EFFECTS ON CONTINUED FOLIC ACID SUPPLEMENTATION DURING THE SECOND AND THIRD TRIMESTERS OF PREGNANCY ON CHILDREN'S NEUROCOGNITIVE DEVELOPMENT AT 11 YEARS.

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HYPOTHESIS

"Folic acid supplementation in late gestation alters DNA methylation in offspring and neurocognitive developmental outcomes are associated with the *ZFP57* genotype".

OBJECTIVES

- Determine the genotypes of the offspring for the *ZFP57* SNP rs365052
- Investigate how the SNP affects DNA methylation and its regulation
- Investigate the relationship between DNA methylation at *ZFP57* and the IQ of the offspring from EpiFASSTT study
- If any neurocognitive developments found have any links between the *ZFP57* gene, DNA methylation, and the genotype of the informative SNP (rs365052).

OVERVIEW

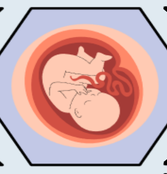
- Background
- Objectives and Hypothesis
- Methods
- Results
- Summary
- Conclusion

BACKGROUND

What is Folic Acid?

- Folate is key to helping form DNA and RNA. It is also involved in the metabolism of proteins
- Has a key role in breaking down an amino acid called homocysteine which can cause harmful effects if it is present within the body in high quantities.
- Folate is also needed for the production of healthy red blood cells (RBC) and is essential during abrupt growth, for example, pregnancy and the development of a foetus. (1)

Why is it important during pregnancy?



- Folic acid (FA) supplementation is known to reduce occurrence of neural tube defects (NTD)
- This is the 2nd most common malformation in humans affecting the development of the central nervous system.
- Remains uncertain whether continuing FA after the first trimester affects offspring neurodevelopment (3)

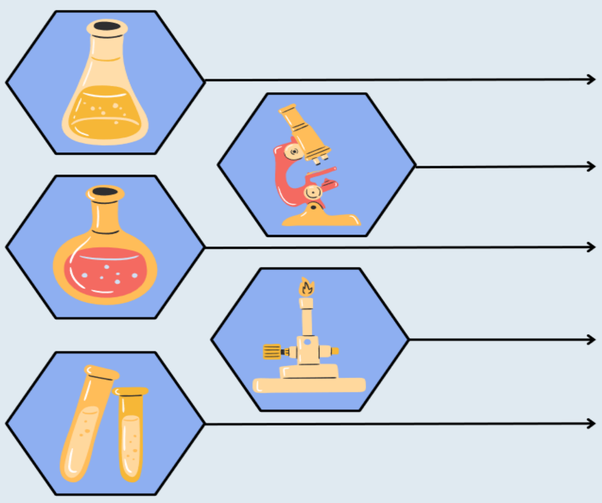
Previous research on neurocognitive development

- A follow-up study of mothers who had participated in a randomized controlled trial FASSTT in pregnancy and who had received 400 micrograms/day FA or placebo from the 14th gestational week until the end of pregnancy.
- This showed that the FA supplemented group offspring at 3, 7, and 11 years scored significantly higher than the placebo group in word reasoning and cognition. (5)

POSITIVES & NEGATIVES OF FA ON PREGNANCY

POSITIVES	NEGATIVES
FA has been proven to have many positive effects on not only pregnancy but also overall health (1)	Over-supplementation of FA, may have negative effects on the health of the mother (8)
Folate in the body increases the stability of DNA, which is a crucial process for the synthesis and repair of DNA (6)	Not getting enough FA leads to the decrease of the maturation of RBCs which in turn would lead to anaemia (4)
Folate also disallows the commencement of DNA oxidation by free radicals (7)	Too much FA intake leads to impaired fetal growth, increased risks of asthma and autism, and promoting the growth of some cancer cells. (2)

METHODOLOGY



Bisulfite Conversion	Converts unmethylated Cytosines to Uracil	Can easily distinguish between methylated from unmethylated cytosines
PCR Reaction	Converts the Uracils to Thymine at target sequences	Amplifies target sequence potentially hundreds of thousands of times
Gel Electrophoresis	Separates the amplified DNA fragments based on size	Determines the presence/absence of the target sequence within the sample
Methylation Pyrosequencing	Measures the % of DNA methylation at CpG sites of interest	Gives overall area of methylation
Genotyping Pyrosequencing	Uses DNA to detect allele presence of SNP	Shows % allelic presence and the genotype of the SNP

