Pigmentation and the cutaneous response to ultraviolet radiation

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Declaration

I declare that I have composed this thesis and this thesis is my own work. The experiments described herein were the unaided work of the author except where acknowledgement is made by reference. No part of this work has been previously accepted for any other degree, nor is any part of it being submitted concurrently in candidature for another degree.

Terence H Wong

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Abstract

Pigmentation and the cutaneous response to ultraviolet radiation

Variation in pigmentation of hair and skin is one of the most striking forms of human diversity. Human pigmentation and sun sensitivity is a complex trait. The melanocortin 1 receptor gene (*MC1R*) (OMIM 15555) has been shown to be a key determinant of hair and skin colour. Recently a number of other genes have been implicated in human pigmentation.

This thesis presents the relationship between human pigmentary phenotypes and genetic variation at *MC1R* and 34 other candidate loci from 159 individuals. The relationship between experimentally induced cutaneous erythemal and facultative pigmentary response to UVB radiation and *MC1R* and other pigmentation genotypes was investigated in a subset of 98 individuals. Some of this work involved the development of novel methods of assaying phenotype.

I present a detailed description of human pigmentation and facultative pigmentation with respect to a number of key variables (e.g. sex, site, freckling, skin type) and seek to explain the variation in pigmentation in relation to these factors.

The effect of *MC1R* on hair colour is large, but *MC1R* explains a smaller amount of the variation for skin colour.

I found that a number of loci including *MC1R*, oculocutaneous albinism type 2 *OCA2* (OMIM 611409), *KIT* oncogene ligand *KITLG* (OMIM 184745) and the Hermansky-Pudlak syndrome 3 *HPS3* (OMIM 606118) are determinants of pigmentary phenotype. Some of these findings are in keeping with previous work and some are novel.

I present data showing novel SNPs in genes Hermansky-Pudlak syndrome 3 (*HPS3*) and *KIT* ligand (*KITLG*) to be associated with human skin and hair colour variation. Association of *HPS3* to eye colour was also found and has to be confirmed in another population. The possible putative mechanisms for the novel association finding in *HPS3* are discussed. I am in the process of confirming these positive significant findings in collaboration with another laboratory in Denmark. Further experiments are proposed to confirm other associations and phenotypes.

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List of abbreviations

°C	Degrees Celsius
A_{600}	Absorbance at 600nm
ACTH	Adrenocorticotrophic hormone
ASIP	Agouti signalling protein
α-MSH	Alpha–melanocyte stimulating hormone
C	Celsius
cAMP	Cyclic adenosine monophosphate
DCT	Dopachrome tautomerase
DHI	Dihydroindole
DNA	Deoxyribonucleic acid
DdH_20	Double distilled water
dNTP	Deoxynucleotide triphosphate
DOPA	3,4-dihydroxyphenylalanine
EDR	Erythemal Dose Response
EDTA	Ethyldiaminetetra-acetic acid di-sodium salt
EI	Erythemal Index
FP	Fluorescence polarization
g	Gramme
HGU	Human Genetics Unit
Hm	Homozygous
Ht	Heterozygous
KITLG	KIT ligand
LD	Linkage disequilibrium
µ	Micro
MC1R	Melanocortin 1 receptor gene
MED	Minimal Erythemal Dose
MDR	Melanogenic Dose Response
MI	Melanin Index
MITF	Microphthalmia-associated transcription factor
OCA	Oculocutaneous albinism
OMIM	Online Mendelian Inheritance in Man
PAR2	Protease activated receptor-2
PCR	Polymerase Chain Reaction
PKA	Protein kinase A
POMC	Pro-opiomelanocortin
SDS	Sodium dodecyl sulphate
TAE	Tris, acetic acid, EDTA
TBE	Tris, boric acid, EDTA
TE	Tris, EDTA
TYR	Tyrosinase
TYRP1	Tyrosinase-related protein 1
TYRP2	Tyrosinase-related protein 2
UV	Ultraviolet
WT	Wildtype

Chapter 1 Introduction

1.1 Skin

Skin is the most conspicuous part of human body. It is the largest organ in humans, measuring 1.94-1.95 square metres in area (Boyd, 1935; Dubois and Dubois, 1916) and weighing approximately 4 kg. Imagine a life without skin, our muscles, bones and internal structures would be freely exposed to various environmental insults. Skin therefore has numerous functions in humans including protection of our internal organs against injury, thermoregulation through sweating, innate immune response against pathogenic organisms, barrier against fluid loss and protection from the damaging UV radiation from the sun (Fitzpatrick, 2003). In addition, skin has other roles including

i) Sensory; the detection of light touch, temperature, pressure, itch and pain stimuli by mechanoreceptors e.g. Meissner's corpuscles, Pacinian corpuscles and the nerve fibres within.

ii) Homeostasis by acting as an external covering and barrier to integrate other deeper structures.

iii) The biochemical role of skin involving vitamin D synthesis and therefore the prevention of rickets and may be other diseases. This was previously of more importance where dietary vitamin D was not plentiful. Nowadays that food is regularly fortified with vitamin D, the cutaneous contribution is less essential (Holick, 2008). Loomis argued that variation in UV radiation from different latitudes away from the equator sun might favour lighter pigmented individuals against vitamin D deficiency and rickets (Loomis, 1967, 1970) whereas, UV radiation at the equator favoured darker pigmented individuals who possessed enough melanin to protect against burning and skin cancers (Harrison, 1973). Bodmer and Cavalli-Sforza hypothesized that lighter skin pigmentation prevented rickets in higher latitudes by encouraging higher levels of vitamin D production and also allowed individuals to retain heat better than someone with darker skin (Bodmer WF, 1976).

iv) Role in human sexual selection (Darwin, 1859; Frost, 1988, 2007; Madrigal and Kelly, 2007).

v) Social appearance. Despite the various roles, the macroscopic appearance of our skin together with other phenotypes including hair colour and eye colour form our external image.

1.2. Skin layers

Skin varies in thickness. Our eyelids have the thinnest skin whereas our palms and soles have the thickest. Microscopically, our skin consists of 3 layers – epidermis, dermis and subcutis. The stratum corneum is the outermost horny layer. Dividing cells from the deepest layer stratum basale (basal cell layer) move upwards, through stratum spinulosum (prickle cell layer) and stratum granulosum (granular cell layer).

1.2.1. Skin levels and cell types

Keratinocytes are keratin-producing cells at the stratum spinulosum. They are held together and to each other via gap junctions and a range of desmosomal proteins including desmogleins, desmoplakins and desmocollins (Dusek *et al.*, 2007). Melanocytes are melanin-producing cells of neural crest origin (Boissy, 1988; Vancoillie *et al.*, 1999). Each melanocyte is associated with about 36 keratinocytes (Fitzpatrick, 2003). They account for our skin colour, pigmentation and tanning. Melanocytes transfer melanin that is packaged within membrane-bound organelles called melanosomes. Langerhans cells are dendritic cells originating from the bone marrow. They have a role in immune mediated responses and antigen presentation. Merkel cells are cells that mediate fine touch sensation (Winkelmann, 1977; Winkelmann and Breathnach, 1973). They are located around the stratum basale.

Africans are darkly pigmented because their melanocytes produce larger and more melanosomes, not because they have more melanocytes (Szabo *et al.*, 1969). The number of melanocytes for both Caucasians and Africans are the same.

1.3. Ultraviolet radiation

Human skin is exposed to various stimuli and insults. It is our first contact to the outside world, including UV radiation. Johann Ritter first discovered the UV region of the solar spectrum in 1801 (Hockberger, 2002). Charcot first determined that UV radiation caused erythema in 1858 (Urbach, 2001) and Unna determined that UV

radiation can cause pigmentation in 1894 (Hockberger, 2002). Unna also related changes in the stratum corneum, epidermis and dermis to chronic UV radiation exposure. Miescher in 1930 showed that stratum corneum thickening provided photoprotection to further UV radiation (Diffey, 1991).

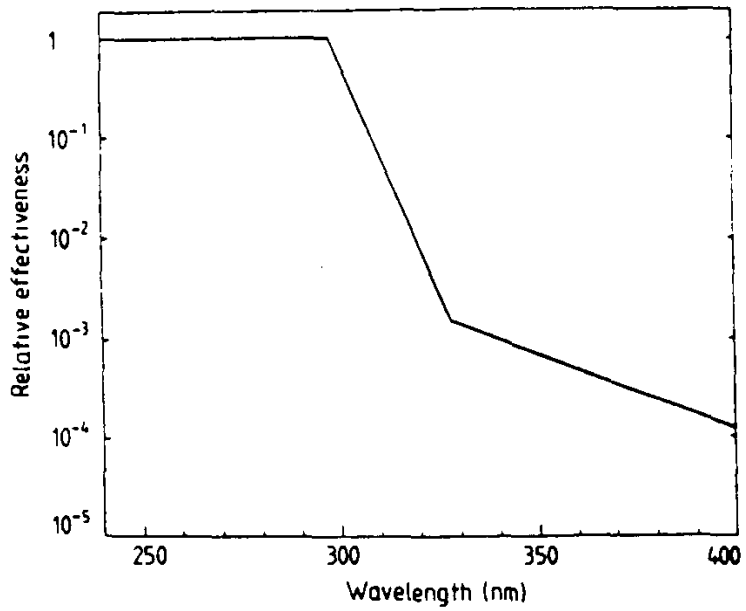
UV radiation is divided into 3 regions: UVA 320-400nm, UVB 290-320nm, UVC 200-290nm. These are arbitrary divisions and differ depending on the discipline involved (Diffey, 1991). UVB is capable of penetrating the epidermis and up to superficial dermis. It stimulates the production of prostaglandin E_2 (PGE_2) (Black *et al.*, 1978), leukotrienes (Sondergaard *et al.*, 1985), histamine (Soter, 1990), interleukin-1 (IL-1) (Granstein and Sauder, 1987; Oxholm *et al.*, 1988) and TNF-alpha (Bashir *et al.*, 2009; Kock *et al.*, 1990) and iNOS (Chang *et al.*, 2003; Seo *et al.*, 2002).

