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Collagen Q – A potential target for autoantibodies in myasthenia gravis

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ABSTRACT

Myasthenia gravis (MG) is an autoimmune disorder caused by autoantibodies targeting proteins expressed at the neuromuscular junction (NMJ). In most cases the targets are acetylcholine receptor (AChR), muscle-specific tyrosine kinase (MuSK), or occasionally low-density lipoprotein receptor-related protein 4 (LRP4), but there is still a group of patients, often called seronegative MG (SNMG), with unknown antibody targets. One potential target is collagen Q (COLQ), which is restricted to the NMJ and is crucial for anchoring the NMJ-specific form of acetylcholinesterase (AChE). 415 serum samples with a clinical diagnosis of MG and 43 control samples were screened for the presence of COLQ autoantibodies using a cell-based assay (CBA) with HEK293 cells overexpressing COLQ at the cell surface. COLQ antibodies were detected in 12/415 MG sera and in one/43 control samples. Five of the COLQ-Ab + individuals were also positive for AChR-Abs and 2 for MuSK-Abs. Although the COLQ antibodies were only present at low frequency, and did not differ significantly from the small control cohort, further studies could address whether they modify the clinical presentation or the benefits of anti-cholinesterase therapy.

1. Introduction

Myasthenia gravis (MG) is a classic autoimmune disorder resulting from the presence of autoantibodies targeting neuromuscular junction (NMJ) proteins, and leading to defects in neuromuscular transmission with fatiguable muscle weakness. In most cases the antibodies recognise acetylcholine receptor (AChR) or muscle-specific tyrosine kinase (MuSK) [1], but there are still individuals, often referred to as seronegative MG (SNMG), in whom AChR and MuSK autoantibodies are not detected in present assays. Additional antibody targets include low-density lipoprotein receptor-related protein 4 (LRP4) [2–4], agrin (AGRN) [5] and acetylcholinesterase (AChE) [6]. Despite some evidence of antibodies to these proteins [2–6], there remain patients presenting with an autoimmune MG with no specific antibody identified.

Collagen Q (COLQ) is a protein crucial for anchoring and concentrating AChE at the NMJ, where its expression is restricted to the extracellular matrix and accessible to circulating antibodies. Indeed, mutations in *COLQ* can underlie one form of congenital myasthenic syndrome (CMS) [7,8]. Although COLQ possesses a number of features which make it a potential target for autoantibodies in MG, the presence of these have not been reported.

Traditionally autoantibodies have been detected by radioimmunoprecipitation assays (RIA) or in some cases by enzyme-linked immunosorbent assays (ELISA) or fluorescence immunoprecipitation assays (FIPA). These assays can be sensitive and highly specific, but do not necessarily detect the most pathogenic antibodies. Recently cell-based assays (CBAs) have been established in order to look for antibodies that bind to the extracellular domains of proteins that are naturally expressed on the cell surface. To apply this technique to proteins that are not membrane tethered, it is necessary to fuse them with a transmembrane protein or domain. Here we expressed COLQ fused with the transmembrane domain of contactin-associated protein-like 2 (CASPR2) and looked for antibodies in MG patients and controls.

2. Materials and methods

2.1. Ethics statement

The MG samples were archived from therapeutic plasmaphereses in the 1980s and 1990s when written consent was not required, but verbal was obtained. Ethical approval for use of pre 2006 stored patient samples without patient written consent was obtained from the Oxfordshire REC C 09/H0606/74. Samples from healthy individuals were obtained with written consent and ethical approval from the Oxfordshire REC Rf 07/Q1604/28.

2.2. Cloning of pcDNA-COLQ-CASPR2TM

Construct encoding *COLQ* in pcDNA™3.1/Hygro(+) (Invitrogen, V87020) was kindly provided by Dr Janet Kenyon. COLQ cDNA was engineered into pcDNA-*Lgi1-CASPR2TM* to replace the leucine-rich glioma inactivated 1 (*Lgi1*) cDNA. The C-terminus of COLQ was chosen for the fusion with the transmembrane domain (TM) of contactin-associated protein-like 2 (CASPR2TM). A construct with a Myc tag,

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introduced immediately downstream the N-terminal signal peptide, was also cloned to control for the cell surface expression of the protein.

2.3. HEK293 cell culture and transfection

Human embryonic kidney cells (HEK293) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma, D6429) supplemented with 10% foetal calf serum (Sigma, F2442) and antibiotics (Invitrogen, 15240-062). Transfections were performed using polyethylenamine (PEI). Transfection mixes used for one well of a 6-well plate contained 3 μg DNA, 1.25 μl 20% glucose, 1.5 μl PEI, nuclease-free water up to 6.5 μl and 1 ml growth medium.

2.4. A cell-based assay for the detection of COLQ antibodies

Cells plated on poly-L-leucine-coated coverslips were transfected with pcDNA-*COLQ-CASPR2TM*, pcDNA-*Myc-COLQ-CASPR2TM* or pcDNA™3.1(+) vectors, respectively. 2 days after the transfection, the cells were incubated for 1 hour with sera diluted 1:20 in blocking solution (DMEM, 1% BSA, 20 mM HEPES) or a mouse monoclonal anti-Myc antibody (Cell Signalling, 2276), fixed with 3% paraformaldehyde and then incubated for one hour with Alexa Fluor 568 secondary antibodies (1:500) against human or mouse IgG, respectively. The immunostaining of the cells was analysed by widefield or confocal fluorescence microscopy using an Olympus X71 Fluorescence Microscope and SimplePCI software, or a Zeiss 780 Inverted Microscope and ZEN lite software, respectively.

