Correlation of Major Iron Metabolism Related Genes Single Nucleotide Polymorphisms (SNPs) with Autism Spectrum Disorder (ASD) in Palestinian Patients.

Sabha Rabaya
Supervised by: Prof. Hisham Darwish
Arab American University
Agenda

- Introduction
  - ASD
  - Iron
  - Relationship

- Hypothesis
  - Research question
  - Hypothesis

- Methodology
  - Patients vs Controls
  - Approaches

- Results
  - Lab results
  - Statistical analysis

- Conclusion
  - Research conclusion
  - Recommendations
Autism Spectrum Disorder (ASD) and Iron

- **Neurodevelopmental**
  - Autism
  - Asperger’s syndrome

- **Symptoms**
  - Communication
  - Behavior
  - Interaction

- **Diagnosis**: 1 to 3

- **Prevalence**
  - World wide: 1 per 160
  - Palestine
  - Male: Female 4:1

- **Etiology**
  - Genetic
  - Non-genetic

**Why iron:**
- Vital Roles in brain
- Tightly regulated
- Oxidative stress in autism
- Genetic variation in Iron genes affect iron homeostasis

**Vital metal**
- Oxidation Reduction reactions
- Cofactor for a lot of enzymes
- Oxygen transport

**Vital Roles in brain**
- Tightly regulated
- Oxidative stress in autism
- Genetic variation in Iron genes affect iron homeostasis

**Autism Spectrum Disorder (ASD)**
Question and Hypothesis

Genetic variations in specific major iron metabolism related genes maybe associated Autism Spectrum disorder (ASD) development
Iron metabolism

Knutson MD., 2017
Iron metabolism

Small Intestine
- Dietary Fe: ~15 mg/d
- Non-heme
  - DMT1
- Absorbed Fe: ~1-2 mg/d
  - FPN

Bone Marrow
- Fe taken up by bone marrow: ~25 mg/d
- Erythrocyte precursor → Mature RBC
  - Erythropoiesis
- Hemoglobin

Liver
- Inflammation
- Iron stores
- Hepcidin
- BMP6
- Sinusoidal endothelial cell
- Hepatocyte

Reticuloendothelial System
(liver, spleen, bone marrow)
- Fe released from RES: ~25 mg/d
- FPN
- Senescent RBC
- Macrophage

Knutson MD., 2017
Iron metabolism

Knutson MD., 2017
Genes and SNPs of interest

- **TFRC**: Tfr1 → Uptake
- **SLC11A2**: DMT1 → Uptake
- **SLC40A1**: FPN → Export
- **HAMP**: Hepcidin → Regulation
Different polymorphisms associated with many disorders including, age-related macular degeneration, Alzheimer’s disease, microcytic anemia, Parkinson’s disease, and Wilson’s disease.

Rs224589 Parkinson’s Disease

Intronic variant might affect the transcription, post-transcription, and ultimately the mRNA translation of SLC11A2 gene.
SLC11A2 gene (DMT1) (rs1048230)
RS1048230 → Parkinson’s disorder

An Exonic variant → does not results in amino acid substitution
TFRC gene (Tfr1)(rs11915082)
Reduced or decreased expression of TFRC have been linked with many pathological conditions.

Linked to age-related macular degeneration risk.

Promoter region to affect the regulation and level of expression.
SLC40A1 gene (FPN)(rs1439816)
SLC40A1 gene (FPN)(rs1439816)

Several mutations and defects linked with iron overload and hemochromatosis type 4 (HFE4)

rs1439816 correlated with iron dysregulation in hereditary hemochromatosis (HH), linked with Alzheimer’s disease risk
HAMP gene (rs10421768)
Defects in HAMP gene have been associated with iron dysregulation in many pathological conditions.

Rs10421768 iron overload and dysregulation, Thalassemia
## Genes and SNPs of interest

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP reference</th>
<th>Position (GRCh38.p12)</th>
<th>Gene region</th>
<th>Variation</th>
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<td>Rs11915082</td>
<td>chr3:196082268</td>
<td>Promoter</td>
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<td>Intron Variant/5’ UTR variant</td>
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Methodology

Control

88 ASD patients: 48

Samples collection
DNA extraction
SNPs determination

Statistical analysis
Bioinformatics analysis

Choose the analysis you need:

- Single site analysis
- Pair-loci D'/r2 value
- Haplotype analysis

Number of sites:

Selected sites for haplotype analysis:

e.g. "1 1 0 1" to choose 1st, 2nd & 4th sites for hap-analysis when there is 4 sites in total. Error input will be recognized as default -- "1 1 1 1..." (all chosen).

Calculate linkage disequilibrium in:

- Both case and control

Lowest frequency threshold (LFT) for haplotype analysis:

Default value is 0.03, any number in [0, 1) could be accepted. Haplotype with frequency less than this number will not be considered in analysis.