1.4. Erythema

The erythemal response can be attenuated or blocked completely by prostaglandin inhibitors (Rhodes *et al.*, 2001). Nitric oxide is also involved as a mediator (Rhodes *et al.*, 2001).

An action spectrum is a plot of the relative effectiveness of radiation of different wavelengths to produce a particular biological effect. Thus an erythema action spectrum shows the erythemal effectiveness of UV radiation.

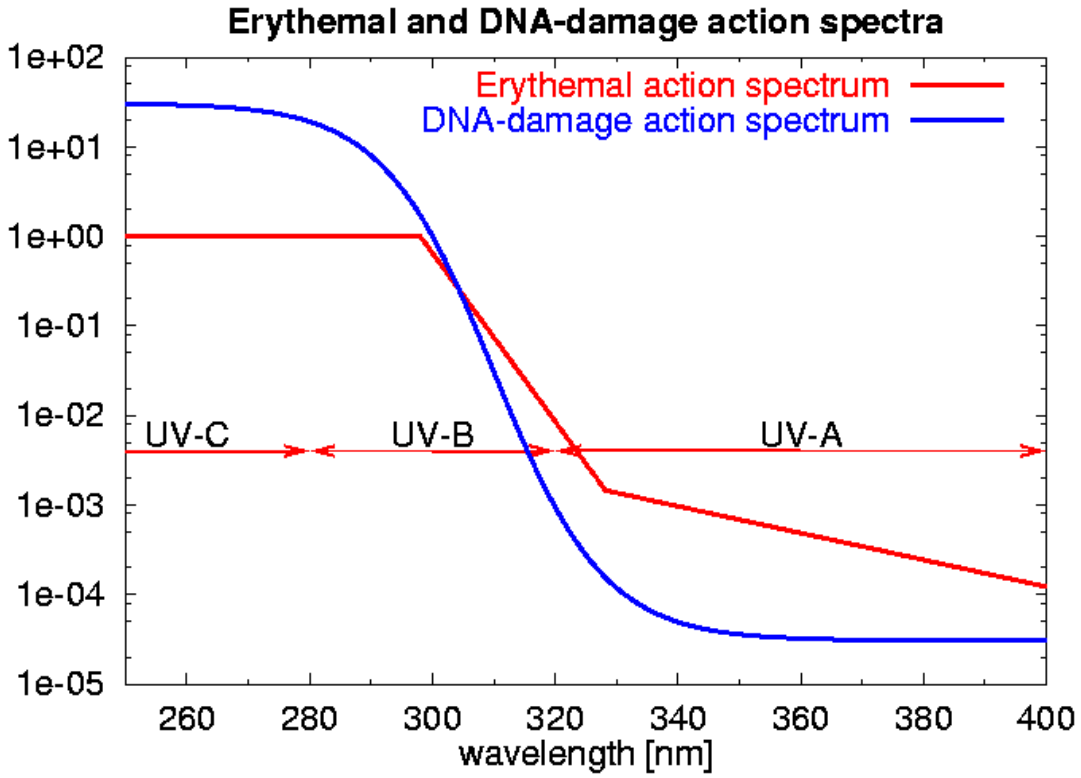
Figure 1.3.1. Erythema action spectrum



The CIE reference erythema action spectrum (McKinlay and Diffey 1987).

Figure 1.3.1 shows the reference CIE erythemal action spectrum and that the effectiveness drops off very quickly at wavelengths greater than 300nm (Diffey, 1991). The erythemal action spectrum and DNA damage action spectrum overlap (Figure 1.3.2), (Parrish *et al.*, 1982) since erythema is a visible trait and is exemplified with burning. This in turn is associated with DNA damage and the erythema action spectrum (McKinley A, 1987) mirrors the DNA action spectrum (Diffey, 1991). DNA is the chromophore for erythema (Young *et al.*, 1998). This in turn linked erythema or sunburn with skin cancer (Elwood, 1996; Kricker *et al.*, 1995; Whiteman and Green, 1994). Thus erythema is a useful endpoint as it is associated with DNA damage.

Figure 1.3.2. DNA action spectrum – the rate of a physiological activity plotted against wavelength of light



Source: www.temis.nl/uvradiation/info/uvaction.html

The effect of UV radiation on the skin can be classified as:

i) acute (sunburn / erythema, tanning, vitamin D production, immunosuppression) and

ii) chronic (photo-ageing and skin cancer).

1.4. Control of tanning

1.4.1. Immediate pigment darkening (IPD)

Immediate pigment darkening (IPD) is a transient darkening of the skin observed after UVA exposure and visible radiation, reviewed in (Young, 2006). The melanocytic system is implicated in its development. It may involve structural changes in melanocytes and keratinocytes (Routaboul *et al.*, 1999) or a chemical modification or redistribution of pre-existing melanin (Brenner and Hearing, 2008; Lavker and Kaidbey, 1982). Darkening intensity is maximal immediately after exposure and decreases rapidly. The maximum efficiency wavelength for induction

of IPD is around 340 nm (Irwin *et al.*, 1993). The phenomenon is inhibited by oxygen deprivation (Auletta *et al.*, 1986). Dose-response curves are linear for doses above 4 J/cm^2 (Chardon *et al.*, 1991). Minimum dose for induction of IPD (MIPDD) varies with the subject according to phototype, melanotype and skin colour (Routaboul *et al.*, 1999). The best criterion for predicting MIPDD seems to be chromametrically determined skin colour. The greater the constitutive skin colour, the greater the ability to exhibit IPD. The biological role of IPD is poorly understood, there are several hypotheses (Routaboul *et al.*, 1999):

Descamps *et al* suggested that IPD may be implicated in the appearance of delayed UVA-induced tanning (Descamps *et al.*, 1990). Black *et al* found that pre-exposure to UVA increases MED and therefore IPD may confer erythemal protection against UVB-erythema (Black *et al.*, 1985).

1.4.2. Delayed pigment darkening (DPD)

This is delayed tanning or facultative pigmentation (tanning) noticeable around 1-2 days after solar exposure and continues to increase for days and may persist for weeks or months (Diffey, 1991). The persistent tanning involves the activation of melanocyte function. Exposure to UV leads to increased expression of MITF (the master transcriptional regulator of pigmentation) and its downstream melanogenic proteins, including tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), melanocyte-specific glycoprotein Pmel17, melanoma-associated antigen recognized by T cells-1 (MART-1) and dopachrome tautomerase (DCT) (Miyamura *et al.*, 2007; Yamaguchi and Hearing, 2005), leading eventually to increases in melanin content. Increased levels of protease activated receptor-2 (PAR2) in keratinocytes also result from exposure to UV, which increases uptake and distribution of melanosomes by keratinocytes in the epidermis (Scott *et al.*, 2001). This is schematically represented in Figure 1.4.2. The release of Diacylglycerol (DAG) (Gordon and Gilchrest, 1989) and arachidonic acid also play a role in the tanning response (Gilchrest *et al.*, 1996).

Figure 1.4.2. Receptors, ligands and factors that regulate pigmentation of human skin

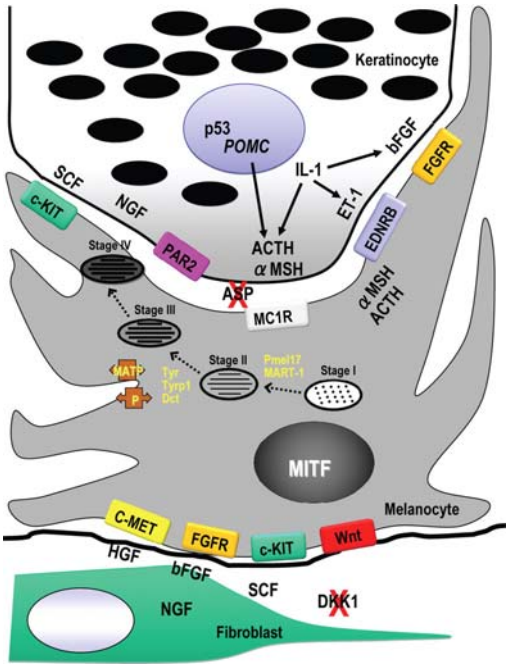


Figure Legend: LRO, lysosome-related organelle; ACTH, adrenocorticotropic hormone; ASP, Agouti signal protein; DCT, DOPAchrome tautomerase; DKK, Dickkopf; DHI, 5,6-dihydroxyindole; DHICA, DHI-2-carboxylic acid; DOPA, L-3,4-dihydroxyphenylalanine; EMI, epithelial-mesenchymal interactions; EMT, epithelial-mesenchymal transitions; bFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; HOX, homeobox; MC1R, melanocortin 1 receptor; MITF, microphthalmia transcription factor; MSH, melanocyte-stimulating hormone; NGF, nerve growth factor; POMC, pro-opiomelanocortin; SCF, stem cell factor; TYR, tyrosinase; UV ultraviolet radiation. Figure adapted from (Yamaguchi *et al.*, 2007).

1.4.3. Sunburn cells and apoptosis

When UV radiation caused severe DNA damage to cells and cannot be repaired, apoptotic pathways are used to remove damaged cells, involving p53 (de Gruijl and Rebel, 2008), upregulation of pro-apoptotic genes Bax and Fas (Banerjee *et al.*, 2005), p21 (Beattie *et al.*, 2005) and Bcl-2 downregulation (Knezevic *et al.*, 2007).

1.4.4. Non-pigmentary photoadaptation

Apart from erythema and tanning, thickening or hyperplasia of the epidermis is a significant component of photoadaptation (Diffey, 1991). A single moderate exposure to UVB can result in up to a 3-fold thickening of the stratum corneum within 1-3 weeks. Diffey *et al* discussed that Miescher in 1930 demonstrated that repeated exposures every 1 to 2 days for up to 7 weeks will lead to 3-5 fold thickening (Diffey, 1991).

