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厚生労働科学研究費補助金

こころの健康科学研究事業

肢帯型筋ジストロフィー１Ｂ型の社会医学的・分子細胞生物学的研究

平成18年度～平成20年度　総合研究報告書

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平成21（2009）年4月

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I. 総合研究報告

厚生労働科学研究費補助金（こころの健康科学事業）
総合研究報告書

肢帯型筋ジストロフィー１Ｂ型の社会医学的・分子細胞生物学的研究

研究代表者　林　由起子　国立精神・神経センター神経研究所室長

研究要旨　LGMD1Bは核膜蛋白質ラミンA/C遺伝子(*LMNA*)変異による疾患である。我々は*LMNA*変異が本邦で3番目に多いLGMD亜型であること、*LMNA*変異が乳児筋炎の原因となりうることを見いだすとともに、類縁疾患について新規疾患関連候補遺伝子を複数見いだした。また、*LMNA*変異患者の新たなスクリーニング法として、抗リン酸化特異的ラミンA/C抗体の有用性を示唆した。一方、種々のモデルマウスを用いた実験から、LGMD1Bの病態形成には遺伝子発現変化や関連タンパク質の局在変化が密接に関与していることを明らかにするとともに、ダメージを受けた核成分の処理機構として、オートファジーが関与していることを細胞生物学的に明らかにした。

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A. 研究目的

肢帯型筋ジストロフィー1B型(LGMD1B)は、核膜蛋白質ラミンA/C遺伝子(*LMNA*)変異による疾患で、筋ジストロフィーに加え、心合併症によって高率に突然死をきたす臨床的に極めて重要な疾患である。本研究は、LGMD1B及びその類縁疾患について、社会医学的、臨床病理学的、分子細胞生物学的特徴を明らかにすることを目的とした。

B. 研究方法

LGMD1Bおよび類縁疾患患者について、詳細な臨床病理学的解析を行うとともに、*LMNA*及び複数の新規疾患関連候補遺伝子について幅広い変異スクリーニングを行った。また、患者およびモデルマウスの組織を用いて詳細な筋病理学的解析を行うとともに、網羅的遺伝子発現解析を行い、核膜病における遺伝子発現変化と臨床病態の関連を検討した。一方、新規診断システムの開発を目的に、抗リン酸化ラミンA/C抗体を作製し、その有用性を検討した。さらに本疾患で特異的に認められる核近傍の空胞形成の機序を明らかにするために、電顕的観察とともに、モデルマウス培養細胞を用いた実験を行った。

（倫理面への配慮）

本研究において使用した全てのヒト検体から得られた情報は、いずれも疾患の確定診断のために病理学的、生化学的、免疫学的ならびに遺伝子レベルの解析が必要であり、かつ患者および家族もこれを希望し、患者および家族の了解を得た上で採取された組織（生検・剖検筋、皮膚、血球など）を用いて得られたものであり、かつ、国立精神・神経センター倫理委員会で承認され

た所定の承諾書を用いて、患者あるいはその親権者から遺伝子解析を含む研究使用に対する検体の使用許可（インフォームドコンセント）を得たものである。遺伝子解析に関しては「ヒトゲノム解析研究に関する共通指針」を遵守した上で、施行されたものである。これらの情報を使用するに当たっては、プライバシーを尊重し、匿名化した上で使用した。

すべての動物実験は、国立精神・神経センター神経研究所動物実験に関する倫理指針に従い行い、国立精神・神経センター神経研究所動物実験管理委員会の審査・承認を得た。研究に使用する際には，必要最小限度の動物を使用するとともに，動物に苦痛を与えないよう最大限の注意を払った。

すべての組み換えDNA実験は、カルタヘナ議定書に基づく「遺伝子組み換え生物等の使用等の規制による生物の多様性の確保に関する法律」と関係省令を遵守し、国立精神・神経センター神経研究所組み換えDNA実験安全委員会の審査・承認を得た。

C. 研究結果

NCNP 骨格筋レポジトリーを用いた *LMNA* の幅広い変異スクリーニングによって、LGMD1Bが本邦で3番目に多いLGMD亜型であることを明らかにし、その具体的臨床病理学的特徴を明確にした。また *LMNA* 変異が乳児筋炎の原因となることも新たに発見するとともに、*FHL1* ならびに複数の新規疾患関連候補遺伝子を見いだした。現在これらの新規遺伝子変異について具体的病態との関連を検討している。

