

Genetic Research in Psychiatry: Update from the Society of Biological Psychiatry and the American Psychiatric Association, New York, May 1990

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Summary – A stimulating variety of papers on genetic and clinical research in psychiatry was discussed at the latest meeting of the American Psychiatric Association and the Society of Biological Psychiatry in May 1990 in New York. Conflicting results point out that extreme caution must be taken in interpreting linkage studies of psychiatric disorders. Difficulties stem from complex models on inheritance as well as the genetic heterogeneity of psychiatric disorders. Progress has been made in the approach to the regulation of receptor genes that have been implicated in the pathogenesis of psychiatric disorders. In some cases, gene regulation may be tissue-dependent, as is suggested by the alternative splicing of D₂ receptor mRNA.

genetics / linkage / manic-depressive illness / Amish / Alzheimer's disease / schizophrenia / D₂ receptors / American Psychiatric Association / Society of Biological Psychiatry

Résumé – **Recherche génétique en psychiatrie: mise à jour de la société américaine de psychiatrie biologique et de l'association américaine de psychiatrie.** Les congrès annuels de l'association américaine de psychiatrie et de la société américaine de psychiatrie biologique se sont tenus en mai 1990 à New York. Les recherches génétiques (études de liaison, études chez des jumeaux, biologie moléculaire des gènes des récepteurs impliqués dans les maladies mentales) ont occupé une place de choix. Les controverses se poursuivent autour des liaisons qui ont été rapportées antérieurement entre la psychose maniaco-dépressive, la schizophrénie, la maladie d'Alzheimer, et des marqueurs génétiques. Ces difficultés sont expliquées par des caractères en partie propres à la psychiatrie, tels que la complexité des modes de transmission génétique, l'hétérogénéité génétique et clinique des troubles, la difficulté de définir les phénotypes et la révélation tardive de certaines affections. La connaissance de la régulation des gènes impliqués dans les maladies mentales, notamment celle des gènes des récepteurs D₂, a aussi progressé notablement.

génétique / liaison / psychose maniaco-dépressive / Amish / maladie d'Alzheimer / schizophrénie / récepteurs D₂ / association américaine de psychiatrie / société américaine de psychiatrie biologique

Introduction

Genetics has emerged as a highly fruitful area of research in psychiatry. It has contributed to the evolution of psychiatric nosology by indicating the etiologic heterogeneity of seemingly identical clinical conditions, and conversely by suggesting the common nature of apparently unrelated disorders.

Research strategies have varied over the years. They have relied on twin and adoption designs, familial segregation analyses and chromosomal studies. More recently, linkage analyses were made

possible by the identification of polymorphic DNA fragments (restriction fragment length polymorphisms). Finally, today's molecular biology aims at identifying receptor genes and unravelling the regulation of their expression in the brain.

The sheer number of presentations at the latest annual meetings of the Society of Biological Psychiatry and the American Psychiatric Association in May 1990 in New York confirms that genetic research is an active field. No particular research strategy is outdated; each one is particularly suited to approaching certain questions. Regardless of the

method used, the field of psychiatry has its own additional complexities, such as the difficulty of identifying phenotypes and cases.

The *fata morgana* of linkage in manic-depressive illness

Manic-depressive illness has been linked with the *X chromosome* in independent samples in the USA, Belgium, Israel and Sardinia (Winokur *et al*, 1969; Mendlewicz *et al*, 1974; Baron *et al*, 1977; DelZompo, 1984). Linkage has been established notably with markers of the terminal fragment (28) of the long arm (q) of the X chromosome, including protan and deutan color blindness, and G6PD deficiency. A report by Egeland *et al* (1987) supported the existence of a gene conferring a strong predisposition to bipolar affective disorder linked to two loci on the short arm of chromosome 11 (11p15), the Harvey-ras-1 oncogene locus (HRAS1) and the insulin (INS) locus.

