

## Deletion of the Williams Beuren syndrome critical region unmasks facioscapulohumeral muscular dystrophy

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### ABSTRACT

Among 1339 unrelated cases accrued by the Italian National Registry for facioscapulohumeral muscular dystrophy (FSHD), we found three unrelated cases who presented signs of Williams-Beuren Syndrome (WBS) in early childhood and later developed FSHD. All three cases carry the molecular defects associated with the two disorders. The rarity of WBS and FSHD, 1 in 7500 and 1 in 20,000 respectively, makes a random association of the two diseases unlikely. These cases open novel and unexpected interpretation of genetic findings. The nonrandom association of both FSHD and WBS points at a gene co-expression network providing hints for the identification of modules and functionally enriched pathways in the two conditions.

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### 1. Introduction

Deletion of an integral number of 3.3 Kb tandemly arrayed repeats, named D4Z4, at the subtelomeric region of chromosome 4, 4q35, is considered the hallmark of facioscapulohumeral muscular dystrophy (FSHD; OMIM 158900) [1]. Although the majority of FSHD affected people carry one D4Z4 allele with 10 or fewer repeats, in the general population there is 3% of healthy individuals carrying D4Z4 allele of the same size [2]. Thus a large number of potentially predisposed people do not develop disease and incidentally discovered carriers of D4Z4 reduced alleles do not report FSHD cases in their families. Reduced penetrance of this molecular defect is also observed in FSHD families [3,4]. There are also scattered reports of patients presenting complex clinical phenotypes, some showing

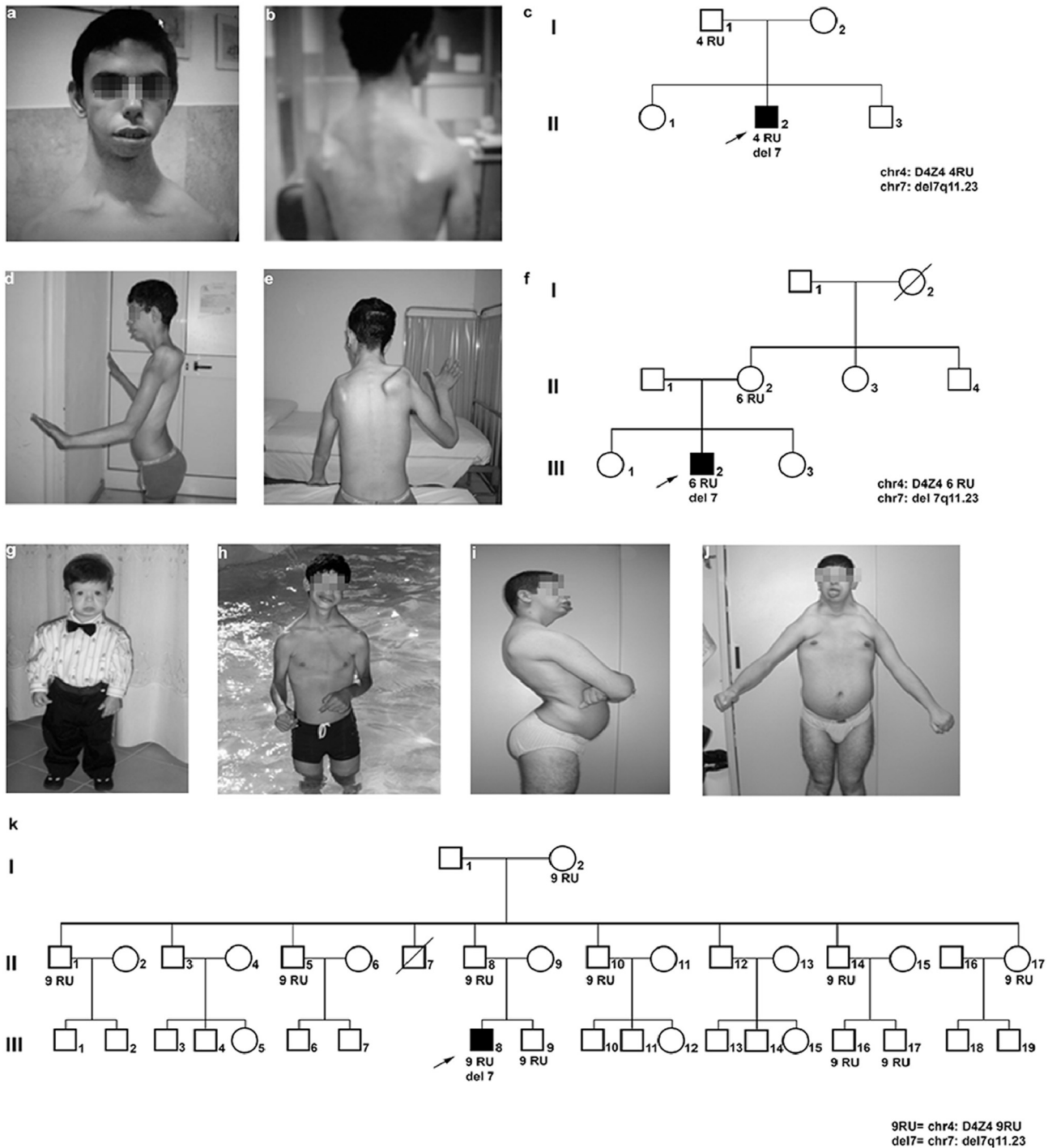
atypical distribution of muscle weakness or atypical presentation of disease onset or progression, some presenting extra-muscular features such as hearing loss, retinal vascular disease, respiratory insufficiency, cognitive impairment or epilepsy [5]. In some cases the atypical features have been attributed to mutations in other genes [6]. All these observations indicate that the wide phenotypic spectrum observed in individuals carrying D4Z4 reduced alleles is determined by the genetic background and, possibly, by environmental factors. We thus reasoned that families including healthy carriers as well as subjects with atypical features might facilitate the identification of genetic components that contribute or interfere with the clinical phenotype.

