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DOTTORATO DI RICERCA IN NEUROSCIENZE SPERIMENTALI E CLINICHE
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TESI

"Clinical characterization of facio-scapulo-humeral muscular dystrophy (FSHD) with short alleles (<20kb)"

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Contents

Chapter 1
Introduction

Facio-scapulo-humeral muscular dystrophy (FSHD; OMIM #158900) is a fascinating and enigmatic genetic disease. It displays an autosomal dominant inheritance and is the third most common inherited muscular dystrophy after dystrophinopaties and myotonic dystrophy, with a prevalence of at least 1 in 20.000 (Padberg, 1982).

1.1 Historical aspects. The earliest recognizable descriptions of FSHD were made in the second half of the 19th century. Up to the middle of 1800s, most physicians held the opinion that chronic muscular atrophy was caused by anterior horn cell disease, causing what we would nowadays recognize as spinal muscular atrophy (SMA). The observation of hypertrophy of some muscles in patients with atrophy of other muscles was such an intriguing finding that it drew the attention of many clinicians. In a series of articles in the "*Archives of Générales de Médicine*" of 1868, Guillaime Duchenne de Boulogne published his "*Recherches sur la paralysie musculaire pseudo-hypertrophyque ou paralysie myo-sclérosique*", describing the condition that now bears his name. In 1852 Cruveilheirs described extreme atrophy of the anterior spinal cord in an adult with proximal weakness, and later facial and lingual involvement. Duchenne quoted this case in recognition of the fact that facial and lingual involvement occurred late in the neurogenic atrophies. However, as noted by Padberg (1982), he failed to comment on a second patient described by Cruveilheirs, a 18-years old man with a severe FSH syndrome who had died in 1848 of variola and on whom autopsy showed the brain, spinal cord and the peripheral nerves to be unaffected. This man had a severe facio-scapulo-humeral syndrome but complete sparing of the central, spinal and peripheral nervous system. This patient almost certainly represents the first description of FSHD in medical literature.

Fig. 1 Landouzy and Dejerine

While Duchenne was concentrating on pseudohypertrophy, in 1884 Vulpian presented a summary of Landouzy and Dejerine's observations *"de la myopathie atrophique progressive, myopathie hereditaire debutant dans l'enfance par la face, sans alteration du systeme nerveux"* at a meeting of the "Académie des Sciences" on January 17th.

One year later (1885a, 1885b), Landouzy and Dejerine (Fig.1) published their first article in the "Revue de Médicine" about "*La myopathie atrophique progressive; myopathie san neuropathie debutant d'ordinaire dans l'enfance, par la face*", in which they described an autopsy performed on a man who died of tubercolosis when he was 24 years old. At the age of three, atrophy of the facial muscles was noted and this was his only symptom. As they observed: "from the age of eighteen little by little the atrophy reached the upper limbs, then all the other muscles of the body". During the subsequent years the atrophy slowly progressed to involve the muscles of the trunk and pelvic girdle. There was no sensory abnormalities and the tendon reflexes were absent. He never had experienced any muscle pains.

Landouzy and Dejerine stressed the clinical and histological integrity of the muscles of the tongue, pharynx and larynx and also of the masseter, the temporal and the pterygoid muscles. The extraocular muscles and the levator palpebrae muscles were unaffected as well. At post-mortem examination the authors found no abnormalities on brain, spinal cord, peripheral nerves and intramuscular nerve endings.

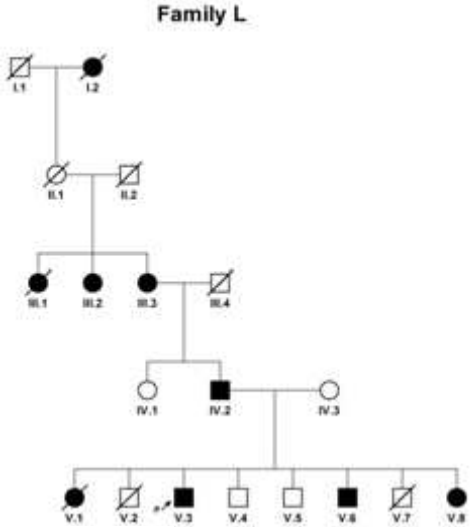

Fig. 2 Family L

They noted the hereditary nature of FSHD since the father, brother and sisters of the proband were affected. The pedigree (Fig.2) showed a definite autosomal dominant pattern of inheritance.

It is interesting to see that the disease seemingly skipped the second generation. Of course it is quite possible that the woman II,1 in the second generation might have represented an abortive case. Furthermore, if someone realizes that the father of the proband developed muscle atrophy in the shoulder girdle at the age of 26 and noted facial involvement when he was 32 years old, all the potential pitfalls involved in the diagnosis of the FSHD are already obvious from the first published pedigree.

The proband fitted the description of Duchenne's "*infantile form of progressive fatty muscular atrophy*". Landouzy and Dejerine assumed that Duchenne's and their own descriptions were about the same disease and that they had proven its myopathic nature. The proband's father and similar familial and sporadic cases described in subsequent articles, led Landouzy and Dejerine to improve the diagnostic criteria of the disorder that they had named "*facioscapulohumeral type of progressive myopathy*". The age of onset was said not necessarily to be in infancy. Furthermore, they stressed that the disease did not always start with involvement of the facial muscles. In such cases shoulder girdle

weakness was the starting symptom; some patients never developed facial weakness. Landouzy and Dejerine described the autopsy of a case that had lacked clinical involvement of the facial muscles, but showed microscopical abnormalities, suggesting a myopathy on examination of these muscles.

Although the concept of a primary muscle disease as a cause of a slowly progressive muscular atrophy was finally accepted by the end of the nineteenth century, the discussion regarding the classification of the human myopathies had only just begun. The introduction of genetical criteria proved to be very useful.

Weitz (1921) was the first to recognize the possibility of autosomal dominant, autosomal recessive and X-linked recessive modes of inheritance of the myopathies. Davidenkow (1930) studied 554 cases of what he called “dystrophia musculorum progressiva”; most of the cases were collected from literature. He was the first to recognize abortive cases of FSHD and he also drew attention to the fact that some affected members of families with FSHD failed to demonstrate facial weakness.

Julia Bell (1942, 1943) studied 1228 cases of muscular dystrophy from literature and 113 records from the National Hospital, Queen Square, London and concluded that all three modes of inheritance seemed to occur. She divided the clinical data into three groups based on two criteria, pseudohypertrophy and facial involvement, hoping to find a certain pattern of inheritance for each group. Her first group consisted of all cases exhibiting pseudohypertrophy of muscles, but cases with facial involvement were excluded. The second group contained all cases that had unaffected facial muscles and no pseudohypertrophy. The third group included all cases with weakness of the facial muscles with or without hypertrophy of muscles. Bell could not ascribe a single pattern of inheritance to each group, perhaps due to ease with which she accepted the diagnosis of reported cases as definitely established and to the fact that in many instances the families were not completely examined, as Tyler (1950) argued. This argument is of particular relevance with respect to FSHD, as all Bell’s 337 cases of group 3 were collected from the literature, because, in the 14-years period covered by the study, no such cases were seen in the National Hospital.

Pseudohypertrophy and facial involvement continued to be decisive criteria in other attempts at classification of the muscular dystrophies, because the age of onset was considered too difficult to established in many cases. Levison (1951) started from clinical criteria and concluded from eight families that the FSH type of muscular dystrophy had an autosomal dominant mode of inheritance. He stressed that he had not seen patients with marked atrophy or paresis of the orbicularis oculi muscles as described by Landouzy and Dejerine. He also distinguished a scapulohumeral type of muscular dystrophy that was

sporadic in five families and present in two brothers of another family. Finally, he discerned an intermediate type between the FSH and scapulohumeral type in which the facial muscles were only slightly involved. The six cases of this type were all sporadic ones. However, it is not stated how extensively the families were examined.

Stevenson (1953) thought an autosomal recessive mode of inheritance to be present in his families with facial involvement and included these families in his group of "autosomal recessive limb-girdle muscular dystrophy", as he judged weakness of the facial muscles an insufficient criterion for separation into two different diseases. Stevenson's examination of the families is certainly open for criticism. His view did not harmonize with the experience of many clinicians who had become accustomed to find an autosomal dominant mode of inheritance in most families with muscular dystrophy and involvement of the facial muscles. Therefore, Walton and Nattrass (1954) encountered little objection when they defined the pattern on inheritance of FSHD being usually autosomal dominant and only occasionally autosomal recessive. These authors were impressed by the occurrence of abortive cases that can obscure the true pattern of inheritance in many families. They stressed that "*the question of minor facial involvement is of the greatest importance and may well be a reason for confusion in published work since many cases which were truly FSH may have been classified as scapulohumeral*".

The classification of the muscular dystrophies given by Walton and Nattrass has proven to be very successful and formed the basis of all other attempts all classification thereafter. It ended several decades of confusion about FSHD.

1.2 Clinical features. Clinical features of FSHD are mainly three: early involvement of the face; subsequent involvement of the upper limb girdle before the lower; and at-times striking asymmetry of muscle involvement.

Facial involvement. Facial muscles hyposteny is, generally, the earliest sign of disease and gives the patient a particular myopathic face.

From the very first descriptions by Landouzy (1874) and Landouzy and Dejerine (1885), it was clear that FSHD was a "*progressive muscular atrophy that starts in childhood with the face*" producing a "*facies particulier*", which was apparent from 3 years of age in their patient. As Walton and Nattrass (1954) point out, however, as many as 2/3 of patients are completely unaware of facial weakness.

FSHD is not the only condition to affect the face, but Landouzy and Dejerine were the first to use the term "*myopathic facies*". In myotonic dystrophy the distribution of involved muscles is quite different (Harper, 2001). In other myopathies, facial involvement is usually

a later feature and often, less severe (Walton and Nattrass, 1954; Ricker and Mertens, 1968; van der Kooi et al., 1996).

Orbicularis oculi involvement often prevents the complete closure of the eyelids. The extrinsic muscles of the eyes is almost never involved. The most striking feature is an aspect of hyperthyroid without pro-ptosis, the limbo of the eye is often visible, especially in the lower eyelid. In milder cases the blinking is often abnormal and the patient tends to rotate upward when the eye blinks (Bell's phenomenon). In rare cases more severe weakness may lead to an exposure keratitis and corneal scarring (Padberg et al, 1995), but this can usually be prevented by appropriate intervention, including surgical insertion of gold weights and lateral tarsorrhaphy (Sansone et al, 1997).

