tumour and metastases, by fold change >2 and statistical significance ($p < 0.01$). Further analysis of the changes occurring between glycoprotein expression in the primary tumour and the lymph node metastases is shown in Figure 4(a). Principal component analysis (PCA) of the changes occurring in glycoprotein expression between primary and LNM pairs was performed to show patterns of similarity between tumours. There is a weak tendency for the primary tumours to group together in the lower half of the figure with the lymph node metastases occurring in the top half. However there is large inter-patient variation, which is the dominant effect. The same effect is seen in the PCA plot in Figure 4(b), showing DM tumour pairs. Here the effect is even more pronounced where tumour pairs are relatively close and no obvious clustering for stage or tissue/relapse site. This is in line with the idea that breast cancer is a very heterogeneous disease and that changes between a primary tumour and the metastases is peculiar to each tumour pair, supporting the rationale for analysing tumours pairwise. It also demonstrates that the tumours do not cluster on tissue, which is a risk when analysing tumours from different sites in the body. Figure 5 shows the two patients that have three samples. Patient 1 (red) has one recurrence in the skin in the ipsilateral breast, and one in the lung, while patient 5 (blue) have two recurrences in the skin. This figure demonstrates that there is a larger difference between the two patients than between different stages of cancer (primary tumour—distant metastases). It also demonstrates that the two skin metastases from patient 5 are very similar, while the two recurrences from patient 1 are more different. This could be explained by that different changes are required for the ability to metastasize to specific sites. Possibly it is the same clone that has metastasized from patient 5 and two different clones from patient 1.

Differences in glycoprotein expression patterns between the primary tumour and metastasis could possibly be modified by any treatment given between the two samples. In this study, several patients received adjuvant treatment (Table 1). However, there were too few patients to study the effect of different treatments and we thus focused on the overall change of glycoproteins. In future studies, it would be of great interest to focus on the effect of specific treatments.

Functional and pathway differences between the primary tumour and metastases

Among the differentially regulated proteins, there are clear groupings of proteins, for example those that are involved in the progression from primary tumour to lymph metastasis and distant metastasis, include many with motility functions. Cell migration-inducing gene 3 is up-regulated and is known to function as a cell migration-inducing gene 3

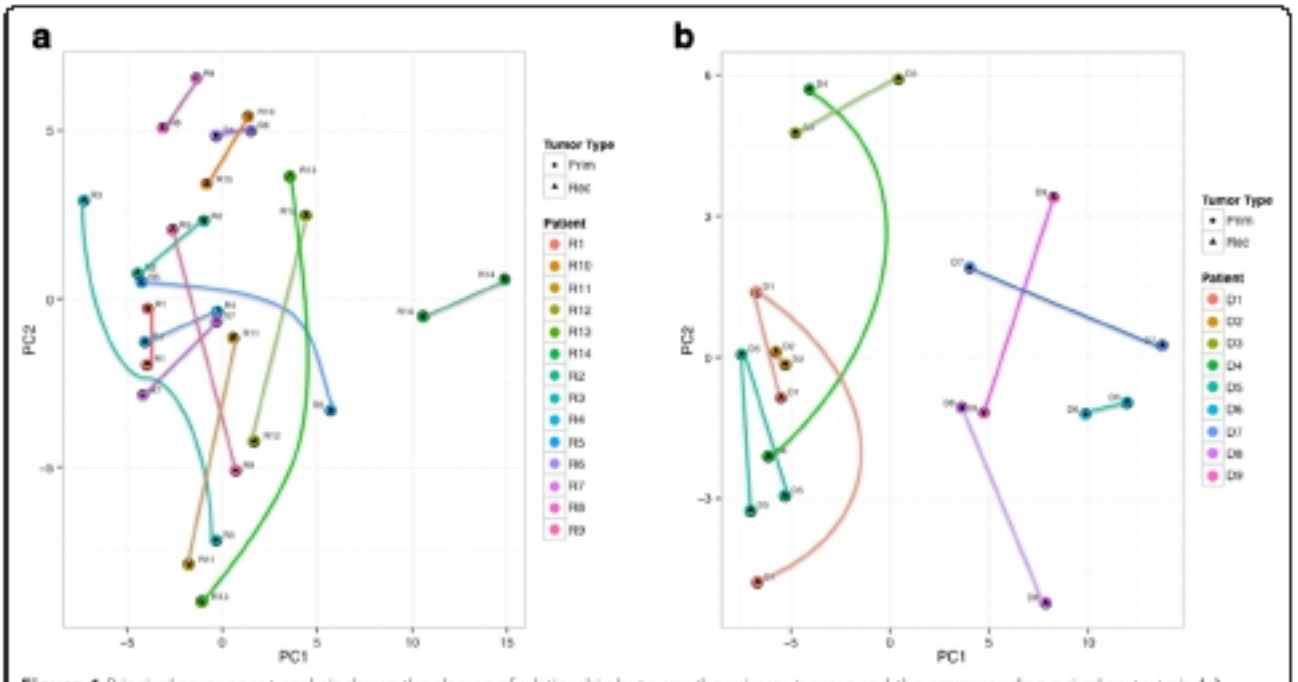

Figure 4 Principal component analysis shows the degree of relationship between the primary tumour and the corresponding paired metastasis. **(a)** Principal Component Analysis of primary and lymph node metastasis pairs. A circle indicates a primary tumour and a triangle the lymph node metastasis. **(b)** Principal Component Analysis of primary and distant metastasis pairs. A circle indicates a primary tumour and a triangle the distant metastasis.