MUTATIONS IN SLC4A11 IN FUCHS ENDOTHELIAL CORNEAL DYSTROPHY- A SYSTEMATIC REVIEW

Pai Divya[1*], D Chaitra[2*], Srinivas SP**
NITTE (Deemed to be University), Deralakatte, Mangalore, Karnataka, India[1*,2*]
Optometry, Indiana University, Bloomington, IN; USA**

[2*]chaitrad@nitte,edu.in

Abstract
Objective: The Fuchs corneal endothelial dystrophy (FECD) is a disorder of the corneal endothelium usually seen after the fourth decade of life. Severe FECD results in the loss of stromal deturgescence, leading to corneal blindness. The present systematic review was carried out to analyze the mutations in SLC4A11 found in some cohorts of FECD. A meta-analysis was also carried out to determine whether the mutations of c.1195G>A allele of SLC4A11 results in FECD.
Methodology: Scientific reports published before September 2018, identified by searching 14 online databases, were included in this review. Title, abstract, keywords, results, conclusions, and references were screened. The statistical analysis to determine the association between the mutations of c.1195G>A allele of SLC4A11 and FECD was carried out using MedCalc software.
Results**:** Mutations in the alleles of SLC4A11 are reported differently in various studies. Three
original articles reported on the role of c.1195G>A of SLC4A11 in FECD. However, a forest plot analysis, suggests that mutation of c.1195G>A of SLC4A11 is not associated with the occurrence of FECD. No heterogeneity and publication bias were noted as indicated by the random effect model results and Begg's test, respectively.
Conclusion**:** There are several pathogenic variants of SLC4A11 that might play a role in causing FECD, but does include the mutations in c.1195G>A allele.

Key words: *FECD, SLC4A11, cornea, endothelium, mutations*

Introduction

The human cornea consists of three cellular layers, viz., epithelium, stroma and endothelium. The two acellular layers - Bowman's membrane and Descemet's membrane (DM), separate the stroma from the epithelium and endothelium, respectively (1). Based on the anatomical location of the defects, the corneal dystrophies are classified into three types: (a) Anterior corneal dystrophy comprises defects in the epithelium, basal lamina, or Bowman's layer. (b) Stromal corneal dystrophy covers defects in the corneal stroma, and (c) Endothelial corneal dystrophy includes defects in DM or the endothelium (2). The Fuchs endothelial corneal dystrophy (FECD), posterior polymorphous corneal dystrophy (PPCD), and congenital hereditary endothelial dystrophy (CHED) are the major endothelial corneal dystrophies that were speculated to be the defects of the neural crest terminal differentiation (3). Corneal endothelial dystrophies follow the Mendelian pattern of inheritance.

Fuchs endothelial corneal dystrophy

Professor Ernst Fuchs, an Austrian Ophthalmologist in the year 1910, was the first to describe FECD. A case series reported by Fuch et al. included 13 cases of epithelial disorders of the cornea where the patients presented with loss of corneal sensation, epithelial bullae, and cloudy central cornea (4). In the year 1920, Kraupa observed that the epithelial changes depended on the changes in the endothelium. In 1921, Vogt described the formation of drop-like excrescences associated with FECD, referred to as guttae (5).

In 1924, Graves was the first to describe the pathology of endothelium that led to the development of FECD (6). Maurice DM, in 1969 described that endothelium was a continuous cellular layer that exhibits lower permeability to salts and hydraulic flow of water (7). The study on the ultrastructure of the corneal Decrements membrane by the transmission electron microscopy revealed that the adult Descemet's membrane consists of two layers – an anterior banded and a posterior non-banded layer. The thickness of anterior banded layer is 3μm and posterior non-banded layer increases in thickness on an average

of 2μm at the age of 10 years and 10μm at the age of 80 years. There is marked increase in the thickness of Descemet's membrane in Fuchs endothelial corneal dystrophy (8).

With the development of new diagnostic techniques and detailed studies, it is now known that genetic defects underlie causation and progression of FECD (9).

Pathological aspects of FECD

The FECD is a progressive disorder marked by weakened and dysfunctional corneal endothelium, typically reported in the fourth decade of life onwards. It is the most commonly inherited corneal dystrophy (4).

The progression of FECD may occur over 2 to 3 decades of life before severe damage to the endothelium results in impaired vision or corneal blindness (10). Initially, FECD manifests with loss of endothelial cell density (ECD) concomitant with stromal edema and a notable decrease in recovery from deliberate stromal edema (i.e., a reduction in percent recovery per hour (PRPH) of stromal thickness following hypoxia-induced stromal edema) (11). The latter causes blurred vision in the morning hours. The reduction in PRPH herald's dysfunctional endothelium.

The FECD occurs in two different forms, i.e., early-onset form and late onset-form. The common inherited for is the late-onset Fuchs endothelial corneal dystrophy (12). The corneal endothelium shows the guttae, which represents an accumulation of extracellular matrix deposits, in addition to loss in ECD and altered morphology. While the late-onset FECD is more common in women than in men, the early-onset form is observed equally in both genders (13). Moreover, the early onset form of FECD clinically becomes evident in the first decade of life and is inherited in an autosomal dominant fashion where one copy of altered gene causes the disorder (13).

The endothelium is responsible for the maintenance of stromal hydration, which is essential for corneal transparency. The cells on the posterior surface of the cornea regulate the corneal transparency and hydration. This is achieved by active ion transport process. (7, fishbarg, riley, srinivas, 14****). In particular, the endothelium carries out fluid-pump while restraining influx of water from aqueous

humor into the stroma (referred to as the barrier function, (2,15). Thus, the stromal edema in FECD could be due to loss of fluid-pump function, barrier function, or both (16). The fluid pump function is dependent on several ion transport systems organized strategically on the apical and basolateral sides of the endothelium (14). These mechanisms elicit a net movement of Na+ and HCO3- into the aqueous humor, which osmotically couple water movement from stroma to the aqueous humor. This loss of water movement, called the fluid pump activity, is essential to counter the continuous fluid movment from the anterior chamber into the stroma because of the imbibition pressure (~55 mm Hg) associated with GAGs dispersed in the stroma. The fluid pump activity is indirectrly dependent on Na^+/K^+- ATPase (referred to as pump sites) for setting Na+ and K+ gradients. In the early stages of FECD, the number of pump sites per endothelial cell has been claimed to be elevated. As the disease progresses, these pump sites are reduced, marking the onset of stromal edema (17). According to Burns et al., an early marker of FECD is the loss in ECD (18). But Wilson SE et al., Bergmanson JP et al. and Mc Cartney MD et al. have suggested that more than the loss ECD, the reduction in pump sites in the corneal endothelial cells play an essential role in the onset and progression of FECD (19, 20, 21).