These studies had a noticeable impact on psychiatric thinking. They came as the logical molecular biology sequel to the adoption and twin studies of the previous decades and suggested that the identification of major susceptibility genes was just around the corner. However, linkage studies of psychiatric disorders have had notorious difficulties withstanding the trial of time. Controversies were revived by the re-analysis of the linkage between chromosome 11p loci and affective disorders in the Old Order Amish, presented at the APA meeting by Kelsoe (1990), in the wake of the article published in the 16 november 1989 issue of *Nature*. As far as is known, the chromosome 11 linkage has not been confirmed. The initial results of Egeland (1987) were based on restriction fragment length polymorphism (RFLP) studies at the HRAS-1 and INS loci in an 81-member pedigree (pedigree 110) with 6 ill probands from the Old Order Amish community in southeastern Pennsylvania. The Amish are an exceptional study environment because their community is extremely endogamous; they are a genetic isolate with minimal heterogeneity. Kelsoe presented results on an extended version of pedigree 110. In addition to the 81 members of the original core pedigree, the new pedigree includes a 31-member (8 affected) "right" extension and a smaller six-member (2 affected) "left extension". Furthermore, two members of the core pedigree had their clinical status changed from unaffected to affected, and the genotyping of 10 unaffected individuals of the core pedigree was completed. With this new data, linkage to an affective disorder locus can be ex-

cluded to 5 centimorgans for either HRAS1 or INS, in the total 120-member pedigree and in the right extension alone. The new data, notably the presence of obligate recombinants in the core pedigree, has caused the lod score to drop from an original value of 4.08 to non-significance (at $\Theta = 0$ in the two-point analysis between HRAS1 and bipolar affective disorder).

Besides the importance of diagnostic follow-up and of at least yearly visits of all the pedigree members, what particular or general conclusions can be drawn from the Amish study? First, some factors are related to the genetic paradigms that are pursued. It is important to bear in mind that linkage normally occurs in relation to a putative major susceptibility gene. Linkage is a strategy directed toward the search for a major Mendelian susceptibility locus, as opposed to additive "Galtonian" polygenic effects. Lack of replication in large populations casts doubt on the universal validity of a single-gene etiology.

Secondly, other factors such as the problems of genetic and clinical heterogeneity, and definition of penetrance and caseness, are closely dependent upon the particular psychiatric disorder that is studied. There are several possible interpretations of the non-replication in the Amish group: either the initial report is simply not reproducible, or else there is genetic heterogeneity, which means that at least two different genes may be responsible for affective illness in the pedigree. It has been suggested by Egeland *et al* (1987) that several genes may be responsible for affective disorder within the Amish population itself, as several of the founding couples had mental illness among their first and second generation descendants.

Thirdly, questions arise concerning the exceptional genetic isolate that is studied and the generalization of linkage results beyond this highly inbred community. The Amish are descended from the Swiss anabaptists who appeared in the wake of the 16th century religious reformation and later emigrated to southern Germany and Alsace to flee persecution. It would be interesting to investigate whether the chromosome 11 linkage could be replicated in the European parent population, which is considerably less inbred. It may be noted that the American Old Order Amish population is exceptionally inbred; genes flow out when subjects marry outside the community, but almost no genes flow in. The very closed American Amish community which is reported here might be expected to differ from its European forefathers by the combined result of two effects: the "founder effects" (only those who emigrated contributed to the new genetic pool) and

the "genetic drift" (the genetic pool of the new community evolved due to different fertility rates).

Three paths to linkage

In general, the search for linkage can be guided by three possible strategies (Gershon, 1990). The first strategy is to investigate linkage with "candidate genes", such as those involved in neurotransmission or neuroreceptors. However, this approach has so far failed to show a major susceptibility locus. For instance, linkage with the D2 receptor region (11q22-23) has been excluded in bipolar and schizophrenic pedigrees. Similarly, Lentjes *et al* (1989) failed to show linkage with beta-1 (chromosome 10q24) and beta-2 (chromosome 5q31) adrenergic receptor genes in manic depressive pedigrees.