また、LGMD1B の簡易診断システムの開発を目的として、抗リン酸化ラミン A/C 特異抗体を作製し、疾患筋におけるラミン A/C のリン酸化の変化を見いだした。

モデルマウスを用いた遺伝子発現解析では、筋萎縮関連遺伝子および TGFβ関連遺伝子の発現亢進が認められ、臨床病理学的変化と一致する結果であった。またラミン A/C およびクロマチン結合タンパク質 BAF の *LMNA* 変異による変化を検討し、臨床症状の多様性との関連を示唆した。

さらに核膜病で共通に認められる核近傍の特異な空胞に注目し、脆弱な核膜構造によって細胞質内へ噴出した核成分が、オートファジーによって処理されうること、その際に巨大な自己貪食空胞が形成されうることを明らかにした。

D. 考察

LGMD1B が頻度の高い疾患であることが明らかになったことは、臨床経過の重要性を考慮すると、厚生労働行政上、特筆すべき点である。さらに *LMNA* 変異は乳児筋炎を含む様々な筋疾患を引き起こす可能性があり、臨床診断上、常にその可能性を念頭に置く必要がある。このような結果からも、*LMNA* 変異例の簡便なスクリーニング法の開発は不可欠であり、抗リン酸化特異的ラミン A/C 抗体の有用性が期待される。一方、特殊な自己貪食機構による核成分の分解現象を哺乳類細胞で初めて明らかにしえたことは、特筆すべき成果である。

E. 結論

本研究成果によって、LGMD1B および類縁疾患の病因・病態の一部を明らかにすることができた。今回得られた結果を基に、さらなる病態の解明、そして治療法の開発へと研究を継続していきたいと考えている。

F. 健康危険情報

突然死予防の観点から、可能性のある患者については、可及的速やかに核膜病の確定診断をおこない、定期的、慎重な心機能評価および経過観察が不可欠である。

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H. 知的財産権の出願・登録状況（予定を含む）

1．特許取得
特になし

2．実用新案登録
特になし

3．その他
特になし

Ⅱ．　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

発表者氏名： 論文タイトル名．発表誌名 巻号： ページ，出版年
原著論文
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Ⅲ.　研究成果の刊行物・別刷

PERGAMON

Neuromuscular Disorders 16 (2006) 256–261

www.elsevier.com/locate/nmd

Rapid and accurate diagnosis of facioscapulohumeral muscular dystrophy

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Received 10 September 2005; received in revised form 9 January 2006; accepted 18 January 2006

Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is a common muscular disorder, but clinical and genetic complications make its diagnosis difficult. Southern blot analysis detects a smaller sized *Eco*RI fragment on chromosome 4q35 in most facioscapulohumeral muscular dystrophy patients, that contains integral number of 3.3-kb tandem repeats known as D4Z4. The problems for the genetic diagnosis are that southern blotting for facioscapulohumeral muscular dystrophy is quite laborious and time-consuming, and the D4Z4 number is only estimated from the size of the fragment. We developed a more simplified diagnostic method using a long polymerase chain reaction (PCR) amplification technique. Successful amplification was achieved in all facioscapulohumeral muscular dystrophy patients with an *Eco*RI fragment size ranging from 10 to 25 kb, and each patient had a specific polymerase chain reaction product which corresponded to the size calculated from the number of D4Z4. Using southern blot analysis, more than 90% of facioscapulohumeral muscular dystrophy patients have a smaller *Eco*RI fragment than 26 kb in our series, and the number of D4Z4 repeats is precisely counted by this polymerase chain reaction method. We conclude that this long polymerase chain reaction method can be used as an accurate genetic screening technique for facioscapulohumeral muscular dystrophy patients.
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Keywords: Facioscapulohumeral muscular dystrophy; Chromosome 4q35; Genetic diagnosis; Southern blotting; PCR; *Eco*RI fragment; D4Z4