The other main factor contributing to the pathogenesis of FECD is oxidative stress. The lifelong exposure to light makes the corneal endothelial cells prone to oxidative stress. There are increased levels of glycated end products and glycated proteins associated with increased cellular oxidative stress in the corneal endothelial cells and DM of FECD patients. Due to the high oxidative stress, the corneal endothelial cells cannot clear the reactive oxidative species and reactive nitrogen species formed. This unbalanced production and clearing rate of reactive oxygen species and reactive nitrogen species is associated with downregulation of oxidative stress related genes. The oxidative stress related environment causes DNA damage leading to the change in cell morphology ultimately leading to apoptosis (22, 23, 24).

Risk factors
The cause for the onset of FECD is mainly correlated with genetic factors. UV radiation, smoking,

hormonal levels and geographical location may add to the risk factors of FECD.

In the pathogenesis of FECD, the role of UV radiations is not clearly understood. Still, guttae formed in FECD usually appear in an interpalpebral region that indicates the environmental exposures in causing the disease (5).

In an epidemiological survey, the study by Kitagawa K et al. suggested that the occurrence of FECD is influenced by the geographic location. In his study survey, Kitagawa et al. included Chinese Singapore and Japanese residents. He proposed that the prevalence of the disease FECD was higher in Chinese Singaporeans than in the Japanese population, which showed that racial and genetic factors were influenced by geographic location (25).

In the study by Zoega et al., the smoking history of more than 20 pack-years is strongly associated to increase the risk of developing FECD by twofold. Further, the study found that higher body weight and body mass index were associated with lower risk of developing corneal guttae. This was corelated with alterations with hormonal levels (26).

Several studies showed an association between smoking and environmental tobacco smoke, causing the increased chance of several ocular diseases, including FECD (27,28,29,30,31). Some studies have proved that prolonged smoking is associated with cell loss, increased cell permeability, and decreased pump function in endothelial cells of FECD compared to the normal endothelial corneal cells (18, 19, 32).

Clinical presentation
In the patients with early-onset FECD, there were no obvious clinical symptoms. But on an Ophthalmic examination, central corneal excrescences were discovered, and the endothelial layer's imaging showed the reduced endothelial cell density. The endothelial cells showed abnormal shapes and cell size variations (33).

The patients with FECD were presented with the symptoms of decreased visual acuity, low night vision, the cornea becomes hazy, and pain while blinking the eye (11). There is marked depletion of the innermost layer of the corneal endothelium. The initial clinical finding is the formation of wart-like excrescences called corneal guttate on the corneal endothelial surface and thickening of the DM (34).

Clinical staging of FECD

The FECD staging is done based on the extent of corneal guttae formation and on the presence of corneal edema (35). The loss of corneal endothelial cells and the formation of guttae in the DM are the characteristics of the early stages of FECD. As the disease progresses, the later stages of FECD involve all the five layers of the cornea. Dr. Jay Krachmer et al. in the year 1978 put the clinical grading scale of FECD forwards. This grading system helped to know the disease progression (36).

Accordingly, there are five stages (9,37):

- Grade 0 (G0): Negative – no apparent disease

- Grade 1 (G1): there is the onset of the disease with 0-12 central, nonconfluent guttata in at least one eye, although asymptomatic

- Grade 2 (G2): the number of central nonconfluent guttata increases to more than 12 in at least one eye

- Grade 3 (G3): zone of central confluent central guttata 1-2mm in the horizontal plane

- Grade 4 (G4): zone of central confluent central guttata 2-5mm in the horizontal plane

- Grade 5 (G5): zone of central confluent central guttata >5mm with or without edema of the corneal stroma and /or epithelium layer

Genetics

The study by various researchers has identified the causal loci for FECD by applying linkage analysis and genome-wide association studies (38).

FECD1: Sundin et al. identified the first loci associated with FECD as FECD1, which was localized to a 26.4-Mb interval between 13pTel 13q12.13 (39).

FECD2: The second locus for FECD was localized to 18q21.2-18q21.32 and was identified as FECD2 by Sundin et al. This locus was found to be more commonly existing on a common locus of chromosome 18 (40).

FECD3: The study by Riazuddin SA et al. identified third loci for FECD was localized to 5q33.1-5q35.2. The single nucleotide polymorphism was localized to chromosome 5(41).

FECD4: Riazuddin SA et al. identified the fourth loci associated with FECD in the region of 9p22.1-9p24.1 (42).

Major Genes associated with FECD

TCF4: This gene is localized in chromosome 18 encodes a transcription factor TCF4. Wieben et al. found an association between intronic thymine-guanine-cytosine trinucleotide repeat sequence in the TCF4 gene of FECD patients (43). Baratz et al. identified that the gene marker rs613872 within an intron of the TCF4 gene has a strong association with FECD (44).

COL8A2: The study by Afshari NA et al., Gottash JD et al, Aldave AJ et al.,. identified the mutations in the collagen gene COL8A2 in the loci chromosomal position 1p34.3-p32.3 (12,13,60). These mutations change DM's composition, which leads to the early onset of FECD (13,46).

ZEB1: This gene encodes the Zinc-finger E-box-binding homeobox 1 transcription factor. The mutation of which is found associated with FECD in the study by Mehta et al. (45). Riazuddin et al. identified the mutation in ZEB1 located on chromosome 9 associated with FECD (41).

LOXHD1: This gene was associated with causing FECD2 located on the locus of chromosome 18 (39).

SLC4A11: The SLC4A11 gene belongs to the SLC4 family of bicarbonate transporters involved in the transport of borate across the cell membranes encodes an 891 amino-acid membrane transporter protein. Mutations in the SLC4A11 result in different endothelial corneal dystrophy (47). Even though SLC4A11 is not a member of the major intrinsic protein family, it facilitates transmembrane water movement. It is localized to the basolateral surface of human corneal endothelial cells (48). FECD is genetically heterogeneous. According to Tang H et al., through the linkage analysis, 4 loci, FECD1, FECD2, FECD3, and FECD4 on chromosomes 13, 18, 5, and 9 are identified associated with numerous linkage peaks and susceptibility loci (49). The SLC4A11 gene

is located at chromosome 20p12 and is highly expressed in corneal endothelium (50). The study by Gottsch JD et al. reported that SLC4A11 is significantly downregulated in the cornea of the patients having FECD (13).

