OSBPL5 methylation abnormalities may be pathogenic in Silver Russell syndrome through genomic methylation analysis

Di Wu, Chunxiu Gong*, Chang Su, Bingyan Cao

Department of Endocrinology, Genetics and Metabolism, Beijing Children's Hospital, Capital Medical University, Beijing, China.
*Corresponding author: Chunxiu Gong E-mail: chunxiugong@sina.com.cn

OBJECTIVES

Background:
SRS is a typical epigenetic disease. 38-62% patients show a hypomethylation in the imprinting cntrol region 1 in 11p15. 7%-10% SRS individuals carry a maternal uniparental disomy of chromosome 7(UPD(7)mat. Approximately 40% of patients can not be detected genetic and epigenetic disturbances.

Objective:
To analysis whether there is unknown genes or imprinted genes associated with pathogenicity of SRS and to detect the fine mapping SRS hypomethylation position through the Illumina Methylation 450K chip to detect genome-wide methylation differences.

METHODS

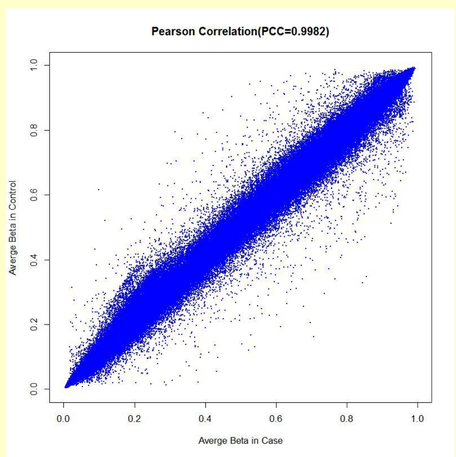
To detect genome-wide methylation sites through the Illumina 450K Infinium Methylation BeadChip chip in 7 cases (two cases were MLPA positive and five cases were negative) of SRS diagnosed in Beijing Children's Hospital and 5 controls matched age. The two methods were validated by using the classical method of sequencing with focal phosphate and digital PCR.

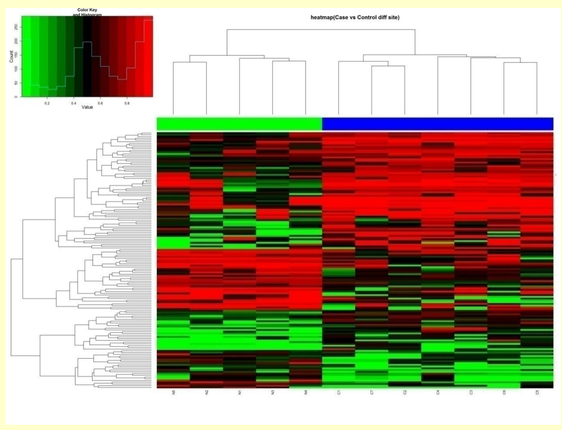
Methylation site probe screening standards meet the following 2 points: (1) adjust Pval < 0.05, if adjust Pval≥0.05, the Pval requires less than 0.05 before correction; (2) case vs control Beta-Difference should be not less than 0.2. That is |Beta-Difference| = 0.2.

