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ORIGINAL ARTICLE

LINE-1 DNA METHYLATION AND CONGENITAL HEART DEFECTS IN DOWN SYNDROME

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Abstract: DNA methylation is a key epigenetic mechanism that plays a significant role in regulating gene activity during cardiac development. Congenital heart defects (CHD) are one of the most common abnormalities occurring in 40% -60% of cases with Down syndrome (DS). The main aim of this study was to establish the association of long interspersed nucleotide element-1 (LINE-1) DNA methylation in children with DS and the presence of CHD. The LINE-1 DNA methylation was investigated in peripheral blood lymphocytes on a sample of 42 people with DS by quantification of LINE-1 methylation using the MethyLight method. No significant differences in global DNA methylation were found according to the presence of CHD ($P=1.000$), but values of LINE-1 DNA methylation were significantly influenced by gender ($R^2=19.1\%$; $P=0.025$). Significant probability of 19.1% was found in women with DS who had lower LINE-1 DNA methylation values than DS male. Gender was a statistically significant predictor of LINE-1 DNA methylation, although the difference was not statistically significant, female subjects had lower LINE-1 DNA methylation values ($P=0.068$). Further research will clarify the role of lower LINE-1 DNA methylation in the formation of CHD among DS females.

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INTRODUCTION

Congenital heart defects (CHD) are among the most common birth anomalies, as well as medically indicated breaks of pregnancy.¹ CHD may occur as isolated malformations or combined with other extracardial malformations.^{2, 3} They are caused by incomplete heart development during the embryonic period.^{4, 5} Chromosomal abnormalities occur in 5-10%, and genetic changes in 3-5% of cases with CHD.⁶ Approximately 2% of CHD cases occur during embryonic development due to alcohol toxicity, and drugs such as lithium, thalidomide, some antiepileptic, as well as the exposure of the mother to x-rays.⁷ It is believed that 85% of CHD has multifactorial etiology and is the result of interaction between mother and child genes as well as numerous external factors that affect the cardiac development of a fetus.⁸⁻¹²

Epigenetic mechanisms contribute to the regulation of many physiological processes during development. As one of the epigenetic mechanisms, aberrant DNA methylation has been clearly associated with CHD.¹³ DNA methylation by the addition of a methyl group on cytosine alters the structure of the DNA and could have an impact on gene expression. Several epidemiological studies have reported that the global methylation level could be a determinant risk factor for CHD, but the results have been inconsistent.¹⁴⁻¹⁶ About 55% of the human genome is made up of repetitive elements. Useful surrogate marker for estimating global genomic DNA methylation are LINE-1, which are highly repeated and widely interspersed human retrotransposons.¹⁷ In addition, LINE-1 DNA hypomethylation is associated with the development of CHD, and particularly of septal defects.^{18, 19}

Down syndrome (DS) is the most common cause of mental retardation in humans, resulting from trisomy of the chromosome 21.²⁰ Clinical signs of DS are well-known, typical craniofacial dysmorphic features and intellectual disabilities are presents in all cases, and other clinical features occur in a certain, greater or lower percentage of DS patients.²¹ CHD are one of the common abnormalities occurring in 40-60% of DS cases. Most commonly these are septal defects, including atrial defect, ventricular septal defect, complete atrioventricular canal, tetralogy Fallot, open ductus Botalli and other cardiovascular malformations.^{21, 22} Causes of clinical differences are still unknown and are the focus of many studies. Evidence shows that phenotypic variability in DS can contribute to various genetic and epigenetic factors such as gene expression variability, transcriptional factor activity encoded at chromosome 21 or elsewhere in the genome, variable number of repeating sequences, activity of different regulatory RNA molecules and DNA methylation.²³ It is known that genes on chromosome 21 can be classified into various functional categories.²⁴ Therefore, apart from the primary effect such as increased gene expression on chromosome 21, there is a secondary effect of trisomy 21 on gene expression located elsewhere in the genome.²⁵⁻²⁷ In addition, epigenetic changes are influenced by external factors; some of them are associated with the onset of CHD.²⁸ Thus, certain types of CHD in DS are associated with smoking tobacco intake and insufficient intake of folate in the preconception period of the mother, as well as the polymorphisms of genes involved in the metabolism of folate and the changed concentrations of metabolites involved in the metabolism of folate in mothers and persons with DS.^{28, 29} Obermann-Borst et al measured the concentration of biometric methylation concentration in the blood of children with CHD and showed a significantly higher concentration of S-adenosylhomocysteine (SAH) and a lower ratio of S-adenosylmethionine (SAM) / S-adenosylhomocysteine (SAM / SAH) in the DS group, which means that DNA hypomethylation had arisen. Similarly, in the mothers of DS and CHD children, a significantly elevated level of total homocysteine and SAH was found, as well as a significantly lower ratio of SAM / SAH compared to the control group.³⁰

Considering that DS is commonly associated with CHDs that have a common pathogenic mechanism of occurrence, and that in many cases septal defects are present, trisomy 21 is also a good model for determining the association of LINE-1 DNA methylation and the development of cardiovascular defects. Therefore, the main aim of this study was to establish the association of LINE-1 DNA methylation in children with DS and the presence of CHD.