1.5. Genetics of red hair and blonde hair

Davenport first studied and described the inheritance of red hair in 1909 at the Cold Spring Harbor Laboratory (Davenport and Davenport, 1909). Other researchers studied and found that red hair appeared to be dominantly inherited due to the high carrier rate in their red hair family studies (Davenport and Davenport, 1909). Reed argued that Schiedt (1925) suggested that red hair was dominant over blonde.

Subsequent studies approximated red hair to an autosomal recessive trait. Reed agreed with Conitzer *et al* and others that red hair was more consistent with a recessive hypothesis (Neel, 1943; Reed, 1952; Singleton and Ellis, 1964). There were subsequent studies on red hair (Barnicot, 1953; Nicholis, 1969; Rife, 1967; Yamamoto and Neel, 1967). There was little uniformity in their methodology of defining "red hair", sometimes with or without the use of a colour standard. Remarkably, little is known about the mode of inheritance of blonde hair.

1.6. Hair greying and age

The greying of hair is an age-related process in humans. It is generally accepted that grey hair is a combination of black and white hairs. It has been described that, as an average, 50% of people have at least 50% grey hair at age 50, in a cohort of Caucasians (Keogh and Walsh, 1965). This greying incidence appears irrespective of sex and initial hair colour. It is therefore important to measure hair colours before they change.

The mechanism of hair greying was described by Nishimura *et al* and involved the genes bcl2 and mitf (Nishimura *et al.*, 2005). A theory involving free radicals has been proposed (Arck *et al.*, 2006). Arch *et al* showed that there was an increased

incidence of hair bulb melanocyte apoptosis associated with oxidative stress in the grey hair from aging individuals. There was also an absence of oxidative stress protectors such as Bcl-s and melanocyte growth factors such as c-Kit. There was a higher frequency of oxidative stress associated mitochondrial DNA damage in grey hair follicles.

The reduction in pigment is due to melanin not being produced at the hair root. Hair colour was found to be lighter in colour during early child life and darkened with age (Matheny and Dolan, 1975). The reason for the darkening is unclear. Redheads have a more fiery shade of red during early childhood.

1.7. Eye colour

Eye colour was initially studied by Davenport in 1907 (Davenport and Davenport, 1907) and Hurst in 1908 (Hurst, 1908). The genetics of eye colour is poorly understood. Genes that are known have limited effects. However a number of loci are implicated in eye colour recently. These include: *OCA2* (OMIM 203200) (Duffy *et al.*, 2007), *HERC2* (Kayser *et al.*, 2008), *SLC24A4* (Sulem *et al.*, 2007), *TYR* (Sulem *et al.*, 2007), *SLC45A2* or *MATP* (OMIM 606202), *ASIP* (OMIM 600201), *TYRP1* (OMIM 115501), *CYP1A2* (OMIM 124060), *CYP2C8* (OMIM 601129) and *CYP2C9* (OMIM 601130).

1.8. Genetics of skin pigmentation

Davenport in 1910 first studied the genetics of skin pigmentation (Davenport and Davenport, 1910a, b). Subsequently other studies investigated the inheritance in other populations (Davenport and Danielson, 1913). Further studies revealed that a number of genes were involved in human pigmentation. This was reviewed in (Barsh, 2003; Rees, 2003; Sturm *et al.*, 2001). Human genetic variation was diverse. The predominant view of genetic variation occurred when major world groups (Europeans, Asians) moved out of Africa (Out of Africa Model), reviewed in (Liu *et al.*, 2006a). The other competing hypothesis is the Multiregional Continuity Model, reviewed in (Stringer, 2002), which differs from the Out of Africa Model in denying a recent African origin for modern humans. The Multiregional Continuity Model emphasizes the role of both genetic continuity over time and gene flow between

contemporaneous populations in arguing that modern humans arose not only in Africa but also in Europe and Asia.

Pigmentation is one of the most obvious and varied human characteristics visible. Why are skin pigmentation and hair pigmentation so diverse? Why do humans differ so much in skin colour and hair colour? Melanocortin 1 receptor (*MC1R*) gene is one of the main genes identified that plays an important role in determining physiological variation in human skin and hair colours (Rees, 2003). How does a single gene give rise to a multitude of phenotypes? How is the diversity achieved at a molecular level? Are there other genetic interactions at play? Other pigmentation genes have been identified which contributed to skin colour in humans, zebrafish *golden* mutation and human homologue Solute Carrier Family 24 (Sodium/Potassium/Calcium Exchanger) Member 5 *SLC24A5* (Lamason *et al.*, 2005). Zebrafish *golden* and human *SLC24A5* explains 25-38% of the skin colour difference between Europeans and Africans (Lamason *et al.*, 2005).

People respond differently to sunlight. Understanding the cutaneous response to ultraviolet radiation (UVR) precisely is of great importance scientifically and medically. The chief environmental cause for melanoma and non-melanoma skin cancers is ultraviolet radiation. Malignant melanoma of the skin was the 12th most common cancer in males and the 8th most common in females in Scotland in 2000 (Scottish Health Statistics 2000). The incidence increased for both males (34.1%) and females (16.4%) over the 10-year period from 1991 to 2000. In 2002 there were 132 deaths. There was no significant change in mortality over the most recent 10-year period. Non-melanoma skin cancer showed a similar increase in incidence (Scottish Health Statistics 2000). Furthermore UVR is increasingly used as a treatment modality in a wide range of skin diseases including psoriasis, atopic eczema and polymorphic light eruption. A better understanding in people's response to UVR would improve the way we utilise UVR as a therapeutic option and our treatment regimes.

1.9. Pigmentation and UVR

There is considerable variation in the cutaneous sensitivity to UVR between persons. Even within a North-European population, a five-fold variation in magnitude is evident (Farr and Diffey, 1984; Ha *et al.*, 2003). The main known determinant of this variation is skin colour due to melanin pigmentation. In turn, skin colour is the main host determinant of skin cancer, and for most skin cancers, rates vary by more than two orders of magnitude depending on skin colour (Marks, 1995, 2000; Marks and Whiteman, 1994; Rees and Healy, 1997). Skin colour is a highly heritable trait (Clark *et al.*, 1981). Understanding these differences in skin colour is therefore of interest for a number of different reasons: as a determinant of skin cancer; as a determinant of sunburn; as a determinant of dose when UVR is given therapeutically for skin diseases such as psoriasis; and finally because understanding the variation in human skin colour is one of the classical evolutionary questions about man's history. Skin colour measurements historically involved the use of colour standards e.g. Von Luschan tiles or coloured papers by Gates *et al* (Gates, 1952) and visual matching. This is limited by subjectivity and the problem of giving discrete values to a continuous variable.

1.10. Melanocortin 1 receptor (MC1R)

The *MC1R* (OMIM 155555) maps to chromosome 16q24.3 (Gantz *et al.*, 1994; Magenis *et al.*, 1994) and codes for a 317 amino acid product of 7-pass transmembrane G-protein coupled receptor (Chhajlani and Wikberg, 1992; Mountjoy *et al.*, 1992). The *MC1R* is a member of the Class A Rhodopsin family of G-coupled receptors found within a sub-class of melanocortin receptors – MC1-5R. Two natural ligands are known for the *MC1R*, alpha-melanocyte stimulating hormone (α-MSH) and ACTH. These are produced by sequential cleavage from the larger precursor peptide pro-opiomelanocortin (*POMC*) by prohormone convertases 1 [EC 3.4.21.93] and 2 [EC3.4.21.94] to form ACTH and α-MSH. *MC1R* receptor binding to α-MSH in melanocytes causes activation of Gαs protein, which then activates adenylate cyclase and increases intracellular cAMP production (Chhajlani and Wikberg, 1992; Mountjoy *et al.*, 1992). The exact mechanism whereby the increased cAMP causes

upregulation of tyrosinase is unclear. Bertolotto *et al* provided evidence from murine B16 melanoma cells that increased cAMP level allow binding of microphthalmia protein to tyrosinase gene promoter region (Bertolotto *et al.*, 1996). Yasumoto *et al* also showed that the human homologue microphthalmia-associated transcription factor (*MITF*) can bind and upregulate tyrosinase promoter (Yasumoto *et al.*, 1994). Tyrosinase-related protein 1 (*TYRP1*), tyrosinase-related protein 2 (*TYRP2*) levels and other pathways may also play a role. All these in turn control the regulatory switch between eumelanin synthesis and phaeomelanin synthesis. *MC1R* is therefore a key regulator determining the regulatory switch point in the synthesis of eumelanin and phaeomelanin (Aroca *et al.*, 1993; Hunt *et al.*, 1994; Kuzumaki *et al.*, 1993). To date, over 70 allelic variants of the human *MC1R* have been identified in the world population (Box *et al.*, 1997; Rees, 2000; Smith *et al.*, 1998; Valverde *et al.*, 1995; Wong and Rees, 2005).

Candille *et al* identified a role for β-defensin in pigmentation (Candille *et al.*, 2007). A mutation at the K^B locus was shown to be the cause of dominant inheritance of black coat colour in dogs (Kerns *et al.*, 2004). The gene at the locus is *CBD103*, which is the canine ortholog of human DEFB103, which encodes the human β-defensin 3 (HBD3). Human β-defensin 1 (HBD1) and HBD3 both bind to MC1R (Pazgier *et al.*, 2006). Together these suggest that β-defensins interact with MC1R to mediate pigmentary, immunological and antimicrobial effects (Harder *et al.*, 2001).

