Metadata Analysis

Metadata analysis of the squalene epoxidase gene in dermatophytes.

Fatima Ismail¹, Iqra Ahmad² & Eqaza Javed³
Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur-Pakistan.

Abstract

Background: Squalene epoxidase gene is an azole antifungal target enzyme in the ergosterol biosynthesis pathway in fungi—the inhibition of enzyme aggregate squalene, a type of ergosterol derivative depletion that leads to fungal growth inhibition. Squalene epoxidase widely diverges in three fungal groups of dermatophytes Trichophyton, Microsporum and Epidermophyton.

Methodology: The study has demonstrated a variety of squalene epoxidase genes in the dermatophyte genome. Squalene epoxidase gene was also studied for possible single nucleotide polymorphism (SNPs) in the Trichophyton group’s homologs and found multiple SNP positions that induce non-synonymous mutation and change the sequence of amino acid into protein structure that can change phenylalanine to leucine.

Results: Squalene epoxidase is widely present in dermatophytes. Mutation in the squalene epoxidase gene can mislead normal sterol formation in the fungal cell membrane that induces fungal resistance against several drugs, including azoles and polyenes. Squalene epoxidase gene carries 2 - 3 transcripts and 2 - 3 number of exons. Squalene epoxidase gene has FAD-dependent oxidoreductases and NADP binding domain conserved domains in fungal groups of dermatophytes.

Conclusion: This study analyzed the abundance of the squalene epoxidase gene, suggesting that squalene epoxidase gene resistant mutants can occur naturally. Thus squalene epoxidase gene should be extensively studied in order to increase the potential of available antifungals.

Keywords

Squalene Epoxidase, Resistant Mutant, Trichophyton, Microsporum, Epidermophyton.
Introduction

Dermatophytes are a very specialized group of keratinophilic fungi responsible for superficial mycoses of humans and animals. Dermatophyte infections are a worldwide major health concern, specifically in an immunocompromised individual. They may pose severe risks. Dermatophytes are divided into three genera: Microsporum, Trichophyton and Epidermophyton, although the precise taxonomic associations of this fungal group are far from being fully determined. Regardless of the common occurrence of dermatophyte infections, little is known about these pathogens' molecular biology. Dermatophytes are slow-growing as compared to other more significant fungal pathogens. They are not very well studied for their metabolic reactions and stress adaptive mechanisms. However, several natural mutants are investigated in clinical and agricultural practices.

Squalene epoxidase is a monooxygenase that catalyzes the conversion of squalene to 2,3-oxidosqualene. It is a microsomal membrane-associated enzyme that functions as a significant regulator in the biosynthetic pathway of sterols. Ergosterol helps in the photo-oxidation of fungi. This enzyme needs oxygen, Nicotine amide, Adenine dinucleotide, Phosphate hydrogen and Flavin adenine dinucleotide for stimulation. Previously, terbinafine-resistant isolates have been reported in increasing number. Terbinafine inhibits the Squalene epoxidase (SE) enzyme and interferes with the ergosterol biosynthesis, an integral cell membrane constituent. Mutations have been identified within the squalene epoxidase gene like clinical terbinafine-resistant isolates.

Squalene epoxidase gene sequence

Squalene epoxidase gene from the dermatophytic fungi Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton interdigitale, Microsporum canis, Aspergillus Versicolor, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans and Candida glabrata obtained from NCBI gene sequences. Squalene epoxidase and related proteins were explored within acquired amino acid sequences of a FAD-binding domain. In the next step, FAD-binding proteins were searched against a transmembrane domain NADB. Squalene epoxidase contains a putative FAD-binding site and is a crucial enzyme in the sterol biosynthetic pathway. Putative transmembrane regions are found in the proteins C-terminus. For the detection...
of some unpredicted genes, we explored raw genomic sequences using NCBI nucleotide blast. Sequences of all known Squalene epoxidase genes were disguised from genomic sequences. All amino acid sequences of previously identified Squalene epoxidase genes were used as queries in nucleotide sequence blast.

For all Trichophyton species, similarity ranges from 44.66%-45.34%. Query cover ranges from 95%-99%. For all Microsporum and Aspergillus species, similarity ranges from 37.34%-45.53%. The range of query cover is 84%-99%.