Whistling or puffing cheeks, drinking from a straw acts become almost impossible because of orbicular oris muscles and zygomatic muscles involvement; the smile is flat and transverse to the deficit of the laugh muscles and savings of the platysma muscle, lips have a particular tendency to protrude and flaccidity. The lower lip tends to be the more prominent and both together somewhat protuberant described by some as “bouche de tapir” (Padberg, 1982; Brooke, 1986).

The temporalis and masseter muscles, in contrast to myotonic dystrophy, are usually spared.

Like the limbs, asymmetric facial involvement is not unusual, being most evident on smiling.

Most clinicians recognize that not all patients will develop facial weakness, and the diagnostic criteria produced following a European Neuromuscular Centre workshop allow 50% of individuals within a family not to have facial involvement (Padberg, 1992).

Upper limb involvement. More than 80% of all patients notice shoulder girdle weakness as the first symptom of the disease, when they blame a difficulty abducting the arms.

The chief muscles involved are latissimus dorsi, serratus anterior, pectoralis, subscapularis, rhomboids, trapezius, supraspinatus and infraspinatus and deltoid.

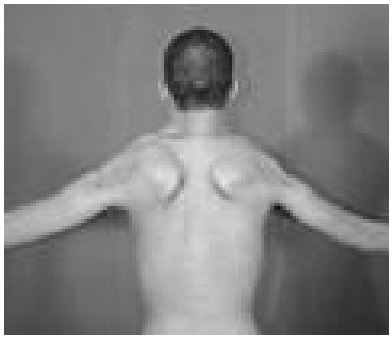

Fig. 3 Scapular winging

The weakness of the muscles of the scapula fixators (serratus anterior, lower trapezius muscle, rhomboid muscles) prevents the complete lifting of the arms and promotes the appearance of scapular winging (Fig.3). The upper fibres of trapezius tend to be spared until very late in the course of the disease, contributing to the superior movement of the winged scapula. Anteriorly, the clavicular head of the pectoralis major is

one of the first muscles to be involved producing a localized atrophy and additional typical axillary fold pointing towards the manubrium sterni. Involvement of the deltoid muscle is also characteristically localize to the proximal fibres, producing at-times strikingly visible shelf where the distal muscle is relatively spared.
In later stages, the dystrophic process extends to the biceps and triceps brachii muscles, with relative sparing of the muscles of the forearm.

Lower limb involvement. Classical forms of FSHD can be completed with the appearance of weakness of the muscles of peroneal compartment and the onset of foot drop (Padberg, 1982).
Subsequently, the weakness tends to spread to the muscles of the pelvic girdle, which contributes, in association with deficiency of abdominal muscles, the appearance of the lumbar lordosis.
The appearance of foot drop may occur earlier in the disease and early involvement of the pelvic girdle is quite rare.
To return to the more typical presentation, following involvement of the shoulder girdle, there are two common modes of progression. In both, weakness is likely to spread simultaneously down the arm to involve the biceps, triceps and brachioradialis. At about the same stage, in the lower limb, a little more commonly, involvement of the pelvic girdle is first noted (Becker, 1953; Walton and Nattrass, 1954; Chung and Morton, 1959). Also fairly commonly, the pelvis may be spared and the weakness may “jump” or “skip” to involve the peroneal muscles first (Tyler and Stephens, 1950; Chyatte et al, 1966; Kazakov et al, 1974; Padberg, 1982). It is an important diagnostic feature that involvement of the upper limb girdle is virtually always earlier and more severe than the lower limb girdle.

Abdominal muscles. In many cases, an upward movement of the umbilicus to the simple bending of the neck (Beevor's sign), due to deficiency of the abdominal rectus muscles, allows us to highlight the involvement of the abdominal muscles (Awerbuch et al, 1990).

Extramuscular involvement. The most common extramuscular manifestations of FSHD is probably hearing loss, which occurs in about 75% of the patients. Hearing impairment has been reported to be the presenting symptom of FSHD in some families. (Taylor et al, 1982).
FSHD is, moreover, associated with retinal telangiectasias, a blood vessel disorder of the retina, in 60% of cases (Tawil and Van Der Maarel, 2006). Coats’ disease, a severe exudative vascular retinopathy, has been described by a number of authors in association

with FSHD. According to Manschot and de Bruijn (1967), Coats re-defined this disease in his second German paper as a condition which is usually unilateral, non-hereditary, with an exudative vascular retinopathy consequent upon an underlying telangiectasias of small retinal blood vessels. It usually affects juvenile or infant males “in the absence of systemic disease” (Coats, 1912). It is often associated with massive retinal exudates, consequential retinal detachment and blindness.

The first description of an association with FSHD is generally accepted to be that of Small in 1968 (Small, 1968). The clinical cases presented by Small as “muscular dystrophy” are certainly convincing as cases of childhood-onset FSHD. For the next 25-30 years, over 55 cases of severe infantile FSHD were described, many with retinal vasculopathy (reviewed by Brouwer et al, 1991; Fitzsimons et al, 1987).

In spite of myotonic dystrophy, Emery-Dreifuss muscular dystrophy (EDMD) or Xp21 dystrophies or Limb Girdle Muscular Dystrophy type 1 (LGMD1), mild cardiac abnormalities were observed only in 5% of FSHD population (Laforêt et al, 1998). Tyler and Stephens (1950) in their large Utah FSHD family noted a high prevalence of cardiac disease. The prevalence of rheumatic fever was high in Utah anyway but the prevalence of “*signs suggestive of rheumatic disease*” was 12 times higher at 87 per 1000 members of the family. Other early studies were hampered by the probable inclusion of other disparate diagnoses now known to be associated with cardiac disease such an EDMD and scapulo-peroneal muscular dystrophy with cardiomyopathy.

Manning and Cropp (1958) compared standard 12-lead electrocardiograms of 28 cases of juvenile muscular dystrophy with 10 patients who had adult onset muscular dystrophy or FSHD. They found that more than 70% of the juvenile group had abnormal ECGs but in spite of the inclusion of other diagnoses still found “little or nothing abnormal” in the FSHD group. Clinical description suggests that only two of these cases fitted as FSHD phenotype. Of these, one exhibited ECG changes compatible with an incomplete right bundle bunch block (RBBB).

Stevenson et al (1990) attempted to find evidence of atrial standstill in FSHD. On standard 12-lead ECG, they found minor *p* wave abnormalities in 60% (18/30) of patients. Twelve patients underwent intracardiac electrophysiological studies with direct stimulation of the atria inducing arrhytmias in 80% of patients compared to 17% of controls. Three patients had mild prolongation of the PR interval, two patients exhibited right bundle bunch block and one left anterior hemiblock. The authors concluded that in patients with FSHD the heart was unusually sensitive to inducible arrhythmias but they were unclear about the clinical significance of this findings.

The Dutch group of de Visser et al. (1992) compared Becker and FSHDs with Bethlem Myopathy, performing standard ECG, M-Mode and two-dimensional ecocardiography, and, in the cases of FSHD, also 24-hours Holter-monitoring. Apart from one patient with mitral valve prolapse, cardiac changes were absent in the remaining 27 FSHD patients. Thallium-201 single-photon-emission computed tomography (T1-201-SPECT), in conjunction with a pharmacologically induced (dobutamine) stress test, was used to evaluate 15 members of a family with molecularly confirmed FSHD (Faustmann et al, 1996). Only the affected members of the family showed a reduced thallium-201 uptake reflecting, in the author's opinion, cardiomyogenic changes related to FSHD. Following these two studies, Laforêt et al. (1998) assessed 100 FSHD patients from 90 families with molecularly confirmed FSHD . Surface ECG showed no abnormality in 53% of patients. Minor abnormalities were seen in 38 patients including RBBB, sinus bradycardia, short PR interval, repetitive supraventricular extrasystoles and others. Conduction abnormalities were induced by intracardiac stimulation in two patients; two other patients had symptomatic cardiac disease, one with symptomatic bradycardia and the other with nocturnal atrial dysrhythmias. A fifth patient had a right ventricular cardiomyopathy and related ventricular tachycardia. There was no correlation between severity of FSHD, age of onset or size of double-digest fragment. In absence of other cardiovascular risk factors the authors concluded that 5% of their patients exhibited FSHD-related cardiac disease. More recent studies suggest that although symptomatic cardiac disease is still rare in FSHD, possibly 5% of patients show clear evidence of cardiac abnormality. Most of these appear to be rhythm disturbances.

Pulmonary dysfunction is not a well-recognized feature of FSHD. Even in severely affected patients, respiratory involvement is rare. It can occur in extremely severe cases and this may be secondary to skeletal complications (more likely do to rare cases of scoliosis rather than the commoner hyperlordosis). In other cases, especially in infantile-onset FSHD with very short p13-E11 fragments, presumed primary diaphragmatic and/or intercostal muscle involvement may very rarely cause respiratory failure (Nakagawa et al, 1996 and 1997).

In 1988 Yasukohci et al described two sibling cases of FSHD. One was characterized by sensorineural hearing loss, marked tortuosity of retinal arterioles, an early onset and progression of severe restrictive-type pulmonary dysfunction, and cor pulmonale. The other had a mild course of FSHD without involvement of any other organ than muscles at the time of diagnosis.

In a recent study sixteen patients with moderately advanced FSHD and 16 healthy controls were evaluated to establish the prevalence and type of pulmonary and respiratory muscle

dysfunction in FSHD. Lung function tests showed an increased residual volume in five patients. There was a significant difference in global respiratory muscle function in patients versus controls; weakness was mild, and it affected expiratory more than inspiratory muscles. There was no significant difference in the diaphragm inspiratory action of patients versus controls. The dystrophic process that underlies FSHD did not significantly involve the muscles of the diaphragm, but it caused mild global respiratory muscle weakness that affected expiratory more than inspiratory muscles. (Stübgen et al, 2009).

Clinical variability and progression. There is a wide variability in the spectrum of disease among patients, ranging from subjects with very mild muscle weakness, who are almost unaware of being affected, to those who are wheelchair-dependent. This great variability in clinical expression is present even within the same family and its explanation remains an open question. A mild gender effect has been noted by several authors; women tend to be more often asymptomatic, have a slightly later onset and a somewhat milder course of the disease (Tawil et al, 2006).