1.11. The identification and cloning of the *MC1R* gene

The discovery of *MC1R* owes its origins stemming from mouse fancy communities since 18th century which developed into the basis of modern mouse coat colour genetics. A large number of murine coat colour variants (e.g. yellow, pink-eyed dilution, silver) provided the initial tools for fuelling the development and discovery of pigmentary genetics. To date, around 100 genes have been identified that influence murine coat colours (Barsh, 1996; Jackson, 1994; Silvers, 1979). Two genetic loci have been the focus of attention; *extension* and *agouti*, despite they have been known for years that they control the regional distribution of brown-black eumelanin and yellow-red phaeomelanin pigments in animal coating and along hair shafts (Searle, 1968). There are four extension alleles: *wildtype* (E^+), *sombre* (E^{so},

$E^{so\text{-}3J}$), *tobacco* (E^{tob}), and *recessive yellow* (*e*). Loss of function mutations at the *extension* locus result in mice with yellow hair, while dominant gain of function mutations result in black hair (Robbins *et al.*, 1993). On the contrary, wild type agouti mice have subapical yellow hair and that overexpression of murine agouti product results in yellow haired mice (Barsh, 1996; Jackson, 1994). The *MC1R* gene, previously known as the melanocyte stimulating hormone (MSH) receptor gene, has been cloned independently by two groups in 1992 (Chhajlani and Wikberg, 1992; Mountjoy *et al.*, 1992). The *extension* locus in mice underlies the human MC1 receptor.

1.12. Evidence implicating *MC1R* as a candidate gene

The discovery and progress from murine coat colour studies, the cloning of the human *MC1R*, followed by the demonstration of the action of melanotropins (MSH) in man (Lerner, 1993), namely their pigmentary actions, suggested and pointed to MSH receptor as a candidate gene for red hair and pigmentation. Shortly after *MC1R* had been cloned, confirmatory experiments were also reported relating *Mc1r* mutation with murine phenotype of *recessive yellow* (*e*) mouse (Robbins *et al.*, 1993). Subsequently a number of studies reported on the various human phenotypes associated with mutations in human *MC1R*.

MC1R allelic variants affect pain and analgesia in mice and humans (Mogil *et al.*, 2005). Humans with *MC1R* allelic variants showed reduced sensitivity to pain stimuli and increased analgesic responsiveness to mu-opioid morphine metabolite (Mogil *et al.*, 2005). Females with two variant *MC1R* alleles showed greater analgesia to kappa opioid (Mogil *et al.*, 2003).

Melanocortin 2 receptor (MC2R) is the physiological receptor for adrenocorticotropin hormone (ACTH) and is responsible for glucocorticoid function (Clark *et al.*, 1993).

Melanocortin 3 receptor (MC3R) (Gantz *et al.*, 1993) is expressed in brain, placenta and gut tissues and is involved in energy homeostasis (Chen *et al.*, 2000).

Melanocortin 4 receptor (MC4R) is primarily localized to the brain and is involved in obesity and energy homeostasis (Branson *et al.*, 2003; Chambers *et al.*, 2008).

MC5R has a potential role in sebum production and exocrine gland function (Chen *et al.*, 1997).

Melanotropins have various actions by acting on human melanocortin 1-5 receptors: pigmentation, anti-inflammation, steroidogenesis, lipogenesis and energy homeostasis (Bohm *et al.*, 2006; Brzoska *et al.*, 2008).

1.13. Allelic studies and *MC1R* phenotypes

The first report describing a functional role of *MC1R* in man was a case control study associating several *MC1R* variant alleles with red hair and fair skin (Valverde *et al.*, 1995). Some parts of the human *MC1R* were sequenced from 30 Northern European redheads with a strong family history of red hair, pale skin and burning easily in response to UVR and compared them against control individuals without these traits. Interestingly *MC1R* allelic variations are common (>65%) and the allelic frequency of variants was higher in redheads. Subsequent study by Smith *et al* confirmed that over 75% of Irish population possesses *MC1R* allelic variants (Smith *et al.*, 1998). Some *MC1R* variants seem to be more common in individuals with red hair e.g. Arg151Cys, Arg160Trp, Asp294His (Box *et al.*, 1997; Smith *et al.*, 1998; Valverde *et al.*, 1995). Although many redheads have two *MC1R* allelic variants, some showed only one. Furthermore some individuals have more than one *MC1R* sequence variant on the same allele.

Other genetic association studies have expanded this data to include a larger number of *MC1R* alleles. These include twin analysis (Box *et al.*, 1997), several large population studies (Bastiaens *et al.*, 2001b; Box *et al.*, 2001; Kennedy *et al.*, 2001; Palmer *et al.*, 2000; Smith *et al.*, 1998), a family inheritance study (Flanagan *et al.*, 2000) and have the following findings: The red hair pale skin freckling phenotype is commonly associated with *MC1R* variant allele homozygosity or compound heterozygosity. Red hair can also manifest in single variant allele carriers, or even in a dark skin background (McKenzie *et al.*, 2003). There is a dosage effect for red hair phenotype. The presence of *MC1R* allelic variants is necessary but not sufficient for the red hair phenotype (Box *et al.*, 1997). Other loci may be involved in expressing or masking of the phenotypic trait.

What about other *MC1R* alleles? Arg142His, 86insA and 537insC mutations are found to be associated with the RHC in red hair kindred, whereas Val92Met and Arg163Gln variants seem to be genetically neutral (Sturm, 2002). *MC1R* gene variants can modify the phenotype of oculocutaneous albinism (OCA) type 2 (King *et al.*, 2003). King *et al* (King *et al.*, 2003) found *MC1R* mutations to be responsible for red rather than yellow/blond hair in six patients with OCA type 2. One of the patients was a compound heterozygote for R151C and R160W, as well as for two mutations in the P gene (King *et al.*, 2003). However *MC1R* is not the only gene mutation that leads to red hair. Two siblings with red hair, early onset obesity and adrenal insufficiency were shown to be homozygous or compound heterozygotes for loss of function *POMC* gene [OMIM 176830] mutations (Krude *et al.*, 1998).

As highlighted previously, studies have shown that there is an association between sequence variation of the coding region of *MC1R* locus and hair colour (Box *et al.*, 1997; Flanagan *et al.*, 2000; Smith *et al.*, 1998; Valverde *et al.*, 1995). Three particular *MC1R* variants Arg151Cys, Arg160Trp and Asp294His are strongly associated with red hair and most subjects being homozygous or compound heterozygote for one of the three alleles. What about other hair colour phenotypes? Box *et al* reported an association between the *MC1R* allelic variant Val60Leu and blond/light brown hair and/or fair skin (Box *et al.*, 1997). The agouti signaling protein gene (G allele: position 8818) carriage appears to be more common in individuals with dark hair (OR 1.8 95% CI 1.2-2.8) and brown eyes (OR 1.9 95% CI 1.3-2.8) (Kanetsky *et al.*, 2002). The inheritance of blond hair is unclear at present. One mechanistic problem with measuring hair colour phenotype is the lack of quantitative methodology. Naysmith *et al* recently showed that a quantitative approach can be used, and hair colour phenotype can be objectively, quantitatively measured with the effect of *MC1R* assessed (Naysmith *et al.*, 2004).

1.14. Family studies

Subsequent genetic studies have extended to include a larger number of *MC1R* alleles, including a twin family study with red hair (Box *et al.*, 1997) and 174 individuals from 11 families (Flanagan *et al.*, 2000). These confirmed the *MC1R* allelic variants associated with red hair. The inheritance of red hair approximates to a

Mendelian recessive model (Davenport and Davenport, 1909; Michelson, 1934; Neel, 1943; Reed, 1952; Singleton and Ellis, 1964; Smith *et al.*, 1998). Interestingly the Val60Leu variant may act as a partially penetrant recessive allele in these families, and so may Asp84Glu with regards to the whole population (Ringholm *et al.*, 2004; Schioth *et al.*, 1999).

1.15. Functional Ligand Binding Transfection studies

Human *MC1R* transfection into cultured cells in vitro has allowed ligand binding studies to study the function of *MC1R* receptor. These are mainly based on natural and synthetic ligand binding or displacement of radiolabeled MSH analogue (Chhajlani and Wikberg, 1992; Mountjoy *et al.*, 1992; Ringholm *et al.*, 2004; Robbins *et al.*, 1993; Schioth *et al.*, 1995; Schioth *et al.*, 1999). In vitro site directed mutagenesis studies have been performed on the murine and human *MC1R* with several residues mutated and the resultant mutants tested for binding to α-MSH and NDP-α-MSH (Frandberg *et al.*, 1994). NDP-α-MSH gave binding affinities similar to wildtype, whereas α-MSH showed greatly reduced binding affinities for Asp117Ala (267-fold), His260Ala (132-fold). Other studies have been performed to investigate the functional status of *MC1R* allelic variants. Expression of *MC1R* variants in heterologous cell cultures reduced the functional coupling of the *MC1R* to adenylate cyclase (Frandberg *et al.*, 1998; Koppula *et al.*, 1997; Schioth *et al.*, 1999). Franberg *et al* identified a Arg151Cys variant of the *MC1R* gene in genomic DNA of a person with red hair and skin type I (Frandberg *et al.*, 1998). This variant bound to radiolabeled α-MSH analogue with identical affinity as wildtype *MC1R* but could not be stimulated to produce any cyclic AMP, rendering the *MC1R* completely non-functional (Frandberg *et al.*, 1998). Transfection assays for Val60Leu allelic variant showed diminished signaling (Schioth *et al.*, 1999).

The conclusions of functional status of *MC1R* from transfection experiments may be limited. The limitations arise because the relative amounts of peptides in vivo may differ from those in vitro. Stable transfection assay into B16G4F melanoma cells showed that cAMP response was still compromised in the variant MC1R transfected clones with similar MC1R numbers per cell (Robinson and Healy, 2002).

