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PARKINSON'S DISEASE

Guilt by genetic association

Certain sequence variants of the α-synuclein gene are linked to the risk of Parkinson's disease. An analysis of these variants using gene-editing technology provides a possible explanation for this increased risk.

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Genome-wide association studies have identified swathes of the human genome in which DNA sequence changes are associated with an altered likelihood that an individual will develop a given disorder, such as Parkinson's disease[1]. But the implicated DNA regions typically contain many tightly linked sequence variants that are co-inherited through the generations, and most of these are probably not involved in disease. It may therefore be impossible to identify the true culprit or culprits using genetic association studies alone. For this reason, although multiple variants in *SNCA*, the gene that encodes α-synuclein, have been associated with an increased lifetime risk of Parkinson's disease[2], the mechanisms by which they alter risk have remained enigmatic. To uncover the effects of two such *SNCA* variants, Soldner *et al.*[3] have turned to the sophisticated gene-editing technique CRISPR–Cas9. In a paper online in *Nature*, they report that one sequence variant increases *SNCA* expression in human neurons by reducing DNA binding of proteins that inhibit transcription.

Single bases that vary between individuals are called single nucleotide polymorphisms (SNPs). The SNP variants in *SNCA* that have been most strongly associated with sporadic Parkinson's disease increase lifetime disease risk by around 30% (ref. 2). More than half of the world's population carries these risk-associated *SNCA* variants[4], making an understanding of their effects of paramount importance to public health.

None of the SNPs commonly associated with sporadic Parkinson's disease are predicted to alter the amino-acid sequence of the α-synuclein protein — in contrast to rare familial forms of the disease, which can be caused by changes in protein-coding regions of *SNCA*. It has therefore been proposed[5] that the common variants might instead modify gene expression. Consistent with this theory, high levels of α-synuclein accumulate in the brain tissues of people with Parkinson's disease, in abnormal neural aggregates called Lewy body inclusions that typify the disorder. Furthermore, some familial forms of the disease are caused by duplications of the entire *SNCA* gene, which leads to greatly elevated expression levels.

In pursuit of SNP variants that underlie an increased risk of Parkinson's disease, Soldner *et al.* focused on a suspect non-coding region within *SNCA*. A previous analysis[6] of human brain tissue charted molecular modifications to DNA-binding proteins that might alter gene expression and found that this region contained footprints characteristic of regulatory elements called enhancers, which influence gene expression. Soldner and colleagues investigated the region in a manner reminiscent of the precise forensic reconstruction of a crime scene, making use of CRISPR–Cas9 technology. This allows precise deletion and replacement of specific DNA sequences[7].

The investigators started with human embryonic stem cells (which can give rise to all bodily cell types) taken from an individual presumed to be unaffected by Parkinson's disease. Using CRISPR–Cas9 editing, they precisely excised a 500-base-pair stretch of DNA containing the suspect enhancer region from each of the cells' two copies of *SNCA*, which lies on chromosome 4. There are two known risk-associated SNPs in this region, called rs356168 and rs3756054. At each SNP, one variant seems to be associated with a higher risk of Parkinson's disease, whereas a different base is associated with a lower risk. Soldner *et al.* reintroduced any one of four possible SNP combinations into one of the two *SNCA* copies before inducing the human embryonic stem cells to differentiate into either neural precursors or neurons.

Next, the authors interrogated the genetically re-engineered cells using an innovative approach that precisely quantified the relative level of *SNCA* messenger RNA transcribed from each chromosome. The variant at rs3756054 had no effect on expression. But, remarkably, expression was 10–20% higher from chromosomes harbouring the high-risk-associated rs356168 variant than from those with the low-risk variant or those in which the enhancer was deleted (Fig. 1). Two inhibitory transcription factors, EMX2 and NKX6-1, normally bind to the DNA around this SNP, and the researchers report evidence to suggest that increased *SNCA* expression might be a direct consequence of reduced binding by these proteins to the risk variant.

Taken together, Soldner and colleagues' findings support a model whereby levels of *SNCA* expression — whether increased subtly by the presence of the high-risk variant at rs356168 or drastically, as in rare familial gene duplications — are highly correlated with the risk of Parkinson's disease. Another exciting aspect of the study is that it offers a general framework for dissecting the mechanisms underlying common

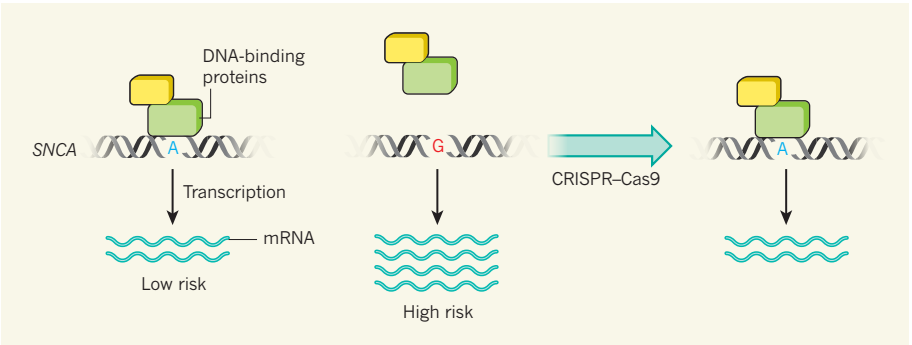

Figure 1 | A CRISPR cross-examination. At one nucleotide in a non-protein-coding region of *SNCA*, the gene that encodes α-synuclein, the presence of the base adenine (A) is protective against Parkinson's disease, whereas the presence of another, guanine (G), confers increased risk. Soldner *et al.*[3] report that this region regulates *SNCA* expression levels. If the two copies of the chromosome in a human cell each contain a different base at this site, gene expression is significantly higher from the risk-variant chromosome, owing in part to a reduction in the attachment of DNA-binding proteins that inhibit transcription. Using CRISPR–Cas9 gene-editing technology to remove the G and replace it with A reduces *SNCA* expression.

disease-linked genetic variants in humans.

The work provides several avenues for further investigation. For instance, there are many SNP variants in *SNCA* that are strongly associated with Parkinson's disease but that were not interrogated in the current study. As such, Soldner *et al.* cannot rule out the possibility that the risk-associated variant at rs356168 is simply an innocent bystander. This SNP alone does not fully explain the disease risk associated with the *SNCA* region[2], and so probably has accomplices — these may have more marked effects on gene expression.

Another limitation is that Soldner and colleagues do not analyse whether their risk-associated SNPs also modulate *SNCA* expression through non-transcriptional mechanisms. For instance, disease-associated SNPs in the non-coding 3′ region of *SNCA* have been reported to regulate the processing or translation of mRNA[8]. Finally, a fundamental question is whether the SNP-dependent regulation of *SNCA* transcription seen in the authors' cell-based model is truly at work in the human brain. This could potentially be investigated by analysing brain tissue obtained at autopsy from cohorts of unaffected individuals who carry either the risk-associated or protective SNP variants.

It remains unclear how elevated levels of *SNCA* expression ultimately lead to Parkinson's disease. Nonetheless, Soldner and colleagues' findings support the pursuit of therapeutic strategies that suppress *SNCA* expression. Such efforts would complement current strategies that focus largely on improving the clearance of accumulated α-synuclein protein aggregates — for example, through the use of therapeutic antibodies. ■

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The authors declare competing financial interests.
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