Natural history studies show a rate of progression that is generally slow and steady (The FSH-Dy study, 1997; Stubgen et al, 2010). So far progression of FSHD has been described as if it were inevitable that all FSHD patients go on to develop a relentless progressive dystrophy involving all muscles of the limbs. However, many patients appear to have long static phases, where progression is so slow that they appear to be in a plateau phase. Most authors would agree that FSHD does not have true plateau phases, but rather that progression in some patients decelerates to such an extent that is appears to almost stop.

For many patients there is, therefore, no further apparent progression, and in 30% remain only mildly affected throughout life (Lunt and Harper, 1991). Thus in mild cases, there may only be very slight facial weakness, only noted by an inability to whistle, and mild weakness of shoulder abduction. More typically there is slow but just-perceptible deterioration over 20-30 years.

Although FSHD is often regarded as a benign, mild condition (Bell, 1942; Walton and Nattrass, 1954, Lunt and Harper, 1991) noted that 19% of heterozygotes will be wheelchair-bound by the age of 40 years. This figure increases further with increasing age.

Life expectancy is typically not compromised.

Instrumental examination. Serum levels of CK in this form of myopathy is increased slightly and sometimes normal.

The EMG shows the signs of a primitive myopathy (polyphasic potentials of low amplitude and short duration). The muscle biopsy shows dystrophic changes beyond the features,

such as variability in fiber size, necrosis, regeneration and fibrosis, the presence of small angulated fibers, as denervation. Initially these were interpreted as a possible sign of distress associated with neurogenic muscle, has recently been shown that these small fibers contain myosin, suggesting the possibility that it is regenerating fibers. It is also frequently the response of moth-eaten. Mononuclear cell infiltrates can be seen in more than 30% of biopsies and may erroneously be incurring in the diagnosis of polymyositis (Padberg, 1982).

Recently Reilich et al. (2010) described five patients who carried the pathogenic FSHD mutation on chromosome 4q35, whose muscle biopsies revealed numerous rimmed vacuoles and filamentous cytoplasmic inclusions in all cases, suggesting to consider FSHD in the differential diagnosis of adult-onset distal myopathies with rimmed vacuoles.

1.3 Early-onset FSHD. The infantile form (also known as early-onset FSHD) was first described in 1977 by Brooke who described it as a special form of the disorder and even suggested a specific clinical course and mode of inheritance.

In 1994 Brouwer et al. confirmed that despite the wide range of phenotype, FSHD is a genetically homogeneous disorder, and defined criteria for the infantile form of FSHD as (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.

In addition to muscle weakness, infantile FSHD may be associated with sensorineural hearing loss and retinal vasculopathy. Mental retardation and epilepsy have been reported in severe cases (Funakoshi et al 1998; Miura et al 1998; Saito et al, 2007; Trevisan et al, 2008; Grosso et al, 2011).

Infantile FSHD is described as a severe and rapidly progressive disease. Its incidence is low making up for 4% of the total FSHD population (adults and children) but 58% of the FSHD population <18 years (Klinge et al, 2006). In a series of 7 infantile patients followed for between 9 and 25 years at the Newcastle Neuromuscular Centre, only two patients had a de novo mutation which previously was believed to be the main underlying genetic defect in infantile FSHD (Jardine et al, 1994).

Somatic mosaicism of a parent might have been underrepresented in the previous studies of infantile FSHD which date back to the early 1990s. It is now known that almost half of de novo FSHD cases arise through a mitotic rearrangement either in the unaffected carrier parent of an affected non-mosaic child or in the affected individual (van der Maarel et al, 2000; Kohler et al, 1996). While a positive family history is rare, patients with infantile FSHD may frequently have a parent with somatic mosaicism for the mutation who was unlikely to have presented with any symptoms at the time that the child was diagnosed.

Whereas the severity and progression in adult FSHD may vary considerably, the variability in the infantile form is less marked. The presenting symptom could be usually incomplete eye closure during sleep or the inability to smile. Therefore, Möebius syndrome is the main differential diagnosis in infants with this presentation.

In infantile FSHD weakness in the shoulder girdle may be preceded by pelvic girdle weakness. There have been no reports on asymmetry of weakness in infantile FSHD.

Respiratory insufficiency has been reported to be rare in FSHD affecting about 1% of the Dutch population (Wohlgemuth et al, 2004). Anyway, spirometry values have to be interpreted with care since facial weakness prevents tight mouth closure during the assessment and may lead to falsely low results.

Funakoshi et al. in 1998 examined 140 Japanese FSHD patients from 91 unrelated families, of whom twenty patients had small (10-11kb) EcoRI fragments and a high frequency of epilepsy (44%) and mental retardation (89%). These authors concluded that FSHD patients with a large deletion in the FSHD region tend to have a higher chance of clinical phenotypes being associated with central nervous system abnormalities. Miura et al. (1998) reported 2 sporadic cases of early-onset scapulohumeral muscular dystrophy with mental retardation and epilepsy in unrelated, severely affected females. In both cases, Southern blot analysis of the EcoRI-digested genomic DNA, using 2 probes, detected 10-kb EcoRI fragments, the shortest reported to that time. Patient 1 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 patient 2, lack of facial expression was noticed since the age of 1 year, and at 4 years she was noted to have loss of upward gaze bilaterally. 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. She had moderate sensorineural hearing loss, a loss of upward gaze bilaterally, and tongue atrophy. Their IQs were 33 and 45, respectively. They suggested that mental retardation and epilepsy may be part of the clinical spectrum of FSHD, especially in very early-onset patients with large deletions.

Up to now data on the life span of infantile patients are also limited but death in infancy or early adolescence does not seem to be common.

Bailey et al. (1986) reported on four patients of one family who all died in adolescence, while McGarry et al. (1983) reported one patient with death at the age of five years due to respiratory insufficiency caused by recurrent pneumonias. Her creatine-phosphokinase, lactate dehydrogenase and aldolase were all elevated four to ten-fold on different occasions. There was no family history of any neurological or muscle disorders.

1.4 Genetic defect and molecular diagnosis

FSHD is a disease with a unique epigenetic etiology. It is not caused by structural mutations within the disease gene as commonly seen in monogenetic traits, but rather involves a complex cascade of epigenetic events following contraction of a subtelomeric macrosatellite repeat.

Linkage studies. Linkage for FSHD was established in the early 1990s by a genome-wide microsatellite scan in a few Dutch families with autosomal dominant FSHD. When polymorphic markers became available, it was possible to search for genes responsible for or associated with genetic diseases by association analysis and linkage analysis.
Unfortunately, none of the 35 markers present at that time showed linkage to FSHD (Padberg et al, 1984). Next, the search for the FSHD locus was continued by use of restriction fragment length polymorphisms (RFLP) (Lunt et al, 1989), but also these analyses did not yield any evidence for linkage. By the early nineties, almost 95% of the human genome was excluded (Sarfarazi et al, 1989), but the FSHD locus had not been found. Then marker technology shifted from RFLPs to (CA)n type microsatellite markers (Gelehrter et al, 1998; Litt et al, 1989; Weber et al, 1989).
One of these markers, Mfd22 (Weber et al, 1990), displayed positive linkage and located the putative FSHD locus to chromosome 4 (Wijmenga et al, 1990).
Subsequently, a number of chromosome 4 markers were tested to identify markers in closer linkage or flanking marker Mfd22. A variable number of a tandem repeat marker pH30 (Milner et al, 1989), showed high linkage corresponding to locus D4S139 and located the FSHD locus to 4q35 (Wijmenga et al, 1991). Afterward the cosmid 13E, distal to the D4S139 locus, was isolated. This clone contained multiple copies of a 3.3 kb repeat sequence. An 800 bp probe termed p13E-11 (D4F104S1), proximal to the repeat array, was identified. The probe p13E-11 could be used for hybridization of Southern blots of human DNA digested with restriction enzyme EcoRI. The use of this probe displayed that, in FSHD families, EcoRI fragments, shorter than 28 kb, co-segregated stably with the disease through generations (van Deutekom, 1993; Wijmenga et al, 1992a). This probe also displayed short EcoRI fragments in sporadic FSHD patients, whereas it was absent in the clinically unaffected parents, revealing a de novo DNA rearrangement (Wijmenga et al, 1992b). Once the rearranged EcoRI fragment had arisen, it could stably be transmitted to the next generation in association with the disease (Wijmenga et al, 1992a). Additional genetic studies on FSHD families confirmed these findings (Cacurri et al, 1994; Zatz et al, 1995).

In some of the FSHD families without linkage to 4q35, markers on chromosome 12 were also tested flanking the regions of two diseases with clinical features similar to FSHD, as scapuloperoneal muscular dystrophy and scapuloperoneal muscular atrophy (Tim et al, 2001). Both disease regions were excluded by extensive linkage analysis (Tim et al, 2001). In addition, mutations in the gene myotilin, which has been identified for one of the autosomal dominant forms of limb girdle muscle dystrophy, were also eliminated as a cause of non-4q linked FSHD (Hauser et al, 2002).

Further genomic screening has identified a region on chromosome 15 consistent with linkage in one non-4q linked FSHD family (Randolph- Anderson et al, 2002). Sequence analysis ruled out a possible candidate gene, POLG on 15q25 (Bastress, et al 2004; Randolph- Anderson et al, 2002), in which mutations are responsible for progressive external ophthalmoplegia (Napoli et al, 2001).

Other possible candidates in this region were also evaluated (Bastress et al, 2004), but no mutations were observed in desmuslin, an intermediate filament protein that may play an important role in maintaining muscle integrity (Mizuno et al, 2001; Napoli et al, 2001) nor in chromodomain helicase DNA binding protein 2 that may be involved in chromatin structure regulation and gene transcription (Napoli et al, 2001; Woodage et al, 1997). The sequences of three proteins recently identified to bind D4Z4 (YY1, nucleolin and HMGB2) (Gabellini et al, 2002), were also excluded as candidate genes (Bastress et al, 2004).

In 2007, the apparent absence of the contracted D4Z4 repeat associated with FSHD was explained through the finding of a deletion in the region proximal to the D4Z4 repeat array that encompasses the p13E-11 (D4F104S1) probe-binding site used in the DNA diagnosis. The frequency of such proximally extended deletions has been estimated approximately around 3%, but to date, few patients have been described due to the difficulties in the molecular identification of such cases (Deak et al., 2007).

D4Z4 units. Not much later the identification of the FSHD polymorphic EcoRI fragments, the genetic defect in FSHD was established as a partial deletion of an integral number of repeat KpnI fragments, designated D4Z4 (van Deutekom et al, 1993).

