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Research Article

EVOLUTIONARY RELATIONSHIP BETWEEN AQP2 & AVPR2 AND THEIR MUTANT SEQUENCES

[*]Monica Soni[1] and Arvind Pareek[2]
[1]Mewar University, Gangrar, Chittorgarh, Rajasthan, India
[2]Vardhaman Mahaveer Open University, Kota, Rajasthan, India
**Author for Correspondence*

ABSTRACT

Mutations in AQP2 and AVPR2 genes play an important role in the pathogenesis of nephrogenic diabetes insipidus. NDI is of three different types on the basis of inheritance mechanism. AVPR2 is the gene in which mutations are known to cause X-linked nephrogenic diabetes insipidus, whereas AQP2 is the gene in which mutations are known to cause autosomal recessive and autosomal dominant nephrogenic diabetes insipidus. Evolutionary relationship study was done with help of Clustal Omega between candidate gene and mutant gene. Clustal Omega is a global multiple sequence alignment programme for proteins and nucleotides. MSA is a set of sequences can provide information as to the most likely regions in the set via cladograms. Cladogram showed the evolutionary relationship within the sequences.

Keywords: *ADH, Alignment, Clustal Omega, BLAST, Phylogram*

INTRODUCTION

Aquaporin2 is the Arginine Vasopressin (AVP) dependent water channel of the collecting duct. AVP regulates the body's retention of water; it is released when the body is dehydrated and causes the kidney to conserve water, thus concentrating the urine and reducing urine volume (Lim *et al.*, 2004). The AVPR2 gene encodes the arginine vasopressin receptor-2 (AVPR2), is a G protein-coupled receptor. The V2 receptor activates adenyly cyclase by interacting with G protein (Birnbaumer, 1992). This process is the molecular basis of the vasopressin-induced increase in the osmotic water permeability of the apical membrane of the collecting tubule. The activated V2R will induce an increase of intracellular cAMP levels via stimulatory Gs protein and adenylate cyclase, which will eventually lead to activation of protein kinase A and to phosphorylation of aquaporin-2 (AQP2) water channels.

Mutations in two genes are known to cause NDI, these genes are; AQP2 and AVPR2. Mutations in the arginine vasopressin receptor 2 (AVPR2 or V2) genes are responsible for congenital nephrogenic diabetes insipidus (NDI) in approximately 90% of patients. Mutations in the x-chromosomal V2 receptor result in X-linked NDI (Hochberg *et al.*, 1997). Mutations in the AQP2 gene are responsible for the autosomal Recessive NDI and Autosomal Dominant NDI. Deen *et al.*, (1994) demonstrated compound heterozygosity for inactivating mutations in the AQP2 gene located on chromosome 12q12–13 in a patient with autosomal recessive NDI. Nucleotide deletions in the coding region of the C-terminus of AQP2 were reported to cause dominant NDI.

Mutations in AQP2 and AVPR2 define the changes in their DNA or in gene promoter region. The mutant sequences follow the theory that all contempory genetic material has one ancestral ancient DNA (Sangita, 2006). During the way of evolution mutation occurred, creating differences between families of contemporary species. Multiple sequence alignment between AQP2 & AVPR2 and their mutant sequence have insertion (an insertion of a letter or several letters to the sequence) and deletion (deleting a letter or more from the sequence). Multiple sequence alignment reviews similar alignment method in the context of phylogenetic analysis. Alignment sequence positions subjected to phylogenetic analysis represent a prior phylogenetic conclusion because the site themselves are effectively assumed to be gene logically related or homologous (Baxevanis, 2002).

MATERIALS AND METHODS

The present study was conducted on aquaporin2 (AQP2) and arginine vasopressin receptor2 (AVPR2) gene sequences, because mutations in both gene sequences play a major role in the pathogenesis of Nephrogenic Diabetes Insipidus. Present study of both gene sequences and mutant sequences has been carried out with the help of following databases and tools -

