## Paratumoral gene expression profiles: promising markers of malignancy in melanocytic lesions

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MADAM, Gene expression analysis is becoming a useful tool for a better definition of neoplasms at diagnostic, prognostic and predictive levels. An example of these applications appears in a recent issue of the *BJD*, which focuses the attention on a noninvasive procedure for this purpose.[1] This attractive proposal takes us to some biological and practical considerations.

As the samples for analysis are taken from the corneal layer overlying the lesions, two biological aspects are essential for this process: how the tumour messenger RNA (mRNA) gets there and the functionality of this mRNA. Most cases of malignant melanomas will not show a sufficient number of superficial tumour cells to explain the positive findings, as confirmed by the authors in their results and discussion.[1] In this context, the RNA cannot be directly provided by the tumour cells but by keratinocytes through a transfer process similar to what happens with melanin. Although the paper does not provide experimental documentation on this issue, microvesicles (exosomes) containing mRNA and microRNA (miRNA) can be taken up by normal host cells, such as keratinocytes or endothelial cells, and translated by recipient cells.[2] Exosomes are specialized membranous nano-sized vesicles derived from endocytic compartments that are released by many cell types. Tumour-derived microvesicles therefore serve as a means of delivering genetic information and proteins to recipient cells in the tumour environment. It has been suggested that microvesicles shed by certain tumour cells hold functional mRNA that may promote tumour progression. Purified exosomes contain functional miRNAs and small RNA, but little mRNA is detected. Exosomes are specialized in carrying small RNA including the class 22–25 nucleotide regulatory miRNAs. Both the evidence provided from studies on exosomes and the lack of the expected phenotypic changes in keratinocytes from the expression of putative melanoma markers in the study support a transfer of predominant short RNA rather than functional mRNA.[1,2] No experimental data are provided on electron microscopic analysis of microvesicles/exosomes or on the comparative gene expression between superficial epidermal samples and direct tumour samples from the same cases. These experimental data will clearly evaluate these possibilities. The biological heterogeneity in melanomas and dysplastic melanocytic lesions would potentially make these assessments more difficult.[3–5] We only have to consider that deep tumour compartments provide more accurate prognostic information in melanomas, and dermal compartments rather than the junctional compartments better define dysplastic melanocytic lesions.

From a practical perspective, the data clearly segregate malignant from benign melanocytic lesions. However, the analysis needs a 17-gene classifier to achieve acceptable specificity and sensitivity, a complexity that is far greater than the standard histopathological evaluation. Using this approach 13 of 89 lesions classified as malignant by the test did not show histological evidence of malignancy. This result gives a positive predictive value of 85%, while the negative predictive value is 100%. To argue that the melanocytic naevi clustered with melanomas were mainly atypical with mild to severe dysplasia does not change the facts without knowing the distribution of dysplastic lesions in the benign cluster. The biological heterogeneity of intraepithelial and invasive melanocytic lesions is well known at the morphological, kinetic and genetic levels, an issue that has not been addressed in this paratumoral gene expression analysis and should warrant future studies. The gene expression markers should be evaluated in the appropriate biological context. Gene expression profiles are determined by a gene regulatory network comprising a regulatory core of genes represented most prominently by transcription factors and miRNAs, that influence the expression of other genes, and a periphery of effector genes that are regulated but not regulating.[6] Most studies do not differentiate between these two essential groups, which can also be useful in selecting surrogate markers for a given condition.[6,7] There is a general concept to keep in mind for gene expression analyses: the amount of information is overwhelming and the number of variables included in the studies significantly outnumbers the cases. In this scenario, the significant variables can be the result of a statistical selection rather than the expression of a biologically significant process for a particular neoplasm. Supporting this aspect, significant variables frequently include genes of the general metabolic activation associated with the neoplastic transformation,[7,8] rather than tissue- or differentiation-specific gene variables.

The identification of these markers and the negative predictive value (100%) make this test an excellent way for the screening and selection of atypical melanocytic lesions to be biopsied. However, the nature and biological meaning of these markers still need further studies and clarifications: origin of the tested RNA, mechanism of RNA transference, utility for subclassification of melanocytic lesions.

