The emperors of the schizophrenia polygene have no clothes

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A substantial body of research literature, identified by nine out of ten papers on genetics in the recent ISI research front on schizophrenia, claims to have established associations between aspects of the disease and sequence variation in specific candidate genes. These candidatures have proven unreplicated in large sibling pair linkage surveys and a targeted association study. Even if the case for an association be regarded as a lucky guess (assuming one gene in 30 000 was guessed right) the large linkage and association studies provide no evidence of sequence variation relating to psychosis at any of these gene loci. Thus this body of work must be regarded as an indicator of the extent to which the 'eye of faith' is able to discern meaning in complex data when none is present.

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Introduction

A recent Institute of Scientific Information (ISI) citation analysis identified the 20 most cited papers in research on schizophrenia in the epoch 2005–2007. Ten papers (cited between 35 and 212 times) were in the field of genetics (Table 1), nine of which drew positive conclusions concerning a basket of 12 candidate genes. The authors of the ISI review relate these papers to a 'research front leader' (Harrison & Owen, 2003; cited 222 times) that argued in support of seven of these candidates. The reader is lured by the thesis that (1) a major thrust of psychosis research is now genetic, (2) there is substantive progress, and (3) genes that contribute to predisposition have already been identified.

On the contrary it can be argued that (1) the trend depicted in the ISI analysis reflects the triumph of optimism over sober appraisal, (2) there are no grounds for regarding these genes separately or in combination as having any specific relation to schizophrenia, and (3) the emphasis on polygenes of small effect that permeates this literature obscures a challenge with implications within and outside the boundaries of psychosis research.

What I tell you seven times is not necessarily true

No casual reader of this body of work or the ISI analysis will be aware of the fragility of the data.

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A chorus of reviews with titles including 'The discovery of susceptibility genes for mental disorders' (Cloninger, 2002), 'Genes for schizophrenia? Recent findings and their pathophysiological implications' (Harrison and Owen, 2003), 'The molecular genetics of schizophrenia: new findings promise new insights' (Owen et al. 2004), 'Schizophrenia – genes at last?' (Owen et al. 2005), 'Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence' (Harrison and Weinberger, 2005), 'The genetics of schizophrenia and bipolar disorder: dissecting psychosis' (Craddock et al. 2005), and 'The genetic deconstruction of psychosis' (Owen et al. 2007) pervades the literature and will convince all but the most inquisitive that a solid foundation of evidence supports the pathophysiological relevance of these candidate genes.

With one or two possible exceptions (COMT, G72) each candidature originated in a genetic linkage study. All have later been reported as supported ('confirmed') by a genetic 'linkage' or 'association' study, in most cases several.

Draining the pond dry

The distinction between 'linkage' and 'association' (also referred to as linkage disequilibrium or LD) is important. Both strategies use genetic variation (polymorphisms) to identify the location of a disease-predisposing gene at a particular site within the genome. Whereas *linkage* refers to the co-transmission *within families* of variants at a given site and disease (the 'phenotype'), *association* refers to the relationship

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Emperors (with ISI rank 2005–2007)	Cites	BDNF	NRG1	DTNBP1	COMT	DISC1	RGS4	G72	DAO	Mitogenes
Harrison & Weinberger (2005) (2)	201		0 & 0	0 & 0	0 & 0	0 & 0	0 & 0	0 & 0		
Craddock et al. (2005) (4)	82		0 & 0	0 & 0		0 & 0	0 & 0	0 & 0	0 & 0	
Callicott et al. (2005) (6)	66					0 & 0				
Millar et al. (2005) (8)	59					0 & 0				
Neves-Pereira <i>et al.</i> (2005) (13)	42	0 & n.d.								
Cannon et al. (2005) (14)	39					0 & 0				
Iwamoto et al. (2005) (15)	39									N.D. & N.D.
Fan <i>et al.</i> (2005) (16)	38				0 & 0					
Petryshen et al. (2005) (17)	38		0 & 0							
Green et al. (2005) (19)	35		0 & 0							
Hashimoto et al. (2005) (20)	35	0 & n.d.								
Research front leader										
Harrison & Owen (2003)	222		0 & 0	0 & 0	0 & 0		0 & 0	0 & 0	0 & 0	

Table 1. Lack of evidence for the candidate genes identified in the 10 studies in the ISI most highly cited list for 2005–2007

BDNF, Brain-derived neurotrophic factor; NRG1, neuregulin; DTNBP1, dysbindin; DISC1, disordered in schizophrenia 1; RGS4, regulator of G-protein signalling 4; G72, D-amino-acid oxidase activator; DAO, D-amino-acid oxidase; mitogenes, mitochondrial genes.