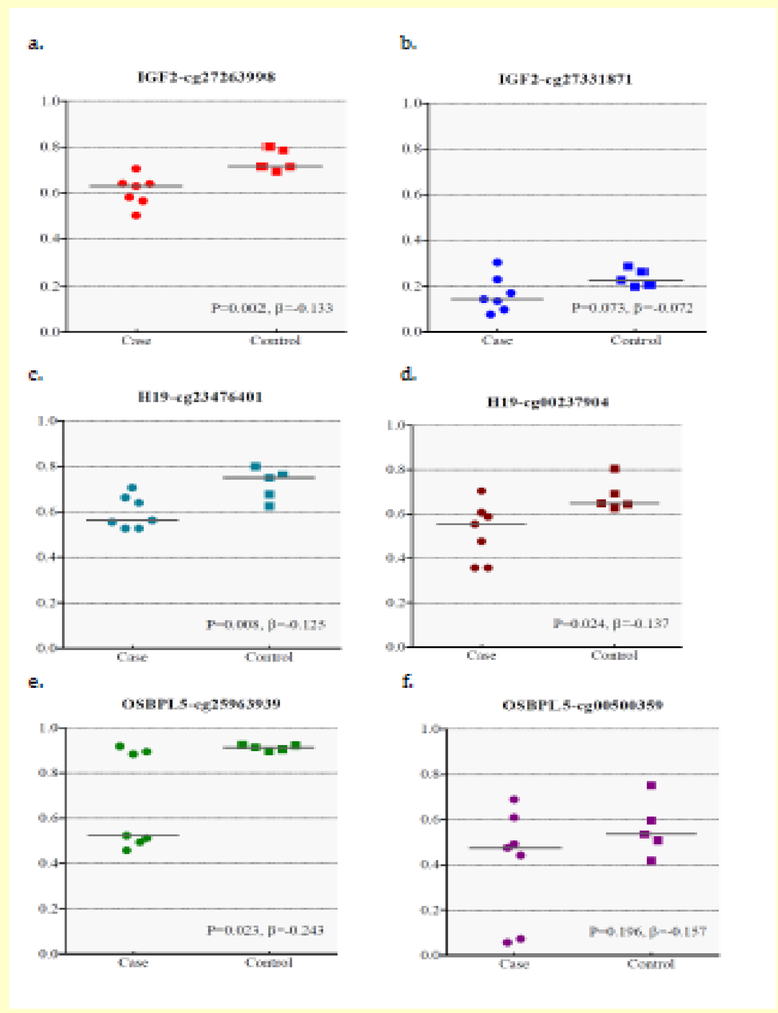
Graphs and tables

OSBPL5 ID	C1	C2	C3	C4	C5	C6	C7	Mean C	P Value	Beta Difference
cg25963939	0.919	0.884	0.513	0.895	0.458	0.495	0.524	0.670	0.023	-0.243
	N1	N2	N3	N4	N5			Mean N		
	0.925	0.913	0.905	0.897	0.923			0.913		

Case group	sex	Age (y)	11p15 MLPA	UPD(7) mat	microarray assay to detect methylation	Pyrosequencing analysis of percent of CpG in OSBPL5	Digital PCR analysis of percent of CpG in OSBPL5
C1	M	1.75	(+)	(-)	0.919	79	80
C2	F	2.67	(+)	(-)	0.884	83	54
C3	F	2.92	(-)	(-)	0.513	27	33
C4	M	5.58	(-)	(-)	0.895	80	73
C5	F	3.75	(-)	(-)	0.458	23	32
C6	M	4.83	(-)	(-)	0.495	24	35
C7	M	3.92	(-)	(+)	0.524	29	35
Normal group (Control group)							
N1	F	1.17			0.925	83	56
N2	F	2.50			0.913	84	52
N3	M	3.08			0.905	80	52
N4	M	4.17			0.897	82	55
N5	F	5.67			0.923	79	59
Comparison between 2 groups					t=-2.969	t=-2.416	t=-0.759
					P=0.025	P=0.036	P=0.475
Compared with C3-C7, N1-N5					t=-4.171	t=-4.113	t=-1.654
					P=0.014	P=0.014	P=0.137







RESULTS

Screening out 116 differential methylation sites in 484821 probes. Through the GO Pathway enrichment analysis, found the cg25963939 site of OSBPL5 was the most significant methylation difference in case group and normal control group (P=0.023,β= -0.243). The 2 methods were validated by using the classical method of sequencing with focal phosphate and digital PCR. And the gene is located on 11p14 5 'UTR region, it is quite possible pathogenic.

This study also found that TGF beta 3, HSF1, GAP43, NOTCH4, MYH14 these 5 genes have some sites which were significant differential methylation between the experimental group and the control group, through the pathway GO function analysis, there may be related to SRS. These 5 genes were located at chromosome 3rd, 6th, 8th, 14th, 19th .

CONCLUSIONS

Through whole genome methylation chip detection, we found the imprinted gene OSBPL5, located on chromosome 11p14, which was detected a significant differential hypomethylation site in 5'UTR area. The two methods were validated by using the classical method of sequencing with focal phosphate and digital PCR. So OSBPL5 may be related to the pathogenicity of SRS. Through the detection of Illumina 450K Infinium high density microarray methylation, we confirmed that the most important epigenetic methylation changes of SRS are located in the 11p1. This is consistent with traditional classical methods such as MS-MLPA.

References

1. Eggermann T (2010) Russell-Silver syndrome. Am J Med Genet C Semin Med Genet 154C: 355-364.
2. Eggermann T, Spengler S, Gogiel M, Begemann M, Elbracht M (2012) Epigenetic and genetic diagnosis of Silver-Russell syndrome. Expert Rev Mol Diagn 12: 459-471.
3 Carrera IA, de Zaldivar MS, Martin R, Begemann M, Soellner L, et al. (2016) Microdeletions of the 7q32.2 imprinted region are associated with Silver-Russell syndrome features. Am J Med Genet A 170: 743-749.
4 Prickett AR, Ishida M, Bohm S, Frost JM, Puszyk W, et al. (2015) Genome-wide methylation analysis in Silver-Russell syndrome patients. Hum Genet 134: 317-332.
5 Azzi S, Steunou V, Tost J, Rossignol S, Thibaud N, et al. (2015) Exhaustive methylation analysis revealed uneven profiles of methylation at IGF2/ICR1/H19 11p15 loci in Russell Silver syndrome. J Med Genet 52: 53-60.
6. Kagami M, Mizuno S, Matsubara K, Nakabayashi K, Sano S, et al. (2015) Epimutations of the IG-DMR and the MEG3-DMR at the 14q32.2 imprinted region in two patients with Silver-Russell Syndrome-compatible phenotype. Eur J Hum Genet 23: 1062-1067.

55ESPE 827--P1
Syndromes : Mechanisms and Management
DI WU
DOI: 10.3252/pso.eu.55ESPE.2016
Poster presented at: ESPE 2016 PARIS 10-12 SEPTEMBER
PosterSessionOnline.com