PharmacologyOnLine

Archives • 2021 • vol.2 • 1070-1086

# MUTATIONS OF SLC4A11 IN CONGENITAL HEREDITARY ENDOTHELIAL DYSTROPHY : A SYSTEMATIC REVIEW

D Chaitra[1*] Adiga Usha*; Adiga Sachidananda [*], Pai Divya [°] Srinivas SP[**]
*°NITTE (Deemed to be University), Deralakatte, Mangalore, Karnataka, India
**Optometry, Indiana University, Bloomington, IN; USA

*divyas@nitte.edu.in

## Abstract

### Background

Mutations in the gene SLC4A11, which encode for an isoform of the solute carrier 4 (SLC4) family of proteins, cause congenital hereditary endothelial dystrophy (CHED). Here, we provide a systematic review of the mutations in SLC4A11 and also evaluate the association of its alleles with CHED.

### Method

Two investigators conducted the literature search and data extraction independently from November 2020 to January 2021 using SLC4A11 and congenital hereditary endothelial dystrophy as keywords. Out of twelve original articles included in the review, six were chosen for a meta-analysis. Six alleles of SLC4A11 were analyzed to determine their association with CHED. In the selected studies, families with diagnosed CHED cases were screened for the gene mutations. Random effect models were used to calculate the odds ratio (OR) and confidence interval (CI). Heterogeneity and publication bias were studied using Forest and Funnel plots, respectively. The heterogeneity assumption was checked by $\chi^2$-based Q test.

### Results

Mutations in the different alleles of SLC4A11 were found to be associated with CHED. However, mutations in alleles c.2264G>A, c.2528 T>C, and c. 2263C>T could not conclusively be associated with CHED because of heterogeneity and inconsistencies in the studies. Nonetheless, a positive association was observed for mutations in three alleles, c.2605C>T, c.1156T>T, and c.22440 1 G>A and CHED, with all three squares and two diamonds being on the left side of the "line of no effect" in the Forest plot.

### Conclusion

CHED patients exhibit several point (missense and nonsense) and frameshift mutations of SLC4A11. A meta-analysis of six alleles suggests a positive association of mutations in three alleles, c.2605C>T, c.1156T>T, and c.22440 1 G>A alleles of SLC4A11 with CHED.

**Keywords:** *CHED, SLC4A11, Mutations, Cornea*

## Introduction

The cornea is the main refractive element of the eye. Hence, its transparency is critical for acute vision. It consists of five layers: viz. epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium (1, 2). The anterior epithelium, 50 µm in thickness, is stratified and forms a barrier to the entry of solutes, harmful substances, and water from the tear film into the stroma. (3) The Bowman's membrane, 15 µm in thickness, lies between the basal lamina of epithelium and stroma. It is made up of I, III, V, and VII collagens (4). The stroma, which is ~ 450 µm, forms the bulk of the cornea (5). It consists of several lamellae (200 -250 lamellae) of collagen fibrils (Collagen I and V) held together by proteoglycans (chondroitin sulfate and keratin sulfate), which are responsible for corneal hydration. A precise arrangement is attributed to corneal transparency (**). Thus, when stromal deturgescence (i.e., state of dehydration of the stroma) is adversely affected, the corneal transparency is lost. The Descemet's membrane is comprised of extracellular matrix proteins secreted by the corneal endothelium (8). It provides structural support to the endothelium (9). Finally, the endothelium is a monolayer resting with a characteristic hexagonal mosaic and is non-regenerative in humans. The endothelial cell density (ECD) and morphology change throughout life. In particular, ECD declines gradually between 20-80 years of age at a constant rate of 0.52% per year (10, 11). Moreover, the corneal endothelial dystrophies lead to a decrease in ECD, disruption of the endothelial monolayer, and loss of endothelial functions. Since the endothelium is vital for maintaining stromal deturgescence, the endothelial dystrophies result in stromal edema, leading to a decline in visual acuity (13). This review focuses on congenital hereditary endothelial dystrophy (CHED), the main form of endothelial dystrophy.

CHED manifests in two forms: viz. slowly progressive autosomal dominant CHED1 and non-progressive autosomal recessive CHED2. CHED1, which appears within the first two years of age, is characterized by defective vision (without nystagmus), epiphora, and photophobia (16). CHED2 is noticed at birth or in the neonatal period, and it is characterized by impaired vision, nystagmus, and a hearing deficiency (14). CHED2 is generally more severe than CHED1. Patients diagnosed with CHED2 do not complain of epiphora or photophobia. The CHED corneas show a characteristic ground glass-like corneal appearance concomitant with extensive stromal edema. Given its role in the maintenance of stromal deturgescence, the endothelium is disrupted in CHED. Not ECD only is very low at birth, but the endothelial cells show a fibrotic morphology. Descemet's membrane is also relatively thick in CHED (19). With disturbance to vision, CHED patients develop varying degrees of amblyopia (18).

Mutations in the SLC4A11 gene are attributed to the pathophysiology of CHED (20). In particular, CHED patients may show any of the 80 mutations in 17 of the 18 exons of SLC4A11 (27). SLC4A11 gene encodes for a membrane protein that belongs to the solute carrier 4 (SLC4) protein family. These are integral membrane proteins associated with Cl-/HCO3- exchange or Na+ coupled HCO3- cotransport across the plasma membrane. In particular, SLC4A11 functions as an electrogenic Na+ coupled borate cotransporter. It is expressed in the corneal endothelium, cerebral cortex, epididymis, gall bladder, small intestines, testis, kidneys, breast, cerebellum, lungs, ovaries, pancreas, and stomach. In humans, depending on the mRNA splicing, SLC4A11 shows three variants: (a) SLC4A11 variant 1(A; 918 amino acids), (b) SLC4A11 variant 2(B; 891 amino acids), and (c) SLC4A11 variant 3(C; 875 amino acids). (22).

Recent studies have also claimed a role for SLC4A11 in a Na+ independent H+ permeation pathway in contradiction to Na+ coupled borate transport. SLC4A11-B is suggested to be a water channel instead of a transporter for borate or other ions. SLC4A11 is also thought to function as an EIPA inhibitable Na+ OH- cotransporter. In any case, the role of these different mechanisms remains unknown in the fluid pump activity of the corneal endothelium, although mutations are implicated in a loss of endothelial fluid pump function (24). Apart from anomalies resulting from loss of transport functions, mutations in SLC4A11 may induce cell biological effects indirectly. For example, some

missense mutations are believed to result in defective trafficking of the protein to the cell surface, leading to their ER retention (25)(26). Alternatively, mutations in SLC4A11 are thought to have an adverse impact during the growth and terminal differentiation of neural crest cells into the endothelial monolayer. Despite a clear understanding of the mechanisms of SLC4A11 on endothelium, it has been demonstrated that functional loss in SLC4A11 underlies endothelial cell death, loss of barrier function, and stroma edema (28).

