

Commentary for *Biological Psychiatry*

ZNF804A: Insights from the First Genome-wide Significant Schizophrenia Gene

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1225 words, 10 references.

In 2008, the first substantive genome-wide association study (GWAS) of schizophrenia identified one locus, implicating one gene: zinc finger protein 804A (*ZNF804A*) (1). Now we have over 100 loci, implicating hundreds of genes, and many more still to come (2). Nevertheless, the story of *ZNF804A* over the intervening nine years, culminating in the paper by Deans and colleagues (3; this issue), remains an interesting and valuable one (4). It sheds light specifically on how *ZNF804A* may modulate disease risk, and raises broader issues relevant to all GWAS findings in our field (5).

ZNF804A was the antithesis of a candidate gene for psychosis. As O'Donovan and colleagues said in their original description of the association: 'The encoded protein is uncharacterized and has no known function...' (1). As well as drawing attention to the limitations of the candidate gene strategy (and the benefits of the GWAS approach), this paucity of information immediately raised a question for researchers interested in trying to understand the role of the gene in schizophrenia: Where do we start? The only real clue was the 'C2H2' zinc finger domain encoded in the sequence, which gave the gene its name and suggested it might function as a zinc finger transcription factor. Even this clue was a weak one, since *ZNF804A* contains only one C2H2 domain, whereas most other zinc finger proteins contain several. On the plus side, at least the risk SNP (rs1344706) resided within the middle of *ZNF804A* and hence implied that this gene was the source of the genetic signal (1) – in contrast to the majority of schizophrenia loci which encompass many or no known genes and thus do not provide such a clear steer. The subsequent, much larger, GWAS studies confirmed the association of *ZNF804A* with schizophrenia, and extended it to a broader psychosis phenotype including bipolar disorder. Completing the picture, copy number variants affecting the *ZNF804A* locus have also been identified in some cases of psychosis and autism spectrum disorder. In the larger GWAS data sets, rs1344706 remains genome-wide significant, but several other *ZNF804A* intronic SNPs are also significant (2,4). Hence at present the evidence is extremely strong that *ZNF804A* is a psychosis risk gene, but the causal variant(s) remain(s) to be identified unequivocally.

So what have we learnt about the functions of the gene? Three main themes are apparent (4,5). The first comes from a number of imaging genetics papers, starting in 2009, which show that rs1344706 impacts on various parameters of brain activation, functional connectivity, and cognition, thereby suggesting *ZNF804A* has a role in these processes (see 4,6). Second, evidence has emerged to support the possibility that *ZNF804A* might act as a transcription factor, regulating the expression of other genes, including some implicated in schizophrenia (7,8). Third, the expression of *ZNF804A* in human brain has been studied and has revealed interesting and unanticipated findings. Hill and Bray (9) reported that rs1344706 influences *ZNF804A* mRNA in fetal human brain, with risk allele carriers having reduced expression, and providing a clue that the gene might be exerting its psychosis-

relevant effects at that stage of brain development. Tao and colleagues (10) confirmed and extended this finding, showing that *ZNF804A* is expressed as a novel, short transcript isoform, and that it is this isoform rather than canonical *ZNF804A* mRNA which is influenced by rs1344706 in fetal brain. They also showed that *ZNF804A* immunoreactivity in human prefrontal cortex is abundant, especially in layer III pyramidal neurons, and is not limited to the nucleus (as would be expected if *ZNF804A* were functioning solely as a transcription factor), but in fact was more prominent in the cytoplasm, including dendrites.

Set against this background, the work reported by Deans and colleagues (3) significantly advances our understanding of the function of *ZNF804A* and helps link together the prior findings. They used human induced pluripotent stem cells, neurons derived from neural progenitor cells, and rat primary cortical neurons, to study in detail the localization of *ZNF804A*. They replicated the finding that *ZNF804A* is not limited to the nucleus but extends to the somatodendritic compartment (especially in the differentiated neurons) and further showed that it co-localizes with the post-synaptic markers GluN1 and PSD-95. Similarly, in the mature primary neurons, *ZNF804A* was found – using super-resolution microscopy – in dendritic spines, clustered in nanodomains together with post-synaptic proteins, as well as pre-synaptically in some axonal processes. These findings implied that *ZNF804A* might be involved in synaptic structure and function. To investigate this possibility, the authors used siRNA to knock down *ZNF804A* in order to investigate its role in formation of neurites, dendritic spine morphology, and in responses to synaptic activity. They found that *ZNF804A* knockdown decreased neurite growth (mediated in part via an effect on the synaptic adhesion molecule neuroligin-4) and reduced dendritic spine density. Knockdown also impaired responses to activity-dependent stimulation of the neurons induced by chemical long-term potentiation. Together the findings revealed novel and unexpected roles of *ZNF804A* in neuronal growth, dendritic spine maintenance, and synaptic function.

