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Differential gene expression analysis in patients with primary hyperhidrosis

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Abstract

Hyperhidrosis, a condition of excessive sweat generation, is believed to be genetic, but it is yet not known well whether primary hyperhidrosis is regulated by a single gene or multiple genes. The prospective genes that regulate primary hyperhidrosis are known to be *ITPR2*, *TMEM16A, FOXA1* and *AQP5.* Of these four genes, *ITPR2* gene plays a significant role in primary hyperhidrosis; thus in the current research sequence, analysis of this gene was carried out. Following inclusion and exclusion criteria, whole blood samples were taken from four patients inflicted with primary hyperhidrosis and two from healthy persons as control. From the blood samples, white blood cells were separated by reacting the samples with ammonium chloride (NH_4Cl) solution to lyse red blood cells. Then total RNA was extracted using the trizol reagent. For synthesizing cDNA, one script reverse transcriptase cDNA synthesis kit was employed. The cDNA samples were quantified using a Qubit Flourometer and their quality was appraised on agarose gel. Real time PCR was performed to carry out the quantitative analysis of these four genes. Of four genes, the expression of three genes, i.e., *TMEM16A, ITPR2* and *FOXA1*, was found to be significantly high in the primary hyperhidrosis patients, whereas low expression of the gene *AQP5* was noted compared to that of the control samples. Microarray data analysis on anhidrosis was done using GEO2R database as well as the anhidrosis datasets available on Gene Exression Omnibus (GEO). From this analysis, the low expression of *FOXA1* in anhidrosis was confirmed. Based on the highest

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expression of *ITPR2* in hyperhidrosis, it was selected for PCR amplification as well as sequencing. The sequence analysis of *ITPR2* showed 100% identity without any mutation.These key genes will further help us to devise new treatment modalities.

Introduction

The term sweating inappropriately alludes to several different phenomena. Sweating may allude to the passive loss of water through the skin, much of the time alluded to as "insensitive" sweating (Quinton, 1983)**.** In hot situations, sweating is the chief method for thermoregulatory heat indulgence in people (Cramer et al., 2022). The rise of essential temperature signals the hypothalamus, which at that point directs vasomotor and sudomotor functional units to disperse body heat in an organized autonomic response (Cheshire and Low, 2007; Cramer et al., 2022).

Sweat vaporization is essential for human heat dissemination; heat sicknesses and fluid/electrolyte inequality can result from unusually directed sweating (Baker, 2017). Sweating dysregulation can be systemic or focal, and can result in anhidrosis (absence of sweating), hypohidrosis (imperfect sweating) or hyperhidrosis (excessive sweating) in light of a proportionate thermal or pharmacologic stimulus (Metzler-Wilson and Wilson, 2016). Anhidrosis is the absence of sweat production in a company of applicable incentive. Anhidrosis is uncommon, however, it might be caused by an assortment of diseases (Sil et al., 2022). It might be acute or progressive, and it might start incidentally or centrally.

Hyperhidrosis is a condition characterized by excessive sweat production by the body beyond what is required by homeostasis (Lima et al., 2015; Wohlrab et al., 2023). Hyperhidrosis can significantly impact a patient's quality of life, causing a significant deterioration in daily activities, social contacts and word-related activities (Haider and Solish, 2005; Wohlrab et al., 2023). It is usually described as primary in case of isolation (the most common form) or secondary when it occurs as a result of another disease or treatment regimen (Nyamekye, 2004; Kisielnicka et al., 2023).

The most common cause of secondary hyperhidrosis is the underlying disease that prompts general sweating, including most body parts (Kreyden and Scheidegger, 2004; Nyamekye, 2004; Collercandy et al., 2022). Obesity, menopause, and drug use (antidepressants) may be linked to secondary hyperhidrosis (Mijnhout et al., 2006) and endocrine diseases (reading hyperthyroidism, diabetes, pheochromocytoma, carcinoid, systemic mastocytosis), yet additionally happens with malignancy and neurological conditions including autonomic dysregulation (Odderson, 1998). Prescribed medication can likewise cause generalized sweating (Nyamekye, 2004; Kisielnicka et al., 2023).

The most commonly recognized type of localized hyperhidrosis is focal hyperhidrosis, located on the palms, soles, axils or face. Typically, focal hyperhidrosis is primary (idiopathic) and emotionally stimulated (Stolman, 2003). It has no known reason and is defined by excessive sweating due to the sympathetic nervous system's hyperactivity, which intrudes on the sweat glands (Metzler-Wilson and Wilson, 2016). Primary hyperhidrosis may be hereditary and it typically occurs during childhood or adolescence, in contrast to generalized hyperhidrosis (Ro et al., 2002; Dunford et al., 2022). Axillary hyperhidrosis can deliver skin maceration and wet clothing, prompting continual clothing changes among the day. Palmar hyperhidrosis can meddle with shaking hands when one meets individuals and furthermore with school work and activities that require dry hands. Plantar and craniofacial hyperhidrosis can cause physical and social uneasiness (Benson et al., 2013; Parashar et al., 2023).

The pathophysiology of hyperhidrosis is not well understood, although, it is thought to be the result of an overly central response to normal emotional stress or environmental stimuli. Sweating cools the body by the loss of heat, usually in hyperhidrosis. Although there is some overlay, there are in fact, two types of sweating: thermal and emotional sweating (Schwarck et al., 2019). Thermoregulation production of sweat is controlled by three organ systems: the cerebral cortex, the anterior hypothalamus and the sympathetic nervous system (Hornberger et al., 2004; Khalifa et al., 2018). Thermal sweating is likely to occur on the trunk at any time, but emotional sweating does not occur during sleep and mainly occurs on the palms and soles (Schieman et al., 2010). More than 2 million sweat glands are estimated to be unevenly distributed throughout the body surface (Kaufmann et al., 2003; Baker, 2019). There are three types of sweat glands secreted: eccrine, apocrine and apoeccrine (Scrivener and Cribier, 2002; Baker, 2019). The eccrine is the most abundant type in which the highest densities occur on the feet, palms, axillas and face soles. These glands are stimulated by a sympathetic nervous system with acetylcholine as the main neurotransmitter. It is the only component of the essential adrenergic sympathetic nervous system and does not use norepinephrine as a neurotransmitter (Shargall et al., 2008). In primary hyperhidrosis, sweat secreted from the eccrine gland is thin, clear and hypotonic to plasma. This dysfunction is likely to occur in areas where there is a higher intensity of eccrine sweat glands (Kreyden and Scheidegger, 2004).

Primary hyperhidrosis (PH) is idiopathic. It occurs as focal or multifocal (Stolman, 2003). The

multifocal PH was described as more common than the focal form (Glaser et al., 2016). Excessive sweating beyond that is essential to cool the body influences between 0.6% and 1% of the general population (Quraishy and Giddings, 1993). The rate is higher among infants, adolescents, and young adults (Dohn and Zraik, 1969). Palmar hyperhidrosis is already in infancy, while axillary hyperhidrosis occurs in adolescence or later (Hornberger et al., 2004; Kisielnicka et al., 2022). In hyperhidrosis, both sexes are affected equally, while females may find the disorder less acceptable and present more as often as possible for treatment as males (Leung et al., 1999).

Hyperhidrosis is a genetically transmitted disorder. However, it is still unclear whether primary hyperhidrosis is a single gene defect or a multifactorial disorder (Ro et al., 2002; Parveen et al., 2023). There is a certain consensus that hyperhidrosis is often hereditary, possibly autosomal dominant inheritance, and the online database (OMIM) defines hyperhidrosis as “a clear familial disorder” (Kaufmann et al., 2003; Kisielnicka et al., 2022).

The genes involved in primary hyperhidrosis are known to be *ITPR2*, *TMEM16A, FOXA1* and *AQP5,* however, their expression in different individuals varies to a great extent. Our primary objective of the present investigation had been to examine differential expression of only *ITPR2* gene, one of the key genes involved in primary hyperhidrosis.

Materials and Methods

Sample collection

Whole blood samples were collected from five patients of primary hyperhidrosis and two control samples in EDTA tubes according to the inclusion and exclusion criteria.

Isolation of white blood cells

White blood cells were isolated using NH_4Cl solution (0.82 g of ammonium chloride + autoclaved distilled water 90 mL).

At room temperature, an aliquot of 36 mL of ammonium chloride solution was added for each 4 mL of blood to lyse the red blood cells. The solution was then incubated on a rotator for 5 minutes. It took more than 5 minutes for the white blood cells to start lysing. The solution was centrifuged at 2000 rpm for 5 minutes. The supernatant was aspirated and the pellet was suspended in 10 mL cold 1X PBS. That procedure was repeated for 2-3 times until the pellet became white. Lastly, PBS was discarded, and 0.5 mL of the trizol reagent was added to the Eppendorf tube (pipette up/down) until the pellet was dissolved.