The objective of this study is to review the role of mutations of the SLC4A11 in CHED. We also assess the association of the mutations of various alleles of the SLC4A11 gene with CHED.

## Materials and Methods

The present systemic review was carried out in January 2021 at our institution (K S Hegde Medical Academy, Nitte Deemed to be University) in Mangaluru, Karnataka, India.

Journal articles published on or before 2020 were included if they were quantitative, written in English, and discussed the role of the SLC4A11 gene causing congenital hereditary endothelial dystrophy (CHED). This is an updated search from the original date specified in the registered protocol; the eligibility criteria remained the same through all search terms. The focus of this review was CHED. Therefore, FECD, X-linked endothelial corneal dystrophy diseases, and their conditions were excluded.

Five online databases that were searched for identification of articles, PUBMED, DOAJ, GOOGLE SCHOLAR, Open Grey Greylit, and PROQUEST. Search terms were generated initially for PUBMED use and adapted for the other databases SLC4A11 gene-Mutations-CHED framework was used to develop these search terms mutations of the SLC4A11 gene SLC4A11 and CHED, Clinical condition of CHED.

Two investigators conducted search and data extraction independently from November 2020 to January 2021, with keywords' SLC4A11 AND congenital hereditary endothelial dystrophy. All the duplicate articles were removed. Articles were then screened for title, abstract, keywords, and publication year to identify the studies useful for the systematic review. The remaining systematic review articles were screened by reading the full text. A list of references was filtered (Figure 1).

In the selected articles, diagnosis of CHED was made based on characteristic mosaic corneal haze with corneal edema present since birth by history. It was confirmed by the complete clinical ophthalmic examination (slit lamp, intraocular pressure measurements, cyclopaedic retinoscopy, and indirect ophthalmoscope). A detailed history was recorded for all subjects, including the recording of any family history and duration of onset of symptoms. Peripheral blood samples were collected for DNA analysis. DNA amplification was done by PCR using suitable primers. Whole-genome sequencing was carried out for SLC4A11.

The statistical analysis was carried out using the software Medcalc. The association between the mutations of various alleles of SLC4A11 and CHED was assessed.

The association of occurrence of CHED and c.2264 G>A, c.2528 T>C, c.2263 C>T, c.2605C>T, c. 1156 T>C and c.2024+1G> alleles of SLC4A11 were assessed by pooled odds ratio (OR) with 95% Confidence Interval (CI). Random effect models were used to calculate OR and CI. Heterogeneity assumption was checked by $\chi$2-based Q test. A p-value more than 0.10 for the Q test indicated a lack of heterogeneity among the studies, and the summary OR estimate of each report was calculated by the random-effects model (Der Simonian and Laird method) as well as by fixed-effect model (Mantel-Haenszel method). In the absence of individual heterogeneity, all the points were expected to lie within.

The confidence bounds. Between-study heterogeneity was assessed using the Q test and inconsistency by I2. Publication bias was evaluated using a funnel plot and the standard error of log (OR). Statistical significance was defined as $p<0.05$.

## Results and Discussion

The relevant articles related to the search words obtained from each database were as follows: MEDLINE (17), DIRECTORY OF OPEN ACCESS

JOURNAL (4), and GOOGLE SCHOLAR (5), PROQUEST (3).

There were no relevant articles found in HIGH WIRE, INDIAN SCIENCE ABSTRACT, SCIENTIFIC INFORMATION DATA, SCIRUS, and SCOPUS. No other papers were identified following the citation searching. A total of 57 full-text articles were obtained that were screened for the title, abstract, and keywords. The number of duplicate reports was 17, and they were excluded from the study. The remaining 40 articles were screened for the title and abstract. Out of 27 articles, eight articles reviewed articles on the SLC4A11and CHED, and hence they were excluded. Nineteen original articles were screened. It was found that seven articles were on primary studies on the molecular basis of SLC 4A11, hence excluded. The remaining twelve articles were the original studies, case-control and cohort studies being three each, which met the inclusion/exclusion criteria for the final inclusion in the review. Out of 12, a meta-analysis of four articles on c.2264G>A, three articles on c.2528 G>an allele, four articles on c.2263C>T, three articles on c.2605C>T, three articles on c1156T>C, three articles on c.2240G>A were carried out.

The summary of the selection of the articles is depicted in figure 1. The papers with the same research objectives, a mutant form of SLC4A11, type of the study, results, and outcome of the study were the characteristics that were considered to select the articles as inclusion criteria for this review article. The methodology employed in various papers was, this factor was considered weak to be included in our study.

Studies may have heterogeneity due to patients of different ethnicity, outcome definition, and study design. The random-effect model and the fixed effect models were used to assess the association between the mutations of different alleles of SLC4A11 and CHED alleles.

While interpreting the forest plot, each study is represented by a square area directly proportional to the weight of the study. The diamond represents the overall weight of the study, the width of which represents 95% CI for the estimated OR.

Forest plot by random effect model [Fig 3] for the association of c.2264G>A of SLC4A11 and CHED showed that three studies represented by squares on the left side and both the diamonds for random and fixed effect models on the left of the line of no effect, suggested that this mutation of SLC4A11 may have an association with the occurrence of CHED. As the size of individual squares was directly proportional to the study's weight, all the three studies of significance were given equal and highest weight. However, Q test for heterogeneity was significant (p=0.0046) (table 2), suggesting the heterogeneity among the studies.I2 test was also suggestive of inconsistency. Hence the association of c.2264G>A of SLC4A11 with CHED is not conclusive.

The forest plot for the association of the allele c.2528T>C with CHED showed three squares on the left, one big square on the right, and both the diamonds on the left side of the line of no effect (fig 4). However, significant heterogeneity and inconsistencies were observed in the studies selected (table 2). So our analysis could not confirm the association of the mutations of the allele c.2528T>C and CHED.

