Alternative Splicing in FMR1 Premutation Carriers

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Background: The FMR1 gene, as many housekeeping genes, undergoes alternative splicing. Using Single Molecule, Real-Time (SMRT) sequencing and qRT-PCR we have recently reported that the relative abundance of all FMR1 mRNA isoforms is significantly increased in the premutation group compared to controls. However, there is a disproportionate increase, relative to the overall increase in mRNA, in the abundance of isoforms spliced at both exons 12 and 14. In this study, we have further investigated the transcriptional FMR1 isoforms distribution pattern in different tissues including muscle, brain, heart and testes from 3 individuals with a premutation allele and affected by Fragile X-associated tremor/ataxia syndrome (FXTAS) and compared them to the isoform mRNA profile of age-matched controls. Here we report on the identification of novel isoforms which might play a role in the pathogenesis of FXTAS.

Materials and Methods: Tissue samples from 6 subjects including three premutation male carriers and 3 age-matched male controls were used. A combination of PCR and Southern blot analysis was used to measure the CGG repeat size of the FMR1 alleles. Total RNA from postmortem tissues were isolated with Trizol. RNA integrity was checked using the Agilent 2100 Bioanalyzer. cDNA synthesis was performed as previously described (Tassone 2000). PCR was carried out using specific primers for the different group of isoforms. SMRT sequencing and qRT-PCR were used to proportionally measure FMR1 isoforms mRNA expression levels in the different tissues.

Results

- Using SMRT sequencing, we obtained 23,000 to 28,000 full-length non-chimeric (FLNC) reads in different tissues for each of the premutation carrier samples and between 10,000 to 12,000 FLNC reads for each of the control samples (Table 1).
- Using SMRT sequencing, 48 different isoforms were identified, 45 were observed in premutation carriers compared to 19 in the normal group. Three of them were unique to the normal group (Table 2, Figure 1).

We identified a number of novel isoforms including one that contains “a coding region” between exons 9 and 10 (coordinate: chrX:147014919-147015059). This region was absent in normal tissues and it was found only in samples from premutation carriers (Table 3, Figure 2).

- Preliminary data indicates the existence of transcriptional signature of the FMR1 gene which differs between premutations and controls (Figure 3).

Our findings suggest that an abnormal alternative splicing process is present in individuals with premutation alleles. The characterization of expression levels of the different FMR1 isoforms is fundamental for understanding the regulation of the FMR1 gene as well as for elucidating the mechanism(s) by which “toxic gain of function” of the FMR1 mRNA may play a role in FXTAS and/or in the other FMR1-associated conditions.

References