The second strategy is the exploration of markers in the region of a rare cytogenetic event found in rare pedigrees. This has been the case in schizophrenia where a report of a trisomy implicating the proximal long arm of chromosome 5 (5q11-13) inherited with schizophrenia (Basset *et al*, 1988) was followed by the finding that schizophrenia might be linked to a gene locus with a dominant susceptibility allele on chromosome 5q in British and Icelandic families (Sherrington *et al*, 1988). However, negative findings were published at the same time by other researchers in a Swedish population (Kennedy *et al*, 1988). Kennedy *et al* (1990) presented additional results at the American Psychiatric Association meeting, and confirmed the absence of linkage to schizophrenia of the genes for tyrosine hydroxylase and the D₂ receptor (chromosome 11); other areas of interest were also excluded for linkage in the same Swedish population: the genes for nerve growth factor, pro-opiomelanocortin, and the pseudoautosomal boundary (MIC2) of the sex chromosomes.

The third strategy is systematic mapping, and several papers based on this approach were presented in New York (Berrettini *et al*, 1990; Byerley *et al*, 1990; Polymeropoulos *et al*, 1990). Systematic mapping, leaving no region unexplored, is now possible, mainly because of the availability of polymorphic informative markers distributed throughout the genome and the ability to screen the whole genome in a less costly and more automated manner. It had been estimated as early as 1980 (Botstein) that 150 evenly spaced markers would ensure that no disease locus could be more than a 10% recombination distance from a marker locus. Preliminary results on systematic mapping of genes for manic-depressive disease were reported in May

in New York. Berrettini *et al* (1990) studied a series of 20 bipolar pedigrees consisting of 375 informative subjects (160 bipolars, unipolars or schizoaffectives) under the assumption of a dominant gene, 1% frequency and 85% penetrance. Results with 20 DNA markers exclude linkage to 5p (short arm of chromosome 5), 5q11-q13, 5q32-34, 11p15 (region reported to contain the susceptibility gene for bipolar disease in the 1987 Old Order Amish study) and 11q22-23 (D2 receptor region). In the same line, Byerley *et al* (1990) conducted a systematic genomic mapping study using over 250 DNA markers and also reported absence of linkage on chromosome 5, 11 and 19 in eight manic-depressive kindreds and eight schizophrenic families.

Late onset of Alzheimer's Disease hinders linkage

Several linkages have also been reported for Alzheimer's disease (AD). A number of authors examined chromosome 21 because Alzheimer-like neuropathology develops among patients with Down's syndrome. St George-Hyslop *et al* (1987) reported linkage of the rare disorder Familial Alzheimer Disease (FAD) to anonymous polymorphic DNA sequences mapping to the proximal long arm of chromosome 21. Positive LOD scores on 21q were also reported in FAD families in England by Goate *et al* (1989). However, some authors could not confirm these results; others even excluded linkage to the same markers by mapping other pedigrees (Pericak-Vance *et al*, 1988; Schellenberg *et al*, 1988). More recently, Roses *et al* (1990) reported positive LOD scores between AD and several markers on the long arm of chromosome 19 in an enlarged set of families. In this case too, genetic heterogeneity, *ie* multiple predisposing genes resulting in indistinguishable phenotypes, has been suggested to account for these discrepant or negative linkage results. In addition, clinical heterogeneity has been suggested. One form of the illness may be caused by a genetic defect on chromosome 21 and show early onset, typically in the fifth decade, while another more common form of the disorder may be a different genetic defect and show later onset, typically around the age of 60.