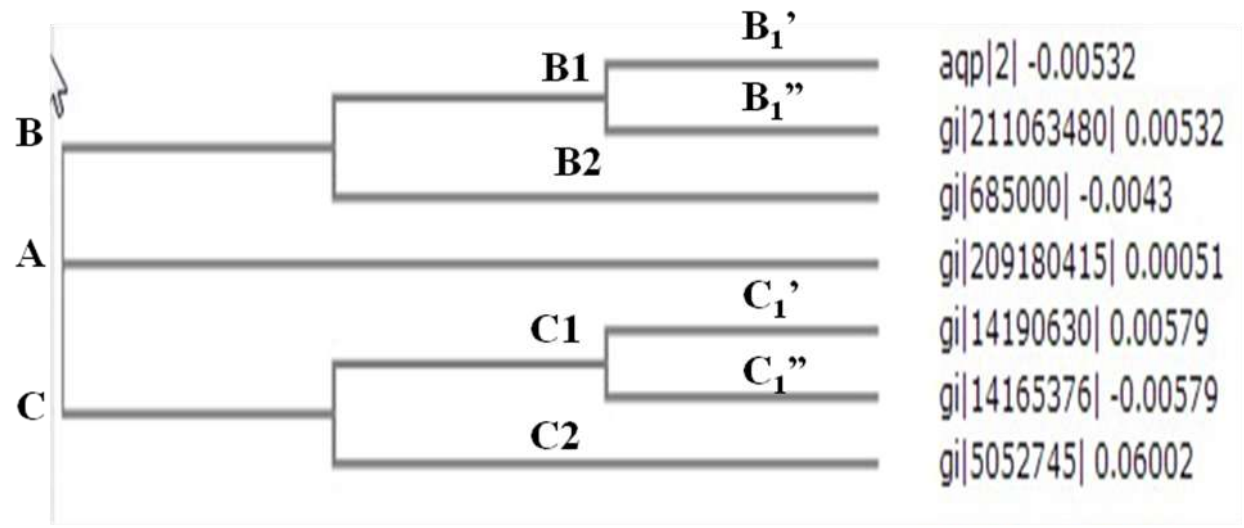
Kyoto Encyclopedia Genes and Genomes (KEGG): Both the gene sequences were assorted from the KEGG database for the current study. For the present study Gene's orthology, disease pathway, drug target and motif information all were collected from KEGG database.

National Centre for Biotechnology Information (NCBI): Mutant sequences were collected in FASTA (Fast Alignment Sequence Analysis Tool) format; extracted from the NCBI.

Clustal Omega: Multipal Sequence Alignments (MSA) and Phylogenetic relationship study was done by Clustal Omega. Clustal Omega uses "Hidden Markov Model (HMM)" for the alignment. CLUSTAL Omega provides more realistic alignments that should reflect the evolutionary changes in the aligned sequences and the more appropriate distribution of gaps between conserved domains. There are 23 homo sapience mutant nucleotide sequences of AQP2 and AVPR2 gene was found in NCBI; from which 6 homo sapience mutant sequences of AQP2 and 7 homo sapience mutant sequences of AVPR2 were selected for the study. The first 6 similar hits; >gi|209180415|, >gi|211063480; >gi|14190630|; >gi|14165376|; >gi|5052745|, >gi|685000| of AQP2 mutant sequences and 7 similar hits >gi|225903384|, >gi|225903383|, >gi|225903386|; >gi|208973254|; >gi|3004498|; >gi|27469609| and >gi|51873895| of AVPR2 mutant sequences obtained after the blast identitiy results regarding candidate genes.

Multiple sequence alignment was done between candidate genes & known mutant nucleotide sequences. MSA of a set of sequences can provide information as to the most likely regions in the set via cladograms. Cladogram showed the evolutionary relationship within the sequences.

For this study, the identified sequences were submitted along with the candidate genes in Clustal Omega; which was based on a Hidden Markov Model (HMM). After the multiple sequence alignment, align sequences sequentially, guided by the phylogenetic relationship indicated by the tree. This phylogenetic analysis by Clustal Omega predicted a cladogram that provided the result of possible ancestral relationship between the sequences as shown in figure 1 and 2.



Figur 1: Showing Cladogram Tree shows relation between AQP2 & mutant sequences.

