Journal of Autoimmunity 82 (2017) 62–73

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Methylome and transcriptome profiling in Myasthenia Gravis monozygotic twins

Shimrat Mamrut, PhD [a], Nili Avidan, PhD [a], Frédérique Truffault, MS [b], Elsebeth Staun-Ram, PhD [a], Tarek Sharshar, MD [c], Bruno Eymard, MD [d], Mélinée Frenkian, PhD [b], Jiri Pitha, MD [e], Marc de Baets, MD [f], Laurent Servais, MD [g], Sonia Berrih-Aknin, PhD [b, 1], Ariel Miller, MD/PhD [a, h, *, 1]

[a] Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, 31096, Israel
[b] INSERM - U974/CNRS UMR7215//UPMC UM76/AIM, Institute of Myology Pitie-Salpetriere, Paris, 73013, France
[c] General Intensive Care Medicine, Assistance Publique Hôpitaux de Paris, Raymond Poincaré Hospital, University of Versailles Saint-Quentin en Yvelines, 92380, Garches, France
[d] Department of Neuromuscular Disorders, CHU Salpêtrière, Paris, 75013, France
[e] Department of Neurology and Clinical Neuroscience Center, 1st Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic
[f] Neuroimmunology Group, Division of Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands
[g] Institute of Myology, Groupe hospitalier Pitié-Salpêtrière, AP-HP, Sorbonne Universités, UPMC Universités Paris 06, INSERM, Paris, 75013, France
[h] Division of Neuroimmunology, Lady Davis Carmel Medical Center, Haifa, 34362, Israel

A R T I C L E I N F O

Article history:
Received 21 February 2017
Received in revised form
11 May 2017
Accepted 15 May 2017
Available online 24 May 2017

Keywords:
Autoimmunity
Biomarkers
Disease chronicity
Epigenetics
Monocyte function
Predisposition
Resolution of inflammation

A B S T R A C T

Objective: To identify novel genetic and epigenetic factors associated with Myasthenia gravis (MG) using an identical twins experimental study design.
Methods: The transcriptome and methylome of peripheral monocytes were compared between monozygotic (MZ) twins discordant and concordant for MG, as well as with MG singletons and healthy controls, all females. Sets of differentially expressed genes and differentially methylated CpGs were validated using RT-PCR for expression and target bisulfite sequencing for methylation on additional samples.
Results: >100 differentially expressed genes and ~1800 differentially methylated CpGs were detected in peripheral monocytes between MG patients and controls. Several transcripts associated with immune homeostasis and inflammation resolution were reduced in MG patients. Only a relatively few genes differed between the discordant healthy and MG co-twins, and both their expression and methylation profiles demonstrated very high similarity.
Interpretation: This is the first study to characterize the DNA methylation profile in MG, and the expression profile of immune cells in MZ twins with MG. Results suggest that numerous small changes in gene expression or methylation might together contribute to disease. Impaired monocyte function in MG and decreased expression of genes associated with inflammation resolution could contribute to the chronicity of the disease. Findings may serve as potential new predictive biomarkers for disease and disease activity, as well as potential future targets for therapy development. The high similarity between the healthy and the MG discordant twins, suggests that a molecular signature might precede a clinical phenotype, and that genetic predisposition may have a stronger contribution to disease than previously assumed.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Myasthenia gravis (MG) is a relatively rare autoimmune neuromuscular disorder, clinically characterized by weakness and fatigability of skeletal and extraocular muscles [1]. MG is a B-cell driven, T-cell dependent, complement- and antibody-mediated

* Corresponding author. Division of Neuroimmunology, Department of Neurology, Lady Davis Carmel Medical Center, Michal St. 7, Haifa, 34362, Israel.
E-mail addresses: milleras@netvision.net.il, ariel_miller@clalit.org.il (A. Miller).
[1] Equally contributing authors.

http://dx.doi.org/10.1016/j.jaut.2017.05.005
0896-8411/© 2017 Elsevier Ltd. All rights reserved.

disease, resulting from autoantibodies directed against molecules at the post-synaptic membrane of the neuromuscular junction, including antibodies against autoantigens at the acetylcholine receptor (AChR) (85% of patients), the muscle-specific kinase (MuSK) or the lipoprotein-related protein 4 (LRP4) [2]. The binding of anti-AChR antibodies to their target impairs neuromuscular transmission by blocking the receptor, inducing its internalization and by complement-mediated destruction of the postsynaptic membrane [2]. MG predisposition is assumed to be influenced by both genetic and environmental factors [2,3]. Accumulating evidence supports the contribution of epigenetic modifications, DNA methylation in particular, in the development of autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Sjögren's Syndrome and Multiple Sclerosis (MS) [4–6].

Monozygotic twin studies indicate a concordance rate in MG of 30–40%, similar to MS, Inflammatory Bowel Disease, and SLE, compared to 4–5% in dizygotic (DZ) twins [7]. The high discordant rate of MZ MG twins suggests that despite identical DNA and shared in-uterus and childhood environment, unknown factors contribute to disease development [7]. Thus, the study of discordant and concordant MZ twins provides an attractive model to investigate gene-environment interactions, including epigenetic contributions to disease pathogenesis. However, due to the rarity of autoimmune diseases such as MG, related publications have contributed only limited epidemiological data and brief clinical case reports.

