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# A rapid PCR method for genotyping the Large$^{myd}$ mouse, a model of glycosylation-deficient congenital muscular dystrophy

Claudia A. Browning[a], Prabhjit K. Grewal[a,b], Christopher J. Moore[a], Jane E. Hewitt[a,*]

[a]*Institute of Genetics, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK*
[b]*Howard Hughes Medical Institute, 9500 Gilman Drive-0625, University of California, San Diego, La Jolla, CA 92093, USA*

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**Abstract**

The myodystrophy (Large$^{myd}$) mouse has a spontaneous loss of function mutation in a putative glycosyltransferase gene (*Large*). Mutations in the human gene (*LARGE*) have been described in congenital muscular dystrophy type 1D (MDC1D). Mutations in four other genes that encode known or putative glycosylation enzymes (*POMT1*, *POMGnT1*, *fukutin* and *FKRP*) are also associated with muscular dystrophy. In all these diseases hypoglycosylation of α-dystroglycan, and consequent loss of ligand binding, is a common pathomechanism. Currently, the Large$^{myd}$ mouse is the principal animal model for studying the underlying molecular mechanisms of this group of disorders. Over-expression of LARGE in cells from patients with mutations in *POMT1* or *POMGnT1* results in hyperglycosylation of α-dystroglycan and restoration of laminin binding. Thus, *LARGE* is a potential therapeutic target. Here, we define the intronic deletion breakpoints of the Large$^{myd}$ mutation and describe a simple, PCR-based diagnostic assay, facilitating the study of this important animal model.


*Keywords:* Muscular dystrophy; Glycosylation; Dystroglycan; Genomic rearrangement; PCR; Genotyping

## 1. Introduction

We previously showed that the myodystrophy mouse (*myd*, now designated Large$^{myd}$) has a loss of function mutation in a putative glycosyltransferase gene (*Large*) [1]. α-Dystroglycan (α-DG), a central component of the dystrophin-associated glycoprotein complex (DGC) is hypoglycosylated in muscle and brain tissues of homozygous mutant mice [1,2]. This hypoglycosylated form of α-DG fails to bind the extracellular matrix ligands laminin, agrin and neurexin [2,3].

LARGE is one of five genes that encode known or putative glycosylation enzymes and are implicated in various forms of muscular dystrophy [4]. Mutations in the human gene (*LARGE*) have been identified in a patient with congenital muscular dystrophy type 1D (MDC1D) [5]. Walker–Warburg Syndrome (WWS) and muscle–eye–brain disease (MEB) are caused by mutations in two genes involved in *O*-mannosylation, *POMT1* and *POMGnT1*, respectively [6,7]. Fukuyama type congenital muscular dystrophy (FCMD) is due to mutations in *fukutin*, a putative phospholigand transferase [8,9]. Congenital muscular dystrophy type 1C and limb girdle muscular dystrophy type 2I are allelic, both being due to mutations in the gene encoding fukutin related protein (*FKRP*) [10,11]. This group of muscular dystrophies is also associated with aberrant glycosylation of α-DG [4]. Over-expression of LARGE can hyperglycosylate α-DG and rescue its laminin-binding activity in cells from WWS and MEB patients (which do not have mutations in LARGE but in other glycosyltransferase genes), identifying it as a potential therapeutic target [12].

The spontaneous myodystrophy mutation was identified in 1963 at the Jackson Laboratory, USA. Affected mice have abnormal shuffling gaits, a diffuse and progressive myopathy, and a shortened lifespan [13]. Homozygous mutant mice can be recognised at 12–15 days by their small size and abnormal posturing of their hind limbs. At 6–8

* Corresponding author. Tel.: +44 115 849 3229; fax: +44 115 970 9906.
*E-mail address:* jane.hewitt@nottingham.ac.uk (J.E. Hewitt).

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weeks of age, a thoracic kyphosis is evident which becomes progressively worse [13,14].

Muscle histology demonstrates a myopathy with focal areas of acute necrosis and clusters of degenerating and regenerating fibres in affected homozygotes, while heterozygotes show no pathological changes in muscle. Foci of degeneration are characterised by size variation, loss of striation and central migration of nuclei. The dorsal and ventral spinal roots of Large$^{myd}$ mice contain groups of axons completely devoid of ensheathing Schwann cells or myelin [15]. Examination of the sarcolemma from Large$^{myd}$ muscles shows reduced levels of several membrane cytoskeletal proteins, including dystrophin and β-spectrin, at the costameres and loss of the dystrophin complex between costameres [16].

