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The exon 55 deletion in the nebulin gene - one single founder mutation with world-wide occurrence

Vilma-Lotta Lehtokari[1,*], **Rebecca S. Greenleaf**[2,*], **Elizabeth T. DeChene**[2], **Mutsumi Kellinsalmi**[1], **Katarina Pelin**[3], **Nigel G. Laing**[4], **Alan H. Beggs**[2], and **Carina Wallgren-Pettersson**[1]

[1] The Folkhälsan Institute of Genetics and the Department of Medical Genetics, University of Helsinki, Helsinki, Finland [2] Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research, Children's Hospital Boston, Harvard Medical School, Boston USA [3] Department of Biological and Environmental Sciences, Division of Genetics, University of Helsinki, Helsinki, Finland [4] Centre for Medical Research, University of Western Australia, West Australian Institute for Medical Research, Queen Elizabeth II Medical Centre, Nedlands, Australia

Abstract

Anderson and co-workers (2004) reported a homozygous 2,502 bp deletion including exon 55 of the nebulin gene in five Ashkenazi Jewish probands with nemaline myopathy (NM) [1]. We determined the occurrence of this deletion in a world-wide series of 355 NM probands with no previously known mutation in other genes and found the mutation in 14 probands. Two of the families were not of known Ashkenazi Jewish descent but they had the haplotype known to segregate with this mutation. In all but two of eight homozygous patients, the clinical picture was more severe than in typical NM.

Keywords

nebulin; mutation; nemaline myopathy; Ashkenazi Jews

Introduction

Nemaline (rod) myopathy (NM) (OMIM 161800, 256030 and 605355) can be caused by mutations in at least six different genes encoding thin filament proteins of the striated muscle sarcomere (OMIM 161650, 102610, 191030, 190990, 191041, and 601443). Recessive mutations in the nebulin gene (*NEB)* and new dominant mutations in skeletal muscle α-actin *(ACTA1)* are the most frequent causes [2].

The nebulin gene (*NEB*) comprises 183 exons spanning 249 kb of genomic sequence [3]. To date we have described 63 different, mostly frameshifting, mutations of *NEB* in 54 families with recessive NM [4,5]. In 2004 Anderson and co-workers reported a homozygous 2,502 bp deletion encompassing exon 55 of *NEB* (g.84897_87399del, p.Arg2478_Asp2512del,

Corresponding authors: Alan H. Beggs (E-mail: beggs@enders.tch.harvard.edu), Carina Wallgren-Pettersson (E-mail: carina.wallgren@helsinki.fi).
*These authors have contributed equally to the work

GenBank NT_005151.12 and P20929) (del55) in five Ashkenazi Jewish probands [1]. Removal of exon 55 results in an in-frame deletion of 35 amino acids from the protein that likely disturbs the super-repeat structure of the protein [5] and possibly impairs nebulin-tropomyosin interactions. In a random sample of 4,090 Ashkenazi Jewish persons the carrier frequency of this mutation was one in 108 [1].

Patients and Methods

After the publication of this deletion, the European Neuromuscular Centre International Consortium on Nemaline Myopathy used the PCR method of Anderson and co-workers (2004) [1] to screen 355 probands with no previously known mutation in other genes of NM sample collections in Helsinki, Finland, Boston, USA and Perth, Australia. We haplotyped families 7 and 13 using microsatellite markers D2S2277, D2S2275, and D2S2299 and SNPs in *NEB* exons 10, 57, 116, 146, and 181 which we had used to define the haplotype of the Ashkenazi del55 mutation.

Results and discussion

Seven homozygous and seven heterozygous probands were identified, totalling 17 patients including siblings (Table 1). Two of these had been independently ascertained and included in the previous report by Anderson and co-workers (2004) [1]. These 14 probands represent 2.0% of the total of 702 NM probands included in this study. The proportion of mutation-positive probands was highest in the North American cohort, 5/125, or 4%, likely related to the fact that this population has the greatest proportion of individuals with Ashkenazi Jewish heritage. Families 7 and 13 were not known to be of Ashkenazi Jewish origin. The haplotyping demonstrated that the del55 mutation in these families was associated with the same haplotype seen in Ashkenazi Jewish families with *NEB* del55 (data not shown).

Two of the eight patients who were homozygous for the deletion and for whom clinical data were available had the “typical” form of NM. The others had more severe features, ranging from the most severe form of NM to the slightly milder, intermediate form of NM (Table 1) suggesting that some of the clinical variability seen likely results from environmental, genetic and/or epigenetic influences.

Both parents of all the homozygous patients, and one parent each of five of the seven heterozygous patients, knew themselves to be of Ashkenazi Jewish descent. Those who did not were, however, found to share the same haplotype as the other families; It is unlikely that identical deletions should have occurred more than once on the same haplotype.

