An association study of inflammatory cytokine gene polymorphisms in Fabry disease

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ABSTRACT. ***Background*. Fabry disease is an X-linked disorder associated with early-onset stroke, cardiomyopathy, and progression to end-stage renal failure. Correlations between inflammatory cytokines have been shown in other lysosomal storage diseases. The aim of the study was to evaluate functional gene polymorphisms of key pro- and anti-inflammatory cytokines and to correlate them to a clinical score to assess the potential role of inflammation in Fabry disease.** ***Design*. Genotyping for IL-10[819C/T; -592C/A]; IL-1β[+3954 C/T; –511C/T]; IL-1α[-889C/T]; and TNF-α[-308G/A] was performed in 76 patients and correlated with MSSI sub-scores and with enzyme (alpha-galactosidase A) levels. Fifty, normal, age- and sex-matched volunteers were also genotyped.** ***Results*. Of 76 patients, 31 (41%) were males and 45 (59%) were females. There was no correlation between enzyme levels and any cytokine levels. Statistically significant differences were found in prevalence of TNF-α [-308G/A] genotypes: 84% GG in patients *versus* 63% GG in controls ($p = 0.038$) and for IL-1α [-889C/T] genotypes: 94% CC in patients *versus* 21% CC in controls ($p < 0.001$). Statistically significant differences were found in the ratio between the two polymorphisms of IL-10 ($p < 0.0001$), between the two polymorphisms of IL-1β ($p = 0.001$); between IL-1α [-889C/T] and IL-1beta [3954C/T] ($p = 0.002$); and between IL-10[-592C/T] and IL-1β [3954C/T] ($p = 0.041$). Correlations between TNF-α [-308G/A] and both kidney and neurological MSSI sub-scores (both: $p = 0.06$) and between IL-10[-819C/T] and the MSSI neurological score ($p = 0.03$) were noted. The majority of patients with Fabry disease have therefore a profile of low TNF-α (increased frequency of GG genotype of the TNF-α[-308] polymorphism), high IL-10 production (preponderance of the C allele of the wild type or heterozygous state for the polymorphisms of IL-10 [819; -592], but simultaneously increased production of the pro-inflammatory cytokines IL-1β and IL-1α usually associated with a preponderance of the C allele of the wild type or heterozygous state for the polymorphisms of IL-1β [3954; -511] and of IL-1 α[-889].** ***Conclusions*. We speculate that sequence variations of important inflammatory genes of the interleukin inflammatory family are associated with differential effects in Fabry disease, and with increased sample size, haplotype blocks might be constructed.**

Key words: Fabry disease, inflammatory cytokine, polymorphism, Mainz Severity Score Index, enzyme replacement therapy

Fabry disease, an X-linked disorder caused by a deficiency of the lysosomal enzyme α-galactosidase-A, results in endothelial dysfunction and damage to peripheral and smooth muscle cells of the vascular system, glomerular and tubular cells of the kidney, myocardial cells, and brain stem structures, because of abnormal storage material [1]. Fabry disease is a multi-system disorder, albeit with considerable phenotypic heterogeneity in onset and severity [2]. Recently, it has been suggested that the later-onset of the renal, cardiac, and ischemic stroke variants may be due to specific mutations that induce missense changes rather than null enzymatic forms [3]. Nonetheless, because of the myriad of mutations, many of which are novel, identification of mutations in the gene alone cannot predict disease severity, although some structural changes may be related to the disease [4].

In the past decade, enzyme replacement therapy has been shown to alter the natural history of the disease by improving the progress of some clinical features [5, 6]. The Mainz Severity Score Index (MSSI) was devised in order to track clinical parameters and their response to treatment. This score awards points on the basis of increased severity for each of four categories: general, neurological, cardiac, and kidney involvement. The combined "total" score of sub-scores can then be used to compare severity over time in the same patient and also between patients or groups of patients. For each category, 1-20 points is considered mild involvement; 21-40 points is considered moderate, and > 41 points is severe involvement [7]. A further advantage of this particular scoring system is the ability to compare sub-scores separately, which is particularly relevant when disease parameters are unequally affected and possibly unequally responsive to treatment. Nonetheless, although it has been shown that missense mutations may be correlated with age, and genotype with severity in these cases [8], genetic markers are not included in the MSSI.

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Table 1
Primers used for restriction analysis

Gene poly-morphisms	Forward primer (5′-3′)	Reverse primer (5′-3′)	Restriction enzyme
TNF-α [-308G/A]	AGGCAATAGGTTTTGAGGGCCAT	AGGAGGGACGAGGCTAAGGC	NcoI[a]
IL-10 [819C/T]	AGACAACACTACTAAGGC TTCTTGAGGA	AGGTAGTGCTCACCATGACC	MslI[a]
IL-10 [592C/A]	CTCAGTTAGCACTGGTGTAC	TGTTCCTAGGTCACAGTGAC	RsaI[a]
IL-1β [+3954]	GCTTTTTTGCTGTGAGTC CCG	CTCAGGTGTCCTCGAAGAAATCAAA	TaqI[b]
IL-1β [-511C/T]	TGGCATTGATCTGGTTCATC	GTTTAGGAATCT TCCCACTT	AvaI[b]
IL-1α [-889C/T]	AAGCTTGTTCTACCACCTGAACTAGGC	TTACATATGAGCCTTCCA	NcoI[a]

[a] New England Biolabs, MA, USA.
[b] Fermentas, USA.