Through linkage analysis or genome-wide association studies by Weiss JS et al., Nanda GG et al., the study stated eight different loci are found associated with FECD (44, 50). FECD is categorized as FECD 1-8 by the International committee to classify corneal dystrophies (IC3D) (50).

Due to increased methylation, there is a considerable decrease in the expression of SLC4A11 in the corneal endothelium that promotes FECD. Because of the methylation, there is impaired transport of Na^+ coupled with OH^- transport, $NH3:2H^+$ cotransport (51, 52). The study by Zhang W et al. reported that SLC4A11 is essential to transport the ammonia for the generation of ATP using the Na^+/K^+ ATPase system (53). This mechanism serves as an additional source of energy for the corneal endothelial cells (34). In the study by Geroski et al., in the very early stage of FECD disease, the corneas with moderate guttae showed significantly increased Na^+/K^+ ATPase pump density (17). The corneas showing high-density guttae formation showed reduced Na^+/K^+ ATPase pump density. The cornea becomes edematous (21,53). The functional activity and efficiency of the protein coded by SLC4A11 are affected when there is a mutation of the gene SLC4A11 (54). However, the study by Hemadevi B et al. of the SLC4A11 gene screening did not observe any pathological mutations (55). Riazuddin SA et al., in the cohort study, identified seven mutated variants of the SLC4A11 gene associated with the adult FECD (56). Vithana EN et al., in their study, identified four heterozygous SLC4A11 gene sequence alteration cause FECD. Ten variants of SLC4A11 gene sequence alteration were non-pathogenic and are identified in both controls and FECD patients. Five missense mutations were identified in both management and FECD patients (57). Skorodumova LO et al. identified four variant mutations of the SLC4A11 gene associated with the late onset of FECD (58). Soumitra N et al. identified four mutant variants of the SLC4A11 gene associated with late-onset FECD (15). Tang H et al. identified 14 mutant variants of the SLC4A11 gene that caused the late-onset FECD(49).

Objectives:
The systematic review's objective was to assess the association of SLC4A11 mutations and Fuchs endothelial corneal dystrophy by analyzing the research papers available that throw light on the current perspectives of the gene SLC4A11 and FECD. We also aimed to study the association of mutations of c.1195G>A allele of SLC4A11 in the causation of FECD by meta-analysis.

Methods
The review was conducted using published original articles, with a case-control and cohort study design.

Eligibility criteria:
The journal articles published before September 2018 were included for our systematic review. The articles written in the English language and discussed the mutations of the SLC4A11 gene in association with Fuchs endothelial corneal dystrophy were included for the study.

Study selection, data extraction, and critical appraisal:
Searches and data extraction were done by two independent investigators from November 2020 to January 2021, with keywords' SLC4A11 AND Fuchs endothelial corneal 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).

All patients included in the articles had undergone complete ophthalmic examination, including fundoscopy and slit-lamp examination and confocal specular microscopy to document corneal guttata.

The diagnosis of FECD was based on the presence of greater than 2 mM of confluent central corneal endothelial guttae in each eye or histopathologically confirmed FECD after performing a penetrating keratoplasty or endothelial transplantation. 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 or single nucleotide polymorphisms by PCR-RFLP were carried out for SLC4A11. The controls of this study had a

normal cornea on ophthalmic examination and no family history of FECD.

Information source and search terms:

Fourteen online databases were searched for the identification of the articles: Directory of open access journal, EBSCO host research database, Google scholar, High wire, Indian science abstract, J Stage, Koreamed, Pubmed, Open J gate, Proquest, Sage, Scientific information data, Scirus, Scopus.

Statistical analysis

The statistical analysis was carried out using the software Medcalc. The association between the mutations of c.1195G>A allele of SLC4A11 and FECD were assessed.

The association of occurrence of FECD and c.1195G>A allele of SLC4A11 was assessed by pooled odds ratio (OR) with 95% Confidence Interval (CI). Random effect models were used to calculate OR and CI. The significance of summary ORs were determined with a Z test. 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 study 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 2
the confidence bounds.

Between-study heterogeneity was assessed using 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), GOOGLE SCHOLAR (5), PROQUEST (3).

There were no relevant articles found in HIGH WIRE, INDIAN SCIENCE ABSTRACT, SCIENTIFIC INFORMATION DATA, SCIRUS, SCOPUS. No other papers were identified following the citation searching. A total of 29 full-text articles were obtained that were screened for the title, abstract, and keywords. The number of duplicate reports was 8, and they were excluded from the study. The

remaining 21 articles were screened for the title and abstract. Out of 21 articles, ten articles reviewed articles on the SLC4A11 gene and Fuchs endothelial corneal dystrophy, and hence they were excluded. Eleven original articles were screened. It was found that five articles concluded that the mutation of SLC4A11 causing congenital hereditary endothelial dystrophy leads to FECD. The remaining six 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. 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. As the methods and methodology were based on various factors in different papers, this factor was considered weak to be included in our study.

The study included six original articles that assessed the mutations of SLC4A11 in FECD patients compared to normal individuals without FECD; the alleles of SLC4A11 found to be mutated were different in different studies. However, three of the original articles reported the role of c.1195G>A of SLC4A11 in FECD, which were further included in the meta-analysis. The total sample size for the meta-analysis was 238 FECD cases and 594 controls without FECD.

Studies may have heterogeneity due to patients of different ethnicity, outcome definition, and study design. Both the random effect model and the fixed effect models were used to assess the association between the mutations of c.1195G>A of SLC4A11 and FECD. The fixed-effect model assumed that the mutation effect is the same in both the FECD cases and controls without FECD.

Forest plot by random effect model [Fig 2] for the association of bad c.1195G>A of SLC4A11 and FECD showed that all the studies were passing the line of no effect, suggested that mutation of c.1195G>A of SLC4A11 has no association with the occurrence of FECD. As the size of individual squares was directly proportional to the weight of the study, all the three studies of significance were given the equal and highest   weight.

Diamonds of both fixed and random effect models were on the line of no effect, which clearly suggested that mutations of c.1195G>A allele of SLC4A11 has no influence on FECD. Z values for both the random and fixed effect models were 1.839, CI values being 0.941-6.76, p=0.066 for both the models.