RNA isolation by trizol

Trizol reagent, a complete ready-to-use reagent for the isolation of high-quality total RNA, was used for RNA isolation. Voltex preserved white blood cells (resuspended in trizol) for 30 sec, then left at room temperature for 10 min and then 200 µL chloroform were added and vigorously shaken the tube for about 15 sec. After that, again left that solution in Eppendorf tube at room temperature for 10 min and then centrifuged at 12,000 rpm for 10 min at 4 °C. At this point, there were three layers in each tube: the top layer was clear, transparent, and aqueous, the middle layer/interphase white precipitated DNA and the bottom layer was pink organic. From those three layers, the aqueous phase was carefully shifted using a pipette to leave some of the aqueous phase behind (approximately 1 mm above the DNA layer to avoid DNA contamination). After that, 500 µL isopropanol were added to the aqueous phase, mixed gently and then left at room temperature for 10 min. The mixture was centrifuged at a maximal speed (14,000 rpm) for 20-30 min and placed the samples on ice. At this point, the pellet was barely visible at the base of each tube. Next, isopropanol was poured off, and 200 µL of 75% ethanol were added and mixed gently. The samples were then centrifuged at 12,000 rpm for 5 min at 4 °C. After centrifugation the ethanol was poured off and the pellet was left to air-dry. After that, approximately 40 µL of injection water were added to the RNA pellet

cDNA synthesis

The reverse transcription reactions were assembled in an RNase-free environment. cDNA samples were synthesized using one script reverse transcriptase cDNA synthesis kit (Catalogue no: G234). The following reaction mixture was prepared on ice:

A variable volume (1 ng -2 µg/rxn) of total RNA was used and the oligo dt and random primers were used with a volume of 1 µL, making the final concentration as 0.5 µM. dNTP was mixed with 1 µL of volume and a final concentration of 500 µM was prepared.

The mixture was heated at 65 °C for 5 min on veriti PCR and incubated on ice for 1 min. A brief centrifugation was employed to collect all the components. After that, the following components were

added: 5X RT buffer with 4 µL (final Concentration 1 X), 0.5 µL of RNaseOFF Ribonuclease Inhibitor (final concentration 20 U/ rxn) and One Script® RTase with 1 µL volume (200 U/rxn). The components were mixed thoroughly, and brief centrifugation collected all components (20 µL). CDNA was synthesized by incubating the tubes at 42 °C for 50 minutes. The reaction was stopped by heating it for 5 minutes at 85 °C. The newly synthesized cDNA of the first strand was then ready for immediate downstream applications or long-term storage at -20 °C.

Quantification of cDNA

cDNA was quantified using an Invitrogen Qubit 2.0 Fluorometer. A 0.5 mL thin-walled PCR tube was prepared for each sample. A working solution of (1 X *n*) µL Quant-iT reagent with (199 x *n*) µL Quant-iT buffer was prepared in a clean plastic tube, where *n* equaled (number of samples + 2). The samples were vortexed for 2-3 seconds. Standard solutions 1 and 2 for nucleic acid assays were prepared by combining a working solution of 190 µL with 10 µL of an appropriate storage solution from the kit. A 1 µL sample was mixed with a working solution 199 µL to give a final volume of 200 µL. All the sample tubes were vortexed for 2-3 seconds. The tubes were incubated at room temperature for 2 minutes. The Qubit was turned on. The value given by the Qubit fluorometer was noted.

Concentration of sample = (QF Value) X 200/x

QF value = the value given by the Qubit fluorometer, X= the number of microliters of sample added to the assay tube.

Primer designing

For the amplification of *ITPR2, AQP5, TMEM16A and FOXA1* genes, both forward and reverse primers were designed using bioinformatics tools such as NCBI and *in silico* PCR (**Table 1**).

Table 1: Gene-specific primers

Gene	Primer	Sequence	Nucleotide Size	% of GC content	Melting temp (°C)
ITPR2	F Primer	5'-AAGCCGTGACTAGCCTTTCA-3'	20	50	58.4
	R Primer	5'-ATGCATTGCGAATGTGTGAT-3'	20	40	54.3
AQP5	F Primer	5'-TCCATTGGCCTGTCTGTCAC-3'	20	55	60.5
	R Primer	5'-CTTTGATGATGGCCACACGC-3'	20	55	60.5
TMEM16A	F Primer	5'-GGCCACGATGAGGGTCAACG -3'	20	65	64.6
	R Primer	5'-CCTGTAGCTATGCCAGCGGG-3'	20	65	64.6
FOXA1	F Primer	5'-ACAGCTACTCGCAGACACG-3'	20	55	60.5
	R Primer	5'- CCCAGGCCTGAGTTCATGTT-3'	20	55	60.5

Quantitative PCR (qPCR)

Bright Green 2X qPCR Master Mix (Mastermix-S) was used for the qPCR. Firstly, the master mix, template DNA, primers, and nuclease-free water were thawed on ice, and each solution was mixed thoroughly. The components containing Bright Green 2X qPCR, such as MasterMix, Forward Primer and Reverse Primer (10 µM), Template DNA (≤ 500 ng/reaction) and nuclease-free H_2O were set up for qPCR, and the reaction volume mixture was 10 µL. The qPCR reactions were performed using the following cycling program: The standard duration of enzyme activation was 10 mins, fast duration was 20 sec, with only one cycle, and temperature 95 °C. The standard duration of denaturation was 15 sec, fast duration was 3 sec, and the temperature was 95 °C. Annealing/extension took 10 mins for its standard duration. Fast duration was 30 sec and temperature 60 °C.

Polymerase chain reaction (PCR)

PCR was performed using 2X PCR Taq Mastermix (Cat. No. G013). Following components were used: ~2 ng/µL final concentration with the volume of template DNA ~100 ng, 200 nM – 500 nM of the final concentration of forward primer (10µM) with a volume of 1-2.5 µL, 200 nM – 500 nM of final concentration reverse primer (10 µM) with a volume of 1-2.5 µL and 1X final concentration 2X PCR Taq MasterMix without dye 25 µL and up to 50 µL nuclease-free H_2O. All the tube contents were added to a sterile 0.2 mL PCR tube and then mixed and centrifuged briefly. The tubes were incubated at 94 °C for 3 minutes in a thermocycler to denature the template completely. 30-35 cycles of PCR amplification were performed as follows: denaturation was 94 °C for 30 sec, annealing: 45 – 72 °C for 30 sec, extension: 72 °C for 1 min/1 kb template and additional final extension was 5 mins at 72 °C. The samples were stored at -20 °C until use.

Agarose gel electrophoresis

The samples were analyzed using gel electrophoresis. One percent (1%) agarose gel was prepared

using 0.45 g of agarose dissolved in 45 mL of 1X TAE buffer and the solution was heated using a microwave oven to completely dissolve agarose in water. Ethidium bromide solution (3 µL) was added and after mixing it well, the solution was poured into a gel caster tray with an adjusted comb in it. The solution was left open to cool and dry for 10-15 mins and after its solidification the gel comb from the gel was taken out. The caster tray was fitted in the tank of the gel and the tank was supplied with 250 mL of 1X of TAE buffer. The samples of DNA were prepared for loading by adding 5.0 µL of DNA sample with 1 µL of loading dye, and these samples were loaded to wells of agarose gel. Likewise, an aliquot of 4.0 µL of DNA ladder was loaded in a separate well to estimate the size of genomic DNA. The speed of the gel electrophoresis was adjusted to 100 V for 30 minutes during its run. The gel electrophoresis results were analyzed using an ultraviolet transilluminator.

Sequencing and sequence analysis

After the gel electrophoresis, unpurified PCR products were sent to a commercial private facility along with a forward primer of ITPR2 for determining the sequencing.

Results

Isolation of white blood cells

The results of white blood cell pellets of primary hyperhidrosis patients and controls are presented in Figure 1**.** The whole blood was taken according to the inclusion criteria. White blood cells were isolated as described in materials and methods. **Figure 1, a-e** shows different stages of WBC isolation.