1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a common autosomal dominant muscular disorder characterized by its distinct clinical presentation. It often involves weakness and atrophy of facial muscles, followed by shoulder-girdle, the scapula fixators, and the upper arm muscles. Subsequently, pelvic girdle and lower limbs are also affected. About 20% of the patients eventually become wheelchair-bound by 40 years of age [1]. Difficulties of whistling, eye closure, or arm raising are common initial symptoms. Prominent scapular winging and horizontally positioned clavicles are also observed. Facial or shoulder girdle weakness usually appears during adolescence, but signs may be apparent on examination even in early childhood. Asymmetry of muscle involvement is often observed in apparently affected patients, but this is unrelated to handedness [2]. Weakness is relatively mild and the progression is usually slow with frequent association of subclinical hearing loss and retinal vasculopathy. The clinical diagnosis of FSHD is sometimes difficult because the onset of illness and the phenotypic expression is extremely variable, both within and between families [3,4].

The gene locus for FSHD has been identified on chromosome 4q35 wherein an array of tandem repeat units is located. Each repeat is a 3.3-kb *Kpn*I digestable fragment designated as D4Z4 (Fig. 1) [5–7]. The disease is usually associated with a deletion of this repeated region, however the responsible gene has not yet been identified, and the underlying molecular mechanism is still enigmatic. Southern blot analysis using the probe p13E-11 (D4F104S1) [6] is usually performed in the genetic diagnosis of FSHD. Normal individuals have *Eco*RI digested fragments containing D4Z4 repeats which varies from 40 kb to more than 300 kb in size, however, most of the FSHD patients have a smaller sized fragment from 10 to 35 kb. The clinical severity is often correlated to the fragment size, and patients with the smallest *Eco*RI fragment show very early onset and can be associated with epilepsy and mental retardation [8,9].

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doi:10.1016/j.nmd.2006.01.008

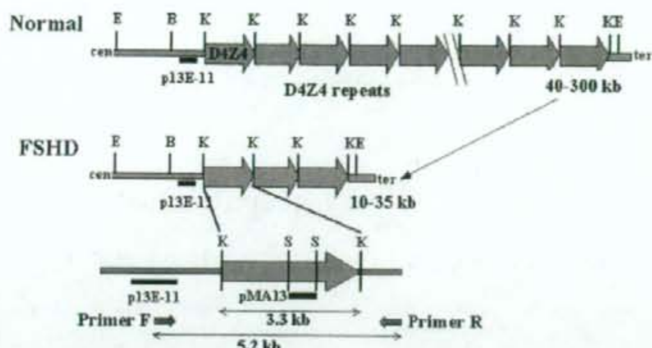


Fig. 1. A schematic diagram of the FSHD gene region on chromosome 4q35 showing the relative locations of primers and the probes used in this study. The primer set has been designed in the non-repeated region, and is expected to produce a 5.2 kb PCR amplified product when template genomic DNA contains one D4Z4 repeat. Cen, centromeric side of the gene; ter, telomeric side of gene; E, *Eco*RI; B, *Bln*I; K, *Kpn*I; S, *Sal*I.

Presently, the accuracy of the molecular diagnosis for FHSD using southern blot is up to 98% [10], however, several factors make this method cumbersome, and more than a week-length of time is required to obtain the results. In the conventional southern blotting method, it is difficult to resolve fragment size over 50 kb, and pulsed-field gel electrophoresis (PFGE) is sometimes taken together to increase resolution. Somatic and germline mosaicism is frequently observed in which more than three different sized *Eco*RI fragments on chromosome 4q are identified [11,12]. Furthermore, homologous 3.3-kb repeat-like sequences are also identified on many other chromosomes such as chromosomes Y and 3p [13,14]. In addition, chromosome 10q26 also contains 3.3-kb *Kpn*I digestable tandem repeats with 98% nucleotide identity to D4Z4 on chromosome 4 [15,16]. Consequently, there is a high incidence of inter-chromosomal exchange between 4q35 and 10q26, which is observed in about 20% of normal individuals [17,18]. In southern blot analysis, the probe p13E-11 used is not specific only to recognize *Eco*RI fragment from chromosome 4q but can also identify *Eco*RI fragment on chromosomes 10q26 and Y. This would require double restriction enzyme digestion using *Eco*RI and *Bln*I to be performed to distinguish 4q35-derived D4Z4 (*Bln*I-resistant) from 10q26-derived repeated units (*Bln*I-sensitive) [19]. From these complexities, there is an urgent need to develop a more simplified and reliable method for the diagnosis of FSHD.

