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Human homeostatic iron regulator gene polymorphism in autistic population of India; a case-control study

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ABSTRACT

Background: Autism is a heterogeneous neurodevelopmental disorder. Human homeostatic iron regulator (HFE) codes for HFE protein. HFE protein is very essential for inhibitory regulation of the endocytosis of iron.
Objective: Present study aims to screen C282Y and H63D polymorphism of the HFE gene in autistic children.
Method: 30 autistic children and 30 healthy age-matched control children were included in the study. TXRF analysis was performed for quantification of Iron in plasma. Genomic DNA was extracted using peripheral blood samples and targeted SNPs were screened using Restriction fragment length polymorphism. Genotype, allelic frequencies and risk ratio were calculated using the statistical method.
Results: TXRF analysis shows a significantly very low concentration of iron in autistic children compared to the control group [1039.6 ± 28 μg/L vs 2372.2 ± 35 μg/L, *p*-value 0.001]. Genetic Study shows that all the 30 controls and 28 autistic cases showed homozygote C/C allele. Two heterozygote C/Y alleles and no homozygous Y/Y allele were observed for C282Y polymorphism for autistic cases. 29 control and 23 autistic cases showed a homozygote H/H allele. 01 control and 07 autistic cases showed heterozygote H/D allele for H63D polymorphism. C282Y and H63D polymorphisms of HFE gene for heterozygous condition showed non-significant evidence of risk for causing autism OR = 5.35, 95%CI = 0.25–116.3, *P*-value-0.29 and OR = 8.8, 95%CI = 1.0–76.9, P-value = 0.05 respectively.
Conclusions: Present study found that C282Y and H63D were not found to be the risk factor for autism in the targeted study cohort.

1. Introduction

Autism is a complex heterogeneous neurodevelopmental disorder by the influence of genetic and environmental factors. It is characterized by impaired social interactions, deficient communication, restricted interests and stereotyped activity (Hegde et al., 2021a). It predisposes at the early onset of the age of three. In the general population, it occurs approximately 0.6 to 1.2% (Sudarshan et al., 2016). The male to female ratio for autism is estimated at 4:1 (Laumonnier et al., 2004). Different studies on twins, high risk infant siblings, population, families have estimated concordance rates of 60–70% in monozygous twins and 5–30% in siblings (Yoo, 2015). No prevalence data for autism is available from India for the general population. Oxidative stress plays a major role in the aetiology of several neurological diseases and neurodevelopmental disorders such as Alzheimer's disease, Parkinson's disease, Schizophrenia, Autism and ADHD (Goldani et al., 2014). Increased oxidation stress caused by either increased production or decreased elimination of oxygen free radicals was associated with cell damage (Gebril and Meguid, 2011). Recently many studies have shown trace elements like zinc, copper, iron and heavy metals like mercury, cadmium is implicated as potential risk factors for autism. Iron is one of the most studied trace elements in autism that maintains the integrity and

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functioning of the central nervous system (Baj et al., 2021).

Hereditary hemochromatosis is an autosomal recessive condition characterized by an excess of iron in different organs, particularly the liver. The mutations, C282Y and H63D in the histone family E1 [HFE] gene on chromosome 6p21.3 are the most common causes of hemochromatosis (Lakshmiprabha et al., 2019). HFE has an extracellular peptide binding region [alpha 1 and alpha 2], an Ig-like transmembrane domain [Alpha3], as well as a short cytoplasmic tail. The alpha3 domain's functional three-dimensional structure is maintained by disputed bridges between cysteine residues (Mikhaĭlova et al., 2003).

The human homeostatic iron regulator is a major histocompatibility complex (MHC) class I-like gene that codes for HFE protein combine with b2-microglobulin (ß2M) to compete with transferrin (Tf) for binding to the transferrin receptor (TfR). Normal HFE protein is very essential for inhibitory regulation of the endocytosis of iron (Enns, 2006; Bennett et al., 2000). Deregulation of iron metabolism leads to Hemochromatosis. The two most common SNPs in the HFE gene that cause Hemochromatosis are C282Y (rs1800562) and H63D (rs1799945). The C282Y polymorphism inhibits HFE from reading the cell surface, blocking interactions with hepcidin and TFRs. Unlike C282Y, H63D do not disrupt the binding with B2M. H63D may disrupt iron hemochromatosis and causes iron accumulation when it occurs simultaneously with C282Y (Katsarou et al., 2016).

