Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders

Project: Identification of Disease Genes in Bipolar Affective Disorder

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Introduction

Bipolar affective disorder (BPAD [MIM 125480]) is a major psychiatric disorder characterized by episodes of mania and depression. It affects approximately 0.5-1.5% of the world's population, and constitutes a major public health problem with significant morbidity and mortality (World Health Organization, World Health Report 2002). Furthermore, projections from this report indicate that the contribution of BPAD to the global burden of disease will increase by 10-15% over the next 20 years.

Although, BPAD is a distinct nosological entity according to DSM IV, there is increasing evidence for the existence of a *bipolar spectrum* of milder bipolar disorders that have less severe manic episodes but which, nonetheless, cause substantial morbidity and include many individuals that have previously been regarded as unipolar depressive (Akiskal 2003; Angst et al. 2003).

Family, twin and adoption studies provide strong evidence of the importance of genes in predisposing to BPAD (reviewed by Sklar 2002). Eight of the family studies have included control subjects and show an approximately sevenfold risk increase to first-degree relatives of BPAD probands. Twin studies also support a substantial genetic contribution to BPAD, the proband-wise concordance rates vary between 36-79% for monozygotic co-twins and 0-8% for dizygotic co-twins. In addition, two adoption studies are reported, which found significantly greater risk of affective disorder (bipolar, unipolar, and schizoaffective) in the biological parents of bipolar adoptees compared with the adoptive parents.

Although in occasional families a single gene may play a major role, the findings support the view that no single gene explains the majority of cases of BPAD and demonstrate features that are expected and are being found in the search for genes involved in complex genetic disorders.

Identification of genes involved in complex genetic disorders is challenging but using a positional cloning approach of linkage analysis – genome-wide and fine-mapping linkage studies – followed by association analysis –SNP-based linkage disequilibrium (LD) mapping and LD-based association studies – there have been encouraging recent reports of gene identification in several complex genetic disorders. The aim of our project is to gain insights into the aetiology of BPAD. Within the present NGFN2 Network our project attempts to identify BPAD disease genes using a positional cloning approach.

Results/Project Status

Genome-wide Scans

Our project builds on findings from genome-wide linkage analyses which we have performed in two independent family samples with BPAD: Our first linkage set (sample A) comprised 75 families of German, Israeli, and Italian origin. In the parametric and non-parametric analyses we found evidence for linkage to several chromosomal regions including 1p36, 3p14, 4q27, 6q15-q22, 8p21, 8q24, 10q25-q26, and 13q12 (Cichon et al. 2001). Most recently we completed our genome-wide linkage study in the second BPAD linkage set (sample B) including 52 multiplex families of Andalusian, Bulgarian, and Roma descent. In the non-parametric analysis we observed evidence for linkage to the chromosomal regions 1p36, 2p22, 4q25-q31, 6q21-q24, 11p15, 13q12-q13, and 19p12-p13.

Fine mapping analysis

Since linkage findings for the 1p36, 4q25-q31, and 6q21-q24 regions were also obtained in our linkage sample A as well as found by independent BPAD studies these three genomic regions were selected for further fine mapping linkage studies. In addition to the non-parametric analyses, we performed multipoint MOD-score analyses using the newly developed program GENEHUNTER-MODSCORE (Strauch et al. 2003) and maximized parametric LOD scores with respect to the disease model parameters.

On chromosome 1p34-p36, we genotyped 27 additional STR markers, covering a region of 58.3 cM. In the combined sample, an NPL score of 3.69 was obtained at 45.9 cM. The best sub-sample score was observed in the Spanish families (NPL=3.97 at 45.9 cM). The highest MOD score value of 3.40 was obtained in the combined sample at 45.9 cM under a broad disease definition and a near dominant mode of inheritance. The data suggest that the susceptibility allele at this locus has a high frequency (16%) and incomplete penetrance (53%). The linkage region defined by the NPL and MOD score analyses is well circumscribed and covers less than 5 cM between markers D1S478 and D1S493 on chromosome 1p35-p36. This region has not been implicated in BPAD previously. The nearest region on 1p, for which linkage to BPAD or any affective disorder has been reported previously, is more telomeric, at marker D1S1597 (23.2 cM). A multipoint LOD score of 3.60 for this region was obtained in a sample of 81 families with UPR (Zubenko et al. 2003).