**Squalene epoxidase protein prediction**
The number of genes contained FAD and NABD binding domain, which was similar in all species of dermatophytes. These domains have an alpha-beta-alpha configuration. NAD binding involves numerous hydrogen and van der Waals contacts.

**Transcript Exon and intron analysis**
Several exons and introns of different species of dermatophytes were obtained from NCBI. A single gene can produce multiple different RNAs (transcripts). Similarly, the numbers of transcripts were also obtained from the CBI database (https://www.ncbi.nlm.nih.gov/).

**Squalene epoxidase gene Phylogenetic Analysis**
A sequence alignment was done with ClustalX. Squalene epoxidase genes were determined by a conserved domain database. Phylogenetic analysis was made in MEGA6. Phylogenetic analysis was done using maximum possibility and neighbourhood joining method.

**Single nucleotide polymorphism**
Single nucleotide polymorphism (SNPs) analysis was found in the Squalene epoxidase gene of the Trichophyton group of dermatophytes. SNPs were obtained from NCBI and compared with the reported.

**Results**

**Bioinformatics survey of Squalene epoxidase gene**
The squalene epoxidase gene was analyzed using different bioinformatics tools. The comparison was made based on percentage similarity, exon count, conserved domains and query cover. Sequences were aligned, and phylogenetic trees were constructed. The squalene epoxidase gene in dermatophytes has an average sequence length of 489 amino acids.

**Squalene epoxidase protein prediction**
NAD binding involves numerous hydrogen-bonds and van der Waals contacts, particularly H-bonding of residues in turn between the first strand and the subsequent helix of the Rossmann-fold topology. Characteristically, this turn exhibits a consensus binding pattern. The first 2 glycines participate in NAD(P)-binding, and the third facilitate close packing of the helix to the beta-strand (https://go.usa.gov/xARNY).

**Prediction of conserved domains of Squalene epoxidase**
In this study, we used genomic sequences of all available dermatophyte species. The first step is the identification of all Squalene epoxidase genes within their genomes. Squalene epoxidase consists of two types of domain, FAD and NADB. The identified genes were containing FAD and NABD binding domain. The E value of 1e-135 was used. Query cover ranges from 95% to 99%. The length of the amino acid sequence was 489 bp. Percentage similarity was very low such as 45.34% for Trichophyton rubrum. Trichophyton rubrum has the highest percentage similarity to other Trichophyton species, as shown in table 1.
Table 1: Paralogs of Squalene epoxidase gene

| S. no | Species                        | Accession no  | Query cover | E-value | Percentage similarity | Amino acid sequence length | Conserved domains                                      |
|-------|--------------------------------|---------------|-------------|---------|------------------------|---------------------------|--------------------------------------------------------|
| 1     | squalene epoxidase [Trichophyton mentagrophytes] | APQ47162.1   | 99%         | 5e-136  | 44.90                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 2     | squalene epoxidase [Trichophyton mentagrophytes] | ATA67033.2   | 99%         | 7e-136  | 44.90                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 3     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32792.1   | 99%         | 1e-135  | 44.90                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 4     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32790.1   | 99%         | 4e-135  | 44.90                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 5     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32789.1   | 99%         | 5e-135  | 44.69                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 6     | squalene epoxidase [Trichophyton mentagrophytes] | ATA67030.2   | 99%         | 6e-135  | 44.69                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 7     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32791.1   | 99%         | 6e-135  | 44.69                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 8     | squalene epoxidase [Trichophyton mentagrophytes] | QHO61327.1   | 98%         | 1e-134  | 44.66                  | 487                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 9     | squalene epoxidase [Trichophyton mentagrophytes] | QGA31127.1   | 95%         | 1e-129  | 44.82                  | 453                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 10    | squalene epoxidase [Trichophyton mentagrophytes] | QIB02548.1   | 95%         | 2e-129  | 44.82                  | 453                       | FAD-dependent oxidoreductase, NADP binding proteins     |
| 11    | squalene epoxidase [Trichophyton mentagrophytes] | XP_00323384 5.1 | 99%    | 5e-136  | 45.34                  | 489                       | FAD-dependent oxidoreductase, NADP binding proteins     |
|   | Squalene epoxidase [Trichophyton rubrum] | AAQ18216.1 | 99% | 1e-135 | 45.12 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
|---|----------------------------------------|-------------|------|--------|-------|-----|--------------------------------------------------|
| 12|                                        | AAZ08563.1  | 99%  | 4e-135 | 45.12 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
| 13|                                        | ATA67047.1  | 99%  | 1e-135 | 44.90 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
| 14|                                        | ATA67045.1  | 99%  | 2e-135 | 44.90 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
| 15|                                        | ATA67048.1  | 99%  | 2e-134 | 44.69 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
| 16|                                        | AVR57463.2  | 99%  | 1e-135 | 44.90 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
| 17|                                        | AVU05317.1  | 99%  | 3e-135 | 44.69 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
| 18|                                        | AVU05318.1  | 99%  | 5e-135 | 44.69 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |
| 19|                                        | QBL54211.1  | 98%  | 2e-134 | 44.66 | 487 | FAD-dependent oxidoreductase, NADP binding proteins |
| 20|                                        | QBL54209.1  | 98%  | 5e-134 | 44.66 | 487 | FAD-dependent oxidoreductase, NADP binding proteins |
| 21|                                        | EGD99021.1  | 99%  | 7e-136 | 44.90 | 489 | FAD-dependent oxidoreductase, NADP binding proteins |