An association of the allele c.2263 C>T and CHED mutations was analyzed using a forest plot, which showed three squares on the left, one big square on the right, and both the diamonds on the left of the line of no effect. Significant heterogeneity and inconsistencies were observed (table 2), which do no support the possible association between the allele and the disease.

Contrary to the above results, clear associations were established between the mutations of the different alleles of SLC4A11, c.2605C>T, c.1156T>T, and c.22440 1 G>A and CHED without any heterogeneity and inconsistency. A clear positive association was observed for all the three alleles and CHED mutations with all three squares and both the diamonds being on the left side of a line of no effect.

The funnel plots for publication bias for all the alleles were symmetrical, inverted funnel-shaped, boundaries being straight lines for all the alleles [fig 2]. This suggests that studies were symmetrically distributed in the plot, suggesting no publication bias (table 2).

Brejchova et al., carried out molecular genetics investigations in six probands on CHED in six European Czech and European British families. Eleven mutations of SLC4A11 were identified, of which c.1237G>A, c.2003T>C, c.1216þ1G>A, and c.2240þ5G>A were novel. The mutation of c.2240+5G>A variant resulted in aberrant pre-mRNA splicing. c.2240+1G>A mutation was also likely to cause aberrant pre-mRNA splicing because of their location in canonical splice sites (29).

Hand et al., carried out a study on members of a large Irish consanguineous CHED2 family and observed the mutations of the SLC4A11 gene. The study reported homozygous DNA sequence variant c.2528 T>C (p.Leu843Pro) in exon 18 in all eight affected individuals. This pathogenic change has previously been described in compound heterozygotes with Harboyan syndrome/CDPD in two distinct families (30).

Aldamesh et al., investigated ten CHED patients, reported seven mutations, out of which five were novel mutations and resulted in reduction or loss of bicarbonate transporter-related protein 1(BTR-1). The homozygous missense mutation p.Gly394Arg was caused by a c.1228G_C transversion in a single proband; a missense mutation (c.1253G_A, p.Gly418Asp) was identified and is predicted to disrupt TMD 2.11 a splice-site mutation (c.2114_1G_A) was also identified in all affected members of the family (15).

Tannanuvat et al., studied eight patients from seven unrelated families of the Karen population affected by harbor a syndrome reported that patients with SLC4A11 mutations had CHED and sensorineural hearing loss SLC4A11 mutations were not related to the onset and severity of hearing loss or outcome of keratoplasty. The homozygous base substitution c.2264 G>A (rs121909387) was observed. A single-base substitution was predicted to cause amino acid change p.Arg755Gln. The single-base deletion c.2127delG was novel, which caused a frameshift mutation p.Leu710Cysfs.The homozygous single-base substitution c.2263 C>T (rs757553189) was found only in a Thai patient, and the single-base substitution was predicted to cause amino acid change p.Arg755Trp. The c.2264G>A (p.Arg755Gln) mutation in SCL4A11 was detected in unrelated Karen tribe patients. (31).

In a study done by Romero et al., on 3 Chilean families, five patients were reported homozygous for the mutation in the SLC4A11 gene. The sequence of the 19 exons of the SLC4A11 gene of all the affected patients showed homozygous eight nucleotide sequence duplication (c.2233_2240dup TATGACAC, p. (Ile748Metfs*5) at the end of exon 16(27).

Seven novel mutations in SLC4A11 were reported by the study by Ram Prasad et al. These mutations included two nonsense, (p.Trp240 X), (p.Gln800 X), three missense (p Glu143 Lys, p.Cys386 Argo, p.Arg755 Tarp), and two splice-site mutations (c.2240 + IG>A; c.2437-IG>A). c.1156T>C mutation caused missense mutation by substituting cysteine by arginine at codon 386.Transition mutation in c.2605C>T led to the substitution of arginine by cysteine at position 869, a mutation in c.2263C>T that leads to a substitution of arginine by tryptophan at position 755 (p.Arg755Trp).

And a mutation in c.2264G>A resulted in the substitution of arginine by glutamine at the same position of the protein (p.Arg755Gln) (32).

Desir et al., studied seven families of eastern Europe, Dominican Republic, Morocco, south American Indian, Netherlands, Sephardi Jewish, and India reported mutations of SLC4A11 in CDPD (Harboyan syndrome), showed mutations of the SLC4A11.According to the study results, missense mutations (p.Arg488Lys and p.Val824Met), a 4 bp deletion/1 bp insertion (c.1378_1381delTACGinsA) and an 8 bop deletion (c.473_480delGCTTCGCC) resulted in a truncated protein of 160 residues. Mutations in the non-consanguineous families were an eight bp duplication (c.2233_2240dup TATGACAC), resulting in an aberrantly truncated protein of 751 residues; a 32 bp deletion (c.2423_2454del) resulting in an aberrantly truncated protein of 916 residues (26).

A study done by Puangsricharen observed 68kb deletion flanked by repeat sequences which may lead to aberrant recombination resulting in loss of intervening sequence in CHED patients. These microdeletions could be the disease-causing alleles responsible for CHED2. The study also identified a novel c.778A>G mutation resulting in lysine to

glutamic acid substitution (p.K260E) at codon 260 (34).

Jiao et al., suggested that loss of SLC4A11 results in loss of sodium and bicarbonate transport resulting in edema. It may also cause abnormality in cell division by virtue of its function as a borate transporter. These functions were constant with a clinical phenotype of CHED2. Nonsense mutations observed were p.Arg605X (g.8298CRT, families 73015 and 73026), and p.Glu632X (g.8379GRT, family 73035). Three frameshift mutations (p.ArgR82ArgfsX33 (g.2943delTTinsA, family 73024), p.His568HisfsX177 (g.8118delCT, family 73013) and p.Leu807ArgfsX71 (g.9200delTinsGG, family 73004)) resulted in the loss of a large number of amino acids from the carboxy terminus of the protein and would also replace these sequences with long novel amino acid sequences encoded by the shifted reading frame (35).

Mehta et al., in his study reported a homozygous sequence variation (c.2264G.A) in exon 17 caused the amino acid substitution R755Q, sequence variation c.1813C.T caused a premature stop at codon 605 (p.R605X). 4-bp deletion (c.353_356delAGAA) resulted in an aberrant truncated protein of 128 residues. A c.2605C.T transition lead to the p.R869C amino acid substitution was observed (36).

