

Genome-Wide Expression Analysis in Vitiligo

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SPECIFIC AIMS AND OBJECTIVES

Vitiligo is a debilitating skin disease with areas of pigment loss that affects one or two of every 100 people. The cellular and molecular mechanisms leading to the destruction of melanocytes in this ailment have not yet been elucidated. The three proposed pathways of disease pathogenesis are the immune hypothesis, the neural hypothesis, and the self-destruct hypothesis. According to the existing data, it is likely that the loss of epidermal and follicular melanocytes in vitiligo may be the result of several different pathogenetic mechanisms. High density DNA microarrays, or so-called "gene chips," are a compelling tool for profiling complex diseases in terms of gene expression and discovery of novel disease-related genes which can help to further elucidate the molecular basis of disease. The specific aims of this project are: 1) To document patterns of gene expression in normal vs. depigmented skin in vitiligo using oligonucleotide microarrays. 2) To identify specific up- or down-regulated sequences in depigmented skin vs. normal skin using rigorous statistical algorithms. 3) To confirm the validity and functional relevance of differentially expressed sequences in vitiligo by alternative platforms. 4) To systematically examine gene expression through microarray-based data and identify genes that can be responsible for causing the disease.

PROGRESS TO DATE

Recruitment of subjects: Patients identified by established clinical and histopathologic criteria as having vitiligo were recruited at our outpatient dermatological clinics into this study. Thus far, we have recruited 7 patients with vitiligo through the dermatology outpatient clinics at New York Presbyterian Hospital-Cornell. Each patient was counseled on the risks and benefits of the study and signed an informed consent.

Gene Expression Profiling: Human Genome U95A arrays (Affymetrix#900303) containing probes for the detection of 12,656 human full-length genes and ESTs were used. RNA was extracted from biopsies of affected and unaffected skin from 7 vitiligo patients and hybridized to Hu95A GeneChips. Following hybridization, each array was loaded into the Affymetrix fluidics station, washed and stained using a streptavidin-phycoerythrinconjugate. Image data were acquired using the GeneArray Scanner and subsequently analyzed using MicroArray Analysis Suite v5.0 (Affymetrix) and GeneSpring' v4.2.1 (Silicon Genetics) software. Identification of Differentially Expressed Genes: 7129 out of 12,656 genes were found to be present in all samples analyzed. Statistical comparison of gene expression in depigmented vs. nonlesional skin identified 73 differentially expressed genes: 43 genes are up-regulated (fold changes ranging from 1.5 to 9.7) and 30 genes are down-regulated (fold changes ranging from -1.6 to -6.6). Chromosomal

Location of Differentially Expressed Genes: We find that 5 out of the 73 genes that are differentially expressed map to one of the putative susceptibility loci for vitiligo (17 p 13, 6p21, 6p22).

Classification of Differentially Expressed Genes: Classification of the 73 differentially expressed genes based on ontology (revealed that 12 genes are involved in signal transduction. Seventeen genes are involved in transcription and RNA processing, and 8 are related to host response mechanisms. Four of the functionally characterized genes are involved in DNA replication and repair. Ten genes encode known enzymes and five genes encode for structural proteins or cellular transport molecules. We also identified 17 ESTs that have not yet been functionally classified.