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Research Article

ATP-SENSITIVE POTASSIUM CHANNEL SUBUNITS ARE EXPRESSED IN HUMAN SCALP HAIR FOLLICLES

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ABSTRACT

Hair plays a vital role in a person's appearance and self-image throughout life, and is perceived as a source of strength and sexuality. Consequently any pathological abnormality in hair growth can be psychologically distressing, whether abnormal hair loss such as androgenetic alopecia, or excessive hair growth such as hirsutism. The study aimed to investigate whether the gene expression of K_{ATP} channels subunits occurred in the anagen stage of the hair cycle of human scalp hair follicles. The limited understanding of hair follicle biology has meant that not all current treatments for these disorders are fully effective. Presently, minoxidil is the most commonly used non-hormonal treatment for hair loss. It belongs to a group of drugs known as ATP-sensitive potassium (K_{ATP}) channel openers. It is not explicitly known how minoxidil functions to stimulate hair growth. The general assumption is that it stimulates the follicular blood supply and/or K_{ATP} channels. Occipital scalp anagen hair follicles were microdissected individually from each skin sample. This followed by RNA extraction and cDNA synthesis. The gene expression was investigated using RT-PCR, real-time PCR and the products were confirmed by sequencing. The molecular biological investigation into K_{ATP} channel subunits revealed that SUR1, SUR2B, Kir6.1 and Kir6.2 are expressed in scalp hair follicles, while SUR2A was not expressed. These findings would aid more enhanced testing and better understanding of the mechanism of action of K_{ATP} channel openers and blockers for treatment of hair disorders. Therefore, this would increase our knowledge of hair biology and help the development of new improved therapies for hair pathologies.

INTRODUCTION

Androgenetic alopecia is a common form of hair loss in the region of the scalp for both men and women. In men, this condition is also known as male-pattern baldness. This form of disorder entails the gradual transformation of thick, pigmented hairs to thinner, shorter, non-pigmented vellus hairs, in both men and women. As the disorder progresses, the anagen phase shortens with the telogen phase remaining constant. The loss occurs in a precise, distinctive pattern on the scalpand the severity of the hair loss graded from prepubertal scalp through to the progressive recession of the bitemporal hairline and thinning on the vertex (Thomas, 2005). Though it is possible for male pattern balding to occur in women, a different pattern of hair loss is more common as the gradual hair loss in the crown with preservation of the frontal hair line (Sinclair *et al*., 1999; Price, 2003). Androgens and genetic disposition are the two main factors in the pathogenesis of androgenetic alopecia. Research evidence supports the involvement of local androgens in the development of androgenetic alopecia. For example androgenetic alopecia does not occur in men castrated prior to puberty, and for men castrated after puberty baldness ceases to progress (Hamilton, 1960, Randall, 2008). Although a number of genes have been investigated for an association with androgenetic alopecia, no specific gene or set of genes have been identified (Nyholt *et al*., 2003).

A range of treatments for androgenetic alopecia can be undertaken, such as wigs and hairpieces, surgery, hormonal and non-hormonal therapy. The surgical treatment involves the relocation of follicles from the non-balding areas to the sites that are bald, relying on the intrinsic responses of hair follicles to androgens (Orentreich and Durr, 1982). Though this treatment endures for a long time, it is expensive and painful; furthermore it may require further surgery as the hair loss develops around the transplanted region. Hormonal treatments include antiandrogens and 5α-reductase inhibitors. Antiandrogen treatment blocks the androgen binding to the androgen receptor, which has impractical effects on male masculinity (McPhaul, 2004). 5α-reductase inhibitors, however, such as finasteride, block the conversion of testosterone to 5α-dihydrotestosterone, which has been found to both slow down gradual hair loss, and promote hair growth

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in men under 42 years of age (Kaufman *et al*., 1998). 5α-reductase inhibitors remain as the main hormonal treatment for hair loss in men, however similar to all hormonal treatments, it is subject to continuation. The most commonly used non-hormonal treatment is minoxidil, which is a widely used topical treatment for hair loss in men and women. It belongs to a group of drugs known as potassium (K^+) channel openers. Initially developed as a treatment for hypertension, it was however, discovered to have the interesting side effect of hypertrichosis, excessive hair growth (Shapiro and Price, 1998; Dawber, 2000), making it unacceptable to patients and it was remarketed as a hair loss treatment. Nevertheless it is unclear how minoxidil functions to stimulate hair growth.