METHODS

Patients

Hemizygote and heterozygote patients with Fabry disease were included in this study. All patients were evaluated by physical examination and then ascribed an MSSI score [7]. Institutional Review Board (Helsinki Committee) approval was received for this study.

Genotyping

Blood samples were drawn for enzymatic activity of α-galactosidase-A, for DNA extraction for analysis for α-galactosidase-A mutations, and for the following cytokine polymorphism genotyping: IL-10[819C/T; -592C/A]; IL-1β[3954 C/T; –511C/T]; IL-1α[-889C/T]; and TNF-α[-308G/A]. The primers and restriction enzymes used for each polymorphism are presented in *table 1*.

Fifty, age- and sex-matched Caucasians were also genotyped for the promoter gene polymorphism. Results of each polymorphic genotype were compared to these controls.

Patient polymorphic genotypes were also compared to age, sex, use of enzyme replacement therapy, CRP levels, and to each of the sub-categories of the MSSI.

Statistical analysis

Hardy-Weinberg equilibrium analysis could not be performed because of the small sample size. Chi-square analysis with the Pearson Continuity Correction was used to compare sub-tests of the MSSI with each of the polymorphic variants and with the use of enzyme replacement therapy. Similarly, the chi-square was used to compare plasma levels of CRP, IL-6, and TNF- α with use of enzyme replacement therapy. Fisher's Exact test was applied when wild-type variants were cross-tabbed in a two-tailed model *versus* homozygous plus heterozygous for the mutated alleles. A p value below 0.05 was considered statistically significant.

RESULTS

The demographic data of the patients are presented in *table 2*.

There was no correlation between the use of enzyme replacement therapy and plasma levels of CRP, IL-6, or TNF-α. There were also no statistically significant correlations between the sub-tests of the MSSI with use of enzyme replacement although there was a trend to a significant relationship between the neurological score and treatment with enzyme.

Statistically significant differences were found in prevalence of TNF-α [-308G/A] and IL-1α [-889C/T] genotypes between patients and controls (*table 3*).

Statistically significant associations were found between four sets of the two polymorphisms (*table 4*). The implication of this type of association is illustrated by the following correlation ($p < 0.0001$) between the two IL-10 variants: the association occurred in 46 of 64 patients (71.9%) and 50% of patients who were wild type for IL-10[819C/T] were also wild type for IL-10[-592C/A], whereas < 10% were homozygous (AA or TT respectively) for either of these polymorphisms.

Furthermore, in the associations between sets, based on the genotype of one variant, different "behaviors" are predictable for the variant of the other polymorphism, e.g. 77% of those heterozygous (CT) for IL-1β[3954], were CC (wild type) for IL-10[-592]; in contrast, only 39% of patients CC (wild type) for IL-1β[3954], were CC for IL-10[-592].

A further difference in associations between polymorphic variants among patients heterozygous (CT) for IL-1β[3954], was that half were CC and half CT in IL-1β[-511]; but 86% of patients homozygous for the mutation

Table 2
Demographic data of 76 patients with Fabry disease

Mean age (yrs ±SD)	41±18
Females	45 (59.2%)
α-galactosidase A enzyme (as% normal levels±SD)	0.43±0.07
Patients treated with enzyme therapy	48 (63.2%)
Patients with cardiac hypertrophy	28 (36.8%)
Patients with neurological pain	54 (84.2%)
Patients with signs of stroke (TIA or CVA)	12 (15.8%)
Patients with renal involvement (proteinuria > 500 mg/dL)	50 (65.8%)
Patients with renal failure	15 (19.7%)

Table 3
Significant differences in inflammatory gene allele distributions between patients with Fabry disease *versus* controls

Polymorphic variant	Genotype	Patients	Controls	p value
TNF-α [-308]	GG	84%	63%	0.038
IL-1α [-889]	CC	94%	21%	<0.001

(TT) in IL-1β[3954], were CC for IL-1β[-511]. Finally, 53% of patients wild type (CC) for IL-1β[3954], were heterozygous (CT) for IL-1β[-511].

Another significant association was between IL-1β[3954C/T] and IL-1α[-889C/T], because 64.4% of all patients were heterozygous for both polymorphisms, but of patients wild type (CC) for IL-1α [-889], 90% were also wild type (CC) for IL-1β [3954].

