CORRESPONDENCE

Correction: Functional analysis of BRCA1 M1628V variant

Germline-inactivating mutations in the breast and ovarian cancer susceptibility gene *BRCA1* are associated with a substantial increase in the risk of cancer.¹ One of the possible outcomes of genetic testing for *BRCA1* is the finding of an alteration in the *BRCA1* coding region for which the risk of cancer has not

been determined. These alterations have been termed unclassified variants (UCVs) or variants of uncertain significance. Most of these alterations are missense changes (Breast Cancer Information Core database, <http://research.nhgri.nih.gov/bic/>). We have developed a functional assay that aids in the determination of the association of cancer with *BRCA1* UCVs.^{2,3} The assay measures the ability of a heterologous fusion of *BRCA1* (or its mutants) to activate transcription.^{4,5} UCVs that activate transcription to levels comparable to the wild-type protein represent neutral variants, whereas UCVs that abrogate or

drastically reduce activity are associated with a predisposition to cancer.^{2,3} In a recent paper, we reported that *BRCA1* variant M1628V presented markedly reduced activity in a transcriptional assay, which suggested that it represented a deleterious variant.²

During the course of additional experiments, we noticed that constructs containing the M1628V variant also included a deletion of nucleotides 5388–5582 (Entrez nucleotide accession U14680), leading to a truncated protein. Therefore, we generated new constructs for this variant using the primers and procedures described previously.² We then repeated the transcription assays in yeast and mammalian cells (fig 1). Contrary to what was reported,² this variant displayed ~80% of the wild-type activity, suggesting that it corresponds to a neutral variant. We regret this error and apologise for any confusion or inconvenience it may have caused.

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Figure 1 Functional assay of the M1628V variant of the *BRCA1* gene. (A) Quantitative transcriptional assay in yeast. Cells were cotransformed with a LexA-responsive β -galactosidase (β -gal) reporter gene (diagram shown above the graph) and a LexA DNA-binding domain (DBD) fusion to residues 1396–1863 of wild-type (WT) *BRCA1*, or the same fragment carrying the M1628V variant (M1628V). We used the WT and the S1613G neutral polymorphism as positive controls. Deleterious mutations M1775R and Y1853X were used as negative controls. Three independent yeast clones were tested in triplicate. (B) To control for possible variations in protein expression levels, samples were analysed by western blot using anti-LexA DBD monoclonal antibody. Levels are comparable in all samples with the exception of the Y1853X truncation, which shows lower levels owing to protein instability. (C) Quantitative transcriptional assay in mammalian cells. Cells were cotransfected with a GAL4-responsive firefly luciferase reporter gene (diagram shown above the graph), a *Renilla* luciferase driven by a constitutive promoter (internal control, not shown), and a GAL4 DBD fusion to residues 1396–1863 of WT *BRCA1*, or the same fragment carrying the M1628V variant. Controls are the same as described above but fused to GAL4 DBD. Measurements were taken in triplicate and normalised against the internal controls. The activity of the construct with WT *BRCA1* was expressed as 100%, with the other results placed on this scale. (D) Western blot using anti-GAL4 DBD monoclonal antibody. M1628V levels are comparable to WT and S1613G controls. Controls M1775R and Y1853X display lower levels owing to instability.

References

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