Are CHCHD10 mutations indeed associated with familial amyotrophic lateral sclerosis?

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Sir,

A recent study by Bannwarth et al. (2014) in Brain identified a novel mutation (c.176C > T; p.Ser59Leu) in the CHCHD10 gene that segregates in a family presenting with ataxia, myopathy, hearing loss as well as motor neuron disease and frontotemporal dementia (FTD). Furthermore they identified a second family with ALS/FTD harbouring the identical mutation making this a gene of interest in the pathogenesis of ALS/FTD. For this reason we read with great interest the letter by Müller et al. (2014) that describes the identification of two novel variants in CHCHD10 in three pedigrees with familial amyotrophic lateral sclerosis (ALS). The authors state that their findings provide strong support for CHCHD10 being a novel ALS gene. Although their findings are highly interesting and their conclusion is appealing, we feel obliged to make several remarks concerning the genetic evidence provided to support this statement.

The definition of genetic evidence, driven by the problem of non-replicating findings, is a matter of concern over the past years and has led to multiple consensus definitions for different study designs (reviewed by Pulit et al., 2014). A landmark paper published nearly 20 years ago defined segregation of the gene with the studied phenotype within a pedigree resulting in a LOD score \( \geq 3 \) as robust genetic evidence (Lander and Kruglyak, 1995). For genome-wide association studies the problem of multiple testing was overcome by adopting a significance threshold of \( P < 5 \times 10^{-8} \) on a genome-wide level and \( P < 5 \times 10^{-7} \) on an exome-wide level (Dudbridge et al., 2008; Pe’er et al., 2008; Do et al., 2012). Although these measures might seem conservative and are hard to meet in smaller studies, relaxing these thresholds in the past has led to an unfortunately high number of false positive associations: of 166 associations with more than two follow-up studies, only six (3.6%) replicated (Hirschhorn et al., 2002).

In the study by Müller et al. (2014) whole exome sequencing data for 125 index patients from different ALS pedigrees was obtained, which is a common start-off point in a hypothesis-generating study to find new ALS genes (Müller et al., 2014). The results presented, however, include the results for CHCHD10 only. The question is to what extent this study truly represents a replication approach since the authors investigate all variants in CHCHD10 among familial ALS cases and the report by Bannwarth et al. (2014) identified one specific CHCHD10 mutation in a very distinct sub-phenotype of ALS/FTD. Furthermore, since it is unlikely that this data set will be used to test this hypothesis exclusively, their results should be seen in the context of a large exome-wide hypothesis-generating search that goes along with the burden of multiple testing requiring stringent control of false positive findings.

After identifying two novel mutations (p.Arg15Leu and p.Gly66Val) in three different pedigrees the authors state that there is an autosomal dominant mode of inheritance with incomplete penetrance. Unfortunately, the authors do not provide LOD score calculations, taking potential non-penetrance into account, which could support segregation within these pedigrees. Indeed, screening in pedigree B identified the mutation in four out of six unaffected family members and pedigree A includes three obligate carriers that are apparently unaffected. Although it is not impossible for the p.Arg15Leu mutation to be the causal variant in these pedigrees, one could argue that the high number of unaffected carriers pleads against this and raises the question...
whether there are other non-tested variants that show better segregation and higher penetrance.

Furthermore, the authors describe that the novel variants were not observed in public databases such as the 1000 Genomes and Exome Variant Server (EVS) databases nor in an in-house database of exome variants. Although the presence of variants in these public databases of supposedly healthy individuals has proven to be a powerful filtering tool, absence of a variant in these databases does not provide evidence for pathogenicity. This is especially true for rare variants since they are geographically clustered and therefore more likely to be absent in the public databases containing individuals from unmatched populations (Nelson et al., 2012).

To illustrate the aforementioned points we investigated how many genes fulfill these criteria in an equally sized set of 125 non-related population-based Dutch control individuals. These individuals were randomly sampled from a larger data set that was whole-genome sequenced on the Illumina HiSeq2500 platform using 100 bp paired-end reads at minimal 30X coverage. After sequence alignment, variant calling was performed using the Isaac Variant Caller. Stringent quality control was performed for all variant calls (details available on request). In total we found 458 genes that harboured a novel (i.e. not in 1000 Genomes or EVS database) non-synonymous variant shared by two control subjects in combination with a second novel mutation in a third subject. These 458 genes represent 0.88% of the currently known RefSeq genes and it is thus unlikely that the 1070 novel variants found in these genes are highly pathogenic considering the studied individuals did not share a pre-defined phenotype by definition.

Finally, the assumption that the novel variants in CHCHD10 indeed cause ALS/FTD in these families is derived from the fact that these variants are well conserved across different species. Conservation of codons, however, does not provide evidence for an association with ALS/FTD, but merely provides circumstantial evidence that it may be important to the protein’s function. Indeed, as many as 38.3% of the novel variants in our control population are also conserved (phyloP ≥ 2).

In conclusion, we acknowledge the need to further investigate the role of CHCHD10 mutations in ALS/FTD and the data set presented by Müller et al. (2014) is extremely valuable in an effort to find new genes associated with ALS. Moreover, we cannot provide solid evidence that the two described CHCHD10 mutations are not the cause of ALS in the described families. We merely argue the absence of convincing evidence thereof at this time. Studies designed to test a single variant in whole exome sequencing data sets are the product of a study that tests multiple hypotheses and therefore need adequate error control. The field of ALS genetics is moving towards the discovery of rare variants with incomplete penetrance, which are a challenge to unequivocally link to a disease. Nevertheless, we therefore encourage the efforts to provide solid genetic evidence by means of true segregation in families, where both affected and unaffected subjects are tested, or genome/exome-wide significant associations in well-powered cohorts also including patients from pedigrees with more than one person affected with ALS/FTD.

References