rubrum CBS 118892]
Prediction of exon count and number of transcripts

An exon is a coding region of a gene that contains the information necessary to encode a protein. Numbers of exons of different species of dermatophytes were obtained from NCBI. A single gene can produce multiple different RNAs (transcripts). All squalene epoxidase genes generally contain 2 to 3 transcripts. Similarly, the numbers of transcripts were also obtained from NCBI's genomic regions, as shown in table 2.

| S. no | Species | Accession no | No of exons | Transcript no |
|-------|---------|--------------|-------------|---------------|
| 1     | squalene epoxidase [Trichophyton mentagrophytes] | APQ47162.1 | 2           | 3             |
| 2     | squalene epoxidase [Trichophyton mentagrophytes] | ATA67033.2 | 1           | 2             |
| 3     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32792.1 | 2           | 3             |
| 4     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32790.1 | 2           | 3             |
| 5     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32789.1 | 2           | 3             |
| 6     | squalene epoxidase [Trichophyton mentagrophytes] | ATA67030.2 | 1           | 2             |
| 7     | squalene epoxidase [Trichophyton mentagrophytes] | QIJ32791.1 | 2           | 3             |
| 8     | squalene epoxidase [Trichophyton mentagrophytes] | QHO61327.1 | 2           | 2             |
| 9     | squalene epoxidase [Trichophyton mentagrophytes] | QGA31127.1 | 2           | 3             |
| 10    | squalene epoxidase [Trichophyton mentagrophytes] | QIB02548.1 | 2           | 3             |
| 11    | squalene epoxidase [Trichophyton rubrum CBS 118892] | XP_003233845.1 | 2 | 3             |
| 12    | squalene epoxidase [Trichophyton rubrum] | AAQ18216.1 | 2           | 2             |
| 13    | squalene epoxidase [Trichophyton rubrum] | AAZ08563.1 | 2           | 3             |
| 14    | squalene epoxidase [Trichophyton rubrum] | ATA67047.1 | 2           | 3             |
| 15    | squalene epoxidase [Trichophyton rubrum] | ATA67045.1 | 2           | 3             |
| 16    | squalene epoxidase [Trichophyton rubrum] | ATA67048.1 | 2           | 3             |
| 17    | squalene epoxidase [Trichophyton interdigitale] | AVR57463.2 | 1           | 2             |
| 18    | squalene epoxidase [Trichophyton interdigitale] | AVU05317.1 | 2           | 3             |
| 19    | squalene epoxidase [Trichophyton interdigitale] | AVU05318.1 | 2           | 3             |
| 20 | squalene epoxidase [Trichophyton interdigitale] | QBL54211.1 | 2 | 2 |
| 21 | squalene epoxidase [Trichophyton interdigitale] | QBL54209.1 | 2 | 2 |
| 22 | squalene epoxidase [Trichophyton tonsurans CBS 112818] | EGD99021.1 | 2 | 3 |

**Figure 1:** Multiple sequence alignment of amino acid sequences of 22 species were performed using **clustalx**. Phylogenetic tree of paralogs of squalene epoxidase protein sequences of various dermatophytes. The construction of the tree was performed with Mega 6. Sequences are annotated with their GenBank accession numbers.
Orthologs of Squalene epoxidase

Similarly for orthologs, Squalene epoxidase gene was compared with Microsporum canis, Aspergillus versicolor, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans and Candida glabrata. Conserved domain database search demonstrated the existence of conserved domain of squalene epoxidase gene. Another functional domain found was NADP binding domain, also recognized as Rossmann fold. Amino acid sequence length varies among different species from 405 bp to 512 bp. For orthologs, Aspergillus fumigatus has highest similarity for ERG1. Percentage similarity was low compared to paralogs of ERG1, and query cover was 98%, as shown in table 3.