The del55 mutation is thus likely to be a founder mutation. Given the ease of detection of this mutation, we recommend starting molecular genetic investigations in NM probands by checking for this deletion, and for mutations in *ACTA1*, regardless of whether they know themselves to have Ashkenazi Jewish ancestors. If the del55 is found in the heterozygous state, more extensive mutation analysis of *NEB* is recommended. Subsequent mutation analyses in cases negative for *ACTA1* mutations and del55 should be considered *in casu*.

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Table 1

Data on probands with deletion g.[84897_87399del], p.Arg2478_Asp2512del of nebulin gene exon 55 (del55). Mutations are reported based on the DNA sequence GenBank NT_005151.12 using the first nucleotide of the nebulin translation initiation site in exon three as the starting point, and on the protein sequence GenBank P20929. Clinical categories of the patients were determined according to the criteria published by the ENMC International Consortium on Nemaline Myopathy.

Patient number	Form of NM	Mutation in *NEB* (M) and predicted effect of it on nebulin protein (P)	Clinical features
Patient 1-1	Severe	M: Homozygous del55 P: 35 aa missing, representing one repeat domain, possibly impaired tropomyosin binding	Progressive respiratory insufficiency after surgery at 8 yrs. Walked without support for very short distances aged 7, but mainly uses wheelchair or stroller. Current age 15 yrs. Mechanically ventilated
Patient 1–2	Severe	M: Homozygous del55 P: See Pt 1	Arthrogryposis at birth. Did not achieve walking. Mechanically ventilated, currently aged 5 yrs.
Patient 2-1[1]	Severe	M: Homozygous del55 P: See Pt 1	Did not achieve walking. Permanently ventilated from age 11 months, deceased at 19 yrs.
Patient 2-2[1]	Severe	M: Homozygous del55 P: See Pt 1	Did not achieve sitting or walking. Mechanically ventilated since the age of 4 months, current age 21 yrs.
Patient 3-1[1]	Typical	M: Homozygous del55 P: See Pt 1	Markedly weak antigravity movements at birth. Requires mechanical ventilation at night since the age of 7. Ambulant at 16 years, uses wheelchair only occasionally.
Patient 3-2[1]	Not classified	M: Homozygous del55 P: See Pt 1	No data available
Patient 4	Intermediate	M: Homozygous del55 P: See Pt 1	Able to sit but did not achieve walking. Tracheostomy at 2 years, night-time ventilation. Current age 10 yrs.
Patient 5	Intermediate	M: Homozygous del55 P: See Pt 1	Able to walk using walker. Uses night-time ventilation. Current age 7 yrs.

Patient number	Form of NM	Mutation in *NEB* (M) and predicted effect of it on nebulin protein (P)	Clinical features
Patient 6[¤]	Typical	M: Homozygous del55 P: See Pt 1	Acute respiratory distress at 3 months, found to have isolated Complex I deficiency. Ambulant.
Patient 7	Severe	M: Homozygous del55 P: See Pt 1	Died at the age of 1 month.
Patient 8	Severe	M: Heterozygous del55[*] P: See Pt 1	Ventilator dependent and tube fed, little spontaneous movement.
Patient 9	Severe	M: Heterozygous del55[*] P: See Pt 1	Died at the age of 2.5 months.
Patient 10	Intermediate	M: Intron 23, g.42331G>C P: Abnormal splicing of exon 24 M: Del55 P: See Pt 1	An affected twin brother died at the age of 9 months from infection. Patient permanently ventilated from age 9 months, current age 7 yrs, did not achieve walking.
Patient 11	Intermediate	M: Intron 4, g.3543G>A P: Skipping of exon 4 p.Tyr13_Glu26del, 14 aa missing in the TMOD-binding region M: Del55 P: See Pt 1	No hypotonia in the neonatal period, achieved sitting but not walking, died at 3.5 yrs from respiratory infection
Patient 12	Typical	M: Intron 79, g.122400G>C P: Skipping of exon 79, p.His3693_Gln3727del M: Del55	Not walking at 15 months of age. Does not require assisted ventilation.

Patient number	Form of NM	Mutation in *NEB* (M) and predicted effect of it on nebulin protein (P)	Clinical features
		P: See Pt 1	
Patients 13-1 and 13-2	Unclassified	M: Intron 151 g.207199_207207delGTAAGTGAT P: Skipping of exon 151, p.Asn5650_Asp5686del, 37 aa encoding one repeat domain missing, ?impaired tropomyosin and desmin binding	No data available
		M: Del55 P: See Pt 1	
Patient 14-1	Severe	M: Heterozygous del55*	No spontaneous antigravity movements. Died at the age of 5 months.
		P: See Pt 1	

*The second mutation awaits identification,

[1]published by Anderson et al. (2004),

[¤]Patient 6 was described by Lamont and co-workers in 2004 [6]