In the present study, we analysed C282Y (rs1800562) and H63D (rs1799945) polymorphisms in autistic children to investigate the association of these polymorphisms with autism because iron metabolism has its known biological effect on autism and no previous attempt was made to study these two SPNs in Indian autistic population.

2. Materials and methods

The present case-control study included 30 autistic children [mean age: 11.5 ± 3.1, $n_{male} = 20$, $n_{female} = 10$] and 30 healthy age-matched children as control group [mean age: 12 ± 2.5, $n_{male} = 20$, $n_{female} = 10$]. All the autistic children were diagnosed and included as per the Diagnostic and Statistical Manual of Mental Disorders [DSM–5] [https://www.psychiatry.org/psychiatrists/practice/dsm] and International Classification of Diseases-10[ICD-10] [https://www.who.int/classifications/icd/icdonlineversions/en/] criteria. Associated medical conditions such as fragile-X syndrome, metabolic disorders and chromosomal aberrations were excluded from the study. Written informed consent was obtained from parents/guardians. Ethical approval for the study was obtained from Karnataka Institute for DNA Research, Dharwad.

2.1. Fe element analysis

Minimal sample preparation was required for total reflection x-ray fluorescence for the analysis of Fe elements in plasma. To 495 µl of plasma and 5 µl of Internal Standard [IS] gallium [Ga] solution [500 mg/L] was added to get concentration 5 mg/Sample was then vertex slightly and 10 µl of the sample was deposited as a single drop over a quartz glass sample carrier. Samples were allowed to dry for 5 min. After drying, the sample carrier was inserted into the TXRF instrument [Bruker S2 Picofox TXRF, Bruker AXS GmbH, Germany] and analysis was carried with an integration time of 1000s for each sample with 50 kV voltages and 698 µA Current. Each sample was prepared and analysed in triplicate.

2.2. Genotyping

1 ml of peripheral blood was drawn into EDTA coated vacutainer (BD, Vacutainer) for genomic DNA isolation. Genomic DNA was isolated using a Blood and tissue DNA isolation kit (QIAgen, Germany) as per the manufacturer's instruction. PCR amplification for C282Y SNP was carried out using 95 °C for 0.5 min followed by the 35 cycles of 95 °C for 0.5 min, 62.2 °C for 01 min, 68 °C for 01 min, and final extension of 68 °C for 05 min, hold at 04 °C and the same PCR condition was used for H63D SNP except annealing temperature being 64.4 °C. Forward Primer sequence for C282Y was 5'-TGGCAAGGGTAAACAGATCC-3′ and reverse primer sequence was 5'-CTCAGGCACTCCTCTCAACC-3′. For H63D forward primer sequence was 5'-ACATGGTTAAGGCCTGTTGC-3′ and the reverse primer sequence was 5'-GCCACATCTGGCTTGAAATT-3′ (Ogouma-Aworet et al., 2020; Leão et al., 2014). Amplified products were electrophoresed on 2% agarose gel and the amplified product was visualised.

The Candidate SNPs were identified using standardised restriction endonuclease mixtures *RsaI* (New England Bio Labs, USA) for C282Y and MobI (New England bio labs, USA) for H63D. The digested products were electrophoresed on 3% agarose gel and visualised after staining with ethidium bromide by using a gel documentation unit (Cleaver Scientific, UK).

2.3. Statistical analysis

Descriptive statistics were used to calculate the mean and standard deviation (±SD) for numerical data. The student's *t*-test was used to assess the statistical significance of the difference between two study group means. The chi-square test was used to determine whether the allelic and genotype frequencies were distributed in accordance with Hardy-Weinberg equilibrium (HWE) or not. *P*-value <0.05 is considered statistically significant. Statistical analysis was carried by using SPSS software version 15.

