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Exploring Potential Association between Autism Spectrum Disorder, Genetic Deletions in GSTT1, GSTP1, GSTM1, and Heavy Metals Found in Hair Samples

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ABSTRACT

The aetiology of ASD is complex and likely involves a combination of genetic and environmental factors. The increasing prevalence of ASD has led to an increased interest in environmental factors and their potential influence. In this study, we investigated the association between genetic deletions of the GSTT1, GSTP1, and GSTM1 genes analyzed by Array-CHG and levels of heavy metals in hair samples of children with ASD analyzed by inductively coupled plasma optical emission spectrometry. We analyzed a total of 50 children (aged 2-8) of which 25 had been diagnosed with ASD and had genetic deletions in one or more of the analyzed genes (GSTT1, GSTP1, and GSTM1), and 25 control samples from children from the same geographic area exposed to the similar environmental conditions but with no deletions in these genes. We found that children with deletions in these genes had significantly elevated hair levels of aluminium (Al) in 100% of the examined children, and in some cases in combination with, mercury (Hg) – 1 case, lead (Pb) – 2 cases, cadmium (Cd) – 1 case, barium (Ba) - 8 cases, and nickel (Ni) – 2 cases. In the control group of children, 23 out of 25 had normal levels of heavy metals in their hair, while one child had a borderline value of aluminium (Al) and another had an increased level of thallium (Tl). Our findings suggest that genetic deletions in detoxification and antioxidant enzymes like GSTT1, GSTP1, and GSTM1 might be associated with increased levels of heavy metals thus leading to possible complex interactions between genetic and environmental factors, influencing the level of expression of symptoms and impairment in people with ASD.

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Introduction

Copy number variation is an important factor of phenotype variation, especially in the case of complex disorders. Numerous neurodevelopmental disorders including intellectual disability and autism spectrum disorders have CNVs as important predisposition factors. There is emerging evidence for an etiological role of some de novo and rare inherited CNVs in ASD. The genetic deletion in GSTT1, GSTP1, and GSTM1 genes, as well as the presence of heavy metals, has been a topic of interest in the field of neurodevelopmental disorders, particularly autism. Several studies have demonstrated a correlation between autistic symptoms and genetic and environmental risk factors, such as pollutants, pesticides, and metals like aluminium, mercury, and lead. Previous research has indicated a potential connection between deletions in the GSTT1, GSTP1, and GSTM1 genes and heavy metal exposure, suggesting that heavy metals may exert epigenetic influences on genes related to neurodevelopmental diseases.

The glutathione S-transferases (GST) are a superfamily of phase II metabolizing enzymes that catalyze the conjugation of reduced glutathione, to a multitude of electropholic and hydrophobic compounds Located on at least seven chromosomes, and divided into five main classes: alpha, mu, pi, theta, and zeta. Their role has been considered to be in detoxication reactions, and they also play a part in the antioxidant defense mechanism. Aluminium has been determined as a neurotoxic agent that causes destabilization of the cell membranes, more precisely, it has been determined that the principal target of aluminium toxicity is the astrocytes, which are responsible for the blood-brain bareer. Due to its high reactivity, aluminium interferes with the enzymatic activities in multiple metabolic pathways. It has been also shown by (citation) that aluminium's toxicity may be increased by oxidative stress, that would further lead to progression of ASD.

This study aims to further investigate the relationship between the genetic deletion of GSTT1, GSTP1, and GSTM1 and the presence of heavy metals in hair samples of children with autism.

Materials and Methods

To conduct this study, a sample of 25 children with autism, who did not have known genetic variants associated with autism, were selected. Selection of the patients was based according to variants described [1]. The genetic deletion of GSTT1, GSTP1, and GSTM1 genes was analyzed using array-based comparative genomic hybridization (aCGH) genetic analysis by gilent Genome CGH Microarray 44B with resolution ~45 Kb and analysis

software: CGH Analytic 3.4.40 (Agilent). In addition, 25 healthy children without genetic deletion in GDTT1, GSTP1 and/or GSTM1 were analysed as control group.

Hair samples from these children were collected and analyzed using inductively coupled plasma optical emission spectrometry with the Avio 200 from PerkinElmer, to determine the levels of heavy metals, including Arsenic (As), Aluminium (Al), Barium (Ba), Cadmium (Cd), Nickel (Ni), Lead (Pb), Mercury (Hg), Strontium (Sr) and Thallium (Tl). The result of measured total heavy metals in the hair samples of the unaffected children are shown in table 1, while the measured concentration of the affected children are shown in table 2.

Table 1: Concentration in ppm of the heavy metals Arsenic (As), Barium (Ba), Aluminium (Al), Cadmium (Cd), Nickel (Ni), Lead (Pb), Mercury (Hg), Strontium (Sr) and Thallium (Tl) in the hair samples of the unaffected children.