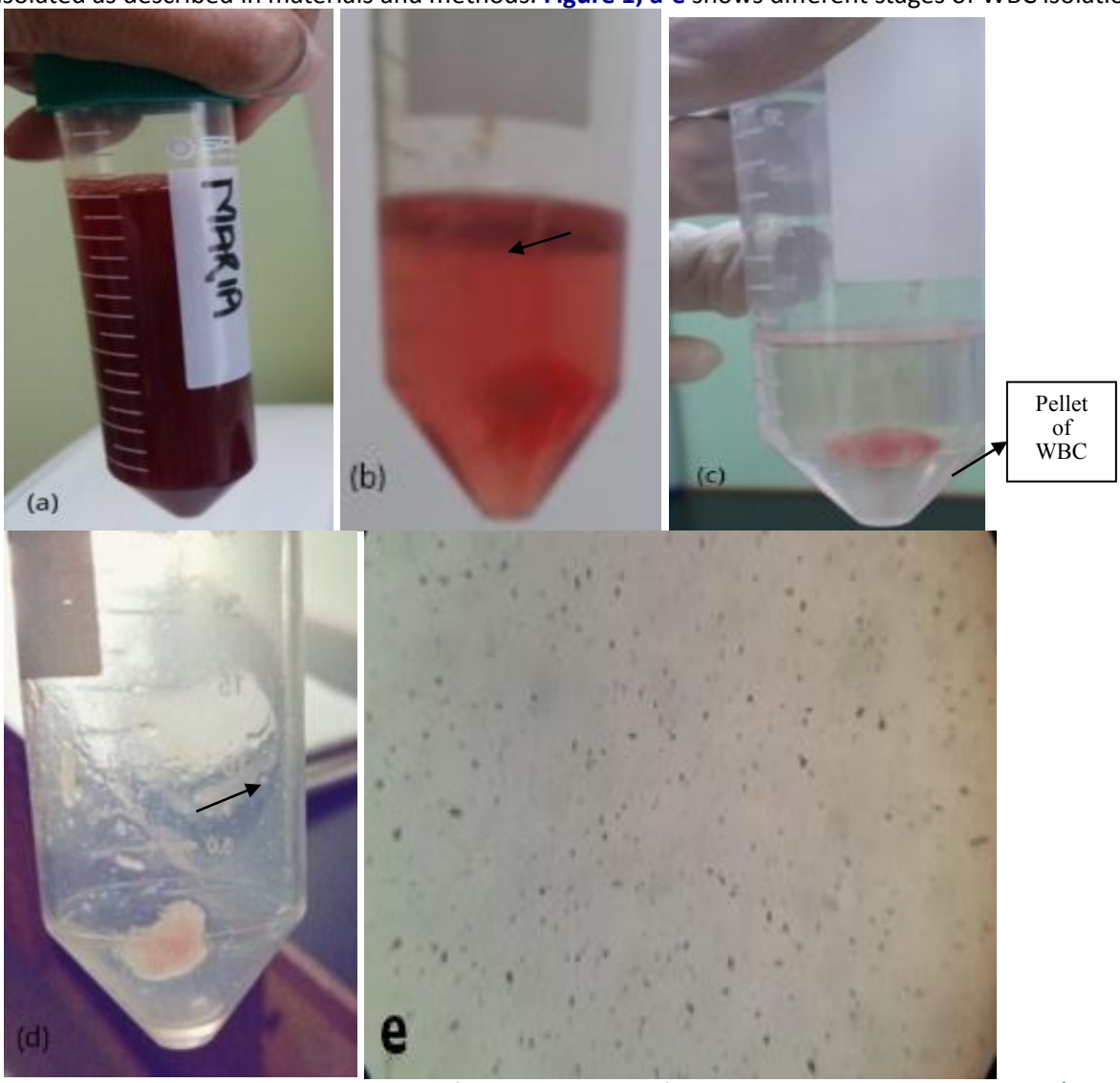


Figure 1: The images show the isolation of white blood cells from human whole blood samples. a) represents image of whole blood samples with NH_4Cl solution. (b) shows the first pellet of white blood cells after centrifugation with NH_4Cl solution. (c) exhibits the second visible pellet of WBCs. (d) Last clear white pellet of white blood cells after resuspending into PBS 2-3 times. (e) Microscopic image of white blood cell pellet

cDNA synthesis

As described earlier the cDNA samples were synthesized using one script reverse transcriptase cDNA synthesis kit (Catalogue no: G234) and the optimization of the volume of cDNA components was achieved (**Table 2**).

Table 2: Optimized values of components, and volume and final concentration used for the synthesis of cDNA

Components	Volume (µL)	Final concentration
Total RNA	11.5	1 ng – 2 µg/rxn 1 pg – 2 ng/rxn
Oligo (dT)	1	0.5 µM
or Random Primers	1	0.5 µM
dNTP Mix	1	500 µM
5X RT Buffer	4	1X
RNaseOFF Ribonuclease Inhibitor	0.5	20 U/rxn
One Script® RTase	1	200 U/rxn

cDNA quantification through Qubit

Seven cDNA synthesized samples (5 samples of primary hyperhidrosis and 2 samples of healthy controls) were quantified before RT-PCR using the Invitrogen Qubit 2.0 Fluorometer with the dsDNA HS Assay kit.

Quantitative PCR (qPCR)

As described earlier, the Bright Green 2X qPCR Master Mix (Master Mix-S) was used for qPCR and the given component volumes were optimized for the quantification of differential gene expression (**Table 3**).

Table 4 indicates temperature, duration and cycles programmed into Bio-Rad qPCR. The steps involved in the qPCR reaction, in which enzyme activation was optimized at 95 °C for 3.0 minutes, required 1 cycle. Annealing temperature was optimized based on bioinformatically designed primers of different genes and extension required at 72 °C. These steps were completed in 40 cycles.

Table 3: Optimized quantities of components and their final concentration used for the total reaction of qPCR.

Components	Quantity (µL)	Final Concentration
2X qPCR MasterMix	5	1X
Forward Primer (10 µM)	0.5	300 nM
Reverse Primer (10 µM)	0.5	300 nM
Template DNA	2	≤ 500 ng/reaction
Nuclease-free H_2O	2	

Table 4: PCR conditions

Steps	Temperature	Duration	Cycles
Enzyme activation	95 °C	3.0 min	1
Denaturation	95 °C	30 sec	1
Annealing	ITPR2 48 °C AQP5 57 °C TMEM16A 55.5 °C FOXA1 55.5 °C B-Actin 57 °C	1 min	40
Extension	72 °C	1 min	

Polymerase chain reaction of beta actin

PCR was performed on synthesized cDNA samples to optimize reference gene *B-ACTIN* annealing temperature. After that, 1% agarose gel was prepared using optimum conditions for running the gel at 100 V, and the required band was obtained that was exactly the size of *β-Actin* (**Figure 2**).

Differential gene expression

Real Time PCR was performed for the identification of differentially expressed genes (*ITPR2, AQP5, FOXA1* and *TMEM16A*) in the pathogenesis of primary hyperhidrosis (**Figure 3**).

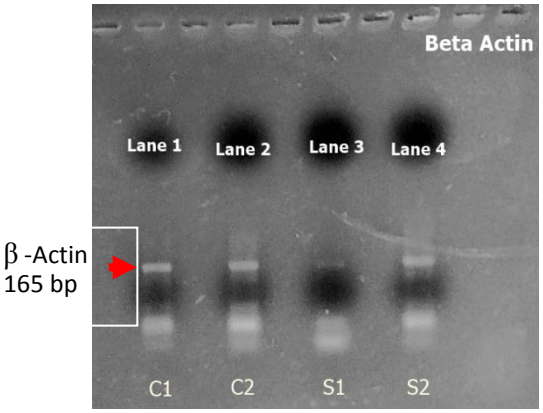


Figure 2: Lane 1 and 2 show PCR amplification of control samples, lane 3 and 4 show PCR amplification of hyperhidrosis patients by using beta-actin primer at temperature 57 °C.

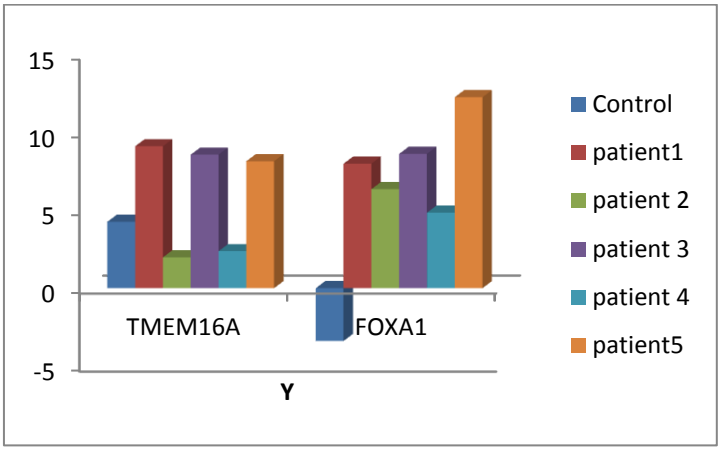


Figure 3: Expression of *TMEM16A* and *FOXA1*: Y-axis depicts the ΔCT calibrate and ΔCT test and X-axis shows different genes of hyperhidrosis patients. In this Figure healthy control has been shown as dark blue bar, and bars of different colors indicate diseased patients of hyperhidrosis.

From this RT-PCR, we investigated the gene expression of primary hyperhidrosis patients from freshly isolated human white blood cells. **Figure 4** illustrates that *TMEM16A* gene is highly expressed in patient 1, patient 3 and patient 5, and low expression in patient 2 and patient 4 as compared with the control sample. Correspondingly, *FOXA1* gene was found to be upregulated in all five hyperhidrosis patients 1 to 5 and the gene down-regulated in the control sample.