Bacterial artificial chromosome (BAC) rescue assay of *MC1R* homozygous null mice has also been performed to define the nature of *MC1R* allelic variants (Healy *et al.*, 2001). These alleles were found not to be complete loss of function but rather impair signaling to various degrees, e.g. D294H with greater functional impairment than R151C or R160W. The functional status of D84E is still unclear with different findings (Bastiaens *et al.*, 2001b; Healy *et al.*, 1999; Kennedy *et al.*, 2001; Valverde *et al.*, 1996). Further functional studies are required to assess cellular responses in relation to MC1R gene variants and the different phenotypic traits.

Beaumont *et al* showed that melanocytic cells exogenously or endogenously expressing MC1R show strong surface localization of the wildtype and D294H alleles but markedly reduced cell surface expression of the R151C and R160W receptors. D84E and I155T variants demonstrated a reduction in plasma membrane receptor numbers. These results raised the possibility that MC1R gene variants can be linked to an altered cell surface or cellular localization of MC1R numbers (Beaumont *et al.*, 2005).

What is the true null phenotype of *MC1R* in humans? This question has been debated but due to the rarity of *MC1R* null alleles, they have only been found in the heterozygous state until recently. Recently the case of a homozygous *MC1R* null individual with two *MC1R* loss of function alleles (86_87insA, 537_538insC resulting in frameshift stop codons), indicated that red hair and fair skin is the null phenotype of *MC1R* in the absence of *MC1R* function (Beaumont *et al.*, 2008).

1.16. Phenotype definition and skin type

There has been much progress with *MC1R* genetics despite crude phenotypic definition currently. In order to relate the multitude phenotypic traits (e.g. hair colour, skin colour, eye colour, skin cancer, freckling, moleliness, immune phenotypes and others) relevant to *MC1R* genotypes and the cutaneous response to UVR, one has to attempt to define these phenotypes precisely, quantitatively, objectively, easily, quickly, accurately and reproducibly. To put it slightly differently as an overview, we have a gene - *MC1R* - that varies between individuals and the variation in expression results in a number of phenotypic endpoints. How do we

explain these? The difficulty in attempting to explain the precise relationship also stems from the inherent problems associated with the definition of the skin type.

1.17. Fitzpatrick Skin Type

Skin type has been assessed routinely according to the Fitzpatrick classification (Fitzpatrick, 1975, 1988) as: I, always burns and never tans; II, always burns and then tans; III, always tans and sometimes burns; IV, always tans and never burns. In addition to white skinned persons, brown and black skinned persons were included later in the classification to give skin types V and VI (Pathak MA, 1976). A separate system was introduced by Satoh and Kawada in 1986 for the classification of Japanese skin type (Satoh, 1986). Although Fitzpatrick classification has been utilized widely in clinical practice, there have been suggestions that this has low reproducibility (Rampen *et al.*, 1988). This classification only deals with the 2 variables erythema and tanning (pigmentation) together. The 2 variables "erythema" and "tanning" may confound each other. It would be better to be able to measure each of these variables separately and quantitatively. Also this ordinal classification based on anamnestic recall has limitations for quantitative phenotypic use.

1.18. Cancer syndromes

There are a number of cancer syndromes with special clinical relevance to pigmentation, genetics and UV radiation.

Naevoid basal cell carcinoma (Gorlin) syndrome (OMIM 109400)

This syndrome involved 9q22-31, the PTCH gene. The gene was mapped to 9q22-31 and identified as a human homologue of the Drosophila gene *patched*.

Ferguson Smith syndrome (self healing epitheliomata of Ferguson Smith) (OMIM 132800)

This syndrome is characterized by lesions that resemble SCCs but they resolve spontaneously. The gene was mapped to same locus (9q31) as naevoid basal cell carcinoma (Gorlin) syndrome.

Bazex-Dupre-Christol syndrome (OMIM 301845)

This syndrome is an X-linked dominant condition characterized by congenital hypotrichosis, follicular atrophoderma and basal cell carcinomas from the second decade. This maps to Xq24-27.

Rombo syndrome (OMIM 180730)

This syndrome is characterized by vermiculate atrophoderma, milia, hypotrichosis, trichoepitheliomas, basal cell carcinomas and peripheral vasodilation with cyanosis (Michaelsson *et al.*, 1981; van Steensel *et al.*, 2001).

Muir Torre syndrome (OMIM 158320)

This syndrome is the association of sebaceous skin tumours with internal malignancy (Muir *et al.*, 1967; Torre, 1968). The gene locus was mapped to 3p21.3 and 2p21-22 and caused by mutations in the DNA mismatch repair genes MLH1 (Bapat *et al.*, 1996) and MSH2 (Kruse *et al.*, 1996) genes respectively.

Epidermodysplasia verruciformis (OMIM 226400)

This syndrome is associated with a high risk of skin cancer and results from an abnormal susceptibility to specific related HPV genotypes, causing intraepithelial carcinomas and squamous cell carcinomas on photoexposed sites. The gene was mapped to 17q25 and caused by mutations in either EVER1 or EVER2 (Ramoz *et al.*, 2002).

Dyskeratosis Congenita (OMIM 305000)

This syndrome is caused by mutation in the gene dyskerin *DKC1* and characterized clinically by cutaneous pigmentation, nail dystrophy, oral leukoplakia, lacrimal duct atresia-induced lacrimation, often thrombocytopenia, anaemia and testicular atrophy.

Xeroderma Pigmentosum (OMIM 194400, 133510, 216400, 278700-278720-278730-278740-278780-278800, 314700, 610551, 610651)

This is a group of autosomal recessive disorders that comprises of DNA repair gene polymorphisms. XP is characterised by extreme photosensitivity to UV radiation and

early appearance of non-melanoma and melanoma skin cancers. These patients have an exaggerated erythemal response too.

1.19. Project objective

This project aims to assess clinical phenotypes quantitatively and the effects of the skin's response to UVR in order to relate to human genotypes in man. Objective measures are used to see how sensitive skin is to UVR and to measure objectively hair and skin colour, and study changes in the *MC1R* and other genes that may influence such sensitivity. Key questions include:

What is the difference between heterozygote individuals with red hair and homozygotes with red hair?

Define the pigmentary effects of the V60L (and other less common alleles) on skin's response to UVR and hair colour and hair melanins.

Do genetic differences at the agouti locus account for objective pigmentary characteristics that are different between individuals with identical *MC1R* genotypes?

Search for further SNPs in the G-proteins thought to be downstream of *MC1R*, and ask if these can account for different phenotypes in individuals with the same *MC1R* genotypes.

How does variation within European populations (with varying *MC1R* status) fit within the range of objective responses seen in those with different genetic ancestries with respect to response to irradiation?

The goal is a quantitative understanding of the skin's response to ultraviolet radiation.

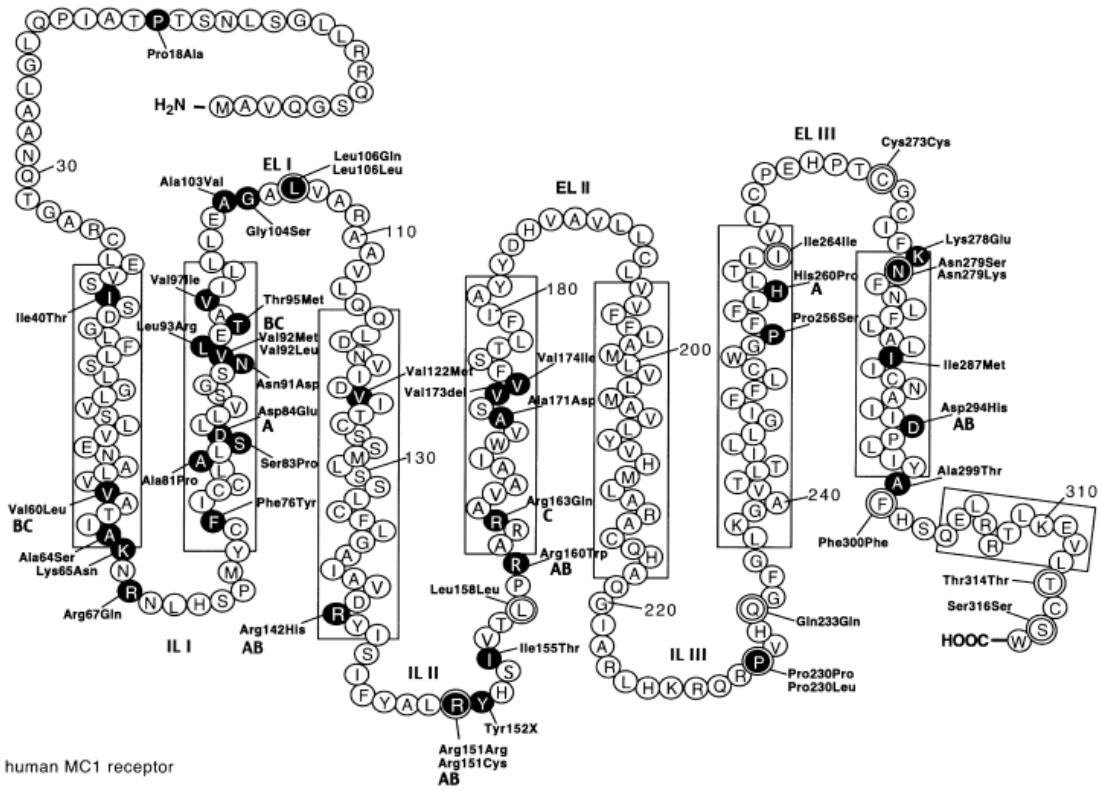
1.2. Thesis Outline

This project aims to quantitatively measure human pigmentary phenotypes including hair colour, skin colour and eye colour and then quantify the cutaneous facultative pigmentary change after UVB irradiation. The detailed quantitative phenotypes were studied in relation to *MC1R* and other genotypes using a candidate gene approach.

In this thesis, the phenotype of 159 individuals was investigated. The static phenotypes were quantitatively measured and will be described in Chapter 3. The

induced phenotypes from a subset of 98 individuals who underwent UVB irradiation will be described in Chapter 4. Their facultative pigmentary response will be detailed in Chapter 5. *MC1R* genotypes will be described in Chapter 6. The other candidate gene loci genotypes will be described in Chapter 7. Blondes will be discussed in Chapter 8.