3. Results

Present study population included 30 autistic children [mean age: 11.5 ± 3.1, $n_{male} = 20$, $n_{female} = 10$] and 30 healthy age-matched children as control group [mean age: 12 ± 2.5, $n_{male} = 20$, $n_{female} = 10$]. Demographical characters of autistic children were tabulated in table no.1 (Table 1). Results from Statistical tests showed that the children with ASD showed a significantly lower concentration of Iron [Fe] compared to the control group (1039.6 ± 28 µg/L vs 2372.2 ± 35 µg/L,

Table 1
Demographic characteristics of autistic children and healthy control children.

Demographic character	Autistic children	Healthy children
No of children	30	30
Sex		
• Male	20	20
• Female	10	10
Mean age	11.5 ± 3.1	12.0 ± 2.5
Ethnic origin	Indian	Indian
The average age of the father at child's birth	38 ± 2.0	31 ± 2.3
The average age of mother at child's birth	33 ± 1.5	28 ± 1.2
Consanguineous marriage		
• Yes	18	06
• No	12	24
Prenatal factors		
• Preeclampsia	04	01
• Maternal Hyper thyroidism	01	01
• Hypertension	01	00
• Gestational problem	02	00
Postnatal factor		
• Labour complication	06	02
• Forceps mediated delivery	01	01
• Birth asphyxia	02	00
• Feeding problem	01	01
• Delayed crying	01	00
Intelligent quotient [IQ]	30 ± 10	60 ± 5
Severity		
• Mild to moderate	18	00
• Severe	12	00
Co-morbid condition	None	None
Family history of the neurodevelopmental condition	None	None

p-value 0.001) [Table 2]. Iron showed a significantly slight higher concentration in ASD Females compared to ASD males (1043.2 ± 6.0 μg/L vs 1030.2 ± 10.5 μg/L) [Table 3].

3.1. C282Y genotyping

The PCR products (390 bp) of C282Y SNP were used for genotyping. The SNP analysis for allele C282Y was determined after restriction digestion with RasI. The C allele generates two fragments of 250 and 140 bp while the Y allele generates three fragments of 250, 111 and 29 bp (Fig. 1). In our study, 30 controls and 28 autistic cases showed homozygote C/C allele, two heterozygote C/Y allele and no homozygote Y/Y allele was detected in any of the autism cases (Table 4).

The Chi-Square test analysis did not show any significant difference between genotype frequencies and allelic frequencies of C282Y polymorphism of the HFE gene among cases ($p > 0.05$). The percentage of C/C and C/Y genotypes among autism cases was 93.33% and 6.67% respectively. The Control group shows 100% of C/C genotypes. The allelic frequencies of C and Y alleles were 96.77% and 3.33% respectively in cases. Among controls, 100% were C alleles (Table 4). 3 genetic models [codominant, dominant, and recessive] significant were applied to analyse the association between C282Y (rs1800562) and autism risk using logistic regression. A statistically non-significant risk of autism was observed for Y/Y genotype compared to wild-type C/C genotype [OR = 1.0, 95% CI = 0.02–52.1, P-value = 1.0] under codominant model. Genotype C/Y-Y/Y was also non-significant for the risk of autism risk under the dominant model [OR = 5.35, 95% CI = 0.25–116.3, P-value = 0.29] [Table 5].

3.2. H63D genotyping

The PCR products (208 bp) of H63D SNP were used for genotyping. The SNP analysis for allele H63D was determined after restriction digestion with MobI. H allele generates 2 fragments of 138 bp and 70 bp. D allele does not generate any fragments (no cutting sites are available for MobI restriction) (Fig. 2). In our study, 29 control and 23 autistic cases showed a homozygote H/H allele. 07 autistic cases and 01 control showed heterozygote H/D allele and no cases showed homozygote D/D allele (Table 4).

The Chi-Square test analysis did not show any significant difference between genotype and allelic frequencies of H63D polymorphism of the HFE gene among cases ($p > 0.05$) and control ($p > 0.05$). The percentage of H/H and H/D genotypes among autism cases was 76.67% and 23.33% respectively. The Control group shows 96.67% H/H genotypes and 3.33% of H/D genotypes. The allelic frequencies of H and D alleles were 88.33% and 11.67% respectively. H and D alleles were found in 98.33% and 1.67% of control groups, respectively (Table 4). under the codominant model, the D/D genotype was found to have a statistically non-significant risk of autism when compared to the wild-type H/H genotype [OR = 1.0, 95% CI = 0.02–52.0, P-value = 1.0]. Under the dominant model, genotype H/D-D/D was also non-significant for the risk of autism [OR = 8.8, 95% CI = 1.0–76.9, P = 0.05] [Table 5].