The underlying pathogenic mechanisms of MG have mainly been attributed to anti-AChR autoreactive T cells, pathogenic autoantibody-producing B cells as well as impaired regulatory T-cell (Treg) function [8–10]. The function of immune cells is further modified by the inflammatory milieu, which is part of the autoimmune cascade, and associated cytokines from both the adaptive as well as innate immune system. Monocytes/macrophages (Mo/Mφ) of the innate system play an important role in regulating inflammation and induce immunity mainly through their hallmark function of phagocytosis and subsequent antigen presentation [11], and are important sources for cytokines and chemokines, which attract T and B cells and regulate the inflammatory response [12,13]. Dysregulation of Mo/Mφ has been associated with several autoimmune diseases, but has scarcely been studied in MG. Reduced phagocytosis in monocytes in RA [14], defective phagocytosis and aberrant activation of the Mo/Mφ system in SLE [12], and unbalanced pro-inflammatory macrophage (M1) and anti-inflammatory (M2) phenotype in diabetes [15] has been described. Thus, monocytes might have an important role in MG pathophysiology, yet to be fully elucidated.

The aim of this study was to analyze the transcriptome and methylome of five immune cell subsets in MZ twins discordant for MG compared with concordant MZ twins, MG singletons and healthy controls. We hypothesized that by using the unique study design of MZ twins and purified immune cells, novel disease-associated genes would be detectable, including genes associated with predisposition and other genes associated with disease course. Due to data complexity, this article is focused on the analysis of data from monocytes.

2. Materials and methods

2.1. Subjects

7 female twin pairs diagnosed with MG, 4 discordant and 3 concordant, age 20–66 years (y) were recruited at the Institute of Myology, France. All twins were tested for auto-antibodies against AChR, and if negative, for antibodies against MuSK. Furthermore, 5 singleton MG patients, age 24-59y, 9 healthy females, age 23-52y and 6 healthy males, age 32-50y were recruited, and our previous obtained datasets from 5 healthy females, age 30-50y included [16]. The study was conducted following the approval of the institutional Ethical Committee (Ref afssaps B100384-30), and all participants signed a written informed consent.

2.2. Cell separation, DNA/RNA extraction and BeadChip hybridization

Blood was collected from each female participant, 5 cell-subsets (CD4 and CD8 T cells, CD25^{+} Tregs, B cells, CD14 monocytes) were purified, RNA and DNA extracted, and RNA quality control performed as previously described [16]. This multi-step protocol was done in parallel for each twin pair to reduce technical noise. Subsequently, RNA and DNA samples were hybridized to Illumina HumanHT-12 v4 (BD-103-0204 Illumina) or to Human-Methylation450 BeadChip (WG-314-1003 Illumina) respectively, according to manufacturer's protocol. Data generation was performed by the Genomics Core Facility at the Rappaport Faculty of Medicine, Technion, Israel. The data was submitted to Gene Expression Omnibus (GEO): GSE85649.

2.3. Statistics and pathway analysis

The raw gene expression data from monocytes was exported from GenomeStudio and imported into JMP Genomics V7.1 software (SAS Institute Inc, Cary, NC). Quality control and analysis in JMP Genomics was done on log2 transformed data, after filtering for non-expressed genes (detection p-value<0.01), and for low variance transcripts across samples (variance <5%). Initially, 3 discordant twin datasets were compared, however high similarity between the healthy discordant twins and the MG twins was found. Thus, in order to increase sample size, additional expression and methylation profiles were generated from 3 MG singleton patients and 3 healthy controls, and combined with the discordant and 3 concordant MZ MG twin datasets, as well as with previously collected monocyte datasets from 5 healthy controls [16], all females. In order to reduce batch effect between sets, two samples from the twin dataset and from the GSE71245 dataset were replicated, and data normalized accordingly. For gene expression the data was analyzed using one-way ANOVA, and MG- associated differentially expressed genes (DEGs) were defined as transcripts with more than 1.2-fold change of expression at a less than 0.05 False Discovery Rate (FDR) adjusted P-value [17]. For the methylation datasets, the methylation β-values were extracted directly from iDAT files using ChAMP R pipeline, that also performs extensive data normalization and reduction of systemic technical bias [18]. Statistical analysis was performed using both limma program within ChAMP pipeline and JMP-genomic software using ANOVA. Given the relatively small sample size and number of probes under consideration, we adopted a pragmatic approach and defined differentially methylated CpGs (DMGs) at $\geq$ 5% difference in methylation β-value at uncorrected p-values of <0.01. The DMG list was evaluated against lists of defined regulatory elements, such as an enhancer annotation list from Broad Institute, ENSEMBL Regulatory Track, FANTOM and VISTA databases to define possible functional relevance [19]. Differentially methylated regions (DMRs) were defined using Probe lasso embedded with ChAMP R pipeline [20] and the relation between the DEGs list and DMRs list was evaluated using Genomic Regions Enrichment of Annotations Tool (GREAT) [21] and illumina annotation file. In addition, pathway analysis of DEGs was performed using Ingenuity Pathway Analysis tool (IPA 8.0, Ingenuity_ Systems, and Redwood City, CA) and Advaita Bio's iPathwayGuide (http://www.advaitabio.com/ipathwayguide). We considered upstream regulatory or biological

Download English Version:

https://daneshyari.com/en/article/5667872

Download Persian Version:

https://daneshyari.com/article/5667872

Daneshyari.com