The D4Z4 units are members of a large family of 3.3 kb tandem repeat loci that are located on the short arm of the acrocentric chromosomes, the pericentromeric regions (especially on chromosome 1), and the telomeric regions of the long arms of chromosomes 4 and 10 (Hewitt et al, 1994; Lyle et al, 1995).

The organization of the D4Z4 repeat is rather unusual (Fig. 4), in particular the presence of two homeobox sequences within the same open reading frame. Homeobox genes, encoding homeodomain transcription factors, often play important roles in embryonic

development. The predicted homeodomains from D4Z4 are both most closely related to the Mix and paired families.

Because of the potential of D4Z4 to encode a protein, several groups have focused on searching for transcripts from D4Z4. Although cDNAs and RT-PCR products containing closely related sequences have been identified, none originate from chromosome 4q35. For examples, several related cDNAs clones representing transcripts from acrocentric chromosome were isolated. However, all of these cDNAs contained in-frame stop codons within the predict homeodomains (Hewitt et al, 1994, Lyle et al., 1995). Subsequently, clones that could encode such double homeodomain proteins have been identified; again these do not represent transcripts from D4Z4 but rather are from other loci (Ding et al, 1998; Gabriels et al, 1999; Beckers et al, 2001). Thus, there is currently no conclusive evidence that D4Z4 is transcribed.

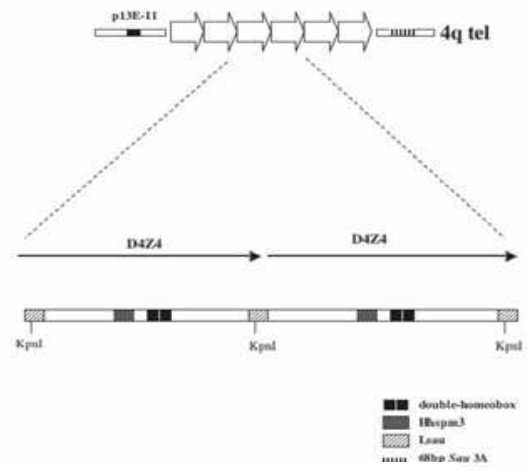

Figure 4. D4Z4 units

Within each D4Z4 repeat, the homeoboxes are flanked by two classes of repetitive DNA; a GC-rich low copy repeat originally named hhspm3 (Zhang et al, 1987) and LSau (Agresti et al, 1987 and 1989; Meneveri et al, 1993). LSau repeats are dispersed, preferentially found in regions of heterochromatin and are often associated with 68bp (or β) satellite DNA (Meneveri et al, 1993). On chromosome 4q35, 68 bp satellite DNA is not interspersed between repeat units, but present as a block of about 8 kb immediately distal to D4Z4 (van Geel et al, 2002). This satellite is only present on one variant (4qA) of the 4q telomere.

Screening of large series of control individuals and FSHD patients showed that the D4Z4 repeat normally varies between 11 and 100 units, giving rise to EcoRI fragments of 40>300 kb, while >95% of FSHD patients carried one allele of 1–10 units (EcoRI fragments of 10–38 kb) (Lunt, 1998) . FSHD patients with one allele completely devoid of D4Z4 units have never been reported, and monosomy 4q does not cause FSHD, predicting a critical role for D4Z4 in FSHD pathogenesis (Tupler et al,1996).

Molecular diagnosis. Over recent years, it has become apparent that there are many complications to this relative straightforward diagnosis of FSHD.

First, it was shown that an almost identical and equally polymorphic repeat resides in the subtelomere of chromosome 10q as a result of an ancient duplication (Bakker et al, 1995; Deidda et al, 1995). This region at 10q26 shares numerous homologies with the 4q35 subtelomeric region. The repeat element at 10q is 98% homologous to D4Z4, and the size

of 10q EcoRI alleles varies between 11 and 300 kb. Moreover, 10% of these alleles are shorter than 35 kb (Bakker et al, 1995, 1996), overlapping the 4q alleles, and clearly this can complicate the molecular diagnosis of FSHD. However, the presence of a BlnI restriction site within the 3.3 kb element associated with chromosome 10q allows the discrimination between 4q and 10q alleles (Deidda et al, 1996). As a result, Southern blot hybridization of EcoRI and EcoRI/BlnI digested genomic DNA has been used for the molecular diagnosis of FSHD (Lunt 1998). Repeat contractions on chromosome 10q are nonpathogenic (Lemmers et al, 2001; Zhang et al, 2001).

Second, apparently unconstrained exchanges between repeat units on both chromosomes can be encountered in the population and although generally the precise allele constitution for all chromosomes 4 and 10 can be precisely defined, even for the most complicated repeat exchanges, it needs sophisticated follow-up experiments often not available in diagnostic centres. Repeat exchanges between chromosomes 4 and 10 can be encountered in approximately 10% of the population, but despite this dynamic behaviour, FSHD is uniquely linked to chromosome 4 and contractions of translocated 4-type repeat units on chromosome 10 have never been observed in FSHD (Lemmers et al, 1998; van Deutekom et al, 1996a; van Overveld et al, 2000).

Third, 5−10% of subjects showing FSHD clinical features do not carry D4Z4 deleted alleles. Possible explanations for such anomalous cases include a different mutational mechanism at 4q35 and the presence of other mutations not linked to the FSHD locus at 4q35. In some cases, however, the apparent absence of the pathognomonic short EcoRI fragment was due to the presence of a deletion of the region proximal to the D4Z4 repeat array that encompasses the p13E−11probe. The frequency of such extended deletions has been estimated around 3% (Lemmers et al, 2003). Interestingly in one FSHD family, which was previously linked to a chromosomal locus on chromosome 15 (Randolph−Anderson et al, 2002), the deletion of p13−E11 region was demonstrated (Deak et al, 2007). Further studies targeting a cohort of patients failing to exhibit smaller EcoRI/BlnI fragment should define the frequency of such deletions in the FSHD population.

In addition to a contraction of D4Z4, at least one yet to be identified cis-element seems to be necessary to develop FSHD. A 4qter polymorphism distal to D4Z4 was recently described, giving rise to two distinct 4q chromosome ends: 4qA and 4qB (van Geel et al, 2002) (Fig. 5).

Within 4qA there is an 8-kb region of 68bp satellite DNA immediately distal to the D4Z4, and adjacent to this is a 1-kb divergent (TTAGGG)n array. None of these repeats is present within the 4qB sequence. In 4qB the terminal 3.3-kb repeat contains only the first 570 bp of a complete unit, whereas in 4qA the terminal repeat is divergent 3.3-kb repeat (pLAM) (van Deutekom et al, 1993). Although initially the elements that distinguish 4qA from 4qB were assigned to the region distal to D4Z4, a region of approximately 60 kb ending in the telomere repeat, more recent studies show that these sequences distal to D4Z4 are in strong linkage disequilibrium with sequences immediately proximal to D4Z4.

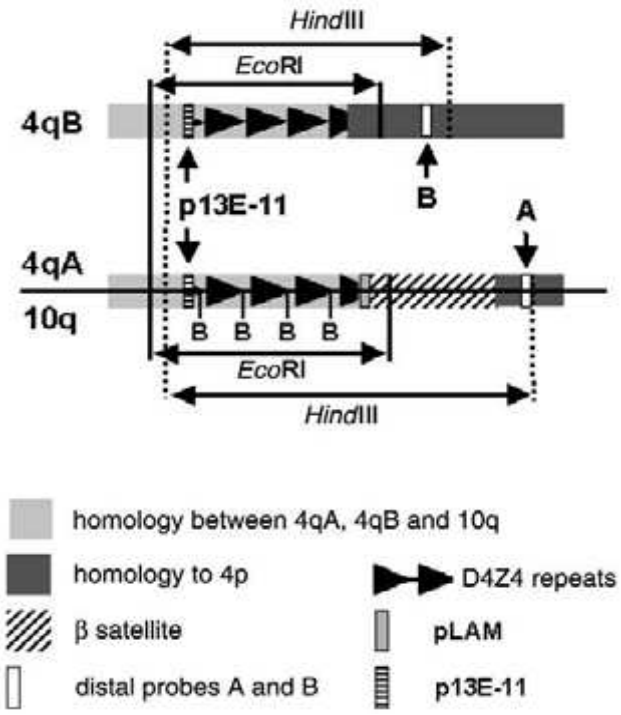

Figure 5. 4qA/4qB polymorphism

These studies have also indicated that 4qA and 4qB alleles infrequently exchange material in the regions proximal and distal to D4Z4 feeding the assumption that both alleles are functionally different (Lemmers et al, 2004). The distribution of these alleles in the control population is controversial: Lemmers and colleagues (2004), analyzing 80 control individuals, have observed an almost equal frequencies of 4qA and 4qB alleles (42% and 58%, respectively), while in an another study is reported an overrepresentation of 4qA telemeres (68% for 4qA and 32% for 4qB from a total number of 66 control individuals) (Rossi et al, 2007). Nevertheless, FSHD alleles are apparently always of the 4qA type (Lemmers et al, 2002; van Geel et al, 2002; Rossi et al, 2007). By studying three families (approximately 1% of Dutch patients with FSHD) that were ascertained by the unusual observation that all probands carried two short D4Z4 alleles, Lemmers and colleagues (2004) provided strong evidence that 4qB chromosomes carrying short D4Z4 repeats, do not cause FSHD, suggesting the presence of elements that prevent FSHD pathogenesis. The almost exclusive association between FSHD disease-expression and the presence of small (<11 repeats) D4Z4 repeat arrays located on small 4qA-defined 4qter subtelomeres has been confirmed in a large cohort of 164 unrelated patients with FSHD from Turkey and the UK (Thomas et al, 2007). Finally, a third possible 4q subtelomeric polymorphism has been found as sequence lacking hybridization with 4qA and 4qB probes in a small number of cases of both affected and healthy subjects (Thomas et al, 2007; Buzhov et al, 2005).

Recently, by analyzing four different polymorphic markers in 86 FSHD patients and 222 unrelated healthy individuals of European descent, it has been demonstrated that the subtelomeric domain of chromosome 4q can be subdivided into nine distinct haplotypes, three of which carry the distal 4qA variation (Lemmers et al, 2007). The authors have examined the repeat-size variation of D4Z4 and sequence variations in the FSHD locus, including a G/C single nucleotide polymorphism (SNP) within the first unit of D4Z4 repeat (AF117653:g.6045G→C), the 4qA/4qB polymorphism distal to D4Z4, and a relatively stable simple sequence-length polymorphism (SSLP) located 3.5 kb proximal to D4Z4 between positions 1532 and 1694 of AF117653. Furthermore, Lemmers and colleagues have shown that repeat contractions in two of the nine haplotypes are not associated with FSHD, and in particular, in two independent families, they have shown the presence of 4qA-type FSHD sized D4Z4 repeats in multiple unaffected relatives, indicating that these alleles are non-pathogenic.