On chromosome 4q28-q31, we analyzed 13 additional STR markers between D4S1615 (129.7 cM) and D4S2982 (157.5 cM). In the entire fine mapping sample, we obtained evidence of linkage at 151.1 cM, with an NPL score of 3.18. The strongest evidence and the best result overall in the study was produced by the Romany sub-sample (NPL=5.49 at 148.4 cM). This was also the region for which MOD score analysis yielded the most significant result. In the combined sample, including the Romany, German and Bulgarian families, a MOD score of 4.24 was obtained at 147.2 cM under a medium disease definition, a disease allele frequency of 4.5 % and a near additive mode of inheritance. Application of the two other phenotype definitions supported this region, with a MOD score of 3.22 at 147.6 cM for the narrow, and of 2.76 for the broad disease definition. Our findings confirm the findings of genome-wide analyses of other investigators. Ekholm et al. (2003) observed a 3-point LOD score of 3.60 at 152 cM (D4S1629) in a sample of 41 BPAD families from an isolated Finnish population using a dominant model of inheritance and a broad diagnostic definition. In another genome-wide scan of 65 European-US families with BPAD, an NPL of 2.80 and a LOD score of 1.9 were reported, again at 152 cM (D4S1629) under a broad phenotype model (McInnis et al. 2003). A genome-wide study of 40 BPAD pedigrees from the USA and Israel obtained a 2-point LOD score of 3.16 under a dominant model and broad phenotype definition, with a maximum at 140 cM (marker D4S1625) (Liu et al. 2003). The peak identified in our fine mapping NPL and MOD score analysis spans around 10 cM, and is situated precisely in the D4S1625-D4S1629 interval supported by the above mentioned genome-wide analyses.

Fine mapping of the 6q15-q24 region, flanked by D6S1570 (101.5 cM) and D6S1633 (170.2 cM), was performed with 51 additional STRs. This large region is of particular interest as several previous studies have reported 'suggestive' and 'significant' evidence of linkage to BPAD. Rather than narrowing down the region, the fine mapping analysis led to the identification of three well-defined positive areas in each sub-sample, all of which have been reported previously in BPAD, namely 6q16, 6q23, and 6q24.

Evidence supporting 6q16 was mainly obtained from the German sub-sample, with an NPL score of 2.55 at 112.0 cM. Weak linkage evidence was found in the full fine mapping sample at the same position (NPL=1.42). Our results support the suggestion of evidence for linkage to 6q16 produced by the large NIMH study of 250 families (Dick et al. 2003; Schulze et al. 2004). They reported a LOD score of 2.26 at D6S1021 (107 cM), using a broad disease definition. At the same marker, Ewald et al. (2002) found a LOD score of 2.59 in two multiplex BPAD families from Denmark under a narrow phenotype definition.

We also obtained evidence of linkage to 6q23 (full fine mapping sample; NPL=2.85 at 135.1 cM), a region which was implicated in the Danish BPAD study (Ewald et al. 2002) with a 2-point LOD score of 2.49 at D6S1009 (139 cM) using a narrow disease definition. At the same marker, Rice et al. (1997) found a 2-point LOD Score of 2.08 in 97 US families of European descent. In the latter study, all markers on 6q between 112 cM and 155 cM yielded positive LOD scores under a broad affected status model.

The third linkage region, 6q24, was supported by the combined sample, producing an NPL of 3.18 at 147.68 cM. The strongest evidence was produced by the Romany sub-sample (NPL=4.87 at 152.0 cM), which was furthermore the second highest score in the study overall. As this was the best finding on chromosome 6q, and due to computational constraints, MOD score analysis was limited to the 6q24 region only. In the combined sample, including the Romany, German, and Bulgarian families, this analysis identified two peaks, at 146.1 and 152.5 cM, with MOD scores 3.59 and 3.10 under a broad disease definition. For both peaks, the best genetic model was nearly additive, with low disease allele frequencies and low penetrance. Using the medium and narrow phenotype definitions, we obtained MOD scores of 3.46 at 145.2 cM and of 3.32 at 147.6 cM. While previous support for this region has been modest (Rice et al. 1997, see above), strong evidence for a 6q24 BPAD susceptibility locus was provided recently by a genome-wide linkage study of an isolated population in northern Sweden (Venken et al. 2005). The study identified a 29 cM region on 6q (133-162 cM), with an MPLOD of 2.48. Follow-up fine mapping analysis, under a recessive, affected only model led to a 3 cM candidate region (144-147 cM) and an MPLOD of 3.25 at 146 cM, which matches the peak identified by our MOD score analysis under the narrow and medium phenotype definitions (Fig. 3). Collectively, the data provide strong support for a BPAD susceptibility locus on 6q24.