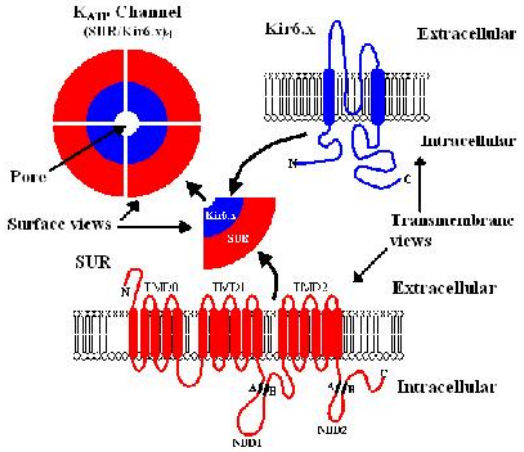

Fig 1. Structure of the K_{ATP} channels. K_{ATP} channels are composed of an ATP-binding cassette protein family, sulfonylurea receptor SUR and an inward rectifying potassium channel subunit, Kir6.x. Note the presence of two intracellular nucleotide binding domains (NBD), consisting of Walker A and B motifs joined by a conserved linker sequence in the SUR protein.

ATP-sensitive potassium channels (K_{ATP}) are formed by two distinct protein subunits (fig 1), a sulfonylurea receptor (SUR) and an inwardly rectifying potassium channel subunit (Kir6.x). They were first discovered in cardiac myocytes (Noma, 1983), and subsequently in various other tissues including the pancreatic β-cells, skeletal muscle, neurons, arterial smooth muscle, kidney and in the inner mitochondrial membrane and secretory granules (Rorsman and Trube, 1985; Bernardi *et al*., 1988; Standen *et al*., 1989; Thevenod *et al*., 1992). The SUR protein acts as a regulatory subunit, which is the site of interaction for a majority of drugs, whilst Kir6.x subunits form the channel pore, through which ions pass. The expression of both sub-units is necessary to form a functional channel (Inagaki *et al*., 1995). K_{ATP} channels are regulated physiologically by the levels of intracellular nucleotides and pharmacologically by K_{ATP} channel openers and blockers such as sulphotransferase (Meisheri *et al*., 1993). Intracellular nucleotides create polar reactions in K_{ATP} channels. Intracellular ATP (ATPi) inhibits the channels, whilst intracellular Mg^{2+} bound adenosine diphosphate (MgADPi) activates the channel (Cook and Hales, 1984; Gier *et al*., 2009). The lack of a precise treatment for hair loss is mainly due to insufficient knowledge of normal hair follicles and the mechanism of action of potassium channel openers and blockers. Thus this study aims to enhance knowledge of this field by investigating further the expression of potassium channel subunits in human scalp hair follicles including kir6.1, kir6.2, SUR1, SUR2A and SUR2B.

MATERIALS AND METHODS

Biological *materials*

Human skin samples were obtained from healthy donors undergoing elective cosmetic dermatological surgeries, with full written donor consent. For molecular biological investigations, tissues were collected from occipital regions of three men (aged 28, 33, anf 39 yr) and two women (aged 34 and 38 yr), were placed individually into sterile universal tubes (10 ml) containing RNA stabilization solution, RNA *later* (Sigma-Aldrich Ltd., UK), to inhibit RNases. The samples were stored in the fridge at 4°C overnight to allow the RNA *later* to penetrate the tissues. Human anagen hair follicles from non-balding scalp were micro dissected individually from each skin samples under a leica MZ8 dissecting microscope (Wetzlar, Germany) using sterile equipment and plastic ware. Each skin sample was transferred to a petri dish containing RNA*later*for molecular biological investigations. The skin sample was cut at the junction between the epidermis and dermis using a sterile scalpel blade. The hair follicles were pulled from the skin gently using fine forceps and then transferred into another petri dish containing fresh RNA*later*kept on ice. The isolated hair follicles were cleaned of any dermis or fat debris under a higher magnification using sterile syringe needles (27G1/2 tuberculin syringe; Sigma).

Total RNA isolation and amplification

Total RNA was extracted from scalp anagen follicles from each individual immediately after microdissection using the RNeasy Mini Kit (Qiagen, Crawly, UK). The extraction process was performed in an area cleaned before use with 70% (v/v) ethanol and RNase Zap solution (Sigma). The quality of total RNA was checked by agarose gel electrophoresis 1.5% (w/v) before further purification to isolate poly (A) RNA (i.e., mRNA) using GenElute mRNA Miniprep Kit (Sigma). Due to the limited starting materials, the isolated total RNA was amplified using the Smart™ RNA amplification kit (Clontech laboratories, USA) to synthesize high quality RNA for use in molecular biological investigation. The amplification procedure was done in accordance to the manufacturer's instructions in the following successive steps:

First-strand cDNA synthesis: RNA sample (3 µg) was added to cDNA synthesis (CDS) primer II (12 µM) and a suitable amount of nuclease-free water. The contents were mixed and centrifuged briefly at 8000 g for few seconds in eppendorf 5415 microcentrifuge. The tube was incubated in a thermal cycler for 3 minutes at 70ºC and then the temperature was reduced to 42ºC for 2 minutes. The master mix was prepared by combining the following components in RNase-free eppendorf tube, 5X First-Strand Buffer (25 mMTris-HCl, pH 8.3, 15 mMMgCl_2, 20 mMspermidine, 35 mMKCl), DTT (100 mM), RNase inhibitor (1 U/µl), SMART™ T7 Oligonucleotide (10 µM), 50X dNTP mix (dATP, dGTP, dCTP, dTTP; 10 mM), smartscibe reverse transcriptase. Following a brief centrifugation, the master mix (5.75 µl) was added to the reaction tube prepared previously and returned immediately to

the thermal cycler and incubated at 42ºC for 1.5 hr. The reaction was terminated by heating at 68ºC for 10 minutes.