Patient	As	Ba	Al	Cd	Ni	Pb	Hg	Sr	Tl
AA	0,03200	0,63000	6,19000	0,09200	0,79000	1,67000	0,02700	2,17000	0,00010
MV	0,03200	0,19000	8,57000	0,06700	0,25000	0,59000	0,02700	0,43000	0,00010
MG	0,03200	0,25000	9,59000	0,10100	0,18000	3,06000	0,02700	0,68000	0,00010
UH	0,03200	0,18000	6,33000	0,14600	0,25000	2,22000	0,02700	0,27000	0,00010
MM	0,03200	0,37000	12,65000	0,10600	0,18000	1,68000	0,02700	0,51000	0,00010
AM	0,03200	0,32000	12,41000	0,12300	0,14000	3,41000	0,02700	0,45000	0,00010
DF	0,03200	1,88000	8,98000	0,18000	0,14000	2,73000	0,02700	1,59000	0,00010
EX	0,03200	0,52000	9,61000	0,03500	0,40000	1,62000	0,02700	1,84000	0,00010
FV	0,03200	0,17000	6,41000	0,07000	0,14000	1,98000	0,17200	0,28000	0,00010
GA	0,03200	0,18000	32,42000	0,01600	0,14000	1,86000	0,47700	0,33000	0,00010
FP	0,03200	0,71000	26,51000	0,08000	0,20000	1,25000	0,16000	0,59000	0,00010
ER	0,03200	0,15000	4,20000	0,08500	0,89000	1,25000	0,02700	0,52000	0,00010
TG	0,03200	0,30000	5,75000	0,05600	0,35000	1,58000	0,02700	0,47000	0,00010
OP	0,03200	0,32000	6,15000	0,10000	0,19000	1,52000	0,02700	0,65000	0,00010
AS	0,03200	0,41000	8,17000	0,12500	0,27000	0,69000	0,02700	0,28000	0,00010
DE	0,03200	0,17000	5,14000	0,12000	0,18500	2,00000	0,02700	0,55000	0,00010
VV	0,03200	0,71000	4,23000	0,11000	0,15800	2,50000	0,02700	0,47000	0,00010
GN	0,03200	0,69000	4,14000	0,09800	0,14000	1,25000	0,02700	1,68000	0,00010
MK	0,03200	0,56000	3,13000	0,00500	0,52000	1,65000	0,02700	1,95000	0,00010
MS	0,03200	0,58000	3,56000	0,06800	0,15000	1,47000	0,02700	0,35000	0,00010
KS1	0,03200	0,15000	4,58000	0,07200	0,01000	0,95000	0,02700	0,45000	0,00010
KS2	0,03200	0,90000	8,12000	0,09000	2,14000	0,85000	0,02700	0,45000	0,00010
KO	0,03200	1,12000	9,12000	0,15000	0,25000	1,45000	0,02700	0,65000	0,00010
KA	0,03200	0,12000	6,25000	0,17000	0,35000	1,58000	0,02700	0,57000	0,00010
AF	0,03200	1,11000	2,23000	0,12000	0,33000	2,53000	0,02700	0,75000	0,00010

Table 2: Concentration in ppm of the heavy metals Arsenic (As), Barium (Ba), Aluminium (Al), Cadmium (Cd), Nickel (Ni), Lead (Pb), Mercury (Hg), Strontium (Sr) and Thallium (Tl) in the hair samples of the affected children

Patient	As	Ba	Al	Ni	Ni	Pb	Hg	Sr	Tl
AC	0,03200	0,36000	13,05000	0,24300	0,44000	2,94000	0,02700	0,50000	0,00010
AH1	0,03200	3,30000	79,34000	0,20000	0,90000	2,97000	0,02700	7,92000	0,00010
AH2	0,03200	2,71000	13,55000	0,11400	1,02000	2,55000	0,02700	10,55000	0,00010
AK	0,03200	0,63000	14,02000	0,03300	0,22000	0,97000	0,02700	0,38000	0,00010
AP	0,03200	0,44000	20,51000	0,09700	0,49000	2,77000	0,02700	1,49000	0,00010
BG	0,03200	0,76000	15,86000	0,07800	0,25000	3,18000	0,02700	1,47000	0,00010
EX	0,03200	2,88000	20,24000	0,16700	0,99000	2,25000	0,02700	0,75000	0,00010
MZ	0,03200	1,07000	21,92000	0,15400	0,69000	2,96000	0,02700	3,20000	0,00010
VA	0,03200	0,95000	18,23000	0,16100	0,96000	3,37000	0,02700	3,11000	0,00010
RJ	0,03200	0,35000	32,92000	0,15800	0,47000	2,42000	0,02700	0,38000	0,28350
MO	0,32000	0,94000	30,89000	0,29500	0,85000	5,39000	0,02700	2,65000	0,00010

