Biomarkers in children with Autism: A case control Study

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INTRODUCTION

- Detailed neuro-pathogenesis of ASD is not yet very well elucidated
- Different studies on putative biomarkers of ASD have yielded conflicting results further complicating the overall conclusion
- Our study sheds light on a group of biomarkers, which can later be evaluated in at risk population for prediction of development of ASD
- These biomarkers by revealing the intricate neuropathogenic pathways can open new windows for potential therapeutic options in ASD

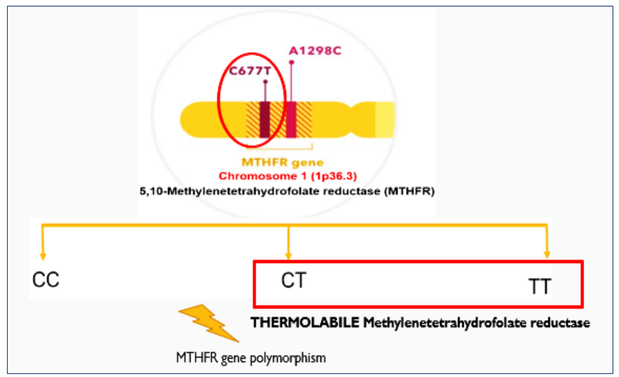

Fig 1 - The C677T polymorphism at the position 677 on MTHFR gene

OBJECTIVES

- To compare the levels of methylation pathway biomarkers and advanced glycation end-products
- To correlate these levels with severity of autism (CARS score), sensory issues (SP2 score), comorbidities (CBCL score) and DQ/IQ (MISIC/ VSMS/ BKT)

MATERIALS AND METHODS

- **Study design:** Case- control study,
- **Participants:** Children with ASD between 2-18 years fulfilling DSM-5 criteria as cases and age and sex matched typically developing children as controls
- 4 ml blood and urine sample collected for biomarkers
- CARS 2, ABC, CBCL, CSHQ and sensory profile 2 was performed

Table 1- Hardy Weinberg Equilibrium Testing

CONTROLS (n=50)		
GENOTYPE MTHFR C677T	**OBSERVED**	**EXPECTED (HARDY WEINBERG Principle)**
Common Homozygotes (CC)	43	42.32
Heterozygotes (CT)	6	7.36
Rare Homozygotes (TT)	1	0.32
X^2 = 1.70		
CASES (n=100)		
Common Homozygotes (CC)	84	82.81
Heterozygotes (CT)	14	16.38
Rare Homozygotes (TT)	2	0.81
X^2 = 2.10		

RESULTS

- 100 cases (male - 82) and 50 controls (male - 41) were enrolled
- The C677T polymorphism was not associated with increased ASD risk **[Fig 1]**
- The frequency of CC, CT and TT genotypes in ASD group was 84%, 14% and 2% respectively and in control group was 86%, 12% and 2% respectively **[Table 1]**
- Dityrosine level was also higher in ASD cases as compared to controls (p=0.01) **[Table 2]**
- The median level of serum homocysteine in ASD group was 9 µmol/L (95% CI: 7-16 µmol/L) which was significantly higher than median level in the control group 7 µmol/L (95% CI: 4-11 µmol/L) (p=0.01)[Table 3]

Table 2- Comparison of Blood levels of advanced glycation end products

Variable (Mean ± SD)	Cases (n=100)	Control (n=50)	p
Urine uric acid/ creatinine ratio	0.69 ± 0.16	0.65 ± 0.13	0.57
N - Carboxymethyl lysine (ng/ml)	8.16 ± 3.58	8.23 ± 2.13	0.89
N – Carboxymethyl arginine (Counts per second)	20.79 ± 18.01	18.91 ± 12.71	0.56
Dityrosine (Counts per second)	17.48 ± 17.62	4.99 ± 5.81	0.001

CONCLUSIONS

- Serum Homocysteine levels & Prevalence of Hyperhomocysteineima is significantly higher in ASD compared to controls
- There was no significant difference in Serum Folate & Vitamin B 12 between case & controls, indicating high homocysteine not a mere marker of vitamin deficiency but may be an independent risk factor or an indicator of disease
- Results from our study infer that MTHFR C677T polymorphism is neither a risk nor a protective factor for Autism spectrum disorder.
- Increased circulating concentrations of dityrosine is thought to be a consequence of oxidative stress, our study demonstrated that the concentration of dityrosine is elevated among the children with ASD compared to controls

Table 3 - Comparison of various amino acids

Variable (Mean ± SD)	Cases (n=100)	Control (n=50)	P value
Cysteine (µmol/L)	268.55 ± 28.91	272.18 ± 29.85	0.43
Methionine (µmol/L)	23.90 ± 3.95	23.46 ± 5.20	0.56
Homocysteine (µmol/L)	10.53 ± 2.65	8.70 ± 2.77	**0.01**

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