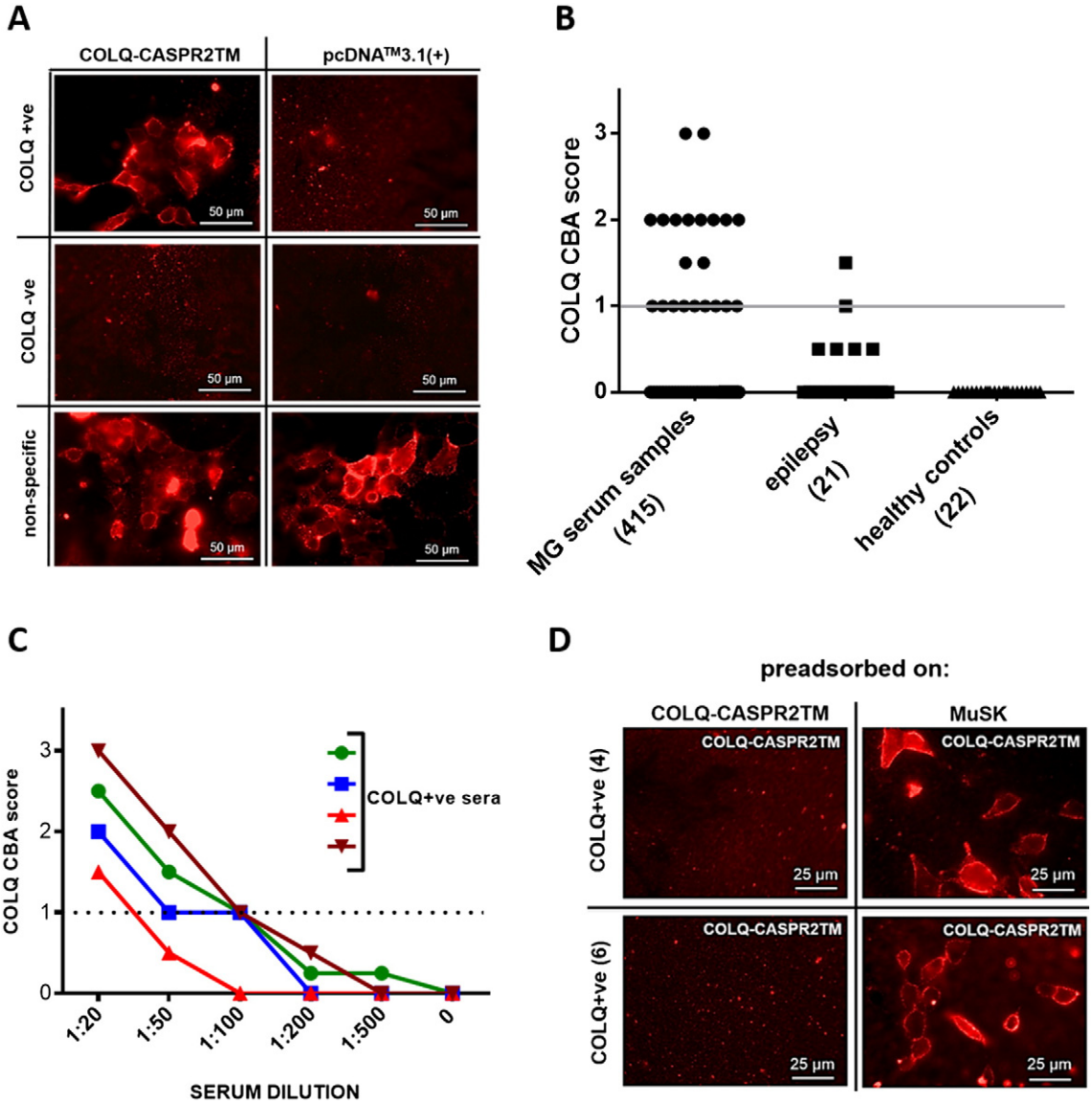

Fig. 1. CBA for the presence of anti-COLQ autoantibodies in patient sera. A. HEK293 cells were transfected with pcDNA-*COLQ-CASPR2TM* or pcDNA™3.1(+) vectors, respectively, and stained with patient sera, and an Alexa Fluor 568 anti-human IgG secondary antibody. The panel shows example staining of COLQ + ve, COLQ-ve serum samples or the ones exhibiting unspecific cell surface binding. B. COLQ CBA scores in the cohort of serum samples. The dot plot presents the binding scores, reflecting the intensity of the fluorescence obtained with the patient and control sera. The numbers in brackets indicate a number of individuals in a particular subgroup. The grey line indicates the cut-off of the CBA (score = 1), based on mean ± 3SDs of all controls. C. Titration of COLQ + ve sera. HEK293 cells were transfected with the pcDNA-*COLQ-CASPR2TM* plasmid, stained with anti-COLQ sera diluted 1:20, 1:50, 1:100, 1:200, and 1:500, and binding detected with Alexa Fluor 568 anti-human IgG secondary antibody. The intensity of the fluorescence was scored (0–3). Different symbols represent serum samples from different individuals. The dotted line indicates the cut-off of the CBA (score = 1), based on mean ± 3SDs of all controls. D. Preadsorption of anti-COLQ antibodies from COLQ + ve sera. HEK293 cells were transfected with COLQ-CASPR2TM-encoding plasmid and incubated with two different COLQ + ve serum samples (from patients #4 and #6), preadsorbed against either COLQ-CASPR2TM or MuSK expressing cells, respectively. Binding of the patients' antibodies to the COLQ-CASPR2TM expressing cells was not found after adsorption against COLQ-CASPR2TM-expressing cells, but adsorption against MuSK-expressing cells did not reduce binding of the patients' antibodies.