LETTERS

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Examination of the possible role of biologically relevant genes around FBN1 in systemic sclerosis in the Choctaw population

To the Editor:

Previous studies have demonstrated an association between a particular microsatellite haplotype on human chromosome 15 and systemic sclerosis (SSc) in Choctaw Indians, a population with a high prevalence of SSc (1). While genetic and functional studies have implicated the gene encoding fibrillin 1 (FBN1) as a candidate gene for SSc, a defect in this gene has yet to be found in human SSc (2–5). One possible explanation is that FBN1 is actually a marker that is in linkage disequilibrium with another gene on the chromosome 15 haplotype.

Indeed, this region contains several other genes relevant to extracellular matrix (ECM) metabolism, including thrombospondin 1 (THBS1) on 15q15, fibroblast growth factor 7 (FGF7) on 15q15-q21.1, microfibrillar-associated protein 1 (MFAP1) on 15q15-q12, and furin (FUR), or paired basic amino acid–cleaving enzyme (PACE), on 15q25-26. THBS1 encodes a secreted protein that associates with the ECM and modulates cell–matrix interaction. Thrombospondin 1 interacts with cell-surface receptors, cytokines, and proteases and can activate latent transforming growth factor β, an important cytokine that mediates fibrosis (6,7). FGF7 encodes a growth factor specific for epithelial cells, with predominant activity in keratinocytes, and is important in wound healing (8). MFAP1 is located in the same chromosomal region as FBN1, and its gene product localizes to fibrillin 1, containing 10–12 nm microfibrils in the ECM (9). Finally, FUR encodes a subtilisin-like protease that is responsible for the proteolytic processing of profibrillin 1 by cleavage of the carboxy-terminal domain into fibrillin 1 (10). The present study was undertaken to determine whether these ECM genes located near FBN1 might contribute to SSc susceptibility in the Choctaw population.

Thirteen single-nucleotide polymorphisms (SNPs) in THBS1, 7 SNPs in FGF7, and 6 SNPs in FUR were obtained by querying the National Center for Biotechnology Information dbSNP database. The SNPs were genotyped in 20 Choctaw SSc cases and 48 matched controls, by automated sequencing. They were then tested for disease association at the level of individual SNPs and at the haplotype level. Haplotypes were inferred from the genotype data (Table 1).

Table 1. THBS1, FGF7, and FUR haplotype frequencies in Choctaw Indians with systemic sclerosis (SSc) and in controls

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Choctaw with SSc</th>
<th>Controls</th>
<th>Overall P</th>
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<tbody>
<tr>
<td>THBS1 haplotypes*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CATTTT</td>
<td>0.0750</td>
<td>0.0952</td>
<td></td>
</tr>
<tr>
<td>TGCCCC</td>
<td>–</td>
<td>0.0119</td>
<td></td>
</tr>
<tr>
<td>TGCCCT</td>
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<td>0.8890</td>
<td>0.511</td>
</tr>
<tr>
<td>TGCTCT</td>
<td>0.0500</td>
<td>0.0328</td>
<td></td>
</tr>
<tr>
<td>GCCCTT</td>
<td>0.0200</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>FGF7 haplotypes†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CACTCTG</td>
<td>0.3611</td>
<td>0.4149</td>
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<tr>
<td>CACTCGG</td>
<td>0.4722</td>
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<tr>
<td>AGCTCGA</td>
<td>0.1111</td>
<td>0.0745</td>
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</tr>
<tr>
<td>AGTCAGA</td>
<td>0.0278</td>
<td></td>
<td></td>
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<td>CACTGA</td>
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<td>0.1000</td>
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<td>CATTGG</td>
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<tr>
<td>AACTCGG</td>
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<td>0.0319</td>
<td></td>
</tr>
<tr>
<td>CGTCTCG</td>
<td>–</td>
<td>0.0319</td>
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<tr>
<td>FUR haplotypes‡</td>
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<td></td>
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<tr>
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<td>0.521</td>
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<td>CTCGGG</td>
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<td>GCCAGT</td>
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<tr>
<td>CTCATG</td>
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<tr>
<td>CCCGGG</td>
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<td>0.010</td>
<td></td>
</tr>
<tr>
<td>GCCGGG</td>
<td>–</td>
<td>0.016</td>
<td></td>
</tr>
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</table>

* Reference single-nucleotide polymorphisms (SNPs): rs2292305, rs755399, rs755398, rs1904209, rs20664141, rs22282633.
† Reference SNPs: rs28099435, rs28099436, rs2413945, rs952127, rs952126, rs2413959, rs2413958.
‡ Reference SNPs: rs2071410, rs1573643, rs1048346, rs4702, rs6225, rs6224.

