ABSTRACT

Predicting the role of RNA binding proteins interaction with the mRNAs transcribed from 15q11.2 BP1-BP2 microdeletion region

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The Burnside-Butler susceptibility area, a microdeletion region in 15q11.2 BP1-BP2, is linked to neurodegenerative disorders. The four conserved, non-imprinted protein-coding genes in this region are *TUBGCP5, CYFIP1, NIPA1*, and *NIPA2*. This study aims to investigate the interaction of RNA-binding proteins (RBPs) with the mRNAs of four genes in the microdeletion area and their probable function to better understand the pathogenic effects when deleted. In-silico study was done by intersecting the eCLIP data from ENCODE with the genomic position of the four genes located in 15q11.2 BP1-BP2 susceptibility region to find out the RBPs binding to the region. Then the significant RBPs were filtered for each gene and the binding of RBPs FASTKD2 and EFTUD2 with the mRNAs encoded from *CYFIP1* and *TUBGCP5* genes were done using in-vitro WEMSA experiments. Our result indicates that most of the RBPs interacting with the susceptibility region are involved in post-transcriptional regulation of the concerned genes. RBPs binding to UTRs, coding region, and junction region were found. The fact that these proteins bind to exon-intron junctions implies that they may be involved in the splicing process. In addition to their functional relevance in normal development, or lack thereof in neurodevelopmental disorders, this work may contribute to a better understanding of the complex connection between RBPs and mRNAs within this area. This insight will aid in the development of improved treatment techniques.

Keywords: 15q11.2 microdeletion, WEMSA, BP1-BP2, eCLIP, RBPs

Introduction

The 15q11.2 microdeletion region is situated in chromosome 15 which contains five breakpoints such as BP1, BP2, BP3, BP4, and BP5 in the proximal end of the long arm of the chromosome. The 15q11.2 BP1-BP2 microdeletion region is approximately 500 kb long and belongs to the Burnside-Butler Syndrome. This region consists of four non-imprinted genes such as *NIPA1*, *NIPA2*, *CYFIP1*, and *TUBGCP5*. These genes are individually found to be participating in neuro-developmental processes and any alteration leads to neurodegenerative issues. Since RBPs play crucial role in the function of RNA, hence, it is important to explore the various RBP interaction with the mRNAs in the susceptibility are to understand the cause of the microdeletion which then leads to the neurodegeneration problems.

Conclusion

Highest number of RBPs are binding to CYFIP1 RNA and the lowest is for TUBGCP5 RNA which may be due to their gene size.

Among the top 3 RBPs which are binding to NIPA1, NIPA2, CYFIP1 and TUBGCP5, most of them are known to be involved in splicing such as FAM120A, RBFOX2, PRPF8, etc. RBPs like PUM2 and KHSRP are known to be associated with neurological processes.

WEMSA experiment proves the binding of FASTKD2 and EFTUD2 RBPs with CYFIP1 and TUBGCP5 RNA.

References

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Predicting the role of RNA binding proteins interaction with the mRNAs transcribed from 15q11.2 BP1-BP2 microdeletion region

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Objectives

Objective 1: In-silico analysis of RNA-RBP interaction on 15q11.2 BP1-BP2 microdeletion region.

Objective 2: In-vitro validation of RNA-RBP interaction on 15q11.2 BP1-BP2 microdeletion region.

Methodology

In-silico analysis of the RBPs interacting with the RNAs present in the 15q11.2 BP1-BP2 microdeletion region

Bed file with chromosome no., genomic coordinates, and gene names

eCLIP data from ENCORE RNA ELEMENTS

Intersection

RBPs binding at 15q11.2 BP1-BP2

Filter

RBPs with $-\log_{10}$P value ≥ 3, $\log_2$fold enrichment ≥ 2

In-vitro Experiments

In-vitro transcription

RNA purification

RNA-RBP binding

WEMSA

Results

RNA	Total RBPs	Significant RBPs	Unique RBPs
NIPA1	2707	137	52
NIPA2	1898	75	30
CYFIP1	4778	317	62
TUBGCP5	1195	54	15

Table 1: Total no. of significant RBPs binding to the RNAs in 15q11.2 BP1-BP2 microdeletion region.

