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Non-synonymous single nucleotide polymorphisms in genes for immunoregulatory galectins: Association of galectin-8 (F19Y) occurrence with autoimmune diseases in a Caucasian population

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ABSTRACT

Background: Galectins are potent immune regulators, with galectin-8 acting as a pro-apoptotic effector on synovial fluid cells and thymocytes and stimulator on T-cells. To set a proof-of-principle example for risk assessment in autoimmunity, and for a mutation affecting physiological galectin sensor functions, a polymorphism in the coding region of the *galectin-8* gene (rs2737713; F19Y) was studied for its association with two autoimmune disorders, i.e. rheumatoid arthritis and myasthenia gravis.
Methods: A case–control analysis and a related quantitative trait-association study were performed to investigate the association of this polymorphism in patients (myasthenia gravis 149, rheumatoid arthritis 214 and 134 as primary and repetitive cohorts, respectively) and 365 ethnically matched (Caucasian) healthy controls. Distribution was also investigated in patients grouped according to their antibody status and age at disease onset. Comparative testing for lectin activity was carried out in ELISA/ELLA-based binding tests with both wild-type and F19Y mutant galectin-8 from peripheral blood mononuclear cell lysates of healthy individuals with different genotypes as well as with recombinant wild-type and F19Y mutant galectin-8 proteins.
Results: A strong association was found for rheumatoid arthritis, and a mild one with myasthenia gravis. Furthermore, the presence of the sequence deviation also correlated with age at disease onset in the case of rheumatoid arthritis. The F19Y substitution did not appear to affect carbohydrate binding in solid-phase assays markedly.
General significance: This is the first report of an association between a galectin-based polymorphism leading to a mutant protein and autoimmune diseases, with evidence for antagonistic pleiotropy.

1. Introduction

During the past years numerous susceptibility alleles have been described for a series of human autoimmune diseases. It has become clear that such alleles can often occur in more than one autoimmune disorder [1]. As a consequence, their identification can help to explain why certain autoimmune diseases such as myasthenia gravis (MG), a rare autoimmune disease affecting neuromuscular transmission and rheumatoid arthritis (RA), a highly disabling disease that causes destruction of the joints, are often diagnosed in the same patient [2]. Of course, the development of these diseases will be associated with

Abbreviations: AChR, acetylcholine receptor; ANOVA, analysis of variances; BMLA, Bayesian multilevel analysis; BSA, bovine serum albumin; CCP, cyclic citrullinated peptide; CD, cluster of differentiation; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; ELLA, enzyme-linked lectin assay; Exp(B), exponentiation of the B value; HLA, human leukocyte antigen; HWE, Hardy–Weinberg equilibrium; LGALS8, human galectin-8; MG, myasthenia gravis; OD, optical density; OR, odds ratio; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline; RA, rheumatoid arthritis; SD, standard deviation; SE, standard error of mean; SNP, single nucleotide polymorphism

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a complex pattern of inherited traits, with an array of genes being involved in their development and progression [3,4]. Among the genetic factors, HLA-DRB1, for instance, accounts for approximately 30% of the genetic susceptibility in the case of RA [5], while a series of HLA associations were delineated among MG patients [6]. Using the sophisticated tools of molecular genetics, genome-wide association studies are unveiling "common" non-HLA autoimmunity genes, giving current research to define molecular mechanisms of disease manifestation a clear direction [7]. Obviously, it is a promising aim to identify new susceptibility genes and risk-associated single nucleotide polymorphisms (SNPs). Based on the increasing body of evidence for a role of glycan–protein (lectin) interactions in inflammatory pathways [8–10], we here test the hypothesis of a respective potential for a family of adhesion/growth-regulatory proteins, also known to be strongly immunomodulatory, i.e. the galectins [11,12].

These endogenous lectins are known to target distinct glycan and peptide motifs (signals) and hereby trigger biosignaling for growth control/mediator release or modulate adhesion/migration of cells [10–12]. What attracted our attention was that the tandem-repeat-type galectin-8 (for a review on its structure and physiological roles, please see [13]) is an inducer of apoptosis of synovial fluid cells, an activity neutralized by a distinct glycan in the rheumatoid version of CD44 (CD44vRA) [14], and that auto-antibodies against this lectin are present in the sera of patients with systemic lupus erythematosus (23%), rheumatoid arthritis (16%) and septicaemia (20%) [15]. By virtue of its remarkable affinity to α2,3-sialylated N-glycans, distinct sulfated N-acetyllactosamine forms and histo-blood group B epitope, assigned to either the N- or C-domain [16–22], this galectin could have a special function in autoimmune regulation. Such a role is intimated by its T cell-stimulatory activity, assumed to enhance otherwise borderline immune responses [23], acting in a concentration-dependent manner on proliferation and as co-stimulator of antigen-specific responses [23,24]. In order to reveal a hypothetically postulated relationship, we focused on an SNP in the coding region of the *LGALS8* gene of patients with RA and MG, in relation to healthy controls. The examined non-synonymous mutation affects amino acid 19 in exon 1, causing a substitution of phenylalanine (F) to tyrosine (Y). Since the case study of human galectin-1 mutant with serine replacing cystein at position 2 has taught the lesson that a seemingly minor change can have structurally long-range effects with an impact on entropic contributions in the thermodynamic balance sheet of ligand binding in the β-sandwich fold [25], the quest to define clinical associations, if successful, will give ensuing structural work a clear direction.

