"IDENTIFICATION OF MODY2 GENE MUTATIONS AND ITS CLINICAL CORRELATION IN INDIAN FEMALES AFFECTED WITH GESTATIONAL DIABETES"

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By

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6.SUMMARY & CONCLUSION

This study was conducted to identify *GCK* mutations or variants present in a cohort of North Indian females diagnosed with GDM. In parallel, we also studied the status of *HNF4A* (MODY1) and *HNF1A* (MODY3) variants in a subset of GDM females. Another objective was to study the role in inflammation in GDM development.

Salient findings of this work can be summarised into below sections-

6.1 Analysis of *GCK* gene variants in women with GDM

A total of 76 women diagnosed with GDM were analysed for GCK gene variants and we have identified one novel variant, *c.1030G>T* (p.Asp344Tyr) on exon 9 of the *GCK* gene in one female diagnosed with GDM. It was predicted to be "damaging" by the in-silico tools. Family segregation of the women harbouring this variant showed that this variant was also present in the brother and father of the women, who were diagnosed with pre-diabetes and diabetes and had mild hyperglycemia. This woman was not treated on insulin and was managed on diet alone, which resulted in good pregnancy outcomes, which emphasized on the pathogenicity of this variant. Two intronic variants, c.1256+8C>T (rs2908274) and c.1256+49G>A (rs13306387), which are already reported in relation to MODY2 in the Caucasian population were observed in our study population and are reported in Indian population for the first time.

6.2 Analysis of variants of *HNF1A and HNF4A* genes in GDM affected women

We identified one novel variant, c.1501+1G>A in the splice donor site at the junction of exon 7-intron 7 of *HNF1A* gene and one novel variant, c.224G>A (p.Arg75Lys) at exon 2 of the *HNF4A* gene. Both the variants were predicted to be "damaging" by the in-silico tools. Two already reported variants of *HNF1A* (rs193922586) and *HNF4A* (rs113308087) in relation to

type 2 diabetes are hereby also reported from India for the first time in this study. One of the females with GDM was positive for a known "likely pathogenic" variant, rs142204928, of *HNF4A* gene reported in Indian patients with MODY.

6.3. Risk factors of GDM

Prevalence of GDM was observed as 15.7% in our study. It was highest in women of age between 30-34 years. Parameters such as age>30 years, having one of the parents with diabetes, GDM in a previous pregnancy, gestational weight gain, hypothyroidism and PCOD were identified as factors increasing the odds of having GDM.

6.4. Serum IL-6 and CRP estimation in women with GDM

In our study significantly raised levels of circulating serum IL-6 were observed in the samples of women with GDM. On categorising the samples based on BMI, it was found that highest levels of IL-6 were present in women in the BMI range of 25-29.9 Kg/m^2. IL-6 was positively correlated with pre-pregnancy BMI, showing its association with obesity which is an established rish factor of GDM. GDM samples (n=60) also had elevated serum CRP levels than the non-GDM samples (n=60), but the difference was not statistically significant.

6.5. Bioinformatics analysis of the three novel variants

Homology modelling for the three novel variants (*GCK*, *HNF1A* and *HNF4A*) was performed and the 3D structures of the proteins were generated, to have an insight of the spatial arrangement of important residues in the protein. This analysis also helped us to understand how the structure and stability of the protein was affected by the mutation. The i-Mutant2 tool showed that stability of the protein of the *GCK* and *HNF4A* variant decreases due to the mutation. Other in-silico tools predicted the variants to be "disease-causing" or "damaging".

In conclusion, we hereby report three novel "likely pathogenic" variants of the *HNF1a*, *HNF4a* and *GCK* genes. The bioinformatics analysis of these novel variants showed that these mutations have an effect on the protein's structure and stability and further studies are required to find out the functional impact. Our findings suggest that further large-scale screening studies for the three common MODY subtypes (MODY1, 2 and 3) could possibly derive their actual prevalence in women of Indian origin who are primarily diagnosed with GDM. Identifying underlying MODY in such women would provide an opportunity to optimally decide their treatment regime, which in turn improve feto-maternal outcomes. This is the first of its kind report from India specifically studying *GCK* variants in gestational diabetes mellitus. Secondly, we also conclude from our findings that IL-6 might have an important link in causing insulin resistance and it could be responsible for causing GDM. Moreover, obesity and inflammatory markers may have an association with GDM.