Bioinformatics analysis and construction of eukaryotic expression plasmid of Cx50 V64G mutation

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Received: 2008-10-28 Accepted: 2008-12-15

Abstract
• AIM: To construct and analyze eukaryotic expression plasmid inserted by Cx50 with V64G mutation through bioinformatics software.
• METHODS: The full coding domain sequence of Cx50 with V64G mutation was acquired from the blood of patients with cataract and was cloned into pcDNA3.1/Amp (+). The constructed plasmid was identified with PCR, enzyme digestion and sequencing. The analysis of Cx50 with V64G mutation was performed with bioinformatics software.
• RESULTS: Cx50 with V64G mutation was successfully amplified and its eukaryotic expression plasmid was constructed. Valine-64 is well conserved in the first extracellular loop of connexin 50 in different species and also in different human α-type gap junctional proteins.
• CONCLUSION: The successive reconstruction and verification of eukaryotic expression plasmid containing Cx50 with V64G mutation established the foundation for further studying the mechanism of cataract.
• KEYWORDS: Cx50; bioinformatics analysis; eukaryotic expression plasmid


INTRODUCTION

Congenital cataract, a clinically and genetically highly heterogeneous eye lens disorder, is one of the significant causes of visual impairment. In the world 20 million children under the age of 16 suffer from cataract and 1.4 million of them are blind[1]. Hereditary cataracts are most commonly inherited in an autosomal dominant manner and are phenotypically and genotype heterogeneous, showing considerable inter- and intrafamilial variability[2]. At least 35 loci have been linked with various forms of congenital and developmental cataracts, and mutations in at least 15 genes have been identified for isolated congenital cataracts [3]. These genes encode crystallins, gap junction proteins, the major intrinsic protein (MIP) of lens fiber and cytoskeletal proteins [4]. Gap junctions are membrane specializations containing clusters of channels that allow intercellular passage of ions, metabolites, and secondary messengers up to 1 kDa [5]. The channels that make up a junction are formed from the contribution of one hemichannel or connexon from each apposed cell surface. Hemichannels are themselves oligomers formed of six subunit proteins of largely a-helical structure called connexins.

Connexin 50 encoded by GIAS located on chromosome 1q21.1 is comprised of two exons, the coding sequence being encompassed entirely by the second exon (NM-005267). It is an integral membrane protein containing four transmembrane domains, two extracellular loops, and an intracellular loop with both the amino and carboxyl termini located in the cytoplasm. In the former study [6], we identified a five generation family having bilateral nuclear congenital cataract. Upon sequencing analysis of GIAS we identified a heterozygous mutation T>G at position 191 (c.191 T>G) resulting in the transition of valine to glycine at codon 64 (V64G). The change cosegregated completely with the disease phenotype, thus suggesting this as the causative mutation in the present family. This is a novel mutation which hasn't been reported previously with congenital cataract.

MATERIALS AND METHODS

Bioinformatics Analysis of Connexin50 V64G Mutation
Using the program of the Clustal et al. [7], we know the degree of the conservation of valine in the first extracellular loop of connexin 50 in different species (Figure 1) and also in different human α-type gap junctional proteins (Figure 1). Secondary structure predictions of the altered protein were analyzed using the Bioinformatics tool of the DeepView/ Swiss-PdbViewer3.7.

Cloning of Mutant Human Cx50 DNA
Mutant human
**RESULTS**

**Bioinformatics Analysis of Connexin50 V64G Mutation**

Using the program of Clustal et al, we found that Valine-64 was well conserved in the first extracellular loop of connexin50 in different species (Figure 1) and also in human α-type gap junctional proteins (Figure 1). Using bioinformatic tool of DeepView/Swiss-PdbViewer3.7, we found there were changes of α-helix, H-bond and the energy (Figure 2) in the altered protein, which might be the pathogenesis. The transmembrane domains of the connexins are essential for the correct transport of the protein into the plasma membrane. It has been identified that pore lining residues lie in the first transmembrane domain and are essential for the formation of the pore and therefore channel permeability.

**Cloning of Mutant Human Cx50 DNA**

The full-length sequence of Cx50 was amplified by PCR (Figure 3). The sequencing data indicated that the construction of expression plasmid with T>G at position 191 was correct (Figure 4).
Cx43 (GJA1), and Cx50 (GJA8) have been associated with certain types of deafness, skin disease, peripheral neuropathy, heart defects, and cataracts.

The former study described the identification of a novel V64G substitution in Cx50 segregating solely in 7 affected members of a five generation family, having nuclear cataract with Y-sutural opacities. The observed V64G substitution lies within the first extracellular loop of Cx50 and represents a non-conservative amino acid change as valine is a polar amino acid while glycine is a nonpolar amino acid. The transmembrane domains of the connexins are proposed to participate in the oligomerization into connexin hemichannels and are also essential for the correct transport of the protein into the plasma membrane. It has been identified that pore lining residues lie in the first transmembrane domain and are essential for the formation of the pore and therefore channel permeability. The V64G mutation may influence the correct transport of proteins into the plasma membrane. Valine-64 is well conserved in the first extracellular loop of connexin50 in different species and also in different human α-type gap junctional proteins. So far, at least eight congenital cataract families have been linked with Cx50 and significant inter-familial phenotypic variability has been observed. It seems that in cataract, one major gene is involved but variants in other genes, involved in lens development, growth, and maintenance. The variants might cause phenotypic variability.

The successive reconstruction and verifying of eukaryotic expression plasmid containing Cx50 V64G gene established the platform to further pursue the mechanisms of Cx50 gene mutation leading to cataract.

Acknowledgements: We gratefully acknowledge the numerous sample donors for making this work possible.

REFERENCES