Second-strand cDNA synthesis: Following first-strand cDNA synthesis, the master mix for second-strand cDNA was prepared by combining the following components; nuclease-free water, 10X Advantage® PCR buffer (10 mMTris-HCl, 15 mM$MgCl_2$, 50 mMKCl), 50X dNTP mix (dATP, dGTP, dCTP, dTTP; 10 mM), T7 extension primer (10 µM), RNase H (10 U/µl) and 50X Advantage® polymerase mix. The contents were mixed by vortexing and the tube centrifuged briefly at 8000 g for few seconds. The mixture was added to the reaction tube containing the first-strand cDNA obtained from the previous step. The reaction tube was placed in a thermal cycler using the following program: 37ºC for 15 minutes, 95ºC for 2 minutes, 60ºC for 1 minute, 68ºCf or 10 minutes.

Purification of double-strand cDNA: Prior to performing *in vitro* transcription, the double-strand cDNA was purified using the AtlasNucleospin® Extraction II kit (Clontech). Two volumes of buffer NT, contained chaotropic salt, was added to one volume of sample. The mixture was centrifuged for 1 minute at 11,000 g; the flow-through was discarded. Buffer NT3 was added to the reaction mixture and centrifuged at 11,000 g for 1 minute. Buffer NE (50 µl, 5 mMTris-HCl; pH8.5) was added to the column and was left to stand for 1 minute, before the tube was centrifuged at 12,000 g for 1 minute. The eluted cDNA was centrifuged at 12,000 g for an additional 3 minutes. The supernatant was transferred to a new collection tube. Linear acrylamide, sodium acetate and 100% ethanol were mixed and added to the reaction tube which was placed at -20ºC overnight to precipitate the cDNA. The next day the tube was centrifuged for 20 minutes at 12,000 g. The supernatant was removed and the pellet washed once in 70% ethanol for 10 minutes before dissolving in nuclease-free water. The purified double-strand DNA was used immediately or stored at -20ºC until required.

Synthesis of cRNA (in vitro transcription): Following purification of double-strand cDNA, *in vitro*cRNA synthesis was carried out. The transcription master mix was prepared by combining the following components at room temperature; 10X T7 transcription buffer (20 mMTris-HCl; pH 7.5, 50 mMNaCl, 10 mMspermidine, 30 mM$MgCl_2$), 3X rNTP mix (ATP, CTP, GTP, UTP; 10 mM), RNase inhibitor (1 U/µl) and T7 RNA polymerase (1,000 U/µl). The tube was mixed by vortexing and centrifuged briefly at 8000 g for few seconds in the microcentrifuge. The transcription master mix was added to the purified double-strand cDNA and mixed by vortexing before incubating at 37ºC for 12 hr. The resulting cRNA was purified as described below.

Purification of cRNA: The cRNA was purified to remove any unicorporatedribonucleotides and small cDNA and RNA fragments. The purification process was carried out using the NucleoSpin® RNA II purification kit (Clontech). Buffer RA1 was added to cRNA in RNase-free eppendorf tube and mixed well by pipetting. Ethanol (100%) was added to the mixture and mixed thoroughly. The sample was loaded into a Neucleospin column already inside a 2 ml collection tube. The tube was centrifuged for 60 seconds at 8000 g, and the flow-through discarded. The cRNA was washed by adding buffer RA3 to the Nucleospin column and centrifuged at 14,000 g for 1 minute. The RNA was eluted by adding nuclease-free water to the centre of the column. After elution, the spin column was discarded and the eluted RNA was transferred to the mixture. The purified cRNA was either used immediately or stored at -80ºC until required.

Checking the quality of purified cRNA: The quality and purity of the resulting RNA was evaluated using the NanoDrop spectro-photometer (Thermo Scientific, UK). The automatic path length capability of the NanoDrop spectrophotometer allowed for measurement of samples using the instrument's standard method, with no special sample preparation required. All samples were measured on the NanoDrop without dilution. The elution solution was used as a control to set the background as zero and then each RNA sample was used for measurement of the RNA concentration. The NanoDrop created a graph for each sample measured.