Marker names (please use space to split variation names):

Input data of control

Caution: The format of input data should be:

- ID1 G A 1 1 a b ...
- ID2 A G 1 2 b b ...
- ID3 G G 1 1 c c ...

Here, the first column refers to the sample ID;
the second & third column refer to the alleles of the 1st site;
fourth & fifth for the 2nd site;
sixth & seventh for the 3rd site;
......etc.
Pls use "0" for the missing alleles.

Input data of case

Activate Windows
Results and outcomes

1. **rs11915082**
   - Image A: Gel electrophoresis showing a band at 306 bp.
   - Image B: Sequencing traces for AG, GG, and AA genotypes.

2. **rs10421768**
   - Image A: Gel electrophoresis showing a band at 244 bp.
   - Image B: Gel showing bands at 244 and 187 bp.
   - Image C: Sequencing traces for different genotypes.
SLC11A2 gene (DMT1) (rs224589)

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<td>C</td>
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<td>A</td>
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P = 0.584
SLC11A2 gene (DMT1) (rs224589)

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<td>AA</td>
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P = 0.194
SLC11A2 gene (DMT1) (rs1048230)

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<td>C</td>
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P = 0.635
SLC11A2 gene (DMT1) (rs1048230)

### Summary Table

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<td>TC</td>
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<td>CC</td>
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\[ P = 0.223 \]
TFRC gene (Tfr1) (rs11915082)

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<tr>
<td>A</td>
<td>26.00%</td>
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<td>G</td>
<td>74.00%</td>
<td>58.00%</td>
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P = 0.008
TFRC gene (Tfr1) (rs11915082)

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<tr>
<td>AG</td>
<td>35.40%</td>
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<td>GG</td>
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<td>AA</td>
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P = 0.041
SLC40A1 gene (FPN) (rs1439816)

Control

ASD

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<td>G</td>
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P = < 0.0001
SLC40A1 gene (FPN) (rs1439816)

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<tr>
<td>GG</td>
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<td>CC</td>
<td>47.90%</td>
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P = 0.000
HAMP gene (Hepcidin) (rs10421768)

Control

ASD

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<td>AG</td>
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P = 0.028
Results and outcomes

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<th>S.E.</th>
<th>Wald</th>
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<td><strong>Step 1</strong></td>
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<tr>
<td>TFRC</td>
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<td>3.913</td>
<td>2</td>
<td>.141</td>
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<td>TFRC(1)</td>
<td>.729</td>
<td>.733</td>
<td>.987</td>
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<td>TFRC(2)</td>
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<td>.724</td>
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<td>FPN</td>
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<tr>
<td>FPN(1)</td>
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<td>.542</td>
<td>13.168</td>
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<td>.629</td>
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<td>HAMP</td>
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<td>Constant</td>
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a. Variable(s) entered on step 1: TFRC, FPN, HAMP.
<table>
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<tr>
<th>Gene</th>
<th>SNP</th>
<th>Self Behavior (P value) n=39</th>
<th>Language Skills (P value)</th>
<th>Self Expression (P value)</th>
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<tbody>
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<td>TFRC (Tfr1)</td>
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<td>SLC11A2(DMT1)</td>
<td>Rs1048230</td>
<td>0.60</td>
<td>0.18</td>
<td>1.00</td>
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<tr>
<td>SLC11A2(DMT1)</td>
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<td>0.74</td>
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<td><strong>0.026</strong></td>
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<td>SLC40A1(FPN)</td>
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<td>HAMP(Hepcidin)</td>
<td>Rs10421768</td>
<td>0.25</td>
<td>0.17</td>
<td>0.79</td>
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In Silico analysis

RegulomeDB

HaploReg V4.1
TFRC gene Tfr1 (rs11915082)
SLC40A1 gene (FPN) (rs1439816)
HAMP gene (Hepcidin) (rs10421768)

RegulomeDB 2.0

Searched Coordinates
PEAKS
RANK
SCORE
RS10421768

chr19:35772898-35772899
28 peaks
0.67002

A=0.7933, G=0.2067 (GnomAD)
A=0.8411, G=0.1589 (1000 Genomes)
A=0.8047, G=0.1953 (TOPMED)

6 more

Regulatory motifs altered

<table>
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<tr>
<th>Position Weight Matrix ID (Library from Kheradpour and Kellis, 2012)</th>
<th>Strand</th>
<th>Ref</th>
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<td>11.8</td>
<td>10.1</td>
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</table>
Conclusion

First study to draw the attention toward the role of iron dysregulation in ASD pathogenesis.

Polymorphisms within iron metabolism genes, Tfr1, FPN, Hepcidin, may be linked to ASD phenotype.

Possible novel genetic markers for ASD susceptibility that can be incorporated in early diagnostic genetic analysis of ASD.
Recommendation

Research on a larger group, other genes

Hematological Parameters
Thank you
References


Brain Iron metabolism