LS	0,03200	1,36000	32,61000	0,21300	0,14000	3,48000	0,02700	2,38000	0,00010
IM	0,03200	0,94000	30,89000	0,29500	0,85000	5,39000	0,02700	2,65000	0,00010
DK1	0,03200	0,50000	11,79000	0,14900	9,72000	2,38000	0,46600	1,73000	0,00010
BH	0,03200	0,54000	13,94000	0,10800	0,40000	1,48000	0,02700	0,70000	0,00010
EC	0,03200	0,51000	13,34000	0,04100	0,20000	4,36000	0,02700	1,01000	0,00010
NH	0,03200	4,66000	39,26000	0,93400	0,14000	5,11000	0,02700	10,08000	0,00010
FT	0,03200	0,36000	30,05000	0,23100	0,40000	4,20000	0,02700	0,47000	0,00010
AZ	0,03200	0,22000	15,62000	0,19600	0,47000	3,39000	0,02700	0,41000	0,00010
AS	0,03200	0,69000	22,90000	0,12100	1,22000	1,75000	0,02700	0,71000	0,00010
MA	0,03200	0,46000	18,70000	0,04000	0,31000	0,36000	0,02700	0,65000	0,00010
VZ	0,03200	2,12000	36,40000	0,19900	1,90000	2,36000	0,02700	4,23000	0,00010
MS	0,03200	3,91000	33,41000	0,11000	0,14000	2,37000	0,02700	4,27000	0,00010
DB	0,03200	0,83000	44,53000	0,06600	0,58000	4,13000	0,02700	0,90000	0,00010
DK2	0,03200	1,05000	17,42000	0,05200	0,22000	1,35000	0,02700	3,22000	0,00010

Results

Overall concentrations of the measured heavy metals in the hair samples of the 25 children with genetic deletions in the GSTT1, GSTP1, and GSTM1 genes was significantly higher than in the children without deletion in GSTT1, GSTP1, and GSTM1 genes (Figure1)

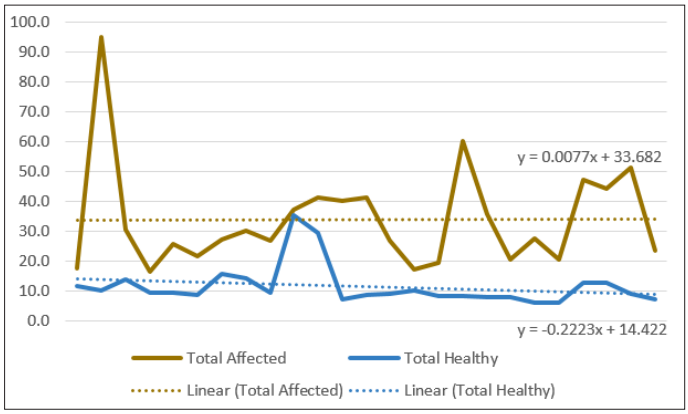

Figure 1: Graphical representation of cumulative concertation of heavy metal in affected and unaffected children.

To assess the presence of heavy metals in hair samples we made graphical representation of cumulative concentrations of different heavy metals in the hair samples of the affected children (Figure 2). We found significantly elevated levels of Aluminium in all 25 children with genetic deletions in the GSTT1, GSTP1, and GSTM1 genes. In some cases, beside Aliminium the test revealed elevated levels above physiological level of mercury (Hg) – 1 case, lead (Pb) – 2 cases, cadmium (Cd) – 1 case, barium (Ba) - 8 cases, and nickel (Ni) – 2 cases. In the control group of children, 23 out of 25 had normal levels of heavy metals in their hair, while one child had a borderline value of aluminium (Al) and another had an increased level of thallium (Tl).

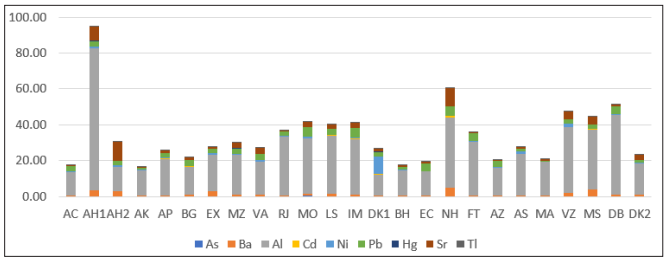

Figure 2: Graphical representation of the total concentration of heavy metals in hair samples of the affected children.