The procedure of RT-PCR

RT-PCR was used to investigate the expression of mRNA for Kir6.1, Kir6.2, SUR1, SUR2A and SUR2B, in anagen scalp hair follicles. In order to ensure that the RNA samples to be used for cDNA synthesis were free of any contaminating DNA, the sample was treated with the DNA amplification Grade I Kit (Invitrogen Ltd., UK). cDNA synthesis was carried out by using an Avian Myloblastosis Virus (AMV) reverse transcription system (Promega, Southampton, UK) to produce single strand cDNA from DNase-treated poly (A) RNA. PCR ampilification was performed using c DNA in 50 µl reaction volume containing 0.5 µM concentrations of forward and reverse primers (Sigma-Genosys Ltd., Pamisford, UK), 10X PCR reaction buffer (200 mMTris-HCl, pH 8.4, 500 mMKCl; Invitrogen), 200 µM concentrations of each dNTP (Promega), 2.5 mM$MgCl_2$, and Taq DNA polymerase (5 units/ µl; Invitrogen).

For each PCR reaction, a negative control was set up replacing the cDNA with nuclease free water. To prevent evaporation of the reaction mixture, one drop of mineral oil (Sigma) was added on the top of the mixture. The PCR products were analyzed by gel electrophoresis on 1.5% Tris-Acetate EDTA (TAE) agarose gel (Invitrogen). PCR products were visualised using the Uvitec gel documentation system (Uvitec Limited, Cambridge, UK) at 312 nm wavelength and the image captured. Sequence analysis was used to confirm the identity of PCR products.

Real-time PCR procedure

Real-time PCR was performed using the MyiQ™ single-colour real-time PCR detection system (Bio-Rad, UK) and SYBER® Green PCR Master Mix (Applied Biosystem, USA). For each real-time PCR reaction, the following reaction mix was prepared and used: SYBER® Green PCR Master Mix, the forward and reverse primers of the target gene, the cDNA template and nuclease-free water. The reaction mixture was then transferred into an optical 96-well reaction plate (Applied Biosystem). Each well was tightly covered using specific optical caps. The highly expressed housekeeping protein, GAPDH, was used as an endogenous control. The plate was then placed into the real-time PCR machine.

Real-time PCR was performed using specific forward and reverse primers for each cDNA target sequence. The annealing temperature for each target primer set was initially optimized using the cDNA template, synthesized from the universal human reference RNA (Stratagene, UK) which composed of total RNA isolated from 10 cell lines representing different human tissues which were chosen to ensure a standard broad coverage of human genes. The real-time PCR was performed under the following cycling conditions: 94ºC for 3 minutes, followed by denaturing at 94ºC for 15 seconds, annealing (gradient) of 55ºC to 63ºC for 30 seconds, followed by 72ºC for 15 seconds; this was repeated for 40 cycles. Real-time PCR data and the differences between samples and controls were calculated using the Genex database software based on the comparative (ΔΔCt) equitation method to calculate relative quantities of a nucleic acid sequence. The Ct is the threshold cycle during which a reaction emits the threshold level of fluorescence. The detectable amount of fluorescence when a signal is significantly greater than background is known as the threshold. Data was normalized to the corresponding values of an endogenous control, GAPDH.

RESULTS

Checking the quality of RNA

Total RNA quality for each sample was checked by agarose gel electrophoresis using 1.5% (w/v) agarose gel. Total RNA was mixed with blue/orange loading dye to assist loading and monitor the resolution of the sample. Intact total RNA should have sharp 28S and 18S rRNA bands (Skrypina *et al*., 2003), with the 28S rRNA band should be approximately twice as intense as the 18S rRNA band (fig 2). This 2:1 ratio (28S:18S) is a good indication that the RNA is intact. Furthermore, partially degraded RNA will not exhibit a 2:1 ratio whereas completely degraded RNA will appear as very low molecular weight smear (Ambion, USA). The results for all five scalp follicle samples showed clear bands with much stronger bands for 28S than 18S (fig 2). The quality and purity of amplified RNA was evaluated using the NanoDrop spectrophotometer (fig 3). One single peak of absorbance occurred at the wavelength 260 nm for each sample indicating that the RNA is intact as degraded RNA shows more than one peak. All RNA samples exhibited a 260/280 ratio close to 2 indicating high quality resulting RNA.

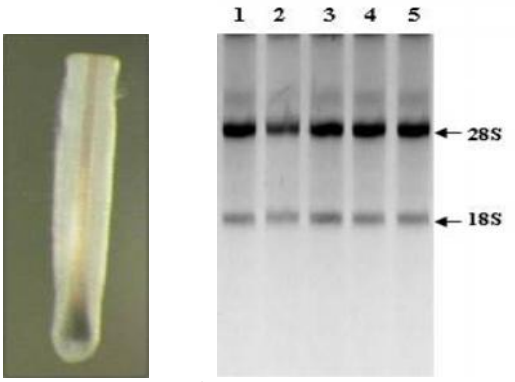

Fig 2. Gel electrophoresis of total RNA. Total RNA samples (1-5) from isolated scalp hair follicles, leftt panel, were loaded on 1.5% agarose gel, right panel. The rRNA bands (28S and 18S) exhibited 2:1 ratio and are clearly visible in the total RNA gel.