Four novel mutations of the SLC4A11 gene (p.Thr262Ile, p.Gly417Arg, p.Cys611Arg, and p.His724Asp) were identified in a study by Kodaganur et al., causing CHED. Most of these mutations were caused by missense followed by insertion and deletions (38).

Hemadevi et al., screened 20 families with CHED2 and revealed 10 mutations. Seven were missense, one was nonsense and two were frameshifts. CHED2 displayed allele (perhaps locus) heterogeneity, which was explored using extensive linkage analysis. They identified, missense mutations, (p.Leu873Pro) owing to the transition of T_C at c.2618, p.Arg125His (c.374G_A), p.Ala160Thr (c.478G_A), p.Cys386Arg (c.1156T_C), and p.Arg755Trp (c.2263C_T), p.Ala269Val (c.806C_T) was found in two families. It was observed that c.2318C_T in exon 17 (leading to p.Pro773Leu) was a premature stop mutation (14).

An Indian study by Paliwal et al., studied the mutation spectrum and genotype-phenotype correlation in CHED2 patients. The study detected one novel, and three already reported mutations. A homozygous missense mutation, c.1156T>C, led to the substitution of a cysteine residue at amino acid position 386 with arginine, also identified a homozygous splice site mutation c.2240+1G>A, which showed a variable phenotype. (39).

According to Vilas et al., SLC4A11 mutation [R125H] resulting in CHED causes protein retention in endoplasmic reticulum due to misfolding, as a result R125H is transported to cell surface as wild type[WT].This shows that mutation is resulting in the disease by compromising function rather than impaired protein localization in cell surface. In fact, SLC4A11 does not facilitate water influx which specify that CHED occurs due to the incompetence of SLC4A11 to facilitate water flux across the plasma membrane (24).

Guha et al., in her study reported that mutant SLC4A11 cells are prone to oxidative stress.SLC4A11 gene with oxidative stress is required for NRF2 activation under oxidative stress conditions in human corneal endothelial cells. Inducing antioxidant gene expression might help in overcoming the oxidative stress in corneal endothelial cells (33).

SLC4A11 gene mutations cause retention of protein in the cytoplasm and generate increased reactive oxygen species. Mutant SLC4A11 cells are more vulnerable to oxidative and mitochondrial damage and are less able to overcome oxidative stress by expressing sufficient levels of antioxidant genes and are prone to apoptotic death (37).

Patients with CHED2 had corneal haze since birth or shortly after histology and electronic microscopy study showed thickened descendant's membrane, especially the non-banded zone, and the molecular study showed novel homozygous in-frame deletion mutation. High interfamilial and intrafamilial phenotypic variability was seen in the cohort of patients (39).

Histopathological findings of CHED i.e. degenerated and dysfunctional corneal endothelium

characterized by increased permeability and abnormal and accelerated descement's membrane secretion leading to swelling of corneal stroma (40).

Moazzeni et al., in his study suggested that SLC4A11 mutations are the cause for CHED among the study done in 21 patients of CHED , along with this they also found that MPDZ mutations in minority of CHED(41).

## Conclusion

The systematic review identified various point mutations (missense as well as nonsense) as well as frameshift mutations of SLC4A11 in CHED patients. A meta-analysis of six different alleles suggested a positive association of three alleles' mutations, c.2605C>T, c.1156T>T and c.22440 1 G>A alleles of SLC4A11 and CHED.

**Acknowledgments:**

We thank S P Srinivas and Dr. Usha S Adiga for their guidance and comments to improve the manuscript.

## References

1. Willoughby C E., Ponzin D., Ferrari S., Lobo A., Landau K., Omidi, Y. et al (2010). Anatomy and physiology of the human eye:, effects of mucopolysaccharidoses disease on structure and function–a review. Clin Exp Ophthalmol, 2-11.
2. Del Monte DW., Kim T. (2011) Anatomy and physiology of the cornea. J Cataract Refract Surg , 37, 588-598.
3. Torricelli A A., Singh V., Santhiago M R., Wilson S E. (2013). The corneal epithelial basement membrane: structure, function, and disease. Invest Ophthalmol Vis Sci, 54, 6390-6400.
4. Wilson S E., Hong J W. (2000). Bowman's layer structure and function: critical or dispensable to corneal function? A hypothesis. Cornea , 19, 417-420.
5. Meek K M., Knupp C. (2015). Corneal structure and transparency. Prog Retin Eye Res , 49, 1-16.
6. Meek K M., Boote C.(2004). The organization of collagen in the corneal stroma. Exp Eye Res , 78, 503-512.
7. Torricelli AA., Wilson S E. (2014). Cellular and extracellular matrix modulation of corneal stromal opacity. Exp Eye Res, 129, 151-160.
8. Johnson D H., Bourne WM Campbell R J. (1982). The ultrastructure of Descemet's membrane. I. Changes with age in normal corneas. Arch Ophthalmol , 100, 1942-1947.
9. Eghrari A O., Riazuddin S A.,Gottsch J D. (2015). Overview of the Cornea: Structure, Function, and Development. Prog Mol Biol Transl Sci ,134, 7-23.
10. Tuft S J., Coster D J. (1990).The corneal endothelium. Eye (Lond) 4 , ( Pt 3), 389-424.
11. Sanchis-Gimeno JA., Lleo-Perez A.,Alonso L., Rahhal M S .,Martinez Soriano F. (2005) Corneal endothelial cell density decreases with age in hemitropic eyes. Histol Histopathol , 20, 423-427.
12. Vincent A L. ( 2014). Corneal dystrophies and genetics in the International Committee for Classification of Corneal Dystrophies era: a review. Clin Exp Ophthalmol , 42, 4-12.
13. Malhotra D et al SLC4A11 in Blinding Endothelial Corneal Dystrophies: Characterization, Molecular Defect and Potential Therapeutic Strategy
14. Hemadevi B., Veitia RA., Srinivasan M., Arunkumar J., Prajna NV., Lesaffre C., Sundaresan P. (2008). Identification of mutations in the SLC4A11 gene in patients with recessive congenital hereditary endothelial dystrophy. Arch Ophthalmol , 126:700-8.
15. Aldahmesh A M., Khan O A., Meyer F B., Alkuraya S F. (2009) Mutational Spectrum of SLC4A11 in Autosomal Recessive CHED in Saudi Arabia; Investigative Ophthalmology & Visual Science, September , Vol. 50, No. 9;4142-4145.
16. Ciralsky J., Colby K. (2007). Congenital corneal opacities: a review with a focus on

genetics. Semin Ophthalmol , 22:241-246. Review.