**Table 3: Orthologs of Squalene epoxidase gene.**

| S. no | Species name                      | Accession no. | Query cover | E-value | Percentage similarity | Amino acid sequence length | Conserved domains                  |
|-------|----------------------------------|---------------|-------------|---------|------------------------|---------------------------|-----------------------------------|
| 1     | squalene epoxidase [Microsporum canis CBS 113480] | XP_002849431.1 | 84%         | 1e-106  | 42.86                  | 425                       | FAD-dependent oxidoreductases, NADP binding proteins |
| 2     | squalene epoxidase [Microsporum canis] | BBC18146.1    | 84%         | 4e-105  | 42.35                  | 425                       | FAD-dependent oxidoreductases, NADP binding proteins |
| 3     | squalene monooxygenase [Nannizzia gypsea CBS 118893] | XP_003170113.1 | 99%         | 2e-134  | 44.57                  | 489                       | FAD-dependent oxidoreductases, NADP binding proteins |
| 4     | hypothetical protein ASPVEDRAFT_52898 [Aspergillus versicolor CBS 583.65] | OJJ02152.1    | 98%         | 1e-114  | 43.11                  | 1427                      | FAD-dependent oxidoreductases, NADP binding proteins |
| 5     | squalene monooxygenase Erg1 [Aspergillus flavus NRRL3357] | XP_002378894.1 | 98%         | 1e-128  | 42.98                  | 480                       | FAD-dependent oxidoreductases, NADP binding proteins |
| 6     | squalene epoxidase [Aspergillus flavus] | RAQ67502.1    | 98%         | 2e-128  | 42.98                  | 480                       | FAD-dependent oxidoreductases, NADP binding proteins |
| 7     | squalene monooxygenase Erg1 [Aspergillus flavus AF70] | KOCl2150.1    | 98%         | 4e-128  | 42.76                  | 480                       | FAD-dependent oxidoreductases, NADP binding proteins |
| 8     | squalene epoxidase [Aspergillus flavus] | RAQ47162.1    | 98%         | 8e-128  | 42.76                  | 480                       | FAD-dependent oxidoreductases, NADP binding proteins |
| 9     | squalene monooxygenase Erg1 | XP_753858.1 | 98%         | 2e-130  | 44.93                  | 481                       | FAD-dependent oxidoreductases, |
| 10 | Squalene monooxygenase Erg1  
[Aspergillus fumigatus Z5] | KMK59426.1 | 98% | 4e-130 | 44.71 | 481 | FAD-dependent oxidoreductases, NADP binding proteins |
| 11 | Hypothetical protein  
CNMCM8812_000993  
[Aspergillus fumigatus] | KAF4275551.1 | 98% | 5e-128 | 44.93 | 481 | FAD-dependent oxidoreductases, NADP binding proteins |
| 12 | Squalene epoxidase 1  
[Aspergillus fumigatus] | AAS60234.1 | 79% | 5e-106 | 45.53 | 405 | FAD-dependent oxidoreductases, NADP binding proteins |
| 13 | Squalene epoxidase  
[Aspergillus niger CBS 513.88] | XP_001390258.1 | 98% | 2e-130 | 45.41 | 512 | FAD-dependent oxidoreductases, NADP binding proteins |
| 14 | SE-domain-containing protein  
[Aspergillus niger CBS 101883] | XP_025456429.1 | 98% | 8e-131 | 45.41 | 478 | FAD-dependent oxidoreductases, NADP binding proteins |
| 15 | Squalene monooxygenase Erg1  
[Aspergillus fischeri NRRL 181] | XP_001259905.1 | 98% | 1e-126 | 44.93 | 481 | FAD-dependent oxidoreductases, NADP binding proteins |
| 16 | Squalene monooxygenase  
[Candida albicans P94015] | KGQ90519.1 | 99% | 3e-115 | 38.71 | 496 | FAD-dependent oxidoreductases, NADP binding proteins |
| 17 | Squalene monooxygenase  
[Candida albicans SC5314] | XP_711894.1 | 99% | 3e-115 | 38.71 | 496 | FAD-dependent oxidoreductases, NADP binding proteins |
| 18 | Squalene monooxygenase  
[Candida albicans Ca529L] | RLP62132.1 | 99% | 3e-115 | 38.71 | 496 | FAD-dependent oxidoreductases, NADP binding proteins |
| 19 | Uncharacterized protein  
CAGL0D05940g  
[[Candida glabrata]] | XP_445667.1 | 98% | 1e-106 | 37.34 | 489 | FAD-dependent oxidoreductases, NADP binding proteins |
| 20 | Squalene monooxygenase  
[Candida tropicalis MYA-3404] | XP_002551185.1 | 98% | 9e-115 | 38.98 | 497 | FAD-dependent oxidoreductases, NADP binding proteins |
Predicting exon count, a number of transcripts and chromosome location.
Numbers of exons were obtained from NCBI from a genomic context. All aspergillus species have only one gene transcript. Numbers of transcripts were also obtained from genomic regions from NCBI. The chromosome of almost all species was unknown except Aspergillus fumigatus, located on chromosome no 5 and Candida albicans are located on chromosome no 1 as shown in table 4.

