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Interleukin-23 receptor (IL-23R) gene polymorphisms in acquired aplastic anemia

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Abstract

Acquired aplastic anemia (AA) is a rare disease with a complex pathogenesis. In most cases, T-cell mediated immune destruction of hematopoietic cells results in peripheral blood pancytopenia and bone marrow hypoplasia. A subset of the heterodimeric interleukin-23 receptor gene (*IL-23R*) is significantly associated with autoimmune-mediated diseases. To examine whether *IL-23R* single nucleotide polymorphisms (SNPs) might contribute to AA, we selected three *IL-23R* SNPs with amino-acid changes (rs11209026: p.Arg381Gln; rs41313262: p.Val362Ile; rs11465797: p.Thr175Asn), and compared their frequencies in 279 AA patients and 184 ethnically matched healthy controls. The three SNP prevalences were similar between the AA patients and controls. The Arg381Gln variant, which has a strong protective effect against inflammatory bowel disease, showed no association with AA. Further, IL-23 levels in sera were measured in the AA patients and in controls, and there were no significant differences among them. Our results indicate that these three *IL-23R* SNPs and serum IL-23 level have no apparent impact on susceptibility to AA.

Keywords

aplastic anemia; *IL-23R*; autoimmune disease; polymorphism

INTRODUCTION

Acquired aplastic anemia (AA) is the paradigm of the human bone marrow failure syndromes [32]. In most cases, peripheral blood and marrow of AA patients contain elevated numbers of activated cytotoxic lymphocytes, which decrease with successful immunosuppressive therapy, implicating T-cell mediated immunity in hematopoietic cell destruction. In almost all AA patients, a decreased number of regulatory T cells (Tregs) has been observed, as in other autoimmune diseases [25]. Increased levels of T-bet (a member of the T-box transcription family) in patients' T cells are responsible for the increased interferon- γ (IFN- γ) levels and the T helper type 1 (Th1) shift of CD4⁺ T cells in AA [24]. T-bet mediated IFN- γ elevation would be a trigger for endogenous stimulation in T cells of AA patients as IFN- γ is a master cytokine of the Th1 immune response.

Interleukin-23 (IL-23), a member of the IL-12 family, has proinflammatory and immunoregulatory activities. IL-23 signaling pathway molecules may be important candidate genes for autoimmune disease [13,27]. The IL-23 receptor is a heterodimeric complex formed by an IL-12R β 1 subunit (shared with IL-12) and a unique *IL-23R* subunit [16]. Although IL-23 and IL-12 as well as their receptors share structural and functional similarities, IL-23 has biological activities in the T-cell mediated immune response similar

to but distinct from IL-12. IL-23 promotes the development of T helper cells (Th17) producing IL-17, a proinflammatory cytokine with many targets and multiple downstream mediators [3,12]. Elevated IL-17 levels have been observed in sera and target tissues of various autoimmune diseases and inflammatory conditions, including rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE), asthma, the two common forms of inflammatory bowel disease (IBD) (Crohn's disease (CD) and ulcerative colitis (UC)), bacterial pneumonia, and in murine experimental autoimmune encephalopathy [32].

A recent genome-wide association study revealed that several single nucleotide polymorphisms (SNPs) in the *IL-23R* gene (chromosome 1p31; GenBank Accession: NM_144701.2, GeneID: 149233) were significantly associated with IBD: an uncommon coding variant (rs11209026, c.1227G>A, p.Arg381Gln) conferred strong protection against CD [8]; several other non-coding variants in the *IL-23R* gene also were associated with CD and UC. Subsequent studies also have provided more evidence for an association of *IL-23R* gene with IBD in both adult and child cohorts [4,7,14,15,19,26,29], and further with other immune-mediated disorders [6], such as psoriasis and ankylosing spondylitis [5,21]. Accumulating data indicate that genetic risk factors may be shared among various autoimmune diseases. This study is the first attempt to assess the possible contribution of *IL-23R* gene polymorphisms to AA.

