LETTER TO THE EDITOR

Dominant LGMD2A: alternative diagnosis or hidden digenism?

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Sir,

We read with great interest the work recently published in Brain (Vissing et al., 2016), in which the findings on limb girdle muscular dystrophy families from the UK, Denmark and Sweden are described. The cases showed a segregation pattern compatible with a dominant transmission. All the affected cases except for one share a heterozygous mutation in the CAPN3 gene previously described in recessive forms of limb girdle muscular dystrophy 2A (LGMD2A) (Richard et al., 1997; Groen et al., 2007). The mutation located within a common haplotype, suggests a common ancestral origin of these families, probably spread over a territory that shared a past Viking settlement as described for other mutations (Pliner et al., 2014).

The possibility of an alternative inheritance pattern of the same phenotype, associated with mutations in the same gene, is a well-known scenario in several myopathies. Besides the myotonia congenita, the collagen-related myopathies and the desminopathies referred to by the authors (Vissing et al., 2016), there are also other well characterized examples such as RYR1 mutations associated to a central core myopathy (Klein et al., 2012; Snoeck et al., 2015) and mutations in the TTN gene responsible for dominant distal myopathy or a recessive variant of limb girdle muscular dystrophy (LGMD2J) (Hackman et al., 2002, 2003; Udd et al., 2005). Furthermore, in other recessive limb girdle muscular dystrophies, heterozygous carriers may present subclinical symptoms [creatine kinase (CK) elevations and/or alterations in MRI] associated with a reduction of the protein (Fischer et al., 2003; Brummer et al., 2005; Illa et al., 2007).

In this context, the understanding of this phenomenon in LGMD2A would be of great interest to shed some light on the knowledge of its pathophysiology, as it remains unclear. However, we believe that the information provided by the authors presents some gaps that may raise doubts about the plausibility of their hypothesis, and therefore, deserves to be clarified or at least constructively discussed.

From a clinical point of view, the information provided by the authors is not uniform: only Families 1, 2, 3, 4 and 7 accomplish clearly with a dominant pattern of transmission. In Family 5, Case III-2 is 21 years old and only presents myalgia with unknown levels of CK. From Family 6, data for only one patient are available (despite the referred clinical antecedents of myopathy in his mother and grandmother). In Families 8 and 9, the dominant pattern is supported by information on family history without data. The clinical features for Case 9/I-2 are very doubtful: a female aged 85 with a muscle biopsy with unspecified myopathic changes, normal CK and proximal weakness in legs (Family 9). Relevant clinical information is also missing in Case 10/I-2 (Family 10). Regarding the apparently more affected cases, there is a great clinical variability among a subgroup of subjects (Cases 9/II-2, 8/III-3, 2/I-2 and 1/II-7) and the rest of the patients. This group is composed of aged patients (except for Case 2/I-2 with unknown data) with clinical onset between 18 and 30 years, high CK levels, myopathic traits in muscular biopsy and a severe clinical course. Conversely, the rest of the cases are mildly affected or asymptomatic and with mild elevations of CK level. MRI of paraspinal muscles corresponds to aged patients...
and no images of these muscles in younger cases (Cases 1/III-4, 1/III-6, 1/III-16 and 1/IV-1) were provided.