These findings suggest that D4Z4 contractions per se are not sufficient to cause disease. As a consequence, FSHD might be only associated with D4Z4 contractions as part of specific haplotypes and researchers propose that future research should be focused on identifying consistent sequence variations for the different haplotypes, since these variations may be essential to FSHD pathogenesis.

Clearly, all those cases of genetically positive, but phenotypically negative patients and vice versa shall be carefully studied, since the definition of “protective polymorphisms” has important implications for genetic and prognostic counselling.

Thus further studies are needed to obtain final validation of these prognostic tools.

1.5 Current treatments for FSHD. Currently there is no treatment or cure for FSHD, but there are aids that can provide symptomatic relief. FSHD patients have a relatively normal life expectancy and therefore sometimes require procedures that will provide prolonged relief.

Many patients with facioscapulohumeral muscular dystrophy eventually have instability of the scapula resulting from weakness of the scapula stabilizers. A subset of patients, however, has sufficient strength in the supraspinatus and deltoid muscles to flex and abduct the arm, if the scapula has been stabilized. Scapulo-thoracic arthrodesis is considered a successful treatment method to limit shoulder motion and scapular winging caused by FSHD. Bunch et al (1993) fixed the scapula to the ribs in twelve FSHD patients and did re-examination from three to twenty-one years after the procedure. Approximately 50% of the individuals examined by Bunch et al. had preserved deltoid function. All the twelve patients obtained solid fusion and all of them experienced a more stable shoulder

while they were carrying or lifting objects. All but one patients was capable of forward flexion and abduction to 90 degrees or more. These patients had an average of 30 degree flexion preoperatively, which increase to 65-125 degrees postoperatively.

Andrews et al (1998) performed scapula-thoracic arthrodesis in six FSHD patients. The raise of flexion and abduction increased. The scapula was clinically and radiologically fused to the chest wall in all the patients. Demirhan et al (2009) evaluated the outcomes of scapula-thoracic arthrodesis using multifilament cables, performed on 13 patients with FSHD (18 shoulders). Solid fusion was obtained in all shoulders and in general active abduction range and anterior flexion significatively increased.

The explosion of molecular genetic information has provided tangible new targets for therapeutic interventions in a number of muscular dystrophies, but despite this, no new effective therapeutic interventions exist for them. In striving to find effective treatments, FSHD offers several advantages, as well as one major disadvantage, over some of the other dystrophies. The one disadvantage is that the gene or genes that mediate the effects of the deletion remain unknown. Consequently, without knowledge of the pathophysiology of FSHD, it's difficult to devise rational, targeted treatment approaches.

On the other hand, FSHD has several advantages over other muscular dystrophies when considering the conduct of therapeutic trials:

1. FSHD is a relatively common dystrophy with clearly defined clinical and genetic diagnostic criteria making recruitment of patients straightforward.
2. FSHD has few clinically important extramuscular manifestations that would preclude the use of certain pharmaceutical agents
3. The biggest advantage is the fact that, other than Duchenne, FSHD is the only dystrophy whose natural history has been prospectively studied and in which sizable randomized controlled trials have been performed (Personius et al, 1994; Tawil et al, 1994; The FSH-DY Group, 1997).

Therapeutic trials in FSHD

Corticosteroids. Mononuclear inflammatory infiltrates are seen in as many as 40% of muscle

biopsy samples of patients with FSHD (Padberg, 1982). At times this inflammation is intense enough to suggest an inflammatory myopathy (Bates et al, 1973). Case reports of FSHD patients treated with corticosteroids showed either improvement or no effect (Munsat et al, 1972; Bates et al, 1973, Wulff et al, 1982). Some of the patients treated with prednisone initially improved in strength (Munsat et al, 1972), though this improvement quickly waned and progression resumed (Munsat and Bradley, 1977). The effects of

prednisone on FSHD were subsequently studied in a prospective, 3-month, open-label trial (Tawil et al, 1997). Prednisone (1,5 mg/kg/day) was administered for 12 weeks to eight patients with FSHD. There were no significant changes in measures of muscle strength or muscle mass. Given the limited power of the study, no conclusions regarding the effects of prednisone in slowing or arresting disease progression could be reached. However, the authors concluded that no further trails with prednisone could be justified given the risk versus limited benefit of lifelong use of corticosteroids.

Albuterol. Compounds that demonstrate skeletal muscle anabolic properties are logical putative therapeutic agents for any muscle-wasting disease. Beta 2 agonists exert a number of effects on muscle metabolism and function including proliferation of satellite cells, increased muscle protein synthesis and inhibition of muscle proteolysis (Benson 1991; Matlin and Delay, 1992; Matlin et al, 1992). A positive 3-month, open-label trial of a sustained-release albuterol in FSHD led to the conduct of a 1-year, randomized, placebo-controlled trial (Kissel et al, 1998 e 2001). In this study 90 FSHD patients were enrolled and randomized to receive two different doses of sustained-release albuterol (8mg b.i.d. and 16 mg b.i.d., twice daily). Although a trend towards improved muscle strength was seen at 3 months in those groups receiving active drug, no difference between the treatment groups was noted at 1 year, even if muscle mass, estimated using dual-energy X-ray absorptiometry, was significantly increased at 1 year in the active drug groups in a dose-dependent fashion. In a subsequent controlled trial of sustained-release albuterol (8 mg twice daily) a similar positive effect on muscle mass, measured by computerized tomography scan, was noted after 26 weeks, along with a nodest and statistically significant improvement in strength in several muscles. (Van der Kooi et al, 2004)

Creatine monohydrate. How creatine-P, which is the immediate source of energy during muscle contraction, can improve strength in longstanding dystrophic conditions remains unclear. There is evidence that phosphocreatine stores are depleted in some dystrophic muscles and that creatine may have cellular protective characteristics (Kemp et al, 1993; Pulido et al, 1998). A randomize double-blind, cross-over trial, in a mixed population of dystrophies, including 12 FSHD patients, demonstrated slight improvement in overall strength following short-term (8-week) supplementation with creatine monohydrate (10.6 g/day for 10 days followed by 5.3 g/day thereafter) (Walter MC et al, 2000). Given the size of the trial and the mixed subjects population, little can be concluded as to the utility of creatine supplementation in FSHD.

Acid folic and methionine supplementation. The marked hypomethylation of the shortened D4Z4 allele or the finding of the hyopomethylation in both alleles of FSHD patients without contraction suggest that hypomethylation plays a critical role in the pathogenesis of FSHD. Folic acid and vitamin B12 are essential for the synthesis of methionine, required in the maintenance of DNA methylation. Van der Kooi et al (2006) undertook a pilot study to test the hypothesis that folic acid and methionine supplementation in FSHD can alter the methylation level at D4Z4. No such effect was noted in either FSHD or controls after 12 weeks of supplementation.

Chapter 2
Clinical protocol and FSHD score

FSHD variable onset and unpredictable progression, as well as its high clinical variability, even within the same family, and the presence of non-penetrant gene carriers, suggest that additional factors can influence the disease outcome.

As described above, the number of D4Z4 units, in the 4qter region, is considered diagnostic for the disease. However, correlation between D4Z4 repeats number and disease severity is difficult to establish and no prognostic tools are available, so far.

To accurately correlate the molecular defect with the clinical outcome a clinical data questionnaire was designed (Lamperti et al, 2010) to evaluate FSHD patients and their relatives. The main purpose of the questionnaire is to gather information about the onset and evolution of the disease and correlate the disease history with events occurring in the patients' life.

Several questions have been introduced, based on sporadic observations collected by the diverse clinicians.

A clinical evaluation form was also generated for determining the FSHD clinical score of each subject carrying a D4Z4 pathogenic allele. The clinical evaluation scale is divided into six independent sections that assess the strength and the functionality of (I) facial muscles (scored from 0 to 2); (II) scapular girdle muscles (scored from 0 to 3); (III) upper limb muscles (scored from 0 to 2); (IV) distal leg muscles (scored from 0 to 2); (V) pelvic girdle muscles (scored from 0 to 5); and (VI) abdominal muscles (scored from 0 to 1). The functional examination of six different groups of muscles, integrated with the result of the functional quantification of muscle weakness, generates the FSHD clinical score. The total score can range from 0, when no signs of muscle weakness are present, to 15, when all muscle group tested are severely impaired. The FSHD evaluation scale has been designed based on the Brooke et al. (1981) evaluation scale modified by Ricci et al. (1999) and Trevisan et al. (2006). The evaluation scale has been specifically adapted for FSHD.

To assess the reliability of the clinical evaluation protocol the concordance of the FSHD score generated by diverse clinicians through the examination of a single patient was tested. To this purpose, five meetings were organized.

Certified neurologists from 11 participating Neuromuscular Institutions were involved. For each patient, the functional test was administered by clinician and simultaneously evaluated by at least five qualified neurologists (participating at a single meeting) from the 11 Institutes of the Italian Consortium for FSHD. A functional score was assigned for each

muscle group according to the evaluation scale. All tests and evaluations were performed in a blind manner with respect to the results of molecular analysis of D4Z4 locus.

Stati stic analysis demonstrated the robustness of the clinical evaluation protocol that can therefore be used by different neurologists in large cooperative clinical studies (Lamperti et al, 2010). The relevance of having developed the FSHD score is that such a tool allows for translating what is called clinical impression of the progressive involvement of specific muscle groups into a number.

The use of the FSHD score might provide information to define the natural history of the disease throughout time. Importantly, the definition of the clinical involvement of specific muscle groups by a number will allow the identification and characterization of atypical cases. Moreover, the FSHD score can represent a useful way to gauge the efficacy of any potential treatment for FSHD (Attachment 1).

Because of the FSHD variable onset and unpredictable progression, as well as to better understand the phenotypical assessment of both the adult and the infantile form of this disease, we also designed a clinical data questionnaire about the infantile informations of FSHD patients.

We designed it on the basis on data reported in literature about the early-onset form of FSHD. The questions are related to medical conditions or events that could have been associated with an infantile onset of the disease, through which we aim to better understand the evolution and the prognosis of this form (Attachment 2).

