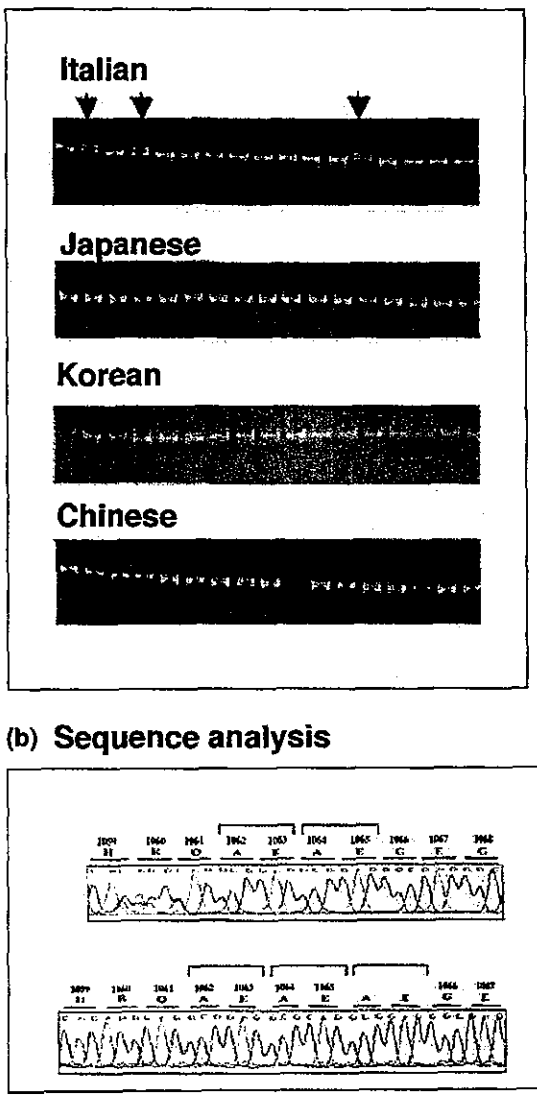

Figure 2 Six basepair insertion in exon 30. (a) Fifteen per cent of Italian control individuals had a heterozygous 6-bp insertion polymorphism in exon 30 (arrows). This polymorphism was absent in East Asian populations of Japanese, Korean and Chinese origin. (b) Sequence analysis of the 6-bp insertion. This insertion caused two additional amino acids AE following AEAE.

be a powerful method for *DYSF* mutation analysis. In fact, all the mutations identified in this study showed abnormal shifted patterns by PCR-SSCP.

We identified 11 possible mutations in 10 families. All missense mutations were observed only in the affected patients, but not in the control individuals. Distribution of the mutations in *DYSF* varied along the molecule, and no hot spot was observed. The 4376G > A missense mutation was observed in two unrelated patients (patients 5 and 7), and the 5-bp frame-shift deletion mutation observed in patient 10 was the same as previously reported in an Italian MM patient (Aoki *et al.*, 2001). These mutations may be relatively common in Italian patients. One nonsense mutation of 5358C > G observed in one family (F1) was within the fifth putative C2 domain. This patient lacked the last C2 domain, but the clinical severity was intermediate. The large deletion we found in one family (F9) contained two dysferlin domains, whose functions are not yet known. This deletion mutation produced two different truncated transcripts, the larger one being a major product corresponding to the deletion from exons 25 to 29, while the shorter one from exons 25 to 30. PCR of genomic DNA failed to amplify exons between 25 and 29, but not 30, and sequence analysis showed no abnormality of the exon–intron boundary of exons 24 and 30. Haplotype analysis showed that the patient was homozygous in the *DYSF* gene region. These results suggest that the patient has a homozygous deletion, including exons from 25 to 29, and two transcriptional variants, although detailed genomic sequence of the breakpoints and Southern blotting analysis were not performed. Both the truncated transcripts were in-frame, although immunoblotting analysis showed no detectable band. Further, dysferlin protein was either greatly reduced or absent in the muscles of patients with missense mutations. The truncated or altered protein produced by mutations in *DYSF* may degrade rapidly and was therefore barely detectable in biopsied muscles.

In one family (F6), the father and two sons had the same homozygous mutation, although there was no consanguinity reported in this intriguing family. Haplotype analysis also showed a homozygous pattern in all three patients. We could not perform genetic analysis on the mother, but she could be a mutation carrier as the father and the mother in this family were from the same small village. Interestingly, the two siblings had a very active myopathy, while the father with the identical mutation showed no apparent clinical symptoms up to the age of 58 years, except for an elevation of serum CK levels, and had only mild myopathic changes at muscle biopsy. In addition, the younger brother (patient 6c) showed predominant distal weakness and the elder brother (patient 6b) revealed both proximal and distal muscle involvement. Different clinical features were also observed in F2, in which the brother showed proximal dominant muscle involvement, while the sister showed distal myopathy. Although it is unclear as to why clinical features were different among the same family with the same mutation, similar findings have often been observed (Matsumura *et al.*, 1999; Weiler *et al.*, 1999; Illarioshkin *et al.*, 2000; Nakagawa *et al.*,

2001). Additional genetic and epigenetic factor(s) may play a role in modifying clinical symptoms.

In the four heterozygous patients in our study (patients 4, 5, 7, 10), only one allelic mutation was identified, although all 55 exons were sequenced directly. No truncated transcript was observed by RT-PCR, either. These patients may have a mutation in the promoter region or a regulatory region of an intron of *DYSF*.

The 6-bp insertion in exon 30 was frequently observed in Italian controls heterozygously. Two families with dysferlinopathy also had this insertion, and additional possible mutations were identified in these families that were not observed in 60 control individuals. Furthermore, an unaffected mother from F3 had this insertion homozygously, while an affected patient had it in one allele. Hence, the 6-bp insertion is considered to be a polymorphism. This 6-bp insertion occurred after two repeats of the same 6-bp AGGCGG, and the amino acid sequence was changed from AEAE to AEAEAE. It is interesting to speculate on the functional difference, if any, of the dysferlin molecule with this insertion. This 6-bp insertion was observed only in an Italian population, but not in East Asian populations of Japanese, Korean and Chinese origin. It is important for the mutation analysis to choose control samples from individuals with a similar genetic background.

