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GUEST EDITORIAL

DISC1 as a genetic risk factor for schizophrenia and related major mental illness: response to Sullivan

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In a recent guest editorial,[1] Patrick Sullivan questioned several aspects of the original Scottish t(1;11) family in which the *DISC1* gene was discovered.[2–4] He challenged the wider significance of the original genetic finding and questioned the relevance of DISC1 biology to psychiatry, casting doubt on the validity of the 'integrative' approach. Sullivan prefaced his strong views on *DISC1* by welcoming the 'uncompromising statistical rigour and replication' that is now being applied to his area of expertise in genome-wide association studies (GWAS). In his opinion, the evidence in support of *DISC1* falls short of the mark, but the methods he champions are based on the assumption of the 'common disease; common variant' hypothesis, and are not valid tests of the 'common disease; rare variant' hypothesis of which *DISC1* is a prime example. If we were to apply his criteria universally, we would, for example, be obliged to ignore everything learnt about Alzheimer's Disease through *APP*, *PSEN1* and *PSEN2*, none of which show up in GWAS, yet these clear-cut, family-based rare variant discoveries have provided profound insights into the disorder.[5] We would likewise have to ignore much of the insights that have been gained from copy number variant analysis in autism[6,7] and, indeed, schizophrenia.[8,9] Denying an important role for rare variants makes no sense and contradicts theoretical considerations;[10] insights from exceptional cases and families have repeatedly served medical research well across the full spectrum of conditions generally considered 'common and complex'.

So why does Sullivan pick out *DISC1* for special criticism? This is not the time or place to reiterate the wealth of evidence in favour of *DISC1* and of the DISC1 pathway, but readers might find it useful to consult recent reviews[11–14] in addition to those he cites.[1] Here, we summarise his Table 1, answer his main questions and expand upon these in the main text.

Sullivan queries the presence of a Robertsonian t(13q;14q) translocation reported in a small branch of the Scottish t(1;11) pedigree, and the chromosome 1 constriction that is more prevalent but predominantly in branches of the family that do not carry the t(1;11). The former was in fact discussed in the Lancet study[2], and neither materially alter the conclusion that the t(1;11) robustly tracks the high risk of psychiatric illness in this family. The logarithm of the odds (LOD) score for schizophrenia alone meets stringent genome wide significance, whilst for schizophrenia plus bipolar disorder and major depressive disorder it substantially exceeds genome-wide significance (MLOD = 7.1). Regarding the alleged disparity between the pedigree in the original 1970 report[15] and the follow-up report in 2001,[3] there is nothing suspicious here. Any clinician who has followed up families in their care over such a long period of time will fully understand that not all of the original members will still be willing or able to participate and that new members and new cases may become available. That the follow-up study confirmed and substantiated the original study should give comfort not concern. For the avoidance of any further doubt, we can confirm that the pedigree presented in the 2001 study focuses only upon branches of the family carrying the t(1;11); the t(1;11) shows unequivocal linkage to the high burden of major mental illness in this family, and the t(1;11) has been scored both by classical chromosome banding and by fluorescence *in situ* hybridisation[16]. The molecular cloning of the t(1;11) breakpoint[4] with sequence confirmation has more recently allowed us to develop a PCR-based assay spanning the breakpoint; we routinely apply this test to validate samples in contemporary studies (Thomson, unpublished). Finally, regarding the Scottish t(1;11) pedigree, a major third wave of follow-up has recently been completed with brain imaging added to the clinical phenotype: this will be submitted shortly for publication.