Q test for heterogeneity was 0.000024, the degree of freedom, DF=1 and p= 0.99, suggesting that there was not much heterogeneity between studies. There was no inconsistency, I2=0% 95% CI=0.00-0.00. OR was 2.523 for random and fixed effect models [Table 1].

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. All the squares and diamonds were found to lie on the line of no effect, suggesting that mutations of c.1195G>A allele of SLC4A11 did not have any role in the causation of FECD.

The funnel plot for publication bias was symmetrical, inverted funnel-shaped, boundaries being straight lines. This suggests that studies were symmetrically distributed in the plot, suggesting no publication bias is present [Fig-3].

Egger's test showed an intercept 95% CI -0.02819. Begg's test shows Kendall's Tau of – 1.0 at p=0.317, which suggested no significant publication bias while analyzing the association of mutation of the c.1195 G>A allele of SLC4A11 and FECD [Table 1].

There are no meta-analyses or systematic reviews available on this topic to the best of our knowledge. The study seems to be the first systematic review on the association of SLC4A11 mutations and FECD. The systematic review has revealed the role of various pathogenic alleles of SLC4A11, and meta-analysis suggests that c.1195G>A allele of SLC4A11 did not have any role in the occurrence of FECD.

This systematic review was focused on evaluating the different loci of the mutated form of the SLC4A11 gene and their association in causing Fuchs endothelial corneal dystrophy.

Vithana et al. conducted the mutation analysis of 89 subjects (57). 64 subjects had Chinese ethnicity, and 25 subjects were of Indian origin. In the Chinese cohort, the subject's ages ranged between 52 to 91 years, and eight patients had a family history of FECD. The remaining patients were classified as late-onset FECD cases. In the Indian cohort, the subject's age ranged between 28 to 81 years. In this, two patients had a family history of FECD, and the rest were classified as late-onset FECD cases. The study identified four heterozygous mutations in the SLC4A11 sequence associated with FECD. A missense mutation was observed in the Chinese cohort group. There were ten silent and five non-pathogenic missense mutations. A transition mutation in c.1195G>A allele resulted in the substitution of glutamic acid by glycine at the position of 399, which could have resulted in the disease. However, our study suggests no association between the above allele and the disease. The study also noted minor frequency changes in single nucleotide polymorphs in FECD cases and the control group, which was insignificant.

In the study by Soumittra N et al., 45 late-onset and four early-onset FECD patients identified a missense mutation (15). The age range between late-onset FECD was between 42 – 81 years. The age range for early onset of FECD was between 22-39 years. These mutations contribute to 11% of late-onset FECD. There were five cases with four variations in the exons. These revealed that the mutations cause FECD. The study was designed to identify mutations of SLC4A11, causing early as well as late-onset FECD. In this study, researchers identified three mutations that were previously unreported, c.1519G>A being one of them. The allele c.1519G>A was reported to have a role in late-onset sporadic FECD. The missense and frameshift mutations of SLC4A11 are described by the studies done by Vithana et al. (57), Minear et al. (59), Aldave AJet al. (60). These missense mutations showed different domains of SLC4A11 proteins, which did not show any genotype-phenotype associations in the causation of FECD. As per the study by Soumittra et al., the newly identified mutation c.1519G>A led to the inactivation of the protein resulting in a significant reduction in its functional activity, even though the cell surface abundance was normal. In the present study. Out of 45 late-onset sporadic FECD cases, five were identified to have a mutation of the SLC4A11 gene., This contributed to 11% of the disease pathogenesis.

The study by Skorodumova L et al., in a case-control study that included 100 cases and 100 controls, identified four pathogenic mutant variants of the SLC4A11 gene that caused late-onset FECD (58). The study did not detect any mutation in the four alleles of SLc4A11 in the patient group. There was no mutation in c.1195G>A.

Hemadevi B et al., in a case-control study of 80 patients and 100 controls, found that they were all sporadic cases of FECD, and no family history of the disease was observed (55). The subjects were in the age group between 17-76 years. The screening of SLC4A11 mutations did not identify any pathogenic mutation. This study found no significant association of mutation of the SLC4A11 gene with Fuchs endothelial corneal dystrophy. In the survey, exons and their flanking splice junctions of SLC4A11 were analyzed in FECD patients. The research could identify two novel variants and three already reported variants of SLC4A11. However, there was no statistically significant association between the mutation and FECD.

A cohort study done by Riazuddin SA et al. sequenced SLC4A11 in 192 sporadic and late-onset FECD families. This study found seven heterozygous missense mutations that were pathogenic and caused FECD (56). The study suggested that heterozygous mutations of SLC4A11 contribute somewhat to the pathogenesis of FECD. This study was conducted to hypothesize that both the dominant and recessive variants implicated in the early onset of FECD may contribute to the late-onset disease. Hence all the coding sequences and intron-exon junctions were screened to detect mutations. Seven missense mutations were noted in the SLC4A11 gene, all leading to amino-acid substitutions in the functional domains of SLC4A11, namely bicarbonate and transmembrane domains.

A study of 191 individuals of Chinese ethnicity without any ophthalmic complications was considered controls. A multi-generational family with late-onset Fuchs endothelial corneal dystrophy was considered. There were 14 variants of the SLC4A11 gene that caused FECD (49). The researchers studied SNP's of 14 allelic variants of SLC4A11, out of which 3 were exonic, and 11 were intronic. All exon variants were previously reported. Only a single intronic

polymorphism was freshly detected. However, its causative role in FECD is uncertain.

Limitations
Due to the paucity of published studies in this particular field, this study could include only six original articles for the systematic review. Only three articles were qualified to be fit for the meta-analysis.

Conclusion
It could be concluded from our study that there are several pathogenic variants of SLC4A11 that might play an essential role in causing FECD. But the exact position of these mutations is still unclear. Mutations in c.1195G>A allele of SLC4A11 is not associated with FECD.

References
1. Labetoulle, M., Baudouin, C., Calonge, M., Merayo-Lloves, J., Boboridis, K. G., Akova, Y. A., Aragona, P., Geerling, G., Messmer, E. M., & Benítez-Del-Castillo, J. (2019). Role of corneal nerves in ocular surface homeostasis and disease. *Acta ophthalmologica*, 97(2), 137–145.

2. Vincent A. L. (2014). Corneal dystrophies and genetics in the International Committee for Classification of Corneal Dystrophies era: a review. *Clinical & experimental ophthalmology*, 42(1), 4–12.

