Dear Editor,

Please find enclosed the revised version of the manuscript by Palomba et al., entitled "Origin and distribution of the BRCA2-8765delAG mutation in breast cancer" (MS# 1276153713441403). Revision of the manuscript was based on the Reviewers’ remarks. Changes have been highlighted into the paper and here listed point-by-point:

Reviewer 1 (Abeliovich)
Major Revisions
1. In the new FIGURE 2, pedigrees of analyzed BRCA2-8765delAG positive families are reported. As specified into the FIGURE 2 LEGEND, the family members who underwent haplotype analysis from Sardinian and Jewish-Yemenite families have been indicated. In RESULTS (page 14, paragraph 1), it has been specified that, for each of the two French-Canadian families, one proband with the two parents was analyzed. These information are helpful to clarify how the haplotype was determined in families from the three populations (as required by Reviewer 1, point 1) but also may satisfy the query of Reviewer 2 (at points 1 and 2) to show the pedigrees and family individuals undergoing the haplotype analysis (respectively);
2. In DISCUSSION (page 9, paragraph 1), the word ancient has been removed since this affirmation is not dependent on the results from this study. In our previous paper (Palmieri et al., 2002), it was described that pedigrees were evaluated up to 1700 in order to search for common ancestors. Since we did not find any common ancestor in all Sardinian families positive to BRCA2-8765delAG mutation included into the study (therefore they were classified as unrelated, as indicated in MATERIALS AND METHODS at page 5, end of paragraph 1 - this answers also the question asked by Reviewer 2 at point 4), we may hypothesize that appearance of the BRCA2-8765delAG mutation in our population was at least before 1700 and, thus, such a variant may be indeed classified as an ancient founder mutation;
3. In MATERIALS AND METHODS (page 6, paragraph 1), we have indicated that marker loci were ordered as in the Ensembl map at http://www.ensembl.org (a physical map), instead of the previous Marshmed map (a genetic map). In Table 1 (which derives from the old Figure 2), it has been specified the physical position of each marker as well as of the BRCA2 gene, according to the Ensembl map. On this basis, in RESULTS at page 7-paragraph 2, the word "intragenic" has been consequently changed in "closely flanking" BRCA2 marker loci. However, the right position of some marker loci in comparison to that of the BRCA2 gene (intragenic or intergenic) is still controversial [see Geck P, Sonnenschein C, Soto AM. The D13S171 marker, misannotated to BRCA2, links the AS3 gene to various cancers. Am J Hum Genet. 2001; 69:461-3]
4. In RESULTS (page 7, paragraph 1), it has been specified that "presence of the BRCA2-8765delAG mutation in all family probands from the three populations was confirmed by direct sequencing";
5. In RESULTS (page 8, paragraph 2), information about familial breast cancer in mutation-negative patient has been provided;
6. In RESULTS (page 7, paragraph 1), it has been specified that "The BRCA2-8765delAG mutation was absent in blood DNA from 103 unrelated healthy individuals (corresponding to 206 chromosomes), originating from the same geographical area and used as normal controls".
Minor Revisions
1. In DISCUSSION (page 10, paragraph 2), the section has been rewritten in order to better clarify the content. Moreover, this paragraph as well as the second one at page 9 have been shortened in order to avoid redundancies and, consequently, reduce the length of the entire DISCUSSION;
2. The old FIGURE 2 has become the new TABLE 1 in this revised version of the manuscript.

Reviewer 2 (Schmitt)
1. Pedigrees of analyzed BRCA2-8765delAG positive families are shown in the new FIGURE 2 (see also answer to point 1 of Reviewer 1);
2. The analyzed family members are also shown in the new FIGURE 2 (see also answer to point 1 of Reviewer 1);
3. Throughout the text we substituted the term genotype with the term haplotype, as rightly suggested by the Reviewer 2;
4. In MATERIALS AND METHODS (page 5, paragraph 1), it has been specified that "Sardinian families were unrelated since they did not present any common ancestor after evaluation of pedigrees up to 1700" (see also answer to point 2 of Reviewer 1);
5. The number of unselected patients from South Sardinia who underwent BRCA2-8765delAG mutation analysis has been increased during the past few months. Indeed, results for additional 214 BC cases from South Sardinia are now available and have been included into the manuscript (all of them were negative for the BRCA2-8765delAG mutation, further supporting the statement that such a variant is "recurrent in North Sardinia but absent in South Sardinia").

Reviewer 3 (Hamann)
Regarding the criticism about the lack of novelty of the present study in comparison with our previous publications and the paper by Manning et al (2001), we would like to underline the following two issues:
- As now better stated in DISCUSSION (page 9, paragraph 2), "our results indicate that carriers of the BRCA2-8765delAG variant may have an independent origin and, although already suggested for French-Canadians and Yemenite-Jews [13], allow to exclude the presence of a common ancestor in Mediterranean area". This latter point is helpful to dispel the doubt that a roughly homogeneous (or, more appropriately, less heterogeneous) genetic background/environment of the populations facing the Mediterranean Sea may contribute to generate the same disease-causing mutation in breast cancer (as already demonstrated for other diseases - most of them monogenic, but some of them also complex diseases);
- Data from the mutation analysis among a large collection of unselected and consecutively-collected BC patients (with the additional results of this new version of the manuscript, the number of 1,141 analyzed cases is highly significant and their geographical distribution is much more homogeneous - 648 patients from North Sardinia and 493 patients from South Sardinia) allow to identify the presence of such a genetic heterogeneity in a well-known genetically-homogeneous population like the Sardinian one. As underlined in DISCUSSION, our findings further support the hypothesis that patients’ origin may determine different susceptibility roles of the candidate cancer genes even in so small geographical area.
Some errors have been corrected throughout the text.
Hoping to have addressed all issues and looking forward to receiving good news from You.
Sincerely Yours,
Giuseppe Palmieri