LETTER TO THE EDITOR

Reply: Dominant LGMD2A: alternative diagnosis or hidden digenism?

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Sir,

We thank Drs Sa´enz and Lo´ pez de Munain for their interest in our recently published paper describing a dominant transmission of calpainopathy associated with a muscle disease, resembling limb girdle muscular dystrophy type 2A (LGMD2A) (Vissing et al., 2016). The authors, however, challenge some of the conclusions of our paper and suggest alternative genetic explanations for our findings, which we will respond to in the following.

First, the authors notice a great variability in the phenotype of our patients, and that dominant inheritance could only clearly be demonstrated in half of the 10 reported families. Variability of phenotype is to be expected if the dominant form of calpainopathy resembles the recessive type, which was what we found. Indeed, LGMD2A is one of the recessive limb girdle types (LGMDs) with the greatest clinical variability. It may present as a classical LGMD2 with symmetric limb weakness starting in the lower limbs and with elevated creatine kinase (CK), but unlike other LGMDs, it is often asymmetric and can start with upper limb weakness first (Angelini et al., 2010), and CK doesn’t have to be elevated (Luo et al., 2012). Furthermore, it may even present with distal weakness (Burke et al., 2010; Angelini et al., 2010; Luo et al., 2012). The age of onset of symptoms is also highly variable, and has been shown to be associated with the genotype (Angelini et al., 2010; Luo et al., 2012; Richard et al., 2016). Thus, there is nothing strange about a great clinical variability of calpainopathies. The pattern of affection in the dominant form of calpainopathy we report (Vissing et al., 2016), closely resembles that of the recessive LGMD2A, but with a later onset and milder phenotype, which is in accordance with numerous other myopathies with a similar dual pattern of inheritance. The authors criticize missing clinical information on some of the patients, which is inevitable when data are gathered from multiple generations in families from multiple sites in four countries. On a similar note, the authors criticize that definite dominant inheritance could only be established in half of the reported families. We agree that some families could not prove the dominant inheritance alone, although they were all suggestive of such inheritance, but they were of course included to support the main conclusions, because the findings in these families were all supportive of a dominant form of calpainopathy. We think the inclusion of these smaller families strengthens the study, although they cannot stand alone. In fact, with an allele frequency of the mutation of 0.006% among individuals of European decent, it would be highly unlikely to identify these families just by chance, emphasizing the association between heterozygosity of c.643_663del21 and muscle disease.

The authors, although acknowledging similarities to LGMD2A in terms of muscle findings on MRI, still question whether our patients could have been affected in some other genes than CAPN3, of which they mention the titin gene as a possibility, in part because titinopathies may have secondary CAPN3 deficiency. First, the titin gene (TTN) is located on chromosome 2 whereas CAPN3 is located on chromosome 15. The two genes are thus expected to segregate independently. What is the likelihood that an aberration in TTN would segregate in 36 individuals from the 10 families in an identical pattern as demonstrated for the CAPN3 deletion we report? It is not only unlikely, but bordering on impossible from a statistical point of view. Furthermore,
TTN was examined in two of the reported patients, in whom no pathogenic TTN variants could be found.

The authors state that paraspinal muscle imaging was shown only in older patients (age 49–74 years), possibly inferring that the findings could be an age phenomenon. However, the images showed an almost total replacement of paraspinal muscles in all of the cases, which is never observed in a healthy person.

The authors also suggest that calpain 3 deficiency could have been found just by chance. It is true that calpain 3 deficiency is variable even for patients affected by LGMD2A, and that downregulation of calpain 3 expression may occur in other myopathies. Still, a severe downregulation of calpain 3 to estimated 15% of normal or less was found in all nine patients in whom western blot of muscle was performed in our study. This is highly suggestive of a disease affecting calpain 3.

The authors acknowledge that a second mutation in CAPN3 likely could not have been missed in all the unrelated, tested subjects, with which we agree, but they still argue that a second CAPN3 mutation should have been ruled out. This was in fact done in several patients, in whom cDNA analyses of CAPN3 showed normal expression of the other allele, which was also reported in the paper. Moreover, the molecular analyses were performed in several independent laboratories, reducing the risk of a systematic failure.

The authors also suggest that an exonic enhancer, either placed in or near the mutated CAPN3 or in another gene, could have downregulated CAPN3 from the healthy allele. Again, as with the suggestion that an entirely different gene aberration could be responsible for the disease, the statistical likelihood for such an enhancer segregating with the disease in the 10 families, as the in-frame CAPN3 deletion did, is negligible, and identifying a putative polymorphism placed elsewhere in the genome would have been exceedingly difficult, and virtually impossible to reach a conclusion from. As described and referred to in our paper, cDNA analysis of muscle mRNA did not indicate any biased allele expression nor did we identify any additional suspicious variant, which argues against the speculative idea, that a second linked variant within CAPN3 is the main causative variant. Even if this would be the case, it still results in a dominantly inherited form of calpain 3 deficiency, which is the main point of our paper.

Further to the issue of exonic modifiers, the authors suggest investigating previously reported families affected by the 21-base pair deletion of CAPN3 in combination with other CAPN3 variants to look for possible modifiers by sequencing CAPN3 in all and complementing with genome-wide association study to identify potential associations. First, these families are not available to us, and even if they were, we find it speculative that exonic modifiers should segregate, in all 10 families, identically to the CAPN3 mutation we reported. It is however important to assess carriers of c.643_663del21 clinically, as they might exhibit unrecognized symptoms of LGMD.

Finally, LGMD2 is per definition always a recessive form of muscle disease, and therefore the title of the letter of ‘dominant LGMD2A’ is inherently contradictory. Dominant calpainopathy or LGMDII is in better keeping with the current nomenclature.

In conclusion, it is our opinion that although some clinical data are missing from the 10 reported families, and although some of the families do not alone prove a dominant inheritance of a myopathy linked to the 21-base pair deletion of CAPN3, then collectively the total body of evidence proves a dominant inheritance of this aberration, giving rise to a myopathy resembling its recessive counterpart, LGMD2A, albeit in a milder form. We think some of the concerns have been addressed by our response, and welcome further molecular studies in this new disease entity in the future, and look forward to the reporting of other cases of dominantly inherited calpainopathy cases, of which we are currently aware of one other mutation in CAPN3 giving rise to this dominant form of calpainopathy.

References