Attachment 1. FSHD classic anamnestic records and score

Referring Hospital: *Referring physician:*
Date:
Patient's code: *Initials:*
Date of birth: *Sex:* □ M □ F
Family geographical origin:

Clinical history
Previous evaluation in other center(s) and which:

Onset

Age:
Muscle group:
□ facial muscles □ pelvic girdle muscles
□ abdominal muscles □ lower limb muscles

Asymmetry □ Yes □ No

Triggering event (if any)

Associated diseases:

Diabetes
□ Yes □ No □ Not evaluated
□ type 1 □ type 2 Age of onset: Therapy:

Thyroid Hormones alterations
□ Yes □ No □ Not evaluated
□ hypotiroidism □ hyperthiroidism Therapy:

Hepatitis
□ B □ C □ Toxic □ Not evaluated
Age of onset: Therapy:

Other disease(s)
□ Not evaluated □ Other:

Drugs:

Statins
□ No □ Yes
Type: Dosage: Duration of the treatment:
Modification of the disease: □ None □ Worsening □ Improvement

Interferon

□ No □ Yes

Dosage: Duration of the treatment:

Modification of the disease: □ None □ Worsening □ Improvement

Other treatments (chronic treatments were only considered)

□ anabolic steroids □ creatine

□ vitamins □ estroprogestinic

□ antitumoral □ other

- molecule: - molecule:

Pregnancy

□ No □ Yes Number of pregnancies:

Modification of the disease: □ None □ Worsening □ Improvement

Spontaneous abortion

□ No □ Yes

Modification of the disease: □ None □ Worsening □ Improvement

Menopause

□ No □ Yes

Physiological menopause: □ Yes □ No Age:

Hormonal therapy: □ Yes □ No

Modification of the disease: □ None □ Worsening □ Improvement

Physical activity

□ No □ Yes

Kind of sport: □ agonistic □ not agonistic

Modification of the disease: □ None □ Worsening □ Improvement

Physiokinesitherapy (PKT)

□ No □ Yes

Modification of the disease: □ None □ Worsening □ Improvement

Traumatic events

Surgical operations:

Modification of the disease: □ None □ Worsening □ Improvement

Year:

Anesthesia: □ General □ Local □ Epidural Molecule:

Family history (pedigree):

PART B

Functional evaluation:

Face:

Ability to close eyes:

□ normal □ partial □ unable to close eyes

Ability to protrude lips:

□ normal □ partial □ unable to protrude lips

Ability to puff out cheeks:

□ normal □ partial □ unable to puff out cheeks

Scapular girdle:

Ability to abduct arm:

□ whole (180°) □ complete but abnormal

□ incomplete but >45° □ incomplete but ≤45°

Pelvic girdle:

Ability to climb 4 stairs:

□ without support

□ without support but abnormally/posterior leg muscle hypotrophy

□ with support □ taken more than 12 sec/unable

Ability to walk:

□ without support □ with support □ unable

Ability to stand up from a chair

□ without support □ with support □ unable

Use of wheelchair:

□ not necessary □ with manual control

□ with electric control □ bed bound

Legs:

Ability to walk on tiptoes and/or heels:

□ normal □ on tiptoes only □ on heels only □ unable

Beevor's sign:

Beevor' sign was considered positive if, when the patient was examined in the supie position, there was an upward/downward deflection of the umbilicus on neck flexion.

□ positive □ negative

PART C

MRC score:

Scores range from 0 to 5, with .5 increments (e.g. 3, 3.5, 4, 4.5, etc)

Muscle	**Right**	**Left**
Triceps		
Biceps		
Common finger extensors		
Wrist extensors		
Long fingers flexor		
Wrist flexors		
Quadriceps		
Tibialis anterior		

Others: (trophism, lordosis, abdominal muscles, peculiarity, asymmetry)

Specific tests:

Electrocardiogram (ECG):

Pulmonary function tests (PFT) (only in wheelchair patients):

Creatine phosphokinase (CPK):

Sign:

I – Facial weakness

0 - no weakness
1 - moderate weakness:
partial ability to do at least one of the following tasks:
- to close eyes
- to protrude lips
- to puff out cheeks

2 - severe weakness
unable to do at least one of the following tasks:
- to close eyes
- to protrude lips
- to puff out cheeks

II – Scapular girdle involvement

0 - no involvement
1 - mild involvement with no limitation of arm abduction
2 - arm abduction > 45°
3 - arm abduction ≤ 45°

III – Upper limbs involvement*

0 - no involvement
1 - at least two muscles affected with MRC >3
2 - at least two muscles with MRC ≤3

*the following 4 muscles will be assessed on each side: 1. triceps; 2. biceps; 3. common finger extensor and wrist extensors; 4. long finger flexors and wrist flexors.
Only the weaker muscles will be considered for evaluation.

IV – Legs involvement

The ability to walk on tiptoes and heels will be assessed on each side:

0 - no involvement
1 - unable to walk on tiptoes or heels (only one task impaired)
2 - unable to walk on tiptoes and heels (two tasks impaired)

V – Pelvic girdle involvement

0 - no involvement
1 - able to walk and to climb stairs without support but abnormally/because of posterior leg muscle hypotrophy
2 - able to walk unaided, to climb stairs or to stand up from a chair with support
3 - able to walk unaided but unable to stand up from a chair or to climb stairs without support/more than 12 seconds
4 - able to walk with support
5 - wheelchair bound

VI – Abdominal muscle involvement

0 - no involvement
1 - presence of Beevor's sign

Attachment 2. FSHD infantile anamnestic records

Referring Hospital: *Referring physician:*
Date:
Patient's code: *Initials:*
Date of birth: *Sex:* □ M □ F
Family geographical origin:

Pregnancy

Active fetal movements	□ Yes	□ Reduced	□ Normal
	□ No		
Fetal cardiac beats	□ Normal	□ Altered	
Placental dysfunction	□ Yes	□ No	weight:
Diseases/infections	□ Yes	□ No	
Ultrasound	□ Yes	□ No	
	Routine ultrasound	□ Normal	□ Altered
	Morphological ultrasound	□ Normal	□ Altered
Therapy during pregnancy	□ Yes	Drugs:	
	□ No		

Birth

Partum	□ Eutocic	□ Dystocic	week:
Fetal position	□ Normal	□ Podalic	
Weight	Lenght	Apgar	
Revived	□ Yes	□ No	
Cianosis	□ Yes	□ No	
Mechanical ventilation	□ Yes	□ No	
Clubfoot	□ Yes	□ No	
Neonatal jaundice	□ Yes	□ No	

Perinatal period/ months after

Breastfeeding	□ Yes	□ No	
Breast Suction	□ Vigorous	□ Normal	□ Reduced
Facial nerve palsy's diagnosis	□ Yes	□ No	
Moebius syndrome's diagnosis	□ Yes	□ No	
Hip dysplasia	□ Yes	□ No	
Upper limbs	□ Normal	□ Altered	
Facial hypomimia	□ Yes	□ No	
Floppy	□ Yes	□ No	

Psychomotor development

Sitting alone: age	(months)			
Self-standing: age	(months)			
Walk alone: age	(months)			
Social smile	□ Yes	age	(months)	□ No

Familiarity

□ Yes □ No □ Maternal line □ Paternal line □ Brothers/sisters

Data collection

Mechanical ventilation	□ Yes (age:)		□ No
Vision problems	□ Yes (age:)		□ No
Hypoacusia	□ Yes		□ No
Academic success	□ Yes		□ No
Cognitive impairment	□ Yes		□ No
Loss of walking	□ Yes (age:)		□ No

First year of life

Neurological examination

Hypotonia	□ Yes	□ No
Facial mimicry	□ Normal	□ Absent
Antigravity limb movements	□ Possible	□ Impossible
Hospital admission	□ Yes	□ No
	Clinical report:	

After the first year of life

Neurological examination

See *FSHD clinical form*

Lagophtalmos	□ Yes	□ No
Gowers	□ Yes	□ No
Waddling gate	□ Yes	□ No
Steppage	□ Yes	□ No
Scapular winging	□ Yes	□ No

Instrumental tests

Blood tests	CK	UI/L		
Electrophysiological tests	EMG/ENG		□ Yes	Results:
			□ No	
	BAERs	□ Yes		Results:
			□ No	
	EEG		□ Yes	Results:
			□ No	
Neuroradiological tests	Cerebral MRI		□ Yes	Results:
			□ No	
	Muscular MRI		□ Yes	Results:
			□ No	
Eye examination	Fundus oculi		□ Yes	Results:
			□ No	

	ERG	□ Yes	Results:
		□ No	
	Fluorescein angiography	□ Yes	Results:
		□ No	
Ear examination	Audiometry	□ Yes	Results:
		□ No	

Chapter 3

Reasearch aim

A patient usually recognizes the disease in his teens but an extreme variation in onset is reported, ranging from early infancy to late fifties, even within families with all affected members carrying the same genetic lesion.

While much is known about the clinical course of adult FSHD, data on the ‘‘infantile phenotype’’ and especially on the progression of the disease in early-onset patients are limited.

The evaluation of the size of the D4Z4 alleles is the diagnostic test for FSHD. As shown in Figure 6, the presence of alleles EcoRI less than 38 Kb is considered diagnostic for the disease.

A correlation between the length of the EcoRI fragment and the clinical course was evaluated in several studies and was shown that the more severe phenotype is associated with smaller D4Z4 alleles (Zatz et al, 1995, Lunt et al, 1995; Tawil et al, 1996, Ricci et al, 1999).

Units	EcoRI size
1 U	11.9 Kb
2 U	15.2 Kb
3 U	18.5 Kb
4 U	21.8 Kb
5 U	25.1 Kb
6 U	28.4 Kb
7 U	31.7 Kb
8 U	35 Kb
9 U	38.3 Kb
10 U	41.6 Kb

Fig.6 D4Z4 units and EcoRI size

Studies extended to a larger group of patients showed that the number of repeats is a critical determinant of the age of onset and disease severity. Indeed, it was seen that alleles containing from 1 to 3 D4Z4 units are associated with a severe form of disease, those containing from 4 to 7 units are found in the most common form of the disease and alleles containing from 8 to 10 D4Z4 units are found in the most attenuated and in those with reduced penetrance (Zatz et al, 1995, Lunt et al, 1995; Tawil et al, 1996, Ricci et al, 1999).

The aim of this research is to characterize, in our cohort of FSHD patients with short EcoRI fragment (allele <20 kb), phenotypical features to better understand the clinical variability, to add data on the progression and to acquire useful informations for both diagnosis and prognosis of the early-onset form of FSHD.