Checking the purity of cDNA

Prior to investigating the expression of K_{ATP} channel subunits, the quality of the cDNA samples was investigated by PCR using specific primers for β-actin. The detection of this housekeeping gene would indicate that the isolated RNA is of sufficient quality for RT-PCR to be performed effectively. PCR products from all hair follicle samples corresponded to the anticipated size of β-actin 838 bp (fig 4). Therefore, the successful amplification of β-actin on all cDNA samples verified that the cDNA samples were of adequate quality for further RT-PCR analysis to be performed, investigating the expression of K_{ATP} channel subunits.

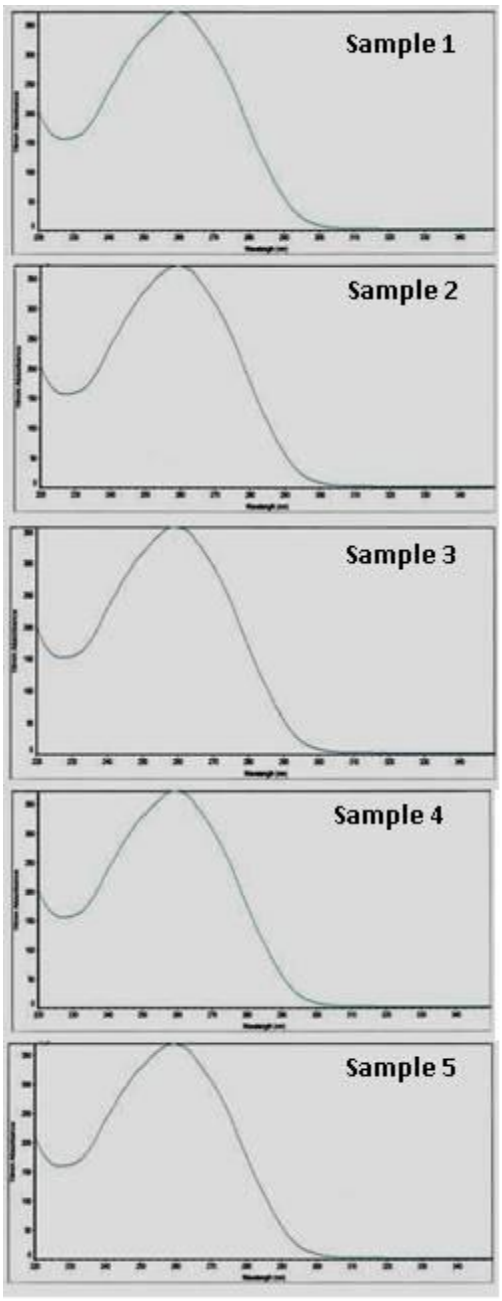

Fig 3 Checking the quality of amplified RNA. RNA obtained from scalp follicle samples (n=5) were evaluated using the NanoDrop spectrophotometer. The single peak in each sample at the wavelength 260 nm indicates that the RNA is intact. The x axis indicates wavelength (nm), the y axis indicates absorbance.

K_{ATP} channel subunits are expressed in scalp hair follicles

The RT-PCR results demonstrated the expression of K_{ATP} channel subunits in anagen scalp hair follicles. All five isolated hair follicle cDNA samples produced PCR products of the expected size 336, 301, 291 and 312 bp for the genes of Kir6.1, Kir6.2, SUR1 and SUR2B respectively (fig 4). The gene expression for SUR2A was not detected in anagen follicles. In the negative control, where the template cDNA was excluded from the reaction mix, no PCR products were present, indicating that the amplification of the cDNA synthesised from the mRNA samples produced the PCR products. In addition, it also demonstrated that no DNA contamination occurred in the reactionmix. To confirm the identity of the PCR products, sequence analysis was used. The sequenced PCR product of each gene was compared to the known expected human sequence. Therefore, sequencing verified all genes against their relevant human sequences in Genebank. All PCR products of scalp hair follicles cDNA exhibited more than 95% homology to the known human sequence.

