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KCTD17 is a confirmed new gene for dystonia, but is it responsible for *SGCE*-negative myoclonus-dystonia?

Over the past decade, the increasingly widespread use of next-generation sequencing (NGS), and in particular of whole-exome sequencing (WES), has allowed the identification of genes responsible for Mendelian diseases at an extraordinary and unprecedented pace.

In these times of relatively easy and fast gene discovery, a frequent pitfall of NGS is false assignment of pathogenicity. What this means is that not all published studies reporting novel causal associations between genes and Mendelian diseases are based on robust and correct conclusions, an issue that bears obvious and negative consequences not only for patients (i.e. incorrect genetic diagnosis, prognosis and family and career planning) but also for correct allocation of disease-oriented research funds.

As far as it concerns rare Mendelian disorders, false assignment of pathogenicity derives mostly from the fact that small or moderately sized pedigrees, which would have not been amenable for rigorous mapping studies through linkage analysis in the pre-NGS era, are now easily studied thanks to WES. In such pedigrees, it is not uncommon that WES analysis may detect candidate mutations in biologically plausible and compelling genes, though without the statistical corroboration of linkage analysis. However, one should keep in mind that some of these mutations could simply segregate by chance, while the true causative mutation is actually missed, either due to limitation of WES (i.e. mosaicisms, copy-number variants, repeat expansions, intronic mutations affecting expression, heteroplasmic mutations of mitochondrial DNA) or because it was not prioritized being located in a "less compelling" gene (i.e. genes with uncharacterized function).

Caution and rigorous scrutiny should be therefore applied before causally linking a novel candidate gene to a disease. Several criteria have been proposed to reduce the likelihood of false assignment of pathogenicity, including the identification of multiple segregating mutations in independent pedigrees, assessment of the variant frequency in large datasets of genetic variation and/or in ethnically matched controls and functional validation of the effect of the mutations on the function of the encoded proteins [1].

Ultimately, the most convincing piece of evidence for validating a novel disease-gene association would always derive from the replication of the finding by independent research groups. Unfortunately, this step may not always be straightforward, the main reason being the rarity of some of these findings.

The field of dystonia genetics has been no exception to this scenario; in the NGS era dominant and recessive mutations in several genes, including *CIZ1* [2], *ANO3* [3], *GNAL* [4], *HPCA* [5], *COL6A3* [6], *VPS16* [7] have been linked to isolated dystonias, a type of dystonia where other movement disorders or involvement of other neurological systems are not observed [8]. Furthermore, *KMT2B* mutations have been recently recognized as a prominent cause of generalized dystonia with onset in childhood, often accompanied by other minor clinical features, such as short stature, mild dysmorphisms and intellectual disability [9].

Myoclonus-Dystonia Syndrome (MDS) is a particular subtype of combined dystonia, characterized by dystonic movements associated with prominent non-epileptic myoclonic jerks [10]. The major genetic cause of MDS are heterozygous loss-of-function mutations in *SGCE*, which are detected in 20–50% of cases [11,12]. However, a substantial number of cases remains genetically undiagnosed in spite of a highly similar phenotype. Mutations in the genes *KCTD17* [13], *CACNA1B* [14] *and RELN* [15] have been recently proposed to be novel disease-causing genes for MDS. Furthermore, mutations in some other dystonia-genes involved in pre-or post-synaptic dopamine signaling (e.g. *TH* [16] or *ADCY5* mutations [17]) have also been linked to a MDS-like presentation.

In the field of dystonia, the identification of *PRKRA* mutations represents a perfect example of the struggle discussed above. In 2008, a *PRKRA* homozygous mutation was first described and reported in few distantly related families with autosomal recessive dystonia of Brazilian descent [18]. However, we had to wait more than 6 years before other unrelated cases appeared in the literature [19]. It is easy to conceive how this slowness may leave several novel gene-disease associations in a limbo: wrong gene or simply too rare to be replicated?

Amongst the recently reported genes linked to monogenic dystonias, *GNAL* [20], *ANO3* [21], *KMT2B* [22] and more recently also *HPCA* [23], have all been conclusively confirmed to be disease causing. On the other hand, the pathogenicity of some others has been seriously questioned by replication studies (i.e. *CACNA1B* [24] and *COL6A3* [25]). For some others, the jury is still out (i.e. *CIZ1*, *RELN, VPS16*).

A heterozygous missense mutation in *KCTD17* (c.434 G > A; p.R145H) was recently described in two pedigrees with a clinical diagnosis of *SGCE*-negative MDS. The mutation was initially identified thanks to a combination of traditional linkage analysis and WES in a large dominant British pedigree comprising 8 affected subjects. The same amino acid change was then also detected in a dominant German family with MDS. Haplotype analysis excluded a common founder, strongly suggesting the mutations had arisen independently in the two families and further supporting the causal nature of the *KCTD17* mutation.

In this issue of Parkinsonism and Related Disorders, two independent manuscripts by Graziola and colleagues [26] and Marcé-Grau and colleagues [27] report the identification of two novel cases affected by a combination of myoclonus and dystonic features carrying *de novo* mutations in *KCTD17*. Almost 4 years after the initial report, these reports confirm that heterozygous mutations in this gene are a

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bona fide cause of dystonia.

Importantly, the two new cases contribute to define and expand the clinical presentation caused by pathogenic *KCTD17* mutations. Both cases share several clinical features with the previously reported patients with the missense p.R145H mutation. The movement disorder starts early in life (range of age at onset 3–10 years) and, at least in the initial phases, the most prominent disease feature appears to be limb non–epileptic myoclonic jerks of subcortical origin (as documented by the EEG-EMG studies conducted by Graziola et al.) associated distal choreatic movements and relatively mild dystonic features.