17. Vithana E N., Morgan P., Sundaresan P., Ebenezer N D., Tan DT., Mohamed M.D. et al. (2006) Mutations in sodium-borate cotransporter SLC4A11 cause recessive congenital hereditary endothelial dystrophy (CHED2). Nature Genetics , 38, 755-757.
18. Feizi, S. (2018). Corneal endothelial cell dysfunction: etiologies and management. Ther Adv Ophthalmol , 10, 2515841418815802.
19. Malhotra D., Casey R J et al. (2020). Molecular Mechanisms of Fuchs and Congenital Hereditary Endothelial Corneal Dystrophies. Physiol Biochem Pharmacol.
20. Sultana A..,Garg P., Ramamurthy B., Vemuganti G K .,Kannabiran C. 2007 Mutational spectrum of the SLC4A11 gene in autosomal recessive congenital hereditary endothelial dystrophy. Mol Vis ,13, 1327-1332.
21. Liu J., Seet F L., Koh W L., Venkatraman A., Venkatraman D.,Mohan RR, et al. (2012). Depletion of SLC4A11 Causes Cell Death by Apoptosis in an Immortalized Human Corneal Endothelial Cell Line; IOVS, June ,3270-3279.
22. Vilas G. L., Loganathan K S., Quon A., Sundaresan P., Vithana N E., Casey J,et al. (2012). Oligomerization of SLC4A11 Protein and the Severity of FECD and CHED2 Corneal Dystrophies Caused by SLC4A11 Mutations, 419 -428.
23. Kao L., Azimov R., Abuladze N., Newman D., Kurtz I. (2014).Human SLC4A11-C Functions as a DIDS-Stimulatable H+ (OH- 1 ) 2 Permeation Pathway: Partial Correction of R109H Mutant Transport, Am J Physiol Cell Physiol;176-188.
24. Vilas G L., Loganathan S K., Liu J., Riau A K., Young J D., Mehta J S, et al. (2013). Transmembrane water-flux through SLC4A11: a route defective in genetic corneal diseases. Hum Mol Genet , 22, 4579-4590.
25. Loganathan SK., Casey JR. (2014). Corneal Dystrophy-Causing SLC4A11 Mutants: Suitability for Folding-Correction Therapy. Hum Mutat , 35, 1082-1091.
26. Desir J., Abramowicz M et al.(2008) .Congenital hereditary endothelial dystrophy with progressive sensorineural deafness (Harboyan syndrome); Orphanet Journal of Rare Diseases. 3-28.
27. Romero T P., Donoso R., Lopez P.,Miranda A, Rodriguez L., Chrzanowsky D et al, (2019) .Clinical features and possible founder mutation of the 8bp duplication mutation in the SLC4A11 gene causing corneal dystrophy and perceptive deafness in three South American families; ophthalmic genetics ,1-9.
28. Vithana EN., Morgan P., Sundaresan P., Ebenezer ND., Tan DT., Mohamed MD., Anand S., Khine KO., Venkataraman D.,Yong VH., Salto-Tellez M., Venkatraman A et al. 2006. Mutations in sodium-borate cotransporter SLC4A11 cause recessive congenital hereditary endothelial dystrophy (CHED2). Nat Genet 38:755-7.
29. Brejchova K., Dudakova L., Skalicka P., Dobrovolny R., Masek P., Putzova M, et al (2019) . IPSC-Derived Corneal Endothelial-like Cells Act as an Appropriate Model System to Assess the Impact of SLC4A11 Variants on Pre-mRNA Splicing Invest Ophthalmol Vis Sci. 60: 3084–3090.
30. Hand C K., McGuire M., Parfrey A N ., Murphy CC . (2016). Homozygous SLC4A11 mutation in a large Irish CHED2 pedigree, Ophthalmic Genetics.1-4.
31. Tananuvat N., Tananuvat R ., Chartapisak W.,Mahanupab P., Hokierti C., Srikummool M,et al (2020). Harboyan syndrome: novel SLC4A11 mutation, clinical manifestations, and outcome of corneal transplantation. Journal of Human Genetics, 1-11.
32. Ramprasad VL., Ebenezer ND., Aung T., Rajagopal R., Yong VH., Tuft SJ, et al. 2007. Novel SLC4A11 mutations in patients with recessive congenital hereditary endothelial

dystrophy (CHED2). Mutation in brief #958. Online. Hum Mutat 28:522-3.

33. Guha S., Chaurasia S., Ramachandran C.,Roy S, et al. (2017). SLC4A11 depletion impairs NRF2 mediated antioxidant signaling and increases reactive oxygen species in human corneal endothelial cells during oxidative stress. Scientific Reports 1- 11.
34. Puangsricharen V., Yeetong P., Charumalai C., Suphapeetiporn K., Shotelersuk V, et al. (2014). Two novel mutations including a large deletion of the SLC4A11 gene causing autosomal recessive hereditary endothelial dystrophy.Br J Ophthalmol October , 98: 1460-62.
35. Jiao X., Sultana A., Garg P., Ramamurthy B., Vemuganti KG., Gangopadhyay N, et al. (2007). Autosomal recessive corneal endothelial dystrophy (CHED2) is associated with mutations in SLC4A11. J Med Genet . 44:64–68.
36. Mehta S J., Hemadevi B., Vithana N E., Arunkumar J., Srinivasan M., Prajna V., et al. (2010).Absence of Phenotype–Genotype Correlation of Patients Expressing Mutations in the SLC4A11 Gene; Cornea _ Volume 29, Number 3, March ,302-6.
37. Roy, S., Praneetha, D. C., & Vendra, V. P. (2015). Mutations in the Corneal Endothelial Dystrophy-Associated Gene SLC4A11 Render the Cells More Vulnerable to Oxidative Insults. *Cornea*, *34*(6), 668–674
38. Kodaganur G S., Kapoor S., Veerappa M A., Tontanahal J S., Sarda A., Yathish S et al. (2013). Mutation analysis of the SLC4A11 gene in Indian families with congenital hereditary endothelial dystrophy 2 and a review of the literature, Molecular Vision;1694-1706.
39. Paliwal, P., Sharma, A., Tandon, R., Sharma, N., Titiyal, J. S., Sen, S., Nag, T. C., & Vajpayee, R. B. (2010). Congenital hereditary endothelial dystrophy - mutation analysis of SLC4A11 and genotype-phenotype correlation in a North Indian patient cohort. *Molecular vision*, *16*, 2955–2963
40. Ehlers, N., Módis, L., & Møller-Pedersen, T. (1998). A morphological and functional study of Congenital Hereditary Endothelial Dystrophy. *Acta ophthalmologica Scandinavica*, *76*(3), 314–318.
41. Moazzeni, H., Javadi, M. A., Asgari, D., Khani, M., Emami, M., Moghadam, A., Panahi-Bazaz, M. R., Hosseini Tehrani, M., Karimian, F., Hosseini, B., Nekuie Moghadam, T., Hassanpour, H., Akbari, M. T., & Elahi, E. (2020). Observation of nine previously reported and 10 non-reported *SLC4A11* mutations among 20 Iranian CHED probands and identification of an *MPDZ* mutation as possible cause of CHED and FECD in one family. *The British journal of ophthalmology*, *104*(11), 1621–1628