We screened the Italian National Registry for FSHD (INRF) for cases with complex phenotypes in families with reduced

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**Fig. 1. Representative photographs and pedigrees from subjects with concomitant facioscapulohumeral muscular dystrophy and Williams-Beuren Syndrome.** Photographs of Case 1 obtained at 14 years show pouty lips, sloping shoulders and straight clavicle (A) and winging and asymmetric shoulders, scoliosis (B). Family Pedegree of Case 1 (C). Photographs of case 2 obtained at 12 years show lumbar hyperlordosis (D); asymmetric scapular winging with periscapular hypotrophy and limited abduction (E). Family Pedegree of Case 2 (panel F). Photographs of case 3: at the age of 2 years the patients shows full cheeks and pouty lips (G); at the age of 10 he displays years straight clavicles, axillary plicae and bilateral elevation of trapezius muscle are evident (H), at the age of 29 years he has severe lumbar hyperlordosis (I) and marked difficulty in raising arms with polyhill sign (panel J). Family pedigree of Case 3 (panel K). RU, Repeat Units.

penetrance. By reviewing 1339 unrelated index cases and 1271 relatives from the INRF, all carrying at least one D4Z4 deleted allele we found three cases presenting both FSHD and Williams-Beuren syndrome (WBS; OMIM 194050) due to the typical 7q11.23 deletion.

## 2. Methods

### 2.1. Genetic studies

We performed standard molecular diagnostic procedures for WBS and FSHD. Our institutional ethics committee approved the

**Table 1**  
Synopsis of overlapping clinical features in FSHD and WBS.

Clinical feature	FSHD	WBS
musculoskeletal weakness (muscle hypotonia)	++++	++
Myopathy	++++	++
down sloping shoulder	+++	+++
Hyperlordosis	+++	++
Scoliosis	++	++
Pectus excavatum	+++	++
elevated serum creatine phosphokinase	+++	++
global cognitive impairment	+	++++
mild-to-moderate high-tone sensorineural hearing loss	++	++
narrow face	+	++++
hypoplasia of the zygomatic bone	+	+++
malar flattening	+	++
Micrognathia	+	++++
thick lower lip vermilion	++	++++
Dysarthria	+	+
joint contraction (adult)	+	+

List of selected symptoms described in WBS cases from the Human Phenotype Ontology (HPO) database. Frequency of symptoms are detailed as follows: ++++ 80%–99% of people have these symptoms; +++ 30%–79% of people have these symptoms; ++ 5%–29% of people have these symptoms; + sporadically observed.

study, Informed written consent was obtained from the patient's parents in accordance with the ethical standards of the 1964 Declaration of Helsinki.

