DOI 10.18699/SBB-2022-235

Alternative splicing of human *FMR1* gene in peripheral blood: making a single-gene transcriptome

Lan F.H.[1, 2]*, Qiu P.[3], Yan A.Z.[1, 2], Xu Y.J.[1, 2], Fu X.G.[4], Yang W.J.[5], Liao J.[6], Guo X.Y.[7], Zhang D.[1, 2], Zheng D.Z.[1, 2]

[1] *Center for Medical Genetics, Dongfang Hospital, Xiamen University School of Medicine, Fuzhou, China*
[2] *Fujian Key Laboratory of Transplantation Biology, Fuzong Clinical College, Fujian Medical University, Fuzhou, China*
[3] *Department of Laboratory Medicine, Yichang Central Hospital, Yichang, Hubei Province, China*
[4] *Central Laboratory, Ningde Municipal Hospital, Ningde, Fujian Province, China*
[5] *Department of Laboratory Medicine, Zhongshan Hospital of Fudan University, Shanghai, China*
[6] *Department of Laboratory Medicine, Fujian People's Hospital, Fujian University of Traditional Chinese Medicine, Fuzhou, China*
[7] *Department of Laboratory Medicine, Fuzhou Second Municipal Hospital, Xiamen University School of Medicine, Fuzhou, China*
** fhlan@xmu.edu.cn*

Key words: alternative splicing, *FMR 1* gene, transcriptome, fragile X syndrome

Background and Aim: Alternative splicing represents an important mechanism by which a eukaryotic organism diversifies the products from each single gene and aid a further layer to the network of gene regulation [1]. Fragile X mental retardation 1 gene (*FMR1*), the disease-determining gene of fragile X syndrome (FXS), which encodes the fragile X mental retardation protein (FMRP), is one of first human genes having the most abundant spliced products [2-6], with up to 20 FMRP isoforms being found before us [2]. The present study aims to further identify more spliced variants from *FMR1* gene in human peripheral blood, and the significance of single-gene transcriptome would be discussed.
Methods and Algorithms: RT-PCR and clone-sequencing technology was employed to detect the types of alternatively spliced products in the peripheral blood from 10 normal individuals and their expression abundance was analyzed. Bioinformatics to analyze the splice sites and predict the structure of new splice isoforms was carried out by visiting the corresponding open-source servers. Potential functions of a splice isoform were initially studied by its lentiviral vector-based over-expression in HEK293T cells, followed by tracking the expression patterns of relevant genes.
Results: A total of 50 alternatively spliced products, including 27 novel ones, were identified from 505 recombinant plasmids of PCR product. The alternatively spliced products with the highest abundance are ISO 17 and ISO 7, both of which involve exon 12 skipping, followed by ISO 1 (containing the full-length sequence of the coding region) and ISO 13 (using the second acceptor site of exon 17), while the frequency of other types of spliced products is less than 5.0 % (Fig. 1).The novel alternatively spliced variants identified in this study involved the skipping of exons 3, 4, and 11, insertion of 30bp and 87bp sequences from the middle of intron 7, and 46 bp and 140bp sequences from the middle of intron 9. Bioinformatics analysis of the splice sites was performed, justifying the splicing of these vatiants. The structure of three novel spliced isoforms ISO51-ISO53 was predicted by I-TASSER server, showing that the intracellular location of truncated FMRP protein encoded by the novel transcripts was

altered. Over-expression of one novel FMRP isoform encoded by a spliced transcript with the insertion of the 46bp from amid intron 9 of human *FMR1* gene demonstrated that xCT might be a new interacting protein of FMRP.

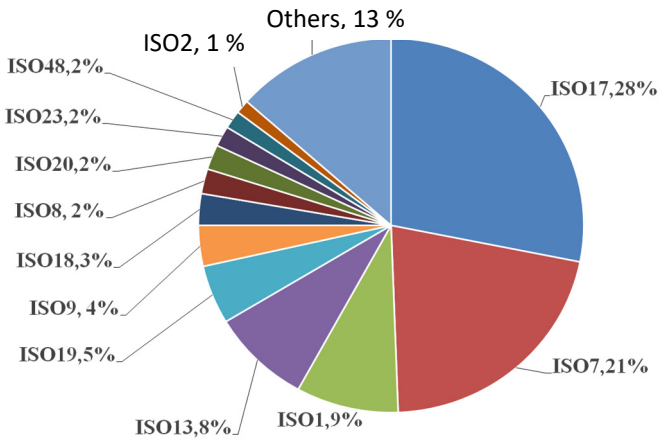

Fig. 1. Expression abundance of spliced variants from human *FMR1* gene in peripheral blood

Conclusion: This study not only enriched our understanding of the complexity and diversity of alternative splicing of *FMR1* gene, but also laid the foundation for exploring the effects of alternative splicing on the function of FMRP and the pathogenesis of FXS.

Acknowledgements: This study was supported by Key Scientific Projects of Fujian Province, P. R. China (2010Y0045, 2016Y0071) and Fujian Provincial Natural Science Foundation of China (2018J01341).

References

1. Blencowe B.J. Alternative splicing: new insights from global analyses. *Cell.* 2006;126(1):37-47.
2. Sittler A. et al. Alternative splicing of exon 14 determines nuclear or cytoplasmic localization of fmr1 protein isoforms. *Hum Mol Genet.*1996;5(1):95-102.
3. Fu X. et al. Alternatively spliced products lacking exon 12 dominate the expression of fragile X mental retardation 1 gene in human tissues. *Mol Med Rep*. 2015;12(2):1957-1962.
4. Tseng E. et al. Altered expression of the FMR1 splicing variants landscape in premutation carriers. *Biochim Biophys Acta Gene Regul Mech.* 2017;1860(11):1117-1126.
5. Fu X.G. et al. Splicing of exon 9a in FMR1 transcripts results in a truncated FMRP with altered subcellular distribution. *Gene.* 2020;731:144359.
6. Yang W.J. et al. Further identification of a 140bp sequence from amid intron 9 of human FMR1 gene as a new exon. *BMC Genet.* 2020;21:63-72.