Here, we introduce a new method to count the numbers of D4Z4 repeats on chromosome 4q35 by using long PCR amplification, which is quite useful for the rapid and accurate genetic diagnosis of FSHD.

2. Materials and methods

All clinical materials used in this study were acquired with informed consent. One hundred and five patients with a 4q-linked small *Eco*RI fragment from 10 to 35 kb (Table 3), and seven healthy individuals were examined. Genomic DNA was carefully and gently extracted from blood lymphocytes using a standard method. Southern blot analysis using the probe p13E-11 was performed as previously described [12].

For a long PCR amplification, a 50 μl reaction mixture was used. This mixture contains 400–600 ng of genomic DNA, 25 μl of 2× GC Buffer I (TAKARA BIO INC. Japan), 7.5 μl dATP/dTTP/dCTP mixture (10 mM each), 2.5 μl dGTP/7-deaza-dGTP mix (2:3), 1 μl (10 pM/μl) of each primers, and 0.5 μl (5 U/μl) LA Taq HS (TAKARA BIO). The primers were designed based on the human genomic sequences from GenBank (Accession Numbers D38025 and U74497). The primer sequences are F: 3′-GGCCAGAGTTT-GAATATACTGTGGTCATCTCTGCTCCAG-5′, R: 3′-CAGGGGATATTGTGACATATCTCTGCACTCATC. Amplification was performed using GeneAmp PCR System 9700 (PerkinElmer Japan Co., Ltd, Japan) with the following conditions; 1 min at 94 °C for the initial denaturation, followed by 10 cycles of 10 s at 98 °C and 20 min at 64 °C, and an additional 23 cycles of 10 s at 98 °C, 20 min with autoextension of 20 s per cycle at 64 °C, and 10 min at 72 °C for final elongation. The PCR products were separated by electrophoresis using 0.4% SeaKem HGT agarose gel (FMC BioProducts, ME) in 1× TAE with 0.5 μg/ml ethidium bromide at 3 h. High Molecular Weight DNA Marker (8.3–48.5 kb) (Invitrogen Japan K.K., Japan) and 1 kb plus ladder (Invitrogen) were used. The number of the 3.3 kb *Kpn*I repeated units in the FSHD gene region was calculated by the sequence data from GenBank (Accession Numbers D38024, D38025, and U74497).

In order to ascertain the specificity of the amplified products, we transferred the gels to Hybond N^+ (Amersham Biosciences, Japan) and overnight hybridization at 65 °C was performed with the ^{32}P-labeled probes of p13E-11 and pMA13 (1.3 kb *Stu*I fragment within a D4Z4 unit). The membrane was washed in a stringency of 2× SSC/0.1% SDS for 20 min at 65 °C for two times, followed by

Table 1
Comparison of long PCR and southern blot (SB) analyses

	PCR	SB
Template DNA (μg)	0.4	40
Enzyme digestion	No	*Eco*RI, *Bln*I
Gel size, concentration	11×14 cm, 0.4%	20×20 cm, 0.3%
Required time (h)		
PCR	11	0
Electrophoresis	3	68
Transfer	0	18
Hybridization	0	18
Detection	EB	RI
Total time required	<1 day	7–10 days
Accuracy (%)	90.1[a]	98 [10]

EB, ethidium bromide; RI, radio isotope.
[a] Estimated from the distribution of *Eco*RI fragment size in our series as described in Table 2.

autoradiography for 2 h using BAS2500 image analyzer (Fiji Photo Film, Japan).

3. Results

Table 1 shows the comparison of our newly developed long PCR method and the conventional southern blot analysis. This long PCR method is quite simple, requiring only a small amount of genomic DNA (1/100 of the quantity for southern blotting) and results are rapidly acquired overnight.