AQP5 is the only gene which showed overexpression in the control sample and this gene downregulated in all five hyperhidrosis patients, 1 to 5 (**Figure 5**).

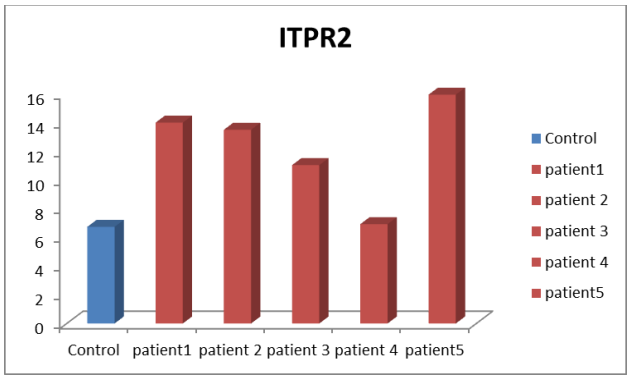


Figure 4: Expression of *ITPR2:* The gene *ITPR2* shows significant high expressions in all patients of primary hyperhidrosis, patient1 to 5, with reference to the control sample which depicts low expression

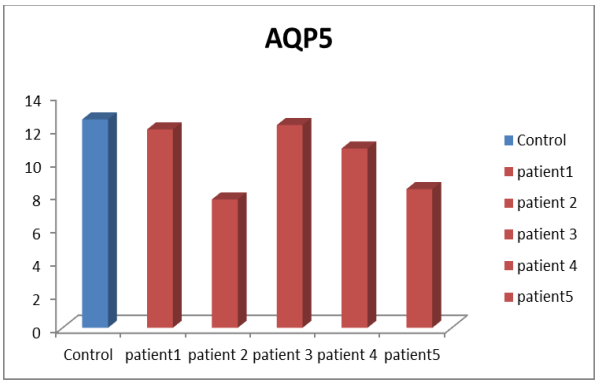


Figure 5: Expression of *AQP5*

Average hyperhidrosis of all patients and control

In **Figure 6** the average of all hyperhidrosis patients with normal control shows that the expressions of all three genes, *TMEM16A, ITPR2* and *FOXA1* were significantly higher in the primary hyperhidrosis patients compared to those of the control samples. Overall, it represents that other than the *AQP5* gene, the mechanism of *TMEM16A, ITPR2* and *FOXA1* genes is involved in primary hyperhidrosis.

Fold change

Figure 7 illustrates that the gene *TMEM16A* showed 1.74-fold increase in the mRNA of hyperhidrosis patients compared to the control sample. The *FOXA1* and *ITPR2* genes showed a minor fold change; the values of $2^{-\Delta\Delta ct}$ of *FOXA1* is 0.18, and *ITPR2* 0.19, and *AQP5* gene 10.48-fold change in the mRNA with respect to its healthy control.

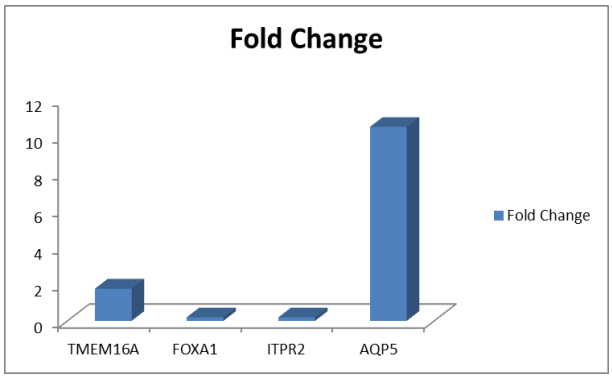


Figure 7: Fold change of gene expression in hyperhidrosis patients. The Y-axis shows the fold change values, and the X-axis shows different genes

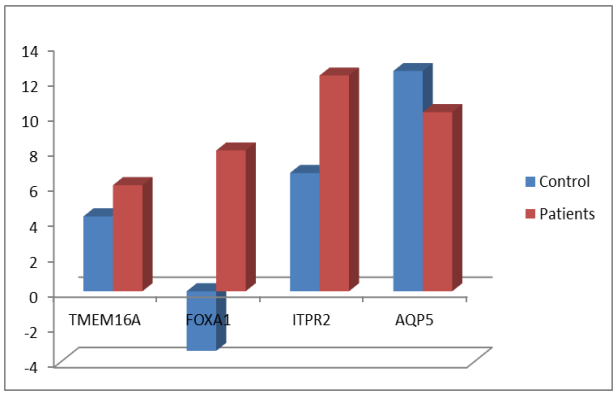
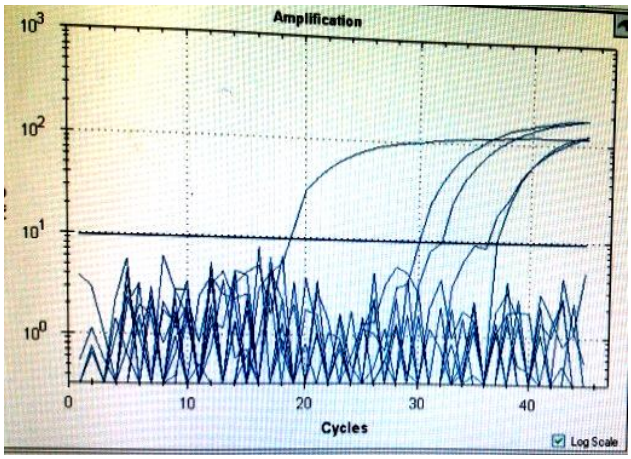
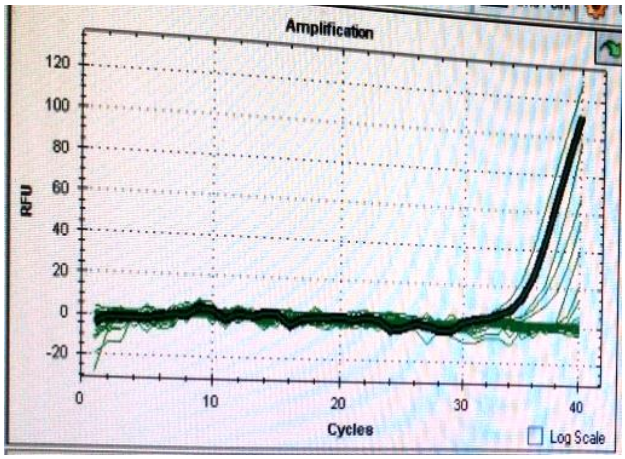


Figure 6: The Y-axis depicts the ΔCT calibration and ΔCT test, and the X-axis shows different genes of hyperhidrosis patients. The average of all 5 primary hyperhidrosis patients is in red, and the healthy control is in blue

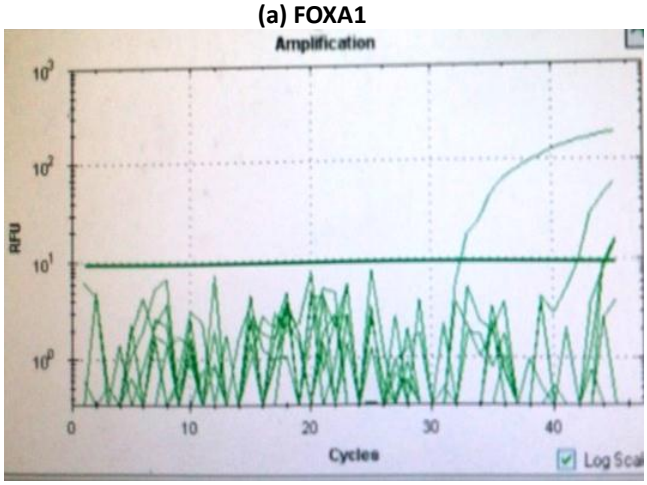
In **Figure 8**, the output of 4 different genes *FOXA1, TMEM16A, ITPR2*, and *AQP5,* is given in which the sample curve was plotted with RFU versus the number of cycles.



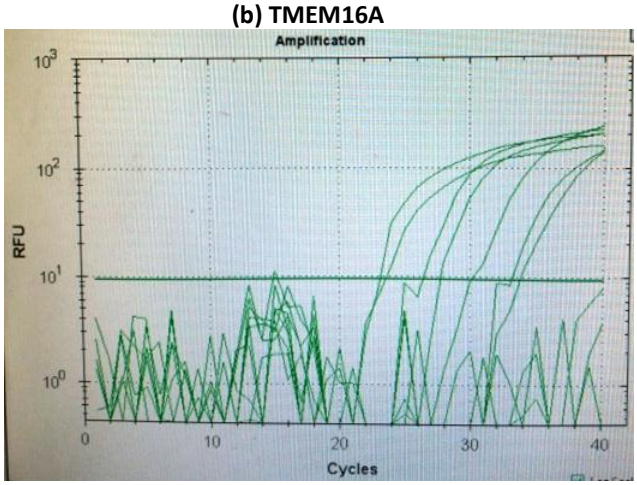
(a) FOXA1



(b) TMEM16A



(c) ITPR2



(d) AQP5

Figure 8: These qPCR curves show different gene expressions of hyperhidrosis patients performed in BioRad-qPCR.