Acknowledgements

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Two novel *CAV3* gene mutations in Japanese families

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Abstract

Caveolin-3 deficiency is a rare, autosomal dominant, muscle disorder caused by caveolin-3 gene (*CAV3*) mutations and consists of four clinical phenotypes: limb-girdle muscular dystrophy type 1C (LGMD-1C), rippling muscle disease, distal myopathy, and familial hyperCKemia. So far, only 13 mutations have been reported. We here report two novel heterozygous mutations, 96C>G (N32K) and 128T>A (V43E), in the *CAV3* gene in two unrelated Japanese families with LGMD-1C. Both probands presented with elevated serum CK level with calf muscle hypertrophy in their childhood but without apparent muscle weakness. However, their mothers showed mild limb-girdle weakness in addition to high CK level. Caveolin-3 was deficient and caveolae were lacking in muscles from both patients. Our data confirm that caveolin-3 deficiency causes LGMD-1C and expand the variability in *CAV3* gene mutations.

Keywords: CAV3; Caveolin-3; Caveolinopathy; LGMD-1C

1. Introduction

Caveolin is an integral membrane protein and is the principal component of caveolae membranes in vivo. Caveolae are vesicular invaginations of the plasma membrane and play a role in vesicular trafficking events and in signal transduction processes. The caveolin gene family consists of caveolin-1, -2, and -3. Caveolin-3 is muscle-specific and is found in both cardiac and skeletal muscles [1].

The first mutation in the human caveolin-3 gene (*CAV3*) was identified in an autosomal dominant limb-girdle muscular dystrophy type 1C (LGMD-1C) [2]. This mutation was an in-frame 9-bp deletion but all the subsequently reported mutations were missense mutations. Muscles from these patients show caveolin-3 deficiency at the protein level. Since monomers of caveolin-3 oligomerize to form the molecular scaffolding of caveolae, it is thought that heterozygous mutations in the *CAV3* gene would have a dominant negative effect in oligomerization and thus in caveolae formation [3].

So far, 13 different heterozygous mutations in the *CAV3* gene have been associated with four different muscle disorders: LGMD-1C, rippling muscle disease (RMD), familial hyperCKemia, and distal myopathy (Table 1) [2,4–17]. Nevertheless, their genotype–phenotype correlations have only been poorly determined because of the rarity of these diseases.

Here, we describe two novel mutations in the *CAV3* gene in two unrelated Japanese families. We also reviewed all the *CAV3* gene mutations reported previously.

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Table 1
Summary of the *CAV3* gene mutations

Mutation	Exon	Position	Nucleotide change	Phenotypes	Reference (ethinic background)
R26Q	1	N-terminal	77G>A	LGMD-1C	[4] (French)
				RMD	[5] (German), [6] (Japanese)
				Distal myopathy	[7] (Japanese)
				HyperCKemia	[8] (Italian)
D27E	1	N-terminal	81C>A	LGMD-1C	[9] (German)
				RMD	[9] (German)
P28T	1	N-terminal	82C>A	RMD	[10] (Bergian)
P28L	1	N-terminal	83C>T	HyperCKemia	[11] (Italian)
N32K	1	N-terminal	96C>G	LGMD-1C	Present report (Japanese)
V43E	2	N-terminal	128T>A	LGMD-1C	Present report (Japanese)
A45T	2	N-terminal	133G>A	LGMD-1C	[12] (German)
				RMD	[13] (Norwegian, German)
A45V	2	N-terminal	134C>T	RMD	[13] (German)
V57M	2	Caveolin-scaffolding domain	169G>A	HyperCKemia	[14] (Spanish)
T63P	2	Caveolin-scaffolding domain	187A>C	LGMD-1C	[15] (Japanese)
TFT63-65del	2	Caveolin-scaffolding domain	186-194del	LGMD-1C	[2] (Italian)
L86P	2	Membrane-spanning domain	215T>C	RMD	[16] (Colombian)
A92T	2	Membrane-spanning domain	232G>A	RMD	[16] (Italian)
F97del	2	Membrane-spanning domain	328-330del	LGMD-1C	[17] (Italian)
				RMD	[17] (Italian)
				HyperCKemia	[17] (Italian)
P104L	2	Membrane-spanning domain	311C>T	LGMD-1C	[2] (Italian), [13] (German)

2. Patients, materials and methods

2.1. Patients

Patient 1 was a 6-year-old Japanese boy. His psychomotor development was normal. However, he started occasionally complaining of fatigability and muscle pain at age 3. On examination, his calf muscles were hypertrophic but otherwise normal. He had neither muscle weakness nor rippling. Serum CK was elevated (3430 IU/l (normal range <200 IU/l)). He had no brother. His 33-years-old mother had noted easy fatigability after his birth. Her calf muscles were hypertrophied. She had mild weakness of MRC 4 level predominantly in the proximal muscles of all four extremities. She was ambulatory but could not run. She showed Gowers' sign. Her serum CK level was elevated to 1700 IU/l. Both did not show either muscle rippling, percussion-induced rapid muscle contractions (PIRCs), or muscle mounding on percussion.

Patient 2 was a 3-year-old Japanese boy unrelated to patient 1. His psychomotor development was normal. Elevated serum CK level (1578 IU/l) was found by chance at age 3. On examination, he had bilateral calf muscle hypertrophy but no muscle weakness. His brother (6-year-old) showed no neurological abnormality and had normal serum CK level. His mother (30-year-old) showed mild weakness in limb-girdle muscles and bilateral calf muscle hypertrophy on examination but did not have any difficulty for her daily activities. His maternal grandfather (age 62) had proximal dominant muscle weakness at MRC 4 level and muscle wasting in limb-girdle muscles. He showed Gowers' sign but was ambulatory. Both had elevated CK levels and often experienced pain in the calf muscles after exercise. Any member of this family did not show muscle rippling, PIRCs, or muscle mounding on percussion.

2.2. CAV3 gene analyses

We sequenced the entire coding region and the exon/intron junctions of the *CAV3* gene. Genomic DNA was extracted from peripheral blood lymphocytes of both patients and affected mothers. We amplified each exon and flanking sequences of the *CAV3* gene by polymerase chain reaction and directly sequenced the amplified fragments using an ABI 3100 Sequencer (PE Applied Biosystems, Foster City, CA) as described previously [7]. We searched for the identified mutations in control DNA from 100 Japanese individuals.