2. Patients and methods

2.1. Patients and controls

We recruited 149 patients with MG (122 women, 27 men, mean age: 50 ± 13.2 years (range:18–78 years), mean age at disease onset: 36.2 ± 13.4 years) from the NEPSYBANK (Hungarian Neurological and Psychiatric Biobank) [26]; 214 RA patients (180 women, 34 men, mean age: 56.7 ± 9.4 years (range: 26–85 years), mean age at disease onset: 53 ± 9 years) treated at the Department of Rheumatology, County Hospital, Kecskemét, Hungary, and 365 ethnically matched Caucasian controls (263 women, 102 men, mean age: 37.5 ± 13.7 years (range: 18–83 years)), without known autoimmune disease. These were blood donors or enrolled during routine check-ups. To add strength to the results, a separate, independent repetitive RA cohort was additionally analyzed in this study, which was composed of 134 patients (26 men, 108 women, mean age: 64.2 ± 11.1 years (range: 29–84)) treated at the Department of Rheumatology, Semmelweis University. Diagnosis in the case of MG was based on standard criteria including symptoms of fluctuating muscle weakness supported by an electromyographic pattern of neuromuscular transmission dysfunction by repetitive stimulation. All RA patients met the American College of Rheumatology/European League Against Rheumatism criteria for rheumatoid arthritis set in 2010 [27].

Determination of age at onset was based on the patient's recollection and/or previous clinical documentation. All patients and controls gave explicit written informed consent. This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, and it was approved by the local ethical committees.

2.2. Prediction of functional SNPs in the human galectin-8 gene

We searched for known *galectin-8* SNPs in exons using dbSNP from the www.ncbi.nlm.nih.gov and HapMap (www.Hapmap.org) websites. By now, 4 missense SNPs have been identified in the *GALS8* gene. Out of these, only rs1041935, rs1041937 and the examined rs2737713 encode changes in putatively functionally relevant sites (localized in the carbohydrate-recognition domains), while the rs2243525 will affect the linker of the long isoform which is the minor species due to prevalent representation of the short linker caused by alternative splicing removing the respective sequence stretch. The rs2737713 A > T SNP of the *LGALS8* gene was selected as it was the only sequence deviation according to the annotation of dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/), which may affect both ligand binding (via a long-range effect first defined for human galectins in the case of the C2S mutant of galectin-1 [25]) and inter-domain interactions. In the Caucasian population, "A" is the major and "T" is the minor allele. Polymorphism of the contingent T allele in the gene causes a missense mutation from F to Y at amino acid 19 (F19Y substitution) in the N-terminal domain of the galectin-8 protein. We tested whether this SNP could contribute to autoimmune disease susceptibility by investigating its distribution in Caucasian MG and RA patients.

2.3. Determination of anti-acetyl-choline receptor (anti-AChR) antibody concentration

The level of AChR-specific antibody was measured by radioimmunoprecipitation (DRG Diagnostics, Marburg, Germany) according to the manufacturer's instructions. Following incubation of the patients' sera with ^{125}I-labeled α-bungarotoxin, complexes of labeled receptor and antibody were immunoprecipitated with anti-human IgG and radioactivity quantitated in a γ-counter. The measured radioactivity was directly proportional to the concentration of anti-AChR autoantibodies of the sample.

2.4. Determination of anti-cyclic citrullinated peptide (anti-CCP) antibodies

Level of serum antibodies reactive to CCP was measured with a commercial ELISA (Eurodiagnostica AB, Malmö, Sweden) according to the manufacturer's instructions.

2.5. DNA isolation and polymorphism analysis

Genomic DNA was extracted from peripheral blood cells using the QIAamp DNA blood kit (Qiagen, Valencia, CA, USA). Genotypes were determined using pre-developed TaqMan Allelic Discrimination Assay (Assay ID: C_1653103_10, the context sequence used was (VIC/FAM) AATTTTTTTTCTTAGGTAATCCCGT[A/T]TGTTGGCACCATTCCTGATCAGCTG; Applied Biosystems, Foster City, CA, USA). Briefly, polymerase chain reactions (PCR) were carried out in a 96-well format with the GeneAmp PCR System 7000 (Applied Biosystems) with aliquots of mixtures consisting of 10 ng of genomic DNA, 5 μl of TaqMan Universal PCR Master Mix, 0.25 μl of 40× assay mix, and double-distilled water up to 10 μl final volume. Thermal cycle conditions were as follows: denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 92 °C for 15 s, and annealing and extension at 60 °C for 1 min. After