Subsequently, as clearly demonstrated by the case by Marcé-Grau et al., dystonia tends to progress significantly, affecting the four limbs, trunk and oromandibular and laryngeal muscle and becoming in adult years the most disabling aspect of the disease.

Interestingly, the adolescent patient reported by Marcé-Grau et al. shows more severe dystonic features than similarly aged cases carrying the *KCTD17* p.R145H. This aspect, together with the fact that both cases with *de novo* splicing *KCTD17* mutations also presented mild delayed motor development, a feature not present in any of the original cases with the p.R145H, suggests that *KCTD17* splice-site mutations may be associated with a more severe presentation.

The question is then whether *KCTD17* mutations cause a movement disorder that truly mimics *SGCE*-MDS and could potentially explain the genetics of some of the several genetically undiagnosed patients with MDS?

Recently, Roze and colleagues have proposed novel diagnostic criteria to define clinically “typical” MDS, based on the phenotype of *SCGE* mutated cases. They also suggest that typical MDS should be differentiated from other similar movement disorders where dystonia and myoclonus co-exist, which they suggest should be referred to as “Myoclonic-Dystonia” [10].

Amongst others, major criteria to define MDS are (i) Myoclonus should be isolated or predominant over dystonia and (ii) Truncal dystonia should be absent. Furthermore, as a minor criterium, they suggest spontaneous improvement of dystonia during childhood or adolescence.

Based on these considerations, it is clear how several differences exist between the phenotype of *KCTD17* and *SGCE* mutated cases. Firstly, the intensity, abruptness and the functional consequence of myoclonus are far milder in *KCTD17*-mutated patients then in patients with *SGCE* mutations. Secondly, the bodily distribution of myoclonus appears to be much less pronounced in proximal upper extremities and neck. Thirdly, dystonia is progressive and functionally disabling in *KCTD17* patients, a very rare finding in *SGCE* cases. Finally, none of the cases with *KCTD17* mutations reported to date show psychiatric comorbidities or improvement of myoclonus with alcohol intake, two hallmarks of *SGCE*-related MDS.

These differences in the clinical presentation may reflect different neural substrates of the two genetic entities; *SGCE*-related MDS is thought to derive mainly from dysfunction of cerebellar networks [28], while it has been shown that *KCTD17* is mostly expressed in striatal tissue [13].

One final clinical remark concerns response of dystonia to deep brain stimulation (DBS) of the internal globus pallidus (GPi) in patients with *KCTD17* mutations. Marcé-Grau and colleagues describe a clear amelioration of tongue and mandibular dystonia resulting in improved speech intelligibility in their 19-year-old patient with an overall reduction of the BFMDRS score of 50% after four months. In the original publication, a 58-year-old patient underwent GPi DBS with marked improvement of cervical dystonia and myoclonus. Furthermore, a 70-year-old member of the original British pedigree with *KCTD17* p.R145H was recently implanted with bilateral GPi DBS with significant improvement of her dystonia (Mencacci, personal communication). Although of limited evidence, these positive findings suggest *KCTD17* mutations as a genetic predictor for a beneficial outcome of DBS.

Finally, these case reports expand the genetic heterogeneity of *KCTD17*-related dystonia to include pathogenic heterozygous splice-site mutations. Interestingly, the two cases carried contiguous point mutations c.508-2A > T and c.508-1G > C (transcript NM_001282684) affecting the same essential acceptor splice-site upstream of exon 5. As expected, both mutations result in the exact same effect on mRNA extracted from patients' fibroblasts, which includes skipping of the first 35 nucleotides of exon 5 and the introduction of a premature stop codon in exon 7. Graziola and colleagues showed reduced KCTD17 protein levels (~50%) in patient's fibroblasts, suggesting haplo-insufficiency as a possible disease mechanism. Marcé-Grau and colleagues did not check protein levels in their case's cells. Intriguingly, gnomAD (the largest publicly available repository of human genetic variation) lists 12 heterozygous loss-of-function mutations (including a combination of frameshift, spice-site and stop-gain variants) in a total of 13 subjects. This observation may have three possible explanations: 1) Symptomatic subjects with pathogenic *KCTD17* mutations are contained in publicly available datasets; 2) Pathogenic *KCTD17* mutations can be associated with incomplete penetrance; 3) Not all loss-of-function *KCTD17* mutations are pathogenic. Similar considerations also apply to a recently described pathogenic heterozygous splice-site mutation found in a family with *ADCY5*-related dyskinesias [29], whilst many other loss-of-function mutations are listed in gnomAD. Future studies are warranted to address whether the disease mechanism of identified pathogenic splice-site mutations involves a dominant negative effect of the mutant protein product on wild-type protein product.

In conclusion, *KCTD17* mutations are a confirmed genetic cause for a combination of dystonia and myoclonus with an early-onset. The phenotype is in many respects different from *SCGE*-related MDS including potential developmental delay and differences in severity, evolution and distribution of both myoclonus and dystonia. Testing for *KCTD17* mutations is thus not only advisable in *SGCE*-negative patients with MDS but should also be considered in patients with early-onset isolated dystonia or myoclonic-dystonia regardless of whether further family members are affected.

Conflicts of interest

The authors report no competing interest.

Financial disclosure

The authors do not have any conflict of interest.

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