Polymerase Chain Reaction (PCR)

Table 5 shows the optimized quantities of components and their final concentrations used for the total reaction of conventional PCR.

Table 5: Optimized quantities of components and their final concentrations used for the PCR reaction

Components	Volume (µL)	Final concentration
Template DNA	4	~2 ng/µL
Forward primer (10 µM)	1	200 – 500 nM
Reverse primer (10 µM)	1	200 – 500 nM
2X PCR *Taq* Master Mix/ without dye	12.5	1X
Nuclease-free H_2O	6.5	

Polymerase chain reaction for sequencing

After real-time PCR, we identified that *ITPR2* was the only gene that was overexpressed in all patients of primary hyperhidrosis as is evident from **Figure 9**.

From the UCSC genome browser database (**Figure 10**), the sequence of *ITPR2* was analyzed using the SnapGene Viewer software and it was revealed that *ITPR2* gene is located on chromosome 12, and there were 6 exons in this gene sequence. The expression of *ITPR2* is evident in 53 different tissues and multiple alignments in different species as shown in **Figure 11**.

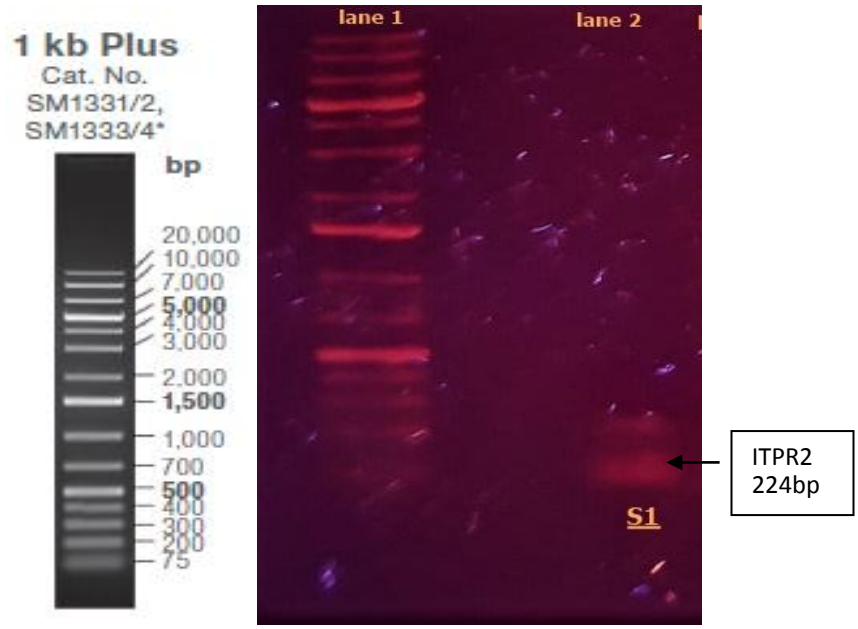


Figure 9: First lane shows the size of DNA ladder; lane 2 shows the results of PCR amplification of cDNA sample of *ITPR2* gene at temperature 47 °C

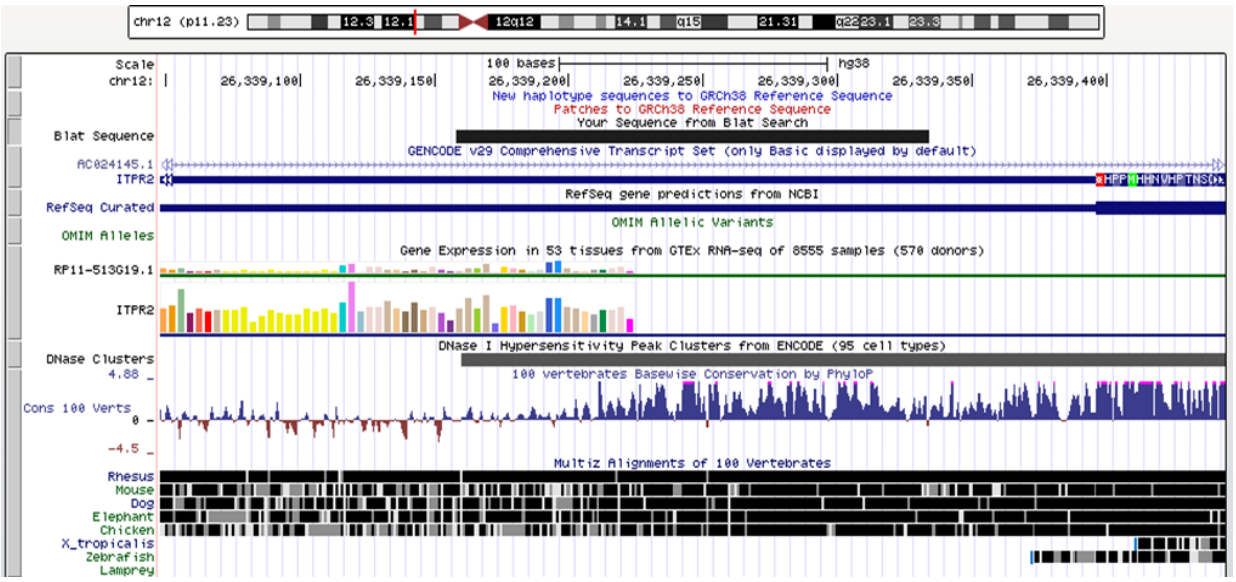


Fig 10: Sequence of *ITPR2* gene from UCSC genome browser

Figure 11 shows gene expression from the NIH Genotype-Tissue Expression (GTEx) project, a data source that is used to study the relationship between genetic variation and gene expression in different human tissues. It shows a significant high expression in adipose tissues, artery-tibial, esophagus, lung, muscle-skeletal, skin-not sun exposed (suprapubic), skin-sun exposed (lower leg), thyroid and whole blood. The sequencing results were obtained in a chromatogram (**Figure 12**) that was analyzed using SnapGene Viewer software. Image shows 197 bp chromatogram of *ITPR2* gene. Out of 12564 bp with 57 exons we selected a specific region of 224 bp with 6 exons for sequence analysis. Nucleotide blast of *ITPR2* gene shows 100% identity and no mutation has been found (**Figure 13**).