MATERIAL AND METHODS

The study included 42 people with a regular type of trisomy 21. Subjects were collected in collaboration with Down syndrome associations in the Republic of Croatia (Rijeka, Split, Pula, Osijek, Zadar, Karlovac, Čakovec and Zagreb) and the Clinic of Gynecology and obstetrics, Clinical Hospital Center Rijeka. Parents and guardians of all participants were familiar with the purpose and methodology of research and gave their written consent. The Ethics Committee for Biomedical Research at the Faculty of Medicine, University of Rijeka approved the research.

Isolation of genomic DNA

Genomic DNA was isolated from peripheral blood (3-5ml) obtained by venipuncture using FlexiGene DNA Kit (QIAGEN GmbH, Hilden, Germany) according to the protocol specified by the manufacturer.

Determination of LINE-1 DNA Methylation by MethyLight

MethyLight is a quantitative real-time polymerase chain reaction (qPCR), high-throughput and high specificity method for detecting sequences that are methylate. Bisulphite modification produces differences in DNA depending on the presence of methylation. Those differences are precisely detected by the MethyLight method, after amplification of the modified DNA, by binding specific fluorescently labeled probe sites to methylated DNA. LINE-1 DNA methylation was determined by quantification of LINE-1 methylation using the modified MethyLight method. This method only detects fully methylated and completely unmethylated LINE-1 sequences, while partial LINE-1 methylation cannot be detected. Methodology and protocols have previously been described in detail.^{17, 18, 31}

Statistical analyses were performed using MedCalc for Windows (MedCalc Software, Ostend, Belgium) and Statistics for Windows (StatSoft, Inc., Tulsa, USA). Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

In this study, the analysis of LINE-1 DNA methylation was performed on a sample of 42 individuals with DS, 20 males and 22 females. The analysis was done on DNA isolated from blood, since DNA methylation is known to differ quite dramatically between tissues. The median of the ages was 14 years [0.08-48]. CHDs were present in 36% (15/42) of participants. The remaining 64% (27/42) of participants were without CHDs. This is lower than the 40-60% documented in literature.^{21, 22}

Table 1. LINE-1 DNA methylation in Down syndrome DS participants (N=42) with or without congenital heart defects (CHD+/-)

DS	LINE-1 DNA methylation (%) Median
*DS-CHD ⁺	98.64 [95.20 - 99.68]
*DS-CHD ⁻	99.02 [95.66 - 99.95]

*Mann-Whitney P=0.227

The main reason for this discrepancy is probably the small number of participants or regional geographic influences. Furthermore, we observed higher frequencies of CHDs among females (53%) than males (47%) but without statistical significance (P=0.409). A predominance of males among children with CHDs in general has been previously observed, but among children with DS and CHDs there is a higher proportion of females.^{22, 32, 33}

Ventricular septal defect alone also showed a higher frequency in the female gender with DS than in the general population.³² It is known that sex differences in cardiovascular diseases result from a complex interaction between genetic, hormonal and environmental factors that provide a profile of individual risk and phenotypic presentation.

No significant differences were found between DS participants according to the presence of CHD in LINE-1 DNA methylation (Table 1). A stepwise multiple regression analysis for LINE-1 DNA methylation was performed and independent variables included age, gender, MTHFR C677T genotype and the presence of CHD. The gender was the only statistically significant predictor of LINE-1 DNA methylation variability. It was established with a statistically significant probability of 19.1% ($\beta=-0.437$, $R^2=0.191$, $P=0.025$) that DS females would have lower LINE1 DNA methylation than DS males ($P<0.05$), despite the fact that LINE-1 DNA methylation values depending on gender were not statistically significant (Table 2).