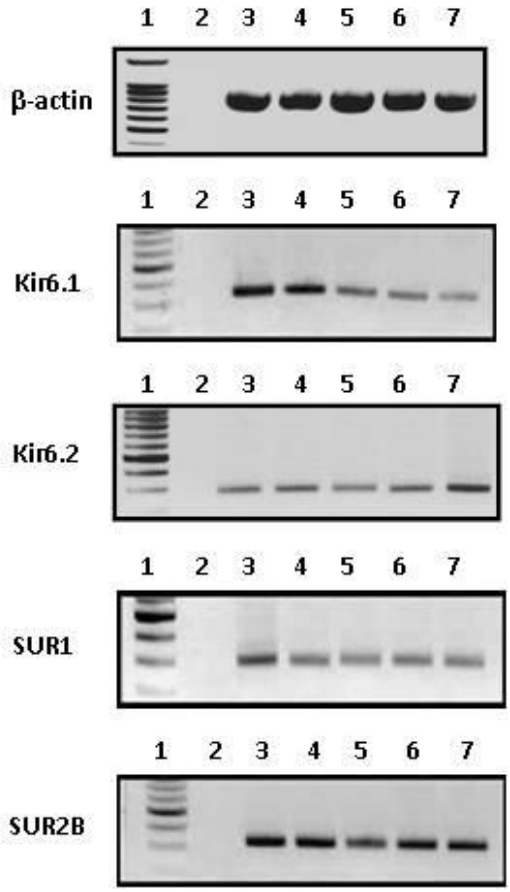

Fig 4 Expression of K_{ATP} channel subunits in scalp hair follicles. RT-PCR demonstrated expression of Kir6.1, Kir6.2, SUR1 and SUR2B in mRNA from five human hair follicle samples. PCR products were applied to 1.5% (w/v) agarose gel for electrophoresis with ethidium bromide staining. DNA was visualized with UV illumination. Lane 1 denotes 100 bp DNA molecular size marker. Lane 2 contained the negative control in which nuclease free water was used as a template instead of cDNA. Lane 3-7 denotes human hair follicle PCR products. The cDNA amplification product was predicted to be 336, 301, 291 and 312 bp respectively.

The relative expression of K_{ATP} channel subunits

To complement the results from the RT-PCR analysis, quantitative real-time PCR was carried out to investigate the relative expression of Kir6.1, Kir6.2, SUR1 and SUR2B in human scalp hair follicles. Since SYBR Green binds to any double stranded DNA, it is necessary to examine the specificity of the resulting PCR products of each gene. Melt-curve analysis allows the identification of any non-specific product which may be amplified with these genes such as genomic DNA contamination and primer-dimers, as the presence of a non-specific product would show up as an additional peak in the melt-curve. The melt-curves for all examined genes Kir6.1, Kir6.2, SUR1 and SUR2B contained only single peaks indicating that these reactions generated only one product for each gene in each of the five samples used, and no contaminating products were present (fig 5). It is clear from the graphs that the inflection point occurred around 80˚C in all investigated genes. Data from all five individual hair follicle samples were collected as cycle threshold (Ct) and the gene expression levels were calculated by normalizing the data against those of the endogenous control GAPDH in each sample. All five scalp hair follicle samples expressed the gene for Kir6.1, Kir6.2, SUR1 and SUR2B, with the relative expression levels of SUR being higher than those of Kir6.1 and Kir6.2 (fig 6).

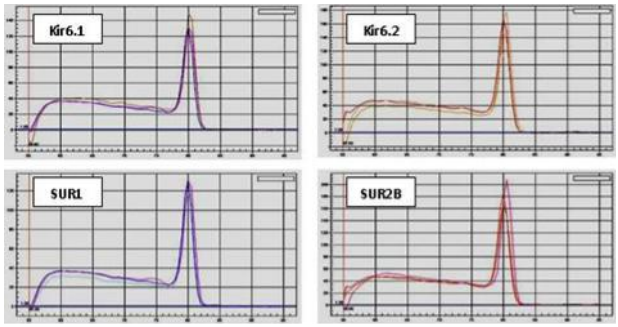

Fig 5 Melt-curve analysis for potassium channel subunits in scalp follicles. Melt-curves were generated by real-time PCR from scalp follicle samples. The melt-curves contain only one peak indicating that no contaminating products are present in these reactions and each reaction generated only one product. The melting temperature of amplified PCR products, the points of inflection, occurred at about 80˚C in. The x axis indicates temperature (˚C), the y axis indicates fluorescence.

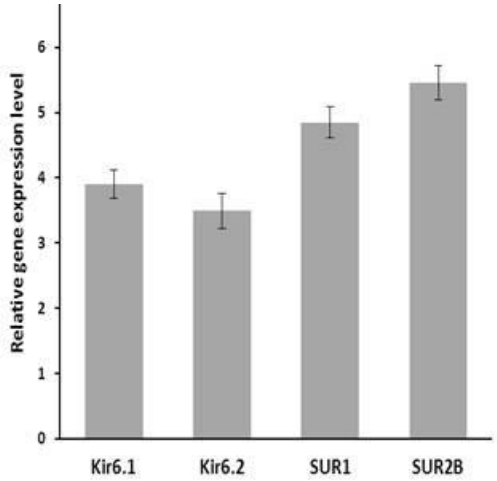

Fig 6. Relative expression levels of potassium channel subunits in scalp follicles. Real-time PCR was performed to analyze the relative expression levels of potassium channel subunits in scalp hair follicles. Expression levels were calculated by normalizing the value against those of the endogenous control (GAPDH). Data are means ± SE from five different individuals.

