GENETIC AND EXPRESSION PROFILES AS PHARMACOGENOMIC MARKERS IN COMPLEX DISEASES

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Introduction

The aim of our study is identification of single nucleotide polymorphisms (SNPs), haplotypes and gene expression profiles of disease susceptibility, drug metabolizing and drug transporter genes associated with treatment outcome in complex diseases including, Inflammatory bowel diseases (IBD), asthma, aspirin intolerance, Chronic obstructive pulmonary disease (COPD), cardiovascular diseases, cancer, psychiatric diseases. In this report we focus on the pharmacogenomics of human inflammatory bowel diseases (IBD), usually classified into Crohn's disease (CD) and ulcerative colitis (UC). Accumulating pharmacogenetic data in IBD strongly support that several genes are important in drug absorption, disposition, metabolism and resulting side effects and treatment outcome in IBD patients for instance mutations in gene coding for thiopurin methyl transferase (TPMT) result in altered metabolism of azathioprine (AZA), drug commonly used in IBD treatment, and are associated with bone marrow suppression and hepatotoxicity. The expression of Multidrug resistance 1 gene (MDR1/ABCB1) coding for ABC transmembrane transporter was higher in peripheral blood lymphocytes in CD patients who required bowel resection for failed medical therapy as compared to controls. Polymorphism in FCG3A gene was associated with response to Inflaximab treatment in CD patients. In this report we provide our pharmacogenomic data on IBD patients resistant to conventional therapy including corticosteroids, antibiotics, and immunosuppressant. We also correlate genetic and gene expression data to efficiency of treatment with novel biological therapeutics Infliximab (antibody against TNF-α) and recently approved biologic drug HUMIRA (adalimumab).

Patients and methods

The DNA was isolated from blood and from paraffin embedded biopsy sections from 355 Slovenian patients with verified chronic inflammation including 139 patients with Crohn disease, 144 patients with ulcerative colitis, including 39 patients with inflammation restricted to rectum (proctitis), and 30 unclassified IBD patients.[1] Informed consent was obtained from all patients enrolled in the study. Therapy and therapeutic outcome was monitored and recorded for all patients for the last 10 years. Twenty four patients with refractory Crohn's disease that failed standard therapy with antibiotics, corticoids and immunosuppressant were enrolled in treatment with anti-TNF alpha (Inflaximab) according to strictly defined criteria. Inclusion criteria for active luminal disease comprised: moderately to severely active CD (11 patients) not adequately responding to prior conventional treatment with 5-aminosalicylates 4.5 g/day given for at least 2 months, antibiotics (metronidazole 1.2 g/day and/or ciprofloxacine 1 g/day) given for at least 1 month, 6-methylprednisolone 42 mg/day given for at least 2 months, and azathioprine (AZA) 2.5mg/kg/day given for at least 6 months.
We use combination of different approaches in our disease association and pharmacogenomic studies including haplotype analysis, single nucleotide polymorphism (SNP) analysis in candidate genes, disease pathway analysis and microarray analysis in Slovenian IBD patients. For genotyping we used the TaqMan allele discrimination assay (Applied Byosystems). Haplotypes were estimated using the Expectation-Maximization algorithm.

Results

We have genotyped 22 SNPs from 4 different genes, including some IBD candidate genes, genes coding for drug transporters, drug metabolizing enzymes and randomly selected

genes in IBD patients and controls. We have identified novel association between multidrug resistance 1 (MDR/ABCB1) gene polymorphisms in intron 13 (rs2235035) and in intron 16 (rs1922242) and haplotypes defined by SNPs in exons 12 (1236 C > A), 21(A893S) and 26 (3435 C > T), and UC patients as well as refractory Crohn disease patients, who do not respond to standard therapy with glucocorticoids, including patients that develop fistulas.

Conclusions *We report here novel correlation of SNPs and haplotypes in MDR1/ABCB1 multidrug transporter with IBD patients not responding to standard treatment with corticoids. We suggest patients not responding to treatment with corticoids for IBD and other autoimmune disease including rheumatoid arthritis should be analyzed for MDR1/ABCB1 polymorphisms. Our results also suggest advantage of haplotype analysis versus single SNP analysis. Our results also suggest the importance of non coding SNPs in pharmacogenetic and disease association studies.*

References

1. Potocnik U, Ferkolj I, Glavac D, Dean M. Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis. Genes Immun 2004; 5: 530–9.