Despite the impressive amount of data reported by Deans and colleagues, inevitably many questions remain to be answered. First, as acknowledged by the authors, some concerns persist about the specificity of the anti-*ZNF804A* antibody; this does not call into doubt any of their main conclusions, but does highlight that limitations of antibodies bedevil many studies of this kind. Moreover, the available antibodies do not clearly distinguish between the isoforms of *ZNF804A*, and hence it is unclear which findings pertain to which isoform. Second, whilst effects of *ZNF804A* at the synapse are a plausible explanation for its involvement in psychosis, and convergent with much other evidence that synaptic pathology is a core part of the disease process, study of other schizophrenia risk genes - notably DISC-1 - shows that many turn out to have multiple and diverse roles. By the

same token, it may be that the synaptic functions of *ZNF804A* are but the tip of the pleiotropic iceberg, and they may or may not prove to be the critical ones *vis à vis* psychosis. Finally, it will be of interest to determine whether and how rs1344706 or other risk alleles influence the properties reported by Deans and colleagues: ultimately studies need to link the biology of the gene with the functional effects of the associated genetic variants if the full picture of the role which *ZNF804A* plays in the psychosis disease process is to be understood.

It is often stated, sometimes glibly, that identification of risk genes for psychiatric disorders will lead to better understanding of disease pathophysiology and provide new drug targets. These are worthy aspirations and, in principle, are correct. However, *ZNF804A* illustrates the challenges in realising this promise. Few if any of the empirical findings summarised here would have been predicted by the *in silico* features of *ZNF804A*, and the discoveries that have been made speak to the essential roles of wet lab science, including the use of human subjects and human material. Science of this kind, carried out with the requisite quality, depth, and breadth, does not happen quickly, nor is it cheap. In these respects, the continuing story of *ZNF804A* provides an important reality check about the speed at which psychiatric genomic discoveries can be turned into clinically significant advances. This should in no way discourage the field, but rather should encourage us – researchers and funders alike - to redouble efforts to achieve these essential goals.

Acknowledgment: I thank Liz Tunbridge for helpful comments.

Financial disclosures: The author is employed by the University of Oxford. His research is funded by Wellcome Trust strategic awards (102616/Z and 098461/Z), UK Medical Research Council (K013092 and P026028/1), and supported by the NIHR Oxford Health Biomedical Research Centre. The views expressed here are those of the author and not necessarily those of the funders. In the past two years, he has received fees for acting as an expert witness in pharmaceutical patent litigation on behalf of Teva, and has received fees or honoraria from Oxford University Press, Wiley Blackwell, Wellcome Trust, Wolfson College (Oxford), and the Society for Biological Psychiatry.

1. O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, *et al.* (2008): Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 40:1053-1055.
2. Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014): Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511: 421-427.
3. Deans PJM, Raval P, Sellers KJ, Gatford NJF, Halai S, Duarte RRR *et al.* (2017): Psychosis risk candidate *ZNF804A* localizes to synapses and regulates neurite formation and dendritic spine structure. *Biol Psychiatry* (this issue)
4. Chang H, Xiao X, Li M (2017): The schizophrenia risk gene *ZNF804A*: clinical associations, biological mechanisms and neuronal functions. *Mol Psychiatry* AOL 14 March, 2017; doi: 10.1038/mp.2017.19.
5. Harrison PJ (2015): Recent advances in schizophrenia genetics and their therapeutic relevance. *J Psychopharmacol* 29:85-96.
6. Cousijn H, Tunbridge EM, Rolinski M, Wallis G, Colclough GL, Woolrich MW, *et al.* (2015): Modulation of hippocampal theta and hippocampal-prefrontal cortex function by a schizophrenia risk gene. *Hum Brain Mapp* 36: 2387-2395.
7. Girgenti MJ, LoTurco JJ, Maher BJ (2012): *ZNF804A* regulates expression of the schizophrenia-associated genes *PRSS16*, *COMT*, *PDE4B*, and *DRD2*. *PLoS One* 10:e0124597.
8. Hill MJ, Jeffries AR, Dobson RJ, Price J, Bray NJ (2012): Knockdown of the psychosis susceptibility gene *ZNF804A* alters expression of genes involved in cell adhesion. *Hum Mol Genet* 21: 1018-1024.
9. Hill MJ, Bray NJ (2012): Evidence that schizophrenia risk variation in the *ZNF804A* gene exerts its effects during fetal brain development. *Am J Psychiatry* 169: 1301-1308.
10. Tao R, Cousijn H, Jaffe AE, Burnet PW, Edwards F, Eastwood SL, *et al.* (2014): Expression of *ZNF804A* in human brain and alterations in schizophrenia, bipolar disorder, and major depressive disorder: a novel transcript fetally regulated by the psychosis risk variant rs1344706. *JAMA Psychiatry* 71:1112-1120.