The long PCR method amplified five different sized products of 5.2, 8.5, 11.8, 15.1 and 18.4 kb which corresponded to the calculated size from the sequence data of the FSHD region containing one to five D4Z4 repeats, respectively (Fig. 2a, Table 3). These PCR products were not digested by *Bln*I, and were exclusively hybridized by the two probes of p13E-11 and pMA13 (data not shown). The same PCR method was performed on 10 individuals with a small *Eco*RI fragment (from 10 to 25 kb) on chromosome 10q26 but no amplified product was identified (data not shown).

Table 2 shows the distribution of the size of small *Eco*RI fragment on chromosome 4q of 263 FSHD families in our series. Table 3 shows the size of the PCR products, the calculated size of the *Eco*RI fragment, the range of the fragment size detected by southern blot analysis, and number of the patients. A 5.2 kb PCR product that contains one D4Z4 repeat was observed in eight patients with a *Eco*RI fragment from 10 to 11 kb. Sequence analysis confirmed that this 5.2 kb fragment contains one D4Z4 repeat on chromosome 4q35. An 8.5 kb band corresponding to the size with two D4Z4 repeated units was detected in 23 patients with 13–17 kb *Eco*RI fragment. An 11.8 kb product (three D4Z4 repeats) was seen in 26 patients with 16–19 kb fragment, a 15.1 kb fragment (four D4Z4 repeats) was seen in 24 patients with 18–22 kb fragment, and a 18.4 kb product (five D4Z4 repeats) was observed in six patients with 23–25 kb *Eco*RI fragment. The PCR products were amplified from all 87 DNA samples of the patients with an *Eco*RI fragment of 25 kb or less. However, DNA from normal individuals and FSHD patients with larger (≧26 kb) *Eco*RI fragments were not successfully amplified/detected by this long PCR method.

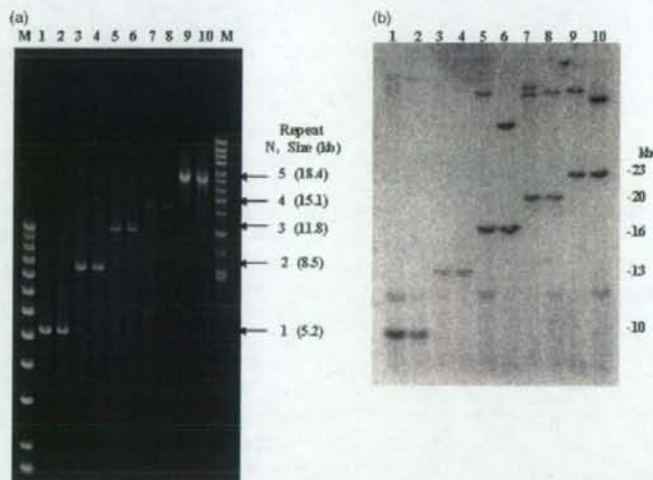
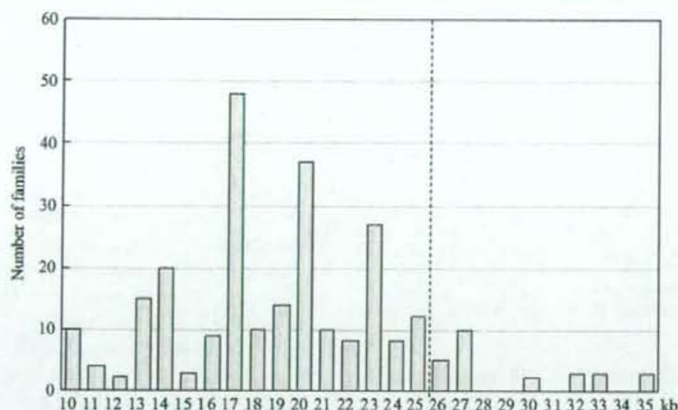


Fig. 2. Long PCR amplification and conventional southern blot analysis using genomic DNA from FHSD patients. (a) A 5.2 kb PCR product was detected on two patients with an *Eco*RI fragment of 10-kb (lane 1), or 11-kb (lane 2) as interpreted from our previous southern blot study. An 8.5-kb band was detected from two patients with a 13-kb (lane 3) or a 14-kb (lane 4) fragment, an 11.8-kb product from two patients with a 16-kb (lane 6) or a 17-kb (lane 7) fragment, a 15.1-kb product from a 20 or a 22 kb fragment, and an 18.4 kb fragment was identified from patients with a 24 and a 25 kb *Eco*RI fragment. These PCR products correspond to the size containing one to five D4Z4 repeated units. (b) Southern blot analysis using the same 10 samples in (a). The samples with the same size of the PCR products showed no difference of the *Eco*RI fragment size, although variable fragment size was previously interpreted.

