52 Whole-exome sequencing identifies novel Machado-Joseph disease-modifying genes and pathways

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Introduction: Machado-Joseph disease (MJD/SCA3), a neurodegenerative polyglutamine disorder, exhibits marked clinical heterogeneity. The size of the (CAG)n at *ATXN3* explains 50-70% of the variation in age at onset (AO), suggesting the involvement of other factors, namely genetic, whose identification remains limited. We aimed to find novel genetic modifiers, analyse their epistatic effects, and identify disease-modifying pathways which contribute to MJD variable expressivity.

Subjects and methods: We performed whole-exome sequencing (WES) in a discovery sample, comprising four AO-concordant and four AO-discordant pairs of first-degree Azorean patients, to identify candidate variants which differed inside each discordant pair, but the same in members of each concordant pair; those variants were then tested in a multi-origin cohort of 282 MJD patients. All genes carrying frameshift and missense variants as well as genes with variants showing a modifier effect in the genotype-phenotype correlations were tested in a transgenic (TG) *C. elegans* model of MJD by silencing the expression of their orthologues using RNAi.

Results: WES analysis allowed the identification of 233 variants; 82 variants in 53 genes were used in downstream analyses. Gene enrichment analyses revealed 18 over-represented pathways relevant for the nervous system, namely the neuregulin signalling and the agrin interactions at neuromuscular junction. Variants at nine genes modulate AO in MJD, with those identified in three genes showing consistent effects across cohorts of different geographical origins. Silencing of five genes significantly restored locomotion deficits and decreased the neuronal aggregation of human ataxin-3 in the *C. elegans* model. Network analyses of the novel 15 MJD modifiers highlighted several important molecular interactions, including genes/proteins previously related with MJD pathogenesis.

Conclusions: We describe novel pathways, modifiers, and their interaction partners, providing a broader molecular portrait of AO modulation in MJD and opening new avenues for the development of disease-modifying therapies.