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Research Article

CHARACTERISATION OF TETRA AMELIA SYNDROME BY SNP BASED ON COMPUTATIONAL GENOTYPING ANALYSIS

LIKITH REDDY P, MANOJ*

Department of Bioinformatics, Sathyabama University, Chennai, Tamil Nadu, India. Email: yucmanoj@rediff.com

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ABSTRACT

Objective: Tetra Amelia syndrome is a congenital disorder, which is mainly caused by the presence of mutation in the *WNT3* region. Being an embryonic developmental disorder, earlier warning of its onset can be predicted by subjecting there single nucleotide polymorphisms (SNPs) to further studies.

Methods: For the identification of the list of mutant WNT3 proteins with the specified (G83X) position, we predicted it through reverse genetics method. This region was further used to determine the SNPs involved in them using the Chi-square test. Finally, we have validated the existence of these SNPs in the *WNT3* gene by multifactor dimensionality reduction analysis.

Results: In Tetra Amelia syndrome, we determined that among the six frames of *WNT3* gene, only the 2nd frame has more identity with WNT3 protein (98%). The mutant amino acid residue was found only at the 83rd position (G83X). Sequence analysis techniques helped to determine 16 SNPs: rs147030694, rs9908452, rs1062339, rs193268056, rs190245126, rs185051815, rs71375364, rs188212517, rs185848450, rs151309556, rs148810526, rs142400306, rs118086135, rs77768380, rs75398055, and rs34608985. These SNPs where validated further and this lead to 3 SNP s, which can be used to genotype Tetra Amelia syndrome.

Conclusion: The present studies of genotyping Tetra Amelia syndrome can help determine congenital disease at earlier stages itself. In future, larger dataset is needed and as well similar methodology can be used on late onset diseases (like Parkinson's) can also be predicted by subjecting there SNPs genotype.

Keywords: Tetra Amelia syndrome, WNT3, Hap map, Multifactor dimensionality reduction, Single, Nucleotide polymorphisms.

INTRODUCTION

Tetra Amelia is a rare autosomal recessive congenital disorder characterized by the absence of all four limbs and other areas of the body are also affected by malformations, such as the face, skull, reproductive organs, anus, and pelvis [1]. The disorder is caused by mutations in the *WNT3* gene [1]. The *WNT3* gene contains 54,249 nucleotides associated with 869 single nucleotide polymorphisms (SNPs). The *WNT3* gene codes for WNT3 protein, which plays a key role in embryonic development of humans [2-5]. If a mutation reflects on WNT3 protein, it inhibits the β-catenin protein of T-cell factor pathway and an amino acid change is triggered at 83rd position that is., G83X to causes disease [4]. *WNT3* mutation occurs at gene level on chr17q21 position between 44,841kb and 44,896kb [6-8]. Based on previous reports Tetra Amelia is an inheritance disorder of single gene i.e. *WNT3* and this gene is not only responsible for causing Tetra Amelia, but it is also involved in late onset of Parkinson's disease in certain cases [7]. *WNT3* has 83% identity with *WNT3A* and it is cluster within *WNT15* on same chromosomal locus 5, 6. In order to obtain the association of significant SNPs, which are responsible for causing the disease, approaches in computational biology are very much useful in analysis of nucleotide sequences using various algorithms.

METHODS

Reverse genetics and sequence analysis

The *WNT3* gene is translated in to 6' frames and aligned with WNT3 protein. After performing the alignment, we have identified that the protein may be expressed from the second frame.

Statistical analysis

Hardy-Weinberg equilibrium was assessed for each SNP using the Chi-square test. The differences in allele and genotype frequencies are calculated based on Chi-square test. SNP association in Tetra Amelia syndrome were identified based on the minor allele frequencies i.e., $p<0.05$ to obtain linkage disequilibrium (LD) between SNPs and finally the SNP-SNP association is done for knowing the maximum risk factor.

Multifactor dimensionality reduction (MDR) software is a non-parametric data mining alternative for detecting and characterizing non-linear interactions among discrete genetic and environmental attributes. MDR method combines attribute selection with attribute construction and cross validation. MDR is used for multi gene interactions but in our case, we have utilized MDR for SNP association in *WNT3*.

RESULTS AND DISCUSSION

Original gene sequence of *WNT3* is translated into 6 frames, among the six frames, only the 2nd frame has more identity with WNT3 protein (98%) and it is estimated that the protein part is present in the mutated region, which is synthesized from the 2nd frame of translated sequence and change in amino acid residue was found only at the 83rd position (G83X) (Fig. 1).

The SNPs of *WNT3* gene determined using alignment techniques with their respective positions are listed in Table 1.

LD analysis was performed with the datasets of Hap map using Haploview (V 4.0) (Fig. 2).

