Summary

To date, mutations in ~75 different genes have been associated with mental retardation (MR), but these likely only represent the tip of the proverbial iceberg. A diminished ability to adapt to the daily demands of a normal social environment, an onset during childhood and an intelligence quotient < 70 define MR.

Anatomical post-mortem examinations have demonstrated that dendritic spines, which are responsible for synaptic connections in the brain, are abnormal in different forms of MR. A large body of evidence shows that learning changes spine morphology and synaptic plasticity through remodelling of the Actin cytoskeleton. Mutations upsetting such remodelling cause MR.

In uniting a pathologic phenotype with a specific genotype, disease-associated balanced chromosomal rearrangements provide a unique opportunity to identify genetic loci relevant to health. In this study, we characterised two novel genes that are disrupted by a *de novo* apparently balanced t(X;8) translocation in a mildly mentally retarded patient. While the autosomal breakpoint disrupts *hFBXO25*, the X-chromosomal breakpoint interrupts *hKIAA1202*.

After characterising the genomic structure of *hFBXO25* and its murine counterpart, establishing their expression patterns and investigating their subcellular localisation, we discovered that hFBXO25 is a *bona fide* F-box protein (FBP), which forms part of the $SCF^{hFBXO25}$ complex. FBPs confer substrate specificity to the SCF E3 ubiquitin ligases, which poly-ubiquitinylate cell cycle regulators for proteasomal degradation. In line with their function, misregulation of and mutations in FBPs have been mostly associated with malignancies; a direct link between FBPs and MR has not yet been reported.

Based on the genomic structure of *hKIAA1202*, and after having confirmed its expression in both foetal and adult human brain, we – and several collaborators – performed an extensive mutation analysis. This effort resulted in the identification of a second patient with mild MR carrying a different *de novo* apparently balanced translocation also disrupting *hKIAA1202*, a silent substitution possibly affecting an exonic splice enhancer in a small family with MR and a c.3266C>T missense exchange segregating with severe MR characteristic of the Stocco dos Santos syndrome. As *hKIAA1202* is subject to X chromosome inactivation (XCI) and both translocation carriers show skewed XCI leading to inactivation of the intact X chromosome, their genetic make-up is predicted to represent a *hKIAA1202* null mutation. However, RT-PCR experiments and generation of an α-hKIAA1202 antibody demonstrated that uncharac-

terised *hKIAA1202* isoforms, which the c.3266C>T transition could affect, are still present in a cell line of the t(X;8) patient. Owing to secondary selection processes, certain mutations result in skewed XCI. Such mutations are often lethal to hemizygous male offspring, resulting in increased frequencies of spontaneous abortion in carrier females. Therefore, the Stocco dos Santos *hKIAA1202* variant could explain the skewed XCI observed in all carrier women and may be the cause of the large number of spontaneous abortions and high rate of infant mortality in the family.

Based on its domain structure, which is compatible with molecular scaffolding and binding of filamentous Actin (F-actin), we have established hKIAA1202 as one of the founding members of the Shroom family of cytoskeleton-associated proteins. By anchoring overexpressed hKIAA1202 to the mitochondria, we demonstrated that it can direct the subcellular distribution of F-actin *in vivo*. We also demonstrated co-localisation of endogenous hKIAA1202 with F-actin at sites of rapid Actin remodelling, such as the leading edge of fibroblasts and the neurites of differentiating neuronal cells. Preliminary *in vitro* studies indicate an interaction between hKIAA1202 and F-actin.

Employing yeast two-hybrid methodology, we identified Vimentin, a cytoskeletal intermediate filament, and several proteins involved in chromatin remodelling and transcriptional regulation as putative hKIAA1202 interaction partners. Interference with transcriptional regulation, mainly through chromatin remodelling, has been established as a mechanism underlying the aetiology of MR.

Taken together, we have provided evidence suggesting *hKIAA1202* as a gene involved in human cognition. Such a role is unlikely for *hFBXO25*.