Letters to the Editor

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Common *BRCA1* Variants and Transcriptional Activation

To the Editor:

Germ-line mutations in *BRCA1* account for a portion of breast and ovarian cancer predisposition (Easton et al. 1993; Miki et al. 1994). *BRCA1* encodes a 1,863–amino acid protein, with tumor-suppression function, since tumors in *BRCA1*-linked families show loss of heterozygosity in the *BRCA1* locus, retaining the mutant allele (Smith et al. 1992; Neuhausen and Marshall 1994). In addition, Scully et al. (1997) have shown that *BRCA1* interacts in vivo with Rad51, suggesting a role for *BRCA1* in the maintenance of genome integrity. Recently, we and others have demonstrated that the *BRCA1* C-terminal acidic region can act as a transcriptional activation domain in vivo when fused to a heterologous DNA binding domain (Monteiro et al. 1996; Chapman and Verma 1996). We have also shown that missense germ-line mutations found in breast and ovarian cancer patients abolish transcription activation. The C-terminus encompasses a domain recently defined as the BRCT domain (Koonin et al. 1996), which is found in several proteins involved in the control of cell cycle. We hypothesize that *BRCA1* can act as a transcriptional activator and that loss of this function predisposes the carrier to disease.

We investigated whether common variants found in the general population and not correlated with disease predisposition would also activate transcription. To do this, we created fusions of GAL4 DNA binding domain (pGBT9 vectors) and *BRCA1* fragments (amino acids [aa] 1560–1863), consisting of the previously identified transactivation domain (Monteiro et al. 1996). We transformed yeast strains (HF7c and SFY526) containing two reporter genes (*HIS3,* which when activated allows growth in the absence of histidine, and *lacZ,* which when activated produces β-galactosidase) with the following constructs (all in the context of aa 1560–1863): (*a*) the wild-type *BRCA1* sequences; (*b*) an unclassified variant suspected to be a disease predisposing mutation Ser1613Cys (Breast Cancer Information Core 1997); (*c*) a polymorphism at the same position Ser1613Gly; and (*d*) a polymorphism Met1652Ile, which does not correlate with disease predisposition (Breast Cancer Information Core 1997). Our previous data have indicated that a yeast-based assay gives the same results as the mammalian transfection system (Monteiro et al. 1996).

As expected, the wild-type *BRCA1* C-terminus activated transcription of both reporter genes (table 1). The Ser1613Cys mutation, which is suspected to be correlated with disease predisposition, did not show activation of either gene. On the other hand, both common variants of *BRCA1* showed wild-type activity in all the reporter genes tested.

Table 1

Transcriptional Activation by Common Variants of BRCA1

	HF7c (Liquid)	HF7c (Solid)	SFY526 (β-Galactosidase)
Wt (aa1560–1863)	+ (1.0)	+	+
Ser1613Cys	− (0)	−	−
Ser1613Gly	+ (1.0)	+	+
Met1652Ile	+ (1.0)	+	+

NOTE.—*S. cerevisiae* carrying the indicated fragments and mutants were assayed for growth in the absence of histidine, both in liquid and on solid medium (HF7c) and also for production of β-galactosidase (SFY526). Cultures were grown as described by Monteiro et al. (1996). Relative activity is shown in parenthesis. Several (4–8) independent clones were assayed for each construct.

It is interesting to note that in a recent report, Dunning et al. (1997) examined whether common *BRCA1* variants could confer modest individual risk as opposed to the highly penetrant mutations found in multiple case families of early onset of breast and ovarian cancer. Examining four polymorphisms, one of which is in the C-terminal region (Ser1613Gly, the same mutation used here), Dunning et al. concluded that haplotypes containing these variants do not contribute significantly to disease predisposition. Our data agree with this analysis, since the construct containing this variant had wild-type activity in our assay.

In conclusion, we show here that two common variants of *BRCA1* that do not contribute to disease predisposition show wild-type transcriptional activity. Moreover, our results strongly suggest that the Ser1613Cys mutation may predispose the carrier to disease. We believe these results provide additional indication that *BRCA1* acts as a tumor suppressor in a transcription-dependent manner. Furthermore, these data validate the use of the yeast transcription assay to predict disease predisposition conferred by mutations found in the C-terminal region.

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The Significance of the 187G (H63D) Mutation in Hemochromatosis

To the Editor:

Carella et al. (1997) have confirmed the report of Feder et al. (1996) that the incidence of the 845A (C282Y; OMIM 235200.0001) mutation in the HLA-H gene is very high in patients with hereditary hemochromatosis. It is of special interest that the 0.01 gene frequency of this gene in the general Italian population is considerably lower than in those of European ancestry who have been studied in the United States and in northern Europe. In agreement with the data from this southern European population, we have recently found that among nearly 400 Ashkenazi Jews the gene frequency of the C292Y mutation in HLA-H was only 0.013, compared with 0.07 in the non-Jewish American white population (Beutler and Gelbart 1997). These findings and those of Carella et al. seem quite consistent with the putative Celtic origin of this mutation (Jazwinska et al. 1995). We sequenced the entire HLA-H coding region of 16 chromosomes in patients with hemochromatosis who did not have the C282Y mutation and, like Carella et al., found no additional mutations. The surprising lack of other mutations suggests the possibility that the C282Y mutation causes a gain in function (Beutler et al. 1997*a*). On the other hand, the accumulation of iron in mice with targeted disruption of β_2 microglobulin