Table 4: Exon count, number of transcripts and chromosomal location of orthologs of Squalene epoxidase gene.

| S. no | Species                                  | Accession no            | No of exons | No of transcripts | Chromosome no |
|-------|------------------------------------------|-------------------------|-------------|------------------|---------------|
| 1     | squalene epoxidase [Microsporum canis CBS 113480] | XP_002849431.1          | 3           | 2                | Unknown       |
| 2     | squalene epoxidase [Microsporum canis]    | BBC18146.1              | 3           | 2                | Unknown       |
| 3     | squalene monooxygenase [Nannizzia gypsea CBS 118893] | XP_003170113.1          | 2           | 2                | Unknown       |
| 4     | hypothetical protein ASPVEDRAFT_52898 [Aspergillus Versicolor CBS 583.65] | OJJ02152.1              | 3           | 1                | Unknown       |
| 5     | squalene monooxygenase Erg1 [Aspergillus flavus NRRL3357] | XP_002378894.1          | 2           | 1                | Unknown       |
| 6     | squalene epoxidase [Aspergillus flavus]   | RAQ67502.1              | 2           | 1                | Unknown       |
| 7     | squalene monooxygenase Erg1 [Aspergillus flavus AF70] | KOC12150.1              | 2           | 1                | Unknown       |
| 8     | squalene epoxidase [Aspergillus flavus]   | RAQ47162.1              | 2           | 1                | Unknown       |
| 9     | squalene monooxygenase Erg1 [Aspergillus fumigatus Af293] | XP_753858.1             | 2           | 1                | Chromosome 5  |
| 10    | squalene monooxygenase Erg1 [Aspergillus fumigatus Z5] | KMK59426.1              | 2           | 1                | Chromosome 5  |
| 11    | hypothetical protein CNMCM8812_000993 [Aspergillus fumigatus] | KAF4275551.1            | 2           | 1                | Unknown       |
| 12    | squalene epoxidase 1 [Aspergillus fumigatus] | AAS60234.1              | 2           | 1                | Unknown       |
| 13    | squalene epoxidase [Aspergillus niger CBS 513.88] | XP_001390258.1          | 2           | 1                | Unknown       |
| 14    | SE-domain-containing protein [Aspergillus niger CBS 101883] | XP_025456429.1          | 2           | 1                | Unknown       |
| 15    | squalene monooxygenase Erg1 [Aspergillus fischeri NRRL 181] | XP_001259905.1          | 2           | 1                | Unknown       |
| 16    | squalene monooxygenase [Candida albicans P94015] | KGQ90519.1              | 1           | 1                | Chromosome 1  |
Figure 2: Phylogenetic tree of orthologs of squalene epoxidase amino acid sequences. The tree was constructed using the neighbour-joining method in MEGA6 software. Protein sequences were obtained from the Gene bank.
Single nucleotide polymorphisms (SNPs) in Squalene epoxidase gene