Fig1: Showing PRISMA

Fig 1: Adapted from the Preffered Reporting Items for Systematic Reviews and Meta-Analyses(PRISMA) flow diagram summarizing screening method and study selection process.

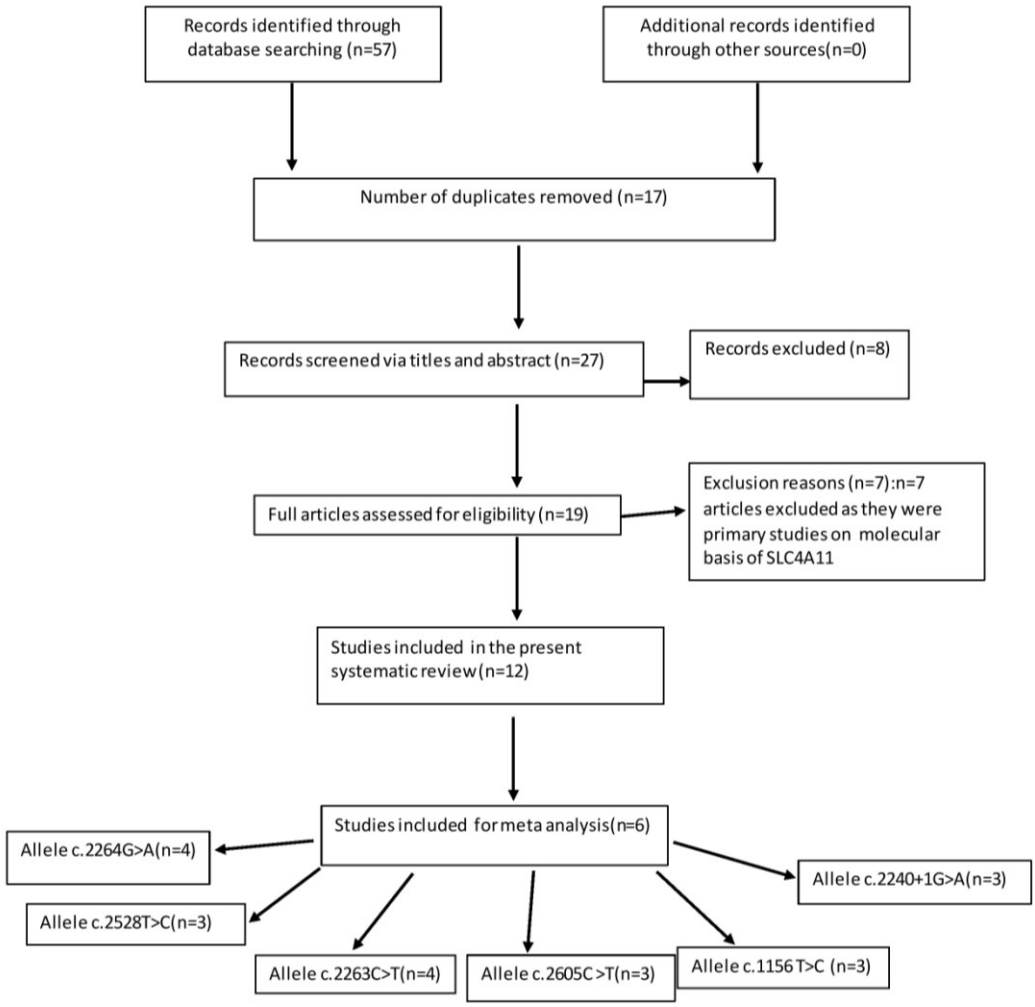

**Table 1: :** Summary of the findings of mutations of SLC4A11 in CHED

| Authors | Year | Allele | Results | Conclusions |
|---|---|---|---|---|
| Brejchova et al[29] | 2019 | c.2411G>A<br>c.2263C>T<br>c.1237G>A<br>c.625C>T<br>c.427G>A<br>c.2003T>C<br>c.2528T>C | Identified 11 pathogenic SLC4A11 mutations, including 4 novel. | This study highlights the potential of using CE-like cells to Investigate the pathogenic SLC4A11 disease-associated variants. |
| Hand et al[30] | 2016 | c.2528 T>C | A homozygous SLC4A11 mutation (Leu843Pro) was detected in large Irish CHED2 pedigree | This is the First report of this SLC4A11 mutation (Leu843Pro) in a homozygous state. |
| Aldamesh et al[15] | 2009 | c.520delGCTTCGC<br>c.1228G_177C<br>c.1253G_177A<br>c.1044_25del19nt<br>2236C_T<br>2236C_T<br>c.2114_1G_A | Seven mutations were identified which Is due to reduction or loss of bicarbonate transporter-related protein 1 (BTR1). | In this study no evidence was found of genetic heterogeneity in CHED and that loss of BTR1 function |
| Tananuvat et al[31] | 2020 | c.2264G>A<br>c.2127delG<br>c.2263C>T | study showed that patients with SLC4A11 Mutations had CHED and sensorineural hearing loss... | The SLC4A11 mutations were not found to be related to the outcome of corneal transplantation and the onset and severity of SNHL.(sensorineural hearing loss) |
| Romero et al[27] | 2019 | c.2233_2240dup TATGACAC, | The dysfunction of the transport of fluid through the endothelium in the eye will lead to inadequate corneal dehydration and the osmotic imbalance in the ear fibrocytes | The five CDPD patients were homozygous for the same mutation in the SLC4A11 gene. |