Table 2
Distribution of *Eco*RI fragment size on chromosome 4q of 263 families in our series



*Eco*RI fragments of <26 kb (dot line) can be amplified by long PCR analysis.

Estimated fragment size from the previous southern blot was not identical among the patients with same numbers of D4Z4 repeats. To determine the inter-individual variability of the fragment size, conventional southern blot analysis was repeated simultaneously. Notably, after the repeated southern blot technique, the *Eco*RI fragment size was similar when the D4Z4 number was the same and this result was consistent with the calculated size (Fig. 2b).

4. Discussions

In this study, we have successfully developed a new method for rapid and specific diagnosis of FSHD by counting the number of D4Z4 repeats via a long PCR amplification technique. This long PCR method can specifically amplify the repeated region from chromosome 4q up to 18.4 kb in size and countable from one to five D4Z4 repeated units.

Table 3
Comparison of the results of long PCR and southern blot (SB) analysis

Number of D4Z4 repeats	PCR product size (kb)	Calculated size of *Eco*RI fragment (kb)	Range of *Eco*RI fragment by SB (kb)	Number of patients examined by PCR
1	5.2	10.2	10-11	8
2	8.5	13.5	13–17	23
3	11.8	16.8	16–19	26
4	15.1	20.1	18–22	24
5	18.4	23.4	23–25	6
6	21.7	26.7		
7	25	30	26–35	18
8	28.3	33.3		(No
9	31.6	36.6		amplification)

D4Z4 repeat has highly GC-rich sequence up to 73% [20]. Difficulties in PCR amplification often arise when GC content of the template DNA exceeds 50%. This difficulty in PCR amplification was overcame in our study by using thermo-stable long accurate Taq, 7-deaza-dGTP, and a higher denaturing temperature (98 °C) followed by a relatively higher annealing/extension temperature (64 °C) for 20 min with autoextension of 20 s per cycle. Therefore 73% of GC-containing repeated region of more than 18 kb in size was amplified with ease. The specificity of each PCR amplified product was ascertained by several ways. First, both probes (p13E-11 and pMA-13) that were used in the hybridization of the PCR products exclusively recognize fragments containing D4Z4 repeats. Second, the restriction enzyme *Bln*I did not digest the amplified fragments and confirmed that the product is apparently different from the repeats derived from chromosome 10q26, wherein 98% homologous *Kpn*I repeated units and franking sequences are known. Third, this long PCR method did not amplify *Kpn*I repeats from 10q26 even though the only difference is one different nucleotide from each of the primer region on 4q35. We also designed 10q-specific primer set and confirmed that only the 10q-derived repeats could be amplified by using this primer set.

The diagnosis of FSHD is sometimes difficult. Clinical symptoms and severity are quite variable between the patients even within the same family. Up to date, genetic diagnosis of FSHD is solely depended on the southern blot analysis since no responsible gene is yet identified within the candidate region. However, such procedure requires a large amount of DNA and would necessitate at least a week-time period to produce results. The requirement for such

amount of time for analysis dwells on the complexity of the experimental protocols in detecting the various fragments, the sizes ranging from 10 to 300 kb, as well as the determination of the existence of homologous regions on the other chromosomes. Determination of the size of *Eco*RI fragment is important since it is usually correlated to the clinical severity. However, identification of the precise fragment size is often difficult in the conventional southern blotting, since only very low concentrated gels of 0.3% is used to detect large sized fragment, and even minor changes in the experimental conditions would produce different results. In fact, in our very own series, DNA samples containing the same number of D4Z4 repeats showed the same *Eco*RI fragment size on one membrane although the estimated size in our previous analysis detected by different membranes were variable. Therefore, the number of D4Z4 units estimated from the *Eco*RI fragment size using Southern blotting could be misinterpreted from its actual number. From the result of the long PCR analysis, we concluded that the number of D4Z4 is countable from the size of PCR products, and the deletion of the FSHD region is certainly caused by the deleted integral number of D4Z4.

