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POST TRANSCRIPTIONAL GENE SILENCING FOR TREATING MEESMANN EPITHILIAL 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 epilthelaial 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 *KRT12* gene using post transcriptional gene silencing by designing siRNA to knock down the mutant gene.

2. MATERIALS AND METHODS

2.1 Sequence Retrieval

The cDNA sequence for *KRT12* gene of *Homo sapiens* was retrieved from NCBI (Accession No: - NM_000223.3) and converted into corresponding mRNA sequence.

2.2 Mutation Analysis

The variations reported to cause MECD were collected from the database OMIM [19].

2.3 Design of siRNA

Whitehead siRNA Imbrute was used to design probable siRNA to target *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 [20].

2.4 Self-Complimentary analysis of antisense strands

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 *via* classic algorithm of Zuker and Stiegler[21] and the centroid structure [22].
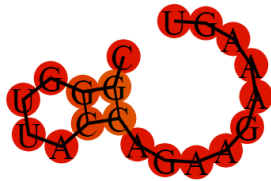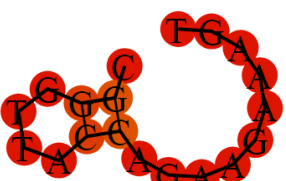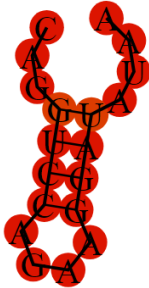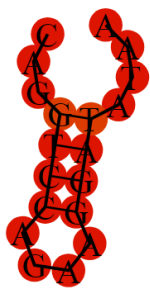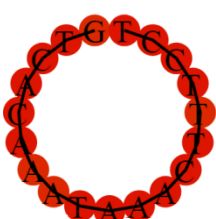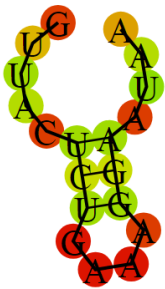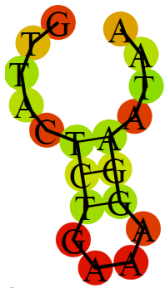
3. RESULTS AND DISCUSSION
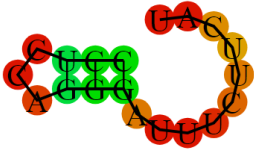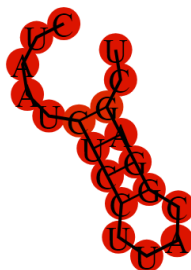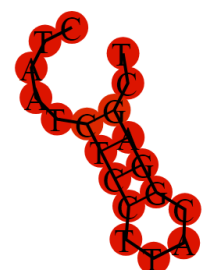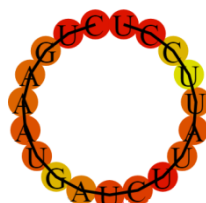
Meesman epithelial corneal dystrophy results from mutations in *KRT12* that are heterozygous, missense and dominant negative mutation that arise *de novo*. Cysts formation is prominent feature in MECD which is formed due to accumulation of mutant protein KRT12 [23].To identify the validated variations known to cause MECD, OMIM database was explored. From the list of hits, gene entry for *Homo sapiens KRT12* having cytogenetic location 17q21.2 and the genomic coordinates 17:40,861,177-40,867,209 was selected [24]. It's most common allelic variant Arg 135 to Thr [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 [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				
Construct	Start position	siRNA Sequence	GC%	Free Energy (kcal/mol)
C1.	106	GGCATACATTTCACATTAT	32.0	-0.03
C2.	202	CGGGTTACCAGAAGAAAGT	47.0	-1.74
C3.	238	CAGGTCCAGAAGGATATAA	42.0	-3.64
C4.	69	CTTCTCTCTATGCTCTTGA	42.0	-0.06
C5.	621	GTCACAAATAAACTTTCCT	32.0	-0.02
C6.	403	GTTACTCTGAAAGGAATAA	32.0	-0.98
C7.	856	CCTCCAGGGATTTCTTCAT	47.0	-1.21
C8.	972	CTAATCTCCTTACGGAGCT	47.0	-4.13
C9.	1063	GCATATCATTGAGGAGCCT	47.0	-0.55
C10.	1390	CTGAAATGATCTTATTCCT	32.0	-0.23

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				
No.	Possible secondary structures (mfe)	Possible secondary structures (css)	Position	Free energy (kcal/mol)
C1.	**GGCATACATTTCACATTAT**			
			106	-0.03
C2.	**CGGGTTACCAGAAGAAAGT**			

			202	-1.74
C3.	**CAGGTCCAGAAGGATATAA**			
			238	-3.68
C4.	**CTTCTCTCTATGCTCTTGA**			
			69	-0.06
C5.	**GTCACAAATAAACTTTCCT**			
			621	-0.02
C6.	**GTTACTCTGAAAGGAATAA**			
			403	-0.98
C7.	**CCTCCAGGGATTTCTTCAT**			

		856	-1.21
C8.	**CTAATCTCCTTACGGAGCT**		
		972	-4.13
C9.	**GCATATCATTGAGGAGCCT**		
		1063	-0.55
C10.	**CTGAAATGATCTTATTCCT**		
		1390	-0.23

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.

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CONFLICT OF INTEREST

There is no conflict of interest related to this study.

REFERENCES

1. Sahrawat TR, Taneja S, Kaur G. Computational Biology Approach for Therapeutic Intervention of Alexander Disease by Post Transcriptional Gene Silencing. International Journal for Computational Biology. 2017; 6(1): 18-24.

2. Leong IU, Stuckey A, Lai D, Skinner JR, Love DR. Assessment of the predictive accuracy of five in silico prediction tools, alone or in combination, and two metaservers to classify long QT syndrome gene mutations. BMC medical genetics. 2015; 16(1):34.