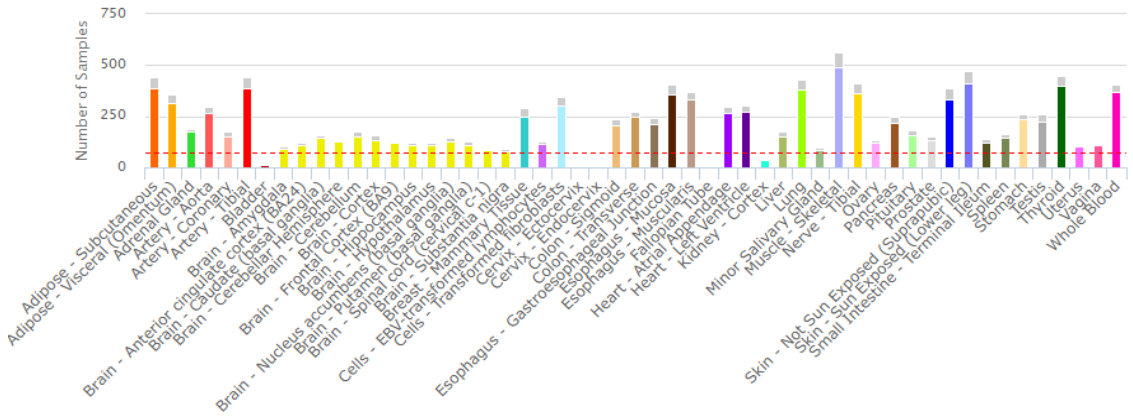


Figure 11: Gene expression of the *ITPR2* gene in 53 tissues

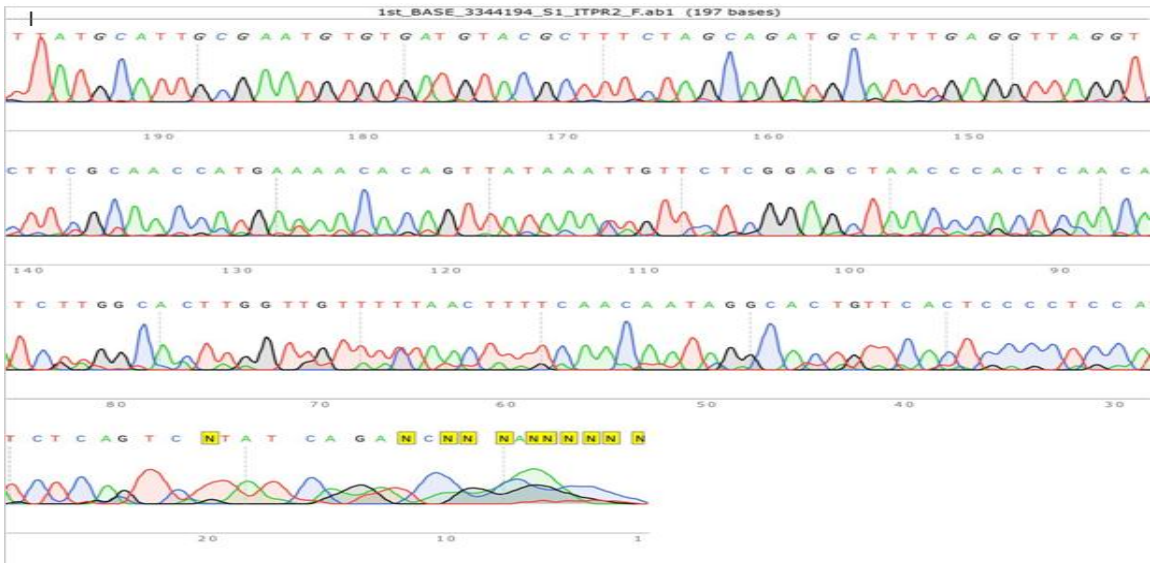


Figure 12: Chromatogram image of hyperhidrosis patients

Range 1: 51 to 224 Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
322 bits(174)	3e-93	174/174(100%)	0/174(0%)	Plus/Plus

```
Query  22   ACTGAGATGGAGGGGAGTGAACAGTGCCTATTGTTGAAAAGTTAAAAACAACCAAGTGCC  81
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  51   ACTGAGATGGAGGGGAGTGAACAGTGCCTATTGTTGAAAAGTTAAAAACAACCAAGTGCC  110

Query  82   AAGATGTTGAGTGGGTTAGCTCCGAGAACAATTTATAACTGTGTTTTCATGGTTGCGAAG  141
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  111  AAGATGTTGAGTGGGTTAGCTCCGAGAACAATTTATAACTGTGTTTTCATGGTTGCGAAG  170

Query  142  ACCTAACCTCAAATGCATCTGCTAGAAAGCGTACATCACACATTCGCAATGCAT  195
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  171  ACCTAACCTCAAATGCATCTGCTAGAAAGCGTACATCACACATTCGCAATGCAT  224
```

Figure 13: Query represents the sequence of hyperhidrosis patient 5, and the subject represents the sequence of primer *ITPR2*

Anhidrosis microarray data analysis

For the *FOXA1* expression profile, hairless forefoot pad skin was collected from *FOXA1* knockout and wild type littermates in P10, P14 and P31 (normal lumen opening, completion of gland development, and homeostasis). Three skin samples from 3 embryos of each genotype from each time point were used for biological replicates. The results showed that the ablation of *FOXA1* in mice resulted in an absolutely lack of sweating.

GEO2R database is used to compare gene knock-out and wild-type groups to identify genes that are differentially expressed under experimental conditions (Table 6).

Table 6: List of top highly expressed genes using the software GEO2R

ID	adj.P.Val	P.Value	GB_ACC	GENE_SYMBOL	SPOT_ID
14265	4.82e-14	1.93e-18	NM_008259.3	Foxa1	
26622	5.82e-04	4.65e-08	NM_172641.1	9930023K05Rik	
36036	2.84e-03	3.41e-07	NM_145388.1	Best2	
20934	1.95e-02	3.46e-06	BC024407.1	Gm1943	
30036	1.95e-02	3.89e-06	NM_133681.2	Tspan1	
38386	6.45e-02	1.77e-05	NM_013891.2	Spdef	
39009	6.45e-02	1.80e-05	NM_001042767.1	Proc	
22321	7.90e-02	2.52e-05	NM_026164.2	Pnpla8	
27257	8.31e-02	3.23e-05	AK016300.1	6530401N04Rik	
12742	8.31e-02	3.32e-05	XM_126991.7	3830431G21Rik	
13981	1.75e-01	7.71e-05	NM_007860.3	Dio1	
7112	2.44e-01	1.17e-04	NM_207136.1	Olfr1349	
16552	2.69e-01	1.42e-04	XM_001003064.1	Pdzrn4	
998	2.69e-01	1.50e-04	NM_030021.1	D730039F16Rik	
34425	6.08e-01	3.64e-04	NM_010726.1	Phyh	
16842	6.48e-01	4.14e-04	NM_011158.3	Prkar2b	
16316	6.77e-01	4.60e-04	NM_008671.1	Nap1l2	
32840	7.14e-01	5.68e-04	NM_026038.1	2810055F11Rik	
36235	7.14e-01	5.70e-04	AK159714.1	Wdr70	
29259	7.14e-01	5.71e-04	NM_009999.3	Cyp2b10	

Figure 14 shows the gene expression of *FOXA1* in mice gene knockout and wild type. The expression defined that *FOXA1* gene expression in the wild type was significantly higher than the gene knock-out with relevantly low expression.

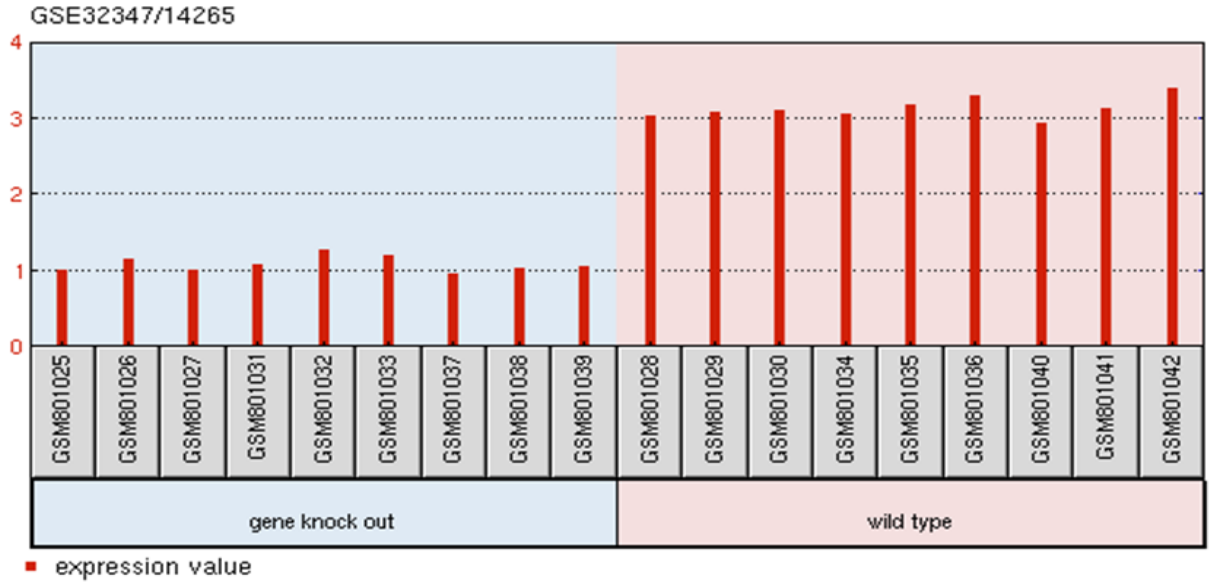


Figure 14: Y-axis shows the expression values of microarray and X-axis shows groups of gene knock-out and wild-type

Comparison of anhidrosis and hyperhidrosis

The anhidrosis study concludes that *FOXA1* is part of a selective evolutionary option to implement human body temperature regulation by sweating accession number (GSE32347), so from the above array analysis, we identified that *FOXA1* gene expression showed in both sweat abnormalities, i.e., in anhidrosis and hyperhidrosis, as this gene showed its low expression in anhidrosis in mice and overexpression in hyperhidrosis patients (**Figure 15**).

