7 OUTLOOK

This project involved a search for genes important in cognitive function, starting from the characterisation of disease-associated balanced chromosome rearrangements. Initial studies centred on the X chromosome and revealed direct evidence supporting a role for the zinc finger gene *ZNF41* in brain function. Several known closely related *ZNF41* splice variants exist, and the predominant transcript codes for a protein with an established transcriptional repressor domain (Senatore et al. 1999). Loss of functional ZNF41 is associated with a severe phenotype in the translocation patient; in a second patient, with a milder phenotype, a splice-site mutation results in absence of specific splice variants. Characterisation of existing transcripts, in particular those that are absent in the patients investigated, and careful analysis of their respective expression in various tissues is of interest. Moreover, two novel *ZNF41* transcripts were introduced in this study. Transcript *ZNF41.9*, amplified by RT-PCR, is a novel variant affected by the splice-site mutation. Its predicted open reading frame lacks the transcriptional repressor domain, suggesting that it may serve a unique function that warrants investigation. The large transcript observed in foetal brain is a second obvious starting point for future investigations. It is of particular interest, for example, to determine if this transcript is also affected by the splice-site mutation. In conjunction, further screening for MR-associated alterations in *ZNF41* is underway; identification of additional mutations may shed light on which aspects of ZNF41 function are specifically important for cognitive development.

While studies on the X chromosome in the two remaining patients with X;autosome translocations did not reveal definitive candidate MRX genes, investigations into the relationship between translocation and disorder merit further attention. In both cases, it is conceivable that the translocation affects one or several X-chromosomal genes that are not in the immediate breakpoint vicinity. Indeed such position effects are possible; there are several document examples (for review, see Kleinjan and van Heyningen 1998). However, it is important to note that in both of these cases, the autosome breakpoint still remains uncharacterised. At least in the case of Patient 1, whose autosome breakpoint lies in chromosome 7q, this is a logical starting point for future investigations that may directly reveal novel candidate genes. In Patient 2, this approach is less likely to prove useful as her autosome breakpoint is in chromosome 15p, where there are no known genes. However, it is

possible that molecular studies on this breakpoint will reveal significant anomalies that could not be detected at the cytogenetic level. Before pursuing studies on undisrupted X-chromosomal genes, therefore, the autosome breakpoints will be investigated.

Our studies on disease-associated autosome translocations also led to the identification of a promising candidate gene, the c-Jun terminal kinase *JNK3*. The truncated JNK3 protein present in Patient 5 may have toxic effects, given its aberrant cellular localisation in over-expression experiments. In addition, it may have a specific dominant negative effect on normal signalling through JNK3, which has direct implications for neuronal apoptosis and neuronal differentiation. Future studies aim to elucidate the molecular and physiological effects of the truncated JNK3 protein. More specifically, we will assess the toxic effects of the aberrant protein by over-expression studies and subsequent cell death assays. Secondly, we will explore the effects of the mutant JNK3 on induced neurite outgrowth in cell culture experiments. We will also attempt to identify JNK3 binding partners, several of which we described in the discussion, that interact preferentially with the mutant protein. Another strategy for investigating the molecular aspects of the patient's disorder is the creation of a transgenic mouse model in which the truncated JNK3 protein is expressed. Such an animal model enables us to address the same questions with *in vivo* studies in the most relevant tissues. Together, these investigations will illuminate the JNK-dependent molecular cascades that are important in cognition.

Finally, it is also plausible that the severe phenotype observed in Patient 4 results from the gene defects that arise from her translocation. In particular, our studies draw attention to a set of novel, uncharacterised genes, one or several of which may play a causal role in the disorder. Especially in light of the severe brain malformations observed in this patient, characterisation of the novel, brain-expressed transcripts that are disrupted by the breakpoint, and studies on the corresponding proteins is a worthy pursuit. Results may provide important insights into the molecular regulation of brain structural development and function.