4. Discussion

The dramatic increase in the prevalence of autism throughout the globe in recent years may be due to an increase in the knowledge and awareness of autism or it may be due to improved diagnostic methods that are being developed during the last decade. Multiple studies throughout the globe showed that more than hundreds of genes are actively involved in autism and these genes are classified into multiple functioning pathways (Gupta and State, 2006). The ferrous ionic form of Iron catalyses the formation of toxic hydroxyl radicals and thus it initiates and exaggerates lipid peroxidation and ultimately leads to brain cell destruction (McCord and Day, 1978). Superoxide dismutase (SOD), ceruloplasm (copper-binding protein), transferrin (iron-binding protein) are the factors that neutralise the oxidative load with a potent role being implicated in autism (Gonzalez-Cuyar et al., 2008; Galaris and Pantopoulos, 2008). Several shreds of evidence have shown that HFE protein plays a major role in iron metabolism (Bennett et al., 2000). Iron deficiency might explain behavioural problems common to people with ASD and is also associated with ASD symptoms and particularly correlates with severity of emotional and behavioural problems and with developmental delay in autism (McCann and Ames, 2007; Saghazadeh et al., 2017). The present study supports this statement by recording a significantly very low concentration of iron in autistic children compared to the control group [1039.6 ± 28 μg/L vs 2372.2 ± 35 μg/L, *p*-value 0.001]. ASD Males showed a low concentration of plasma iron compared to ASD females [Table 2].

In the present study, we examined the two most common potential polymorphisms in the HFE gene (C282Y and H63D) in the autistic population of India for the first time. The idea to include this gene concerning autism came out from many recent studies that showed an excess of iron intake and disturbed iron metabolism as risk factors for autism (Gebril and Meguid, 2011; Padhye, 2003).

In the western population, the C282Y mutation in the HFE gene is a prominent cause of hereditary hemochromatosis (Sumi et al., 2020; Thakkar et al., 2018). In the general population of India, the frequency of C282Y mutation in the HFE gene that causes hereditary hemochromatosis is rare. C282Y is either missing or has a low frequency in non-Caucasian populations, such as Africans, Asians, people from the South Pacific, and Australians (Dhillon et al., 2012). The status of HFE mutations has not been explained well among Indians and currently, no exact incidence and prevalence for autism are available from India for the general population (Poddar, 2006; Hegde et al., 2021b).

The first studied SNP C282Y was very low in our targeted autistic samples and completely absent in controls. Non-significant difference between genotype frequencies of C282Y polymorphism of HFE gene among cases ($p > 0.05$) was observed. Logistic regression analysis of association between C282Y (rs1800562) and autism risk showed statistically non-significant non association for Y/Y genotype compared to wild-type C/C genotype [OR = 1.0, 95% CI = 0.02–52.1, $P = 1.0$]. This result is very similar to a study done by O.H. Gebril et al., 2011 on Egyptian autistic children (Gebril and Meguid, 2011). But heterozygous condition C/Y showed non-significant evidence of association for risk of causing autism OR = 5.35, 95%CI = 0.25–116.3, *P*-value-0.29 in our study population.

In the general population, the frequency of the second common mutation H63D ranges from 9.1% to 13.9%. HH is most common in the Caucasian population [2.6–14%] (Katsarou et al., 2016). H63D SNP was

Table 2
Plasma Iron [Fe] element level (μg/L) in children with autism and controls.

Element (μg/L)	ASD	Control	*P* value
	Mean ± SD	Mean ± SD	
Iron, Fe	1027.6 ± 25	2371.2 ± 30	0.001*

Data presented as Mean ± SD.
* Significant difference as compared to the control values at $p < 0.05$.

Table 3
Sex wise distribution of plasma iron level (μg/L) in children with ASD and control.

