Workshop report
51st ENMC International Workshop:
Nemaline Myopathy
13–15 June 1997, Naarden, The Netherlands

1. Introduction

The second ENMC international workshop on nemaline myopathy was held in Naarden, The Netherlands 13–15 June 1997 in the presence of thirteen participants from eight countries.

Since the first workshop [1], a review paper on nemaline myopathy has been compiled [2], a number of new families have been studied, nemaline myopathy has been established as a cause for the fetal akinesia sequence [3], a new mutation in the gene for slow tropomyosin TPM3 has been discovered [4], the linkage region for autosomal recessive nemaline myopathy on chromosome 2 has been refined and a candidate gene has been assigned to this region [5].

2. Clinical session

Martin Lammens (Leuven, Belgium) presented eight cases from four families where nemaline myopathy was the cause of severe fetal akinesia deformation sequence, including arthrogryposis, lung hypoplasia and craniofacial dysmorphism. The eight patients and one sibling presented with fetal akinesia. Multiple nemaline bodies were found in muscle biopsies of a fetus of 22 gestational weeks. Five out of seven children of a Turkish family died at birth with fetal akinesia sequence. Muscle biopsies of four of them showed multiple nemaline bodies. In one child of a Dutch family with fetal akinesia sequence nemaline myopathy was confirmed on archival paraffin blocks using \( \alpha \)-actinin antibodies. Two children of consanguineous Moroccan parents had neonatally severe nemaline myopathy. Molecular genetic studies of this family indicate no linkage to the region for autosomal recessive nemaline myopathy, NEM2, on chromosome 2q. There were no intranuclear rods present in any of the patients.

Martin Lammens concluded that nemaline myopathy can cause the fetal akinesia sequence with arthrogryposis, that it can be present at 22 weeks of gestation, and that there is a neonatally severe form without intranuclear rods. In the discussion following on Dr. Lammens’ presentation, Caro-
further pregnancies, partial penetrance, phenocopies and new mutations. Out of the families for which details of clinical presentation were known, there were two families with a neonatally lethal form and 17 families with congenital onset of a static or slowly progressive form, including one with autosomal dominant inheritance, and one sporadic case with childhood onset.

Carina Wallgren-Pettersson (Helsinki, Finland) presented data on four new multiplex families. One sib pair with the clinically mainstay form of autosomal recessive nemaline myopathy, NEM2, showed an unusual histological picture (see section on Pathology). This family and all the others with the mainstay form showed linkage results compatible with linkage to the NEM2 region on chromosome 2 [6]. A son of consanguineous parents whose brother had died neonatally of unknown causes was said to be normal at birth, but his motor development was severely impaired and delayed, he never sat, and he died at 21 months from pneumonia [4]. This boy had unusual muscle biopsy findings and was found to have a previously undescribed TPM3 mutation (see below under Pathology and Molecular Genetics). Mutations in the genes for other sarcomeric proteins expressed only in type 1 fibres might give rise to a similar histological picture.

3. Session on pathology, immunohistochemistry and pathophysiology

Kathryn North (Sydney, Australia) presented recent results of her pathological and immunocytochemical analysis of muscle biopsies from patients with TPM3 mutations. There are a number of variable features in the pathology of muscle from patients with nemaline myopathy including the percentage of fibres with rods in them, proportions and relative sizes of fibre types, distribution of rods among different fibre types, expression of fetal myosin isoforms and loss of type 2 fibres or fibre type conversion of the faster fibres. Some active process of degeneration or apoptosis leading to some 2-linked cases of nemaline myopathy. One nemaline myopathy biopsy did exhibit slight variability in the intensity of nebulin staining, but it was unclear whether this reflected a real difference or was simply an artefact of specimen preparation or condition. Alan Beggs (Boston, USA) discussed recent preliminary results from yeast two-hybrid screens using fragments of α-actinin as bait. Since α-actinin is a primary constituent of rods that react with both anti-α-actinin-2 and anti-α-actinin-3 antisera.

A review of 14 biopsies previously studied by North revealed four with the proposed "TPM3 pattern" (mild type 2 predominance, normal diameter of type 2 fibres, type 1 atrophy and rods only in type 1 fibres) suggesting that analyses of these patient’s TPM3 genes should be a high priority.

