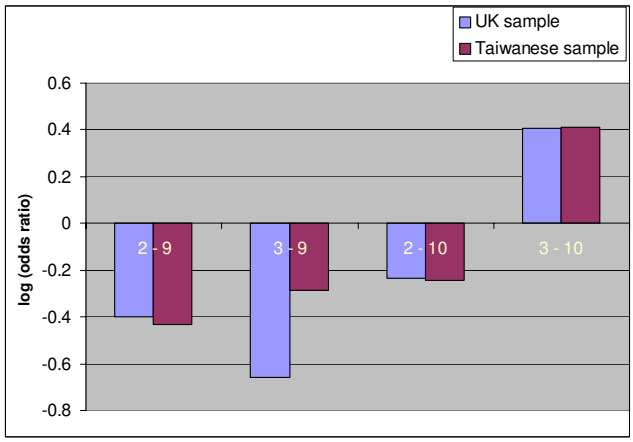

Figure 3
Log of the odds ratios for haplotype specific associations between ADHD and the intron 8 and 3'-UTR repeat polymorphisms in DAT1. Only chromosomes that contained the specific combination of the 3-repeat allele at the intron 8 marker and the 10-repeat at the 3'-UTR marker were over-transmitted from heterozygote parents to their affected offspring with ADHD (adapted from Brookes et al., 2005) [14].

viduals to identify all potential causal variants. Although at this time re-sequencing of all potential genes associated with ADHD is prohibitively expensive, it is envisaged that technical developments will make such an approach feasible within the next decade.

Screening candidate genes for association

The candidate gene approach has been successful in identifying several genetic variants that are associated with ADHD. This has been largely a matter of good fortune, in the sense that the genes investigated initially were selected since they code for protein targets of many treatments used in general psychiatry, including stimulants used to treat ADHD. Such studies are, however, far from complete and in some cases candidate genes have been prematurely described as *not associated* with ADHD. This has occurred for two main reasons.

First, sample sizes used to date are insufficient to reliably detect small effect sizes, similar to those identified so far. Table 1 lists the sample sizes required to replicate the most significant findings reported to date, assuming 80% power of detection and a nominal alpha value of 0.05. We have also listed the amount of power at the same alpha level to detect these genetic effects with a sample size of 200, which is similar to that used in many published studies to date. Our most recent analyses of DAT1 and the dopamine D4 receptor gene (DRD4) in the IMAGE sample illustrate the problem. In a sample of 680 families we just hit nominal significance for the DAT1 association, whereas for DRD4 we required a total dataset of over 1,100 families [17]. For both associations the observed odds ratios were very close to those reported in the meta-analysis by Faraone et al. [1] (Table 1).

Second, few studies have taken a comprehensive approach to the analysis of individual genes by scanning genetic variation across entire gene regions. An example of a comprehensive gene-based approach is reported in a recent study of the noradrenergic transporter gene (NET1) [23]. This initial study aimed to screen the entire region spanning NET1. This was achieved by selecting database SNPs with minor allele frequencies greater than 5% that occurred within known functional regions; upstream promotor region, 5' and 3' untranslated regions, coding regions (exons) and intron sequences flanking each exon. The various sequences that make up the DNA sequence for typical protein coding genes are illustrated in Figure 2. Since NET1 has not been fully sequenced in multiple individuals, we do not know the location of all potential functional variants within the gene, which might for example include regulator elements in non-coding regions of the gene. Additional tagging SNPs were therefore selected, which were predicted to tag polymorphic variants (through LD) that are currently unknown and therefore not available for direct association analysis. In total we identified 26 SNPS and screened these for association with ADHD in case and control samples. Three SNPs were identified that showed nominal significance. Two of SNPs that had previously been reported to show no association with ADHD [24-26] were also negative in this study.

The small effect sizes that we observed for the three nominally associated SNPs led us to conclude that, after adjustment for the 26 SNPs tested, there was no evidence for association between ADHD and NET1. In a subsequent study, Bobb et al. [27] reported on genetic variants that had previously been reported to show nominal association with ADHD and found significant association with two of the three SNPs that we had identified (rs998424 and rs3785157), although with the opposite SNP alleles. The two markers associated with ADHD in both studies are strongly correlated with each other, having an r-square statistic of 0.93 in the UK sample and can therefore be considered to tag a single genetic association. However, despite evidence for association with the same two SNPs in two studies, we cannot be confident in these findings due the different directional effects of the SNP alleles. Further studies will therefore be required to clarify whether the SNP cluster tagged by these two markers is associated with ADHD or whether these are merely chance observations.