Figure 1.4. A two-dimensional model of the human MC1R.



Source: (Ringholm *et al.*, 2004). Amino acids are shown in the circles. The residues shown in filled circles indicate positions for *MC1R* allelic variants, where a mutation results in amino acid exchange. Double circles = synonymous mutations.
Cytoplasmatic loops (IL). Extracellular loops (EL).

Table 1 summarizes the *MC1R* sequence changes associated with phenotypic changes (Wong and Rees, 2005).

Table 1 – *MC1R* sequence changes and phenotypic variation

Codon sequence variation	Cancer	Freckling	Hair and skin colour	In vitro studies	Murine studies
Major					
D84E	++	++	Red hair+++, pale skin+++	b↓↓, AMP↓↓↓	-
R142H	++	++	Red hair+++, pale skin+++	b↓, cAMP↓↓↓	-
R151C	++	++	Red hair+++, pale skin+++	b_N, ↓R, cAMP↓↓↓	Melanogenic response ↓↓↓
R160W	++	++	Red hair+++, pale skin+++	b_N, ↓R, cAMP↓↓↓	Melanogenic response ↓↓↓
D294H	++	++	Red hair+++, pale skin+++	b↓↓↓, cAMP↓↓↓	Melanogenic response ↓↓↓
Minor					
V60L	+	+	Red hair+, pale skin++	b_N, cAMP↓↓	-
V92M	+	+	Red hair+, pale skin+	b↓↓↓, cAMP↓	-
R163Q	-	+	Pale skin+	b↓, $cAMP_N$	-
H260A	-	-	-	b↓	-
H260P	+	+	Red hair+	-	-
Others					
A2V	-	-	-	-	-
P18A	-	-	-	-	-
C35Y	-	-	-	-	-
V38M	-	-	-	-	-
I40T	-	-	-	b↓, cAMP↓	-

L44V	-	-	-	-	-
V51A*	-	-	-	-	-
I63M*	-	-	-	-	-
A64S	-	-	-	-	-
K65N	-	-	-	-	-
R67Q	-	-	-	-	-
R67V	-	-	-	-	-
F76Y	-	-	-	-	-
L80P	-	-	-	-	-
A81P	-	-	-	-	-
S83P	-	-	-	-	-
G89R	-	-	-	-	-
T90S	-	-	-	-	-
N91D	-	-	-	-	-
V92L	-	-	-	-	-
L93R	-	-	-	b↓, cAMP↓	-
T95M	-	-	-	-	-
V97I	-	-	-	-	-
A103V	-	-	-	-	-
G104S	-	-	-	-	-
L106Q	-	-	-	-	-
D117A	-	-	-	b↓	-
I120T	-	-	-	-	-
V122M	-	-	-	b↓, cAMP↓	-
S131N*	-	-	-	-	-
R142C	++	-	-	-	-
I155T	-	-	Fair/blonde hair+	-	-
R162P	-	-	-	b_N, cAMP↓↓↓	-

A164R	-	-	-	-	-
A171D	-	-	-	-	-
V174I	-	-	-	-	-
F179A	-	-	-	Yes	-
V180I	-	-	-	-	-
H209A	-	-	-	Yes	-
A212S*	-	-	-	-	-
R213W	-	-	-	-	-
P230L	-	-	-	-	-
P256S	-	-	-	-	-
V265I	-	-	-	-	-
K278E	-	-	-	-	-
N279S	-	-	-	-	-
N279K	-	-	-	-	-
I287M	-	-	-	-	-
Y298H	-	-	-	-	-
A299T	-	-	-	-	-
A299V	-	-	-	-	-
A306V	-	-	-	-	-
Frameshift Stop Deletions					
Q23X	-	-	-	-	-
29insA	-	-	-	-	-
Y152X	-	-	Red hair+, pale skin+	-	-
Y152OCH	-	-	-	-	-

V173del	-	-	-	-	-
179insC	-	-	-	-	-
F195del / F196del	-	-	-	-	-
Silent					
L106L	-	-	-	-	-
I138I	-	-	-	-	-
R151R	-	-	-	-	-
L158L	-	-	-	-	-
A166A	-	-	-	-	-
P230P	-	-	-	-	-
Q233Q	-	-	-	-	-
I264I	-	-	-	-	-
C273C	-	-	-	-	-
F300F	-	-	-	-	-
T314T	-	-	-	-	-
S316S	-	-	-	-	-

Table 1 – *MC1R* sequence changes and phenotypic variation

Table 1 summarises 81 *MC1R* codon sequence changes reported in the literature to date and their functional significance in relation to phenotypic variations – cancer phenotype, freckling, hair and skin colour, modified from (Wong and Rees, 2005). In vitro data and murine studies are also summarised when available. These codon sequence changes are grouped and interpreted according to the evidence available into major function alleles and minor function alleles for *MC1R*. Other newly reported and unpublished sequence changes are also included. Frame shift, stop, deletion and silent mutations are summarised separately. A one-letter code has been used for the codon sequence changes.

The relative degree of functional impairment is represented by $_{N}$ (normal), + (slight), ++ (moderate) and +++ (severe). - denotes no known studies or evidence available. In vitro data with binding (b), cAMP response (cAMP) and reduced receptor activity (↓R) are symbolised respectively. The relative degree of functional impairment is represented by $_{N}$ (normal), ↓ (slight), ↓↓ (moderate) and ↓↓↓ (severe). Some of these allelic changes (V60L, D84E, R142H, R151C, R160W and D294H) were claimed to have association with fair / blonde hair although with a relatively low risk ratio. Note also that whether the R163Q is a 'r' allele or has equivalent status to consensus sequence is unclear.

4 new sequence variants (V51A, I63M, S131N and A212S) from this study were added into Table 1. * denotes these new unpublished observations.

Chapter 2 Methods and materials

In this chapter I discuss the methodology and materials used for the study.

This was a study involving the precise pigmentary phenotyping of individuals and relating the clinical phenotypes to genotypes. First of all, the detailed pigmentary clinical phenotype of the volunteers was determined. Secondly their genetic phenotype was then ascertained.

2.1 Recruitment of volunteers

2.1.1 Ethics

Ethical approval was obtained from the Lothian Regional Ethics Committee (LREC) (Study number: Derm/03/JLR/6/TW, LREC/2003/4/27, Project ID (Royal Infirmary of Edinburgh): 2003/R/DER/01) in the light of the Declaration of Helsinki (www.wma.net/e/ethicsunit/helsinki.htm) and subsequent amendments (www.wma.net/e), in so far as was possible for studies on volunteers, before commencement of recruitment for this study. All volunteers gave written informed consent (Appendix 1 and 2) prior to taking part in the study.

2.1.2 Volunteers

Volunteers aged 18-40 were randomly recruited to take part in the study. The choice for the age group of 18-40 was intended to reduce likelihood of the hair colour change with age i.e. grey. This project aimed to recruit individuals from Dermatology outpatient clinics, the Medical Research Council Human Genetics Unit, University of Edinburgh and other centres via posters, word of mouth, electronic mail and press coverage. A total of 161 volunteers have participated (see Figure 3.1 flowchart). Volunteers were approached and had at least 24 hours and usually one to two weeks to decide whether to take part in the study. The reasons for individuals who declined to take part in the study were noted. Exclusion criteria include individuals: with photosensitivity, on drugs known to interfere with the cutaneous inflammatory response (such as high dose systemic steroids, ciclosporin, methotrexate and mycophenolate mofetil (MMF), on drugs known to act on the adrenergic pathways (such as Tricyclics, Monoamine oxidase inhibitors (MAOIs)

and beta-blockers) (substudy only), who are breast feeding (substudy only), who are pregnant (substudy only), attempting to get pregnant before or during the study (substudy only) and referred because of acute inflammatory skin disease.

2.1.3 Volunteer Questionnaire

Demographic data were obtained including volunteer name, gender, date of birth, age, address, phone number and their general practitioner. All volunteers completed a questionnaire providing the following background information: Fitzpatrick skin type, natural hair colour, use of hair dye, eye colour, past medical history, drug history, allergies, family history, (sun sensitivity) sun bed use, sunscreen, holidays abroad, sunny countries, (sun exposure) sunburn, freckles, freckling site, resident country, (genetic background) ethnic ancestry, place of birth, parents' place of birth and parents' hair colour (Appendix 3).

Volunteers were asked to attend the outpatient appointment when the procedures were explained to them and demographic details and medical background information and characteristics collected. Volunteer information sheets (Appendix 4) were given and volunteers consented for the initial study:

1) Sample of venous blood
2) Sample of hair
3) Measuring skin, hair and eye colour
4) Exposure to graded doses of ultraviolet radiation affecting 10 small areas of skin less than 1cm^2 in each

And if agreeable and appropriate to return for a substudy involving:

5) Noradrenaline iontophoresis (passage of a small electric current from a 9v battery) using the drug Noradrenaline to make the skin blanch to areas that have developed erythema from ultraviolet radiation exposure

2.1.4 Collection of hair samples

A locket of hair (measuring 3-4cm in length, approximately 0.3mg in weight) was cut with a pair of scissors and stored at room temperature in Minigrip resealable labelled polyethylene 40mm x 65mm bags (Fisher Scientific, UK) until required for the determination of melanin content. Hair was de-identified and planned to be sent

to Professor Ito's laboratory in Japan for biochemical analysis. Hair melanins including eumelanin and phaeomelanin will be assayed using published method (Wakamatsu and Ito, 2002; Wakamatsu *et al.*, 2002). A hair melanin assay (Ozeki *et al.*, 1995) was also performed in an attempt to assay the total melanin content in hair. This made use of new methods to solubilize differentially phaeomelanins and eumelanins, which correlated well with the melanin content in mouse hairs. It made use of a Soluene 350 method to characterise melanins on the basis of eumelanin is insoluble in acid or alkali whereas phaeomelanin is soluble in alkali.