Five of the THBS1 SNPs were located in introns, and 1 SNP encoded a Thr523→Ala523 substitution. All of the FGF7 SNPs were located in introns. Two of the FUR SNPs were synonymous, and the remainder were in the introns and untranslated regions. For THBS1, FGF7, and FUR, no significant differences in individual SNP allele frequencies were observed between the SSc cases and the controls. Moreover, there was a very limited number of recombinational events observed in THBS1, FGF7, and FUR SNPs.

A total of 5 possible THBS1, 8 FGF7, and 8 FUR haplotypes were inferred from the genotype data (Table 1). One THBS1, 2 FGF7, and 2 FUR haplotypes were highly predominant in both groups, implying a significant degree of linkage disequilibrium among the SNPs in these candidate genes. No significant differences between the groups were found at the haplotype level for any of the genes. We noted that for FUR, the frequency of haplotype GCCAGT was higher in SSc cases than in controls (Table 1), although the difference was not significant. In order to investigate whether gene–gene interaction would strengthen association, haplotype combinations for FUR and FBN1 in cases and controls were also inferred. The results showed that the combination of these 2 genes did not further strengthen the association with SSc (data not shown).

1. To the Editor:

Previous studies have demonstrated an association between a particular microsatellite haplotype on human chromosome 15 and systemic sclerosis (SSc) in Choctaw Indians, a population with a high prevalence of SSc (1). While genetic and functional studies have implicated the gene encoding fibrillin 1 (FBN1) as a candidate gene for SSc, a defect in this gene has yet to be found in human SSc (2–5). One possible explanation is that FBN1 is actually a marker that is in linkage disequilibrium with another gene on the chromosome 15 haplotype.

Indeed, this region contains several other genes relevant to extracellular matrix (ECM) metabolism, including thrombospondin 1 (THBS1) on 15q15, fibroblast growth factor 7 (FGF7) on 15q15-q21.1, microfibrillar-associated protein 1 (MFAP1) on 15q15-q12, and furin (FUR), or paired basic amino acid–cleaving enzyme (PACE), on 15q25-26. THBS1 encodes a secreted protein that associates with the ECM and modulates cell–matrix interaction. Thrombospondin 1 interacts with cell-surface receptors, cytokines, and proteases and can activate latent transforming growth factor β, an important cytokine that mediates fibrosis (6,7). FGF7 encodes a growth factor specific for epithelial cells, with predominant activity in keratinocytes, and is important in wound healing (8). MFAP1 is located in the same chromosomal region as FBN1, and its gene product localizes to fibrillin 1, containing 10–12 nm microfibrils in the ECM (9). Finally, FUR encodes a subtilisin-like protease that is responsible for the proteolytic processing of profibrillin 1 by cleavage of the carboxy-terminal domain into fibrillin 1 (10). The present study was undertaken to determine whether these ECM genes located near FBN1 might contribute to SSc susceptibility in the Choctaw population.

Thirteen single-nucleotide polymorphisms (SNPs) in THBS1, 7 SNPs in FGF7, and 6 SNPs in FUR were obtained by querying the National Center for Biotechnology Information dbSNP database. The SNPs were genotyped in 20 Choctaw SSc cases and 48 matched controls, by automated sequencing. They were then tested for disease association at the level of individual SNPs and at the haplotype level. Haplotypes were inferred using the expectation-maximization algorithm and a second algorithm based on the 4-gamete test (3,11). Extremely similar results were obtained with both algorithms. None of the 4 MFAP1 SNPs we initially screened were polymorphic; thus, the 9 exons of MFAP1 were screened for mutations by direct sequencing of genomic DNA from 3 Choctaw SSc cases and 3 matched Choctaw controls. In addition, fibroblast total RNA obtained from 2 Choctaw SSc cases was analyzed by reverse transcriptase–polymerase chain reaction for MFAP1 transcripts, and the products screened for mutations by sequencing and single-strand conformational polymorphism.

