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Design of a SNaPshot assay for simple and cost-effective detection of six variant related to recurrent pregnancy loss

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Background

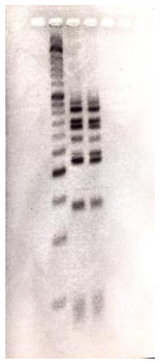
Applying karyotyping as conventional approach is still mainly applicable in laboratory but a considerable proportion of RPL (recurrent pregnancy loss) is related to single-gene alteration such as mutation, insertion and etc. Our aim in this study was to design and development a genetically panel using SNaPshot method beside karyotyping.

Materials and Methods

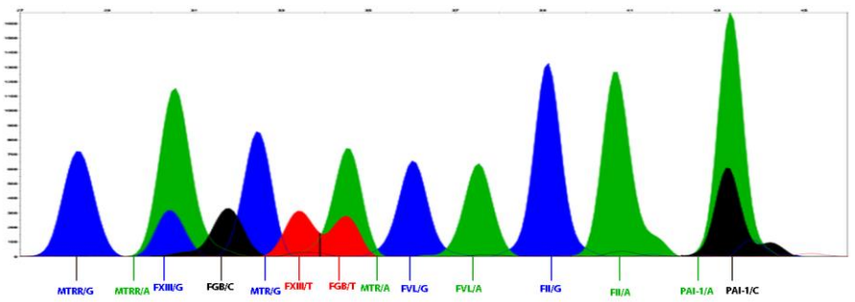
- Six specific primers were designed to amplify target region involved in pathogenesis of RPL through multiplex PCR method. Specific single base extension (SBE) designed in order to minisequencing six mutation site by the SNaPshot simultaneously. The SBE products were electrophoresed in an ABI 3130xl Genetic TM Analyzer) using POP-4 polymer and 35 cm capillary arrays. The GenMapper v3.2 software were used to analyze the resulting electropherograms.

Results

Our pilot study six mutation sites in 16 women with RPL were previously genotyped by real-time PCR, were used to test the accuracy and reproducibility of multiplex SNaPshot assays. The results were compared with the previously analyzed types.



Analysis of six-plex polymerase chain reaction for detection of 6 thrombophilic variant by 3% gel agarose.



Electrophoreogram from Gene Mapper ID, Ver 4.0, analysis of 6 mutations in five thrombophilia- and three folate-related genes in a patient with seven mutations in heterozygous state.

Conclusion

The variant in enzymes involved in one-carbon metabolism lead to elevation of blood levels of homocysteine as a thrombotic risk factor. SNaPshot technique is specific, accurate and inexpensive approach to customized genetically panel and monitoring of frequent pathogenic mutation in RPL. The variant included in this assay have a substantial role in the pathogenesis of spontaneous abortion, which needs to be fully elucidated.

References

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