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Neuromuscular Disorders 16 (2006) 4–13

www.elsevier.com/locate/nmd

Facing the genetic heterogeneity in neuromuscular disorders: Linkage analysis as an economic diagnostic approach towards the molecular diagnosis

M. von der Hagen [a,1], J. Schallner [a], A.M. Kaindl [c,1], K. Koehler [b], P. Mitzscherling [b], A. Abicht [d,1], U. Grieben [c], R. Korinthenberg [e,1], W. Kress [f,1], A. von Moers [g,1], J.S. Müller [d,1], U. Schara [h,1], M. Vorgerd [i,1], M.C. Walter [d,1], C. Müller-Reible [f,1], C. Hübner [c,1], H. Lochmüller [d,1], A. Huebner [b,1,*]

[a] *Department of Neuropaediatrics, Technical University, 01307 Dresden, Germany*
[b] *Children's Hospital, Technical University, Fetscherstrasse 74, 01307 Dresden, Germany*
[c] *Department of Neuropaediatrics, Charité, University Medical School, 13353 Berlin, Germany*
[d] *Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University, 81377 Munich, Germany*
[e] *Department of Paediatric Neurology, Children's University Hospital, 79106 Freiburg, Germany*
[f] *Institute of Human Genetics, Biocentre, Julius-Maximilians-University, 97074 Würzburg, Germany*
[g] *Children's Hospital, DRK-Kliniken Westend, 14050 Berlin, Germany*
[h] *Centre of Neuropaediatrics, Städtische Kliniken Neuss, University of Düsseldorf, 41462 Neuss, Germany*
[i] *Department of Neurology, Neuromuscular Centre Ruhrgebiet, Ruhr-University, 44789 Bochum, Germany*

Received 17 June 2005; received in revised form 20 September 2005; accepted 5 October 2005

Abstract

The identification of an ever increasing number of gene defects in patients with neuromuscular disorders has disclosed both marked phenotype and genotype variability and considerable disease overlap. In order to offer an economic strategy to characterise the molecular defect in patients with unclassified neuromuscular disorders, we designed DNA marker sets for linkage analysis of 62 distinct neuromuscular disorders gene loci, including all known muscular dystrophies, congenital myopathies, congenital myasthenic syndromes and myotonias. Genotyping of marker loci of 140 clinically well-characterised families with unclassified neuromuscular disorders reduced the number of candidates to one or two genes in 49 % of the families. Subsequent mutation analysis and genome-wide scans enabled the determination of the genetic defect in 31 % of the families including the identification of a new gene and a new mutation in an unexpected candidate gene. This highlights the effective application of this approach both for diagnostic strategies as well as for the identification of new loci and genes.

Keywords: Muscular dystrophy; Congenital myopathy; Congenital myasthenic syndrome; Ion channel disorder; Microsatellite marker; Linkage analysis

1. Introduction

Neuromuscular diseases (NMD) constitute a group of phenotypically and genetically heterogeneous disorders. They are characterised by (progressive) weakness and atrophy of proximal and/or distal muscles. Within the last two decades the application of molecular genetic strategies has led to a delineation of subgroups of clinically indistinguishable neuromuscular disorders and disclosed marked disease overlap. The expanding number of molecular defined NMDs requires new strategies to classify overlapping and clinical indistinguishable phenotypes. In this study we present a diagnostic tool, which reduces the extent of costly histochemical and mutation detection techniques.

The Duchenne (DMD), Becker (BMD), Emery–Dreifuss (EMD) and facioscapulohumeral (FSHD) muscular dystrophies and the six autosomal-dominant (LGMD1A-G) and

* Corresponding author. Tel.: +49 351 4582926; fax: +49 351 4584334.
E-mail address: angela.huebner@mailbox.tu-dresden.de (A. Huebner).
[1] Member of German muscular dystrophy network MD-NET (http://www.md-net.org).

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doi:10.1016/j.nmd.2005.10.001

ten autosomal-recessive limb girdle muscular dystrophies (LGMD2A-J) are characterised by progressive weakness predominantly of the limb-girdle muscles. They represent a group of genetically heterogeneous disorders caused mainly by loss of the connection between extracellular matrix and actin cytoskeleton with causative mutations in genes encoding a disparate collection of proteins involved in all aspects of muscle cell biology [1–6].

The congenital muscular dystrophies (MDC) are a heterogeneous group of autosomal recessively inherited diseases, presenting at birth or within the first 6 months of life with hypotonia, muscle weakness and a variable appearance of contractures or brain malformations. Ten genetically distinct entities have been identified [1,4,7–9]. Moreover, the large group of congenital myopathies (CM) with predominant onset at birth or within the first years of life is defined on the basis of structural abnormalities of muscle fibres, visible after staining of muscle biopsy sections with histochemical methods. Different subtypes of myopathies have been suggested: severe neonatal onset, milder congenital, non-progressive and a slowly progressive late or adult onset form. There is however, marked clinical overlap between these groups [1,10–13].