### 3. Results

#### 3.1. Case 1

At the age of 3 years because of aortic and pulmonary valve stenosis, hypotonia, delayed developmental milestones and typical facial features the patient was investigated for suspected Williams-Beuren Syndrome (WBS). Molecular diagnosis revealed the typical 1.55 Mb deletion on chromosome 7q11.23. At 11 years he had difficulty in raising arms and underwent surgical scapular fixation, without beneficial effect. At the age of 14 years he presented thick lower lip vermilion, sloping shoulders, straight clavicles, bilateral scapular winging asymmetric shoulders and scoliosis (Fig. 1A and B), hypotrophy of periscapular muscles with difficulties in raising arms above shoulders, hypotrophy of *pectoralis* and triceps muscles and quadriceps muscles. Neuromuscular examination disclosed *orbicularis oculis* and *oris* muscle weakness, waddling gait with bilateral “steppage”, lumbar hyperlordosis, weakness of neck flexors, *pectoralis*, triceps and wrist extensors muscles; at lower limbs weakness of iliopsoas, quadriceps and tibialis anterior muscle was detected. Deep tendon reflexes were weak at the upper limbs. He had a moderate intellectual deficit (IQ 46) with impaired visuospatial skills and social disinhibition; EEG and brain MRI showed no abnormalities. Echocardiography confirmed the presence of aortic and pulmonary valve stenosis; a depression of the ST segment and the T wave inversion were evident at ECG. Serum Creatine Kinase (CK) was slightly increased (401 U/L). Electromyography showed myopathic pattern. Molecular testing revealed one D4Z4 allele with 4 Repeat Units (RU) on chromosome 4q35, supporting the diagnosis of FSHD. The healthy father carries the same reduced D4Z4 allele (Fig. 1C).

#### 3.2. Case 2

At the age of 16 months, because of craniofacial dysmorphism (esotropia, periorbital puffiness, short nose, full cheeks, thick lower lip vermilion, small chin and bilateral temporal narrowing), delayed motor skills with difficulty in walking and standing up,

with muscle weakness, the finding of depression of the ST segment at ECG and aortic valve stenosis at echocardiography, the patient was investigated for suspected WBS. Molecular diagnosis revealed a 1.55 Mb deletion at 7q11.23, consistent with WBS diagnosis. Walking was achieved at 17 months; at the same age a moderate increase of CK was detected (300–400 UI/L). At 12 years of age, he presented scapular winging and asymmetric shoulders, wasting of the pectoral and upper limb muscles, bilateral axillary plicae, inability to raise arms above 45°, dorsal scoliosis, lumbar hyperlordosis, thick lower lip vermilion and lagophthalmos evocating FSHD (Fig. 1D and E). He had a moderate intellectual deficit (IQ 48), whereas EEG and brain MRI did not reveal significant abnormalities. Molecular analysis showed one D4Z4 allele with 6 RU. The same allele was detected in his mother, to date totally asymptomatic (Fig. 1F).

