POST TRANSCRIPTIONAL GENE SILENCING FOR TREATING MEESMANN EPITHELIAL CORNEAL DYSTROPHY: AN IN SILICO APPROACH

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ABSTRACT: Meesmann epithelial corneal dystrophy (MECD) is a disease that affects the cornea of the eye. It is caused due to a heterozygous missense mutation in KRT12 gene that encodes Keratin K12 protein, which is a major intermediate filament protein present in epithelial cells. MECD is characterized by the formation of tiny round cysts in the corneal epithelium that gradually results in loss of visual acuity. Due to absence of any specific therapeutic intervention of MECD, the present study was undertaken to prevent expression and accumulation of mutant KRT12 using a PTGS (Post transcriptional gene silencing) approach. The dysfunction of KRT12 gene is due to a missense mutation that converts Arg to Thr at position 135 that was verified from the OMIM database. Using various in silico online tools, siRNAs were designed to target KRT12 to effectively silence the mutant gene so as to prevent accumulation of altered protein that results in cornification and keratinisation in the cornea thereby alleviate the pathophysiology of MECD.

KEYWORDS: Gene silencing, in-silico, mutation, Meesmann epithelial corneal dystrophy, computational biology.

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1. INTRODUCTION

The advancement in "omics" has resulted from generation of a large amount of molecular data and systems biology aims to condense and analyze this data to draw meaningful perceptions from it. Through pragmatic computational modelling and theoretical exploration, a robust foundation to formulate and solve crucial biological issues is possible. A number of disorders result from missense mutations. Missense mutations are usually benign while nonsense and frameshift mutations, that
cause premature termination of protein production, have been reported to be pathogenic. The discovery and interpretation of single nucleotide variants (SNVs) is cumbersome, but at the same time results in novel discoveries [1, 2]. In recent years there has been a surge in discovery of variants due to genetic testing and development of *in silico* programs to classify unknown variances into pathogenic or neutral [1, 3]. Missense mutations are a type of non-synonymous substitutions found in DNA and have been reported to cause human diseases such as epidermolysis bullosa, sickle-cell disease, and SOD1 mediated Amyotrophic lateral sclerosis (ALS) since they result in the formation of non-functional proteins [1, 4]. One such autosomal dominant eye disorder that affects the cornea is Meesmann epithelial corneal dystrophy (MECD), characterized by the formation of tiny round cysts in corneal epithelium [5, 6]. Genetic disorders characterized by mutations in specific genes can be therapeutically targeted by siRNA molecule based therapy which has been successfully used to target a number of mutant genes such as *SOD1* associated with ALS [7], viral gene expression in cancerous cells, knockdown of host receptors and co receptors for HIV [8], silencing of hepatitis A [9], hepatitis B genes [10], influenza gene expression [11], and measles viral replication [12]. Post Transcriptional Gene Silencing (PTGS) which has been used for effective and specific inhibition of disease causing genes is based on a natural process used to degrade mRNA transcribed from a specific gene, thereby preventing expression of gene and synthesis of corresponding altered/mutant protein [13]. ‘Genetic medicines’ have been developed for the treatment of nearly 1,800 known monogenic hereditary disorders [12]. PTGS has also been used to treat asthma and metabolic diseases such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis [14]. It has also been reported that bacterial diseases can be prevented by targeting the host genes involved in the immune response caused by infection or those that mediate entry of bacteria into host cells [15-16]. Meesmann corneal dystrophy is a rare disorder whose prevalence is unknown, but is found mainly in population containing MECD. MECD has been recognized in Denmark, Germany, Japan, USA, Saudi Arabia and Poland [5]. The genetic basis of MECD is a negative mutation from Arg135Thr in *KRT12* genes encoding keratins K12 [6]. Keratins are localised in the cytoplasm and form flexible cytoskeleton scaffold of the epithelial cells. They are components of intermediate filaments along with microfilaments and microtubules [17]. The gene *KRT12* is expressed in anterior corneal epithelium’s keratinocyte cells and mutations cause the corneal epithelium to become fragile. These genes encoding human keratins are located in two compact, gene-dense clusters on chromosomes 17q12-q21 (type I keratins) and 12q11-q13 (type II keratins) [5-7]. The clinical presentation of MECD can vary from mild to recurrent epithelial erosions that lead to lacrimation, photophobia and deterioration in visual acuity. The standard method for diagnosis is slit-lamp examination that typically shows changes in cornea seen as surface irregularities and epithelial cysts, which are located most commonly in the interpalpebral zone. Current treatment aims at palliative relief and prevention of deterioration in visual acuity [5, 18], since no specific therapy/treatment for MECD.
is available. In the absence of any therapy/commercially available drug for treatment of MECD, the present study was undertaken to target \textit{KRT12} gene using post transcriptional gene silencing by designing siRNA to knock down the mutant gene.

\section*{2. MATERIALS AND METHODS}

\subsection*{2.1 Sequence Retrieval}

The cDNA sequence for \textit{KRT12} gene of \textit{Homo sapiens} was retrieved from NCBI (Accession No: - NM\_000223.3) and converted into corresponding mRNA sequence.

\subsection*{2.2 Mutation Analysis}

The variations reported to cause MECD were collected from the database OMIM \cite{19}.

\subsection*{2.3 Design of siRNA}

Whitehead siRNA Imbrute was used to design probable siRNA to target \textit{KRT12} gene. It is an online tool that uses various rules to design effective siRNAs such as thermodynamic stability of the double stranded RNA duplex, GC content and presence of SNPs \cite{20}.