Single nucleotide polymorphisms (SNPs) have been identified in the squalene epoxidase gene of the Trichophyton group. Resistance in the gene encoding squalene epoxidase is attributable to point mutations. The mutation was observed in *Trichophyton interdigital* and *Trichophyton rubrum* isolates. *Trichophyton interdigital* nucleotide substitution in the Squalene epoxidase gene was CTC, which was substituted into TTC at 1184. Similarly, another substitution was found at position 1185, leading to the replacement of TTC by CTC. For *Trichophyton rubrum*, a substitution occurred at position 1380, leading to the replacement of TTC by CTC. Another substitution was at position 1175, leading to the replacement of TTC by TTA in the squalene epoxidase gene, as shown in table 5.

**Table 5. SNPs in Squalene epoxidase gene of Trichophyton group.**

| Species                     | Accession no | Nucleotide substitution in SQLE gene | SNPs in this study | Reported 12 (Burmester, Hipler et al. 2020) |
|----------------------------|--------------|-------------------------------------|-------------------|------------------------------------------|
| Squalene epoxidase [Trichophyton mentagrophytes] | MT371234.1   | TTG→TTC                             | 1184              | 1189                                     |
| Squalene epoxidase [Trichophyton mentagrophytes] | MT371233.1   | TTG→TTC                             | 1184              | 1189                                     |
| Squalene epoxidase [Trichophyton mentagrophytes] | MT371235.1   | CTC→TTC                             | 1184              | 1189                                     |
| Squalene epoxidase, partial [Trichophyton interdigital] | QBL54211.1  | TTC→CTC                             | 1185              | 1189                                     |
| Squalene epoxidase, partial [Trichophyton interdigital] | QBL54209.1  | TTC→CTC                             | 1185              | 1189                                     |
| Squalene epoxidase [Trichophyton rubrum] | AAQ18216.1   | CAT→TAT                             | 1380              | 1380                                     |
| Squalene epoxidase [Trichophyton rubrum] | AAZ08563.1   | TTT→TTA                             | 1197              | 1177                                     |
| Squalene epoxidase [Trichophyton rubrum] | ATA67047.1   | TTC→TTA                             | 1175              | 1189                                     |
| Squalene epoxidase [Trichophyton rubrum] | ATA67045.1   | TTC→TTA                             | 1175              | 1189                                     |
| squalene epoxidase [Trichophyton rubrum] | ATA67048.1   | TAT→CAT                             | 1368              | 1380                                     |

**Discussion**

Squalene epoxidase is a monooxygenase that catalyzes the conversion of squalene to 2, 3 oxidosqualene. Terbinafine inhibits squalene epoxidase, a key enzyme in the ergosterol biosynthesis pathway. This inhibition contributes to squalene aggregation, ergosterol depletion and the inhibition of growth of fungus\(^\text{13}\). This study was focused on the abundance of squalene epoxidase in dermatophytes. Gene sequence Percentage similarity ranges were suggested from 44.66%-45.34%. Query cover ranges from 95% - 99%. The percentage similarity of *Trichophyton rubrum* was higher than other species of dermatophytes such as *Trichophyton*. *Trichophyton mentagrophytes* have one exon, while all other species have two exons. The number of transcripts was 2-3 for all species. Chromosomal locations of all species were
unknown except *Aspergillus fumigatus* was located on chromosome no 5\textsuperscript{14}.

The number of detected genes contained FAD and NABD binding domain, which was similar in all genomes. These domains have an alpha-beta-alpha configuration. NAD binding involves numerous hydrogen, and van der Waals contacts\textsuperscript{15}. *Trichophyton rubrum* has a percentage similarity of 45.12 with the squalene epoxidase gene. For orthologs, *Aspergillus fumigatus* has the highest percentage similarity\textsuperscript{16}. The bioinformatics survey of the squalene epoxidase gene revealed that dermatophytes comprise a closely-related and diverged lineage of the squalene epoxidase gene. A variety of divergence of squalene epoxidase gene was found in three fungal groups of dermatophytes, while squalene epoxidase homologs were not found in Epidermophyton\textsuperscript{17}.