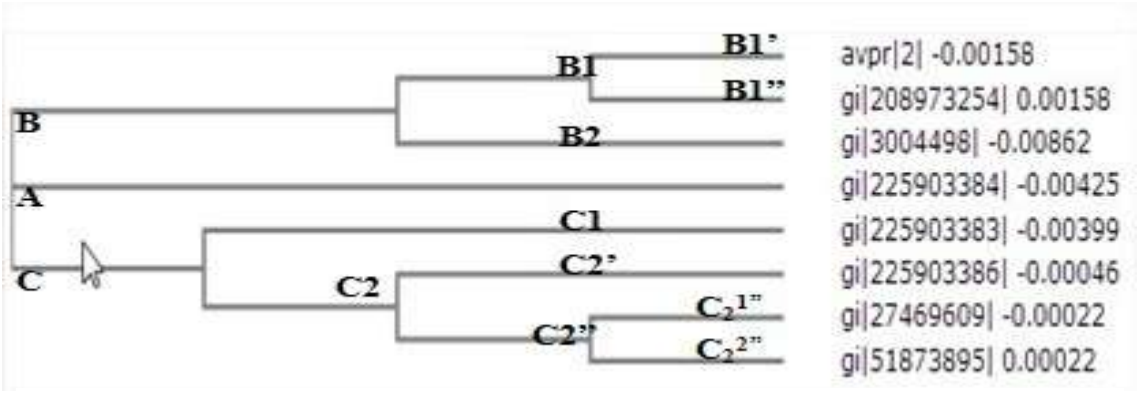


Figure 2: Showing Cladogram Tree shows relation between AVPR2 & mutant sequences

RESULTS AND DISCUSSION

Phylogenetic analysis by Clustal Omega predicts a tree that gave the result of possible ancestral relationship between the sequences. Cladogram is a tree drawing program able to draw any binary tree expressed in the standard phylogenetic tree format. The result shows un-rooted phylogenetic tree. It is a directed tree with a unique node corresponding to the most recent common ancestor of all the entities at the leaves of the tree. Study for Phylogenetic analysis of candidate gene AQP2 & mutant sequences (Figure 1) and also of candidate gene AVPR2 & mutant sequences (Figure 2) with cladogram, constructed with Clustal Omega phylogenetic analysis. Phylogenetic tree were constructed by the Neighbour-Joining (NJ) method. Sequencing analysis exposed that only a single base pair chages were responsible for change in nucleotied sequence and identity between the base pair.

Cladogram for AQP2 and other mutant sequences of the gene did not predict any common ancestry between all these sequences. However it was clear cut indicated that 3 major mutant varient was there between all these 7 sequences (1 candidate AQP2 and 6 mutant sequences). These were named and discussed further with mutant variant A, B and C as indicated in figure 4.13 here. There was further bifurcation in variant B and C. These were named as B_1 & B_2 in variant B and C_1 & C_2 in variant C (figure 1). Out of these B1 & C1 were further bifurcated and named B_1' & B_1'' and C_1' & C_1'' respectively.

This cladogram clearly revealed that only B_1'' have common ancestry with AQP2, which share ancestry of B_2, that is also very much homologus to AQP2. Again entire variant B and A might be of similar ancestry as B_1, B_2 and A have 100% homology between there coding regions. It also revealed that only B_1'' is actual mutant of AQP2 while all others are of different origin. It might be possible that during evolution they all evolved simultaniously and due to structure-function relationship and natural selection, AQP2 evolved as most abundant sequence in population. All other mutant sequences are yet present in population but in heterozygous condition they can not expresse. Varient C also has common ancestry with A and B. Yet mutation in this branch showed in different pattern from varient A & B and developed in major insertion and deletion in coding region of AQP2. Here insertion and deletion term used in relation to sequence of candidate gene AQP2 because actual ancestors of all these sequences is unknown and need further study with all the available sequences showing less or more homology with AQP2, then only actual ancestor of AQP2 can be identified, and insertion and deletion will be find out exactly.

The gene for AVPR2 and mutant sequence did not predict any common ancestry in cladogram. Same as AQP2, 3 major mutant varient was there between all these 8 sequences (1 AVPR2 and 7 mutant sequences). Varient B again bifurcated in B_1 & B_2 and varient C in C_1, C_2 & C_3 (Figure 2). The varient B_1 showed bifurcation in B_1' & B_1'' Same as C_2 in C_2' & C_2'' and again C_2'' in C_2^{1}' & C_2^{1}''. The cladogram of AVPR2 and mutant sequence has shown that varient A and B_1'' & B_2 have similar ancestry between their coding regions. Varient B_1'', B_2 and A have shown definite mutant of AVPR2 on same region. Same ancestry relationship as varient C_2', C_2^{1}' & C_2^{1}'' revealed with AVPR2. Varient C has common ancestry with varient A & B. Major insertion and deletion coding in region AVPR2, showed different pattern of

varient from A & B. Same as AQP2, insertion and deletion term used in relation to sequence of candidate gene AVPR2 for further homology study.

Conclusion

On the behalf of the results obtained by this study, it can be concluded that both the cladograms showed similar type of evolutionary trend revealed that such genes evolved in a same manner. It means further phylogenetic studies of various genes having common or similar type of function or playing role to accomplish a physiological process might have common ancestoral origin.

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