

## Abstract 6

### FUNCTIONAL ANALYSIS OF *UBE2A* C.382C-T MUTATION: INSIGHT INTO THE MECHANISMS LEADING TO MENTAL RETARDATION

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We have previously described a nonsense mutation (c.382C>T) in the *UBE2A/HHR6A* gene (ubiquitin-conjugating enzyme 2A) as the cause of a novel X-linked mental retardation syndrome. It was the first description of an ubiquitin-conjugating enzyme mutation associated with a human disease. *UBE2A* is one of the two human homologs of the *Saccharomyces cerevisiae* RAD6 gene. In humans, the RAD6 ortholog is duplicated, with one X-linked (*UBE2A*) and one autosomal (*UBE2B*) copy, both able to complement the rad6-null yeast strain phenotypes. The proteins encoded by these genes are strongly conserved: the human proteins *UBE2A* and *UBE2B* share about 95% amino acid sequence identity with each other and about 70% amino acid sequence identity with their yeast counterpart. The mutation in our patients introduced a premature stop codon (Q128X) and abolished the 25 C-terminal amino acids of the protein. Here we report on the effect of this mutation on *UBE2A* function. Complementation assays in a rad6-null yeast strain revealed that the defective protein was unable to restore the UV-light sensitivity phenotype and was toxic to the RAD6 knockout strain, but not to the wild type yeast. *In vitro* ubiquitin-conjugating activity assays towards histone H2A showed that, although capable of interacting with the ubiquitin molecule, the defective protein was unable to transfer ubiquitin to histones; both the wild and the defective *UBE2A* proteins were capable of autoubiquitination, the former more efficiently. Our results also showed that ubiquitination and degradation of the defective protein depended on the presence of the paralog *UBE2B* in human or the ortholog RAD6 in yeast. Taken together, these data suggest that *UBE2A/UBE2B* regulate their own degradation. Therefore the presence of the mutated *UBE2A* protein could lead to mental retardation not only directly due to loss of function, but also by becoming a new substrate for *UBE2B*.

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