Placental epigenetic biomarkers for the detection of isolated ventricular septal defect
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Objective
Epigenetics refers to molecular mechanisms for controlling gene function that are not due to gene mutations. DNA methylation is the most studied epigenetic mechanism and it involves the chemical binding of single carbon atoms (“methyl groups”) to cytosine nucleotides in DNA. When this methylation occurs in the cytosines of the coding region of a gene, repression of gene transcription classically results. Environmental factors such as diet, smoking and alcohol exposure profoundly affect DNA methylation. VSD is the most common congenital major heart defect (CHD). The pathogenesis of isolated non-syndromic VSD is largely unknown and population-based screening continues to show low prenatal detection rates using ultrasound. Our prior pilot studies using newborn leucocyte DNA found significant changes in DNA methylation in different types of CHD. Further, screening based on these methylation changes achieved high diagnostic accuracy for CHD detection. This study aims to develop DNA methylation-based molecular biomarkers, using placental tissue, for VSD detection. Additionally, we wanted to use this epigenetic analysis to investigate the pathogenic mechanisms of isolated VSD.

Methods
The Illumina HumanMethylation450 BeadChip assay was used to measure genome wide DNA methylation level in >450,000 cytosine nucleotide loci in approximately 20,000 genes. Placental specimens from 8 isolated non-syndromic VSD cases and 10 unaffected controls were compared. A significant difference in DNA methylation in cytosine loci in case versus controls was stringently defined as ≥2.0 fold increase or ≥2.0 fold decrease in methylation with a False Discovery Rate (FDR) p-value, <0.05. Area Under the ROC Curve: AUC (95% CI) were used to determine the accuracy of methylation markers for detecting isolated, non-syndromic VSD.

Results
We identified 11,000 genes in which there were statistically significant differences (increased or decreased) in cytosine methylation levels in VSD vs controls (FDR p-value <0.01). Using gene methylation levels as a biomarker for VSD prediction, a total of 1,454 genes each accurately detected VSD i.e. AUC ≥ 0.81 (0.61-1.00). Of these 329 had good diagnostic accuracy: AUC ≥ 0.80 to 0.89 and 337 had excellent diagnostic accuracy: AUC 0.90 to 1.00 for VSD detection. Gene ontology analysis revealed significant methylation changes and over-representation of genes involved in heart development, including some responsible for cardiac ventricle (HEY2, FDR p=0.002; ISL1, FDR p=2.61, E-5), cardiac chamber morphogenesis (HEYL, FDR p=0.001), cardiac septum formation (ISL1), cardiac muscle development (ACTC1: FDR p= 7.15 E-12) and others. It appears that the DNA methylation changes alter the expression of multiple genes some of which are known to be critical heart and ventricular development leading to the CHD.

Conclusion
Based on placental analysis, we report novel epigenetic biomarkers that appear highly accurate for VSD detection. Further, the highly significant DNA methylation changes in critical cardiac developmental genes suggest that epigenetic mechanisms play a crucial role in the pathogenesis of non-syndromic VSD. Our findings are very novel and raise the intriguing possibility of the use of trophoblast DNA for the prenatal detection of CHD.