Table 2. LINE-1 DNA methylation in Down syndrome participants (N=42) depending of gender

Gender DS (N=42)	N (%)	LINE-1 DNA methylation (%) Median
Male	22 (52)	99.06 [95.66 – 99.95]
Female	20 (48)	98.31 [95.20 – 99.78]

N- number of individuals; Mann-Whitney P=0.068

In most studies in which LINE-1 DNA methylation was determined by quantification of LINE-1 methylation in peripheral blood lymphocytes, a lower global DNA methylation status was found in female subjects.³⁴ The cause for this gender specific alteration in global DNA methylation is not clear. One of the two

chromosomes in women is greatly methylated and transcriptionally inactivated. It has been suggested that X chromosome inactivation may reduce resources essential for proper methylations of autosomal loci. Lower levels of global methylation in women may also be due to altered levels of dietary folate or other one-carbon nutrients in men and women. Women may also have a different folate requirement than men because of regular red blood cells loss through menstruation.^{35- 37} In conclusion, we found significantly lower LINE-1 DNA methylation in peripheral blood among DS females. The associations are unlikely to be mediated by body composition and other risk factors such as dietary, physical activity, lifestyle. The biological mechanisms underlying these differences warrant further investigation. To explain the possibility that lower LINE-1 DNA methylation in DS females has a role in the development of CHD further research is needed.

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REFERENCES

1. Dolk H, Loane M, Garne E, European Surveillance of Congenital Anomalies (EUROCAT) Working Group. Congenital heart defects in Europe: prevalence and perinatal mortality, 2000 to 2005. *Circulation*. 2011;123:841-849.
2. Grech V, Gatt M. Syndromes and malformations associated with congenital heart disease in a population-based study. *Int J Cardiol*. 1988;68:151-156.
3. Gucer S, Ince T, Kale G, Akcoren Z, Ozkutlu S, Talim B, Caglar M. Noncardiac malformations in congenital heart disease: a retrospective analysis of 305 pediatric autopsies. *Turk J Pediatr*. 2005;47:159-166.
4. Bruneau BG. The developmental genetics of congenital heart disease. *Nature*. 2008;451:943-8.
5. Srivastava D. Making or Breaking the Heart: From Lineage Determination to Morphogenesis *Cell* 2006;126(6):1037-1048.
6. Clark EB. Etiology of congenital cardiovascular malformation: epidemiology and genetics. U: Allen H, Cark E, Gutgesell H, Driscoll D, ed. *Moss and Adams' Heart Disease in Infants, Children and Adolescents*. Philadelphia:Lippincott Williams &Wilkins. 2001:64-79.
7. EUROCAT Special Report. The environmental causes of congenital anomalies: a review of the literature (last updated April 2004; cited October, 2018). Available from: <http://www.eurocat-network.eu/content/Special-Report-Env-Risk-I-and-II.pdf>
8. Terry MB, Delgado-Cruzata L, Vin-Raviv N, Wu HC, Santella RM. DNA methylation in white blood cells: association with risk factors in epidemiologic studies. *Epigenetics*. 2011;6:828-837.
9. Romermann D, Hasemeier B, Metzger K, Gohring G, Schlegelberger B, Langer F, Kreipe H, Lehmann U. Global increase in DNA methylation in patients with myelodysplastic syndrome. *Leukemia*. 2008; 22:1954-1956.
10. Li D, Pickell L, Liu Y, Wu Q, Cohn JS, Rozen R. Maternal methylenetetrahydrofolate reductase deficiency and low dietary folate lead to adverse reproductive