However, proving genetic etiology and showing linkage meet with considerably more difficulties in AD than in manic-depressive illness or schizophrenia. It has been said that a large proportion of sporadic cases argue against the presence of genetic causes in most cases of AD. However, the discussion of results must take into account the particular characteristics of the disorder that is

studied. Breitner *et al* (1988, 1989, 1990, and unpublished data) have emphasized that the very late onset of AD may preclude the occurrence of disease in many relatives whose earlier death has censored the expression of any inherited disease tendency. A characteristic of AD is age-dependent expression and variable age of onset. In the majority of cases, onset occurs in the eighth and ninth decade and therefore only one or two family members are affected. Such a limited number of affected subjects precludes the demonstration of linkage. It has been suggested that most AD cases may in fact be shown to have genetic causes. When appropriate allowance is made for censorship of disease tendency in relatives by other causes of death, the risk of Alzheimer-like dementia in first degree relatives of unselected clinically diagnosed AD probands increases with age and is about 50% over a lifespan of 90 years. One cannot help noticing that this figure is the risk expected in an autosomal dominant Mendelian illness. Because of the late onset of AD, other methods such as twin studies may prove particularly useful. A pilot study in the National Academy of Sciences Registry of aging twin veterans was reported by Breitner *et al* (1990). The study of a sample of 442 pairs (884 subjects) suggests a 60% concordance rate for Alzheimer's disease in monozygotic twin pairs. This concordance rate will likely increase with ongoing longitudinal observation and exceeds prior estimates.

New Approaches to D₂ receptor genes

Our understanding of neurotransmitter action has been refined by molecular biology studies of receptor genes expressed in the brain. This is particularly the case with D₂ receptors, which have been implicated in the pathogenesis of schizophrenia. Some data suggested an increased number of D₂ receptors in the brains of schizophrenics.

Two main types of dopamine receptors, D₁ and D₂, have been identified by pharmacological studies. Bunzow *et al* (1988) have reported the cloning of a rat cDNA (complementary DNA copied from mRNA by the enzyme reverse transcriptase) with the expression characteristics of a D₂ receptor. Todd *et al* (1990) reported that the overall organization of this D₂ gene is similar in rats, mice and man. It contains 8 exons (expressed DNA sequences), a very long first intron (intervening DNA sequence that is not translated in the polypeptide gene product), and spans at least 50 kilobases in the rat. In contrast to the genomic organization of most G-protein-coupled receptors,

this D₂ gene contains multiple introns. The interesting finding was that two forms of D₂ mRNA were detected in various brain regions, and it has been suggested that the D₂ receptor gene identified by Bunzow gives rise to at least two major D₂ receptor mRNA subtypes (Todd *et al*, 1989, 1990). The two forms are generated by alternative splicing of an additional exon (exon 6) within intron 5; consequently the two resulting D₂ proteins differ by the insertion of a 29 amino acid sequence. Splicing refers to the stage of mRNA processing whereby introns are removed. The relative proportion of the two alternative transcripts varies 25-fold in different brain tissues. The exon 6 containing mRNA predominated in areas where D₂ receptor number is highest—basal ganglia, anterior pituitary, and olfactory bulb. These findings imply the presence of tissue-specific factors that control not only D₂ gene transcription, but also alternative splicing of the pre-mRNA. Knowledge of the genetic organization of D₂ receptors is of obvious importance in schizophrenia and neuroleptic drug treatments.

New methods for the approach to neurons expressing D₂ receptors were also discussed. *In situ* hybridization of D₂ receptor mRNA was reported in the primate brain by Meador-Woodruff *et al* (1990) after being reported in rats a short time ago (Meador-Woodruff *et al*, 1989). This technique complements studies using receptor autoradiography and PET; it reveals cell bodies and allows the study of earlier events in receptor synthesis. Regulation at the gene transcription or translation level might thus be explored.

Conclusion

No one doubts that genetics is a promising field in psychiatry. From pedigree studies to the molecular biology of receptor genes expressed in the brain, no particular approach is outdated. Each one is ideally suited toward investigating specific questions. Some results that were first presented a few years ago become the object of renewed controversy as recent findings emerge. Particular caution must be taken in interpreting the results of linkage studies until models of inheritance can be delineated with more clarity.

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