2.3. Muscle biopsy: histochemistry and immunohistochemistry

Muscle biopsies were performed from the biceps brachii muscle in both patients. One portion of each muscle biopsy specimen was frozen in liquid nitrogen-cooled isopentane for histochemistry and the other portion was divided in two for electron microscopy. Transverse serial frozen sections of 10 μm thickness were stained with hematoxylin and eosin (H&E), modified Gomori trichrome and a battery of histochemical methods.

We performed indirect immunofluorescence staining on 6 μm serial cryosections of muscle. These sections were incubated at 37 °C for 2 h with the primary mouse monoclonal IgG antibodies against caveolin-3

(BD Transduction laboratories, Lexington, KY), dysferlin, the C-terminal of dystrophin, α-, β-, γ-, δ-sarcoglycan, α-, β-dystroglycan and laminin α2 (Novocastra, Newcastle Upon Tyne, UK). They were subsequently incubated at room temperature for 1 h with a secondary antibody fluorescein isothiocyanate (FITC)-labeled goat F(ab′)2 anti-mouse IgG (Leinco Technology, St Louis, MO). These sections were examined by fluorescence microscopy. Control specimens were obtained from 10 individuals with morphologically normal muscle.

2.4. Electron microscopy

For electron microscopy, one piece of each biopsy was fixed in buffered 2% isotonic glutaraldehyde at pH 7.4, post-fixed in osmium tetroxide and were embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead nitrate, and examined with an H-7000 electron microscope (Hitachi, Tokyo, Japan). For lanthanum staining, the remaining samples werefixed in 2% glutaraldehyde-2% paraformaldehyde in 0.1 mol/l cacodylate buffer, pH 7.2 for 30 min. The samples were then minced into 1×2 mm pieces, washed three times in buffer, and rinsed overnight at 4 °C in 0.5 mol/l cacodylate buffer. The specimens were postfixed at room temperature by vibratory agitation for 2 h in a medium containing 1% osmium tetroxide in 0.2 M s-collidine buffer at pH 7.2 and 2% lanthanum nitrate. They were dehydrated, washed in 100% propylene oxide and embedded in epoxy resin.

3. Results

3.1. Gene analyses

Patient 1 and his affected mother had a heterozygous C to G substitution on at nucleotide position 96 that is predicted to change neutral amino acid, asparagine, at codon 32 to basic amino acid, lysine (96C>G (N32K)) (Fig. 1). Patient 2 and his affected mother carried a heterozygous T to A substitution at nucleotide position 128 predicted to cause an amino acid change from neutral valine to acidic glutamic acid (128T>A (V43E)). Both mutations were absent in 100 control Japanese individuals.

3.2. Histochemistry and immunohistochemistry

On H&E, both patients showed scattered necrotic and regenerating fibers in addition to mild to moderate variation in fiber size. There were scattered type 2C fibers and mild endomysial fibrosis. By immunohistochemistry, dystrophin, the four sarcoglycans, the two dystroglycans and laminin α2 were normally expressed in the sarcolemma in both patient. However, immunoreactivity of caveolin-3 was almost completely absent and that of dysferlin was markedly reduced compared to a normal control (Fig. 2A–F).

3.3. Electron microscopy

On electron microscopy (Fig. 2G–H), caveolae were identified by their characteristic flask or oval shape and location at or near the plasma membrane of muscle and endothelial cells in a normal control. Plasma membrane was highlighted by lanthanum staining. In contrast, in any of the tested muscle from the patients, we did not find caveolae over the surface of the muscle fibers although the non-muscle caveolae were present in the endothelial cells.

4. Discussion

We have described two novel missense mutations, 96C>G (N32K) and 128T>A (V43E), in the *CAV3* gene in two unrelated Japanese patients with caveolin-3 deficiency. These mutations are likely to be the cause of caveolin-3 deficiency because they are the only nucleotide changes in the *CAV3* gene open reading frame and because both mutations were absent in 100 control individuals.

Both affected mothers showed typical LGMD-1C while the patients had clinically atypical LGMD-1C with no

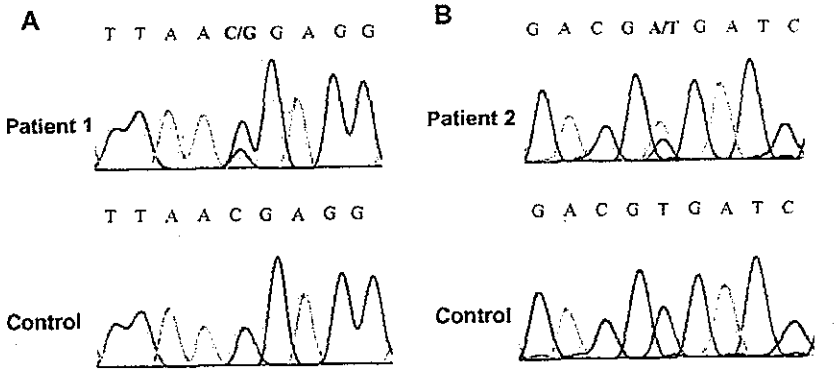

Fig. 1. Direct sequence analysis of the *CAV3* gene. (A) The heterozygous C to G mutation at nucleotide position 96 in patient 1. This change is absent in normal controls. (B) The heterozygous T to A mutation at nucleotide position 128 in patient 2. This change is also absent in normal controls.