On initial screening, 6 of 13 THBS1, all 7 FGF7, and 4 of 6 FUR SNPs were found to be polymorphic in the Choctaw.
not shown). Lastly, no MFAP1 mutations were detected at the genomic or at the transcript level (data not shown).

Polymorphisms in the THBS1, FGFB, FUR, and MFAP1 genes cannot account for the high prevalence of SSC in the Oklahoma Choctaw. These data, together with reports showing functional abnormalities in the fibrillin 1 protein in SSC fibroblasts (5) and disease-specific autoimmune responses directed against fibrillin 1 in SSC (12,13), support the notion that FBN1 is the most likely candidate gene on the chromosome 15 haplotype previously associated with SSC in the Choctaw.

Supported in part by grant 3MO1-RR-02558-12S1 from the National Center for Research Resources, NIH, Specialized Center for Research in Scleroderma Award IP50-AR-44888 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, the RKG Foundation, and the University of Texas LASR 2000 Research Fund.

Letter to the Editor:

Cytomegalovirus (CMV) infection has been associated with antiphospholipid syndrome (APS) or thrombosis in several reported cases (1–3), suggesting that it might play a pathogenetic role. In a study reported in Arthritis & Rheumatism (4) Gharavi et al observed that antiphospholipid antibodies (aPL) induced by immunization of mice with a CMV peptide (TIFII) were pathogenic in vivo. They suggested that in patients with APS, molecular mimicry could be a mechanism for aPL synthesis generated by CMV infection (4). Infection with CMV may trigger aPL production and has been hypothesized to be an etiologic mechanism in APS. Most investigators conclude, however, that aPL in human viral infections have different specificity from those found in APS, and that aPL in systemic autoimmune diseases have no pathogenic function (5).

To investigate Gharavi and colleagues’ hypothesis, we determined the prevalence of IgG and IgM anti-CMV in 25 patients (8 male, 17 female, age range 16–91 years) who were positive for IgG/IgM anti-CMV antibodies. CMV tested for DNA in patients who were positive for IgM antibodies.

The study was carried out during the course of approximately 1 year. Sera were frozen at −20°C until analysis. Control sera were obtained from 66 healthy subjects (37 male, 29 female, age range 17–82 years).

IgG/IgM anti-CMV antibodies were analyzed by immunofluorescence (AxSYM, Abbott, Chicago, IL). IgG/IgM aCL and anti-β2GPI antibodies were determined by enzyme immunoassay (Gentec Diagnostika, Mainz, Germany). CMV DNA viral presence was detected by nested polymerase chain reaction with specific primers for genomic region IRL 1-11. Arlequin version 2.000: a software for population genetic data analysis. Geneva: Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva; 2000.

9. Liu W, Faraco J, Qian C, Francke U. The gene for microfibril-associated protein-1 (MFAP1) is located several megabases centromeric to FBN1 and is not mutated in Marfan syndrome. Hum Genet 1997;99:578-84.
Fifty-four percent of the patients who were positive for IgM aCL or anti-β₂GPI were IgM anti-CMV negative but IgG anti-CMV positive. Only 1 of these patients positive for IgM aCL or anti-β₂GPI had both IgM anti-CMV and CMV DNA. Three of the studied patients had thrombosis and were considered as having definite APS (secondary in 2, primary in 1) according to diagnostic criteria (6). All 3 were IgM aCL or anti-β₂GPI positive but IgM anti-CMV negative.

Our preliminary results do not completely support the notion that acute CMV infection could generate aPL in most patients. In a substantial proportion of the patients in this study, there was no correspondence between the presence of IgM aPL and the presence of IgM anti-CMV. We believe additional studies in human subjects need to be performed before a conclusion regarding a relationship between CMV infection and APS, based on Gharavi et al’s findings in mice, can be drawn.

To the Editor:

Systemic lupus erythematosus (SLE) is a disorder of generalized autoimmunity which is characterized by multisystem organ involvement. SLE pathogenesis is multifactorial and polygenic; in fact, several major histocompatibility complex (MHC) and non-MHC loci were found to be linked or associated with SLE in some populations, but this has been difficult to reproduce in others, suggesting different suscepti-

bility genes among ethnic groups. DNASE1, one of the non-MHC loci (16p13.3) (1), encodes a nuclease which has the potential to remove DNA at the site of high cell turnover. Mice deficient in this enzyme showed SLE-like features (2). Therefore, this enzyme could be involved in the pathogenesis of human SLE.

