Reviewer’s report

Title: MDM2 SNP309 is associated with high grade node positive breast tumours and is in linkage disequilibrium with a novel MDM2 intron 1 polymorphism

Version: 2 Date: 8 May 2008

Reviewer: Diana Eccles

Reviewer’s report:

Overview
This paper investigates the association between a common SNP in the MDM2 gene with breast cancer risk and the same and nearby novel SNPs with clinicopathological characteristics that are known to have prognostic significance. 299 breast cancer patients, 23% premenopausal, mean age and range not given, are compared to 275 controls. The Nottingham Prognostic Index (which does not incorporate ER) and is not widely used outside of the UK is used to assign patients to prognostic groups.

Results
There was no association with breast cancer risk overall and no association with age at diagnosis. The authors found an association between tumour grade and nodal status and the 309 G/G genotype but no association with any other clinicopathological characteristics of known prognostic significance that were examined. Three additional infrequent SNPs in intron 1 of the MDM2 gene were also described.

Major compulsory revisions
Although the authors observed an association of the 309G/G genotype with higher tumour grade, the numbers of patients with this genotype are rather small. A major difficulty with the data is that it is difficult to work out a biological reason why one copy of the SNP (309G/T) should have an opposite effect (low grade) whereas 309G/G leads to the highest average grade – one would expect if the SNP was influencing grade that one would see a progression from low to high grade through the addition of the G allele i.e. T/T lowest through to G/G highest. The small numbers and the lack of progression suggest this could well be a type 1 error (very common in small association studies) and this is not adequately explored in the discussion. The correlation of higher grade (particularly in ER positive tumours which most of them were) with LN positive disease is not surprising. A step further would be to ask if the SNP is having any influence on actual prognosis and is the effect independent of or entirely because of the proposed influence on LN status and grade – this would require a survival analysis. The very low frequency of the additional SNPs identified in MDM2 prohibits any really useful tests of association in such small numbers of cases and by the time it gets down to 3 double homozygotes at 309G/G plus 285C/C it becomes somewhat statistically irrelevant. The small numbers at this stage are
acknowledged.

The discussion is unnecessarily long, paragraph 2 is largely repetition, the discussion in general is not very clear. The authors state that "Taken together the data [does this mean the data in the paper plus the literature?] "suggest that different grades of cancer in fact represent different cancer subtypes", which theory is further expanded in the final sentence in the first paragraph of page 15 into an incomprehensible extrapolation.

The observation of an association between the 309G/G genotype, grade and lymph node status is certainly of interest and worth exploring further. However given the nature of this type of study it is very important not to over interpret the data. It would be helpful in presenting the data to try and come to some sort of tenable hypothesis about how this SNP might be influencing grade given that it does not appear to be an additive effect with each G allele contributing more to grade.

Minor Essential Revisions

Tumour grading is subjective and specialist breast pathologists tend to grade higher than general pathologists so some description of how tumours were graded, and who by (one read or two, consensus etc) would be important given the conclusions that are reached.

ER, PgR scored using IHC, for HER2 the authors state that samples were scored by IHC and FISH – did all samples have both? HER2 scored 2+ on IHC is not usually considered positive without FISH confirmation – the sentence need to be reworded to clarify that.

The number of controls should be stated in the methods section with mean age and range. Selection of controls is always a difficult issue and there is no real consensus on the ideal control group for association studies but the composition of the control group should be clarified – particularly age. If controls are younger than cases they may become cases in future. If older than cases they may be “hypernormal” (ie old age and no cancer) controls. The former could potentially reduce the observation of any difference between cases and controls and the latter could increase the chance of observing an association. Ethnic similarity can be confirmed by genotyping and for an association study would be important to establish or at least make some comment about what is known about the ethnic variation in the population sampled. In small numbers as in this study, an admixture of ethnic minorities in one group or another could easily bias the results especially as the authors comment on the wide variation of 309 genotypes in other ethnic groups and differences in tumour type and prognosis are well described between different ethnic groups, especially black african women.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests