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ScienceDirect

Neuromuscular Disorders 27 (2017) 816–825

www.elsevier.com/locate/nmd

Exome sequencing identifies targets in the treatment-resistant ophthalmoplegic subphenotype of myasthenia gravis

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Received 24 March 2017; received in revised form 13 June 2017; accepted 14 June 2017

Abstract

Treatment-resistant ophthalmoplegia (OP-MG) is not uncommon in individuals with African genetic ancestry and myasthenia gravis (MG). To identify OP-MG susceptibility genes, extended whole exome sequencing was performed using extreme phenotype sampling (11 OP-MG vs 4 control-MG) all with acetylcholine receptor-antibody positive MG. This approach identified 356 variants that were twice as frequent in OP-MG compared to control-MG individuals. After performing probability test estimates and filtering variants according to those 'suggestive' of association with OP-MG ($p < 0.05$), only three variants remained which were expressed in extraocular muscles. Validation in 25 OP-MG and 50 control-MG cases supported the association of $DDX17_{delG}$ ($p = 0.014$) and $SPTLC3_{insACAC}$ ($p = 0.055$) with OP-MG, but $ST8SIA1_{delCCC}$ could not be verified by Sanger sequencing. A parallel approach, using a semantic model informed by current knowledge of MG-pathways, identified an African-specific interleukin-6 receptor (IL6R) variant, *IL6R c.*3043 T>C*, that was more frequent in OP-MG compared to control-MG cases ($p = 0.069$) and population controls ($p = 0.043$). A weighted genetic risk score, derived from the odds ratios of association of these variants with OP-MG, correlated with the OP-MG phenotype as opposed to control MG. This unbiased approach implicates several potentially functional gene variants in the gangliosphingolipid and myogenesis pathways in the development of the OP-MG subphenotype.

Keywords: African; Ophthalmoplegia; Ganglioside; Extreme phenotype; Myasthenia gravis

1. Introduction

In myasthenia gravis (MG) neuromuscular endplate damage occurs most frequently as a consequence of targeted acetylcholine receptor (AChR) antibodies activating complement. Extraocular muscles commonly manifest early, but respond to therapy. While the incidence of MG in sub-Saharan Africa is similar to elsewhere [1], we have identified an ophthalmoplegic subphenotype developing as a consequence of generalised MG that appears almost exclusively in individuals of African genetic ancestry [2]. Individuals with otherwise clinically characteristic generalised MG, most frequently with detectable AChR-antibodies in their sera and juvenile symptom onset, develop severe ophthalmoplegia (OP-MG) that remains treatment-resistant to standard therapy [3,4]. This phenotype can vary from complete ophthalmoplegia and bilateral ptosis to varying degrees of severe partial paralysis of most extraocular muscles (EOMs). Typically in these cases, apart from the EOMs, the remaining muscles affected by MG respond to therapy as expected. OP-MG results in significant morbidity in myasthenic patients with African-genetic ancestry. Based on experimental work by others we speculated that the phenotype results from either excessive endplate damage or altered muscle remodelling in response to the antibody- and complement-mediated injury [5].

Previously, using a candidate gene approach we identified functional African-specific promoter variants in two genes that are associated with OP-MG, decay accelerating factor *(DAF or CD55)* and transforming growth factor beta-1 (*TGFB1*) [5,6]. We found that the *DAF* promoter variant resulted in lower DAF expression levels during LPS-induced immune activation and the TGFB1 promoter variant also lowered TGFB1 expression levels. Since DAF protects cells against complement activation and TGFβ1 has been shown to upregulate DAF in extraocular muscles, these expression traits may result in excessive complement-mediated damage during MG [7]. However, not all individuals with OP-MG harboured the functional variants in *DAF* and *TGFB1*. We therefore hypothesised that there may be other pathways, in addition to excessive complement activation, that are activated by autoimmune MG to result in the OP-MG phenotype.

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http://dx.doi.org/10.1016/j.nmd.2017.06.009
0960-8966/

Here we present the results of an extended whole exome sequencing (WES) study to discover potentially functional gene variants that associate with OP-MG. We used an extreme phenotype sampling approach [8,9] to select a discovery cohort of clinically well-characterised individuals, all with African-genetic ancestry and juvenile-onset AChR-antibody positive MG, representing the group previously defined as being most at risk and differing only in their EOM treatment-resistance (OP-MG vs control MG). This approach increases the power to detect OP-MG associated variants with low frequency and small effect sizes [9,10] and minimises the confounding effect of detecting MG susceptibility gene variants which are likely a background feature of both phenotypic groups.

