Insulin gene promoter methylation status in Greek children and adolescents with Type 1 Diabetes

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Introduction

Insulin (INS) gene is reported to be the most important gene involved in T1DM; its expression is inversely correlated with methylation at CpG sites. Hypermethylated primers are associated with decreased expression.

Methods

Twenty T1DM participants and 20 age-/gender-matched controls were enrolled.

- DNA was extracted from white blood cells
- Genomic DNA(800ng) was modified using the EZ DNA Methylation-Gold Kit.
- Treatment with sodium bisulfite converts unmethylated cytosines into uracyls, whereas methylated cytosines remain unchanged under the same conditions.
- DNA was then amplified by PCR in a total volume of 50 μl targeting a specific sequence of the gene promoters.
- Amplicons were analyzed by electrophoresis (1% agarose gel stained with ethidium bromide) and visualized by ultraviolet trans-illumination.
- PCR products were purified and sequenced with Next Generation Sequencing – Illumina, in order to identify DNA methylation changes
- Comparisons between groups were performed with students t-test or its non-parametric analogue, Mann Whitney U test, as appropriate.

Figure 1. INS forward and reverse primers

	Forward primer	Reverse primer
INS	*5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATTTTGGAATTTTGAGTTATT3'*	*5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACAAAAATCTAAAAACAACAA3'*

Results

Methylation profile at 10 CpGs of the INS promoter, was analyzed. A statistically significant difference in INS gene between the two groups concerning the methylation at position 2-4553 (p=0.046) was detected, while a trend (p=0.06) at position 7-4796 was observed.

Figure 2. INS PCR products of mDNA in diabetic patients and control group

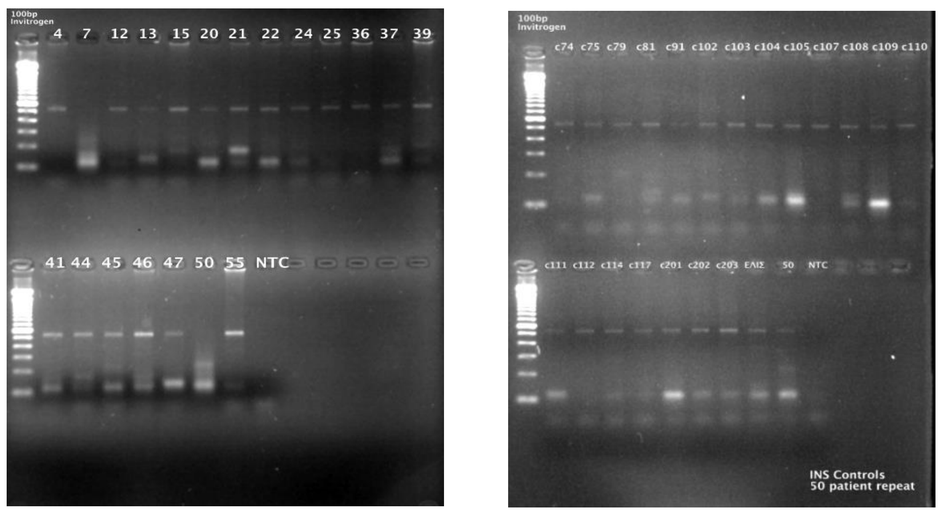

Table 1. Methylated CpGs of the INS promoters in patients and controls

10 CpGs in INS gene	*DNA methylation (%)*		
	T1D (n=20)	*Control group (n=20)*	*p*
	Overall mean methylation percentage		
Mean methylation Range	84.13 ± 3.6 77-92	82.28± 2.8 76-87	0.084
CpG sites			
2-4553	***96.32 ±2.2***	***93.28 ±4.5***	***0.02***
1-4541	*94.00 ±5*	*90.78 ±7.9*	*0.15*
3-4664	*91.02 ±6.3*	*89.58 ±8.4*	*0.65*
4-4692	*63.16 ±8.9*	*62.30 ±9.8*	*0.86*
5-4718	*85.78 ±6.6*	*84.35 ±9.9*	*0.82*
6-4763	*56.65 ±9.8*	*52.82 ±1*	*0.25*
7-4796	***90.01 ±3.6***	***86.53 ±6***	***0.06***
8-4829	*80.28 ±6.2*	*77.72 ±8.4*	*0.32*
9-4879	*91.51 ±5.2*	*89.05 ±9.2*	*0.67*
10-4960	*96.37 ±2.7*	*97.91 ±1.3*	*0.10*

Conclusions

These preliminary data suggest that a tendency for increased methylation in INS promoter already exists in T1D in childhood. Studies with greater number of participants are needed to confirm these findings.

References

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