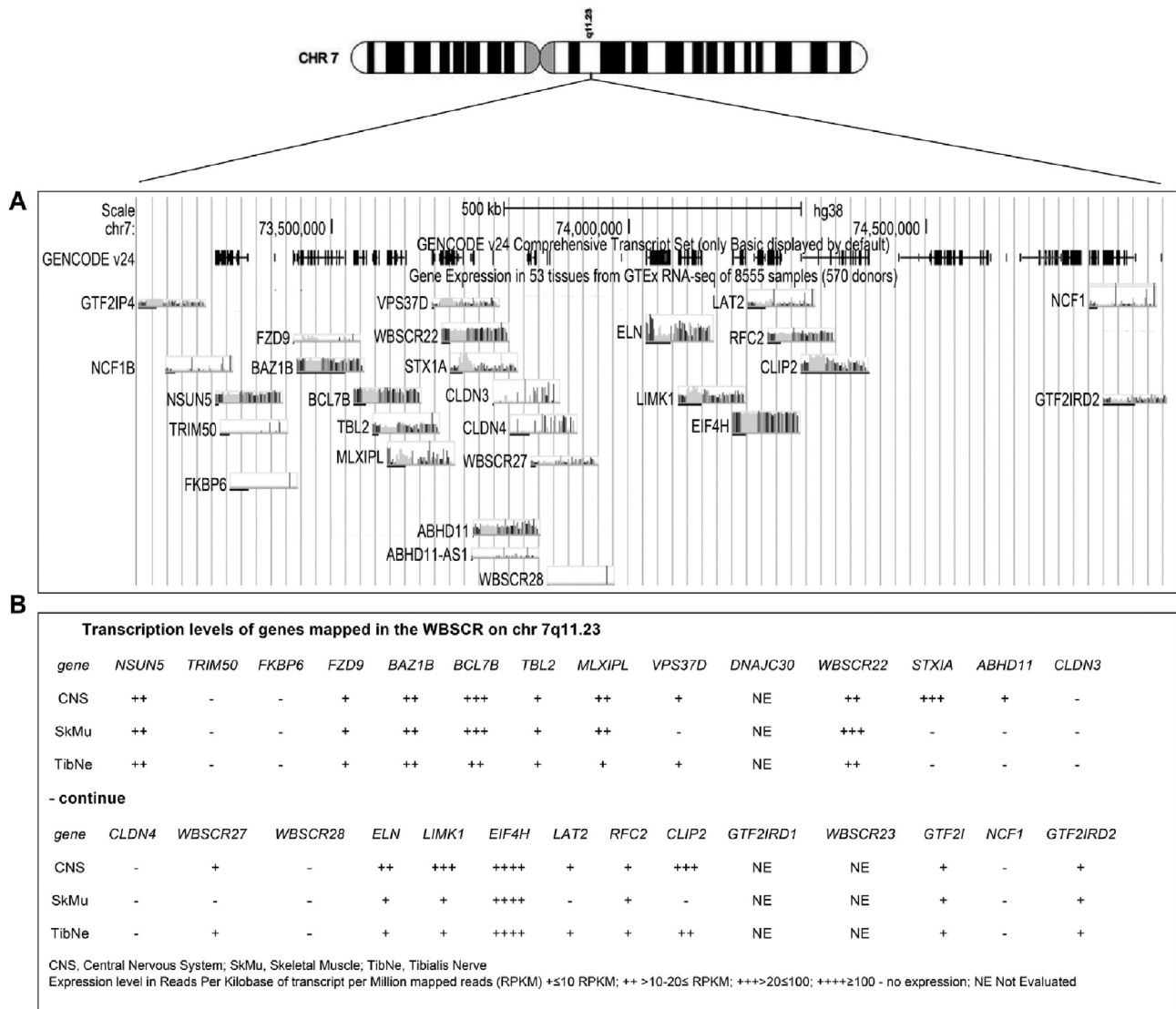
#### 3.3. Case 3

At 11 months of age because of motor skill delay, typical facial dysmorphism (Fig. 1G), aortic and pulmonary valve stenosis, joint hypermobility and fair chest the patient was investigated for suspected WBS. Molecular analysis showed a 1.55 Mb deletion on chromosome 7q11.23. At the age of 10 years he showed asymmetric facial weakness, and moderate muscle weakness, more evident at shoulder girdle, with straight clavicles and axillary fold (Fig. 1H). At this age a moderate intellectual deficit was also evident: I.Q. was 49, with significant discrepancy between the higher level verbal tests (I.Q. 59) and the other performances (I.Q. 42). EEG was normal, brain MRI was not performed. A depression of the ST segment with T wave inversion were present at ECG. Muscular features worsened through time and, at age 29 years, he has a clear myopathy with facial involvement, severed lumbar hyperlordosis and prevalent upper limbs wasting and weakness with difficulty in raising the arms (Fig. 1I and J). CK levels were normal. Molecular testing for FSHD revealed one D4Z4 allele with 9 RU. Several asymptomatic relatives (I.2, II.1, II.5, II.8, II.14, II.17, III.9, III.16, III.17) carry the same D4Z4 allele with 9 RU as shown in Fig. 1K.