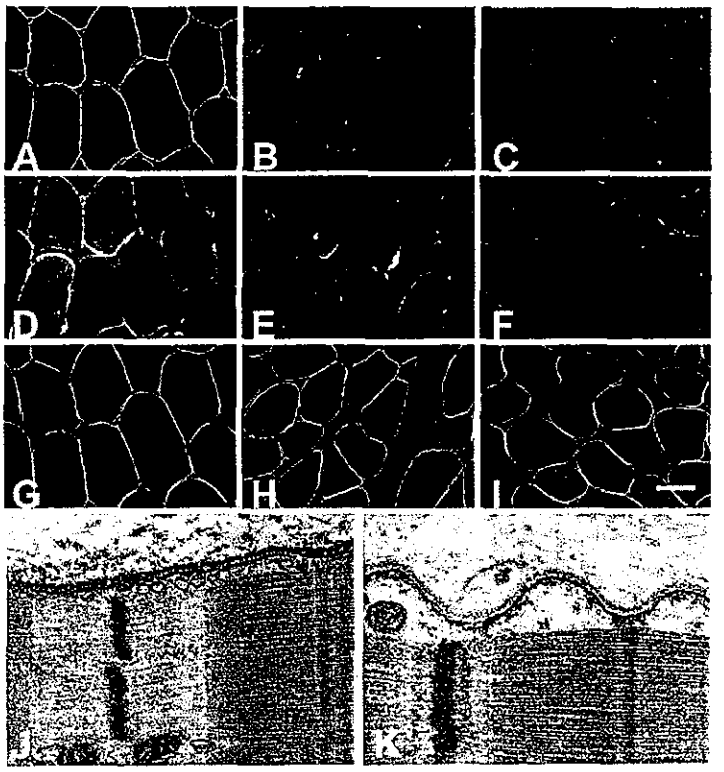

Fig. 2. Immunohistochemistry against caveolin-3 (A–C), dysferlin (D–F) and dystrophin (G–I). Transverse sections of skeletal muscle biopsies from a normal control (A, D, G), patient 1 (B, E, H) and patient 2 (C, F, I) with caveolin-3 deficiency. Normal expression of dystrophin but reduced expression of caveolin-3 and dysferlin at the sarcolemma in both patients, as compared with a control. Electron microscopy. In normal control muscle (J), caveolae are identified by their characteristic flask or oval shape and location at or near the plasma membrane which is accentuated by lanthanum staining. In contrast, in patient 1 with caveolin-3 deficiency (K), there is loss of caveolae in muscle fibers. (A–I) *Bar 40 μm.* (*J and K*) Original magnification, ×10,000.

apparent weakness. Nevertheless, muscle pathology showed dystrophic changes, with marked caveolin-3 deficiency and probable secondary dysferlin deficiency, on light microscopy and loss of caveolae on electron microscopy. In addition, inheritance pattern was compatible with an autosomal dominant trait and both patients had calf hypertrophy. Therefore, both patients were therefore diagnosed as having probable LGMD-1C rather than hyperCKemia, although it was reported that the same mutation may lead to a different phenotypes even within the same family [9].

The secondary dysferlin deficiency in our patients supports the previously proposed hypothesis that dysferlin may play a role in the signaling functions of caveolae by interacting with caveolin-3 [15]. The lack of caveolae in the sarcolemma is most likely due to severe impairment of caveolae formation in the subsarcolemma of muscle fibers, as previously described [18].

Caveolinopathy is a rare muscular disorder [1]; and LGMD-1C is a rare subtype of LGMD as compared to other LGMDs, especially LGMD-2A and LGMD-2B. The *CAV3* gene mutations have been associated with four different muscle diseases; LGMD-1C, RMD, hyperCKemia, and distal myopathy. So far, world-wide, only 13 different *CAV3* gene mutations have been reported as we summarized in Table 1 [2,4–17]. Besides, the G55S, C71W, and R125H were reported in limb-girdle muscular dystrophy patients. However, these mutations are now thought to be polymorphisms rather than pathogenic mutations, because they affected neither the expression nor the localization of the caveolin-3 protein [19]. The R26Q is the most common mutation in the *CAV3* gene and this mutation can cause any phenotype of caveolin-3 deficiency [20]. The mutations associated with LGMD-1C phenotype seem to be scattered throughout the open reading frame.

So far, five Japanese patients with caveolinopathy have been reported including the two LGMD-1C patients in the present study, carrying 96C>G (N32K) and 128T>A (V43E), and two other patients previously reported by our group, one with a peculiar distal myopathy, who had a 77G>A (R26Q) mutation [7] and the other with LGMD-1C who carried a 187A>C (T63P) mutation [15]. The other Japanese patient had RMD phenotype and had a 77G>A (R26Q) mutation [6]. Among these mutations, N32K, V43E, T63P were found only in Japan and all were only associated with LGMD-1C.

However, it is probably too early to define the genotype–phenotype correlation and we will have to await

accumulation of additional patients with genetically-determined caveolinopathy since the number of patients reported so far is too small to characterize this disease. Alternatively, the mutation site may not be the only determinant factor for the different clinical manifestations since all four phenotypes are associated with caveolin-3 deficiency at the protein level.

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14. Unusual clinical features associated with FSHD

Yukiko K. Hayashi

14.1 Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a dominantly inherited myopathy usually associated with a deletion of 3.3 kb *Kpn*I repeated units (D4Z4) on chromosome 4q35 (FSHMD1A; MIM 158900).

Typical clinical symptoms are characterized by unique involvement of muscles, which usually progress in a descending manner, including weakness and atrophy of facial muscles, followed by the shoulder girdle, the scapula fixators, and the upper arm muscles. Subsequently, pelvic girdle and lower limbs are also involved, and eventually, some 20% of the patients become wheelchair-bound by the age of 40 years (Lunt and Harper, 1991). Difficulties in whistling, closing the eyes, or lifting arms overhead are common initial symptoms. Prominent scapular winging and horizontally positioned clavicles are also observed. Facial or shoulder girdle weakness usually appears in adolescence, but signs may be apparent on examination in early childhood. Asymmetry of muscle involvement is often observed in apparently affected patients, but not related to handedness (Tawil *et al.*, 1994). Weakness is relatively mild and the progression is usually quite slow.

The clinical diagnosis of FSHD is sometimes difficult because the onset of the disease and the phenotypic expression is extremely variable, both within and between families (Lunt *et al.*, 1995; Padberg *et al.*, 1995b). One family may show severe disabilities with involvement of organs other than skeletal muscles, whereas others remain almost asymptomatic. Recent reports have shown much broader clinical expression of FSHD than perhaps previously recognized. In this chapter, unusual clinical features of FSHD are reviewed.

14.2 Early-onset form of FSHD

FSHD is generally a benign, slowly progressive myopathy that begins in late childhood or adolescence, and leads to disability only late in its course. However, some

patients exhibit clinical symptoms from infancy or early childhood.

Korf *et al.* (1985) reported six patients in whom facial diplegia occurred in the first year of life, with subsequent development of facioscapulohumeral dystrophy. All had severe progressive disability prior to adolescence. Facial involvement did not include extraocular muscles. All six patients had a sensorineural hearing loss (Korf *et al.*, 1985). Bailey *et al.* (1986) also reported clinical, electrodiagnostic, and biopsy findings in a family with infantile FSHD. Four of eight family members having the disorder, all with onset in infancy, developed severe weakness leading to death in adolescence (Bailey *et al.*, 1986).

