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LETTER TO THE EDITOR

Reply: SCA23 and prodynorphin: is it time for gene retraction?

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Sir,

With the introduction of next generation sequencing into research and the clinic, geneticists now face the problem of variant interpretation in single cases, which is a challenging task due to the lack of data from additional affected family members. For the majority of the variants in our genome we do not know whether they are damaging and pathogenic or just harmless rare polymorphisms. We therefore must rely heavily on *in silico* prediction programs and the information found in large genetic databases such as ExAC [Exome Aggregation Consortium (ExAC), Cambridge, MA] (http://exac.broadinstitute.org) to predict the pathogenicity of variants. The letter from Pedroso *et al.* (2016) addresses this problem.

Pedroso *et al.* (2016) state that many of the reported SCA23 mutations in *PDYN* are present in ExAC and suggest that this brings into question the validity of *PDYN* as the disease-causing gene for SCA23. They point out that the SCA23 mutations were detected in 37 of 60 700 individuals in ExAC, a database that was founded in 2014, 4 years after our original SCA23 report was published, when none of these *PDYN* variations had been detected in controls or any genetic database, supporting our conclusion that *PDYN* is the SCA23 disease gene. ExAC combines multi-ethnic sequencing data from several sources, as it includes sequencing studies on heart disease, type 2 diabetes, schizophrenia, bipolar disorder, and Tourette's syndrome, besides projects such as 1000 Genomes. This means that ExAC clearly must contain disease-causing variants and by definition does not represent the genetic background of the general population, and this may be reflected in higher minor allele frequencies than anticipated for SCA23. SCA23 prevalence reflects only those patients who have been diagnosed with the disease in a population, and are hard to establish given the rarity of the disease. Occurrences of mutations within a population database could reflect everyone who will ever get the disease, including individuals not yet diagnosed and those who will never get a proper diagnosis. Finally, the ExAC browser does not contain clinical information, and the browser disclaimer clearly states it cannot fully exclude the possibility of some samples being of a cancerous origin. Clearly there are very valid reasons to question using ExAC to exclude variations in a gene as being causative.

Pedroso *et al.* also claim all reported *PDYN* variants to be disease-causing, which is not true. We clearly mention in the work described in Jezierska *et al.* (2013), and not in Fogel *et al.* (2012) as claimed by Pedroso *et al.* (2016), that the c.616C > T, p.Arg206Cys variant (MAF ExaC; 0.0001318) and the c.617G > A, p.Arg206His variant might well be rare polymorphisms as the p.Arg206Cys variant did not have any effect on processing of PDYN, and no co-segregation could be detected for p.Arg206His (Jezierska *et al.*, 2013). Therefore, we did not conclude that these variants exhibit pathogenic effects. Indeed, we are quite conservative about reporting pathogenicity for variants that are not reported in ExAC, such as the p.Gly227Asp variant, or for variants that have not shown pathogenicity in functional validation studies, such as the p.Arg206Cys variant. Our work clearly showed that we do not address pathogenicity solely by using *in silico* predictions and database screening, but deliver substantial functional evidence (Bakalkin *et al.*, 2010; Jezierska *et al.*, 2013; Smeets *et al.*, 2015).

Recently, we have clearly demonstrated that mutant *PDYN* causes SCA23 (Smeets *et al.*, 2015). Mice expressing *PDYN* with the p.Arg212Trp mutation exhibited gait deficits at 3 months of age and general loss of motor coordination and Purkinje cell loss at 12 months of age, recapitulating the symptoms of SCA23 patients accurately. These effects were not seen in mice expressing wild-type

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PDYN. Furthermore, while *PDYN* is widely expressed in the CNS, the expression of mutant *PDYN* only causes cerebellar pathology in mice, just like SCA23 patients, mimicking human SCA23 pathology in detail.

Pedroso *et al.* point out that loss-of-function and missense mutations are well tolerated in the heterozygous state. This does not, however, exclude *PDYN* as the disease-causing gene for an autosomal dominant disease such as SCA23. A Fisher's exact test displayed significant enrichment of *PDYN* variants in ataxia cases ($P = 0.0238$), indicating that *PDYN* sequence variation—including loss-of-function and missense mutations—is not well tolerated (Jezierska *et al.*, 2013). This indicates an increased genetic burden of *PDYN* in ataxia cases, and clearly contradicts the statement of Pedroso *et al.* Additionally, the majority of mutations reported for SCA23 are missense mutations, while Pedroso *et al.* deemed them loss-of-function mutations. Mutations are only categorized as loss-of-function after functional validation of the variant—thereby validating pathogenicity. Notably, in autosomal dominant disorders, most missense mutations exhibit a gain-of-function rather than a loss (Roberts *et al.*, 2007; Liu *et al.*, 2010; Giudicessi *et al.*, 2011; Wemhöner *et al.*, 2015), and we have demonstrated a gain-of-function for several of the SCA23 mutations using the increase in dynorphin A peptide levels as the outcome measure (Bakalkin *et al.*, 2010; Jezierska *et al.*, 2013; Smeets *et al.*, 2015).

Furthermore, prior to the identification of *PDYN* as the SCA23 gene, we sequenced many likely candidate genes in the two-point linkage analysis interval but did not detect any mutations (Verbeek, 2009). The odds that a gene in this region other than *PDYN* contains a variation that could cause SCA are extremely low, and these odds are reduced to zero by our validation studies. Recently, MacArthur *et al.* (2014) published guidelines for establishing causality of variants causing human disease and our work on *PDYN* meets important criteria to this end, namely (i) detection of segregation of the p.Ser138Arg variant in a large Dutch pedigree; (ii) identification of multiple independent SCA families carrying additional mutations in *PDYN*; and (iii) experimental validation of the predicted damaging impact of candidate variants in cell models and a mouse model (Bakalkin *et al.*, 2010; Jezierska *et al.*, 2013; Smeets *et al.*, 2015).

In conclusion, our original genetic study and comprehensive functional studies (Bakalkin *et al.*, 2010; Jezierska *et al.*, 2013; Smeets *et al.*, 2015) demonstrate that *PDYN* is the SCA23 disease gene. The fact that we published validation studies of *PDYN* variants that did not show a damaging effect indicates that we (i) do not solely rely on *in silico* predictions and databases to determine pathogenicity of a novel variation; and (ii) do not report all identified variations as pathogenic. While we agree with Pedroso *et al.* that genetic variations should be thoroughly functionally validated before they are reported as disease-causing, and reported variations without functional validation should be critically reviewed, we do not agree that this is the case for *PDYN* and SCA23.

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