

Abstract 7

CHROMOSOME SORTING AND NEXT GENERATION SEQUENCING IN CONSANGUINEOUS FAMILIES WITH AUTOSOMAL RECESSIVE MENTAL RETARDATION: SEPARATING THE WHEAT FROM THE CHAFF

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Homozygosity mapping in 3 patients with moderate to severe non-syndromic mental retardation from a consanguineous Iranian family has enabled us to assign the underlying defect to a single 18.9 Mb interval on chromosome 6p21. This novel interval encompasses 350 gene loci including numerous HLA genes, thereby rendering mutation screening by conventional Sanger sequencing very time-consuming and expensive. After considering various other options, including recently developed hybridization-based enrichment protocols, we decided to use preparative chromosome sorting in combination with next generation sequencing to identify the causative mutation in this family. In the coding regions of this interval, Illumina GA II sequencing revealed a total of more than 5000 single nucleotide variants (SNVs) that are not listed in dbSNP129, including 12 non-synonymous changes in coding regions, but no small (1-2 bp) indels. Large (>100.000 bp) deletions and duplications were excluded by array CGH. After comparison with variants that were also present in recently sequenced genomes of healthy individuals (i.e., Watson, Venter or Yanhuang), 8 changes remained including a homozygous (W to X) nonsense mutation in codon 171 of the *BTN3A3* gene. However, the same homozygous change was also found in two healthy members of the family and could be ruled out as disease-causing mutation. 5 other non-synonymous changes (in the *CCHCR1*, *ATF6B*, *PSORS1*, *MAS1* and *SPDEF* genes, respectively) co-segregated faithfully with the disease but were classified as benign upon PolyPhen analysis. Two changes were classified as probably pathogenic, an H to R exchange in the protein product of *C6ORF89*, a gene of unknown function, and an R to C exchange in the *ANKS1A* gene product. *ANKS1A* is expressed in the brain and structurally similar to *ANK1*, which codes for ankyrin and has been implicated in mental retardation. Currently, the *ANKS1A* mutation and the other 7 remaining variants are being tested in a set of >350 control chromosomes to further evaluate their pathogenetic relevance. To our knowledge, this is the first attempt to identify novel ARM genes by next generation sequencing. Results of the ongoing studies in this family will be presented and possible pitfalls of our approach will be discussed.