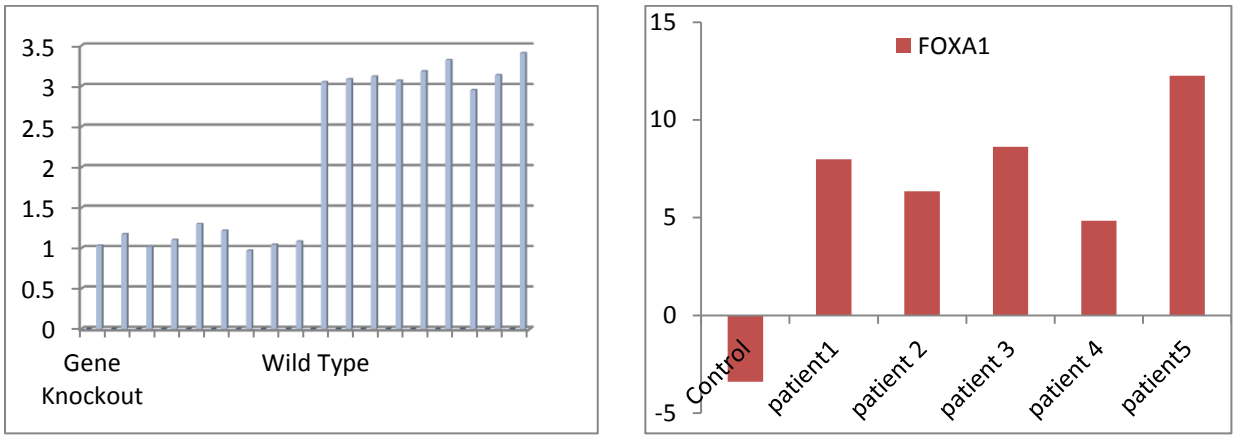


Figure 15: (a) Expression of *FOXA1* gene from microarray data on mice; (b) *FOXA1* gene expression analysis from qPCR on hyperhidrosis patients

Discussion

This is a novel study, because up till now no researches have been conducted on the gene expression from the freshly whole blood samples of primary hyperhidrosis patients. Our study provides the first evidence of differential gene expression of *TMEM16A, ITPR2,* and *FOXA1,* that play a vital role in primary hyperhidrosis. However, we could not find the role of *AQP5* in these hyperhidrosis patients. The expression of *TMEM16A* has been accounted for different cell types and tissues with endogenous Ca^{2+} movement (Yang et al., 2008) demonstrating the functional role of *TMEM16A* in Ca^{2+} subordinate Cl^- secretion. For example, *TMEM16A* has been reported to be expressed by bronchial, intestinal and salivary gland cells (Dulin, 2020). These discoveries upheld the speculation that *TMEM16A* plays just a minor role for Cl^- secretion in aviation route and intestinal cells (Namkung et al., 2011) and that Cl^- conductance may rely upon extra-calcium activated chloride channels (CaCCs), which have not yet been distinguished. In our study, we identified the expression of *TMEM16A* gene from the real time PCR, which showed a high expression in patient 1, patient 3 and patient 5, and low in patient 2 and patient 4 compared with that of the healthy control sample.

In Q-PCR, the fold change was also calculated which shows 1.7 times fold increase in the mRNA with respect to that of the control. Thus, according to its function, overexpression could yield greater sweating per activation of Ca^{2+} dependent Cl^- secretion (Concepcion et al., 2016).

Several earlier studies have shown that *FOXA1* gene can lead to human tumors, such as prostate cancer, breast cancer, and esophageal cancer (Li et al., 2016). Although, ongoing investigations demonstrate that *FOXA1* is associated with normal development and physiological procedures (Friedman and Kaestner, 2006). Cui et al. (2012) found that mouse sweating capacity requires the fork head transcription factor, *FOXA1.* Despite the continuing morphogenesis of the sweat glands, absolute anhidrosis in mice is caused by ablation of *FOXA1*. These discoveries relate to prior perceptions that both sodium/potassium/chloride trade and Ca^{2+} are required for sweating generation (Cui et al., 2012; 2016). Based on the earlier findings, we tried to identify the gene which is also responsible for primary hyperhidrosis in humans, so we performed Q-PCR which showed down-regulation in healthy control sample and overexpression in all five hyperhidrosis patients 1 to 5. The fold change from the RT-PCR shows 0.19 decrease in its fold as related to the control. Overall, these findings clarified the role of *FOXA1* in sweating as already observed elsewhere (Cui et al., 2012) in which complete lack of this gene caused anhidrosis. However, in our study high expression of this gene in hyperhidrosis patients proved that it is involved in both sweat abnormalities. We have also performed anhidrosis analysis from the microarray data of Cui et al. (2012) by using GEO2R Software which gave the similar results as reported earlier by Cui et al. (2012).

AQP5 belongs to subtypes of *AQP* in mammalian cells that allow water to pass through the plasma

membrane via osmosis (Ishibashi et al., 2009; Törnroth-Horsefield, et al. 2022) In acinar cells of adult humans, mice, and rats, *AQP5* has recently been recognized (D'Agostino et al., 2023). Earlier, Du et al. (2016) first time detected the expressions of AQP5 protein and *AQP5* mRNA in human axillary sweat glands. From our study, we could not find the expression of *AQP5* in primary hyperhidrosis patients from the RT-PCR, which showed up-regulation in the control samples, and a significant down-regulation in all five hyperhidrosis patients. This gene also showed 10 times increase in fold change under its control conditions. So far, the main role of *AQP5* in human sweat glands has yet to be clarified. Further studies that identify the relationship between *AQP5* and primary hyperhidrosis will increase our understanding of its pathogenesis.

Inositol 1, 4, 5-triphosphate receptor type 2 (*ITPR2*) is an intracellular Ca^{2+} release channel, and in mammalians, at least three forms of *ITPR* are identified as type 1, 2 and 3, respectively (Yule et al., 2010). Inositol triphosphate receptor-mediated signaling is involved in a number of processes including cell migration, cell division, smooth muscle contraction, and neuronal signaling (Maffucci and Falasca, 2020). A mutation in this gene was associated with anhidrosis which suggested that intracellular calcium release mediated by this protein is necessary for the production of eccrine sweat (Cui and Schlessinger, 2015). In our study, we investigated the expression of *ITPR2* gene which showed overexpression in all patients of primary hyperhidrosis with respect to the healthy control sample which depicted low expression. The *ITPR2* gene shows a minor fold of 0.19 with respect to the healthy control. Our results showed that InsP3R2-mediated Ca^{2+} release plays an important role in the secretion of human sweat and also in primary hyperhidrosis. As this gene shows a significant high expression compared with those of *TMEM16A* and *FOXA1*, out of 5 hyperhidrosis patients, sample 5 was selected for sequence analysis and after PCR amplification the sample was submitted for sequencing with the forward primer of *ITPR2*. The sequencing results revealed 100% identity and no mutation was detected.

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Contribution of authors
Research superior(s): MAJ; Conceptualization and designing the study: MAJ, FM, Conduction of experiment: TP, SU, Moderation of laboratory activities: MUNA ;Resource availability: MAJ; Instrumentation and analysis: AS; Data collection, visualization and interpretation: MAJ, MNT, SU, FM; Graphical representation/visualization: AS; Preparation of initial draft: TP; Intellectual contribution: MO and VA, Proof reading and approval of the final version: TP, MAJ, MNT, SU, MUNA, AS, FM.

Ethical approval
This work was approved by Institutional Ethical Review Board/Committee (IERB/C) of Kunming Institute of zoology, Chinese Academy of Sciences, Kunming, China, under approval number 1245-ERB/M4569 dated 03-27-202

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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References

Baker, L.B. (2019). Physiology of sweat gland function: The roles of sweating and sweat composition in human health. *Temperature (Austin, Tex.)* 6(3):211–259.

Baker, L.B. (2017). Sweating rate and sweat sodium concentration in athletes: A review of methodology and intra/interindividual variability. *Sports Medicine (Auckland, N.Z.)* 47(Suppl 1):111–128. https://doi.org/10.1007/s40279-017-0691-5

Benson, R.A., Palin, R., Holt, P.J.E., Loftus, I.M. (2013). Diagnosis and management of hyperhidrosis. *British Medical Journal* 347:https://doi.org/10.1136/bmj.f6800

Cheshire, W.P., Low, P.A. (2007). Disorders of sweating and thermoregulation. *CONTINUUM: Lifelong Learning in Neurology* 13(6):143-164.

Collercandy, N., Thorey, C., Diot, E., Grammatico-Guillon, L., Thillard, E.M. et al. (2022). When to investigate for secondary hyperhidrosis: data from a retrospective cohort of all causes of recurrent sweating. *Annals of Medicine* 54(1):2089–2101.

Concepcion, A.R., Vaeth, M., Wagner, L.E., Eckstein, M., Hecht, L. et al. (2016). Store-operated Ca^{2+} entry regulates Ca^{2+}-activated chloride channels and eccrine sweat gland function. *The Journal of Clinical Investigation* 126(11):4303–4318. https://doi.org/10.1172/JCI89056

Cramer, M.N., Gagnon, D., Laitano, O., Crandall, C.G. (2022). Human temperature regulation under heat stress in health, disease, and injury. *Physiological Reviews* 102(4):1907–1989.

Cui, C.-Y., Childress, V., Piao, Y., Michel, M., Johnson, A.A. et al. (2012). Forkhead transcription factor FoxA1 regulates sweat secretion through Bestrophin 2 anion channel and Na-K-Cl cotransporter 1. *Proceedings of the National Academy of Sciences* 109(4):1199-1203.

Cui, C.Y., Schlessinger, D. (2015). Eccrine sweat gland development and sweat secretion. *Experimental Dermatology* 24(9):644-650.