Since the original description (Fardeau et al., 1996), subsequently completed by several authors (Urtasun et al., 1998; De Paula et al., 2002; Angelini et al., 2010; Luo et al., 2012; Richard et al., 2016), LGMD2A is described as a myopathy with a preferential onset in adolescence and early youth showing a characteristic topographic impairment and evolutive pattern. Despite that the age at onset determines further evolution (Urtasun et al., 1998), forms with pseudometabolic features at early stages (such as cramps, exercise intolerance or myalgia), as happens in several patients of the series, appear to be associated with a more benign prognosis (Pénisson-Besnier et al., 1998). Although the origin of these phenotypic differences is unclear, there is increasing evidence that suggests a link between the location of the mutation and/or the existence of phenomena of intermolecular complementation (Saéz et al., 2011; Ono et al., 2014), which may affect differently the putative structural or enzymatic function of calpain 3 (Ojima et al., 2011). Regarding the radiological pattern, some of the reported images are certainly suggestive of LGMD2A but other entities could not be definitively ruled out. For example, titinopathies at later stages may present a similar pattern (Díaz-Manera et al., 2015; Fischer et al., 2016). On the other hand, paraspinal involvement in aged subjects (with camptocormia or not) is a frequent event within myopathic or undetermined origin diseases (Ghosh et al., 2015; Witting et al., 2016). It has been described even in a case of heterozygote carrier of a mutation in the CAPN3 gene (Liewluck and Goodman, 2012). Taking this into account, as mentioned for other limb girdle muscular dystrophies, it cannot be ruled out definitively that slightly affected cases reported in several families are symptomatic carriers. Furthermore, the interpretation of the biopsies as proved calpainopathies could be argued given the relatively frequent presence of both false positive and negative cases (Saéz et al., 2005).

From a molecular point of view, we want to point out two controversial aspects: (i) is this a complete study to detect a possible second mutation in the CAPN3 gene (to exclude a pseudodominance)? and (ii) could the apparently dominant segregation be explained by an alternative interpretation?

Regarding the first point, it is not likely that the mutation (associated by chance with a second mutation in all cases) had escaped the usual detection methods except in an environment of great consanguinity, which does not seem the case. On the other hand, the molecular study of the CAPN3 gene should rule out mutations in the promoter and UTR regions of the gene, big deletions, or even mutations in deep intronic regions using RNA (Blázquez et al., 2008).

Another aspect of molecular screening that could be considered incomplete is the possible alteration of other gene regulatory elements, such as exonic enhancers (Ahituv, 2016). These patients could show a reduced expression of the CAPN3 healthy allele as a result of a polymorphism in an exonic enhancer, present either into the mutated CAPN3 allele itself or in another unrelated gene. Given the putative common origin of the families it would be plausible that this polymorphism could be located within the ancestral haplotype ensuring its co-segregation along with the mutation in successive generations. This polymorphism should be able to downregulate the expression of CAPN3 in the healthy allele.

The mutation found in these families has been previously described in combination with at least four other mutations, c.2362_2363delinsTCATCT, c.550delA, c.133G>A and c.1256A>G (Richard et al., 1997; Groen et al., 2007; Vissing et al., 2016). If available, it would be interesting to compare the molecular data (complete sequence of the CAPN3 gene and extended haplotype) between the heterozygote cases of these families and the previously described compound heterozygotes in order to exclude the presence of possible modifiers. This analysis could be completed by the study of a genome-wide associate study that might reveal other alternative changes (the study of Case I/II-4, which does not present any clinical symptoms, could be revealing). Taking into account the common origin of the families, they could be analysed together as previously performed for other neurodegenerative diseases (Moreno et al., 2011).

There is another plausible explanation. These patients, besides the mutation in the CAPN3 gene, may also carry a second mutation in another gene related to a neuromuscular condition transmitted with a dominant pattern. This would justify both the clinical and molecular findings. Among the known genes, the titin (TTN) gene would be an obvious candidate for several reasons; first, for the possibility of a dominant transmission, and second, because its deficit may induce a secondary decrease of calpain 3 in the western blot. The above proposed genome-wide associate study could identify mutations in TTN or alternatively, in other genes that may act as exonic regulators of the healthy allele of CAPN3, as shown in other processes (Van Deerlin et al., 2010).

The authors provide alternative explanations that are interesting but also speculative. For instance, they claim that active CAPN3 is a homodimer, and that due to the altered CAPN3, the complex is rendered inactive. However, as reported by Ono and colleagues (2016), dimerization of full-length CAPN3 molecules has not yet been described.

To summarize, we believe that the work of Vissing and colleagues (2016) is relevant for a better understanding of the pathophysiology of LGMD2A. Nevertheless, to be complete, it would require clarification of some conundrums and drawing on all the practical information that these families could provide.
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