The ISI list is summarized with respect to the genome scan findings in sibling pairs (Crow, 2007), first symbol in each cell, and the association (LD) study of Sanders *et al.* (2008), second symbol. 0, Not supported; N.D., no data. Neves-Pereira *et al.* (2005) and Hashimoto *et al.* (2005) studied BDNF, which was not included by Sanders *et al.*, and Iwamoto *et al.* studied mitochondrial genes that were not covered by either Crow (2007) or Sanders *et al.* (2008). Fan *et al.* (2005) drew negative conclusions regarding COMT. Each of the other papers drew positive conclusions regarding an association between the gene(s) in question and schizophrenia.

between such variation and disease *within populations*. When the same variant (allele) travels with disorder within a given family linkage is said to be present, and the case is strengthened if in another family variation at that same locus also does so, whether the disease-related allele is the same or different from that in the first family. When LD is present a particular allele (sequence variation) is associated with disease above expectation within the whole population. The implication is that insufficient time has elapsed after a 'mutation' for recombination to restore the random association between variants at the marker locus and presence or absence of the mutation in the disease-predisposing gene.

To detect genetic linkage families can be collected from different populations. LD is best detected in homogeneous populations that have remained relatively isolated. Within such populations there is a high probability that a 'founder effect' mutation may have occurred, and account for a significant proportion of the variation related to disease within that population. Linkage can be detected over relatively long genetic distances [measured in centimorgans (cM), say 20–30 or more] whereas linkage disequilibrium implies that the variation is close to the disease locus, perhaps within 1–2 cM. Thus in the classical approach the techniques are used in tandem – linkage to detect the general region within which a disease-causing gene is suspected, LD to locate it with greater precision, and thus to lead to its identification.

The pond is empty

Has the combination of techniques succeeded in psychosis? Many in linkage research thought that success was inevitable – one would 'drain the pond dry' and there would be the genes! However, the reality is that in spite of a plethora of well-hyped findings no linkage claim has proven robust. In each case an apparent finding in a modest-sized population of families that was then used to 'identify' a candidate gene has not been found linked in more systematic and larger

		No. of top 10 papers with positive conclusions	Citations					
	No. of full papers		>500	>200	>100	Most cited primary author(s) (>200 times)		
Dysbindin	105	10		1	2	Straub RE		
COMT	342	6	1	6	18	Egan MF, Lachman HM, Weinberger DR		
Neuregulin	133	9		3	6	Stefansson H, Harrison PJ, Weinberger DR, Owen MJ		
RGS4	52	7			2	Chowdhari KY, Mirnics K		
G72	52	10		1	2	Chumakov I, Hattori E		
DISC1	105	10		1	5	Millar JK		
BDNF	272	10		2	8	Egan MF, Chen B		

Table 2. Analysis of ISI Web of Science data on 'candidate' genes associated with (psychosis or schizophrenia or bipolar or manic or mania) to show numbers of papers, rates of positive conclusions in the most cited papers, numbers of highly cited papers and highly cited authors of papers drawing positive conclusions

In view of the negative findings of the large genome scans and the Sanders *et al.* (2008) study the papers enumerated in column 3 must be regarded as false positives.

studies. Thus the advent of genome scans (unbiased surveys with markers across the whole genome) has not strengthened any of the claims (Table 1). Damagingly two meta-analyses of the genome scans of schizophrenia and bipolar disorder revealed no strong findings and failed to agree on loci of interest even though they examined largely the same body of studies. If one takes the three largest (>300 sibling pairs) genome scans there is little agreement between the studies, and no consistent support for any candidate gene (Crow, 2007).

Three counter-arguments are mounted as follows: (1) the polygenes are more numerous and of smaller effect than had been thought, (2) different genes are present in different populations, (3) linkage evidence will be overtaken by definitive findings from association studies.

Argument 1 flouts Ockham's principle; as an hypothesis it approaches unfalsifiability. Argument 2 gives no account of constancy of incidence across populations (Jablensky *et al.* 1992) or relative uniformity of the spectrum of psychosis, e.g. with respect to sex differences, structural brain change and relationship between form of illness and age of onset (Crow, 1993).