Sullivan finds the spectrum of psychiatric diagnoses seen in the t(1;11) family 'worrying', but what the t(1;11) family showed *par excellence* is evidence for genetic and biological overlap between schizophrenia, bipolar disorder and major depressive disorder. The epidemiological evidence is now clear on this,[17,18] as too is the accumulating GWAS evidence for shared genetic liability across DSM-defined diagnoses.[19,20] The presence of both schizophrenia and major depressive disorder in the t(1;11) family is entirely consistent with recent GWAS-derived estimates of co-heritability: schizophrenia and bipolar disorder (0.68); bipolar disorder and major depressive disorder (0.47); schizophrenia and major depressive disorder (0.43).[21] The variability in presentation in the Scottish t(1;11) family may be accounted for by genetic (independently segregating modifiers) or non-genetic (experiences and exposures) factors, which are under active investigation. In a similar vein, there is nothing 'worrying' about the absence of mental retardation: mental retardation is seen frequently in chromosome deletion conditions without any other psychiatric features,[22] but not of balanced translocations. Finally, it is indeed the case that the original propositus was a male with adolescent conduct disorder: schizophrenia is known to increase in prevalence with an increasing number of conduct problems in childhood.[23]

With regard to the focus on *DISC1*, other possible explanations —a functional effect of the disrupted chromosome 11 locus, an independent or co-dependent effect of the long non-coding RNA antiparallel to *DISC1*, named *DISC2*, and/or a dominant-negative effect of DISC1 fusion proteins—have not been ignored. We and others have considered and actively investigated all of these alternative hypotheses,[24–26] and will continue to do so as the necessary tools, reagents and assays are developed. As part of ongoing work, we are carrying out whole-genome sequencing of t(1;11) family members and will report the findings in due course.

With regard to independent genetic results, the failure of meta-analyses of genome-wide linkage and association to replicate *DISC1* is misleading: it is well understood that the presence and impact of rare or recent genetic events go undetected by these methods.[5] GWAS depends upon genotyped alleles tagging risk loci that are then implicated indirectly by a significant difference in allele frequency between cases and controls. The statistical tests may be highly significant, but the population level odds ratios are typically less than 1.2. Given the reliance on strong linkage disequilibrium at a whole-sample level between the genotyped common allele and the risk allele, this method is neither designed nor suited to the detection of rare risk alleles: this is true even for those of high penetrance and impact, for example structural or coding variants, for which the odds ratios are typically greater

Table 1. Answers to questions from Sullivan about *DISC1*

The pedigree

The classical karyotyping methods developed in Edinburgh by Jacobs *et al.*[42] are robust for tracking the t(1;11) translocation,[2] and were used effectively again in Blackwood *et al.*,[3] but, for convenience, efficiency and utility, have since been supplemented with fluorescence *in situ* hybridisation and now PCR methods: all methods give entirely congruous results. There is no evidence from follow-up studies for any significant change from the original report that the t(1;11) tracks susceptibility to a spectrum of schizophrenia, bipolar disorder and major depressive disorder with genome-wide significance. A comprehensive follow-up on the t(1;11) family, including brain imaging, has recently been completed and will be submitted for publication in due course.

The phenotypes

These are not 'worrying'. The presence of major depressive disorder along with bipolar disorder and schizophrenia is supported by genome-wide association studies (GWAS)-based co-heritability analyses. The absence of mental retardation is unsurprising: mental retardation is a common co-morbid feature of many deletion syndromes, with or without other psychiatric features, but not of balanced translocations.

The focus on DISC1

The focus on DISC1 derives from the remarkable properties attributable to the protein—interaction and modulation of multiple proteins that have been independently linked genetically to psychiatric and neurodevelopmental disorders, and biologically to fundamental neurological processes of signalling, trafficking, synaptic function and neurogenesis. Other molecular genetic consequences of the t(1;11) have been considered and are published. None challenge the primacy of the *DISC1* disruption.