MDR (V 4.2) program is executed for the selected 16 SNPs in mRNA region of *WNT3* by considering them as attributes. After analyzing the cross validation consistency (CVC) of 16 SNPs, we have found that the best model on the basis of specificity and sensitivity values of SNP interaction (Fig. 3).

Fig. 1: Translated sequence with open reading frame (ORF) and ORF is indicated in red

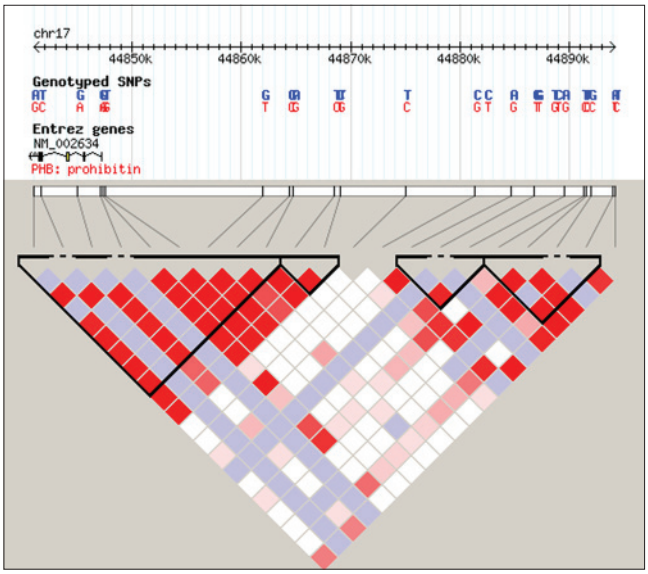

Fig. 2: Linkage disequilibrium plot of 23 single nucleotide polymorphisms (SNPs) of Hap map data (does not include the 16 SNPs that were determined)

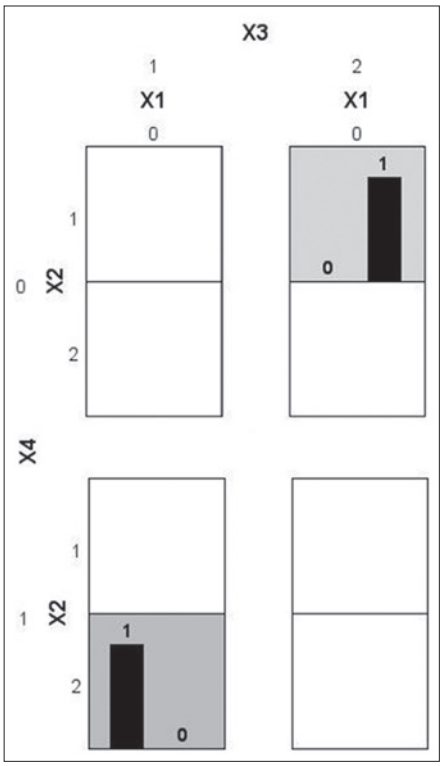

Fig. 3: Attribute data based on the consistent values of specificity and sensitivity

Table 1: List of single nucleotide polymorphisms associated with Tetra Amelia syndrome

S. no	Position	SNPs	Allele
1	44841685-886	rs147030694	/A
		rs9908452	C/G
		rs1062339	C/T
2	44886331-570	No SNP's were found	No Allele
3	44890185-450	rs193268056	A/G
		rs190245126	C/G
		rs185051815	C/G
		rs71375364	A/G
4	44891433-919	rs188212517	C/T
		rs185848450	A/G
		rs151309556	A/C
		rs148810526	C/T
		rs142400306	C/T
		rs118086135	A/G
		rs77768380	A/T
		rs75398055	A/C
		rs34608985	G/T

SNP: Single nucleotide polymorphisms, *All the above mentioned SNP's have more association with Tetra Amelia syndrome

CONCLUSION

In order to validate the association of 16 SNP's which are present in 2nd frame of translated mRNA region; we have used Haploview and MDR software. Haploview shows only LD plot of 23 SNP's other than the 16 SNP's present in 2nd frame of translated mRNA region and Hap map project contains the genotype data of only 3 SNPs. Hence, we performed final validation for model selection based on the CVC of MDR. These results indicate our finding that, with the presently available, sequence data and other datasets like these derived from Hap map project; require more information to completely understand *WNT3* and Tetra Amelia syndrome.

The 16 SNPs determined by sequence analysis have been cut short from over 869 SNPs that are actually present in *WNT3*. Further analysis of these 16 SNPs can highlight its clear association with Tetra Amelia syndrome and future studies based on these 16 SNPs can be useful for screening, detection and other related works in accordance with genotyping of human population to diagnose Tetra Amelia Syndrome and related disorders.

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