MATERIALS AND METHODS

Patients and controls

Peripheral blood (PB) samples were obtained from 279 unrelated consecutive patients diagnosed with AA (133 females and 146 males), according to the criteria of the International Agranulocytosis and AA Study. These AA patients were treated at a single institution (Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health), and composed of four ethnic groups (159 Caucasians (57.0%), 33 African-Americans (11.8%), 49 Hispanics (17.6%), and 33 Asians (13.6%)). Blood samples from ethnically matched 184 healthy donors (117 Caucasians (63.6%), 23 African-Americans (12.5%), 22 Hispanics (12.0%), and 22 Asians (12.0%) from SNP-500 Cancer) [18] served as controls. Patients or their guardians provided written informed consent for genetic testing, according to protocols approved by the institutional review board of the National Heart, Lung, and Blood Institute.

DNA amplification and sequencing

DNA was extracted from PB mononuclear cells using the Qiagen DNA extraction kit and subjected to PCR amplification using the LA *Taq* polymerase kit (Takara, Otsu, Japan) and adequate primer sets in order to obtain DNA fragments containing SNPs (rs11209026, rs41313262, and rs11465797). Primers for rs11209026 and rs41313262: 5'-GAGCAGAGTAAAGAGAATAGTAA-3' (forward), 5'-TGGGCTGAGGACTTAGCCTCTTTAAGCCTC-3' (reverse); primers for rs11465797: 5'-CAAGTAACTGGGATTACAGGCACATG-3' (forward), 5'-CTTTACCTATATCATCCAGGT-3' (reverse). Sequence analysis of the amplified DNA product was performed using the BigDye Terminator version 3.1 ready reaction kit and the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencing primers are 5'-GACATTTGTAGAGAGTTTGGCATG-3' for rs11209026 and rs41313262, and 5'-CTTCCATGCCTAGTGCCTTTGCTG-3' for rs11465797. Sequencing data were aligned and compared using the SeqMan program (DYNASTAR, Madison, WI).

Cytokine enzyme-linked immunosorbent assay (ELISA)

Sera were obtained from 23 AA patients with *IL-23R* polymorphisms, 37 AA patients without polymorphisms, and 21 healthy controls. IL-23 serum levels were measured using the Human Interleukin 23 (Hu IL-23) Heterodimer ELISA kit (BioSource, Camarillo, CA), following the manufacturer's recommendations. All samples were assayed in duplicate.

Statistical analysis

Summary statistics, such as proportions and p-values were used to describe the related variables. Statistical analysis was performed by χ^2 tests with a significance level of $P < 0.05$, for SNPs and for ELISA data. All numerical results were computed using Splus 8 (Insightful Corp, Seattle, WA) and NCSS, PASS (Hintze. J, (2006). NCSS, PASS, and GESS. NCSS, Kaysville, Utah) for Windows statistical software.

RESULTS AND DISCUSSION

To assess the contribution of *IL-23* SNPs to AA susceptibility and phenotype, we first targeted the non-synonymous rs11209026 SNP (c.1142G>A, p.Arg381Gln) because of its strong association with IBD [10]. In addition, we examined allele frequencies of two non-synonymous SNPs, rs41313262 (c.1084G>A, p.Val362Ile) and rs11465797 (c.524C>A, p.Thr175Asn). Genotype frequencies of the three *IL-23R* polymorphisms examined in AA patients and healthy controls are shown in Table 1, together with demographic and clinical features. The p.Arg381Gln variant was present in 6.1% of AA patients and in 6.0% of ethnically matched controls ($P = 0.960$; β -error = 0.16). The carrier frequency of the p.Val362Ile (rs41313262) variant was low, but similar in AA patients (2.2%) and controls (1.6%) ($P = 0.958$); the p.Thr175Asn (rs11465797) variant was not observed in patients or controls. No homozygous carriers of the p.Arg381Gln or p.Val362Ile variant were found in either AA patients or controls. All carriers had only a single SNP, except one with two SNPs.

We performed ELISA analysis to measure IL-23 levels in sera of 23 AA patients with SNP polymorphisms and 37 AA patients without polymorphisms, and compared results with those of 21 healthy controls (Figure 1). There were no statistical differences among the three groups, even after stratifying AA patients according to treatment status.

Collectively, our data indicate that the Arg381Gln SNP did not correlate with susceptibility of AA.