Caroline Sewry (London, UK) described her survey of pathology and immunohistochemistry among cases of nemaline myopathy studied between 1973 and 1997 in London. These included 24 patients from 21 families. Six cases had severe neonatally lethal courses while 18 represented the slowly or non-progressive congenital or childhood-onset form. Fibre size variation was a common feature seen in 16 biopsies and the percentage of fibres containing rods varied widely even within families, ranging from 7 to 100%. Three of these cases had rods only in a population of small fibres, similar to the "TPM3 pattern" described by North.

The six neonatally lethal cases were characterised by very small fibres, presence of satellite cells, a poor number of myofibrils and many heterochromatic nuclei, perhaps suggesting an apoptotic process of cell death. None of them showed intranuclear rods. Fibres expressing fetal myosin were common and were often small.

The severe, neonatally lethal cases, in common with the milder slowly progressive form of nemaline myopathy, show predominance of slow, type 1 fibres. It is unclear whether this represents poor development of fast fibres or some active process of degeneration or apoptosis leading to loss of type 2 fibres or fibre type conversion of the faster fibres.

Studies with isoform-specific α-actinin antisera from Beggs revealed that α-actinin-2-positive rods were present in virtually all rod-containing fibres but α-actinin-3-positive rods were only seen in a subset of fibres. In many of these instances, the α-actinin-3-positive rods were seen in fibres that expressed slow MHC isoforms and variable quantities of α-actinin-3 at the Z lines. This lack of correlation between α-actinin-3 and MHC expression was also seen in biopsies studied by North and Beggs. These observations support the notion that the paucity of fast fibre types may reflect a loss of fast-specific isoforms and conversion to expression of genes for slow type proteins. The presence of residual α-actinin-3-positive rods in slow fibres may indicate that isoform turnover in rods is less than in Z lines.

Finally, immunocytochemical studies of nebulin revealed no demonstrable differences between control and chromosome 2-linked cases of nemaline myopathy. One nemaline myopathy biopsy did exhibit slight variability in the intensity of nebulin staining, but it was unclear whether this reflected a real difference or was simply an artefact of specimen preparation or condition.
both Z lines and nemaline rods, characterisation of new proteins that interact with α-actinin may provide candidate genes for NM and should shed light on mechanisms of rod formation. α-Actinin-2 and α-actinin-3 are muscle-specific isoforms localised at the Z lines of all fibres (α-actinin-2) or a subset of fast, type 2 fibres (α-actinin-3) [8,9]. Subclones containing the α-actinin-2 rod (clone 3c) or the α-actinin-3 EF hand domain (clone 6c) were used to screen a human skeletal muscle cDNA library. Twenty-five to 50 HIS3-positive yeast colonies were characterised for each bait and 10–11 clones from each screen contained prey inserts that specifically activated both HIS3 and lacZ genes only when co-expressed with the 3c or 6c bait clones. DNA sequence analysis revealed that one of the 3c-interacting clones contained a partial α-actinin-2 transcript, consistent with the fact that α-actinin forms head to tail dimers [10]. Three novel bait clones were also identified of which one (3c8) was independently isolated eight times. 3c8 encodes a previously unidentified 1.8 kilobase muscle-specific transcript. Of the ten 6c-interacting clones, four contained α-actinin-2 which suggests that portions of α-actinin-2 and α-actinin-3 may form heterodimers. Three additional new and uncharacterised transcripts were also identified, including one (6c6) that was selected six times.

To confirm these putative interactions, portions of each of the six new genes were cloned into an expression vector to allow in vitro transcription and translation. Preliminary experiments with 3c8 demonstrated that it co-immunoprecipitates with the α-actinin-2 rod domain in these in vitro studies. Further characterisation of this and the other new proteins is continuing.

Kathryn North also presented preliminary results on the generation of transgenic mouse models for nemaline myopathy. In collaboration with Edna Hardeman, Peter Gunning and Nigel Laing, two lines of transgenic mice carrying the Met9Arg TPM3 mutation ([0,1995]) are being produced, and Nigel Laing, two lines of transgenic mice carrying the fast skeletal actin transgene. The TnISLOW-Met9Arg TPM3 transgenes should be expressed in slow fibres, similar to the pattern of native TPM3 expression. In contrast, the fast-skeletal actin promoter is expected to cause expression of the mutant TPM3 in fast type 2 fibres which are particularly abundant in many mouse muscle groups. Since the Met9Arg TPM3 mutation is inherited in a dominant fashion, it is hoped that expressing mice will have similar patterns of pathology to human patients with this mutation.