From the results it has been shown that most frequent heavy metal in the children with genetic deletions in GSTT1, GSTP1, and GSTM1 is aluminium as it is represented with red line in Figure 3 while with the blue line is the total concentration of aluminium in healthy control group. Highest values are 79.34 ppm were detected in the second sample while lowest concentration of 11.79 ppm weas detected in the 14th sample. The gray line represents the upper physiological normal level of aluminium. In the control group of children, 23 out of 25 had normal levels of heavy metals in their hair sample, while one child had an elevated value of aluminium (Al) and another had an increased level of thallium (Tl) and aluminium (Al).

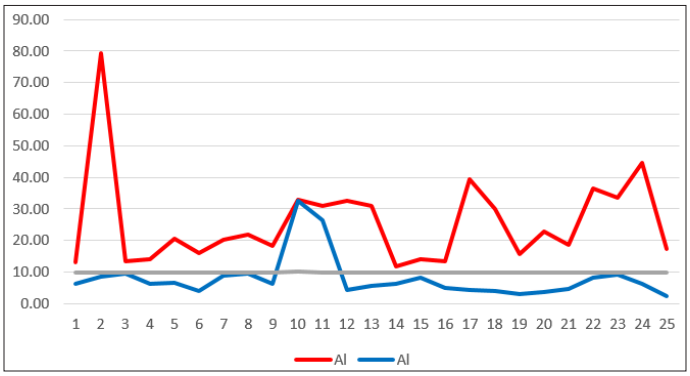

Figure 3: Graphical representation of the hair heavy metal concentration of aluminim in affected children and control group

The F test for two sample variances considering just Aluminium values showed mean value for affected children od 25.6556, while 7.9992 for unaffected children. Calculated variance is 214.2308 for affected while 46.85 for unaffected children (Table 3). With Hypothesis 0 (H_0) set that the Aluminium value would be under 10 ppm and Hypothesis 1 (H_1) set that the Aluminium value would be over 10 ppm we performed t-Test: Paired. Test results showed that for H_0 Rejection Region is Reject H_0 if t>1.71 while the test statistics showed that t=2.553601 with p-value=0.008717. This leads us to Decision/Conclusion - Because t=2.5536 > 1.71 Reject H_0

In addition, we performed reverse statistic for the healthy children (Table 2). The t Stat showed negative value of -9.224 that is significantly lower than t Critical of 1.7109 and negative p-value of 1.16155E-09 showing highly improbable that unaffected children with no GSTT1, GSTP1 and/or GTTM1 mutations will have Aluminum value over 10 ppm. In Figure 2 we represent total heavy metals in affected children with GST genetic deletions with total heavy metals in unaffected children (Figure 2).

Table 3: Calculated variances for the affected children

	Al in affected children
Mean	25,6556
Variance	214,2308
Observations	25
Pearson Correlation	0,181366
Hypothesized Mean Difference	10
df	24
t Stat	2,553601
P(T<=t) one-tail	0,008717
t Critical one-tail	1,710882
P(T<=t) two-tail	0,017435
t Critical two-tail	2,063899

Table 4: Calculated reverse statistic for healthy children

t-Test: Paired Two Sample for Means	
	Al in nonaffected
Mean	7,9992
Variance	46,85050767
Observations	25
Pearson Correlation	0,181365836
Hypothesized Mean Difference	10
df	24
t Stat	-9,22410267
P(T<=t) one-tail	1,16155E-09
t Critical one-tail	1,71088208
P(T<=t) two-tail	2,3231E-09
t Critical two-tail	2,063898562

Conclusion

The findings of this study provide preliminary evidence of a potential link between the genetic deletion of GSTT1, GSTP1, and GSTM1 genes and the presence of heavy metals in children with autism spectrum disorder. This is consistent with previous research suggesting that the null alleles of GSTT1 and GSTM1, associated with reduced enzymatic activity and decreased detoxification capacity, may contribute to increased susceptibility to heavy metal exposure [2]. Additionally, studies have shown that the epistatic effect of GSTM1 and GSTT1 deletion polymorphism may increase the susceptibility to aluminium exposure and other heavy metals, further supporting the hypothesis that these deletions may lead to an impaired ability to metabolize and detoxify such toxins. Moreover, the results of our study align with previous research that has highlighted the role of GSTM1 and GSTT1 deletion polymorphisms in neurodevelopmental disorders and other conditions related to heavy metal exposure. The findings of this study suggest that the genetic deletion of GSTT1, GSTP1, and GSTM1 genes in children with autism may be associated with an increased risk of elevated levels of heavy metals, including Aluminium, Mercury, Lead, Cadmium, and Nickel. The hypothesis underlying this study is that heavy metal exposure can have epigenetic effects on certain genes, potentially affecting their expression and contributing to neurodevelopmental disorders.

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