| | | | | |
|---|---|---|---|---|
| Ram Prasad et al[32] | 2007 | c.[427G>A] + [427G>A]<br>c.[1156T>C] + [1156T>C]<br>c.[2263C>T] + [2263C>T]<br>c.[2264G>A] + [2264G>A] *<br>c.[720G>A] + [720G>A]<br>c. [2240+1G>A] + [?]<br>c.[2398C>T] + [2437-1G>A]<br>c.[2605C>T] + [2605C>T] * | seven novel SLC4A11 mutations were identified include two nonsense, three missense and two splice site mutations. | This study identified seven novel mutations in *SLC4A11* |
| Desir et al[26] | 2007 | c.1378_1381delTACGinsA<br>c.473_480delGCTTCGCC<br>c.2233_2240dup TATGACAC)<br>(c.2423_2454del | Mutations of SLC4A11 in CDPD (Harboyan syndrome), shows allelism with CHED2, and Extends the implication of mutations of this borate transporter<br>To perceptive deafness. | These findings extend the implication of the SLC4A11 borate transporter beyond corneal dystrophy to perceptive deafness. |
| Puangsricharern et al[34] | 2014 | c.778A>G | Mutations in the solute carrier family 4<br>member 11 (SLC4A11) gene have been<br>Identified in most patients with CHED2. | The<br>micro deletions at this region could be<br>The disease-causing alleles responsible for CHED2. |
| Jiao et al[35] | 2007 | g.8298CRT,<br>g.8379GRT<br>g.2943delTTinsA,<br>g.8118delCT<br>g.9200delTinsGG, | Sequencing of SLC4A11 showed homozygotic mutations in<br>Affected members from 12 of 16 families. | results confirm that mutations in the<br>SLC4A11 gene cause autosomal recessive CHED. |
| Mehta et al[36] | 2010 | c.2264G.A<br>c.1813C.T<br>c.2605C.T | Individuals with the same mutation had<br>Different degrees of hearing loss within their respective families. | Corneal endothelial cells are more vulnerable to<br>defects in the functional activity of SLC4A11 than cells of the striae<br>Vascularis of the inner ear. |

| | | | | |
|---|---|---|---|---|
| Kodaganur et al[38] | 2013 | c.1831T>C<br>c.1249G>A<br>c.2170C>G<br>c.785C>T<br>c.2606G>A<br>c.1249G>A | DNA sequence analysis identified four novel (viz., p.Thr262Ile, p.Gly417Arg, p.Cys611Arg, and p.His724Asp) mutations and one known p.Arg869His homozygous mutation in the *SLC4A11* gene. | This study increases the mutation spectrum of the *SLC4A11* gene. |
| Hemadevi et al[14] | 2008 | c.481A_C<br>c.2318C_T<br>c.2618T_C+c.2318C_T_<br>c.2506 C_T<br>c.806C_T<br>c.478G_A<br>c.1156T_C<br>c.374G_A<br>c.806C_T<br>c.2263C_T | This study increases the number of *SLC4A11* gene mutations and confirms the role of this Gene in causing CHED2. | Out of 10 mutations 6 novel mutations were noted .one of it is caused by complete deletion of exon 6 in SLC4A11 |

**Table 2: Results of heterogeneity and publication bias testing for different alleles of SLC4A11**

| Allele | C.2264G>A | C.2528T>C | C.2263C>T | C.2605C>T | C.1156T>C | C.2240+IG>A |
|---|---|---|---|---|---|---|
| Q test | 13.008 | 20.018 | 14.9 | 0.0909 | 0.6925 | 1.56 |
| Degree of freedom | 8 | 2 | 3 | 2 | 2 | 2 |
| P-value | 0.0046 | <0.0001 | 0.0019 | 0.95 | 0.707 | 0.457 |
| $I^2$ | 76.94% | 90.01% | 79.87% | 0% | 0% | 0% |
| 95% CI for $I^2$ | 37.08-91.55 | 73.31-96.26 | 46.66-92.41 | 0.0-26.22 | 0-90.3 | 0.0-95.70 |
| Egger's test | | | | | | |
| Intercept | 12.47 | -41.9645 | -7.0771 | 2.6568 | 2.55 | 2.586 |
| 95% CI | -72.37-97.32 | 399.2-315.28 | -12.36--1.785 | 2.5257-2.787 | 2.388-2.72 | 2.231-2.94 |
| P Value | 0.59 | 0.3758 | 0.0289 | 0.0025 | 0.0033 | 0.0069 |
| Begg's test | | | | | | |
| Kendall's tau | 0.333 | -0.3 | -0.333 | 1 | 1 | 1 |
| P value | 0.4969 | 0.6015 | 0.4969 | 0.1172 | 0.1172 | 0.1172 |

