyne MT, Russo D, Ward BE. BRCA1 IVS16+6T→C is a deleterious mutation that creates an aberrant transcript by activating a cryptic splice donor site. (Submitted.)
8 Langston AA, Malone KE, Thompson JD, *et al*. BRCA1 mutations in a population- based sample of young women with breast cancer. *N Engl J Med* 1996;**334**:137-42.
9 Langston AA, Stanford JL, Wicklund KG, *et al*. Germ-line BRCA1 mutations in selected men with prostate cancer. *Am J Hum Genet* 1996b;**58**:881-5.
10 Takahashi H, Behbakht K, McGovern PE, *et al*. Mutation analysis of the BRCA1 gene in ovarian cancers. *Cancer Res* 1995;**55**:2998-3002.
11 Robledo M, Osorio A, Sentis C, *et al*. The 12 base pair duplication/insertion alteration could be a regulatory mutation. *J Med Genet* 1997;**34**:592-3.
12 Sobczak K, Kozlowski P, Napierala M, *et al*. Novel BRCA1 mutations and more frequent intron-20 alteration found among 236 women from western Poland. *Oncogene* 1997;**15**:1773-9.
13 Shattuck-Eidens D, Oliphant A, McClure M, *et al*. BRCA1 sequence analysis in women at high risk for susceptibility mutations: risk factor analysis and implications for genetic testing. *JAMA* 1997;**278**:1242-50.
14 Neuhausen SL, Mazoyer S, Friedman L, *et al*. Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. *Am J Hum Genet* 1996;**58**:271-80.