Recently, Yasutomo et al identified an A/T transversion (not A/G) in exon 2 at position 172 of the DNASE1 gene coding sequence, which resulted in a lysine-to-stop substitution at codon 5, in 2 female Japanese patients with SLE (3). The patients, both of whom had the heterozygotic mutation, had substantially lower levels of DNASE1 activity in their sera than did patients without a DNASE1 mutation. In addition, both patients had an extremely high IgG titer against nucleosomal antigens. Analysis of the A/T transversion in 1,516 human chromosomes from the UK showed the absence of this mutation in subjects with SLE (n = 182) or Graves’ disease (n = 291) and in healthy controls (n = 285) (4). These contradictory results raise the following question: does the responsibility of the A/T mutation for SLE pathogenesis depend on the ethnic group?

In order to answer this question, we have explored the A/T mutation of DNASE1 in Tunisian patients and controls. Although the Tunisian population is characterized by a high level of consanguinity, it is heterogeneous (5). The strategic geographic location and the genetic exchanges and migration of people around the Mediterranean Sea and the western Sahara for more than 3,000 years could assure an important genetic heterogeneity. This gives us the opportunity to find this mutation at least in Tunisian healthy subjects.

Thirty-nine unrelated SLE patients (3 men and 36 women) were recruited after diagnosis according to the American College of Rheumatology 1982 revised criteria for the classification of SLE (6). The control population consisted of 91 healthy subjects with no clinical evidence or family history of autoimmune and inflammatory joint diseases. All the blood samples were collected after obtaining informed consent from the patients and their responsible family members during the period when the patients’ disease was in remission. Genomic DNA from patients and controls was isolated from peripheral blood using the conventional phenol–chloroform method. DNA from patients and controls was isolated from peripheral blood using the conventional phenol–chloroform method. Three hundred chromosomes from the UK showed the absence of this mutation in subjects with SLE (n = 182) or Graves’ disease (n = 291) and in healthy controls (n = 285) (4). These contradictory results raise the following question: does the responsibility of the A/T mutation for SLE pathogenesis depend on the ethnic group?

Our exploration of 260 chromosomes showed the absence of the A/T DNASE1 mutation. This result can be extrapolated to the population south of the Mediterranean Sea characterized by common genetic features of heterogeneity.

The absence of the A/T mutation in UK and Tunisian populations reflects the absence of the particular form of SLE which was reported by Yasutomo et al. However, we cannot exclude the contribution of the DNASE1 gene to genetic susceptibility to SLE. Indeed, sequence change in or near the DNASE1 gene may alter either its expression or the DNASE1 protein interaction with DNA. Therefore, the genomic sequence of the DNASE1 gene must be explored to definitively establish or exclude its involvement. Analysis of single-nucleo-
Long-term followup needed to define role of infliximab in treatment of renal amyloidosis: comment on the case report by Elkayam et al

To the Editor:

We read with great interest the article by Elkayam and colleagues reporting resolution of proteinuria and stabilization of amyloid deposits in a patient with rheumatoid arthritis treated with infliximab (1). That report is in concert with previous observations and studies suggesting initial improvement rather than an amyloid load response.

Of note is our report on 15 patients with nephropathic amyloidosis of familial Mediterranean fever who had reversal of proteinuria following treatment with colchicine (6). Fourteen of these patients had nephrotic syndrome that resolved completely. Our experience and that of other investigators (7–15) also agree with the present report, that in spite of the favorable clinical results, AA amyloid deposits remain grossly unchanged, and that patients may even present, sometimes many years later, with explosive proteinuria and acute renal failure prompted by a minor insult.

This lack of concordance between the major beneficial clinical response and detectable laboratory evidence for a change in amyloid deposits is poorly understood and very challenging. It possibly matches better with a threshold behavior rather than an amyloid load response. It is, therefore, a little premature to conclude that the renal amyloidosis responded to treatment with infliximab. Long-term followup is imperative before conclusions can be reached regarding the nature of the antiamyloidotic effects of this drug.