\subsection*{2.4 Self-Complimentary analysis of antisense strands}

\textbf{RNAfold tool}

Secondary structures can be predicted from a single sequence or the consensus for a set of aligned sequences using Vienna RNA secondary structure server. The server predicts minimum free energy (mfe) structures of a single sequence \textit{via} classic algorithm of Zuker and Stiegler\cite{21} and the centroid structure \cite{22}.

\section*{3. RESULTS AND DISCUSSION}

Meesman epithelial corneal dystrophy results from mutations in \textit{KRT12} that are heterozygous, missense and dominant negative mutation that arise \textit{de novo}. Cysts formation is prominent feature in MECD which is formed due to accumulation of mutant protein KRT12 \cite{23}. To identify the validated variations known to cause MECD, OMIM database was explored. From the list of hits, gene entry for \textit{Homo sapiens} \textit{KRT12} having cytogenetic location 17q21.2 and the genomic coordinates 17:40,861,177-40,867,209 was selected \cite{24}. It’s most common allelic variant Arg 135 to Thr \cite{25} was selected for design of siRNA using Whitehead siRNA web server. Nineteen constructs were obtained out of which ten constructs (Table: 1) were selected that had a favourable GC content. As per siRNA design guidelines if GC content of one strand is greater than 60\% it cannot become a functional siRNA because bond between guanine and cytosine are much stronger compared to A-U or A-T double bonds and each strand of siRNA can have tendency to form loops by itself \cite{1}. All these ten siRNA constructs were then analysed using RNAfold tool to predict their secondary structures and mfe energy in terms of mfe. Constructs C2, C3, C6 and C8 were rejected as they form self complimentary structures (Table: 2) which may prevent the siRNA construct from binding to their target mRNA and function as effective gene silencers.
Table 1: siRNA constructs obtained from online tool Whitehead siRNA

<table>
<thead>
<tr>
<th>Construct</th>
<th>Start position</th>
<th>siRNA Sequence</th>
<th>GC%</th>
<th>Free Energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1.</td>
<td>106</td>
<td>GGCATACTTCACATTAT</td>
<td>32.0</td>
<td>-0.03</td>
</tr>
<tr>
<td>C2.</td>
<td>202</td>
<td>CGGTTACCAGAAGAAAGT</td>
<td>47.0</td>
<td>-1.74</td>
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<tr>
<td>C3.</td>
<td>238</td>
<td>CAGGTCAGAAAGGATATAA</td>
<td>42.0</td>
<td>-3.64</td>
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<tr>
<td>C4.</td>
<td>69</td>
<td>CTTCCTCTTGCTTCTTGTA</td>
<td>42.0</td>
<td>-0.06</td>
</tr>
<tr>
<td>C5.</td>
<td>621</td>
<td>GTCAACAAATAAATTTTCTC</td>
<td>32.0</td>
<td>-0.02</td>
</tr>
<tr>
<td>C6.</td>
<td>403</td>
<td>GTTACTCTGAAAGGAATAA</td>
<td>32.0</td>
<td>-0.98</td>
</tr>
<tr>
<td>C7.</td>
<td>856</td>
<td>CCTCCAGGGATTTCTTCAT</td>
<td>47.0</td>
<td>-1.21</td>
</tr>
<tr>
<td>C8.</td>
<td>972</td>
<td>CTAACTCTCTTACGGAGCT</td>
<td>47.0</td>
<td>-4.13</td>
</tr>
<tr>
<td>C9.</td>
<td>1063</td>
<td>GCATATCTTGAGGAGCCT</td>
<td>47.0</td>
<td>-0.55</td>
</tr>
<tr>
<td>C10.</td>
<td>1390</td>
<td>CTGAAATGCTTATTTCTC</td>
<td>32.0</td>
<td>-0.23</td>
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The siRNA that do not form secondary structures (internal hairpin and stem loops) can have effective gene silencing roles. Therefore, siRNA construct C1, C4, C5, C7, C9 and C10 were good candidates based on their secondary structures. Lack of internal secondary structures together with a highly negative mfe is indicative of high specificity that are characteristics features of siRNA for effective PTGS. Constructs C7, C9 and C10 with binding positions 856, 1063 and 1390 on KRT12 respectively could be favourable siRNA constructs. But based on binding energies, C7 was found to be the best construct having highest negative free energy of -1.21 kcal/mol with start site binding position of 856 in the KRT12 gene.

Table 2: siRNA secondary structures obtained from RNAfold server

<table>
<thead>
<tr>
<th>No.</th>
<th>Possible secondary structures (mfe)</th>
<th>Possible secondary structures (css)</th>
<th>Position</th>
<th>Free energy (kcal/mol)</th>
</tr>
</thead>
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<tr>
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<td>GGCATACTTCACATTAT</td>
<td></td>
<td>106</td>
<td>-0.03</td>
</tr>
<tr>
<td>C2.</td>
<td>CGGTTACCAGAAGAAAGT</td>
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<td></td>
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C3. CAGGTCCAGAAGGATATAA

C4. CTTCTCTCTATGCTCTTG

C5. GTCAAAATAAACTTTCT

C6. GTTACTCTGAAAGGAATAA

C7. CCTCCAGGGATTTCTTCAT
4. CONCLUSION
In the present study, a computational biology approach has been used to target mutant KRT12 gene with missense mutation of Arg 135 to Thr that has been implicated as a major cause of MECD. A computational biology *in silico* was used to design siRNA were designed to prevent synthesis of mutant protein by targeting the mutant gene using PTGS to prevent formation of cysts in the cornea. Three siRNA constructs designed in the present study *i.e.* C7, C9 and C10 may be further tested for their efficacy in silencing PTGS of mutant KRT12 to therapeutically alleviate symptoms of MECD by preventing the synthesis of mutant cyto-skeletal protein in the cornea.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST

There is no conflict of interest related to this study.

REFERENCES