Chapter 4
Materials and Methods

4.1 Clinical assessment. Our cohort of patients with a molecular diagnosis of FSHD included 35 consecutive index cases with a size of allele D4Z4 <20 kb.

All patients were evaluated according to the two clinical above mentioned questionnaires, focusing on first clinical disturbances manifested by the patient, age of onset and associated-diseases. To avoid potential patient bias, the patient or relatives answered the questionnaire with the assistance of the clinician. Either the patient's own recollection of initial symptoms or the first clinical abnormality observed by relatives, as in the case of children, were considered significant.

The clinical severity of FSHD has been numerically defined as assessed by the FSHD score, obtained by the FSHD clinical form (described above).

4.2 Molecular diagnosis. Molecular analysis was carried out at the laboratory in Modena (MIOGEN-Lab), which provides FSHD molecular diagnosis for the Italian National Health System.

The extraction method involves, at first, the isolation of leukocytes from peripheral blood and their inclusion in agarose; then, the canonical procedures for the purification of DNA are performed, so that it is protected from the agarose to the electrophoresis process. The DNA is analyzed by means of digestion with specific restriction enzymes, in particular, with EcoRI and BlnI. The restriction fragments obtained is separated according to their molecular weight, by pulsed-field electrophoresis (PFGE) on 1% agarose gel. The DNA, after the electrophoresis, is transferred from the gel to a nylon membrane by the technique of Southern blotting. The membrane is hybridized with a specific probe radioactively marked with $^{\alpha}P^{32}$ and, after appropriate washing, subjected to autoradiography. The probe used in molecular diagnostic test is called p13E-11 and recognizes a sequence proximal to the sequence of units D4Z4 repeat (locus D4F104S1). The size of restriction fragments viewed in this way are evaluated by comparing the bands of a molecular weight marker. The molecular weight of each fragment corresponds to a defined number of D4Z4 repeat units. To quantify the end of the molecular weight of less than 4q alleles of 50 kb, the genomic DNA purified by standard methods of DNA extraction with phenol-chloroform must be analysed. The DNA digested with enzymes EcoRI and BlnI restriction, is subjected to agarose gel electrophoresis to the linear concentration of 0.4% and hybridized with the probe p13E-11.

4.3 Statystical analysis. All the continuous variables were summarised by means and standard deviations (i.e. age of onset, age at evaluation, age of walking loss). Both discrete and dichotomous variables were expressed by frequencies (i.e. gender, associated-diseases). The continuous variables were tested for their normal distribution according to the Kolmogorov-Smirnov test. A p value <0.05 was considered significant to reject the null hypothesis that the considered variable is not normal. Non-parametric correlations (i.e. Spearman's correlation coefficient) were used to investigate any possible association between demographic, genetic and clinical characteristics of the patients enrolled. SIGMAPLOT ver. 11.0 (Sigmaplot Software Inc, USA) was used for the statistical analysis. A p value <0.050 was considered statistically significant.

Chapter 5

Results

5.1 Clinical features. Genetic and epidemiological characteristics of the index patients are listed in table 1.

Table 1.

Patient n.	Sex	Case	Fragment size	Age of onset (years)	Age at evaluation (years)
1	F	de novo	14	2	20
2	F	de novo	14	12	24
3	M	de novo	14	0	17
4	F	de novo	14	4	14
5	M	Spor	17	27	60
6	M	de novo	17	12	38
7	M	de novo	16	13	14
8	M	Spor	17	5	44
9	M	de novo	19	10	25
10	M	Spor	17	32	32
11	F	Fam	16	25	27
12	M	de novo	12	10	29
13	M	Fam	17	16	31
14	M	de novo	14	2	6
15	F	de novo	11	16	23
16	F	Fam	17	15	16
17	F	de novo	19	20	47
18	F	Fam	16	7	36
19	F	Spor	17	27	32
20	M	de novo	11	7	10
21	M	de novo	12	1	7
22	M	de novo	11	5	33
23	F	Spor	12	6	50
24	F	Fam	16	4	50
25	M	de novo	17	2	10
26	M	Fam	16	3	22
27	M	Fam	16	13	35
28	M	Spor	14	12	41
29	F	Spor	12	27	41
30	F	Spor	12	11	27
31	F	de novo	17	11	28
32	F	Fam	14	3	12
33	F	de novo	15	8	27
34	F	Spor	14	2	42
35	M	de novo	12	2	22

Eighteen males and 17 females were enrolled. Mean age at evaluation was 28,3 $\pm$13,3 years (range 6-60 years), while the mean age at onset was 10,6 $\pm$ 8,6 years (range 0-32 years). Interestingly, during the anamnestic data collection, in 16 out of the 35 patients (45,7%) signs of muscular weakness were already present before the declared age of onset. The predominant sign of weakness in these cases was a deficit in eye closure, which became most apparent during sleep, as well as during blowing on candles.

Eighteen patients (51,4%) had a de novo mutations, 9 (25,7%%) were sporadic (no molecular data concerning family were available) and 8 (22,9%) were familial cases.

5.2 Distribution of muscle weakness and progression. Clinical characteristics are shown in table 2.

Table 2.

Patient n.	Localization at onset	Asymmetry at onset	Score	Clinical involvement
1	Facial	No	6	
2	Scapular	Yes	13	Clubfoot Breatsfeeding problems
3	Facial	No	12	
4	Scapular	No	15	Breastfeeding problems Hip dysplasia Wheelchaired
5	Facial	No	13	
6	Scapular	Yes	7	Breastfeeding problems
7	Scapular	No	3	
8	Scapular	Yes	14	
9	Legs	Yes	8	
10	Facial	No	2	
11	Legs	No	8	
12	Scapular	Yes	13	Retinopathy
13	Facial	No	2	
14	Facial	No	8	
15	Scapular	No	3	Clubfoot
16	Scapular	No	5	
17	Legs	No	9	
18	Facial	Yes	14	Mental retardation Severe dysphagia Mechanical ventilation Wheelchaired
19	Scapular	Yes	3	
20	Scapular	No	7	
21	Scapular	No	2	Hypoacusia
22	Scapular	No	12	Breastfeeding problems Mental retardation Epilepsy Mechanical ventilation Wheelchaired
23	Scapular	Yes	9	Breastfeeding problems Hypoacusia
24	Facial	No	12	
25	Facial	No	8	Clubfoot Mental retardation Epilepsy
26	Facia	Yes	12	
27	Scapular	Yes	11	
28	Scapular	No	15	Wheelchaired
29	Scapular	No	9	
30	Facial	Yes	14	Retinopathy Hypoacusia Wheelchaired
31	Facial	Yes	12	
32	Facial	No	13	Hypoacusia Wheelchaired
33	Facial	No	13	Hypoacusia Wheelchaired
34	Facial	Yes	13	Hypoacusia Wheelchaired
35	Facial	no	13	Hypoacusia Mental retardation Wheelchaired

Thirteen patients (37,1%) showed asymmetric symptoms at onset, which were represented by:

- facial weakness 16/35 patients (45,7%)
- scapular winging or weakness during shoulder abduction 16/35 patients (45,7%)
- legs' weakness with abnormal gait 3/35 patients (8,6%)

No patients had abdominal involvement at onset.

To better evaluate the FSHD score, we divided our patients in four groups: the first one (score: between 0 and 1) consisted of patients with no significant functional deficits in any muscle group; the second group (score between 2 and 4) was made of patients with a slight involvement in one or more muscles among the facial district, shoulder girdle, upper limbs or abdominal muscles; patients whose score was between 5 and 10 showed a limited ability to raise their arms, walking on toes and/or heels; patients with a score > 10 had a general weakness in all muscle groups, associated with partial or total inability to walk. No patients had a score between 0 and 1; 6/35 (17,1%) had a score between 2 and 4; 11/35 patients (31,4%) showed a moderate severity of disease with a score between 5 and 10. Eighteen patients (51,4%) had a score >10, with a very severe phenotype.

A total of 9/35 patients (25,7%) became wheelchair-dependent at a mean age of 23,7 $\pm$ 8,7 years (range 9-37). The duration between the onset of first symptoms and the wheelchair use was 16,7$\pm$7,4 years (range 5-25).

Only two patient (5,7%) needed mechanical ventilation for a very severe restrictive lung disease (one of these patients showed also a severe dysphagia).

5.3 Additional findings. Four patients had problems during their mothers' pregnancy. In two cases there were placentar dysfunction, in the other two cases there were associated-diseases during pregnancy (i.e. gestosis in the last month of pregnancy and Herpes Symplex infection in the first trimester of pregnancy).

Eight/35 patients (22.9%) had problems during childbirth, as cyanosis and neonatal jaundice.

One patient (2.9%) showed hip dysplasia, while 3 patients (8.6%) had clubfoot that required orthopaedic treatments.

In two patients (5.7%) a retinopathy was diagnosed in childhood or adolescence; two patients (5.7%) had some tortuous retinal vessels but not true teleangectasia by fundoscopy.

Seven patients (20%) had a diagnosis of sensorineural hearing loss, needing in one case hearing aids. Four patients (11,4%) had mental retardation and 2 (5.7%) were epileptic.

Results are shown in Table 3.

Table 3. Results.

N. patients	35	%
M/F	18/17	51,4/48,6
De novo	18	51,4
Spor	9	25,7
Famil	8	22,9
Onset (years)		
≤10	19	54,3
tra 10 e 15	8	22,9
>15	8	22,9
Ancillary signs	16	45,7
Asimmetry at onset	13	37,1
Localization		
Facial	16	45,7
Scapular	16	45,7
Abdominal	0	0,0
Legs	3	8,6
Score		
between 0 and 1	0	0,0
between 2 and 4	6	17,1
between 5 and 10	11	31,4
>10	18	51,4
Problems during pregnancy	4	11,4
Problems during partum	8	22,9
Clubfoot	3	8,6
Hip dysplasia	1	2,9
Breastfeeding problems	5	14,3
Dysphagia	1	2,9
Mechanical ventilation	2	5,7
Retinopathy	2	5,7
Cognitive impairment	4	11,4
Hypoacusia	7	20,0
Epilepsy	2	5,7
Loss of walking	9	25,7

In order to understand if the numbers of D4Z4 alleles can influence either the onset or the severity of disease, we made a correlation between the allele size and both the epidemiological and clinical manifestations in our population. Statystical analysis showed a positive correlation between the allele dimension and the age of onset (p=0,032), the childbirth's problems (p= 0,013), loss of walking (p=0.037) and the hypoacusia (p= 0,018). No correlations were found between allele size and the other clinical signs. The age of onset correlated with hypoacusia (p=0.023) only. Even though 18/35 patients (51,4%%) had a FSHD score >10, there was no correlation with the number of D4Z4 units.

