TO STUDY THE ROLE OF POTENTIAL BIOMARKERS IN AUTISM SPECTRUM DISORDER(ASD)

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INTRODUCTION

Early identification and treatment of individuals with Autism spectrum disorder (ASD) improves outcomes, but specific evidence needed to individualize treatment recommendations is lacking. .Biomarkers that could be routinely measured within the clinical setting could potentially transform clinical care for patients with ASD. This demonstration project employed collection of biomarker data during regular autism specialty clinical visits and explored the relationship of biomarkers with clinical ASD symptoms. But as technology progressed and investigators began to rely more on science, diseases were getting caught by leaving behind the very essence of their being: DNA samples. miRNAs are endogenous regulators produced as small, non coding RNAs. Mature miRNAs sequences are single stranded, ~19–24 nucleotides in length, and are highly conserved among species. Research in various diseases, have found that miRNAs play a role in pathogenesis and have potential as biomarkers and therapeutic agents. MicroRNAs have been implicated in Autism disorder and may contribute to common disease complications. The present study designed to identify candidate miRNA biomarker from plasma of Autistic patients.

AUTISM SPECTRUM DISORDER(ASD)

Autism spectrum Disorder (ASD) is a group of neurodevelopmental disorders including defects in social interaction, communication, repetitive behavior and sensitivity. [1]. Mostly ASD is due to genetic dysfunction with most data suggesting ASD is due to polygenic effects. Because of the complexity of the nature of this disease, most of the studies including classical genetic studies could not identify suitable candidate genes for ASD. In addition, to the genetic factors, other factor including environmental agents also plays an important role in developing ASD [2]. According to the Autism GENOME Project Consortium 2007, approximately 1% of children are affected with autism [3] but are significantly skewed towards boys with a sex ratio of~4:1[4, 5]. The heritability of a phenotype gives an account of the extent to which is controlled by genetic factors &it is estimated to be approximately 90% making the ASD one of the major childhood onset neuropsychiatric diseases [6]. The other clinical conditions associated with autism include epilepsy, anxiety,

intellectual disability and depression [7]. No two Autistic individuals are similar, each Autistic patients have a unique combination of symptoms, variation in severity in the main symptoms and variation in associated clinical conditions. The heterogeneity of disease severity varies from highly impaired individuals who need permanent care to highly functioning patients who fulfill higher education, self efficient proves that autism is a spectrum of conditions, not a single disease [8].

ASD comprises of strict Autism, atypical Autism and Asperger syndrome [3]. According to the current diagnostic and statistical manual of mental disorders [DSV-IV TR] [9], Asperger syndrome, PDD-NOS [pervasive developmental disorder, not otherwise specified] are included under ASD[10]. The symptoms of the ASD can be described as severe, pervasive, and manifested during the first year of life. To identify Autism, diagnosis often made as early as 18 months of age but most of the patients are not diagnosed until 5 years [11]. Autism in monozygotic twins is stated to be 12 times higher than in the normal population. But at the same time Autism rate in dizygotic twins is only 4 times higher than in the general population [12].

GENETICS OF AUTISM

Autism is a highly genetic disorder; indistinguishable autistic disorders are caused by many genetic changes a phenomenon referred to as genetic heterogeneity. The first genes implicated in Autism were associated with broader syndromes. Important clues about the mechanism underlying in Autism come from the monogenic disorders Retts syndrome and fragile X syndrome. Although there are many syndromic forms of Autism and about 40-60% of Autistic children shows some degree of mental retardation [13]. About 7%-10% of Autistic children have a variety of de novo chromosome deletion or duplication [14]. These deletion syndromes cause a spectrum of phenotypes that includes Autism. NLGN3 and NLGN4 genes encoding neuroligins 3 and 4, which are synaptic adhesion molecules. Mutation in NLGN4 causes mental retardation and Autism [15]. SHANK3 gene which encodes a cytoplasmic binding partner of the neuroligins is also deleted in Autism [16]. Chromosomal deletion or translocation involving the NRXN1, neuroxin gene which encodes an extracellular binding ligand for neuroligins is implicated in Autism [17]. Rare changes in CNTNAP2 encoding contactin associated protein like -2 are associated with Autism. The γ-amino butyric acid (GABAA) receptor gene cluster (which contains genes for 3 of the receptor's subunits: GABRB3, GABRA5, and GABRG3) is strongly implicated in the pathogenesis of Autism, given its involvement in the inhibition of excitatory neural pathways and its expression in early development. Chromosomal translocations have also implicated the q22-q33 region of chromosome 7.