2.2 Clinical phenotyping

This study was divided into two parts – an initial study and a substudy. The initial study involved the precise clinical phenotyping and UV irradiation of the volunteers. The substudy involved noradrenaline iontophoresis of UV irradiated skin, this technique will be described in more detail later.

2.2.1 Instruments used in this study

All equipments were compliant with European Union safety standards.

2.2.1.1 Spectrophotometer

An instrument was sought to measure colour, pigmentation quantitatively and objectively. A Minolta spectrophotometer CM-2600d (Photo 2.2.1.1) (Minolta Co., Ltd, Osaka, Japan) was used to measure skin colour and hair colour quantitatively.

Photo 2.2.1.1. Minolta spectrophotometer CM-2600d

The measure of colour using colorimetry and spectrophotometry has been utilised in chemistry and in industrial sectors including colour printing, paints, textiles, food, pharmaceuticals (Fairbrother *et al.*, 1980) and cosmetics (Gillman, 1950). It has also been used for experimental purposes for a number of years in Dermatology (Harvey and Lord, 1978; Little and Wolff, 1981). It consisted of a measuring aperture and a computer for data processing. The aperture contained a polychromatic xenon lamp for illuminating an area, 8mm in diameter, of the surface to be studied. Six silicon photocells, three for measuring the source and three for reflected light, were also situated within the probe. The photocells converted the light they received into current, the voltage of which is proportional to the brightness of the spectral band. This current was converted into a digital signal, which the computer converted to tristimulus values. Prior to making measurements in an experimental session, the spectrophotometer was calibrated with a reference colour (white) plate supplied by Minolta. The measuring end aperture was placed against the skin surface to be measured without exerting pressure to avoid causing any ischaemia of the skin, which may alter the skin colour.

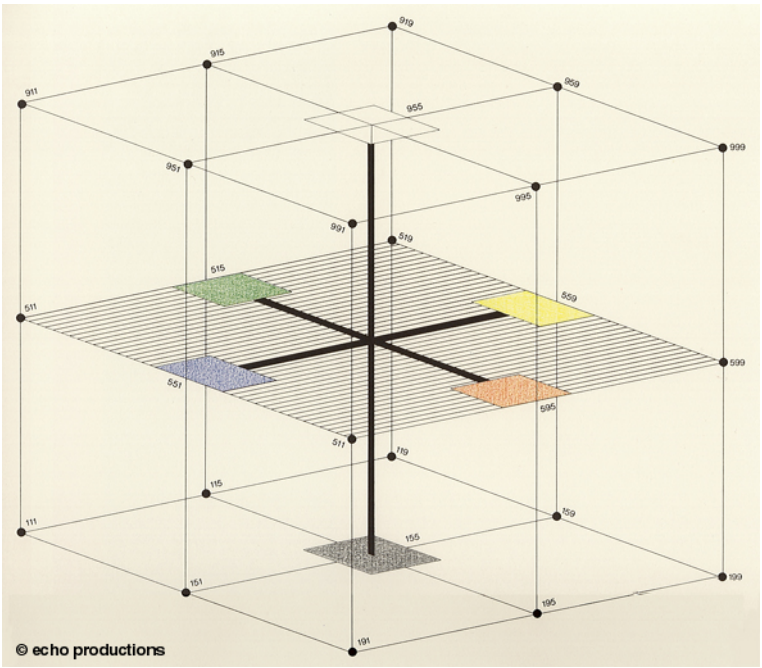
A spectral reflectance graph of 31 readings taken at 10nm increments along the electromagnetic spectrum from 360 – 740nm was obtained. The values were mathematically reduced to 3 values via a calculation that integrated the standard

observer and the light source, resulting in 3 tristimulus values, L* a* b* that was represented into coordinates in the desired colour space.

2.2.1.2 Colour model system

Trichromatic colour theory (Young and Helmholtz) was proposed in the 18th century that colour vision was due to 3 photoreceptors and cone pigments in human retinas. Colour was represented using the L* a* b* system (International Commission on Illumination, Commission Internationale de l'Eclairage CIE 1931), CIE (www.cie.co.at),(Westerhoff, 1995) in which colour was represented as summary values in three dimensions (Figures 2.2.1.2.1-2) designed to be commensurable with human colour perception: L*, representing lightness, on a scale of 0-100 where 0 is black and 100 is white; a*, representing red-green, on a scale from +60 to –60, where positive values indicate increasing shades of red; and b*, representing a yellow-blue, on a scale from +60 to –60, with positive values representing increasing shades of yellow. These three values were plotted and intersected into a three dimensional space to give a numerical value for colour.

Figure 2.2.1.2.1. L* a* b* colour space



Source: www.colorsystem.com

Figure 2.2.1.2.2. L* a* b* 3-Dimensional colour space.

Source: Minolta chromameter operational manual (Minolta, Osaka, Japan)

This method has been previously used in the study of human hair and skin colour and phototype (Naysmith *et al.*, 2004; Park *et al.*, 2002; Takiwaki *et al.*, 1994).

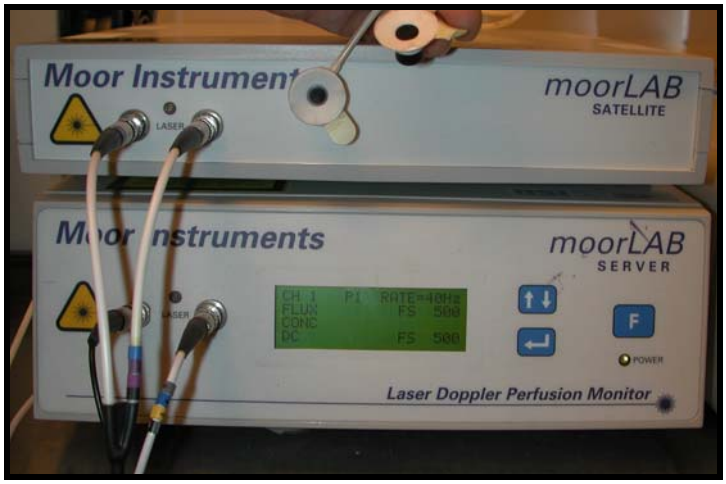
Triplicate measurements were taken for each volunteer. L* a* b* colorimetry and spectrophotometric reflectance data were obtained as described above. Prior to measurements with each volunteer, the spectrophotometer was zero calibrated and white calibrated as per manufacturer's instructions. Appropriate settings were chosen and set (specular component excluded, small aperture). The small aperture was chosen in preference because the diameter of small aperture (0.55cm) was smaller than that of the UVB induced pigmentation whereas the larger medium aperture (1.05cm) would underestimate the degree of pigmentary colour change. The specular component excluded (SCE) option was set in order to estimate the true reading without the specular or gloss component in a spherical spectrophotometer in providing reflectance measurements. Specular reflectance is the light that is reflected without penetrating the stratum corneum. This carries information about the structure and the state of hydration rather than about the underlying chromophores. In mode SCE (Specular Component Excluded), an internal light trap opens up and let the specular reflection of the source lamp onto the sample surface be captured and eliminated from the measurement. Only scattered light from the sample surface is measured. The Total Integrated Scatter (TIS) was measured and related to the micro-

roughness of the surface as: In mode SCI (Specular Component Included), the light trap is closed, therefore the source light bounced off it and got to the sensor: the combination of reflected and scattered lights were measured. Specular reflected light will then come from the calculation of the difference SCI-SCE.

2.2.1.3 Contact Laser Doppler

A contact laser doppler (Photo 2.2.1.3) MoorLab Server Laser Doppler Perfusion Monitor (Moor contact Doppler velocimetry, Moor Instruments Ltd, Millwey, Axminster, Devon, UK) was used to measure blood flux at 24 hours and 1-week post UVB irradiation.

Photo 2.2.1.3. MoorLab contact laser doppler



The blood flux measure was chosen to quantify the amount of erythema post UVB irradiation. This technique has already been widely used in Dermatology (Mustakallio and Kolari, 1983; Oh *et al.*, 2004) and other branches of medicine to measure blood flow (Rushmer, 1977).

This technique was based on the Doppler principle. Christian Johann Andreas Doppler (1803-1853), an Austrian mathematician and physicist, first described the effect in 1842 whereby the change in frequency and wavelength of a wave that is perceived by an observer moving relative to the source of the waves. This was initially on the coloured light from refracted stars and other celestial objects. Hippolyte Armand Louis Fizeau (1819-1896) independently discovered the same phenomenon for electromagnetic waves in 1848. The same effect applies to sound

waves which accounts for the everyday emergency vehicle sirens which start out at a higher approaching pitch than its stationary pitch and then slides down as it passes and continues to go lower than its stationary pitch as it departs further from the observer. In Medicine and in particular Dermatology, Laser Doppler velocimeters were used to measure the Doppler shift in wavelengths of reflections from particles moving with the flow. A low power light from a monochromatic stable laser is scattered by moving erythrocytes (perpendicular to the laser light beam) and as a consequence its frequency broadened. The frequency-broadened light, together with laser light scattered from static tissue, was photodetected and the resultant photocurrent processed to provide a blood flow measurement. One limitation with a single stationary Laser Doppler probe is that these blood flow measurements are relative blood flow values at a particular time point. If absolute blood flow values are required, multiple Laser Doppler probes could be used simultaneously. Melanins in the skin were stationary, and therefore do not affect the Doppler shift caused by the movement of erythrocytes (Stern, 1975). The laser-Doppler blood perfusion monitors output a signal that is proportional to the red blood cell perfusion (or flux). This represents the transport of blood cells through microvasculature and is defined as:

Red Blood Cell Flux = Number of blood cells moving in the tissue sampling volume × Mean velocity of these cells

Microvascular blood perfusion (Flux) is the product of mean red blood cell velocity and mean red blood cell concentration in the volume of tissue under illumination from the probe. For cutaneous measurements, the sampling depth is likely to be in the range 1.0 – 1.5 mm (Holloway and Watkins, 1977). This method is consistent with published guidelines for laser Doppler perfusion imaging (Fullerton *et al.*, 2002).

