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LETTER

DNA microarray analysis of rheumatoid arthritis susceptibility genes identified by genome-wide association studies

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See related review by Clarke and Vyse, http://arthritis-research.com/content/11/5/248

In a recent interesting review, Alex Clarke and Timothy Vyse described the genetics of rheumatic disease [1]. In the past several years, genome-wide association studies (GWAS) have led to the identification of six high-risk rheumatoid arthritis (RA) susceptibility genes – namely, *CD244*, *PADI4*, *SLC22A2*, *PTPN22*, *CTLA4*, and *STAT4* (summarized in [2]). *In vitro* studies using mutant alleles and cultured cells have revealed the individual up-regulation of *CD244*, *PADI4*, *SLC22A2*, and *PTPN22* [2-6]; however, studies on the expression of RA susceptibility genes in RA patients are rare. We therefore investigated the expression of the above-mentioned six RA susceptibility genes in 112 RA patients using DNA microarray analysis. This study aims to clarify whether DNA microarray analysis and GWAS produce comparable results with respect to RA susceptibility genes.

Total RNA extracted from total peripheral blood cells obtained from 112 RA patients and 45 healthy individuals was used to prepare aminoallyl RNA. As a reference, mixed RNA from 45 healthy individuals was used. The aminoallyl RNA of each individual and the reference was subjected to Cy3 and Cy5 labeling, respectively, and was hybridized with an oligonucleotide-based DNA microarray. The data obtained were analyzed by nonparametric statistical group comparison. The intensities of the no-probe spots were used as the background. The median and standard deviation of the background intensity were calculated. The genes with an intensity value that was less than the median plus 2 standard deviation of the background intensity were identified as null. The Cy3/Cy5 ratios of all spots on the DNA microarray were normalized using the global ratio median. Only gene expression data that were collected from at least 80% of samples from each group were selected for further analysis. The unpaired Mann–Whitney test was used to determine statistically significant differences in the mRNA expression levels between the RA and healthy groups. Statistical significance was set at $P < 0.05$.

The results of our DNA microarray analysis showed that the expressions of four out of the six RA susceptibility genes were significantly higher in RA patients than in healthy individuals (1.0×10^{-16} to 2.32×10^{-5}) (Table 1). As described above, the upregulation of these four genes (*CD244*, *PADI4*, *SLC22A2*, and *PTPN22*) has been previously confirmed in *in vitro* studies. We found, however, that *CTLA4* expression levels were similar between the RA and control groups, whereas *STAT4* expression was significantly downregulated in the RA group (1.38×10^{-8}). We investigated the expression of other RA susceptibility genes – namely, *TRF1/C5* [7], *CD40* [8], and *CCL21* [8] – and found that their expressions were similar in both groups. The genetic risk factors for RA were recently reported to differ between Caucasian and Asian (Korean) populations [9]. The samples used in our microarray analysis were derived from the same Asian (Japanese) cohort. The expression profiles for these three genes may therefore not be consistent with the profiles determined by GWAS.

In this study, we revealed the correlation between five out of the six high-risk RA susceptibility genes using DNA microarray analysis. Prostate cancer susceptibility genes identified by GWAS were recently reported to be consistent with those identified by microarray analysis [10]. We therefore concluded that the combination of microarray analysis and GWAS would be a more effective approach for gene identification than the analysis of individual datasets. Moreover, the simultaneous use of both methods would allow for more accurate identification of RA candidate genes.

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Table 1. Candidate genes identified from rheumatoid arthritis genome-wide association studies

Gene	GeneID	PMID	Gene expression (up or down)	Microarray *P* values[a]
CD244	605554	18794858	Up	1.0×10^{-16}
PADI4	605347	12833157	Up	2.32×10^{-5}
SLC22A2	602608	14608356	Up	1.94×10^{-6}
PTPN22	600716	15208781	Up	9.66×10^{-8}
CTLA4	123890	16380915	No change	0.767
STAT4	600558	17804842	Up	1.38×10^{-8}

[a]*P* values determined by comparison between 112 rheumatoid arthritis patients and 45 healthy individuals.

Abbreviations
GWAS, genome-wide association studies; RA, rheumatoid arthritis.

Competing interests
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