To date, there have been 18 pups born with the TnISLOW construct and 7 born carrying the fast skeletal actin transgene. Preliminary RTPCR expression studies of soleus muscles from the TnISLOW-Met9Arg-positive mice have demonstrated that most, if not all, are expressing the transgene. The mice are currently only five weeks old and it remains to be seen what clinical and pathological phenotypes they will express.

4. Molecular genetics session

Nigel Laing (Perth, Australia) reviewed results on screening nemaline myopathy patients for mutations in the α-tropomyosin slow, TPM3 gene. To date 53 nemaline myopathy families have been screened for mutations in the TPM3 gene by single-strand conformational polymorphism (SSCP) analysis of each of the 10 exons which make up the muscle-specific isoform cDNA by amplification of genomic DNA. Only one further patient has shown a mutation in the TPM3 gene and this is a patient who died at 21 months of age from respiratory infection and who had apparently recessively inherited nemaline myopathy. The parents of the boy were first cousins and the boy showed homozygosity for a nonsense mutation at codon 31 of the TPM3 cDNA, approximately 20 codons 3’ of the missense mutation identified in the Australian family with autosomal dominant inheritance.

This result makes TPM3 nemaline myopathy similar to rhodopsin retinitis pigmentosa where missense mutations may give rise to dominant disease and nonsense mutations to recessive disease. Dr. Laing also gave preliminary data on the investigation of nebulin cDNA from illegitimate transcription in lymphocytes of nemaline patients showing possible linkage to chromosome 2.

Carina Wallgren-Pettersson presented linkage data on five new multiplex families, four of which showed results compatible with linkage to the NEM2 region on chromosome 2. Two new key recombinations helped narrow the region down from 13 cM to 4 cM. The candidate region still encompasses the nebulin gene, which is thus a strong candidate as the gene causing the mainstream autosomal recessive form of nemaline myopathy. The fifth family, described clinically by Martin Lammens as showing a severe, arthrogrypotic form of nemaline myopathy, did not appear to show linkage to this or any other known candidate loci.

Alan Beggs updated linkage results in US families with nemaline myopathy, including description of two families with autosomal recessive inheritance and features atypical of the mainstream form which did not show linkage to chromosome 2.

Katarina Pelin (Helsinki, Finland) gave the results of a radiation hybrid study localising nebulin within the linkage region for autosomal recessive nemaline myopathy on chromosome 2 and thus confirming nebulin as a candidate gene for this disease. She also gave preliminary results of a screen of part of the nebulin cDNA, in approximately 1.5 kb PCR fragments, up to 15.3 of the 21 kb of the cDNA, using Southern blots of a number of restriction enzyme digests. No abnormality was seen in 19 patients.

Stefania Millevi (Heidelberg, Germany) described the nebulin cDNA and the start of investigation of the genomic structure of the nebulin gene. The C-terminal 500 residues of the nebulin protein are apparently embedded in the Z disc. Different length thin filaments and Z discs of different thickness occur in different muscle fibres. Investigation of the genomic structure should illuminate the differential pro-
cesses by which this can occur. Preliminary results indicate that the exon boundaries match the repeat structure of the cDNA.

Siegfried Labeit (Heidelberg, Germany) gave an overview of the structure of titin, now localised just telomeric of the recessive nemaline myopathy locus on chromosome 2, including characterisation of an α-actinin binding site within the part of titin embedded in the Z disc.

5. Plans for collaborative efforts

Plans for collaborative ventures within the Consortium include addressing the question of the subclassification of nemaline myopathy in a number of ways: An international database has been set up in Helsinki to collect and analyse detailed clinical, histological and, later on, mutational data. A project is being initiated to find out how common nemaline myopathy is as a cause of neonatal arthrogryposis, with Caroline Sewry, Hammersmith Hospital, London, as the contact person. All new cases are being analysed for the known TPM3 mutations, and all new multiplex families are being tested for linkage to a number of candidate loci. In families showing linkage to the 2q locus, mutations are being sought in the nebulin gene.

6. Workshop participants

Professor Peter Barth, Amsterdam, The Netherlands
Dr. Alan H. Beggs, Boston, USA
Dr. Marc Fiszman, Paris, France
Dr. Siegfried Labeit, Heidelberg, Germany
Dr. Nigel G. Laing, Nedlands, Australia
Dr. Martin Lammens, Leuven, Belgium
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Dr. Kathryn North, Adelaide, Australia
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Dr. Isabelle Penisson-Besnier, Angers, France
Dr. Norma Romero, Paris, France
Dr. Caroline Sewry, London, UK
Dr. Carina Wallgren-Pettersson, Helsinki, Finland

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References