

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. 1997a). On the other hand, the accumulation of iron in mice with targeted disruption of β_2 microglobulin

(Rothenberg and Voland 1996; Santos et al. 1996) implies that failure to display the molecule on the cell surface might be sufficient to cause iron-storage disease.

In the original article by Feder et al. (1996) and in our confirmation of these studies (Beutler et al. 1996), it was clearly shown that there was a highly significant relationship between the other mutation that is commonly found on HLA-H, namely 187G (H63D) and hemochromatosis. But there now seems to be considerable confusion regarding the role of this mutation. Jazwinska et al. (1996) and Jouanolle et al. (1996) both implied that this mutation does not play a causal role in hereditary hemochromatosis, and Carella et al. (1997) refer to its role as being controversial. I suggest that there is no reason for controversy; the population data from all studies published thus far show convincingly a strong relationship.

While Carella et al. (1997) do not reject the possibility that a relationship exists between H63D and hemochromatosis, they suggest that "several lines of evidence indicate that this variant is a polymorphic change" (p. 831).

1. *It attains a similar frequency in patients and controls (and occurs on chromosomes unrelated to the ancestral one).* This, indeed, is the argument that has been put forward by others. For example, Jouanolle et al. (1996) also conclude that the H63D mutation does not bear a relationship to hemochromatosis, reasoning that the frequency of the mutation, 0.16, is the same in the hemochromatosis and control group. Similarly, Carella et al. (1997) point out that in their study the mutation frequency among hemochromatosis patients is 10/150 (0.067) while it is 10/100 (0.10) in the control population. However, one must take into account the fact that no chromosome that bears the C282Y mutation has ever been found to contain the H63D mutation. Of the 150 chromosomes that Carella et al. (1997) analyzed, only 47 are "at risk" for carrying the H63D mutation, and 10 of these chromosomes contain this mutation, a frequency of 0.21, compared to the 0.10 control frequency.

The strong evidence that proves the relationship of the H63D mutation to the disease is the frequency of the H63D mutation in patients with hemochromatosis who carry only one copy of C282Y. If the H63D mutation were unrelated to hemochromatosis, one would also expect the incidence of this mutation to be the same in such patients as in the general population, but this is not the case. In the series of Feder et al. (1996), 8/9 patients heterozygous for the C282Y mutation carried the H63D mutation, whereas in our series it was 8/10 (Beutler et al. 1996); Jouanolle et al. (1996) found 3/3, Borot et al. (1997) 4/8, and Carella et al. now report 5/8. Thus, 28/38, or an impressive 73.7%, of the chromosomes at risk in hemochromatosis patients heterozygous for C282Y who have been analyzed contained the H63D

mutation. The presence or absence of the H63D mutation *trans* to the C282Y mutation in heterozygotes in hemochromatosis patients is shown in table 1. A two-tailed Fischer's exact test gives a remarkable P -value $< 10^{-10}$. It is difficult, in view of such data, to see any cause for controversy regarding the relationship between this mutation and hemochromatosis. While tight linkage with another mutation cannot be ruled out, Feder et al. (1996) found no other candidate genes in a 250-kb region surrounding the gene, and a cause-and-effect relationship is by far the more parsimonious explanation.

The fact that the H63D mutation does not occur on the ancestral chromosome is to be expected, since it represents an independent mutational event and should be, and is, associated with its own haplotype.

2. *Homozygotes for this mutation are rare among patients.* This is true, but neither we nor anyone else had suggested that the homozygous state for the H63D would cause hemochromatosis. There is no reason for assuming that it would merely because the compound heterozygous C282Y/H63D state causes hemochromatosis. However, very recent evidence suggests that H63D homozygotes may be more prevalent in the hemochromatosis population than in the control population (Beutler et al. 1997b).

3. *Compound heterozygotes have been found in the normal population.* Both Feder et al. (1996) and our group (Beutler 1997; Beutler et al. 1996) have pointed out that the penetrance of the compound heterozygous state must be low, because the Hardy-Weinberg equilibrium predicts many more compound heterozygotes in the population than C282Y homozygotes. Feder et al. (1996) estimated a penetrance of 0.5%, our data suggest penetrance of 1.5% (Beutler et al. 1996), and those of Jouanolle et al. (1996) of 0.44%. In their normal population, the gene frequency of H63D is 0.165 and that of C282Y is 0.0288. According to the Hardy-Weinberg equilibrium, the predicted H63D/C282Y compound heterozygote frequency is $0.165 \times 0.0288 \times 2 = 0.0095$; that of C282Y/C282Y is $0.0288^2 = 0.000829$. The ex-

Table 1

Relationship between the Presence or Absence of the 187G (H63D) Mutation and Hemochromatosis in Heterozygotes for the 845A (C282Y) Mutation

	845A/187C	845A/187G	Total
Hemochromatosis	10	28	38
Normal	56	6	62
Total	66	34	100

NOTE.—These data are a compilation of all published data (Beutler et al. 1996; Jouanolle et al. 1996; Borot et al. 1997; Carella et al. 1997; Roberts et al. 1997). Fisher's exact test gives $P < 10^{-10}$.

pected ratio at 100% penetrance is 11.45 in favor of the compound heterozygote, but the observed ratio is 3/59 (0.05). Thus, only 0.05/11.45, or 0.44%, of the expected compound heterozygotes appear in the patient population. The low penetrance of this genotype is supported by the fact that all of the compound heterozygotes with disease we have encountered are males, a known risk factor for the disease.

The relationship between the two HLA-H mutations and hemochromatosis is analogous to that occurring in several other diseases. One example is the interaction of the hemoglobin S mutation with hemoglobin C or D. The compound heterozygotes, HbS/HbC or HbS/HbD, have a disease that is generally milder than the homozygous S state, but neither homozygote (HbC/HbC or HbD/HbD) is affected with a sickling disorder. Similarly, the 1604T glucocerebrosidase Gaucher disease mutation is relatively common in the Jewish population (Allitto et al. 1996), but no 1604T homozygotes with Gaucher disease have ever been encountered. Compound heterozygote with the severe 84GG mutation and even the milder 1226G mutation, however, do manifest the disorder.

Thus, my conclusion from the considerable data that have already been published in the brief time since the Feder et al. (1996) study appeared, including the recent article by Carella et al. (1997), is that the C282Y mutation is the main, but not the only, HLA-H mutation involved in the cause of hereditary hemochromatosis. The compound heterozygote of this mutation with the 187G mutation is also at special risk for the development of hemochromatosis, but with a very low penetrance. It is likely that most of the heterozygotes with mild disease manifestations reported before the discovery of the HLA-H gene will prove, in fact, to be such compound heterozygotes.

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