Trends to statistical significance between pairs of polymorphic variants are noted in *table 5*.

DISCUSSION

There appears to be an association between inflammatory cytokines and Fabry disease activity, although this seems to be unrelated to (α–galactosidase A) enzyme levels. Thus, we show for the first time that there is a genetic discrepancy and/or predilection among patients with Fabry disease relative to the expected frequencies of inflammatory cytokines in healthy individuals.

Patients with Fabry disease generally have the wild type genotypes for both TNF-α[-308] and IL-10[-819], implying decreased production of the pro-inflammatory cytokine TNF-α, but increased production of IL-10.

In addition, we show that there is also a clinical correlation between the GG genotype of TNF-α[-308] and the CC genotype of IL-10[-819] with the neurological MSSI sub-score. However, it should be noted that the MSSI has not been completely validated among cohorts from other countries, and hence this may require further and/or larger patient populations for verification.

The relationship between inflammation and Fabry disease appears to exist on several levels. Firstly, there is a discrepancy in the incidence of polymorphisms for both TNF-α and IL-1α in patients with Fabry disease relative to healthy controls. With regard to TNF-α with its crucial roles in apoptosis and cell survival as well as in immunity, it is clear that this cytokine is involved in antigen-dependent and antigen-independent models of inflammation, defining its role as a pro-inflammatory cytokine [17]. Based on epidemiological studies of inflammatory cytokines [18], the GG polymorphism in TNF-α[-308G/A] [19] has been implicated as a risk factor for (lacunar and ischemic) stroke. And indeed, we report a high incidence of the GG genotype for TNF-α in patients with Fabry disease as well as correlation with the neurological sub-score of the MSSI.

There was a multiplicity of associations among the two IL-10 polymorphisms, the two IL-1β polymorphisms and the one IL-1α polymorphism. This seems to indicate that the majority of patients have at least one C allele (but more often two C alleles) for each of the five polymorphic sites of the anti-inflammatory interleukins.

In the cohort of patients with Fabry disease herein reported, the CC genotype of IL-1α[-889C/T] was significantly more frequent. The IL-1α[-889C/T] CC genotype has been suggested to be associated with the progression of senile/neuritic plaques and neurofibrillary tangles in patients with Alzheimer's disease [20]. There is compelling evidence that β-amyloid deposition in Alzheimer's disease is associated with a local inflammatory response, which is mediated by secretion of cytokines that may contribute to neuronal degeneration and cell death. Hypothetically, the deposition of substrate in Fabry disease may induce a comparable cascade of local inflammatory responses and eventual neurodegeneration.

Table 4
Significant differences between two polymorphic variants or with an MSSI sub-score

Polymorphic variant	Polymorphic variant/score	p value
IL-10 [819]	IL-10 [-592]	<0.0001
IL-1β [3954]	IL-10 [-592]	0.041
IL-1β [3954]	IL-1β [511]	0.001
IL-1α [-889]	IL-1β [3954]	0.002
IL-10 [819]	Neurological MSSI score	0.033

Table 5
Trends to significant differences between two polymorphic variants or with an MSSI sub-score

Polymorphic variant	Polymorphic variant/score	p value
IL-10 [819]	TNF-α [-308]	0.088
IL-10 [819]	IL-1β [3954]	0.069
IL-1α [-889]	TNF-α [-308]	0.095
TNF-α [-308]	Renal MSSI score	0.062
TNF-α [-308]	Neurological MSSI score	0.064

A second interesting association was that half of the patients carried wild type genotypes for the two IL-10 polymorphisms, [819] and [-592] and less than 10% did not have a C allele in either polymorphic genotype. A similar situation was seen in a recent study of patients with type 2 diabetes mellitus, where significantly more patients carried the same preponderance of the C allele for both polymorphisms, both of which were associated with high levels of IL-10 production [21].

Finally, the statistical analysis of incidence of various polymorphic genotypes may allow haplotype block prediction of these inflammatory cytokine genotypes for Fabry disease. We posit that the majority of patients with Fabry disease have a preponderance of the C allele of the wild type or heterozygous state for each of the polymorphisms of IL-10 {819; -592] and IL-1β [3954; -511] and for IL-1α [-889]. This implies increased production of each of these cytokines.

On the other hand, the majority of patients had the GG genotype of the TNF-α[-308] polymorphism which is associated with decreased production of this pro-inflammatory cytokine. The profile of low TNF-α and high IL-10 production is seen in lupus erythematosus [22], in resistance to shock/liver injury [23], in resolved hepatitis C infection [24], and in reactive arthritis joints as in spondyloarthropathies [25].

Author contributions. RS carried out all of the molecular genetic studies. CW and MB assessed the clinical status of all of the patients including scoring, and provided the blood samples. GA and DE conceived the study, participated in its design and coordination. GA also supervised the genotyping. DE wrote all drafts of the manuscript. All authors read and approved the final manuscript. [9-16]

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