### 4. Discussion

WBS and FSHD are two rare autosomal dominant disorders, with an estimated prevalence of 1 in 7,500, and 1 in 20,000 individuals respectively [7,8]. Both syndromes are clinically recognizable and distinguishable: WBS patients present with a combination of specific cardiovascular disease (specifically elastin arteriopathy, peripheral pulmonary stenosis, supravalvular aortic stenosis and hypertension), typical facial features, connective tissue abnormalities, intellectual disability, a specific cognitive profile, unique personality characteristics, growth abnormalities, musculoskeletal abnormalities and endocrine abnormalities [9]. FSHD patients manifest a selective progressive weakness of the facial and scapular girdle muscles with subsequent involvement of the foot dorsiflexors and pelvic girdle muscles [10]. In both WBS and FSHD, wide spectra of phenotypes are observed in association with the molecular defects characteristic of each disease. We describe and discuss some similarities about these two syndromes and we consider the clinical clues for the diagnosis and future perspectives.

Our findings of three cases carrying the hallmark mutations of both FSHD and WBS suggest that there is a non-random association between the two different chromosomal loci. Indeed among 1339 unrelated FSHD index cases carrying D4Z4 reduced alleles accrued in the INRF the prevalence of WBS was 22.4 (4.6–65.5) in 10,000. This value is significantly higher (Poisson exact test  $p$  value = 0.0008) than the expected (1 in 7500) or (1.3 in 10,000), suggesting that they result from a synergistic mechanism. In each



**Fig. 2. Schematic representation of the Williams-Beuren Chromosome region.** (A) The Williams-Beuren syndrome is characterized by interstitial deletion of 1.55–1.8 Mb on chromosome 7, at 7q11.23. An enlarged view of the Williams-Beuren Chromosome Region (WBSR) includes genes within the region and their expression profile in multiple tissues obtained by next-generation sequencing technologies (RNA-seq) as reported in the GTEx RNAseq database. (B) Transcription levels of the WBSR genes in Central Nervous System (CNS), Skeletal Muscle (SkMu) and Tibialis Nerve (TibNe) are detailed as follows: + ≤10 RPKM; ++ >10–20 ≤ RPKM; +++ >20 ≤100; ++++ ≥100; - no expression; NE Not Evaluated. RPKM, Reads Per Kilobase of transcript per Million mapped reads.

one of the three families reported in this study the co-occurrence of the WBS chromosome region deletion not only determines the presence of clinical features of WBS, but it appears to contribute to the development of muscle weakness typical of FSHD in a carrier of a D4Z4 deleted allele. In fact all probands developed very severe muscle impairment at a young age, whereas their relatives carrying the same reduced D4Z4 allele are asymptomatic at adult age. These findings are consistent with data pointing at the possibility that, in the heterozygous state, a D4Z4 contraction might produce a sub-clinical condition that requires other genetic or epigenetic mechanisms or contributing factors to cause an overt myopathy [2].

Another hint for a possible direct interaction of the two mutations on the phenotype comes from some similarities between these two syndromes as summarized in Table 1 A “myopathic picture” (not dystrophic) has been frequently described as part of the spectrum of WBS [11]. In particular, dysmorphic facial features, such as thick lower lip vermillion, horizontal smile, weak orbicularis oculi muscles, and anomalies in the spine curvature, indicating the

presence of muscle weakness, are observed in WBS [12]. Moreover some FSHD subjects present extra-muscular involvement such as retinal vascular disease, high-tone hearing loss, cognitive impairment and epilepsy [5]. Additionally, in the group of patients carrying D4Z4 alleles with 1–3 repeats, we observed cognitive impairment in 35% of FSHD cases presenting extra-muscular comorbidities [5].

