5 SUMMARY

In this study we have characterised the chromosome breakpoints in five patients with mental disabilities and associated balanced translocations, with the aim of identifying both X-chromosomal and autosomal genes critical for cognitive function. In light of the established link between mental disability and X-chromosomal genes, together with the practical advantages of studies that involve the X chromosome, we first focussed on patients with X;autosome translocations. In two out of three patients, molecular analyses provided no evidence for disease-causing gene disruptions on the X chromosome. In Patient 3, however, with a severe phenotype, the zinc finger gene *ZNF41* was disrupted, and full-length transcripts could not be detected in the patient cell line, suggesting an absence of functional ZNF41 protein. Studies on unrelated families with X-linked mental retardation (XLMR) led to the identification of two novel nucleotide exchanges in the *ZNF41* gene that likely play a role in the disease, based on their absence in 400 X chromosomes from controls. One of these mutations results in a proline to leucine amino acid exchange (P111L); the second is an intronic mutation (479–42A>C) that affects a consensus splice-site and results in loss of transcription of specific *ZNF41* splice variants in the patient cell line. Both of these point mutations are associated with mild MR. The *ZNF41* gene is expressed in multiple tissues, including both foetal and adult brain, and it has been implicated in transcriptional repression. Other ubiquitously expressed genes, namely the methyl CpG binding protein encoded by *MECP2* and the helicase-encoding *ATRX*, have also been implicated in both mental retardation and regulation of transcription. Although it is not yet understood how mutations in these three genes exert brain-specific effects, it is conceivable that in neurons, their functions converge on a common critical pathway. In light of our results, *ZNF41* is an especially strong novel candidate for XLMR.

Rearrangements in two additional patients with severe cognitive disabilities and associated autosome translocations were also investigated as part of this study. Molecular cytogenetic analyses indicated the presence of well-characterised candidate genes in the vicinity of the breakpoints for both cases: in Patient 4, the brain-specific forkhead transcription factor *FOXG1B*, and in Patient 5, the predominantly CNS-expressed c-Jun terminal kinase gene *JNK3*. More precise localisation of the relevant breakpoints indicated

that *FOXG1B* was not disrupted; *JNK3,* however, was interrupted within the coding sequence. For this reason, further studies focussed on *JNK3*. JNK3 plays an established role in neuronal apoptosis, and it has recently been shown to directly influence neuronal differentiation. The breakpoint results in translation of a truncated JNK3 protein, which could be detected by western blot in the patient cell line. Over-expression of this mutant protein in various cell lines, including HeLa and Neuro2A, led to protein aggregation, whereas over-expressed wild type JNK3 exhibited diffuse localisation, suggesting that the mutant plays an aberrant role in patient cells. We speculate that the severe neurological phenotype observed in Patient 5 results from a partial loss of JNK3 function in neurite outgrowth, together with a dominant effect on normal GTPase-mediated signalling through JNK3, which could result in modified regulation of neuronal apoptosis. This provides a plausible explanation for the severe phenotype observed in the patient.

Taken together, our results affirm that making use of disease-associated balanced translocations remains an efficient approach for identifying candidate disease genes. In studying five patients, we have uncovered two promising candidate genes for mental retardation, and through both *in silico* and molecular studies have obtained further evidence for their respective roles in cognition. Moreover, our data support the recent hypothesis that both precise control of chromatin remodelling and fine regulation of GTPase-mediated signalling are essential for normal development and function of the brain.