2. Patients and methods

2.1. Inclusion criteria and definition

The discovery cohort comprised 15 individuals all with self-categorised African-genetic ancestry and generalised AChR-antibody positive MG attending the myasthenia gravis clinic at Groote Schuur Hospital, a tertiary teaching hospital attached to the University of Cape Town, South Africa. These individuals were selected to represent the most extreme phenotype of the treatment-resistant ophthalmoplegic subphenotype and/or with the longest observational follow-up. Table 1 summarises clinical characteristics of the 11 OP-MG and four control MG individuals, all with symptom onset <20 years and the classical features of generalised fatigable weakness, pyridostigmine responsiveness as well as improvement on immune therapies in non-ocular muscles [2,3]. Apart from one subject (followed up for 2 years), all subjects have been followed up for ≥7 years (range 2–43 years).

The OP-MG phenotype included patients in whom all 12 EOMs (±ptosis) have 75–100% weakness/pareses (complete OP-MG), or severe partial OP-MG where >50% of the EOMs show moderately-severe weakness (50–100% weakness). Eight of 11 OP-MG individuals selected for the discovery cohort had complete OP-MG (Fig. 1A). The control MG subjects were selected on the following criteria: symptom onset before the age of 20; AChR-antibody positivity; African-genetic ancestry (self-categorisation); prolonged follow-up. The control MG group comprised patients followed up for many years who may have had initial EOM myasthenic weakness, but subsequently responded to standard therapies (one had intermittent fatigable diplopia). All patients were treated with pyridostigmine and immunosuppressive therapies as previously described [2,4]. The validation cohort consisted of 60 individuals, 14 with OP-MG and 46 as control MG (Table 2). Data from normal controls were kindly provided by the South African Human Genome Programme (SAHGP) [12].

The study was approved by the University of Cape Town Health Sciences Faculty Research Ethics committee and all individuals (or their parents if <18 years) signed informed consent to participate.

2.2. Next generation sequencing and bioinformatics

Extended whole exome sequencing (WES) (Agilent SureSelectXT Human All Exon V5 + UTR 71 Mb capture kit) of the coding and untranslated regions (UTRs) was performed on genomic DNA at 50× coverage on the Illumina HiSeq 2000/2500 platform with a 100 bp paired end read length at CLIA accredited Otogenetics Corporation (Norcross, GA, USA). Bioinformatics analysis included alignment of reads to the hg19 human reference genome using the NOVOALIGN short-read aligner (www.novocraft.com), removing the PCR duplicate using Picard (http://sourceforge.net/projects/picard/) and using the Genome Analysis Toolkit [13] to further refine alignments and accurately call variants. ANNOVAR [14] was used to annotate each variant

Table 1
Clinical characteristics of the OP-MG and control MG cases in the discovery cohort used for whole exome sequencing.

Phenotype	Gender	Race	Age at MG onset (y)	MGFA grade*	MG therapies used	Time before therapies (y)	Duration of follow up (y)
OP-MG	F	B	9	4b	Pred, Aza, CsA, PLEX	1	16
OP-MG	M	B	11	5	Pred, Aza, CsA, PLEX	0.1	14
OP-MG	F	M/A	4	3a	Pred, methotrexate, CsA	0.3	43
OP-MG	M	B	3	2a	Pred, methotrexate	1	10
OP-MG	F	B	17	4b	Pred, Aza	5	9
OP-MG	F	M/A	12	5	Pred, Aza, methotrexate	0.3	13
OP-MG	F	M/A	9	2a	Pred, Aza	1	2
OP-MG	M	M/A	10	5	Pred, Aza, PLEX	17	34
OP-MG	M	B	16	5	Pred, Aza, CsA, methotrexate, PLEX, IVIg	0.1	19
OP-MG	F	M/A	2	3a	Pred, methotrexate	2	7
OP-MG	F	M/A	2	3a	Pred, Aza	1	14
Control MG	F	M/A	14	4a	Pred, Aza	0.5	14
Control MG	F	M/A	17	5	Pred, Aza	1.5	9
Control MG	F	B	15	4b	Pred, Aza	12	43
Control MG	M	B	14	4a	Pred, Aza	0.5	26

OP-MG refers to patients with treatment-resistant complete ophthalmoplegia. All subjects had detectable acetylcholine receptor antibodies in their sera. Race was self-categorised according to the South African population census categories; B refers to indigenous black African genetic ancestry and M/A refers to mixed African genetic ancestry.

* MGFA refers to the Myasthenia Gravis Foundation of America maximum clinical severity grade [11]. y – years. Time before therapies refers to time between onset of symptoms and initiation of immunosuppressive therapies. Pred refers to prednisone; Aza refers to azathioprine; CsA refers to cyclosporine; PLEX refers to plasma exchange; and IVIg refers to intravenous immune globulin. All patients were treated with oral pyridostigmine.