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Discussion
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The major aim of the present study is to find suitable markers at the DNA and protein level that can be of use as diagnostic of the risk of REPL seen among south Indian women. Following a strong emphasis by many reports on oxidative stress in reproductive disorders, we started the study by analyzing the detoxification gene polymorphisms. Detoxification systems mediate the interactions between our body and xenobiotics in the environment. During this process, they consume the redox reserves and generate the reactive oxygen species. Hence, a tightly coordinated detoxification network is required for efficient removal of the environmental toxins. Our study revealed a strong association between \textit{CYP1A1} polymorphisms and the risk of REPL. \textit{CYP1A1} polymorphisms alter the enzyme expression as well as activity and may prove fatal to the survival of the first trimester fetus. Aryl hydrocarbon receptor located on the cell membrane binds to the poly cyclic aromatic hydrocarbons and this stimulates the expression of \textit{CYP1A1}. No genetic variants were observed in the aryl hydrocarbon receptor or its repressor. \textit{CYP1A1} is mainly concerned with metabolism of environmental toxins while \textit{CYP2D6} metabolizes pharmacologically active compounds. Two missense polymorphisms were observed exclusively in cases and they showed a strong co-inheritance. Structural analysis revealed that they alter the binding properties of the enzyme to the substrate. Polymorphisms in phase II genes failed to show any association. Since, pregnancy is a complex process mediated by a multitude of events; we considered pregnancy loss as a manifestation of an underlying genetic abnormality in either of the processes necessary for establishment and maintenance of pregnancy.
Endothelial nitric oxide synthase and vascular endothelial growth factor gene polymorphisms were studied as they play an important role in remodeling of maternal vascular network. A novel intronic polymorphism was found in *eNOS* that showed strong association with the risk of REPL. This variation creates a binding site for a transcription factor Ttk 69K involved in embryonic development. Estrogen dynamics play an important role in pregnancy by recruiting hormones such as progesterone, hCG that lead to a successful pregnancy outcome. CYP17 and CYP19 participate in estrogen biosynthesis and polymorphisms in these genes are known to cause pregnancy loss, endometriosis, failure in IVF cycles etc. Intronic repeats in *CYP19* exhibited a significant difference in their distribution among cases and controls. Neither CYP17 nor SULT1E1 showed any association. CAG repeats of androgen receptor code for the poly glutamine tract. The exact number of repeats varies between individuals and is responsible for the androgen responsiveness of an individual. Measurement of repeat lengths suggested that repeat numbers larger than 26 were present at a higher proportion in women with a successful pregnancy history. This probably protects them from the embryo damaging androgen activities. A strong correlation was also seen between the length of the repeats and the choice of X chromosome to inactivate. Lower repeat numbers were exhibiting a tendency to undergo selective inactivation. This corroborates our previous proposition that a selective preference for a low androgen exposure is preferred by the maternal (female, in general) system. Non random X chromosome inactivation is a measure of studying the hidden events that prioritize a particular X for inactivation. A very strong association was found between the degree of skewing and the risk of REPL.
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HLA-G offers the immuno modulation required by the mother to home the semi allogenic fetus. A suppressed cellular immunity marks a successful pregnancy outcome and this is achieved by HLA-G. Novel variations in HLA-G exon 2 and exon 8 were observed and RNA folding studies showed that the mutant forms had lower stability than the wild type. Endocannabinoids play a role via the endocannabinoid receptors and play an important role in implantation. They stimulate processes like muscle contraction that is coupled to the success rates of implantation. Elevated levels of Anandamide, representative of endocannabinoids were seen in pregnancy loss. A synonymous polymorphism in exon 6 of Anandamide hydrolase was found exclusively in women with recurrent abortions. Selective codon choice analysis indicated that the polymorphism leads to a less preferred codon. Hemostatic mechanisms are necessary to meet the increasing demands of the mother as well as the fetus. An increase in blood volume is also accompanied by enhanced thrombotic activities to prevent blood loss. Possible association between factor V, prothrombin and MTHFR were analyzed. The FVL and prothrombin G20210A variations were not observed in any individual while MTHFR failed to show any association.

Platelet proteomic analysis revealed the over expression of a FSD1-like protein that carries a fibronectin type 3 domain. Another protein found at elevated levels in REPL is the variable region of IgM heavy chain. This possibly represents an auto antibody directed against the platelet antigens or a product of alpha granules released upon platelet aggregation. Hence, both the proteins point towards an elevated platelet aggregation as a
risk factor in REPL. This can result in decreased platelet counts seen in gestational thrombocytopenia associated with pre-eclampsia. In conclusion, our study revealed novel genetic markers that can possibly predict the risk of REPL. In addition, screenings for these variants also let us know about possible defect causing the REPL. The results from proteomic analysis need further studies to dissect out components as well as the cascades involved in linking platelet aggregation to REPL.