Following the identification of the mutation, additional aspects of the Large$^{myd}$ phenotype were shown to include cardiac defects and neuronal migration abnormalities in the central nervous system, especially in the cortex and cerebellum [2,3]. These neuronal migration defects are similar, though less severe, than those typically seen in WWS, FCMD and MEB. Although morphologically the eyes and retina of mutant mice appear normal, electroretinograms showed a normal a-wave but increased implicit times and decreased amplitudes of the b-wave, suggesting altered signal processing in the downstream retinal circuitry [3]. Thus, the Large$^{myd}$ mouse is an important animal model of this group of disorders and is the only one currently available.

The human *LARGE* gene was so named because it spans over 660 kb of genomic DNA, although the mRNA is only about 4.4 kb [17]; the mouse gene is a similar size. Thus the exons account for only about 0.6% of the gene sequence, a similar composition to dystrophin [18]. The Large$^{myd}$ mutation is an intragenic genomic deletion extending over many kilobases [1]. This complicates genotyping of the mice. Since the mutation is recessive and affected homozygotes have reduced lifespan and reproductive fitness [13], identification of heterozygotes is important for breeding the strain. We have used a polymorphic $(GT)_n$ marker located within intron 1 of *Large*, but this is not entirely satisfactory as it requires variation between the mutant and wild type allele, limiting its use when crossing Large$^{myd}$ onto different genetic backgrounds. Furthermore there is a chance of recombination between this marker and the mutation as it is located several hundred kilobytes 5′ of the deletion. Here, we define the Large$^{myd}$ deletion breakpoints and describe a simple, PCR-based diagnostic assay for the mutation, facilitating the study of this important animal model.

## 2. Material and methods

### 2.1. DNA preparation

Large$^{myd}$ mice were obtained from Professor R Bittner, University of Vienna, Austria and a colony maintained at Nottingham free of significant laboratory animal pathogens and according to UK 'Code of Practice' requirements. High molecular weight DNA was prepared from the spleens of two heterozygote Large$^{myd}$/+ mice. Tissue was gently homogenised in PBS using a 1.5 ml pestle (Starlab) and digested with proteinase K at 50 °C overnight followed by phenol extraction and ethanol precipitation. Tail snip DNA was prepared using a DNeasy tissue kit (Qiagen).

### 2.2. Preparation of cosmid library and screening

A cosmid library was prepared in the vector sCos-1 (Stratagene). Briefly, the high molecular weight Large$^{myd}$/+ mouse genomic DNA was partially digested with the restriction enzyme *Sau*3AI. DNA fragments were size-fractionated by ultracentrifugation on sucrose gradients; fractions containing fragments >30 kb were pooled and ethanol precipitated. The genomic DNA fragments were then treated with calf intestinal alkaline phosphatase to prevent co-ligation of non-contiguous fragments. The sCos-1 vector was prepared as described in the manufacturer's protocol. Vector and insert were ligated overnight at 4 °C and packaged using Gigapack II XL packaging extract (Stratagene). A total of 200,000 primary transformants were plated onto selective ampicillin plates and replicated onto PALL Biodyne A filters. Filters were hybridised with a $^{32}$P-labelled DNA probe corresponding to mouse *Large* exon 3 (equivalent to exon 4 of the human *LARGE* gene). Positive clones were purified to single colonies by a further two rounds of screening.

### 2.3. Subcloning and sequencing

Cosmid DNA was digested with *Pst*I and the resulting digest was shotgun subcloned into pBluescript II (Stratagene). Random subclones were then sequenced and mapped back to the BAC sequence. *Eco*RI fragments in cosmid 1A5 thought to contain the deletion breakpoint were gel-purified using a Sigma gel purification kit and subcloned into pBluescript II. DNA was sequenced using BigDye v3, sequences were assembled using Sequencher (Genecodes). The partial genomic sequence of *Large* was downloaded from the ENSEMBL database (www.ensembl.org) and from BAC and PAC sequences deposited in GenBank. Sequences from cosmid clones were compared to the available genomic DNA sequence for *Large* using Sequencher. Large$^{myd}$ PCR products were sequenced directly. Intron sequences were screened for repetitive elements using the RepeatMasker program (www.hgmp.mrc.ac.uk).

### 2.4. PCR genotyping

Individual assays for the mutant and wild-type alleles were first designed independently. Primers GT4F and GT4R recognise a 162 bp product found in the wild-type allele only, since these primers are located within exon 6