The number of D4Z4 is specifically countable up to five, which corresponds to the estimated *Eco*RI fragment of 10–25 kb in size. When no amplified product was obtained, southern blot analysis is required. In our series, 9.9% of the 4q-linked small *Eco*RI fragments have 26–35 kb as shown in Table 2, but the percentage may be greater in other countries. In the cases having deletion of p13E-11, no product can be obtained in this PCR analysis, since the forward primer is designed within this region. However, considering the complexity of the southern blot technique, this long PCR analysis is useful for the initial screening of the FSHD patients, and also the genetic test for the other family members with a known D4Z4 repeat numbers from 1 to 5 in an index patient. Obtaining accurate results rapidly is always beneficial for the patient, especially during prenatal test. From the economical point of view, PCR analysis is also beneficial since it costs 1/30–40 for the southern blot analysis.

Both primer sequences we used in this study are 4q-specific, and can amplify fragments even those with zero D4Z4 repeat, if any, producing an estimated 1.9-kb product. We also designed a primer set that can specifically amplify the repeated region on chromosome 10q. Theoretically, by using several combinations of these primers, we should be able to distinguish rare cases with short hybrid repeats on 4q or non-FSHD *Bln*I-resistant fragments on 10q. We concluded that the long PCR method could be used as an accurate genetic screening technique for FSHD.

Acknowledgements

We would like to thank Dr Mina Nolasco Astejada (NCNP) for critically reviewing the manuscript. This work was supported by Health and Labor Science Research Grants, Research on Psychiatric and Neurological Diseases and Mental Health, and The Research Grant (17A-10) for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare, Research on Health Sciences focusing on Drug Innovation from The Japan Health Sciences Foundation, Japan.

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Human Molecular Genetics, 2006, Vol. 15, No. 8 1279–1289
doi:10.1093/hmg/ddl045
Advance Access published on March 10, 2006

Aberrant neuromuscular junctions and delayed terminal muscle fiber maturation in α-dystroglycanopathies

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Received October 15, 2005; Revised and Accepted February 28, 2006

Recent studies have revealed an association between post-translational modification of α-dystroglycan (α-DG) and certain congenital muscular dystrophies known as secondary α-dystroglycanopathies (α-DGpathies). Fukuyama-type congenital muscular dystrophy (FCMD) is classified as a secondary α-DGpathy because the responsible gene, *fukutin*, is a putative glycosyltransferase for α-DG. To investigate the pathophysiology of secondary α-DGpathies, we profiled gene expression in skeletal muscle from FCMD patients. cDNA microarray analysis and quantitative real-time polymerase chain reaction showed that expression of developmentally regulated genes, including myosin heavy chain (*MYH*) and myogenic transcription factors (*MRF4*, *myogenin* and *MyoD*), in FCMD muscle fibers is inconsistent with dystrophy and active muscle regeneration, instead more of implicating maturational arrest. FCMD skeletal muscle contained mainly immature type 2C fibers positive for immature-type MYH. These characteristics are distinct from Duchenne muscular dystrophy, suggesting that another mechanism in addition to dystrophy accounts for the FCMD skeletal muscle lesion. Immunohistochemical analysis revealed morphologically aberrant neuromuscular junctions (NMJs) lacking MRF4 co-localization. Hypoglycosylated α-DG indicated a lack of aggregation, and acetylcholine receptor (AChR) clustering was compromised in FCMD and the myodystrophy mouse, another model of secondary α-DGpathy. Electron microscopy showed aberrant NMJs and neural terminals, as well as myotubes with maturational defects. Functional analysis of NMJs of α-DGpathy showed decreased miniature endplate potential and higher sensitivities to *d*-Tubocurarine, suggesting aberrant or collapsed formation of NMJs. Because α-DG aggregation and subsequent clustering of AChR are crucial for NMJ formation, hypoglycosylation of α-DG results in aberrant NMJ formation and delayed muscle terminal maturation in secondary α-DGpathies. Although severe necrotic degeneration or wasting of skeletal muscle fibers is the main cause of congenital muscular dystrophies, maturational delay of muscle fibers also underlies the etiology of secondary α-DGpathies.

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