Single-nucleotide polymorphism (SNP) is a substitution of a single nucleotide at a specific genome position. Single nucleotide polymorphisms occur within coding sequences of genes, non-coding regions of genes, or in the intergenic regions between genes. SNPs in the coding regions are of two kinds synonymous and non-synonymous SNPs. Synonymous SNPs do not affect protein or DNA sequence, while non-synonymous SNPs change the amino acid sequence of protein or DNA\textsuperscript{18}. SNPs have been identified in squalene epoxidase homologs of Trichophyton group\textsuperscript{19}. Nucleotide substitution within the squalene epoxidase gene was observed in *Trichophyton interdigitale* all isolates that substitutes TTC with CTC at 1185 position\textsuperscript{20}. In Trichophyton rubrum, nucleotide substitution occurred at 1380, leading to the replacement of CAT with TAT\textsuperscript{8}. Another substitution occurred at 1175 position leading to the replacement of TTC by TTA that was near related to previous study\textsuperscript{21} in which the nucleotide and amino acid substitution in the squalene epoxidase gene varies between homologs of the Trichophyton group.

**Conclusion**

Squalene epoxidase is an antifungal targeted key enzyme in the ergosterol biosynthesis pathway of several fungal groups. This inhibition of the gene contributes to squalene aggregation, ergosterol depletion and the inhibition of fungal growth. Studies have demonstrated a variety of squalene epoxidase genes in a major group of dermatophytes. Squalene epoxidase has widely diverged in three fungal groups of dermatophytes Trichophyton, Epidermophyton and Microsporum. Mutation in the squalene epoxidase gene can mislead normal sterol formation in the fungal cell membrane that induces fungal resistance against several drugs, including azoles and polyenes. Squalene epoxidase gene carries 2 - 3 transcripts and 2 - 3 number of exons in dermatophytes. Squalene epoxidase gene has FAD-dependent oxidoreductases and NADP binding conserved domains similar in three major fungal groups of dermatophytes. Squalene epoxidase gene was also studied for possible single nucleotide polymorphism (SNPs) in the Trichophyton group’s homologs and found multiple SNP positions on 1184, 1185 1380, 1175 and 1368 nucleotide positions of TTC→CTC. This position was the cause of non-synonymous mutation, which can change the sequence of amino acid into a protein structure that can change phenylalanine to leucine.

**Conflicts of Interest**

None.

**Acknowledgement**

The authors would like to acknowledge Dr. Sadaf Ambreen for the technical support.

**Funding**

None.

**References**

1. Teklebirhan G, Bitew A. Prevalence of Dermatophytic Infection and the Spectrum of Dermatophytes in Patients Attending a Tertiary Hospital in Addis Ababa, Ethiopia. Int J Microbiol. 2015;653419(10):13.
2. White TC, Oliver BG, Gräser Y, Henn MR. Generating and testing molecular hypotheses in the dermatophytes. Eukaryot Cell. 2008;7(8):1238-1245.
3. Motavaze K, Namvar Z, Emami M, Noorbaksh F, Rezaie S. Molecular characterization of a Squalene epoxidase gene in dermatophyte pathogen Trichophyton tonsurans. Iran Biomed J. 2008;12(1):55-58.

4. Saunte DM, Hare RK, Jørgensen KM, Jørgensen R, Deleuran M, Zachariae CO, Thomsen SF, Bjørnskov-Halkier L, Kofoed K, Arendrup MC. Emerging Terbinafine Resistance in <em>Trichophyton</em> species. Clinical Characteristics, Squalene Epoxidase Gene Mutations, and a Reliable EUCAST Method for Detection. Antimicrob Agents Chemother. 2019;63(10):e01126-19.

5. Osborne CS, Leitner I, Favre B, Ryder NS. Amino Acid Substitution in <em>Trichophyton rubrum</em> Squalene Epoxidase Associated with Resistance to Terbinafine. Antimicrob Agents Chemother. 2005;49(7):2840-2844.

6. Martínez-Rossi NM, Peres NT, Rossi A. Antifungal resistance mechanisms in dermatophytes. Mycopathologia. 2008;166(5-6):369-383.

7. Yamada T, Maeda M, Alshahni MM, Tanaka R, Yaguchi T, Bontems O, Salamin K, Fratti M, Monod M. Terbinafine Resistance of Trichophyton Clinical Isolates Caused by Specific Point Mutations in the Squalene Epoxidase Gene. Antimicrob Agents Chemother. 2017;61(7).