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References


To the Editor:
We read with great interest the case report by Elkayam et al (Elkayam O, Hawkins PN, Lachmann H, Yaron M, Caspi D. Rapid and complete resolution of proteinuria due to renal amyloidosis in a patient with rheumatoid arthritis treated with infliximab. Arthritis Rheum 2002;46:2571–3). The authors described a patient with rheumatoid arthritis (RA) and proteinuria due to AA amyloidosis, whose RA was suppressed by treatment with infliximab. Arthritis Rheum 2002;46:2571–3). The authors describe a patient with rheumatoid arthritis treated with infliximab. We have presented a case of rapid and complete resolution of proteinuria due to renal amyloidosis in a patient with RA. As described, the patient started treatment with infliximab in May 2000 and experienced rapid clinical and laboratory remission. Three years later, the patient is still being treated with infliximab.

This case shows that infliximab is not always able to control the proinflammatory cytokine cascade, which is the cause of hepatic synthesis of serum amyloid A (SAA), as can be seen by the persistence of an increased ESR, elevated levels of CRP and fibrinogen, and increased proteinuria. Probably, the kidney damage in our patient was more severe than that in the patient described by Elkayam et al. In their patient, proteinuria was 300 mg/24 hours at the beginning of the study, and increased to a maximum level of 900 mg/24 hours. In our patient, proteinuria was significant higher, and a renal biopsy revealed a significant deposition of amyloid.

Because Elkayam et al reported that their patient had a history of renal damage following treatment with sodium aurothiomalate in 1996, it is possible that proteinuria in their patient developed earlier than usual, in the presence of a renal burden of SAA that was lower than that in the case we describe. A renal biopsy evaluation of their patient could have been useful to confirm this hypothesis. The treatment of renal amyloidosis in RA needs further investigation, and it is possible that there is a positive role for combination therapy with different biologic agents.

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Reply
To the Editor:
We thank Dr. Tweezer-Zaks and colleagues and Drs. Ciboddo and Idone for their interest in our work and their instructive comments. We have presented a case of rapid and complete resolution of proteinuria due to renal amyloidosis in a patient with RA. As described, the patient started treatment with infliximab in May 2000 and experienced rapid clinical and laboratory remission. Three years later, the patient is still being treated with infliximab, and results of her urinalysis are completely normal, which emphasizes the long-term effect of infliximab on proteinuria.

Tweezer-Zaks et al suggest that AA amyloid deposits “remain grossly unchanged.” However, 123I-labeled serum amyloid P scintigraphy has allowed a quantitative assessment of amyloid deposits, and it has been clearly demonstrated that regression of amyloid deposits occurs frequently in patients in whom a significant reduction of the plasma SAA concentration is achieved by immunosuppressive treatment (1). Although isolated amyloid fibrils are stable in vitro, AA amyloid deposits exist in a state of dynamic turnover, and outcome is favorable in AA amyloidosis when the SAA concentration is maintained below 10 mg/liter (Gillmore JD, Lovat L, Persey MR, Peys M, Hawkins PN. Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentrations of serum amyloid protein. Lancet 2001;358:24–9).

The impressive normalization of proteinuria in our patient was parallel to the clinical improvement of her arthritis and significant reduction of the SAA level. Infliximab may
improve amyloidosis only if it reduces the production of SAA. Although infliximab has emerged as a powerful therapy for RA, studies have shown that up to 40% of patients may not respond to this treatment (Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000;343:1594–602). This is obviously the case in the patient described by Ciboddo and Idone. Treatment with infliximab induced a moderate decrease in synovitis, without influencing the ESR or the CRP level, and probably the SAA concentration.

The reversibility of nephrotic syndrome due to AA also depends on the degree of renal damage. As shown for familial Mediterranean fever, although colchicine is efficient, it is seldom helpful in patients with a serum creatinine concentration of >2 mg/dl. Ciboddo and Idone did not mention whether in their patient with nephrotic-range proteinuria, apparently resistant to infliximab, other causes (e.g., superimposed renal vein thrombosis, a not uncommon complication of nephrotic syndrome) were excluded.

Patients with amyloidosis secondary to RA require treatment that is able to reduce production of SAA, hence reducing amyloid deposition. Anti–tumor necrosis factor therapies may have this effect in a substantial proportion of patients and should therefore be included in the arsenal of drugs used for the treatment of amyloidosis in this setting.

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Erratum

In the article by Raynauld et al published in the February 2003 issue of Arthritis & Rheumatism (pp 370–377), there was an error in Table 3. The value for mean range of motion (degrees) in the intraarticular steroid–treated group should have read 1.33, not 13.3.

We regret the error.