Although these reports have suggested that progressive and severe infantile FSHD is a genetically different form of FSHD, Brouwer *et al.* (1994) proposed them to be part of a wide clinical spectrum of FSHD. They designated the criteria for the early-onset form of FSHD as follows: (1) signs or symptoms of facial weakness before the age of 5 years; and (2) signs or symptoms of shoulder girdle weakness before the age of 10 years (Brouwer *et al.*, 1994).

Clinical features of the early-onset patients are similar but more severe, progressive, and variable (Kilmer *et al.*, 1995; Funakoshi *et al.*, 1998; Yamanaka *et al.*, 2002). Patients present with early-onset facial weakness or diplegia (Shapiro *et al.*, 1991; Jardine *et al.*, 1994a). Gait disturbances are observed before 28 years of age, and significantly earlier than the other group (Shapiro *et al.*, 1991; Jardine *et al.*, 1994a; Yamanaka *et al.*, 2002). Furthermore, the early-onset patients are often accompanied by bilateral sensorineural hearing loss, retinal vasculopathy, mental retardation and epilepsy (Shapiro *et al.*, 1991; Brouwer *et al.*, 1994; Funakoshi *et al.*, 1998; Miura *et al.*, 1998; Yamanaka *et al.*, 2002).

Nakagawa *et al.* (1997) detected the early-onset form in 17% of Japanese FSHMD1A patients. Yamanaka *et al.* (2002) estimated the frequency of early onset type of FSHD to be 13.4% (31/231 Japanese FSHMD1A patients from 145 unrelated families), and that was seen more frequently in sporadic cases. Genetic analysis revealed that they had significantly larger gene deletions on chromosome 4q35, and the patients with the smallest size of *Eco*RI fragment (10–11 kb) were usually of the early-onset type (Kilmer *et al.*, 1995; Funakoshi *et al.*, 1998; Yamanaka *et al.*, 2002).

14.3 Unusual muscle involvement observed in FSHD patients

14.3.1 Facial-sparing scapular myopathy

Scapular winging due to involvement of scapula fixators is a hallmark feature of FSHD, but may also be a prominent finding in other muscular disorders including Emery–Dreyfuss muscular dystrophy, congenital myopathies, myotonic dystrophy and acid maltase deficiency (Barohn *et al.*, 1993; Kissel, 1999). In the absence of facial muscle involvement, a diagnosis of FSHD would be difficult.

Jardine *et al.* (1994b) described a 4q-linked family including seven affected individuals in two generations. The patients showed scapular onset muscular dystrophy without facial involvement. Weakness began in the shoulders between 12 and 40 years of age. There was no distal weakness in the upper or lower extremities and there were no sensory abnormalities. In several cases, there was marked

asymmetry with weakness on the right side more than on the left. There was no demonstrable facial weakness in any of the affected individuals.

Felice *et al.* (2000) performed genetic analysis on 14 patients with facial-sparing scapular myopathy, and determined that 71% of them had a short *Eco*RI fragment of less than 40 kb. These patients were estimated to constitute approximately 15% of FHSD patients. The clinical symptoms of the patients other than facial muscle involvement resembled typical FSHD patients in age at onset, physical characteristics, and association between fragment size and disease severity.

14.3.2 Tongue atrophy

Although involvement of facial muscles occurs in the majority of patients with FSHD, weakness of extraocular, masticatory, pharyngeal and lingual muscles are considered to be the exclusion criteria of the disease. However, some reports described the involvement of the tongue. Shimizu *et al.* (1991) reported a patient with 'congenital FSHD' with tongue atrophy. His father showed similar but milder muscle atrophy of the face and shoulder girdle since adolescence. The patient presented facial muscle weakness since birth, and then developed wasting around the neck, shoulder girdle, upper arms and thighs. Calf hypertrophy was also observed. Hearing disturbance was detected at the age of 6 years, and he also noted atrophy of the tongue and the bilateral thighs at the age of 10. EMG in the extremities and the tongue revealed myopathic changes. Goto and Sugihara (1994) also reported a patient diagnosed as congenital FSHD. Her mother had moderate facial weakness and mild proximal weakness of the upper and lower limbs. The patient presented bilateral facial weakness, tongue atrophy and weakness of the shoulder girdle, upper arms, and thighs, and bilateral mild sensorineural hearing loss.

Yamanaka *et al.* (2001) observed that 4.6% (7/151) of Japanese patients with FSHMD1A had tongue atrophy with abnormal MRI findings and typical myogenic patterns of electromyography (*Figure 14.1*). All seven patients belong to a group of early-onset FSHD and the *Eco*RI fragment size varied from 10 to 17 kb. They suggested that the FSHD patients, especially with a large gene deletion on chromosome 4q35 could have myopathic tongue atrophy.

14.3.3 Head drooping

Ichikawa *et al.* (1996) described three unrelated patients with FSHD showing conspicuous head drooping caused by severe wasting of posterior neck muscles. These patients realized abnormal neck posture much earlier than appearance of obvious gait disability, while they show other characteristic FSHD features. Other affected members from the same families did not show abnormal head drooping.

14.3.4 Abdominal muscle involvement and lumbar lordosis

Awerbuch *et al.* (1990) reported that the Beevor sign is commonly observed in patients with FSHD but not in other types of neuromuscular disorder. This sign was originally proposed by English neurologist C.E. Beevor as an indication of the level of involvement in spinal cord lesions. The umbilicus moves upward when the subject in the supine position raises their head because of weakness of the lower