DISCUSSION

This study aimed to determine whether the gene expression of K_{ATP} channels subunits occurred in the anagen stage of the hair cycle of human scalp hair follicles. Structurally, K_{ATP} channels have been shown to comprise of hetero-octameric complexes of four pore forming (Kir6.x) and four regulatory sulphonylurea receptor (SUR) subunits (Clement *et al*., 1997). Different combinations of K_{ATP} channel subunits (SUR1, SUR2A, or SUR2B and Kir6.1 or Kir6.2) form channels in different tissues with diverse electrophysiological, nucleotide and pharmacological properties (Seino and Miki, 2003). Interaction between K_{ATP} channel openers and the SUR subunit enables potassium ions to exit the cell, instigating hyper-polarisation of the plasma membrane, and as a consequence reduces electrical activity (Ashcroft and Gribble, 2000).

The sensitivity of different SUR subunits to K_{ATP} channel openers varies. For example SUR1/Kir6.2 channels expressed in pancreatic β cells are significantly sensitive to diazoxide, to some extent sensitive to pinacidil, and not at all responsive to cromakalin and nicorandil (Trube *et al*., 1986; Ashcroft and Rorsman, 1989). On the other hand, SUR2A/Kir6.2 channels, expressed in cardiac and skeletal muscles, are sensitive to pinacidil and cromakalin, and only slightly to diazoxide. SUR2A/Kir6.2 channels can be sensitive to diazoxide, when in the presence of MgADP (D'Hahan *et al*., 1999b), while SUR2B/Kir6.2 channels expressed in non-vascular smooth muscle are sensitive to all the K_{ATP} channel openers named above (Gribble *et al*., 1998; D'Hahan *et al*., 1999a). The sensitivity to K_{ATP} channel inhibitors is similarly tissue specific. For example SUR1/Kir6.2 channels found in pancreatic β cells are inhibited by lower concentrations of tolbutamide than channels in cardiac and smooth muscle (Venkatesh *et al*., 1991; Quayle *et al*., 1997). All of these varying sensitivities to K_{ATP} channel openers and blockers support the model that the K_{ATP} channel opener binding site is situated on the SUR subunit (Nichols, 2006).

K_{ATP} channel openers, such as minoxidil, diazoxide, chromakalim, nicorandil and pinacidilare chemically diverse agentsclassified by their ability to directly open ATP sensitive potassium channels (Lawson, 2000). Several of these agents have been identified to cause hair growth as a side effect in human beings (Koblenzer and Baker, 1968; Goldberg, 1988). Different K_{ATP} channel openers have varying effects on the prevalence and severity of hypertrichosis. For example following oral minoxidil treatment 60-80% of adults reported hypertrichosis (Burton and Marshall, 1979; Zins, 1988), whereas, pinacidil treatment caused hypertrichosis in 13% of females and only 2% of males (Goldberg, 1988). During diazoxide treatment most children reported increased hair growth, however only an insignificant 1% of adults reported hypertrichosis (Burton *et al*., 1975). The majority of K_{ATP} channel openers were initially developed for treatment of hypertension and hypoglycaemia. Minoxidil was one of the first to be introduced into the market, in the early 1970s, in the form of an oral treatment directed at hypertension. It was reported to lower arterial blood pressure, via a relaxation of vascular smooth muscle, most likely through the opening of the vascular K_{ATP} channels (Weston and Edwards, 1992), although there was no direct evidence for this.