8. Rudramurthy SM, Shankarnarayan SA, Dogra S, Shaw D, Mushtaq K, Paul RA, Naranj T, Chakrabarti A. Mutation in the Squalene Epoxidase Gene of <em>Trichophyton</em> interdigitale and <em>Trichophyton</em> rubrum Associated with Allylamine Resistance. Antimicrob Agents Chemother. 2018;62(5):e02522-17.

9. Garaiová M, Zambojová V, Simová Z, Griač P, Hapala I. Squalene epoxidase as a target for manipulation of squalene levels in the yeast <em>Saccharomyces cerevisiae</em>. FEMS Yeast Res. 2014;14(2):310-323.

10. Padyana AK, Gross S, Jin L, Cianchetta G, Narayananswamy R, Wang F, Wang R, Fang C, Lv X, Biller SA, Dang L. Structure and inhibition mechanism of the catalytic domain of human squalene epoxidase. Nat Commun. 2019;10(1):01807928.

11. Ruckenstuhl C, Eidenberger A, Lang S, Turnowsky F. Single amino acid exchanges in FAD-binding domains of squalene epoxidase of <em>Saccharomyces cerevisiae</em> lead to either loss of functionality or terbinafine sensitivity. Biochem. Soc. Trans. 2005;33:1197-1201.

12. Yamada T, Maeda M, Alshahni MM, Tanaka R, Yaguchi T, Bontems O, Salamin K, Fratti M, Monod M. Terbinafine Resistance of Trichophyton Clinical Isolates Caused by Specific Point Mutations in the Squalene Epoxidase Gene. Antimicrob Agents Chemother. 2017;61(7).

13. Nowosielski M, Hoffmann M, Wyrwicz LS, Stepniak P, Plewczynski DM, Lazniewski M, Ginalska K, Rychlewski L. Detailed Mechanism of Squalene Epoxidase Inhibition by Terbinafine. J. Chem. Inf. Model. 2011;51:455-462.

14. Singh A, Masih A, Khurana A, Singh PK, Gupta M, Hagen F, Meis JF, Chowdhary A. High terbinafine resistance in <em>Trichophyton</em> interdigitale isolates in Delhi, India harbouring mutations in the squalene epoxidase gene. Mycoses. 2018;61(7):477-484.

15. Unland K, Püttner KM, Vorwerk K, van Deenen N, Twyman RM, Prüfer D, Schulze Gronover C. Functional characterization of squalene synthase and squalene epoxidase in <em>Trichophyton</em> cerevisiae. Plant Dir. 2018;2(6):e00063.

16. Ding C, Zhao CL, Chen ZJ, Wei FG, Song DG, Miao KR. Bioinformatics analysis of the squalene synthase gene and the amino acid sequence in ginseng species. Int J Clin Exp Med. 2015;8(8):12818-12825.

17. Ruckenstuhl C, Lang S, Poschenel A, Eidenberger A, Baral PK, Kohút P, Hapala I, Gruber K, Turnowsky F. Characterization of Squalene Epoxidase of <em>Trichophyton</em> cerevisiae by Applying Terbinafine-Sensitive Variants. Antimicrob Agents Chemother. 2007;51(1):275-284.

18. Su L, Liu G, Wang H, Tian Y, Zhou Z, Han L, Yan L. Research on single nucleotide polymorphisms interaction detection from network perspective. PLoS One. 2015;10(3):e0119146.

19. Pchelin IM, Azarov DV, Chilina GA, Dmitriev KA, Vasilyeva NV, Taraskina AE. Single-nucleotide polymorphism in a local population of <em>Trichophyton</em> rubrum. Med Mycol. 2018;56(1):125-128.

20. Shankarnarayan SA, Shaw D, Sharma A, Chakrabarti A, Dogra S, Kumanan MS, Kaur H, Ghosh A, Rudramurthy SM. Rapid detection of terbinafine resistance in <em>Trichophyton</em> species by Amplified refractory mutation system-polymerase chain reaction. Scientific Reports. 2020;10(1):1297.

21. Burmester A, Hipler UC, Uhrläß S, Nenoff P, Singal A, Verma SB, Elnser P, Wiegand C. Indian <em>Trichophyton</em> mentagrophytes squalene epoxidase erg1 double mutants show high proportion of combined fluconazole and terbinafine resistance. Mycoses. 2020;63(11):1175-1180.