Next Generation Sequencing of autism genes in the Lebanese population and their functional evaluation

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Introduction

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders characterized by marked difficulties in social and verbal communication, and behavior. In Lebanon, ASD prevalence is 1/68 children. Recent findings by our group support a heterogeneous genetic etiology, including rare de novo and inherited mutations, chromosomal rearrangements, as well as double hit mutations. Only a fraction of autism genes have been discovered world-wide. Applying whole exome sequencing (WES) to Lebanese families in order to identify mutations is extremely useful in identifying recessive causes of autism.

AIMS:
• Uncover novel autism risk genes in the Lebanese population
• Characterize functional impact of rare variants in candidate genes
• Investigate effect of a novel frameshift mutation within the Ubiquitin-like domain-containing C-terminal domain phosphatase 1 (UBLCP1) gene.

Methods

Materials:
Eight Lebanese families were chosen because of consanguinity or having 2 affected children. These families did not show any pathogenic aberrations with Affymetrix microarray genechip analysis.

Whole exome sequencing and validation:
Whole-exome sequence (Illumina HiSeq) was obtained with a mean target coverage of 90% at ≥20x and a mean read depth of 106X. Variants were validated by Sanger sequencing in the patient and the available family members in normal Lebanese controls (n=104).

Quantitative real-time PCR:
qPCR was performed in triplicate using specific primers and the SYBR Green Superscript (RoRo). Melting curve analysis was applied and all results were normalized to GAPDH level and calculated using the ΔΔ CT method.

UBLCP1 subcloning:
Normal and mutated UBLCP1 were each subcloned into a pcDNA3.1- myc-HisA vector, containing CMV promoter, a mycynion selection marker, and a C-terminal tag encoding a polystatine metal-binding peptide for rapid purification on nickel-chelating resin.

Cell culture and transfection:
PC12 cells were grown in DMEM supplemented with 10% FBS, 5% NaHCO3, 5% sodium pyruvate in 5% CO2 at 37°C. Transfections were performed with electroporation (Qiagen). Stably transfected cell lines were selected by culturing transiently transfected cells in the presence of 500 μg/ml of G418 for 14 days.

Effect of the novel mutation within UBLCP1

The novel UBLCP1 gene mutation is predicted to generate a stop codon within the sequence encoding the protein phosphatase domain. This is expected to lead to a truncated protein with a dysfunctional phosphatase activity, drastically affecting UBLCP1 protein function.

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Cellular activity

The direct effect of normal and mutated UBLCP1 on proteasome activity will be measured in vitro utilizing a fluorescent synthetic peptide substrate, Suc-LLVY-MCA, which gets converted to a highly fluorescent degradation product, 7-amino-4-methylcoumarin (AMC), upon cleavage by proteasome.

Conclusion

Proposed studies will establish a powerful analysis protocol allowing identification of prioritized lists of potential deleterious variants in the Lebanese population enabling confirmation of ASD susceptibility genes, and uncover novel ones. Extensive functional studies on UBLCP1 and other candidate genes will link novel mutations to ASD.

References


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