**Fig 2: Sample Funnel Plot showing publication bias for the Allele c.2264G>A**

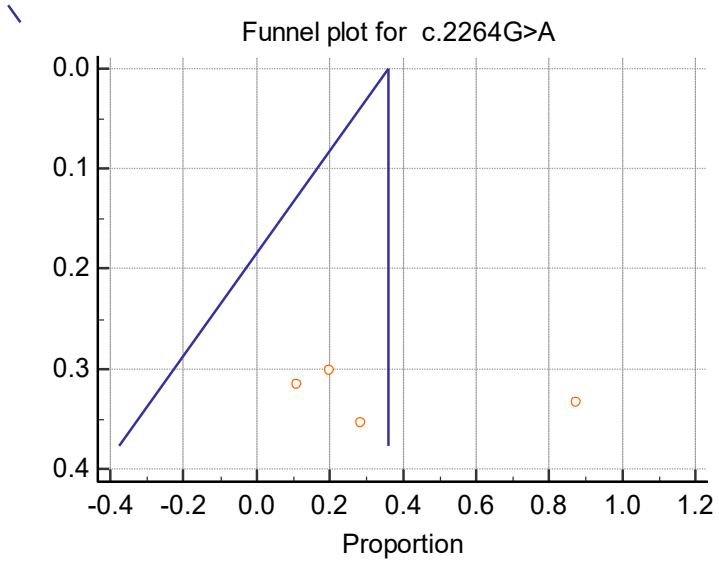


**Fig 3: Forest Plot for c.2264G>A allele of SLC4A11**

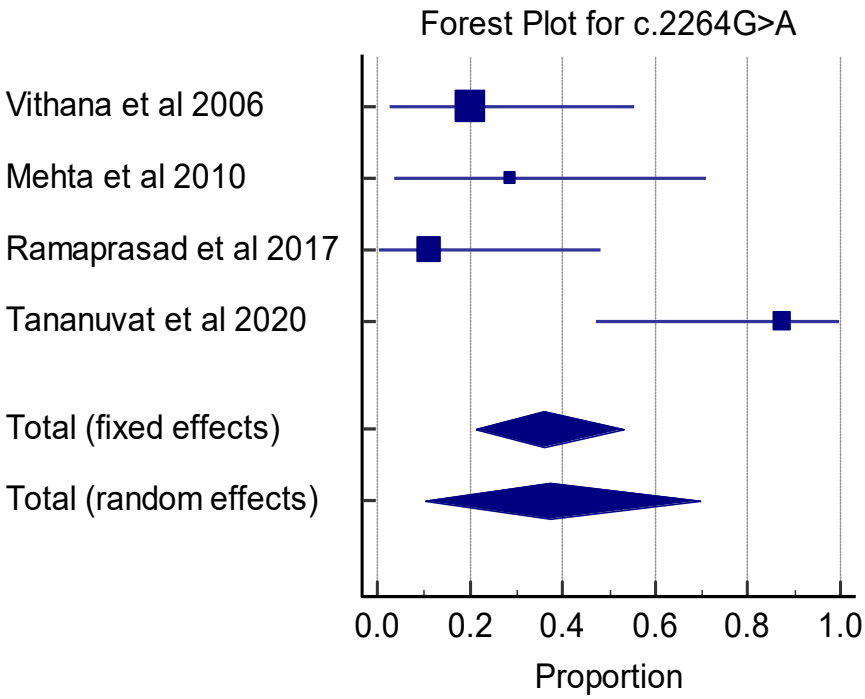

**Fig4: Forest Plot for c.2528 G>A allele of SLC4A11**

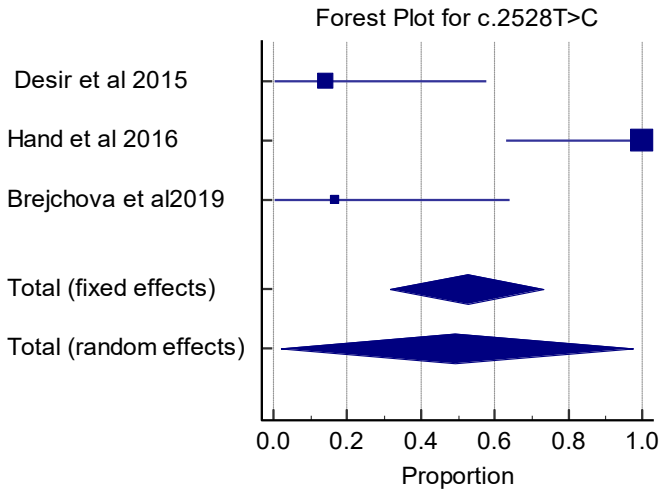


**Fig 5: Forest Plot for c.2263C>T allele of SLC4A11**

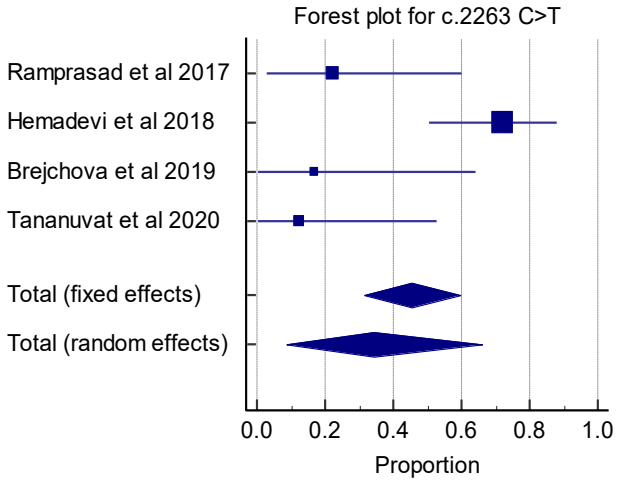

**Fig6:ForestPlotforc.2605C>TalleleofSLC4A11**

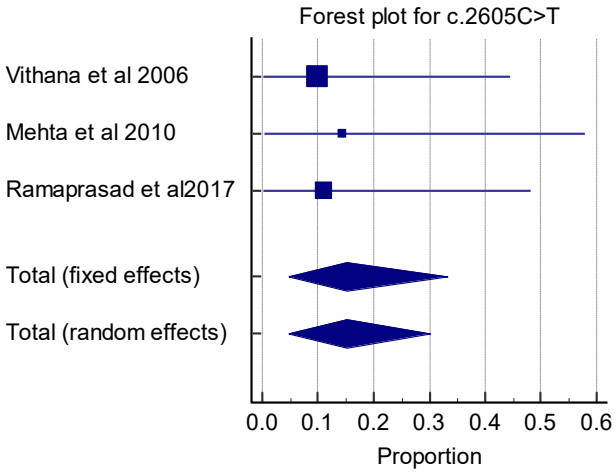


**Fig 7: Forest Plot for c.1156T>C allele of SLC4A11:**

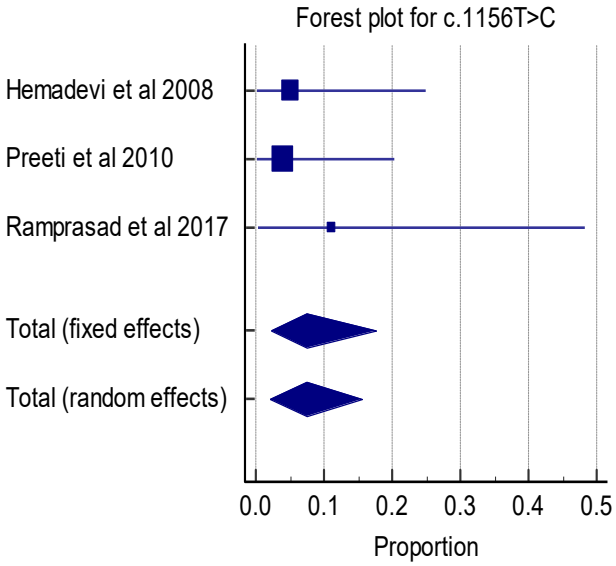

Fig8: Forest Plot for c.22440+1G>A allele of SLC4A11.