SNP-based LD mapping

Within the top linkage peaks of all three regions identified by the fine-mapping step we have started to perform systematic LD mapping using high-throughput SNP genotyping facilities. Since the haplotype block structure for our regions is not yet exactly known, we have adopted approaches that were successful in other LD mapping studies, i.e. spacing of SNPs at an average distance of 20kb. The genotyped markers were chosen from public databases (dbSNP, SNP consortium databases, HGVbase). At the current state of our mapping project the genotyping of 1,876 SNPs is completed. Our association sample consists of 300 BPAD patients and 300 ethnically and age matched controls. For each region we have obtained positive LD findings, in total 146 SNPs are associated at the nominal significance level. The results of the SNP-based LD mapping as well as the exact denotation of the associated markers (and haplotypes) will be presented by upon completion of this analysis step.

Outlook

Few other fields of medical research have experienced such enormous scientific progress as the investigation of complex genetic disorders, particularly of psychiatric disorders. Identifying susceptibility genes for psychiatric disorders will facilitate the understanding of underlying biochemical pathways (functional studies) and the development of more specific therapies (pharmacological studies). In addition, it will provide insights into the interaction of the various etiological factors (gene-gene- and gene-environmental-relationships) and will improve the validity of disease classification (gene-phenotype-relationship). This is likely to have important implications, given that BPAD constitute major public health problems with a significant morbidity and mortality.

*Lit.: **1.** Akiskal HS et al. (2003) Family history validation of the bipolar nature of depressive mixed states. J Affect Disord 73: 113-122 **2.** Angst J et al. (2003) Toward a re-definition of subthreshold bipolarity: epidemiology and proposed criteria for bipolar-II, minor bipolar disorders and hypomania. J Affect Disord 73: 133-146 **3.** Cichon S et al. (2001) A genome screen for genes predisposing to bipolar affective disorder detects susceptibility loci on 8q and 10q and provides evidence for an involvement of imprinted loci. Hum Mol Genet 25: 2933-2944 **4.** Dick DM et al. (2003) Genomewide linkage analyses of bipolar disorder: a new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. Am J Hum Genet 73: 107-114 **5.** Ekholm JM, Kieseppa T, Hiekkalinna T et al. (2003) Evidence of susceptibility loci on 4q32 and 16p12 for bipolar disorder. Hum Mol Genet 12: 1907-1915 **6.** Ewald H, Flint T, Kruse TA et al. (2003) A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24.3 and suggestive linkage to chromosomes 1p22-21, 4p16, 6q14-22, 10q26 and 16p13.3. Mol Psychiatry 7: 734-744 **7.** Liu J, Juo SH, Dewan A et al. (2003) Evidence for a putative bipolar disorder locus on 2p13-16 and other potential loci on 4q31, 7q34, 8q13, 9q31, 10q21-24, 13q32, 14q21 and 17q11-12. Mol Psychiatry 8: 333-342 **8.** McInnis MG, Dick DM, Willour VL et al. (2003) Genome-wide scan and conditional analysis in bipolar disorder: evidence for genomic interaction in the National Institute of Mental Health genetics initiative bipolar pedigrees. Biol Psychiatry 54: 1265-1273 **9.** Murray CJL, Lopez AD (1996) The global burden of disease. Geneva, World Health Organization, Harvard school of public health, World Bank **10.** Rice JP, Goate A, Williams JT, Bierut L et al. (1997) Initial genome scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 1, 6, 8, 10, and 12. Am J Med Genet 74: 247-253 **11.** Sklar P (2002) Linkage analysis in psychiatric disorders: the emerging picture. Annu Rev Genomics Hum Genet 3: 371-413 **12.** Strauch K (2003) Parametric linkage analysis with automatic optimization of the disease model parameters. Am J Hum Genet 73 (Suppl1): A2624 **13.** Venken T, Claes S, Sluijs S et al. (2005) Genomewide scan for affective disorder susceptibility Loci in families of a northern Swedish isolated population. Am J Hum Genet 76: 237-248 **14.** Zubenko GS, Maher B, Hughes HB et al. (2003) Genome-wide linkage survey for genetic loci that influence the development of depressive disorders in families with recurrent, early-onset, major depression. Am J Med Genet 123B: 1-18*