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Results

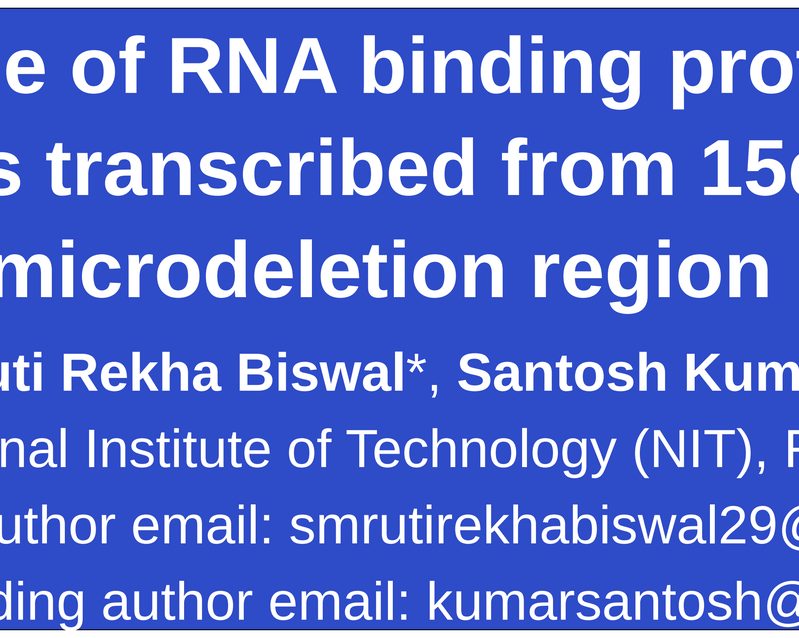

Figure 1: RBPs interacting with the RNAs of the microdeletion region with applied cut-offs. a, b, c, and d shows the RBPs binding with NIPA1, NIPA2, CYFIP1, and TUBGCP5 RNAs and the significant RBPs lie in the second quadrant when cut-off ($\log_2$ fold enrichment ≥ 2, $-\log_{10}$ (P) ≥ 3) is applied.

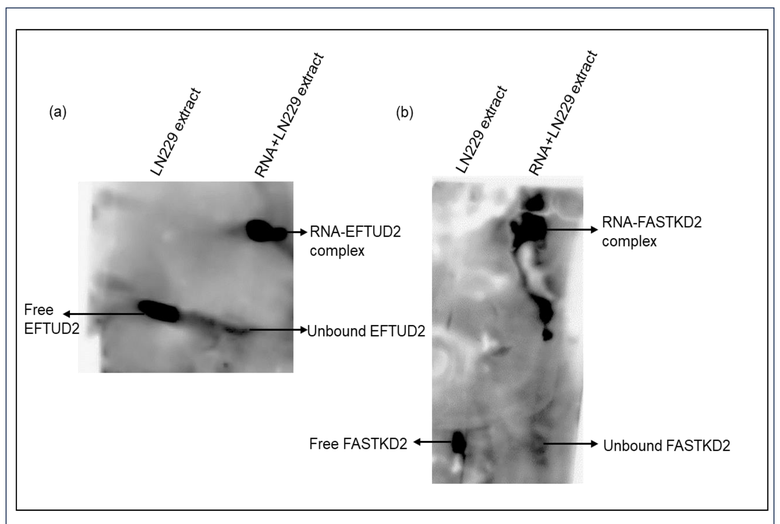

Figure 3: In-vitro analysis showing RNA-RBP interaction through combined western blot and EMSA experiment. **(a)** It shows the binding of TUBGCP5 RNA with EFTUD2 RBP. The band on the first lane shows the protein control, the top right band on the second lane shows the TUBGCP5 – EFTUD2 complex and the lower band shows the unbound EFTUD2. **(b)** The blot image represents the binding of CYFIP1 RNA with FASTKD2 RBP. The band on the first lane shows the protein control, the top right band on the second lane shows the CYFIP1- FASTKD2 complex and the lower band shows the unbound FASTKD2.

Figure 2: IGV image showing the top 3 RBPs with maximum $-\log_{10}$(P) value and $\log_2$ fold enrichment value. Significant binding of the RBPs with RNAs (NIPA1, NIPA2, CYFIP1, and TUBGCP5) is depicted through the peaks. The colored reads belong to the RBPs binding and the grey reads represent their SMInput. **(a), (b)** Positive peaks shows that RBPs bind at plus strand in NIPA1 and NIPA2. **(c), (d)** RBPs bind at minus strand of CYFIP1 and TUBGCP5 hence, the peaks are in downward direction.

Conclusion

- Highest number of RBPs are binding to CYFIP1 RNA and the lowest is for TUBGCP5 RNA which may be due to their gene size.
- Among the top 3 RBPs which are binding to NIPA1, NIPA2, CYFIP1 and TUBGCP5, most of them are known to be involved in splicing such as FAM120A, RBFOX2, PRPF8, etc. RBPs like PUM2 and KHSRP are known to be associated with neurological processes.
- WEMSA experiment proves the binding of FASTKD2 and EFTUD2 RBPs with CYFIP1 and TUBGCP5 RNA.

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