3. Bahn, C. F., Falls, H. F., Varley, G. A., Meyer, R. F., Edelhauser, H. F., & Bourne, W. M. (1984). Classification of corneal endothelial disorders based on neural crest origin. *Ophthalmology*, 91(6), 558–563.

4. Elhalis, H., Azizi, B., & Jurkunas, U. V. (2010). Fuchs endothelial corneal dystrophy. *The ocular surface*, 8(4), 173–184.

5. Eghrari, A. O., & Gottsch, J. D. (2010). Fuchs' corneal dystrophy. *Expert review of ophthalmology, 5*(2), 147–159.

6. Graves B. (1924). Report to the Lang Clinical Research Committee, Royal London Ophthalmic Hospital: A Bilateral Chronic Affection of the Endothelial face of the cornea of elderly persons with an account of the technical and clinical principles of its Slit-lamp observation. *The British journal of ophthalmology, 8*(11), 502–544.

7. Maurice D. M. (1972). The location of the fluid pump in the cornea. *The Journal of physiology, 221*(1), 43–54. https://doi.org/10.1113/jphysiol.1972.sp009737

8. Bourne, W. M., Johnson, D. H., & Campbell, R. J. (1982). The ultrastructure of Descemet's membrane. III. Fuchs' dystrophy. *Archives of ophthalmology (Chicago, Ill. : 1960), 100*(12), 1952–1955.

9. Vedana, G., Villarreal, G., Jr, & Jun, A. S. (2016). Fuchs endothelial corneal dystrophy: current perspectives. *Clinical ophthalmology (Auckland, N.Z.), 10,* 321–330.

10. Waring, G. O., 3rd, Bourne, W. M., Edelhauser, H. F., & Kenyon, K. R. (1982). The corneal endothelium. Normal and pathologic structure and function. *Ophthalmology, 89*(6), 531–590.

11. Wilson, S. E., & Bourne, W. M. (1988). Fuchs' dystrophy. *Cornea, 7*(1), 2–18.

12. Afshari, N. A., Pittard, A. B., Siddiqui, A., & Klintworth, G. K. (2006). Clinical study of Fuchs corneal endothelial dystrophy leading to penetrating keratoplasty: a 30-year experience. *Archives of ophthalmology (Chicago, Ill. : 1960), 124*(6), 777–780.

13. Gottsch, J. D., Sundin, O. H., Liu, S. H., Jun, A. S., Broman, K. W., Stark, W. J., Vito, E. C., Narang, A. K., Thompson, J. M., & Magovern, M. (2005). Inheritance of a novel COL8A2 mutation defines a distinct early-onset subtype of fuchs corneal dystrophy. *Investigative ophthalmology & visual science, 46*(6), 1934–1939.

14. Jalimarada, S. S., Ogando, D. G., Vithana, E. N., & Bonanno, J. A. (2013). Ion transport function of SLC4A11 in corneal endothelium. *Investigative ophthalmology & visual science, 54*(6), 4330–4340.

15. Soumittra, N., Loganathan, S., Madhavan, D. *et al.* (2014). Biosynthetic and functional defects in newly identified *SLC4A11* mutants and absence of *COL8A2* mutations in Fuchs endothelial corneal dystrophy. *J Hum Genet* 59**,** 444–453.

16. Hogan, M. J., Wood, I., & Fine, M. (1974). Fuchs' endothelial dystrophy of the cornea. 29th Sanford Gifford Memorial lecture. *American journal of ophthalmology, 78*(3), 363–383.

17. Geroski, D. H., Matsuda, M., Yee, R. W., & Edelhauser, H. F. (1985). Pump function of the human corneal endothelium. Effects of age and cornea guttata. *Ophthalmology, 92*(6), 759–763.

18. Burns, R. R., Bourne, W. M., & Brubaker, R. F. (1981). Endothelial function in patients with cornea guttata. *Investigative ophthalmology & visual science, 20*(1), 77–85.

19. Wilson, S. E., Bourne, W. M., O'Brien, P. C., & Brubaker, R. F. (1988). Endothelial function and aqueous humor flow rate in patients with Fuchs' dystrophy. *American journal of ophthalmology, 106*(3), 270–278.

20. Bergmanson, J. P., Sheldon, T. M., & Goosey, J. D. (1999). Fuchs' endothelial dystrophy: a fresh look at an aging disease. *Ophthalmic & physiological optics : the journal of the British College of Ophthalmic Opticians*

(Optometrists), 19(3), 210–222.

21. McCartney, M. D., Wood, T. O., & McLaughlin, B. J. (1989). Moderate Fuchs' endothelial dystrophy ATPase pump site density. *Investigative ophthalmology & visual science*, 30(7), 1560–1564.

22. Jurkunas, U. V., Bitar, M. S., Funaki, T., & Azizi, B. (2010). Evidence of oxidative stress in the pathogenesis of Fuchs endothelial corneal dystrophy. *The American journal of pathology*, 177(5), 2278–2289.

23. Jurkunas, U.V., Rawe, I., Bitar, M.S., et al. (2008). Decreased expression of peroxiredoxins in Fuchs endothelial dystrophy. Invest Ophthalmol Vis Sci, 49(7):2956-2963.

24. Buddi, R., Lin, B., Atilano, S.R., Zorapapel, N.C., Kenney, M.C., Brown, D.J., (2002). Evidence of oxidative stress in human corneal diseases. J Histochem Cytochem, 50(3):341-351.

25. Kitagawa, K., Kojima, M., Sasaki, H., Shui, Y. B., Chew, S. J., Cheng, H. M., Ono, M., Morikawa, Y., & Sasaki, K. (2002). Prevalence of primary cornea guttata and morphology of corneal endothelium in aging Japanese and Singaporean subjects. *Ophthalmic research*, 34(3), 135–138.

26. Zoega, G. M., Fujisawa, A., Sasaki, H., Kubota, A., Sasaki, K., Kitagawa, K., & Jonasson, F. (2006). Prevalence and risk factors for cornea guttata in the Reykjavik Eye Study. *Ophthalmology*, 113(4), 565–569.

27. Solberg, Y., Rosner, M., & Belkin, M. (1998). The association between cigarette smoking and ocular diseases. *Survey of ophthalmology*, 42(6), 535–547.

28. Cheng, A. C., Pang, C. P., Leung, A. T., Chua, J. K., Fan, D. S., & Lam, D. S. (2000). The association between cigarette smoking and ocular diseases. *Hong Kong medical journal,*

6(2), 195–202.