- outcomes and congenital heart defects in mice. *Am J Clin Nutr*. 2005;82:188-195.
11. Botto LD, Correa A. Decreasing the burden of congenital heart anomalies: An epidemiologic evaluation of risk factors and survival. *Prog Pediatr Cardiol*. 2003;18:111-21.
 12. Ferencz C, Loffredo CA, Correa-Villaseñor A, Wilson PD. Genetic and Environmental Risk Factors of Major Cardiovascular Malformations. The Baltimore-Washington Infant Study 1981-1989, Futura Publishing, Inc: Mount Kisco, New York: 1997.
 13. Wei L, Liu S, Su Z, Cheng R, Bai X, Li X. LINE-1 Hypomethylation is Associated with the Risk of Coronary Heart Disease in Chinese Population. *Arq Bras Cardiol*. 2014;(37):481-488.
 14. A. Baccarelli, R. Wright, V. Bollati, A. Litonjua, A. Zanobetti, L. Tarantini et al al. Ichemic heart disease and stroke in relation to blood DNA methylation, *Epidemiology*. 21 (2010), 819-828.
 15. Kim M, Long TI, Arakawa K, Wang R, Yu MC, Laird PW. DNA methylation as a biomarker for cardiovascular disease risk. *PLoS One*. 2010;5(3)
 16. Sharma P, Kumar J, Garg G, Kumar A, Patowary A, Karthikeyan G, Ramakrishnan L, Brahmachari V, Sengupta S. Detection of altered global DNA methylation in coronary artery disease patients. *DNA Cell Biol*. 2008;27(7):357-365
 17. Weisenberger DJ, Campan M, Long TI, Kim M, Woods C, Fiala E, Ehrlich M, Laird PW. Analysis of repetitive element DNA methylation by MethyLight. *Nucleic Acids Res*. 2005;33:6823-36.
 18. Chowdhury S, Cleves MA, MacLeod SL, James SJ, Zhao W and Hobbs CA: Maternal DNA hypomethylation and congenital heart defects. *Birth Defects Res A Clin Mol Teratol*. 2011;91: 69-76.
 19. Benjamin EJ, Blaha MJ, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation* 2017; 135: e146-e603.
 20. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongoliens. *C R Acad Sci*. 1959;248:602-603.
 21. Tofts CP, Christianson RE. Anomalies in Down syndrome individuals in a large populations-based registry. *Am J Med Genet*. 1998;77:431-438.
 22. Freeman SB, Bean LH, Allen EG, Tinker SW, Locke AE, Druschel C, Hobbs CA, Romitti PA, Royle MH, Torfs CP, Dooley KJ, Sherman SL. Ethnicity, sex, and the incidence of congenital heart defects: a report from the National Down Syndrome Project. *Genetics in Medicine*. 2008;10:173-180.
 23. Patterson D. Genetic mechanisms involved in the phenotype of Down syndrome. *Ment Retard Dev Disabil Res Rev*. 2007;13(3):199-206.
 24. Gardiner K, Davissou M. The sequence of human chromosome 21 and implications for research into Down syndrome. *Genome Bio*. 2000;1:REVIEWS0002.
 25. Epstein CJ. The consequences of chromosome imbalance. *Am J Med Genet Suppl* 1990;7:31-37.
 26. Epstein CJ. Mechanisms of the effects of aneuploidy in mammals. *Annu Rev Genet*. 1988;22:51-75.
 27. FitzPatrick DR. Transcriptional consequences of autosomal trisomy: primary gene dosage with complex downstream effects. *Trends Genet*. 2005;21:249-253.
 28. Shapiro BL. Down syndrome: A disruption of homeostasis. *Am J Med Genet*. 1983;14:241-269.
 29. Van Driel LM, de Jonge R, Helbing WA, van Zelst BD, Ottenkamp J, Steegers EA, Steegers-Theunissen RP. Maternal global methylation status and risk of congenital heart diseases. *Obstet Gynecol*. 2008;112:277-283.
 30. Obermann-Borst SA, van Driel LM, Helbing WA, de Jonge R, Wildhagen MF, Steegers EA, Steegers-Theunissen RP. Congenital heart defects and biomarkers of methylation in children: a case-control study. *Eur J Clin Invest*. 2011;41(2):143-150.
 31. Babić Božović I, Stanković A, Živković M, Vraneković J, Kapović M, Brajenović-Milić B. Altered LINE-1 Methylation in Mothers of Children with Down Syndrome. *PLoS ONE*. 2015; 10(5):e0127423.
 32. Diogenes TCP, Mourato FA, de Lima Filho JL, da Silva Mattos S. Gender differences in the prevalence of congenital heart disease in Down's syndrome: a brief meta-analysis. *BMC Medical Genetics* 2017; 18:111-117.
 33. Bergstrom S, Carr H, Petersson G, Stephansson O, Bonamy AK, Dahlstrom A, Halvorsen CP, Johansson S. Trends in Congenital Heart Defects in Infants With Down Syndrome. *Pediatrics*. 2016;138(1):e20160123
 34. Zhang FF, Cardarelli R, Carroll J, Fulda KG, Kaur M, Gonzalez K, Vishwanatha JK, Santella RM, Morabia A. Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood. *Epigenetics*. 2011; 6(5):623-629.
 35. Hsiung DT, Marsit CJ, Houseman EA, Eddy K, Furniss CS, McClean MD, Kelsey KT. Global DNA methylation level in whole blood as a biomarker in head and neck squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 2007;16:108-114.
 36. Wilhelm CS, Kelsey KT, Butler R, Plaza S, Gagne L, Zens MS, Andrew AS, Morris S, Nelson HH, Schned AR, Karagas MR, Marsit CJ. Implications of LINE1 methylation for bladder cancer risk in women. *Clin Cancer Res*. 2010;16:1682-9
 37. Zhu ZZ, Hou L, Bollati V, Tarantini L, Marinelli B, Cantone L, Yang AS, Vokonas P, Lissowska J, Fustinoni S, Pesatori AC, Bonzini M, Apostoli P, Costa G, Bertazzi PA, Chow WH, Schwartz J, Baccarelli A. Predictors of global methylation levels in blood DNA of healthy subjects: a combined analysis. *Int J Epidemiol*. 2012;41:126-139.