3. Tavtigian SV, Greenblatt MS, Lesueur F, Byrnes GB, IARC Unclassified Genetic Variants Working Group. In silico analysis of missense substitutions using sequence☐alignment based methods. Human mutation. 2008;29(11):1327-36.

4. Boillée S, Velde CV, Cleveland DW. ALS: a disease of motor neurons and their nonneuronal neighbors. Neuron. 2006; 52(1):39-59.

5. Cao W, Yan M, Hao Q, Wang S, Wu L, Liu Q, Li M, Biddle FG, Wu W. Autosomal-dominant Meesmann epithelial corneal dystrophy without an exon mutation in the keratin-3 or keratin-12 gene in a Chinese family. Journal of International Medical Research. 2013; 41(2):511-8.

6. Irvine AD, Coleman CM, Moore JE, Swensson O, Morgan SJ, McCarthy JH, Smith FJ, Black GC, McLean WH. A novel mutation in KRT12 associated with Meesmann's epithelial corneal dystrophy. British journal of ophthalmology. 2002; 86(7):729-32.

7. Geng CM, ding HL. Double-mismatched siRNAs enhance selective gene silencing of a mutant ALS-causing allele. ActaPharmacologicaSinica. 2008; 29(2):211-6.

8. Martínez MA, Gutiérrez A, Armand-Ugón M, Blanco J, Parera M, Gómez J, Clotet B, Esté JA. Suppression of chemokine receptor expression by RNA interference allows for inhibition of HIV-1 replication. AIDS. 2002; 16(18):2385-90.

9. Kusov Y, Kanda T, Palmenberg A, Sgro JY, Gauss-Müller V. Silencing of hepatitis A virus infection by small interfering RNAs. Journal of virology. 2006; 80(11):5599-610.

10. Jia F, Zhang YZ, Liu CM. A retrovirus-based system to stably silence hepatitis B virus genes by RNA interference. Biotechnology letters. 2006; 28(20):1679-85.

11. Yao-Chen L, Ling-Hong K, Bi-Zhen C, Kang-Sheng L. Construction of influenza virus siRNA expression vectors and their inhibitory effects on multiplication of influenza virus. Avian diseases. 2005; 49(4):562-73.

12. O'Connor TP, Crystal RG. Genetic medicines: treatment strategies for hereditary disorders. Nature Reviews Genetics. 2006; 7(4):261.

13. Yang D, Goga A, Bishop JM. RNA interference (RNAi) with RNase III-prepared siRNAs. Methods Mol. Biol.2004; 252, 471-482.

14. Leung RK, Whittaker PA. RNA interference: from gene silencing to gene-specific therapeutics. Pharmacology & therapeutics. 2005; 107(2):222-39.

15. Lieberman J, Song E, Lee SK, Shankar P. Interfering with disease: opportunities and roadblocks to harnessing RNA interference. Trends in molecular medicine. 2003; 9(9):397-403.

16. Zaas DW, Duncan MJ, Li G, Wright JR, Abraham SN. Pseudomonas invasion of type I pneumocytes is dependent on the expression and phosphorylation of caveolin-2. Journal of Biological Chemistry. 2005; 280(6):4864-72.

17. Allen EH, Courtney DG, Atkinson SD, Moore JE, Mairs L, Poulsen ET, Schiroli D, Maurizi E, Cole C, Hickerson RP, James J. Keratin 12 missense mutation induces the unfolded protein response and apoptosis in Meesmann epithelial corneal dystrophy. Human molecular genetics. 2016; 25(6):1176-91.

18. Corden LD, Swensson O, Swensson B, Smith FJ, Rochels R, Uitto J, McLean WI. Molecular genetics of Meesmann's corneal dystrophy: ancestral and novel mutations in keratin 12 (K12) and complete sequence of the human KRT12 gene. Experimental eye research. 2000; 70(1):41-9.

19. Szaflik JP, Ołdak M, Maksym RB, Kamińska A, Pollak A, Udziela M, Płoski R, Szaflik J. Genetics of Meesmann corneal dystrophy: a novel mutation in the keratin 3 gene in an asymptomatic family suggests genotype-phenotype correlation. Molecular vision. 2008; 14:1713.

20. Yuan B, Latek R, Hossbach M, Tuschl T, Lewitter F. siRNA Selection Server: an automated siRNA oligonucleotide prediction server. Nucleic acids research. 2004; 32(2):130-4.

21. Gruber AR, Lorenz R, Bernhart SH, Neuböck R, Hofacker IL. The vienna RNA websuite. Nucleic acids research. 2008; 36(2):70-4.

22. Ding YE, Chan CY, Lawrence CE. RNA secondary structure prediction by centroids in a Boltzmann weighted ensemble. RNA. 2005; 11(8):1157-66.

23. Ehlers N, Hjortdal J, Nielsen K, Thiel HJ, Orntoft T. Phenotypic variability in Meesmann's dystrophy: clinical review of the literature and presentation of a family genetically identical to the original family. Acta Ophthalmologica. 2008; 86(1):40-4.

24. Bongcam-Rudloff E, Nistér M, Betsholtz C, Wang JL, Stenman G, Huebner K, Croce CM, Westermark B. Human glial fibrillary acidic protein: complementary DNA cloning, chromosome localization, and messenger RNA expression in human glioma cell lines of various phenotypes. Cancer Research. 1991; 51(5):1553-60.

25. Hassan H, Thaung C, Ebenezer ND, Larkin G, Hardcastle AJ, Tuft SJ. Severe Meesmann's epithelial corneal dystrophy phenotype due to a missense mutation in the helix-initiation motif of keratin 12. Eye (Lond). 2013; 27(3):367.