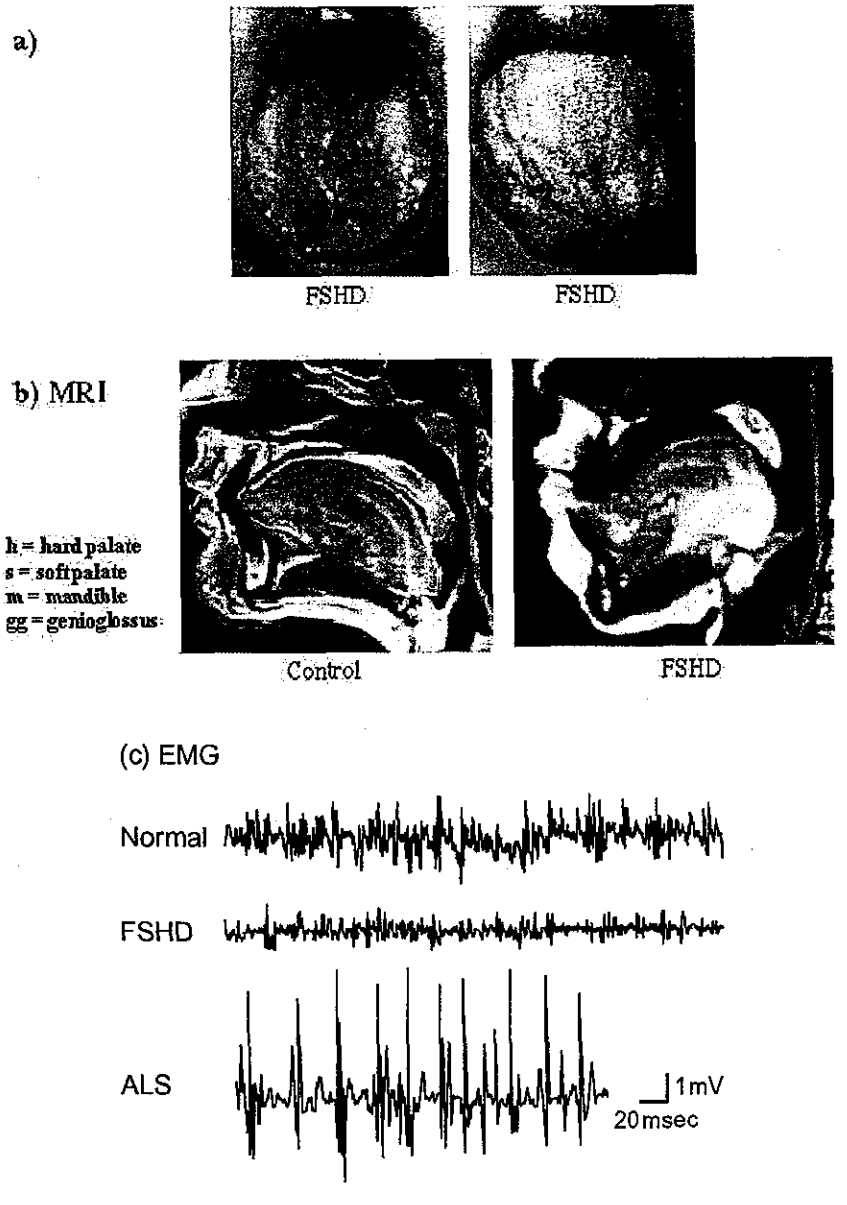

Figure 14.1 (A) Tongue pictures of FSHD patients with tongue atrophy (Yamanaka *et al.*, 2001). (B) Tongue MRI of a normal control and an FSHD patient. The normal tongue virtually fills the entire oral cavity. Its internal structure shows two curvilinear bands (arrows) parallel to the mucosal surface. In FHSD, there are scattered abnormal high-intensity areas (asterisks) in the internal tongue structure. The two curvilinear bands (arrows) are deranged and show disorganization of the tongue architecture. The atrophic tongue produces a space in the upper oral cavity (Yamanaka *et al.*, 2001). (C) In the FSHD patient, EMG showed typical myogenic changes, whereas a patient with amyotrophic lateral sclerosis (ALS) showed neurogenic changes.

rectus abdominis muscles. In FSHD patients, this sign appears even before functional weakness of abdominal wall muscles is apparent.

Early involvement of the abdominal muscles with relative sparing of the psoas major muscle in FSHD patients was detected by CT scan (Horikawa *et al.*, 1992). This may exacerbate lumbar lordosis, the most common form of spinal deformity in the patients (Kilmer *et al.*, 1995).

14.3.5 Limb girdle type muscle weakness

Limb girdle muscular dystrophy (LGMD) is a group of genetically heterogeneous progressive muscular disorders predominantly involved in proximal limb muscles. The genes responsible and their protein products have been identified in at least three autosomal dominant and ten autosomal recessive forms.

Some 4q35-linked FSHD patients were reported to display limb girdle type muscular weakness. The initial symptoms of the patients were weakness of the proximal lower limb muscles, and complaint of difficulty in climbing stairs and walking. Facial muscle involvement was absent or very mild (Nakagawa *et al.*, 1996, 1997; van der Kooi *et al.*, 2000; Felice and Moore, 2001). Although detailed genetic analysis was not performed, Kazakov and Rudenko (1995) also reported a clinically and genetically homogeneous group of patients with autosomal-dominant inheritance manifesting a gradually descending form of FSHD, called facioscapulolimb dystrophy (FSLD).

Reardon *et al.* (1991) reported a 32-year-old male patient who had typical calf hypertrophy and limb girdle type of muscle weakness. These findings suggested Becker muscular dystrophy (BMD), although he presented with sudden onset of facial weakness at age of six. When his daughter showed facial weakness, autosomal dominant FSHD became most likely. Since calf hypertrophy, although rare, has been reported in FSHD, differentiation between FSHD and BMD may also be difficult in an isolated male patient.

14.3.6 Distal myopathy

Some patients with FSHD presented foot drop by virtue of weakness of the foot extensor muscle. Padberg investigated 107 patients and found foot extensor weakness in 8% (Padberg, 1982). Felice and Moore (2001) reported a 78-year-old woman who was followed for 15 years with a diagnosis of late-onset autosomal dominant distal myopathy. The patient showed progressive bilateral foot drop, and later developed difficulties climbing stairs. Although mild eye-closure weakness and late-onset sensorineural hearing loss were observed, the patient showed no other clinical characteristic features of FSHD. Her mother had similar problems with mild facial muscle involvement and hearing loss. The patient was believed to have a form of hereditary distal myopathy, although genetic analysis revealed this proband to have a 30 kb *Eco*RI fragment.

Involvement of calf muscles is presumed to be affected only in later stages of the disease as compared with the anterior tibial muscle. CT scans also revealed a relatively mild involvement of the gastrocnemius and soleus muscles as compared with the tibialis anterior muscle (Horikawa *et al.*, 1992). However, van der Kooi *et al.* (2000) reported a male patient who initially experienced foot pain and inability to

walk on his toes at the age of 50 owing to calf muscle involvement. The *Eco*RI fragment size of this patient was 20 kb.