Genetic results

The examples cited as the 'most rigorously conducted genomic studies' are selective and biased towards meta-analyses of linkage and association, blunt tools to test for rare variants. Positive published findings with genome wide significance have been ignored.

than 2.[10] Family-based linkage studies in relatively isolated populations have the potential to bridge the gap between 'common' and 'rare' variant detection, and this is true for *DISC1*. In a population-based study of 221 Finnish families with schizophrenia, D1S2709, an intragenic marker for DISC1, gave a Z(max) of 2.71 in the combined cohort, increasing to 3.21 when an internal isolate was excluded. Linkage to DISC1 was replicated in an independent set of 70 Finnish families with schizophrenia.[27] Analysis of the combined Finnish samples exceeds genome-wide significant linkage for *DISC1* and schizophrenia (LOD = 3.6).[28] When conditioned on the *DISC1* risk haplotype, the *NDE1* locus was identified as a strong linkage peak (LOD = 3.17), a finding that was replicated by association ($P < 0.011$ after correction for multiple testing).[28] Although *NDE1* does not appear as a top GWAS 'hit', deletions and duplications spanning *NDE1* are among the most common copy number variants in schizophrenia.[29] NDE1 is critical for multiple neurodevelopmental processes: through protein-protein interaction, DISC1 regulates NDE1 function.[30] Thus, consistent with current neurodevelopmental concepts in schizophrenia,[31] the genetic and biological evidence for DISC1 and NDE1 provides evidence for a shared 'risk' pathway.

NDE1 is just one of a large number of proteins for which there is firm evidence for direct interaction with DISC1: several likewise have biological functions that have been independently linked to psychiatric illness.[11–14] None independently meet the PGC criteria for genome-wide significance, but some meet gene-wide significance. Of note, the DISC1 interactor PDE4B encodes a regulator of cAMP signalling that is targeted by the antidepressant/antipsychotic rolipram, and the *PDE4B* gene is directly disrupted by a t(1;16) translocation in two cousins diagnosed with schizophrenia or a psychotic disorder.[13,30,32]

What have we learnt so far from sequencing *DISC1*? Perhaps more than Sullivan suggests.[1] An early sequencing study of *DISC1* identified six ultra-rare non-synonymous amino-acid substitutions in 288 schizophrenia cases, but not in the 288 matched controls.[33] Moens *et al.*[34] reported an increased burden of rare missense mutations in a Swedish cohort. Green *et al.*[35] reported an excess of exon 11 rare variants in schizotypy. In contrast, Crowley *et al.*[36] found no statistical significance on sequencing 2.7 kb of *DISC1* in 727 schizophrenia cases and 733 controls. In a recently published study, we sequenced the full 528 kb of the genomic DISC1 locus in 1562 cases and controls.[37] We found no significant increase in the burden of non-synonymous coding variants in cases, but did find evidence for gene-wide enrichment of conserved, regulatory variants in major depressive disorder. We also reported the ultra-rare variant R37W in a case of major depressive disorder, transmitted to two affected offspring: R37W is one of the ultra-rare variants first reported by Song *et al.*[33] in a case of schizophrenia. We now know that 37W alters the cellular distribution of DISC1, the modulatory role of DISC1 in cellular stress[38] and affects mitochondrial trafficking.[39]

Sullivan's dictum that 'biology does not have a role in establishing a genetic association (but only later in understanding its role)' is not one that we or a large section of the genetics community would agree with, but ironically it does apply to the case for the Scottish t(1;11) family: only once the breakpoint was cloned and *DISC1* identified could the biology begin.[4] It is worth remembering that in 2000 DISC1 was, and still is, a singleton protein with no close homology to any other protein, and thus no neat and meaningful descriptor was available. Naming the protein in the context of its discovery (genome-wide significance for schizophrenia in the t(1;11) family) made sense then and no change is warranted now.

There is no harm and much to gain from reasoned and vigorous debate over alternative approaches to cracking a scientific problem as pressing and challenging as understanding the genetic and biological basis of major mental illness. Indeed, we should take as clear evidence of progress that the relative merits of case, family and population approaches can be debated on the basis of evidence. The value of a biological pathway-based approach is very well established in other fields: in our view, shared by others,[31,40,41] it is critical to the advancement of psychiatric research. DISC1 has already made a valuable contribution to this endeavour. We believe strongly that *DISC1* and the DISC1 pathway paradigm have served the field well, will continue to do so and that the foundational evidence is sound. We will continue to take this systems approach as the one most likely to provide actionable biological insight.

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