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## Investigation of variants of the aromatase gene (*CYP19A1*) in female pattern hair loss

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MADAM, Female pattern hair loss (FPHL) is a common disorder which affects around 12% of women by the age of 30 years, and 30–40% of women by the age of 70 years.[1–3] Although its precise aetiopathogenesis is unknown, reports of familial occurrence strongly implicate genetic factors.[4–6]

Yip *et al*.[7] recently performed a gene-wide study of association between FPHL and the aromatase gene encoding *CYP19A1*, which is known to convert androgens to oestrogens in scalp hair follicles.[8] They examined 61 tagging single nucleotide polymorphisms (SNPs) of *CYP19A1* in an Australian sample of 484 caucasian women with grade 3–5 FPHL (according to the Sinclair scale[9]) and 471 controls.[7] While no significant association was obtained at the allelic level, the CC genotype of rs4646 was significantly more frequent in cases (58·1%) than controls (48·9%) when compared with collapsed AC and AA groups ($P = 0{\cdot}006$), suggesting a recessive genetic effect. Although this result did not withstand experiment-wide correction for multiple testing, its validity was supported by the following lines of evidence: (i) the highest frequency of the CC genotype (68·2%) occurred in women < 40 years, which is consistent with the hypothesis that genetic predisposition is more evident among early-onset cases; and (ii) the rs4646 CC genotype has been associated with higher oestrogen levels than the AA genotype, which is consistent with reports that oestrogen has inhibitory effects on hair growth. Given the lack of experiment-wide significance, the authors were cautious in their interpretation, and stressed the need for replication in independent samples. To our knowledge, the present study is the first to attempt replication of this finding.

The present sample comprised 145 U.K. and 53 German hair clinic outpatients. The inclusion criterion was the presence of grade 2–5 FPHL according to the Sinclair scale,[9] or grade 2–3 according to the Ludwig scale.[10] A total of 53 women (U.K., $n = 42$; German, $n = 11$) presented with severe FPHL (Sinclair grade 4–5, Ludwig grade 3). Eighty-five patients (U.K., $n = 57$; German, $n = 28$) reported an early age at onset (< 40 years). The control groups were 179 U.K. women aged > 60 years without FPHL and 150 German healthy unrelated female blood donors. Ethical approval was obtained from the respective Ethics Committees.

Four SNPs were selected for genotyping: rs4646, rs16964189 and rs2470158, which have shown borderline association in either $\chi^2$ or logistic regression modelling in Yip *et al*.,[7] and one tag SNP (rs28757184), which is not in linkage disequilibrium with the tagging SNPs genotyped by Yip *et al*.[7] Genotyping was performed using the MassARRAY system and a Sequenom Compact MALDI-TOF device (Sequenom Inc., San Diego, CA, U.S.A.). For the association analysis, the following SNP quality criteria were used as exclusion criteria: minor allele frequency < 1%; $P < 0{\cdot}05$ of Hardy–Weinberg equilibrium in controls; and SNP call rates < 95%. After applying these criteria, all four SNPs remained eligible for analysis in all patients and controls. For association testing, the Armitage[11] trend test was used to detect allelic and genotypic effects. A specific attempt was made to replicate the previous association finding by comparing the CC genotype of rs4646 with collapsed AC and AA genotypes. Calculation of power was based on the odds ratio for the CC genotype obtained in the Australian sample. Power was calculated using the power.fisher.exact function in R version 2.0.1 (R Project for Statistical Computing, Vienna, Austria).

In a first step, separate analyses were performed for the U.K. and the German samples. In the U.K. sample, no significant association was found for any of the four variants (Table 1). This finding was unchanged following stratification for disease severity and early age at onset (Table 1). The finding reported by Yip *et al*.[7] of a higher frequency of rs4646 CC genotype carriers among cases was not replicated (Table 2). Negative findings were also obtained in the complete German sample (Table 1). Due to the small sample size, no subgroup analyses were performed in the German sample. A meta-analysis of the U.K. and German data was performed. This generated no significant results.

One possible explanation for the nonreplication is that the association reported by Yip *et al*.[7] was a false-positive finding and that *CYP19A1* is not implicated in the pathophysiology of FPHL. Although inadequate power could explain the negative finding in the present, relatively small German sample (65%), the U.K. sample had sufficient power (96·7%) for replication. It is also possible that another variant in the *CYP19A1* gene is associated with FPHL in U.K. and German populations. However, results for other markers in *CYP19A1* were negative in the Australian sample. Given the ethnic relatedness between white Australian and European populations, in particular the U.K. population, it is unlikely that positive associations would be observed in U.K. or German samples for markers that were