The IL-23/IL-17-mediated inflammatory axis has been implicated in numerous autoimmune diseases and inflammatory conditions. A series of cytokines, including IL-6, IL-21, TGF- β , and IL-23, sequentially or synergistically induce Th17 lineage cells, together with the cooperative action of transcription factors [11]. Conversely, other cytokines, including IL-2, IL-4, IFN- γ , and IL-27, antagonize differentiation of the Th17 lineage and inhibit IL-17 and IL-23 production. In AA patients, increased production of type 1 cytokines (IFN- γ , TNF, and IL-2) from T cells has been observed, implicating that the hematopoietic cell destruction by a Th1 T-cell response [9,23], which includes both the Fas-mediated cell death and the inhibition of hematopoietic stem cell proliferation [30]. Our previous work has suggested that T-bet mediated IFN- γ elevation is responsible for the Th1 shift of CD4⁺ T cells in AA patients. As CD4⁺ effector T cells were shifted to Th1 cells in AA patients, the IL-23/IL-17-mediated inflammatory axis might be suppressed in AA, due to antagonistic effect of Th1 cytokines on Th17 differentiation and development. Therefore, it is consistent that *IL-23R* SNPs have no significant association with AA, although it cannot be excluded that other *IL-23R* SNPs as well as *IL-23R* isoforms (differentially expressed in distinct cell types) might play protective roles.

In other autoimmune diseases such as MS, RA, and SLE, similar results have been obtained, *IL23R* polymorphisms had no significant correlation with susceptibility or severity of disease [2,17,22], in agreement with our results. It has been suggested that the *IL23R* polymorphisms might play a more important role in regulating local inflammation, as in IBD and psoriasis, rather than in systemic inflammation in RA and SLE, supported by some work in mouse models [28]. Further, the IL-23-pathway molecules have no association with SLE; type I cytokines, which are not induced with IL-23, are relevant in the development and maintenance of this disease [1,20]. AA patients may share a common background of genetic risk affecting disease development and severity, which may be similar to some other autoimmune diseases and completely different from others. Further genetic studies are required to investigate as yet unidentified causal genetic alleles responsible for AA.

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Abbreviations

AA	aplastic anemia
SNP	single nucleotide polymorphism
IL-12, 17 or 23	Interleukin-12, 17 or 23
IFN-γ	Interferon- γ
TNF	tumor necrosis factor
IBD	inflammatory bowel disease
CD	Crohn's disease
UC	ulcerative colitis
RA	rheumatoid arthritis
MS	multiple sclerosis
SLE	systemic lupus erythematosus
Tregs	regulatory T cells
Th1	T helper type 1
Th17	T helper cells type 17

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Figure 1.

Comparison of IL-23 levels in sera between AA patients and controls by ELISA. Serum samples were obtained from 23 AA patients with SNPs, 37 AA patients without SNPs, and 21 healthy controls. IL-23 levels were measured using the human IL-23 ELISA assay kit. AA patients with and without SNPs vs. controls: $P = 0.720$; AA patients with SNPs vs. controls: $P = 0.344$; AA patients without SNPs vs. controls: $P = 0.342$.

Table 1

Genotype frequencies of the three *IL23R* SNPs as well as demographic and clinical features in AA patients and controls

Risk Factor	AA patients N (%)	Healthy control N (%)	AA vs. controls	
			OR ^a	P value
Total	279	184	----	----
Diagnosis (%)				
MAA	92 (33.0)	----	----	----
SAA	187 (67.0)	----	----	----
Sex (%)				
Male	146 (52.3)	NA	----	----
Female	133 (47.7)	NA	----	----
Race/Ethnicity (%)				
Caucasian	159 (57.0)	117 (63.6)	----	0.187
African-American	33 (11.8)	23 (12.5)	----	0.943
Hispanic	49 (17.6)	22 (12.0)	----	0.132
Asian	33 (13.6)	22 (12.0)	----	0.967
SNPs (% yes)				
rs11209026	17 (6.1)	11 (6.0)	1.018	0.960
rs41313262	6 (2.2)	3 (1.6)	1.181	0.958
rs11465797	0	0	----	----

rs11209026 (c.1142G>A, p.Arg381Gln); rs41313262 (c.1084G>A, p.Val362Ile);

rs11465797 (c.524C>A, p.Thr175Asn). An amino acid position is relative to the ATG start site; the first nucleotide of the exon 1 start site is designated as position 1 based on the reference sequence GenBank NM_144701.2 for *IL-23R*. AA patients and controls examined carried only one of the three SNPs.

^aOR: odds ratio.