Cui, C.Y., Sima, J., Yin, M., Michel, M., Kunisada, M. et al. (2016). Identification of potassium and chloride channels in eccrine sweat glands. *Journal of Dermatological Science* 81(2):129–131. https://doi.org/10.1016/j.jdermsci.2015.11.001

Dohn, D.F., Zraik, O. (1969). Essential hyperhidrosis--pathogenesis and treatment. Report of seven cases treated by upper horacic sympathectomy. *Cleveland Clinic Quarterly* 36(2):79-83.

Du, Q., Lin, M., Yang, J.-H., Chen, J.-F., Tu, Y.-R. (2016). Overexpression of AQP5 was detected in axillary sweat glands of primary focal hyperhidrosis patients. *Dermatology* 232(2):150-155.

Dunford, L., Clifton, A.V., Stephenson, J., Radley, K., McDonald, L. et al. (2022). Interventions for hyperhidrosis. *The Cochrane Database of Systematic Reviews* 2022(2):CD015135. https://doi.org/10.1002/14651858.CD015135

D'Agostino, C., Parisis, D., Chivasso, C., Hajiabbas, M., Soyfoo, M.S. et al. (2023). Aquaporin-5 dynamic regulation. *International Journal Molecular. Sciences.* 24:1889. https://doi.org/10.3390/ijms24031889

Dulin, N.O. (2020). Calcium-activated chloride channel ANO1/TMEM16A: Regulation of expression and signaling. *Frontiers in Physiology* 11:https://doi.org/10.3389/fphys.2020.590262

Friedman, J.R., Kaestner, K.H. (2006). The Foxa family of transcription factors in development and metabolism. *Cellular and Molecular Life Sciences* 63:2317-2328.

Glaser, D.A., Ballard, A.M., Hunt, N.L., Pieretti, L.J., Pariser, D.M. (2016). Prevalence of multifocal primary hyperhidrosis and symptom severity over time: Results of a targeted survey. *Dermatologic Surgery* 42(12):1347-1353

Haider, A., Solish, N. (2005). Focal hyperhidrosis: Diagnosis and management. *Canadian Medical Association Journal* 172(1):69-75. https://doi.org/10.1503/cmaj.1040708

Hornberger, J., Grimes, K., Naumann, M., Glaser, D.A., Lowe, N.J. et al. (2004). Recognition, diagnosis, and treatment of primary focal hyperhidrosis. *Journal of the American Academy of Dermatology* 51(2):274-286.

Ishibashi, K., Hara, S., Kondo, S. (2009). Aquaporin water channels in mammals. *Clinical and Experimental Nephrology* 13:107-117.

Kaufmann, H., Saadia, D., Polin, C., Hague, S., Singleton, A. et al. (2003). Primary hyperhidrosis: Evidence for autosomal dominant inheritance. *Clinical Autonomic Research* 13:96-98.

Khalifa, F.K., Abdulrasheed, O., Abdulrahman (2018). The physiological role of the hypothalamus in thermoregulation during exercise. *International Journal of Advanced Research in Biological Sciences* 5(11):122-126.

Kisielnicka, A., Szczerkowska-Dobosz, A., Purzycka-Bohdan, D., Nowicki, R.J. (2022). Hyperhidrosis: disease aetiology, classification and management in the light of modern treatment modalities. *Postepy Dermatologii i Alergologii* 39(2):251–257. https://doi.org/10.5114/ada.2022.115887

Kreyden, O.P., Scheidegger, E.P. (2004). Anatomy of the sweat glands, pharmacology of botulinum toxin, and distinctive syndromes associated with hyperhidrosis. *Clinics in Dermatology* 22(1):40-44.

Leung, A.K., Chan, P.Y., Choi, M.C. (1999). Hyperhidrosis. *International Journal of Dermatology* 38(8):561-567.

Li, M., Zhang, W., Liu, C., Shi, Y., Tang, W. et al. (2016). Forkhead box A1 (FOXA1) tagging polymorphisms and esophageal cancer risk in a Chinese population: A fine-mapping study. *Biomarkers* 21(6):523-529.

Lima, S.O., Aragão, J.F.B., Machado Neto, J., de Almeida, K.B.S., Menezes, L.M.S. et al. (2015). Research of primary hyperhidrosis in students of medicine of the state of Sergipe, Brazil. *Anais Brasileiros de Dermatologia* 90:661-665.

Metzler-Wilson, K., Wilson, T.E. (2016). Impact of calcium regulation on eccrine sweating and sweating disorders: The view from cells to glands to intact human skin. *Experimental Physiology* 101:345-346.

Mijnhout, G., Kloosterman, H., Simsek, S., Strack van Schijndel, R., Netelenbos, J. (2006). Oxybutynin: Dry days for patients with hyperhidrosis. *Netherlands Journal of Medicine* 64(9):326-328.

Maffucci, T., Falasca, M. (2020). Signalling properties of inositol polyphosphates. *Molecules* 25:5281. https://doi.org/10.3390/molecules25225281

Namkung, W., Phuan, P.-W., Verkman, A. (2011). Tmem16a inhibitors reveal tmem16a as a minor component of calcium-activated chloride channel conductance in airway and intestinal epithelial cells. *Journal of Biological Chemistry* 286(3):2365-2374.

Nyamekye, I. (2004). Current therapeutic options for treating primary hyperhidrosis. *European Journal of Vascular and Endovascular Surgery* 27(6):571-576.

Odderson, I.R. (1998). Hyperhidrosis treated by botulinum A exotoxin. *Dermatologic Surgery* 24(11):1237-1241.

Parashar, K., Adlam, T. , Potts, G. (2023). The impact of hyperhidrosis on quality of life: a review of the literature. *American Journal of Clinical Dermatology* 24:187–198. https://doi.org/10.1007/s40257-022-00743-7

Parveen, A., Abbas, S., Mehmood, N., Patafi, M.A.M., Wajid, U. et al. (2023). Primary hyperhidrosis: From a genetics point of view. *Journal of Family Medicine and Primary Care* 12(12):3028–3032.

Quinton, P.M. (1983). Sweating and its disorders. *Annual Review of Medicine* 34:429-452. https://doi.org/10.1146/annurev.me.34.020183.002241

Quraishy, M., Giddings, A. (1993). Treating hyperhidrosis. *British Medical Journal* 306(6887):1221.

Ro, K.M., Cantor, R.M., Lange, K.L., Ahn, S.S. (2002). Palmar hyperhidrosis: Evidence of genetic transmission. *Journal of Vascular Surgery* 35(2):382-386.

Schieman, C., Gelfand, G.J., Grondin, S.C. (2010). Hyperhidrosis: Clinical presentation, evaluation and management. *Expert Review of Dermatology* 5(1):31-44.

Scrivener, Y., Cribier, B. (2002). Morphology of sweat glands. *Morphologie: Bulletin de L'association des Anatomistes* 86(272):5-17.

Shargall, Y., Spratt, E., Zeldin, R.A. (2008). Hyperhidrosis: What is it and why does it occur? *Thoracic Surgery Clinics* 18(2):125-132.

Schwarck, J.B., Burdon, C.A., Taylor, E.A., Peoples, G.E., Machado-Moreira, C.A. et al. (2019). Thermogenic and psychogenic sweating in humans: Identifying eccrine glandular recruitment patterns from glabrous and non-glabrous skin surfaces. *Journal of Thermal Biology* 82:242–251. https://doi.org/10.1016/j.jtherbio.2019.04.017

Sil, A., Ghatak, N., Bhanja, D.B. (2022). Congenital idiopathic generalized anhidrosis: A rare cause of recurrent fever of unknown origin in children and review of existing literature. *Indian Journal of Dermatology* 67(2):175–177. https://doi.org/10.4103/ijd.ijd_791_21

Stolman, L.P. (2003). In hyperhidrosis (excess sweating), look for a pattern and cause. *Cleveland Clinic Journal of Medicine* 70(10):896-898.

Törnroth-Horsefield, S., Chivasso, C., Strandberg, H., D'Agostino, C., O'Neale, C.V.T. et al. (2022). Insight into the mammalian aquaporin interactome. *International Journal of Molecular Sciences* 23(17):9615. https://doi.org/10.3390/ijms23179615

Yang, Y.D., Cho, H., Koo, J.Y., Tak, M.H., Cho, Y. et al. (2008). Tmem16a confers receptor-activated calcium-dependent chloride conductance. *Nature* 455(7217):1210-1215.

Yule, D.I., Betzenhauser, M.J., Joseph, S.K. (2010). Linking structure to function: Recent lessons from inositol 1, 4, 5-trisphosphate receptor mutagenesis. *Cell Calcium* 47(6):469-479.

Wohlrab, J., Bechara, F.G., Schick, C., Naumann, M. (2023). Hyperhidrosis: A central nervous dysfunction of sweat secretion. *Dermatology and Therapy* 13(2):453–463. https://doi.org/10.1007/s13555-022-00885-w