Element μg/L	Male		P-value	Female		P-value
	ASD *n* = 20	Control n = 20		ASD *n* = 10	Control n = 10	
Iron, Fe	1030.2 ± 10.5	2381 ± 12.2	0.001*	1043.2 ± 6.0	2355.1 ± 7.8	0.008*

Data presented as Mean ± SD.
* Significant difference as compared to the control values at $p < 0.05$.

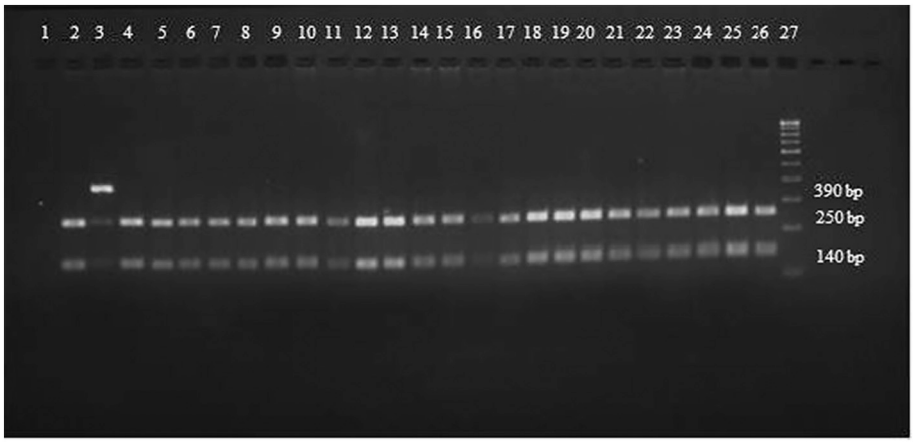

Fig. 1. Restriction analysis of C282Y SNP after digestion with RasI. Lane no:1 Negative control, Lane no: 2–26 autistic samples (Lane no:2 and 4–26 was homozygote C/C allele, Lane no:3 was undigested product), Lane no:27 marker 100 bp.

Table 4
Genotypic and allelic frequency distribution in autistic and control groups for C282Y and H63D polymorphisms of HFE gene.

Group	Genotype frequency (%)			Allelic frequency		Chi-square	p-value
	CC	CY	YY	C	Y		
Cases (*N* = 30)	28 (93.33%)	02 (6.67%)	00	0.97	0.03	0.04	0.98**
Control (N = 30)	30 (100%)	00	00	01	00	–	–

Group	Genotype frequency (%)			Allelic frequency		Chi-square	P-value
	HH	HD	DD	H	D		
Cases (N = 30)	23 (76.67%)	07 (23.33%)	00	0.88	0.12	0.52	0.77**
Control (N = 30)	29 (96.67%)	01 (3.33%)	00	0.98	0.02	0.01	0.99**

N = Number, CC = Homozygous Wild, YY = Homozygous Mutant, CY = Heterozygous (for C282Y polymorphism). HH = Homozygous Wild, DD = Homozygous Mutant, HD = Heterozygous (for H63D polymorphism). *Significant difference as compared to the control values at $p < 0.05$, **non significant difference as compared to the control values at $p > 0.05$.

Table 5
Association between SNPs and the risk of autism under genetic models.

Models	Genotype	Case	Control	OR (95% CI)	P value
C282Y (rs1800562)					
Co-dominant	C/C	28	30	1 (ref.)	–
	C/Y	02	00	5.35 (0.25–116.3)	0.29**
	Y/Y	00	00	1.0 (0.02–52.1)	1.0**
H63D (rs1799945)					
Co-dominant	H/H	23	29	1 (ref.)	–
	H/D	07	01	8.8 (1.0–76.9)	0.05*
	D/D	00	00	1.0 (0.02–52.0)	1.0**

OR = odds ratio, CI = confidence interval. *Significant difference as compared to the control values at $p < 0.05$, **non Significant difference as compared to the control values at $p > 0.05$.