Argument 3 is now directly testable. With technical advances genome-wide association studies are possible with half a million or more markers on populations measured in thousands. The findings of such studies have been preceded by a systematic assessment of the existing list of candidate genes in a population of over 1870 patients and 2002 screened controls (Sanders *et al.* 2008). For a list that includes the above genes (with the exception of BDNF) association was systematically pursued in regions beside each of the candidates. The sample size and density of markers dwarf previous studies. No evidence of an association with any alleles at the polymorphic sites was obtained (Table 1).

Magical thinking in the Clinical Brain Disorders Branch

The most euphoric interpretation of linkage and association findings comes from the National Institute of Mental Health. In a paper entitled 'Schizophrenia genes: famine to feast', Straub & Weinberger (2006) extend the above list of 12 'candidates' to include six more, each of which they describe as 'linked to a gene locus'. These claims cannot be sustained in the findings of the large and systematic linkage studies (Crow, 2007) or the association study of Sanders et al. (2008). They represent the salient manifestation of an exuberant growth in the literature (Table 2), purporting to relate variation at candidate gene loci to the phenomena of psychosis. But if the origin of the relationship was in a genetic linkage, and that linkage has proven not replicable, the claim of significance in relation to psychosis has been built on sand.

At the World Congress of Psychiatric Genetics held in New York in October 2007 separate sessions addressed the state of genome-wide association studies in relation to bipolar disorder and schizophrenia. Full publications are not yet available but it was apparent that no strong findings had emerged, and that such weak associations as were observed were neither in relation to the candidate genes, nor in agreement between different studies. The discussion was sombre. In the morning Francis Collins, Head of the Human Genome Project, had predicted sure future progress with these technical advances. In the afternoon it was seen that just such a strategy had failed to yield decisive findings. Thus association studies have not vindicated the enthusiastic interpretation that many had placed on modest and early linkage findings. Neither technique has succeeded in establishing the location of a gene predisposing to psychosis, still less are they in agreement concerning the identity of such a gene.

What explanations are there for this absence of evidence in the face of twin, adoption and family findings indicating substantial genetic predisposition? Two, and perhaps only two-a high mutation site (Book, 1953) and an epigenetic imprint-are feasible. The former might well escape association searches (different mutations occurring in the same genomic background would add noise to the signal) but would not have escaped linkage (siblings would inherit the same mutation above chance expectation). This leaves variation in modification of the DNA sequence (by methylation), or of the histones (methylation, acetylation or phosphorylation) with which it is associated in the scaffolding of the chromosome, as the single viable explanation. Such might also account for the paternal age effect and discordance for presence or form of illness in twins and other multiple births. Chromosomal rearrangements suggested to have played a role in the origin of the species (Crow, 2002) are susceptible to just such an epigenetic process - now referred to as 'meiotic suppression of unpaired chromosomes' - or MSUC (Turner, 2007).

Conclusion

Claims for 'candidate' genes are not supported either by the larger and more systematic linkage studies or by a targeted association comparison of a population of 1870 patients with 2004 controls. Whole genome association studies reviewed at the IXth World Congress of Psychiatric Genetics suggest that no strong and consistent findings emerge. Thus genetic predisposition to psychosis reflects variation not in the DNA sequence, but in modification of the sequence itself, or more likely of the histone structure with which it is associated within the framework of the chromosome. The failure of the search for psychosis genes by linkage and association therefore reveals the trans-generational reality of the epigenetic imprint.

Declaration of Interest

None.

