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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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
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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 subclinical 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 vermilion, 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

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 Syndrome Chromosome Region (WBSCR) 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 WBSCR 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.
that the telomeric part of the WBSCR has a relevant role in the by multiple genes. Genotype-phenotype studies on WBS suggest indicate that the spectrum of clinical variations in WBS is controlled from a molecular point of view, several observations suggest that the telomeric part of the WBSchr has a relevant role in the study, but no definite gene has been identified. In the field of FSHD there are accumulating evidences that the reduction of D4Z4 repetitive elements is not per se sufficient to cause disease. 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.

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 Elf4H, an important factor for the initiation of translation, are small with reduced endurance to fatigue, and Fzd9 is expressed in developing skeletal muscles during Nm synaptogenesis as an its differential expression influences Achr clustering. 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. In addition Gtf2ird1 may play an important role in fiber-specific muscle gene expression at early stages of muscle development.

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. 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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