29. Lois, N., Abdelkader, E., Reglitz, K., Garden, C., & Ayres, J. G. (2008). Environmental tobacco smoke exposure and eye disease. *The British journal of ophthalmology*, 92(10), 1304–1310.

30. Galor, A., & Lee, D. J. (2011). Effects of smoking on ocular health. *Current opinion in ophthalmology*, 22(6), 477–482.

31. Ye, J., He, J., Wang, C., et al. (2012). Smoking and risk of age-related cataract: a meta-analysis. Invest Ophthalmol Vis Sci, 53(7): 3885–3895.

32. Hatou, S., Shimmura, S., Shimazaki, J., Usui, T., Amano, S., Yokogawa, H., Kobayashi, A., Zheng, X., Shiraishi, A., Ohashi, Y., Inatomi, T., & Tsubota, K. (2011). Mathematical projection model of visual loss due to fuchs corneal dystrophy. *Investigative ophthalmology & visual science*, 52(11), 7888–7893.

33. Sarnicola, C., Farooq, A. V., & Colby, K. (2019). Fuchs Endothelial Corneal Dystrophy: Update on Pathogenesis and Future Directions. *Eye & contact lens*, 45(1), 1–10.

34. Nanda, G. G., & Alone, D. P. (2019). REVIEW: Current understanding of the pathogenesis of Fuchs' endothelial corneal dystrophy. *Molecular vision*, 25, 295–310.

35. Amin, S. R., Baratz, K. H., McLaren, J. W., & Patel, S. V. (2014). Corneal abnormalities early in the course of Fuchs' endothelial dystrophy. *Ophthalmology*, 121(12), 2325–2333.

36. Moshirfar, M., Somani, A. N., Vaidyanathan, U., & Patel, B. C. (2021). Fuchs Endothelial Dystrophy. In *StatPearls*. StatPearls Publishing.

37. Krachmer, J. H., Purcell, J. J., Jr, Young, C. W., & Bucher, K. D. (1978). Corneal endothelial

dystrophy. A study of 64 families. *Archives of ophthalmology*, 96(11), 2036–2039.

38. Iliff, B. W., Riazuddin, S. A., & Gottsch, J. D. (2012). The genetics of Fuchs' corneal dystrophy. *Expert review of ophthalmology*, 7(4), 363–375.

39. Sundin, O.H., Jun, A.S, Broman, K.W., *et al.* (2006). Linkage of late-onset Fuchs corneal dystrophy to a novel locus at 13pTel-13q12.13. *Invest.* Ophthalmol. Vis. Sci. 47(1), 140–145.

40. Sundin, O. H., Broman, K. W., Chang, H. H., Vito, E. C., Stark, W. J., & Gottsch, J. D. (2006). A common locus for late-onset Fuchs corneal dystrophy maps to 18q21.2-q21.32. *Investigative ophthalmology & visual science*, 47(9), 3919–3926.

41. Riazuddin,S.A., Eghrari, A.O., Al-Saif, A., *et al.* (2009). Linkage of a mild late-onset phenotype of Fuchs corneal dystrophy to a novel locus at 5q33.1-q35.2. *Invest. Ophthalmol. Vis. Sci.* 50(12), 5667–5671.

42. Riazuddin, S. A., Zaghloul, N. A., Al-Saif, A., Davey, L, Diplas, B. H., Meadows, D. N., Eghrari, A. O., Minear, M. A., Li, Y. J., Klintworth, G. K., Afshari, N., Gregory, S. G., Gottsch, J. D., & Katsanis, N. (2010). Missense mutations in TCF8 cause late-onset Fuchs corneal dystrophy and interact with FCD4 on chromosome 9p. *American journal of human genetics*, 86(1), 45–53..

43. Wieben, E.D., Aleff, R.A., Tosakulwong, N., et al. (2012). A common trinucleotide repeat expansion within the transcription factor 4 (TCF4, E2-2) gene predicts Fuchs corneal dystrophy. *PLoS One.*7(11):5–12.

44. Baratz, K. H., Tosakulwong, N., Ryu, E., Brown, W. L., Branham, K., Chen, W., Tran, K. D., Schmid-Kubista, K. E., Heckenlively, J. R., Swaroop, A., Abecasis, G., Bailey, K. R., &

Edwards, A. O. (2010). E2-2 protein and Fuchs's corneal dystrophy. *The New England journal of medicine*, 363(11), 1016–1024.

45. Mehta, J. S., Vithana, E. N., Tan, D. T., Yong, V. H., Yam, G. H., Law, R. W., Chong, W. G., Pang, C. P., & Aung, T. (2008). Analysis of the posterior polymorphous corneal dystrophy 3 gene, TCF8, in late-onset Fuchs endothelial corneal dystrophy. *Investigative ophthalmology & visual science*, 49(1), 184–188.

46. Kobayashi, A., Fujiki, K., Murakami, A., Kato, T., Chen, L. Z., Onoe, H., Nakayasu, K., Sakurai, M., Takahashi, M., Sugiyama, K., & Kanai, A. (2004). Analysis of COL8A2 gene mutation in Japanese patients with Fuchs' endothelial dystrophy and posterior polymorphous dystrophy. *Japanese journal of ophthalmology*, 48(3), 195–198.

47. Chaurasia, S., Ramappa, M., Annapurna, M., & Kannabiran, C. (2020). Coexistence of Congenital Hereditary Endothelial Dystrophy and Fuchs Endothelial Corneal Dystrophy Associated With SLC4A11 Mutations in Affected Families. *Cornea*, 39(3), 354–357.

48. Vilas, G. L., Loganathan, S. K., Liu, J., Riau, A. K., Young, J. D., Mehta, J. S., Vithana, E. N., & Casey, J. R. (2013). Transmembrane water-flux through SLC4A11: a route defective in genetic corneal diseases. *Human molecular genetics*, 22(22), 4579–4590.

49. Tang, H., Zhang, W., Yan, X. M., Wang, L. P., Dong, H., Shou, T., Lei, H., & Guo, Q. (2016). Analysis of SLC4A11, ZEB1, LOXHD1, COL8A2 and TCF4 gene sequences in a multi-generational family with late-onset Fuchs corneal dystrophy. *International journal of molecular medicine*, 37(6), 1487–1500.