14.4 Muscle pain

Muscle pain is rarely described as a symptom of FSHD. Some reports described the association of muscle pain and weakness of facioscapulohumeral distribution, but they were unable to distinguish between FSHD and polymyositis (Rothstein *et al.*, 1971; Munsat *et al.*, 1972; Bates *et al.*, 1973; Bacq *et al.*, 1985). van der Kooi *et al.* (2000) reported a patient showing mild shoulder symptoms such as tiredness and pain. The *Eco*RI fragment size exhibited by this patient was 38 kb (van der Kooi *et al.*, 2000). These authors also described another patient with a 20 kb *Eco*RI fragment who initially exhibited foot pain and calf muscle weakness.

Bushby *et al.* (1998) reported four adult patients with FSHD showing muscle pain, which remains their most disabling symptom. Three of them had a short *Eco*RI fragment from 20 to 24 kb, but one manifested only normal-sized fragments. All patients reported between three and seven different pains of varying site and nature, and none had more than one painfree day per month and all complained of disturbed sleep. These myalgic pains could be particularly difficult to control by analgesic or anti-inflammatory therapy. They concluded that muscle pain in FSHD is an under-reported but significant symptom.

14.5 Association with other types of neuromuscular disorders

Tonini *et al.* (2002) reported two unique Brazilian families with FSHMD1A and other forms of muscular dystrophy in the same family. In the first, the 35-year-old male proband had limb girdle muscular dystrophy with proximal weakness, elevated creatine kinase and a myopathic muscle biopsy. All the proteins known to be associated with limb girdle muscular dystrophy were normal. Two of his sisters also complained of muscle weakness. The oldest sister exhibited clinical signs consistent with FSHD, and had a 30 kb *Eco*RI/*Bln*I fragment which was found in another six relatives, but surprisingly not in the affected proband or the other sister. In the second family, a 57-year-old male with a typical FSHD phenotype had a 17 kb *Eco*RI/*Bln*I fragment which was also present in other affected relatives. However, in a 14-year-old severely affected male cousin, confined to a wheelchair since age 12, but without facial weakness, the small fragment was absent. In case of these rare associations, it may be important to perform genetic tests in all affected individuals in a family.

Sakuma *et al.* (2001) reported a male patient with FSHD accompanied by myasthenia gravis. The patient had a 35 year history of FSHD and his mother was also affected. At the age of 50, the proband was admitted to hospital because of acute progression of muscle weakness without any fluctuation. No blepharoptosis or ocular movement disturbance was observed. However, disturbance in chewing and swallowing appeared about a month after admission, an uncommon finding in FSHD. The diagnosis of myasthenia gravis was confirmed by the repetitive stimulation test, edrophonium chloride injection, and by titre of serum anti-Ach receptor antibody (Sakuma *et al.*, 2001).

14.6 Cardiac involvement

Involvement of cardiac muscle and serious electrocardiographic abnormalities are rare in FSHD, and are not considered to be part of the disease (de Visser *et al.*, 1992; Kilmer *et al.*, 1995). However, some reports have described FSHD patients with serious arrhythmia. These patients required pacemaker implantation because of symptomatic atrial tachycardia or complete A–V block (Ohno *et al.*, 1991; Shen and Madsen, 1991).

Also, a unique family with both FSHMD1A and hereditary long QT syndrome (LQT) was reported. In this family, five individuals in three generations were diagnosed as having FSHMD1A by clinical and genetic analysis, and three of the five affected members were also diagnosed as having LQT. LQT constitutes a group of disorders that cause syncope and sudden death from ventricular arrhythmia in an autosomal dominant fashion. One of the loci for LQT (LQT4) was mapped to chromosome 4q25–q27, and possible linkage between FSHD and LQT was speculated upon (Kimura *et al.*, 1997). Recently, an ankyrin B gene mutation was identified in the large French family with LQT4 (MIM:600919) (Mohler *et al.*, 2003). Genetic analysis should clarify the association of FSHD and LQT4 in this family.

Possible cardiomyogenic involvement in FSHD was also reported using Thallium-201 single-photon-emission computed tomography (Tl-201-SPECT). Yamamoto *et al.* (1986) reported abnormal reduced Tl-201 uptake in 71% of FSHD patients that were scattered in all left ventricular wall segments. Faustmann *et al.* (1996) revealed stress-induced reduced Tl-201 uptake in the affected members of a 4q35-linked FSHD family and concluded that careful supervision of cardiac functions may be needed for FSHD patients.

Further, the thoracic deformity observed in FSHD may cause cardiac problems. Nakayama *et al.* (1999) studied electrocardiogram (ECG) and ECG-gated cardiac magnetic resonance imaging (MRI) in eight patients with FSHD. The patients frequently showed ECG abnormalities including elevated P wave and multifocal atrial premature contractions, together with restricted right ventricular movement and enlarged right atrium. Similar changes are often observed in patients suffering from severe 'funnel chest' or 'straight back syndrome'. The authors concluded that the characteristic thoracic deformity may play a primary role in the development of cardiac problems in FSHD (Nakayama *et al.*, 1999).

14.7 Respiratory failure

Pulmonary dysfunction in FSHD is usually mild, if present. However, some patients show progressive, life-threatening respiratory failure. Yasukohchi *et al.* (1988) described two sibs with FSHD. The 8-year-old sister had only muscle manifestations, whilst the brother, aged 13 years, manifested sensorineural hearing loss and marked tortuosity of retinal arterioles. He also showed early onset and progression of severe restrictive pulmonary dysfunction, and cor pulmonale, which led to death. Nakagawa *et al.* (1996) also reported an early-onset FSHD patient with respiratory failure and retinal vasculopathy. Interestingly, the 53-year-old mother

of the proband with the same genetic abnormality had limb girdle type muscular weakness with very mild facial involvement.

Kilmer *et al.* (1995) reported that nearly 50% of the FSHD patients had vital capacity evidence of restrictive lung disease. However, only 13% had severe involvement, and only 22% had a history of pulmonary complications. There was no age or disease duration effect on pulmonary function measurements or complications. They concluded that maximal expiratory pressure measurements on the FSHD patients were more sensitive than other pulmonary function tests, as with the other neuromuscular diseases (Kilmer *et al.*, 1995).

14.8 Central nervous system involvement

FSHD patients usually exhibit little or no cognitive impairment (Sigford and Lanham, 1998). However, the patients with a large deletion in the FSHD gene region tend to have a higher chance of showing severe clinical phenotypes with central nervous system abnormalities. Akiyama *et al.* (1991) reported a female patient with FSHD with sensorineural deafness, retinal vessel abnormality, mental retardation and epilepsy with infantile spasms at 6 months of age.