The 1.8 Mb WBS chromosome region (chr7:73,150,048–74,950,182) contains 28 genes, reported in Fig. 2B. Thirteen of them are expressed in skeletal muscle, central nervous system and tibialis nerve (as reported by the GTEx RNAseq database). Among these genes, *ELN*, which encodes elastin, is the only gene with a definite effect in WBS. Its haploinsufficiency accounts for arteriopathy and heterozygous mutations in *ELN* have been associated with autosomal dominant *cutis laxa* (OMIM 123700) and supravalvular aortic stenosis (OMIM 185700). Interestingly its expression was found specifically reduced in FSHD myoblasts, suggesting a possible involvement of elastin in FSHD cellular

phenotype [12]. Apart from *ELN* the role in disease pathogenic mechanism of genes mapping to the WBS chromosome region remains to be elucidated [13].

Do genes located within WBS chromosome region or D4Z4 deleted alleles act as modifiers of the FSHD or WBS phenotypes? Which traces should we follow? FSHD cases showing atypical clinical features, including mental disability, arterial stenosis, should be considered for further molecular investigations. We also suggest to suspect the presence of one D4Z4 reduced allele in WBS patients, when there is a prominent involvement of shoulder girdle with winging scapulae, peroneal muscle weakness, increased CK levels, marked hyperlordosis, even if facial muscles seems to be spared, in the context of the typical appearance of WBS face.

These three cases with the co-presence of the molecular defects associate with two rare diseases WBS and FSHD provide concrete evidences of the molecular interactions that might underlie the two diseases. From a molecular point of view, several observations indicate that the spectrum of clinical variations in WBS is controlled by multiple genes. Genotype-phenotype studies on WBS suggest that the telomeric part of the WBSCR has a relevant role in the clinical presentation of the syndrome, but no definite gene has been identified [14]. In the field of FSHD there are accumulating evidences that the reduction of D4Z4 repetitive elements is not per se sufficient to cause disease [2]. It is also definite that multiple phenotypes can be found in people carrying the same molecular markers including healthy people or subjects with other diseases [6,15].

This nonrandom association of both FSHD and WBS reveals a gene co-expression network that provides hints for identifying modules and functionally enriched pathways at the basis of the patients' phenotypes. For instance, mice deficient in *EIF4H*, an important factor for the initiation of translation, are small with reduced endurance to fatigue [16]. *FZD9* is expressed in developing skeletal muscles during NMJ synaptogenesis and its differential expression influences AChR clustering [17]. *MLXIPL* is a glucose-responsive transcription factor, which increases its activity in response to changes in glucose levels and promotes myogenesis by inducing the expression of several myokines [18]. In addition *GTF2IRD1* may play an important role in fiber-specific muscle gene expression at early stages of muscle development [19].

The cases described here highlight the possibility that several genes, deleted in WBS, could operate on the same molecular network compromised in FSHD, acting as modifier genes. Indeed, the use of massive parallel sequencing for diagnostic purposes has revealed more than one mutation or copy number variations in individuals with a rare disease. This finding has been interpreted as the co-occurrence of independent mutations [20]. However these findings pose particular challenges for clinical practice, including diagnosis, prognosis, genetic counseling and design of clinical trials. Here we show that a thorough clinical work, including systematic detailed description of phenotypes can open novel and unexpected interpretation of genetic findings. This approach centered on the phenotype analysis has the potential of shedding new light on the complex molecular interactions whose alteration leads to rare diseases and their clinical expression.

#### Author contributions

CR and RT planned, supervised the study, and wrote the manuscript. LPo provided clinical data and contributed to the writing of the manuscript. SP, LB, TB, LPa provided clinical data and

collected the consent forms. TB undertook the data extraction and contributed to the writing of the manuscript. FS performed statistical analysis. SM performed molecular analysis and contributed to the writing of the manuscript.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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