Fig. 2. Restriction analysis of H63D SNP after digestion with MobI. Lane no: 1 Negative control, Lane no: 2–7 autistic samples (Lane no: 2,3,5,6 was homozygote H/H allele. Lane no: 4, 7 was heterozygote H/D allele), Lane no: 8 marker 100 bp.

shown in a few samples but heterozygotic form. No homozygotic mutant form was recorded. This result was slightly high when compared to a study done by O.H. Gebril et al. (2011)). A non-significant difference between genotype frequencies and allelic frequencies of H63D polymorphism of the HFE gene among cases ($p > 0.05$) was observed. Logistic regression analysis of association between H63D (rs1799945) and autism risk showed statistically non-significant non association for D/D genotype compared to wild-type H/H genotype [OR = 1.0, 95% CI = 0.02–52.0, $P = 1.0$]. But heterozygous condition H/D showed significant evidence of association for risk of causing autism OR = 8.8, 95%CI = 1.0–76.9, *P*-value = 0.05 in our study population.

The only pilot study at present on HFE gene polymorphism in autism by Gebril OH et al., 2011 in Egyptian autistic children included 26 patients with autism and their ages ranged between 5 and 15 years (mean age of 6.6 years ±4.4 and 24 males, 2 females). A Control sample consisting of normal healthy children (25 samples) within the same age range was studied. They failed to prove the role of HFE polymorphisms as risk factors for autism (Gebril and Meguid, 2011). Our study provides a similar result in Indian autistic children and supports their findings.

C282Y polymorphism and H63D polymorphism of the HFE gene does not show any evidence of risk for causing autism in our study cohort. Our study is only a preliminary basic research further functional analysis of SNPs is required and also more samples are required to evaluate the prevalence of SNPs in our population that was the limitation of the study.

5. Conclusion

This study is an attempt for the first time in India, to look into the association between HFE gene polymorphisms and autism. It was found that C28Y polymorphism and H63D polymorphism were not found to be the risk factors for autism in our study cohort with a negative association.

CRediT authorship contribution statement

Rajat Hegde and **Smita Hegde** were involved in Sample collection, genetic analysis and data analysis. **Suyamindra S Kulkarni** helped in genetic analysis and over all data analysis and interpretation. **Aditya Pandurangi** helped in identification, screening, counselling and sample collection. **Kusal K Das** and **Pramod B Gai** were involved in overall work design and valuation on results.

Declaration of competing interest

The authors declared no conflicts of interest concerning the research, authorship, funding and publication of this article.

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References

Baj, Jacek, Flieger, Wojciech, Flieger, Michał, Forma, Alicja, Sitarz, Elżbieta, Skórzyńska-Dziduszko, Katarzyna, Grochowski, Cezary, Maciejewski, Ryszard, Karakuła-Juchnowicz, Hanna, 2021. Autism spectrum disorder: trace elements imbalances and the pathogenesis and severity of autistic symptoms. Neurosci. Biobehav. Rev. 129, 117–132.

Bennett, M., Lebrón, J., Bjorkman, P., 2000. Crystal structure of the hereditary haemochromatosis protein HFE complexed with transferrin receptor. Nature 403 (6765), 46–53.

Dhillon, B.K., Prakash, S., Chandak, G.R., Chawla, Y.K., Das, R., 2012. H63D mutation in HFE gene is common in indians and is associated with the european haplotype. J. Genet. 91, 229–232.

Enns, C., 2006. Possible roles of the hereditary hemochromatosis protein, HFE, in regulating cellular iron homeostasis. Biol. Res. 39 (1), 105–111.

Galaris, D., Pantopoulos, K., 2008. Oxidative stress and iron homeostasis: mechanistic and health aspects. Crit. Rev. Clin. Lab. Sci. 45 (1), 1–23.

Gebril, O., Meguid, N., 2011. HFE gene polymorphisms and the risk for autism in Egyptian children and impact on the effect of oxidative stress. Dis. Markers 31 (5), 289–294.

Goldani, A., Downs, S., Widjaja, F., Lawton, B., Hendren, R., 2014. Biomarkers in autism. FrontPsychiatry 5, 100.

Gonzalez-Cuyar, L., Perry, G., Miyajima, H., Atwood, C., Riveros-Angel, M., Lyons, P., et al., 2008. Redox active iron accumulation in aceruloplasminemia. Neuropathology 28 (5), 466–471.

Gupta, A., State, M., 2006. Autismo: genética. Rev. Bras. Psiquiatr. 28 (Suppl. 1), 29–38.