Miura *et al.* (1998) reported two unrelated, severely affected patients with mental retardation and epilepsy. One patient showed infantile spasms at the age of 4 months and localization-related epilepsy at the age of 2.5 years. Muscular atrophy in the face, shoulder girdle and upper arms was observed from the age of 4 years. In the other patient, lack of facial expression was noticed from the age of 1 year, and at 4 years she was noted to have a loss of bilateral upward gaze. She developed localization-related epilepsy at the age of 9 years. From the age of 10 years, weakness of the lower limbs progressed and she became wheelchair-bound at the age of 14 years and 8 months. She had moderate sensorineural hearing loss, a loss of bilateral upward gaze and tongue atrophy. Their IQs were 33 and 45, respectively. Southern blot analysis revealed a 10 kb *Eco*RI fragment in both patients (Miura *et al.*, 1998).

Funakoshi *et al.* (1998) found nine patients with the smallest *Eco*RI fragments (10–11 kb), and reported that all of them were classified as having the early-onset form. These patients exhibited a high frequency of both epilepsy (4/9, 44%) and mental retardation (8/9, 89%). These two reports concluded that mental retardation and epilepsy may be part of the clinical spectrum of FSHD, especially in the early-onset form with a large deletion.

14.9 Psychopathological and emotional examination

Bungener *et al.* (1998) performed psychological studies on patients with 11 FSHD, together with 15 myotonic dystrophy and 14 healthy subjects. A semistructured interview was used to determine DSM III-R criteria for major depressive episodes, dysthymic episodes, and generalized anxiety. The Montgomery and Asberg, and the Hamilton depressive scales, the Covi and Tyrer anxiety scales, the Abrams and Taylor scale for emotional blunting, and the depressive mood scale were all used in the study. The results indicated that the patients with FSHD were the most depressed and most anxious.

14.10 Hearing loss

Meyerson *et al.* (1984) reported sensorineural hearing loss in two sibs with FSHD. Gieron *et al.* (1985) described a mother and three children with FSHD, sensorineural hearing loss, and marked tortuosity of retinal vessels. The deafness, which varied from mild to moderate, was bilateral and early in onset; audiological studies indicated the cochlea to be the site of the abnormality. Matsuzaka *et al.* (1986) reported a sporadic patient with early-onset FHSD, sensorineural hearing loss, mental retardation, and marked tortuosity of the retinal arterioles. Fujimura *et al.* (1989) also reported a sporadic case of a 12-year-old boy with FSHD, sensorineural hearing loss and exudative angioma of bilateral retina. His hearing loss was noted at 9 years, followed by muscle weakness of his right upper extremity at 11 years.

Voit *et al.* (1986) found bilateral sloping high frequency hearing loss of 20–90 dB in 6/10 patients with infantile- or adolescent-onset FSHD. In some patients, the hearing loss was clearly progressive. The outer hair cells of the basal turn were predominantly affected. The authors concluded that cochlear dysfunction is a specific and frequent phenomenon of early-onset FSHD.

Brouwer *et al.* (1991) performed screening audiometry in 56 patients with autosomal dominant FSHD and suggested that the change of hearing function between 4000 Hz and 6000 Hz is part of the disease and may lead to severe hearing loss in some patients. Generally, the patients show bilateral high-tone hearing loss, but some showed also at the lower (speech) frequencies.

Brain stem auditory-evoked potentials were generally normal (Verhagen *et al.*, 1995), but some patients exhibited abnormal increased threshold and prolonged latency (Takeya *et al.*, 1990; Fierro *et al.*, 1997).

The frequency of hearing loss was estimated to be about 25–64% of affected patients, and is now considered to be an important feature of FSHD (Sanchez-Alcon *et al.*, 1994; Padberg *et al.*, 1995a). Age and severity of the myopathy did not have a clear relationship with the hearing loss (Sanchez-Alcon *et al.*, 1994; Padberg *et al.*, 1995a). On the other hand, Rogers *et al.* (2002) undertook detailed pure tone audiometric examination in 21 adult-onset FSHD cases and found no significant difference in the prevalence of hearing impairment. They concluded that hearing impairment is not common in adult-onset facioscapulohumeral muscular dystrophy. Moderate to severe sensorineural deafness is, however, common in early-onset FSHD.

14.11 Retinopathy

Retinal vasculopathy is known to be associated with FSHD. Association of FSHD and Coats' syndrome (exudative retinopathy with telangiectasis, sometimes causing blindness) was reported especially in the severe early-onset form with mental retardation (Small, 1968; Taylor *et al.*, 1982; Voit *et al.*, 1986).

Gurwin *et al.* (1985) reported a 22-year-old FSHD patient with a macular lesion in her right eye and poor central vision, which had been present since early childhood. Fluorescein angiographic examination revealed bilateral peripheral vessel

closure, peripheral retinal telangiectasis, and hyperfluorescence in both foveae. Three affected family members also had clinical deafness and abnormal retinal vasculature, as determined by fluorescein angiography, but none had related visual symptoms. The authors concluded that in young patients with unexplained retinal vascular lesions, the diagnosis of FSHD should be considered (Gurwin *et al.*, 1985).

Fitzsimons *et al.* (1987) found peripheral retinal capillary abnormalities including telangiectasia, closure, leakage and microaneurysm formation in 56 of 75 individuals with clinical or genetic evidence of FSHD. Retinal vasculopathy may present early in life and before there is overt evidence of muscle disease. However, there was no correlation between the severity of the muscle disease and the extent of the retinal vascular abnormality (Fitzsimons *et al.*, 1987). Padberg *et al.* (1995b) also reported similar retinal vasculopathy including telangiectasia and microaneurysms in 49% of patients with FSHD by using fluorescein retinal angiography.

The risk to vision has not been established since there are only few reports of severe visual loss in FSHD. Pauleikhoff *et al.* (1992) reported two cases of young girls who developed FSHD and exudative retinal detachment due to telangiectasis. In the first patient, the severity of the disease precluded visual recovery despite extensive photo- and cryotherapy. In the other, visual acuity in both affected eyes was retained after treatment (Pauleikhoff *et al.*, 1992).

Since visual loss may be preventable, ophthalmic examination should be undertaken on infants and young children at risk of having a deletion of the FSHD region, although visual complications of telangiectasis are rare (Fitzsimons *et al.*, 1987; Pauleikhoff *et al.*, 1992).

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