tion (Pearson r-value 0.463, p = 0.013) between PS2 mRNA and protein expression levels existed.

Conclusions: The presence or absence of ER and PS2 was partially concordant between IHC and microarray analysis. These findings suggest Affymetrix analysis complements IHC and supports the use of this technology for the identification of novel predictive and prognostic markers for breast cancer.

O-26. CONCORDANT LOSS OF HETEROZYGOSITY IN TUMOUR PAIRS OF BREAST CANCER FAMILIES AS A PREDICTOR OF GERMLINE MUTATIONS

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Loss of Heterozygosity (LOH) can arise randomly in sporadic breast cancer due to genetic instability. However, concordant LOH in tumours from related individuals may point to a germline mutation. We looked for LOH at BRCA markers in tumour pairs of at-risk families in order to evaluate its predictive value in selecting those likely to harbour a mutation.

67 tumour pairs and 54 sporadic breast cancer control cases were studied. 5 microsatellite markers flanking and intragenic to each of the BRCA1 and BRCA2 genes, plus a control marker, were evaluated for LOH by analysis of fluorescent labelled PCR products in an automated sequencer.

In familial cancers, the LOH/Informative ratio at BRCA markers was higher than in sporadic controls (54.7% vs. 38.3%, p = 0.001), and higher than at the control marker (54.7% vs. 29.3%, p = 0.004).

7/17 informative families showed concordant homoallelic LOH for 2 or more markers at BRCA1 (O/E = 57.7, p < 0.001) and another 1/7 did so at BRCA2 (O/E = 31.1, p = 0.04). Mutational analysis is ongoing.

Preliminary results indicate that, in tumour pairs, concordant homoallelic LOH at two or more markers may predict the site of a predisposition gene.

O-27. HEAT SHOCK PROTEIN 27 AS A MARKER OF MALIGNANT POTENTIAL IN NORMAL, BENIGN AND INVASIVE BREAST LESIONS

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Heat shock proteins (hsps) occupy a central role in the regulation of intracellular homeostasis. Previously it has been shown that hsp27 is overexpressed in breast cancer. We have investigated its expression in normal and hyperplastic breast lesions that carry a mild increased risk of developing breast cancer. Forty-nine biopsy specimens of normal breast, 47 hyperplasia of usual type (HUT) and 125 primary breast cancers were included in this study. Staining for hsp27 was performed using heat treatment antigen retrieval and a murine monoclonal antibody (Novocastra Laboratories Ltd.). Positive staining was cytoplasmic and quantified by measuring the mean optical density (OD) for each case using a morphometric image analysis system. In addition the % of positive stained cells was estimated manually.

A progressive increase in hsp27 expression was seen from normal through HUT to invasive cancer. In normal breast, the % expression was significantly lower than the expression in HUT and cancers (p < 0.001). The mean % positivity was 6% for normal breast, 23.5% for HUT and 54.9% for invasive tumours. The mean OD was 0.32 for normal breast, 0.49 for HUT (p < 0.001) and 0.57 for cancers (p = 0.01).

Our data show a previously undescribed increase in the expression of hsp27 from normal through precancerous breast to malignancy. This may be an important early event in mammary carcinogenesis.

O-28. DELAY TO FIXATION OF INVASIVE BREAST CARCINOMA: EFFECT ON MITOTIC COUNT, MIB1, ER AND P53 EXPRESSION

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Several small studies have suggested that a delay prior to fixation has an effect on the mitotic count of a tumour. This is an essential component of histological grade of invasive breast cancer and grade itself, as part of the Nottingham Prognostic Index, plays an important role in treatment selection. We have examined the effect of a delay to fixation in 4% Baker's formal calcium of 30, 60 and 120 minutes in a series of 25 breast cancers.

Tumours were received fresh and multiple small portions (approximately 0.5 mm^3) from the periphery sampled. One was immediately placed in formalin; the remainder were placed into fixative after delays of 30, 60 and 120 minutes. After routine processing, H&E stained sections were examined using strictly defined criteria for mitoses by one observer (NB). Sections were also assessed for MIB1, ER and p53 expression.

A significant decrease in mitotic count was seen with a 1 hour delay in fixation (p = 0.016). The mitotic score (1–3) ascribed to the mitotic count and histological grade also tended to be lower with delayed fixation. No significant decrease in MIB1 labelling (p = 0.808) or ER expression (p = 0.079) was seen. A decrease in intensity and percent nuclear staining with p53 was seen after a 2-hour delay in fixation.

A delay in fixation of an only a relatively short time influences mitotic count, with the potential for alteration of histological grade. This effect may be important, not only for fresh samples not immediately placed into fixative but also for tumours within large fatty specimens (for example mastectomy samples) not rapidly penetrated by fixative.