In the present study, the gene expression of all components of potassium channel subunits in isolated scalp anagen hair follicles was investigated. Detection of cytoskeletal protein β-actin confirmed that the isolated RNA from each sample was of sufficient quality for RT-PCR to be performed effectively. Negative controls without the template cDNA in all RT-PCR investigations yielded no PCR products, indicating that the amplification of the cDNAsynthesised from the mRNA samples produced the PCR products. Furthermore, it also demonstrated that no DNA contamination occurred in the reaction mix. The RT-PCR results showed that human scalp hair follicles expressed the gene for kir6.1, kir6.2, SUR1 and SUR2B.The identity of PCR products were confirmed by sequencing which represented more than 95% homology of the human genes. Therefore human scalp hair follicles expressed the genes for both components of K_{ATP} channels in the anagen phase of the hair cycle. The gene expression for SUR2A was not detected. The data obtained from real-time PCR also confirmed the presence of these subunits in scalp hair follicles. This demonstrated that the expression of both types of subunits, the sulphonylurea subunit (SUR) and the pore forming (Kir6.x), are necessary to form a functional K_{ATP} channel (Gribble *et al*., 1997; Yamada *et al*., 1997). Therefore it is most probable that SUR2B forms a K_{ATP} channel with Kir6.1; and SUR1 can form a K_{ATP} channel with Kir6.2, thereby suggesting the existence of a possible two K_{ATP} channels in scalp hair follicles. Furthermore such K_{ATP} channel combinations have been indicated in different tissues, such as SUR1/Kir6.2 K_{ATP} channel in the pancreatic β cells and brain (Sakura *et al*., 1995; Babenko *et al*., 1998). This supports the suggestion that SUR1/Kir6.2 are likely to combine to form a K_{ATP} channel. SUR2B/Kir6.1 has been identified in vascular smooth muscle and SUR2B/Kir6.2 in non-vascular smooth muscle (Isomoto *et al*., 1996; Yamada *et al*., 1997).

These findings are similar to a recent study in human anagen hair follicles, which also indicated the detection of SUR1, SUR2B, Kir6.1 and Kir6.2 subunits (Shorter *et al*., 2008). They also correspond strongly with pharmacological observations in red deer hair follicles, where it was found that both minoxidil and a selective SUR1 channel opener, stimulate hair follicle growth in vitro (Davies *et al*., 2005), indicating the presence of both SUR1 and SUR2 types of channel, both playing a role in hair growth. The detection of SUR2B corresponds with minoxidil's affinity for SUR2B channels (Schwanstecher *et al*., 1998). Taken together these results confirm the existence of at least two types of K_{ATP} channels with SUR1 and SUR2B sulphonylurea receptors in scalp hair follicles in the anagen phase. This is analogous with the findings of both SUR1 and SUR2B K_{ATP} channels in pig urethra (Yunoki *et al*., 2003). However, SUR1/Kir6.1 channels have been suggested not to be physiologically relevant (Babenko *et al*., 1998). An advantage to the fact that SUR2A was not detected in the anagen hair follicle, suggests that the K_{ATP} channels found in the cardiac tissue, formed by SUR2A/Kir6.2, are not expressed in the hair follicle, therefore allowing for the development of treatments for heart conditions, with the employment of the SUR2A

sulphonylurea receptor, that would eliminate the undesirable side effect of hair growth.

Despite the popularity of topical minoxidil as a form of treatment for and rogenetic alopecia, and much research in its field, the mechanism of action by which minoxidil stimulates hair growth remained uncertain. The general accepted view was that it worked via effects on the vasculature to increase blood supply to the follicles (Messenger and Rundegren, 2004). It has been reported that human hair follicles express both the genes and proteins for at least two K_{ATP} channels, SUR1/Kir6.2 and SUR2B/Kir6.1, and showed that the expression was variable within the follicle. Minoxidil has a specific affinity to K_{ATP} channels which contain SUR2A/B subunits, but not those with SUR1. The presence of SUR2B in the dermal papillae suggests that minoxidil affects the human hair follicle via the dermal papillae (Shorter *et al*., 2008). This is supported by their observation that minoxidil increased anagen in human hair follicles in organ culture, an effect inhibited by the potassium channel blocker, tolbutamide, and similar to the effects on the growth of deer follicles in organ culture (Davies *et al*., 2005). This confirms that minoxidil can act directly on hair follicle K_{ATP} channels in human hair follicles and means that its mechanism of action within the follicle merits more investigation to facilitate the development of new treatments to replace the relatively poor effects of minoxidil treatments used currently.

To conclude, the molecular biological investigations into K_{ATP} channel subunits revealed that kir6.1, kir6.2, SUR1 and SUR2B are expressed in human scalp hair follicles. There is a strong correlation in the RT-PCR analysis of K_{ATP} channel sub-units in scalpanagen hair follicles with those obtained from real-time PCR. Both methods showed the gene expression of all four K_{ATP} channel subunits. Thereby exhibiting that both of the essential components, the sulphonylurea receptor and the pore forming sub-unit (Kir6.x) are expressed, enabling a functional channel to be present at the anagen phase of the hair cycle. The absence of SUR2A gene expression in human scalp hair follicles would allow future development of drugs selective for SUR2A K_{ATP} channels, such as those in cardiac muscle without the possibility of hair growth side effects. The detection of SUR1 subunit gene expression in the scalpanagen hair follicle is of key interest, as SUR1 receptors are unaffected by minoxidil, a form of K_{ATP} channel openers. These observations raise the prospect of novel pharmaceutical developments that stimulate hair growth via the SUR1 K_{ATP} channels or combined treatment with minoxidil acting via SUR2B channels that enhance treatments for hair growth. This system may aid the development of better therapies for hair disorders.

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