RESULTS

1.
- Methylation patterns in human blood measured using molecular techniques within *ZFP57* region
- Allele frequency assay provided data on the % that a nucleotide (within the informative SNP) was represented in the population
- Methylation % at CG sites within *ZFP57* region changed depending on the SNP allele present at rs365052

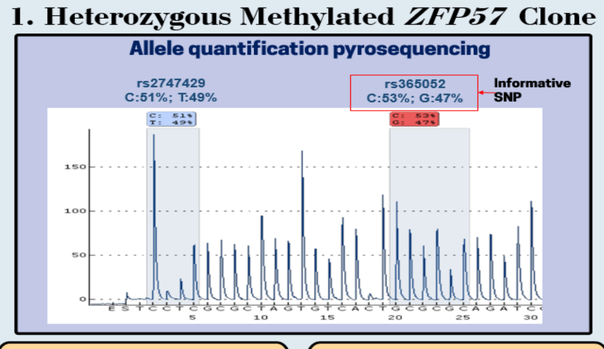
2.
- Samples indicated to have a GG genotype at rs365052 were analyzed further using methylation pyrosequencing.
- These homozygous GG samples displayed nearly 100% methylation within the *ZFP57* region.
- Samples with two G alleles displayed a pattern of elevated regional methylation, indicating a relationship between the genotype of the SNP ad the methylation of the *ZFP57* region

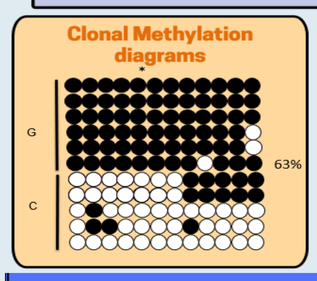
Main points to take from results

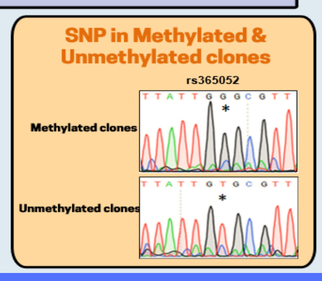
3.
- Data is not normally distributed so a non-parametric test had to be taken (does not assume normality)
- Kruskal Wallis – Asymp. Sig. is <0.05 meaning that we can say there is a statistically significant relationship between methylation and cord blood genotype

4.
- Highest methylation shown in the GG region while lowest methylation shown in the CC region.
- Methylation of the G allele is driven by the G allele
- Gives an indication that the SNP is a mQTL

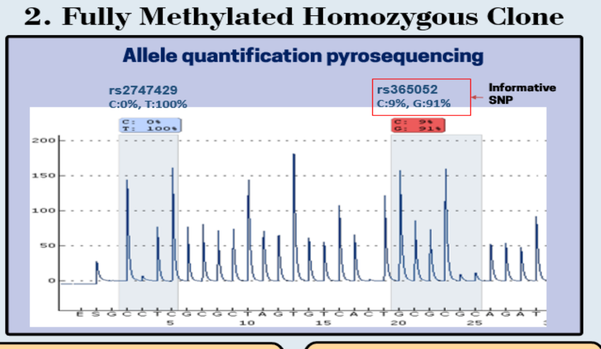
1. Heterozygous Methylated *ZFP57* Clone