Myofibrillar myopathies (MFM) comprise a group of disorders, usually with adult onset and autosomal dominant inheritance, which result in progressive weakening of limb muscles and multisystem involvement. Five different genes have been associated with MFM [1,14–19]. A further group of myopathies with onset in infancy are the congenital myasthenic syndromes (CMS) which represent diseases of the motor end-plate: nine entities are caused by affected genes encoding different receptor subunits (*CHRN*), choline acetyltransferase (*ACHE*), collagenic tail of endplate acetylcholinesterase (*COLQ*), receptor-associated protein of the synapse (*RAPSN*), voltage-gated sodium channel type IV (*SCN4A*) and muscle-specific receptor tyrosine kinase (*MUSK*) [1,20–24].

Non-dystrophic myotonias are inherited ion channel disorders caused by mutations in genes encoding chloride (*CLCN1*), calcium (*CACNA1S*), potassium (*KCNE3*) and sodium (*SCN4A*) channels. Both the absence of dystrophic features on muscle biopsy and their monosystemic nature distinguish the non-dystrophic myotonias from the myotonic dystrophies caused by nucleotide repeat expansions [1,25,26].

The diagnosis of NMDs may be limited, not only by clinical symptoms but also by immunohistochemical analysis and often does not lead to the definite molecular

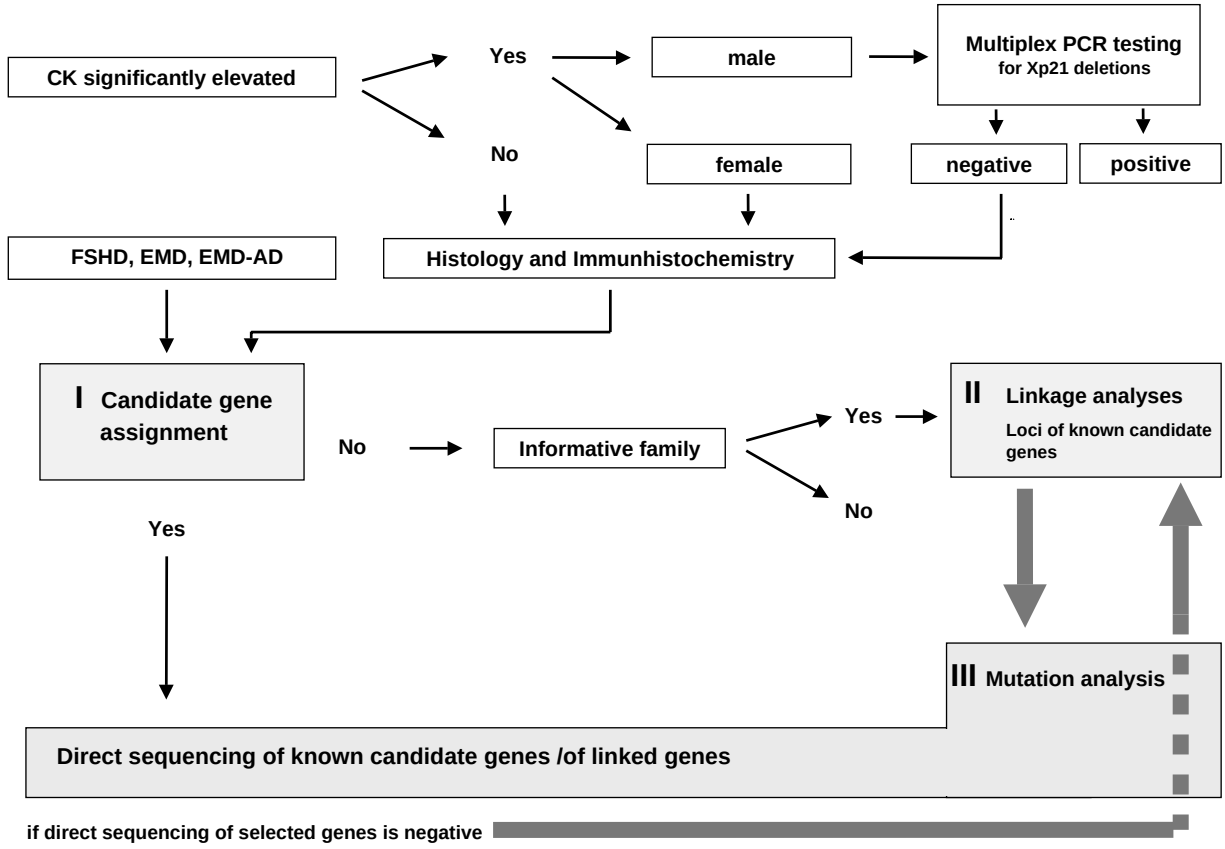

Fig. 1. Diagnostic flow sheet for muscular dystrophies and myopathies with phenotype selection criteria prior to linkage analyses. Abbreviations: CK, creatine kinase; PCR, polymerase chain reaction Nomenclature: gene symbol according to the gene table of this journal [1] or Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM).