

HLA-G gene promoter methylation status in children and adolescents with Type 1 Diabetes

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Introduction

HLA-G gene is involved in the control of immune response. It plays a primary role in immune tolerance and may participate in controlling autoimmune responses serving as a potential independent susceptibility marker. HLA-G has been isolated in some secretory granules and on the cell surface of primary islet cells induced to secrete insulin.

Methods

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

- DNA was extracted from white blood cells
- Genomic DNA(800ng) was then treated with sodium bisulphate using the EZ DNA Methylation-Gold Kit.
- Treatment with sodium bisulfite converts unmethylated cytosines into uracils, whereas methylated cytosines remain unchanged under the same conditions.
- DNA was then amplified by PCR reaction was performed 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 transillumination.
- 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. HLA-G forward and reverse primers

	Forward primer	Reverse primer
HLA-G	5'TCGTCGGCAGCGTCAGATG TGTATAAGAGACAGTTTTGGT TTATGTTGAGAGT3'	5'GTCTCGTGGGCTCGGAGAGATGTGTA TATAAGAGAAGACAGTTACATATATAAA AATAAAATAAAAT3'

Results

Methylation profile at 19 CpGs of the HLA-G promoter, was analyzed. HLA-G gene in T1D did not exhibit significant differences regarding the methylation status within its promoter sites compared to healthy individuals.

Figure 2. HLA-G PCR products of mDNA in T1D patients

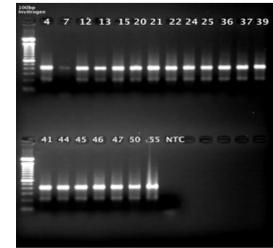


Table 1. Methylation status CpGs of the HLA-G promoters in patients and controls

19 CpGs in HLA-G gene	DNA methylation (%)		
	T1D (n=20)	Control group (n=20)	p
	Overall mean methylation percentage		
Mean methylation	42.99 ± 5.23	43.94 ± 4.31	0.602
Range	36-51	37-52	
CpG sites			
1-5446	84.69 ± 5.6	86.54 ± 5.8	0.31
2-5484	87.07 ± 4.6	89.03 ± 3.8	0.16
3-5550	64.42 ± 8.4	68.55 ± 8.4	0.21
4-5568	96.45 ± 1.5	96.89 ± 1.3	0.12
5-5579	97.29 ± 10	97.42 ± 1	0.56
6-5599	93.09 ± 2.4	93.25 ± 2.5	0.85
7-5629	87.38 ± 5.8	88.28 ± 4.8	0.60
8-5652	12.98 ± 5.2	13.95 ± 5.1	0.58
9-5654	10.83 ± 3.6	11.83 ± 4.1	0.42
10-5690	18.79 ± 7.8	18.92 ± 7.8	0.95
11-5715	13.67 ± 6.5	14.83 ± 5.3	0.46
12-5719	31.28 ± 10.9	32.96 ± 9.9	0.65
13-5772	18.71 ± 6.8	19.21 ± 4.5	0.78
14-5775	32.09 ± 10.4	33.34 ± 8	0.77
15-5778	18.18 ± 6.5	19.59 ± 3.5	0.48
16-5780	14.85 ± 6.6	14.82 ± 4.2	0.98
17-5801	24.35 ± 9.4	24.78 ± 7.1	0.87
18-5813	6.22 ± 2.9	7.69 ± 2.5	0.10
19-5832	4.36 ± 2.68	4.5 ± 1.5	0.35

Conclusions

Despite the described close association of HLA-G with autoimmunity, we failed to find any methylation differences in HLA-G promoter sites between T1D and controls.

References

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