50. Weiss, J.S., Moller, H.U., Aldave, A.J., Seitz, B., Bredrup, C., Kivela, T., Munier, F.L., Rapuano, C.J., Nischal, K.K., Kim, E.K., Sutphin, J., Busin,

M., Labbe, A., Kenyon, K.R., Kinoshita, S., Lisch, W., (2015). IC3D classification of corneal dystrophies–edition 2. Cornea. 34(2):117-59.

51. Jalimarada, S. S., Ogando, D. G., Vithana, E. N., & Bonanno, J. A. (2013). Ion transport function of SLC4A11 in corneal endothelium. *Investigative ophthalmology & visual science*, *54*(6), 4330–4340.

52. Zhang, W., Ogando, D. G., Bonanno, J. A., & Obukhov, A. G. (2015). Human SLC4A11 Is a Novel NH3/H+ Co-transporter. *The Journal of biological chemistry*, *290*(27), 16894–16905.

53. Zhang, W., Li, H., Ogando, D. G., Li, S., Feng, M., Price, F. W., Jr, Tennessen, J. M., & Bonanno, J. A. (2017). Glutaminolysis is Essential for Energy Production and Ion Transport in Human Corneal Endothelium. *EBioMedicine*, *16*, 292–301.

54. McCartney, M. D., Robertson, D. P., Wood, T. O., & McLaughlin, B. J. (1987). ATPase pump site density in human dysfunctional corneal endothelium. *Investigative ophthalmology & visual science*, *28*(12), 1955–1962.

55. Hemadevi, B., Srinivasan, M., Arunkumar, J., Prajna, M.V., Sundaresan, P., (2010) Genetic analysis of patients with Fuchs endothelial corneal dystrophy in India. BMC ophthalmology. 10(3): 1471-1476.

56. Riazuddin, S. A., Vithana, E. N., Seet, L. F., Liu, Y., Al-Saif, A., Koh, L. W., Heng, Y. M., Aung, T., Meadows, D. N., Eghrari, A. O., Gottsch, J. D., & Katsanis, N. (2010). Missense mutations in the sodium borate cotransporter SLC4A11 cause late-onset Fuchs corneal dystrophy. *Human mutation*, *31*(11), 1261–1268. https://doi.org/10.1002/humu.21356

57. Vithana, E. N., Morgan, P. E., Ramprasad, V.,

Tan, D. T., Yong, V. H., Venkataraman, D., Venkatraman, A., Yam, G. H., Nagasamy, S., Law, R. W., Rajagopal, R., Pang, C. P., Kumaramanickevel, G., Casey, J. R., & Aung, T. (2008). SLC4A11 mutations in Fuchs endothelial corneal dystrophy. *Human molecular genetics*, *17*(5), 656–666.

58. Skorodumova, L.O., Belodedova, A.V, Antonova, O.P., Sharova, E.I., Akopian, T.A., Selezneva, O.V., Kostryukova, E.S., Malyugin, B.E., (2018). CTG18.1 Expansion is the best classifier of late-onset Fuch's corneal dystrophy among 10 biomarkers in a cohort from the European part of Russia. Invest Opthal and vis sci. 59:4748-4754

59. Minear, M. A., Li, Y. J., Rimmler, J., Balajonda, E., Watson, S., Allingham, R. R., Hauser, M. A., Klintworth, G. K., Afshari, N. A., & Gregory, S. G. (2013). Genetic screen of African Americans with Fuchs endothelial corneal dystrophy. *Molecular vision*, *19*, 2508–2516.

60. Aldave, A. J., Yellore, V. S., Bourla, N., Momi, R. S., Khan, M. A., Salem, A. K., Rayner, S. A., Glasgow, B. J., & Kurtz, I. (2007). Autosomal recessive CHED associated with novel compound heterozygous mutations in SLC4A11. *Cornea*, *26*(7), 896–900.

