Published in final edited form as:

Nat Neurosci. 2013 July ; 16(7): 774–775. doi:10.1038/nn.3444.

CRESTing the ALS mountain

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that primarily affects motor neurons of the brain and spinal cord. Whereas about 10% of ALS cases are familial (caused by a genetic mutation inherited from one's parents), most are sporadic in nature. Although recent studies have made inroads into unraveling the genomic architecture of the familial form of this disease, only a small fraction of sporadic cases can be attributed to a known genetic factor. In the Brief Communication from Chesiet *al.*¹ appearing in this issue of *Nature Neuroscience*, the authors used trio analysis (Fig. 1) to identify *de novo* variants potentially underlying ALS, finding a mutation in the chromatin regulator *SS18L1*, also known as *CREST*.

To uncover *de novo* variants that may contribute to disease, the authors undertook exome sequencing in 47 ALS trios from North America, each comprising an affected proband with sporadic ALS and both unaffected parents. This elegant and relatively new genetic approach has proven fruitful in developmental and psychiatric conditions, such as autism spectrum disorder and schizophrenia^{2,3}. Lending credence to this approach, several investigators have identified a handful of ALS patients carrying *de novo* mutations in *FUS* and *SOD1*, genes already known to be pathogenic^{4–6}. Until now, however, no one had systematically looked across the genome to discover new disease-associated genes.

Using this formidable genomic approach, Chesiet *al.*¹ pinpointed 25 genes that each harbor a previously unreported, heterozygous, non-synonymous, *de novo* variant. Although 12 trios carried a single variant, 6 harbored more than one hit. Annotation analysis indicated that the 25 genes carrying *de novo* mutations were significantly enriched for chromatin regulators, with 5 encoding proteins involved in this pathway. Applying the same technique to exome sequence data suggested that this enrichment was ALS specific².

Despite the prospective riches residing in these data, not all coding *de novo* variants are necessarily pathogenic. Thus, the authors sought to functionally characterize one of these chromatin regulators, *SS18L1*. They chose this particular gene because one of the trio

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Competing financial interests: Bryan Traynor has a patent pending on the clinical testing and therapeutic intervention for the hexanucleotide repeat expansion of C9ORF72

Competing financial interests: The authors declare competing financial interests: details are available in the online version of the paper (doi:10.1038/nn.3444).

proband harbored a premature stop variant at codon 388 that was predicted to be detrimental to the encoded protein. Examination of exome sequence data from 62 cases of familial ALS revealed a patient carrying a missense variant in *CREST*. This alteration was absent in public databases and in nearly 700 control individuals screened. Such validating genetic evidence in multiple ALS and control cohorts is essential, as the sporadic nature of these trios prevents any attempt to determine whether a genetic variant segregates with the disease phenotype across the family of interest.

To investigate the result of these *CREST* variants, the team leveraged the role of the protein in the neuron-specific chromatin remodeling complex nBAF, which regulates activity-induced dendrite outgrowth⁷. Transient transfection of primary mouse neurons with wild-type *Crest* had little effect on potassium chloride-induced dendrite outgrowth. However, expressing mutant *Crest*1–393 (the mouse ortholog of human *CREST*1–388) significantly inhibited dendrite outgrowth in this system. These functional data demonstrate that expression of the observed *CREST* variants causes neuronal dysfunction.

The authors bolstered the notion that *CREST* variants are pathogenic by showing that mouse *Crest* and *Fus* proteins interact *in vivo*. The importance of this observation stems from the fact that mutations in *FUS* account for approximately 4% of familial ALS, and these individuals develop *FUS*-positive neuronal cytoplasmic inclusions^{8,9}. Using mass spectrometry, Chesiet *et al.*¹ detected *Fus* protein associated with nBAF complexes purified from mouse primary neurons. Immunoprecipitation analyses in nuclear lysates extracted from the same neuronal type demonstrated physical interaction between *Crest* and *Fus*.

Previous work in the literature, including reports from the same research group, has examined prion-like domains in proteins associated with neurodegenerative diseases. These domains^{10,11} may regulate pathogenic aggregation. Such permissive templating might also help explain the apparent spread of pathological changes seen in several conditions, including Parkinson's disease, Alzheimer's disease and ALS¹². The new results further contribute to this hypothesis, as bioinformatic analyses predicted¹ that *CREST* contains prion-like domains.

Much remains to be understood about the role of chromatin modification in ALS. Histone deacetylase inhibitors have shown partial success at reducing motor neuron death and motor dysfunction in ALS mouse models¹³. Furthermore, histone acetylation is now recognized as a critical component of memory formation¹³. Given that mice carrying mutations in the nBAF subunit *Baf53b* show deficits in synaptic plasticity¹⁴, one could speculate that variants in *CREST* or another nBAF component might cause the related neurodegenerative condition frontotemporal dementia. Indeed, the authors point out that several of the identified *de novo* variants fit into a growing narrative describing mutations in nBAF subunits and other chromatin regulator genes that underlie human syndromes characterized by cognitive impairment and/or psychiatric features¹⁵. However, the lack of genetic validation for the other chromatin regulators uncovered in this new study advises caution before concluding that chromatin dysregulation is an overarching mechanism contributing to ALS pathogenesis.

The detected interaction between Crest and Fus is potentially of great importance, but many questions need to be answered. Is this interaction recapitulated in humans? Is it affected by the observed *CREST* variants? Does *CREST* colocalize with *FUS* in ALS inclusions? If so, might this mislocalization perturb normal nBAF chromatin remodeling activity, and could this outcome be central to the pathogenesis of *CREST*-linked ALS? A related question is whether the protein's prion-like domain is involved in any of these effects. These critical issues await further investigation.

Although the genomic architecture of familial ALS is about two-thirds complete, sporadic ALS is still comparatively unresolved, with only about 11% explained. Until now, progress in our understanding of the commoner, sporadic form of ALS had come from mutational screening of familial genes identified elsewhere. This underscores the importance of the genetic data reported by Chesiet *al.*¹ They open the treasure chest of unresolved sporadic ALS by directly screening sporadic cases, independent of our existing knowledge of familial genes. We believe that similar sequencing approaches will yield further riches in the near future.

So where do we go from here? And what of the other 24 *de novo* variants detected in this study? The authors suggest sequencing these hits in larger patient populations. Indeed, large sequencing studies will form the bedrock for elucidating the unresolved genomic architecture of ALS. Certainly more genes will be found, and some of the variants described here may well be validated. Chesiet *al.*¹ have mined the first vein, but undoubtedly many others await discovery.

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Fig. 1.