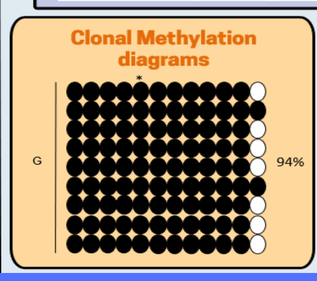


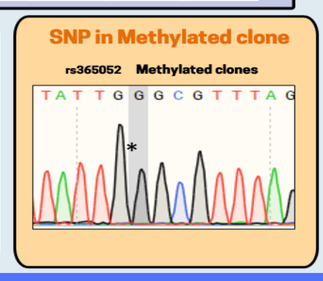




2. Fully Methylated Homozygous Clone



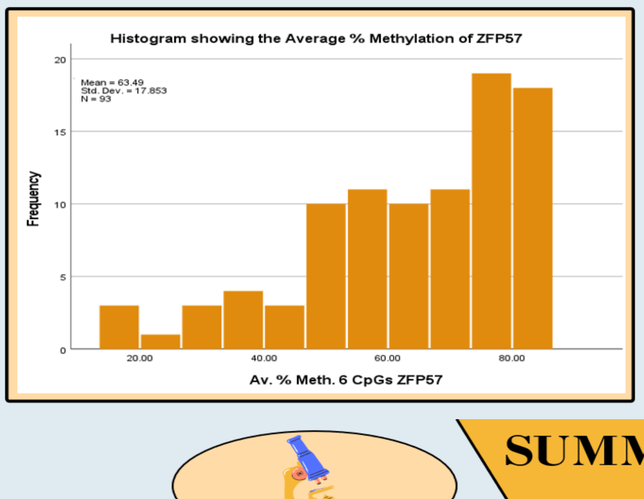




3a. Normal distribution test for *ZFP57* Methylation Data

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Av. % Meth. 6 CpGs ZFP57	.123	93	.001	.911	93	<.001



Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Av. % Meth. 6 CpGs ZFP57	93	41.2%	133	58.8%	226	100.0%

Descriptives

			Statistic	Std. Error
Av. % Meth. 6 CpGs ZFP57	Mean		63.4875	1.85131
	95% Confidence Interval for Mean	Lower Bound	59.8106	
		Upper Bound	67.1644	
	5% Trimmed Mean		64.7729	
	Median		67.2033	
	Variance		318.744	
	Std. Deviation		17.85340	
	Minimum		14.69	
	Maximum		84.96	
	Range		70.27	
	Interquartile Range		24.93	
	Skewness		-.917	.250
	Kurtosis		.198	.495

b. Kruskal-Wallis test

Ranks

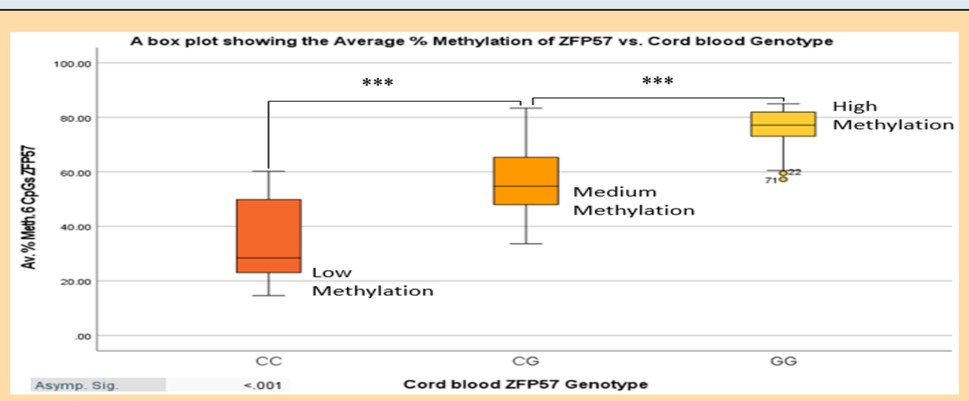
	Cord blood ZFP57 Genotype	N	Mean Rank
Av. % Meth. 6 CpGs ZFP57	CC	13	12.85
	CG	32	31.13
	GG	48	66.83
	Total	93	

Test Statistics[a,b]

	Av. % Meth. 6 CpGs ZFP57
Kruskal-Wallis H	57.804
df	2
Asymp. Sig.	<.001

a. Kruskal Wallis Test
b. Grouping Variable: Cord blood ZFP57 Genotype

4. Statistically significant difference in methylation between genotypes



Case Processing Summary

	Cord blood ZFP57 Genotype	Cases: Valid N	Valid Percent	Missing N	Missing Percent	Total N	Total Percent
Av. % Meth. 6 CpGs ZFP57	CC	13	100.0%	0	0.0%	13	100.0%
	CG	32	100.0%	0	0.0%	32	100.0%
	GG	48	100.0%	0	0.0%	48	100.0%

SUMMARY

Limitations faced

- Lack of laboratory experience due to COVID
- Sample type
- Sample size of the project

Future Research

- Continue looking more into the cognitive relationship between FA and *ZFP57*
- Investigate other SNP's close to the rs365052
- Continue looking at the children as they continue to age

CONCLUSION

- *ZFP57* is expressed in the brain and is a master regulator of imprinting genes so is very important
- *ZFP57* was the top-ranking promoter region effected by FA supplementation showing differential methylation
- From results so far, we can see significant differences in methylation between three genotypes of the SNP
- The SNP has now been recognized as an mQTL

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