Hegde, R., Hegde, S., Kulkarni, S., Pandurangi, A., Gai, P., Das, K., 2021. Genetic analysis of neuroligin 4Y gene in autism population of India. Glob. Med. Genet. https://doi.org/10.1055/s-0041-1736236.

Hegde, R., Hegde, S., Kulkarni, S., Pandurangi, A., Gai, P., 2021. Das K novel frameshift mutation in Indian autistic population causes neuroligin and neurexin binding defect. Gene Rep 24, 101245.

Katsarou, M.S., Latsi, R., Papasavva, M., Demertzis, N., Kalogridis, T., Tsatsakis, A.M., Spandidos, D.A., Drakoulis, N., 2016. Population-based analysis of the frequency of HFE gene polymorphisms: correlation with the susceptibility to develop hereditary hemochromatosis. Mol. Med. Rep. 14 (1), 630–636.

Lakshmiprabha, S., Suganthy, K., Shanmugapriya, V., et al., 2019. HFE gene polymorphism and iron status in preeclampsia. J. Evid. Based Med. Healthc. 6 (52), 3285–3290.

Laumonnier, F., Bonnet-Brilhault, F., Gomot, M., Blanc, R., David, A., Moizard, M., et al., 2004. X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. Am. J. Hum. Genet. 74 (3), 552–557.

Leão, G.D., Freire, J.M., Cunha Fernandes, A.L., Moura de Oliveira, T.M., Leão, N.D., Gil, E.A., de Vasconcelos, R.C., Azevedo, J.P., de Farias Sales, V.S., de Araújo Moura Lemos, T.M., Leão, M.D., do Nascimento Jr., F.F., Maciel Jr., J.F., de Freitas Jr., R.V., de Souza Paiva Jr., A., Cavalcanti Jr., G.B., 2014. Analysis of HFE genes C282Y, H63D, and S65D in patients with hyperferritinemia from northeastern Brazil. J. Clin. Lab. Anal. 28 (3), 178–185. May.

McCann, J.C., Ames, B.N., 2007. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am. J. Clin. Nutr. 85 (4), 931–945.

McCord, J., Day, E., 1978. Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex. FEBS Lett. 86 (1), 139–142.

Mikhaĭlova, S.V., Kobzev, V.F., Kulikov, I.V., Romashchenko, A.G., Khasnulin, V.I., Voevoda, M.I., 2003. Polymorphism of the HFE gene associated with hereditary hemochromatosis in populations of Russia. Genetika 39 (7), 988–995.

Ogouma-Aworet, L., Rabes, J.P., de Mazancourt, P., 2020. A simple RFLP-based method for HFE gene multiplex amplification and determination of hereditary hemochromatosis-causing mutation C282Y and H63D variant with highly sensitive determination of contamination. Dec Biomed. Res. Int. 28 (2020), 9396318.

Padhye, U., 2003. Excess dietary iron is the root cause for increase in childhood autism and allergies. Med. Hypotheses 61 (2), 220–222.

Poddar, Sandeep, 2006. Hereditary hemochromatosis-special reference to Indian scenario. Int. J. Hum. Genet. 6 (1), 73–79.

Saghazadeh, A., Ahangari, N., Hendi, K., Saleh, F., Rezaei, N., 2017. Status of essential elements in autism spectrum disorder: systematic review and meta-analysis. Rev. Neurosci. 28 (7), 783–809.

Sudarshan, S., Gupta, N., Kabra, M., 2016. Genetic studies in autism. Indian J. Pediatr. 83 (10), 1133–1140.

Sumi, Mamta P., Guru, Sameer Ahmad, Sahu, Samantak, Khan, Bazila, Mp, Girish, Saxena, Alpana, 2020. "Role of HFE gene in coronary artery disease" – a study from India. Clin. Epidemiology Glob. Health 8 (2), 69–472.

Thakkar, D.N., Palugulla, S., Selvarajan, S., Dubashi, B., 2018. Frequency distribution of BLMH, XPO5 and HFE gene polymorphisms in the south Indian population and their association with Hodgkin lymphoma. Mar Int. J. Biol. Markers 1, 1724600818766502.

Yoo, H., 2015. Genetics of autism Spectrum disorder: current status and possible clinical applications. Exp. Neurobiol. 24 (4), 257–272.