Parents/guardians completed a demographic and medical history form including birth history, medications, co-morbid medical symptoms, and family medical history. Parents/guardians also completed the Aberrant Behavior Checklist-Community (ABC-C), a 58-item behavioral functioning measure for children and adults with developmental

disabilities that includes five subscales: irritability and agitation; lethargy and social withdrawal; stereotypic behavior; hyperactivity and non-compliance; and inappropriate speech (18). Scoring was completed using the method validated for children with ASD(19).

Research staff reviewed medical records for each participant to identify results of the most recent cognitive testing (developmental or IQ test results). To allow for comparison amongst the various developmental and intellectual assessment measures documented across all participants, only non-verbal cognitive scores (Bayley cognitive score or non-verbal IQ) were analyzed. Study data were collected and managed using REDCap (Research Electronic Data Capture), a secure, web-based application for electronic data capture hosted at the lead site.

TO STUDY THE ROLE OF BIOMARKERS IN AUTISM

Identifying biomarkers for ASD started since in the early 1940s, but no biological biomarkers with enough sensitivity and specificity is identified yet. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [20], disease biomarkers include any measurable characteristic such as DNA sequence variation, MRI imaging, blood and urine parameters etc. Metabolites can be used as an indicator of disease risk, diagnosis or prognostic. Biomarkers may be risk biomarkers, diagnostic biomarker or prognostic biomarkers. Due to the lack of specific pharmacological therapy and clinical heterogeneity of the disease, the researchers are mainly focused on to find out risk biomarkers and markers for diagnosis, useful in early diagnosis of ASD. Many biological markers for ASD have been proposed [21] but none have yet advanced to clinical uses. Some of the reason behind the variability of ASD biomarker results are; small size, small difference between disease and control groups, clinical heterogenecity and disease variability among individuals. Some the proposed biomarkers for ASD include;

Brain imaging biomarker

Structural magnetic resonance imaging (MRI) is useful to identify difference in brain structure associated with ASD . Brain structural variation include , increased frontal lobe volume, increased frontal lobe volume ,structural changes of corpus callosum, basal ganglia, amygdala and cerebellum [22]. Functional MRI is also used as a brain imaging marker.

Other types of biomarkers

Head circumference :

It is one of the mostly investigated early biological markers of Autism used to measure the brain size. Increased head size is one of the clinical characteristics described by Kanner [23].

Serotonin- hyperserotonemia :

It is one of the first blood biomarkers implicated in ASD. Increased level of serotonin in blood is observed in 25%-35% of ASD patients. It is due to the variation in

the serotonin receptor gene SLC6A4 and integrin beta gene [ITGB].

Mitochondrial and metabolic markers :

Biochemical markers of mitochondrial function also altered in ASD patients. ASD with mitochondrial dysfunction may represent a distinct subgroup of ASD[24]

MICRORNA

The story of miRNA starts in 1993 and it was completely unknown before 1993.miRNAs of 19-24 nucleotides in length have now been identified experimentally [25,26] and according to the bioinformatics prediction there are more than 1000 miRNAs in total [27]. The miRNA was first identified by Victor Ambros and colleagues Rosalind Lee and Rhonda Feinbaum. They discovered a gene known as lin-4 known to control the timing of C.elegans larval development , it does not code for a protein instead produces a pair of small RNAs .The importance of miRNA regulated gene expression are coming to focus as more as miRNAs and their regulatory targets and functions discovered. Some of the recently discovered miRNA functions include, control of cell proliferation ,neuronal patterning in nematodes , modulation of hematopoietic lineage differentiation in mammals and control of leaf and flower developments in plants After the discovery of miRNAs the field of miRNA has grown dramatically and within 5 years our idea about miRNA functions, mechanism of regulation of gene expression etc. became more clear. This progress confirmed that miRNA are important post transcriptional regulators of gene expression and also, they identified as a new class of drug targets in therapeutic areas. MiRNAs are approximately 21 nucleotide in length in their mature form. Some of the miRNAs residing in introns are likely to share their regulatory elements and primary transcript with their pre-mRNA host genes. For other miRNAs they transcribed from their own promoters, no primary transcripts have been fully defined. These primary miRNA transcripts called pri-miRNAs are thought to be much longer than the conserved stem loop and currently used to define miRNA genes .

CONCLUSION

In this pilot demonstration feasibility project, collection of multiple biomarkers during a regularly scheduled ASD specialty clinical visit allowed for the examination of associations between biochemical and clinical measures, and identified several findings that suggest direction for future studies. While our findings for individual biochemical and clinical biomarkers should not be viewed as definitive, we found associations between platelet serotonin and melatonin sulfate excretion with patient demographic and clinical characteristics that illustrate the potential of this approach to generate important information about multiple biomarkers and functional domains within a single heterogeneous clinical patient population.

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