Figure 1: PRISMA flow diagram summarising the screening method and study selection process
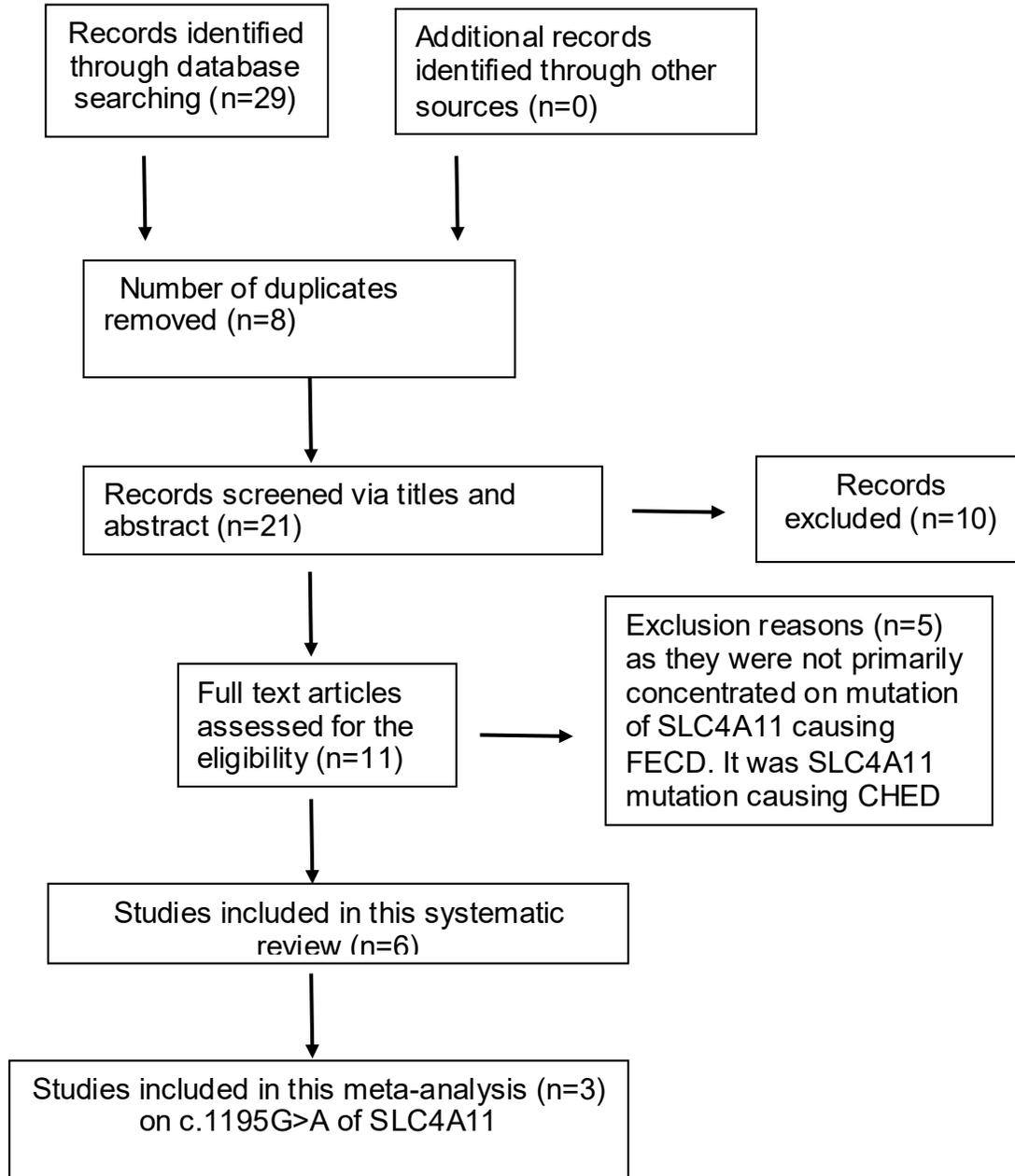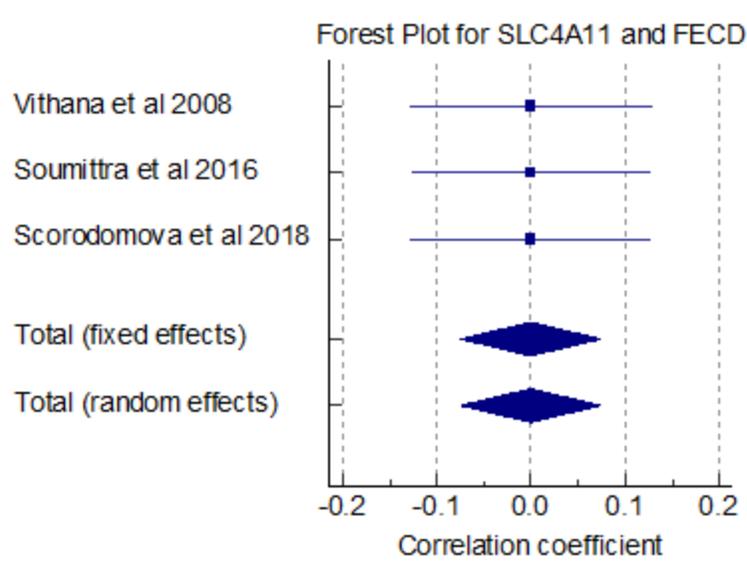
Fig 2: Forest plot for the association of c.1195G>A allele of SLC4A11 and FECD
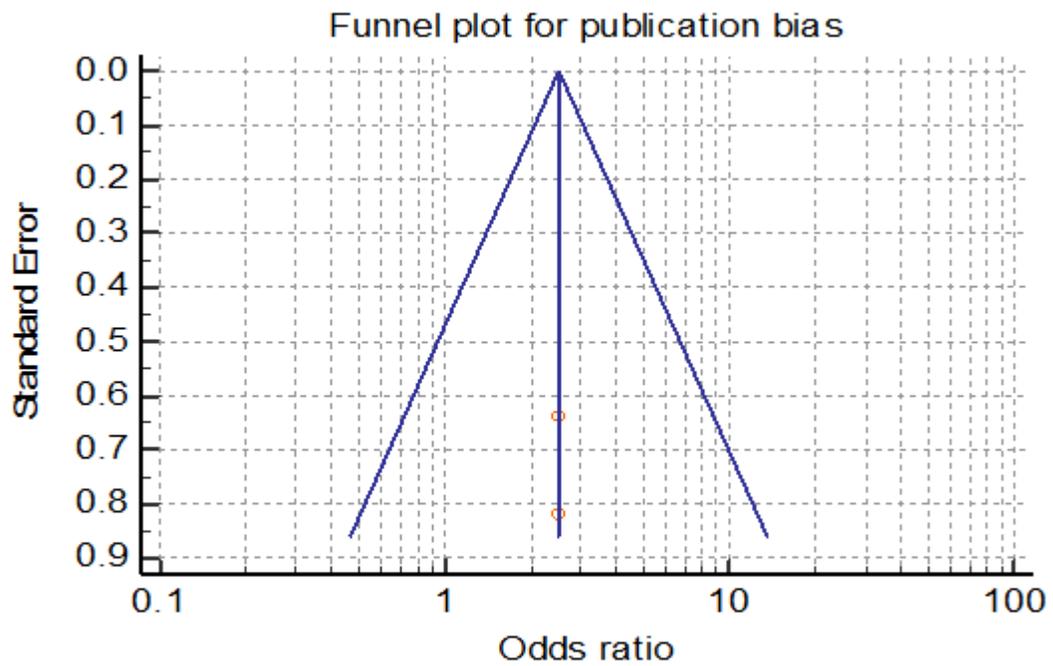
Fig 3: Publication bias assessment by Funnel Plot

Funnel plot for publication bias

Table 1: Showing the results of heterogeneity testing and publication bias

Th

Variable for studies	Study
1. Intervention groups	
Variable for total number of cases	238
Variable for number of positive cases	case_P
2. Control groups	
Variable for total number of cases	594
Variable for number of positive cases	case_P

Study	Intervention	Controls	Odds ratio	95% CI	z	P	Weight (%) Fixed	Weight (%) Random
Vithana et al 2008	3/238	3/594	2.515	0.504 to 12.550			37.64	37.64
Soumittra et al 2016	5/238	5/594	2.528	0.725 to 8.813			62.36	62.36
Scorodomova et al 2018	0/238	0/594	-					
Total (fixed effects)	8/714	8/1782	2.523	0.941 to 6.764	1.839	0.066	100.00	100.00
Total (random effects)	8/714	8/1782	2.523	0.941 to 6.764	1.839	0.066	100.00	100.00

Test for heterogeneity

Q	0.00002466
DF	1
Significance level	P = 0.9960
I^2 (inconsistency)	0.00%
95% CI for I^2	0.00 to 0.00

Publication bias

Egger's test	
Intercept	-0.02819
95% CI	
Significance level	P < 0.0001
Begg's test	
Kendall's Tau	-1.0000
Significance level	P = 0.3173