References

- Book JA (1953). Schizophrenia as a gene mutation. *Acta Genetica et Statistica Medica* **4**, 133–139.
- Callicott JH, Straub RE, Pezawas L, Egan MF, Mattay VS, Hariri AR, Verchinski BA, Meyer-Lindenberg A, Balkissoon R, Kolachana B, Goldberg TE, Weinberger DR (2005). Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. *Proceedings of the National Academy of Sciences USA* 102, 8627–8632.
- Cannon TD, Hennah W, van Erp TGM, Thompson PM, Lonnqvist J, Huttunen M, Gasperoni T, Tuulio-Henriksson A, Pirkola T, Toga AW, Kaprio J, Mazziotta J, Peltonen L (2005). Association of DISC1/TRAX haploytpes with schizophrenia, reduced prefrontal gray matter, and impaired short- and long-term memory. *Archives of General Psychiatry* 62, 1205–1213.
- Cloninger CR (2002). The discovery of susceptibility genes for mental disorders. *Proceedings of the National Academy of Sciences USA* 99, 13365–13367.
- Craddock N, O'Donovan MC, Owen MJ (2005). The genetics of schizophrenia and bipolar disorder: dissecting psychosis. *Journal of Medical Genetics* **42**, 193–204.
- Crow TJ (1993). Sexual selection, Machiavellian intelligence and the origins of psychosis. *Lancet* **342**, 594–598.
- Crow TJ (2002). The Speciation of Modern Homo sapiens. Oxford University Press: Oxford.
- Crow TJ (2007). How and why genetic linkage has not solved the problem of psychosis: review and hypothesis. *American Journal of Psychiatry* **164**, 13–21.
- Fan JB, Zhang CS, Gu NF, Li XW, Sun WW, Wang HY, Feng GY, Clair DS, He L (2005). Catechol-O-methyltransferase gene Val/Met functional polymorphism and risk of schizophrenia: a large-scale association study plus metaanalysis. *Biological Psychiatry* 57, 139–144.
- Green EK, Raybould R, McGregor S, Gordon-Smith K, Heron J, Hyde S, Grozeva D, Hamshere M, Williams N, Owen MJ, O'Donovan MC, Jones L, Jones I, Kirov G, Craddock N (2005). Operation of the schizophrenia susceptibility gene, neuregulin 1, across traditional diagnostic boundaries to increase risk for bipolar disorder. *Archives of General Psychiatry* 62, 642–648.
- Harrison PJ, Owen MJ (2003). Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet* **361**, 417–419.
- Harrison PJ, Weinberger DR (2005). Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Molecular Psychiatry* **10**, 40–68.
- Hashimoto T, Bergen SE, Nguyen QL, Xu BJ, Monteggia LM, Pierri JN, Sun ZX, Sampson AR, Lewis DA (2005). Relationship of brain-derived neurotrophic factor and its receptor TrkB to altered inhibitory prefrontal circuitry in schizophrenia. *Journal* of Neuroscience 25, 372–383.
- Iwamoto K, Bundo M, Kato T (2005). Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Human Molecular Genetics* 14, 241–253.

Jablensky A, Sartorius N, Ernberg G, Anker M, Korten A, Cooper JE, Day R, Bertelsen A (1992). Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization Ten Country Study. *Psychological Medicine* (Suppl.) **20**, 1–97.

Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR, Malloy MP, Chubb JE, Huston E, Baillie GS, Thomson PA, Hill EV, Brandon NJ, Rain JC, Camargo LM, Whiting PJ, Houslay MD, Blackwood DHR, Muir WJ, Porteous DJ (2005).
DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* 310, 1187–1191.

- Neves-Pereira M, Cheung J, Pasdar A, Zhang F, Breen G, Yates P, Sinclair M, Crombie C, Walker N, St Clair DM (2005). BDNF gene is a risk factor for schizophrenia in a Scottish population. *Molecular Psychiatry* **10**, 208–212.
- Owen MJ, Craddock N, Jablensky A (2007). The genetic deconstruction of psychosis. *Schizophrenia Bulletin* **33**, 905–911.

Owen MJ, Craddock N, O'Donovan MC (2005). Schizophrenia: genes at last? *Trends in Genetics* 21, 518–525. Owen MJ, Williams NM, O'Donovan MC (2004). The molecular genetics of schizophrenia: new findings promise new insights. *Molecular Psychiatry* 9, 14–27.

- Petryshen TL, Middleton FA, Kirby A, Aldinger KA, Purcell S, Tahl AR, Morley CP, McGann CP, Gentile KL, Rockwell GN, Medeiros HM, Carvalho C, Macedo A, Dourado A, Valente J, Ferreira CP, Patterson NJ, Azevedo MH, Daly MJ, Pato CN, Pato MT, Sklar P (2005). Support for involvement of neuregulin 1 in schizophrenia pathophysiology. *Molecular Psychiatry* **10**, 366–374.
- Sanders AR, Duan J, Levinson DF, Shi J, He D, Hou C, Burrell GJ, Rice JP, Nertney DA, Olincy A, Rozic P, Vinogradov S, Buccola NG, Mowry BJ, Freedman R, Amin F, Black DW, Silverman JM, Bylerley WF, Crowe RR, Cloninger CR, Martinez M, Gejman PV (2008). No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. *American Journal of Psychiatry* 165, 497–506.
- Straub RE, Weinberger DR (2006). Schizophrenia genes – famine to feast. *Biological Psychiatry* 60, 81–83.
- Turner JMA (2007). Meiotic sex chromosome inactivation. Development 134, 1823–1831.