No correlation between the value of score and genetic data were found, neither with the age of onset of disease, but a correlation between the score and the presence of early signs of muscular weakness was detected (p=0,004).
Statistical analysis' results are shown in table 4, 5 and 6.

Table 4. Correlation between the allele and epidemiological, genetic and clinical characteristics.

		Rho	p
Sex		-0,075	0,666
Case			
	de novo	0,205	0,235
	spor	-0,003	0,984
	fam	-0,241	0,162
Age of onset		**0,362**	**0,032**
Score		-0,189	0,274
Previous signs of weakness		0,069	0,690
Pregnancy		0,195	0,260
Childbirth		**0,416**	**0,013**
Clubfoot		0,097	0,573
Hip dysplasia		0,078	0,654
Retinopathy		0,286	0,095
Cognitive impairment		0,118	0,497
Hypoacusia		**0,397**	**0,018**
Epilepsy		0,062	0,721
Loss of walking		**0,353**	**0,037**

Table 5. Correlation between the age of onset and epidemiological, genetic and clinical characteristics.

		Rho	p
Sex		0,150	0,386
Case			
	de novo	0,329	0,053
	spor	-0,305	0,074
	fam	-0,074	0,670
Allele		**0,362**	**0,032**
Score		-0,270	0,116
Previous signs of weakness		0,082	0,635
Pregnancy		-0,187	0,280
Childbirth		0,179	0,301
Clubfoot		-0,025	0,884
Hip dysplasia		0,128	0,462
Retinopathy		-0,036	0,833
Cognitive impairment		0,303	0,076
Hypoacusia		**0,383**	**0,023**
Epilepsy		0,226	0,190
Loss of walking		0,289	0,091

Table 6. Correlation between the score and epidemiological, genetic and clinical characteristics.

		Rho	p
Sex		0,160	0,357
Case			
	de novo	0,148	0,393
	spor	-0,192	0,266
	fam	0,023	0,891
Allele		-0,189	0,274
Age of onset		-0,270	0,116
Previous signs of weakness		**-0,466**	**0,004**
Pregnancy		-0,062	0,718
Childbirth		0,105	0,545
Clubfoot		0,096	0,578
Hip dysplasia		-0,282	0,099
Retinopathy		-0,282	0,099
Cognitive impairment		-0,184	0,288
Hypoacusia		-0,228	0,186
Epilepsy		0,030	0,859
Loss of walking		**-0,652**	**<0,001**

Chapter 6

Discussion and conclusion

FSHD clinical symptoms may appear in adult ages in most of the cases, but they may appear in early childhood, as well.

In our study, we reported the genetic and clinical data of a population of FSHD patients with the dimension of the allele <20 kb.

Onset and progression. In most of the cases patients become symptomatic in their adolescence, when the individual notices symptoms that reveal shoulder girdle weakness or signs of muscle wasting in this region (Jardine et al, 1994; Lunt et al, 1991; Padberg,1982; Zatz et al, 1998; Orrell, 1999). However, the true age of onset of the disease may appear several years earlier than indicated by patients complaining. In our population the mean declared age at onset was 10,6 years (range 0-32 years), but interestingly during the anamnestic data collection, 16 out of the 35 patients (45,7%) showed signs of muscular weakness before the declared age of onset, being represented in most of them by a deficit in eye closure mainly during sleep or by the inability to puff out cheeks. It represents an interesting observation because it allows us to say that the prevalence of early-onset form of FSHD may be underestimated.

We found a positive correlation between the age of onset and the dimension of the allele, confirming that, as previously reported, smaller EcoRI fragments are associated to an early-onset of the disease. There was no correlation between the age of onset and the score in our population, but we found that the presence of not-recognized early signs of muscular weakness correlates with the score. Eighteen patients (51,4%) had a score >10, with a very severe phenotype. Although the number of patients is small and this result needs to be confirmed in a larger sample, this observation further confirms that the early-onset FSHD is a progressive disease and has a more severe evolution, in accordance with other studies (Zazt et al, 1995; Lunt et al, 1995; Tawil et al, 1996; Goto et al, 1995).

51,4% of our patients were de novo mutations and eight of these (22,9%) had the more severe score (>10). Several controversial observations about the role of new mutation in the early-onset FSHD were published, but recently has been reported that in a series of 7 infantile patients followed for between 9 and 25 years at the Newcastle Neuromuscular Centre, only two patients had a de novo mutation which previously was believed to be the main underlying genetic defect in infantile FSHD (Jardine, 1994). Even though in our population no correlations were found between the disease age of onset, evolution and cases, this finding needs to be confirmed in a larger number of patients.

In our population the 25,7% of patients became wheelchair-dependent at a mean age of 23,7 ± 8,7 years (range 9-37). Seven/9 had an age of onset ≤10 years, while 2/9 had an age of onset respectively of 12 and 11 years (patient number 28 and number 30, respectively), but presented early signs of muscular weakness. Interestingly, the allele size correlated with loss of walking, showing how the early-onset form of FSHD has a more severe progression. In about half of the adult patients weakness is known to gradually descend in a characteristic fashion beginning in the face and slowly progressing to the shoulder and upper-arm musculature and then to the abdominal and foot extensors muscles. In contrast, in infantile FSHD weakness in the shoulder girdle may be preceded by pelvic girdle weakness (Klinge et al, 2006). Just 3 of our patients showed a pelvic and legs' involvement at onset. None of the patients who became wheelchaired-dependent presented legs or pelvic girdle involvement at onset. As leg weakness is easier to detect in young children than facial or shoulder girdle weakness, several parents gave a definite history of onset of legs involvement and maybe the precise sequence of involvement is unclear.

Progressive respiratory failure during childhood or in young adults is rare in patients with FSHD, affecting about 1% of the Dutch population (Wohlgemuth et al, 2004). McGarry et al reported a 5-year-old girl with early-onset FSHD who died of progressive weakness and recurrent pneumonia (McGarry et al, 1983). Yasukohchi et al (1988) reported a 13-year-old FSHD boy with progressive severe restrictive-type respiratory failure and cor pulmonale, leading to death. Nakagawa et al (1997) described 3 early-onset FSHD cases and artificial ventilator support after tracheotomy in 2 cases (respectively at the age of 22 and 41).

Only two of our patients (5,7%) need mechanical ventilation for a very severe restrictive lung disease. Our data confirm that respiratory failure is a rare complication of FSHD, but requires mechanical support to prevent death.

Associated-disorders. Extramuscular manifestations in FSHD represent a controversial clinical issue.

Some studies reported that auditory impairment was present in most FSHD patients but not significantly found to be associated with FSHD by subsequent studies (Rogers MT et al, 2002; Trevisan et al, 2008a). Recent reports pointed out that it tends to occur in those patients carrying a very short allele (Trevisan et al, 2008b). Seven of our patients (20,0%) had hypoacusia, needing in one case hearing aids. We found a correlation between hearing impairment and the dimension of EcoRI fragments (p=0,013), confirming that, as reported in literature, hearing loss tends to occur more frequently in patients with large 4q35 deletions. All but one of our patients who had hearing impairment had an onset

before the age of 10 years. The remaining patient had an onset at age 11, but showed clinical signs previously. Furthermore, the correlation between hypoacusia and the age of onset we found confirms that the hearing loss is more common in early-onset FSHD.

The term Coats' disease is generally used to describe a sporadic unilateral retinovascular disease. Shields et al. (2007) noted that retinal telangiectasia compatible with Coats' disease can be an extramuscular manifestation of FSHD but that most affected patients have asymptomatic retinal telangiectasia found at ocular screening after diagnosis of FSHD. They described a young child who had advanced eye findings of unilateral neovascular glaucoma from bilateral retinal telangiectasia 3 years before FSHD became apparent. Coats' retinopathy has been reported in those FSHD patients with the earliest onset and most severe evolution (Small, 1968; Matsuzaka et al, 1986; Miura et al,1998). Only 2 of our patients had a retinopathy: one patient had a diagnosis of Coats' disease since the age of 5 years, while the other had a diagnosis of generic retinopathy since the age of 17 years. Two other patients had some tortuous retinal vessels but not true teleangectasia by fundoscopy. Although uncommon, since retinal vasculopathy with capillary teleangiectasia is known to be associated with FSHD, every FSHD patients, especially with visual deterioration, should underwent a careful eye examination.

The involvement of central nervous system in FSHD is reported in those patients carrying larger gene deletions. Epilepsy may be observed in the context of muscular dystrophies and it may include both generalized and focal seizures. In Fukuyama congenital muscular dystrophy, epilepsy may be related to brain malformations occurring in this disorder, while in Duchenne or Becker muscular dystrophy, limb-girdle muscular dystrophy type 2A or calpainopathy, the pathogenetic mechanism of epilepsy remains unclear as patients commonly have normal brain MRI. The frequency of mental retardation in FSHD patients is estimated to be less than 2% (Funakoshi et al, 1998). Epilepsy with partial seizures was found in 2 of our patients and in both was associated to mild mental retardation. Other two of our patients had an isolated mental retardation. In all of these cases cerebral MRI was not performed. In literature, the reported cases of epilepsy or mental retardation were confined to a subset of early-onset group with a very small EcoRI fragments (10-11 kb) (Funakoshi et al, 1998). It was hypothesize that the 4q35 deletion induces a central nervous system defect, but only in cases with largest deletions (Bindoff et al, 2006). In particular, the observation that patients with a very short allele are more frequently affected by central nervous system defects, led to the hypothesis that large deletions may determine greater position effect variegation on genes within close proximity. We didn't find any correlation between the allele sizes and these associated-diseases and all our patients had a greater allele dimension (from 12 to 17 kb). Our data, in association with others

reported in literature, led us to consider the possibility that epilepsy and mental retardation are a further clinical expression of the central nervous system involvement in FSHD even in those patients with greater 4q35 fragments.

In conclusion, the early-onset FSHD patients show a more severe progression than the patients with classic form but variable phenotypes. We provide further evidences that extramuscular manifestations may occur in this form, but a close correlation between 4q35 fragments size and clinical severity is not constant. A more accurate anamnestic data collection and a more extensive instrumental screening are required to identify cases with early-onset and improve their management.

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