Perfusion measurements with Flux were obtained from MoorSoft software. Blood flux results were expressed as an increase in blood flux relative to basal cutaneous blood flux. All measurements were taken in triplicate.
The reproducibility of laser doppler flowmetry measurements in a microcirculatory environment was previously tested (Grodzicki *et al.*, 2003).

2.2.1.4 Erythema meter and melanin index measures

Skin pigmentation and ambient lighting influences our perception of erythema (Diffey and Robson, 1992), thus an objective approach is required. A Diastron erythema meter (Diastron, Andover, Hampshire, UK; Photo 2.2.1.4) was used to measure erythema induced by UVB radiation.

Photo 2.2.1.4. Diastron Erythema meter

The basis for erythema index (EI) measurements by erythema meter was fully described in (Diffey *et al.*, 1984). In brief, the principle of the instrument was based on a spectrophotometric technique and the fact that haemoglobin in upper dermal blood vessels is the chief chromophore of green light (510-568nm) in skin. When there is increasing erythema due to vasodilation, more green light (510nm) is absorbed by haemoglobin and less is reflected. However, the amount of red light (650-655nm) absorbed or reflected stays relatively constant. By comparing the quantity of reflected red (660-690nm) and green (530-560nm) light, an 'erythema index' could be obtained which is dependent on the amount of blood. The Erythema index for this instrument can be defined as follows: the logarithm value of the amount of reflected red to green light (Diffey *et al.*, 1984).

$$\text{Erythema index (EI)} = \log_{10}\left(\frac{\text{Intensity of red component of reflected light}}{\text{Intensity of green component of reflected light}}\right)$$

White light from a projector bulb was transmitted along one branch of a fibre optic cable to a conically shaped handheld applicator with an aperture of 21mm diameter,

which was in turn held against the skin to be measured. The aperture of the handheld applicator was greater than the diameter of the light guide (5.5-6mm) such that the instrumental readings were unaffected by changes in applied pressure within reasonable operational limits. Light re-emitted from the skin travels back up two different branches of the fibre optic cable; one to a green interference filter and photodiode, the other to a red interference filter and photodiode. The photocurrents from both diodes were amplified and formed the two inputs to a log ratio module. The output from this module was the erythema index.

The erythema meter was able to record quickly, easily and precisely the degree of erythema at any anatomical site, with a coefficient of variation of about 3% that may be obtained by repeated measurements at a single site. A note of caution when using this instrument – care must be taken not to rest the handheld applicator against the skin to be measured for a prolonged period of time (>5 seconds), as the light from the projecting bulb could warm up the underlying skin, contributing to an artefactually increased erythema index. Although the instrument cannot fully discriminate between erythemal and pigmentary changes, a measurement of the erythema index at a site on the skin before and after irradiation shows that the difference between the two readings is essentially related to an increase in vasodilatation and is independent of the melanin content of the epidermis. UVR induced erythema can therefore be expressed as the erythema index derived from reflectance spectroscopy $\log_{10}$ [672nm: 546nm] × 1000 (Diffey and Farr, 1991; Diffey *et al.*, 1984). A baseline erythema reflectance reading was measured in triplicate from unirradiated skin adjacent laterally to the lowest UVB irradiated dose on the mid back and the buttock. The mean value of erythema index was calculated from the 3 measurements. The baseline erythema index was calculated similarly. The erythema index response was expressed as the change in erythema index after the subtraction of baseline values.

2.2.1.5 Melanin index measurements

Melanin index (MI) was obtained using the same reflectance instrument (Photo 2.2.1.4) to measure pigmentation. The basis of MI is as follows. The MI measurement is a function of the remittance at 632nm (for melanin absorption) and 905nm (reference signal) (Diffey *et al.*, 1984; Feather *et al.*, 1988). For this MI scale

(-150 to 0) increase in pigmentation leads to a higher MI (most Caucasian skins have negative MI numbers) and there is a strong inverse relationship between MI and erythema in response to a UV irradiation dose between individuals (Ha *et al.*, 2003; Wagner *et al.*, 2002b). Thus the MI is a useful predictor of an individual's sensitivity defined as erythema to UVR (Flanagan *et al.*, 2001; Ha *et al.*, 2003). Melanin index (MI) was measured in triplicate before and after UVB irradiation at mid back and buttock at the same locations as the erythema recordings.

2.2.2 Skin colour measurements

Phenotype measurements of these volunteers were performed using tristimulus L* a* b* colorimetry (Clydesdale, 1978; Westerhof *et al.*, 1986) and spectrophotometry by Minolta spectrophotometer CM-2600d (Minolta Co., Ltd, Osaka, Japan) (see Photo 2.2.1.1) to measure their skin colour at multiple sites including forehead, cheek, forearm, medial arm, hand, chest, abdomen, thigh, calf, back and buttock.

Where many previous studies only used upper inner medial arm as the body site for skin colour measurements (Dwyer *et al.*, 1998; Wagner *et al.*, 2002a; Wagner *et al.*, 2002b), this study included a wide range of photoexposed and photoprotected body sites for constitutive skin colour measurements. Before each machine start and experimental session and measurement, the spectrophotometer was zero calibrated according to manufacturing instructions.

2.2.3 Assessment of freckling

Previous methods have used freckling charts (Bastiaens *et al.*, 2001a) as described by (Gallagher *et al.*, 1990), freckling score (Duffy *et al.*, 2004), crude freckling categories (Elwood *et al.*, 1990), presence or absence (Motokawa *et al.*, 2007; Zhang *et al.*, 2004), freckling tendency by history (Naysmith *et al.*, 2004), number of freckling sites by history (Flanagan *et al.*, 2000) to measure freckling. No previous method involves counting the number of freckles. I have chosen to objectively assess the degree of freckling by recording the presence or absence of freckles at 7 freckling sites for ease of recording for this phenotype and due to time constraints.

The presence or absence of freckles at 7 freckling sites including face, shoulders, chest, abdomen, back, arms and legs was recorded. The number of freckling sites was also noted while skin colour measurements were obtained.

2.2.4 Hair colour measurements

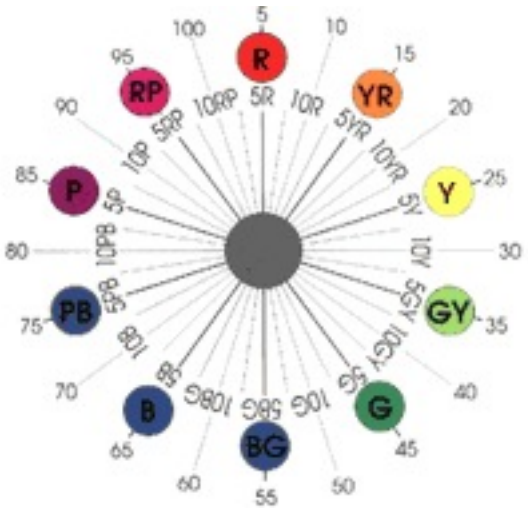
Hair colour was measured similarly using tristimulus L* a* b* colorimetry (Clydesdale, 1978; Westerhof *et al.*, 1986) and spectrophotometry by Minolta spectrophotometer CM-2600d (Minolta Co., Ltd, Osaka, Japan). Hair colour is not the same over the whole scalp from our preliminary studies. Individuals often have different coloured strands of hair within the same scalp. There were intrapersonal variations in hair colour within the same scalp. In order to minimise this, an "average hair colour" was obtained and used. Hair colour was measured at 6 scalp hair sites including: left and right frontal (8cm superiorly from supraorbital ridge), left and right temple (8cm laterally from supraorbital ridge), left and right occipital (5cm laterally from occiput). All measurements were obtained in triplicate. These sites were chosen in an attempt to obtain a true "average hair colour". The sites were chosen from anatomical points so that it was reproducible.

2.2.5 Eye colour measurements

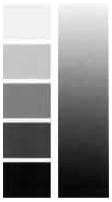
Previous methods have used crude classification, photography, computerised image analysis of photographs (Takamoto *et al.*, 2001) and colour matching, comparison to standard photographs (Mitchell *et al.*, 2003), purpose-developed chart (Semes *et al.*, 2006), 3-CCD video camera with computer software (Niggemann *et al.*, 2003) to assess eye colours. This method has been used previously (Sparrow *et al.*, 1988). An indirect method was therefore used to measure eye colour. The colour of the iris were noted and matched using Munsell colour atlas (X-Rite Ltd, UK), based on the Munsell colour system (Munsell, 1912), with over 1500 colour plates under standardised daylight illumination (custom-made) fitted with two (2 feet) Philips F20W/54 Daylight fluorescent tubes (colour temperature 6500K) (Philips, UK). A myopic vision technique was used such that any variation in iris colours was blended to one uniform iris colour due to myopic vision. The observer, having spectacles

removed, performed eye colour matching of the uniform iris colour to the resultant Munsell colour plate in the Munsell colour atlas. Colour vision of the observer was previously evaluated by using the Farnsworth-Munsell 100 Hue Test (Macbeth, New Windsor, New York). The resultant Munsell colour plate was recorded as a complete Munsell notation: Hue Value / Chroma i.e. H V/C. There were 5 main hues: red, yellow, green, blue and purple, with 5 intermediate hues midway between the main hues.

These 10 steps were broken into 10 substeps which resulted in 100 hues with integer values.



Value or lightness ranges from black (value 0) to white (value 10).



Chroma is a measure of saturation or colour purity, with less pure having a lower number.

Figure 2.2.5. The Munsell Colour System

Source: www.uni-mannheim.de/fakul/psycho/irtel/colsys/Munsell.html

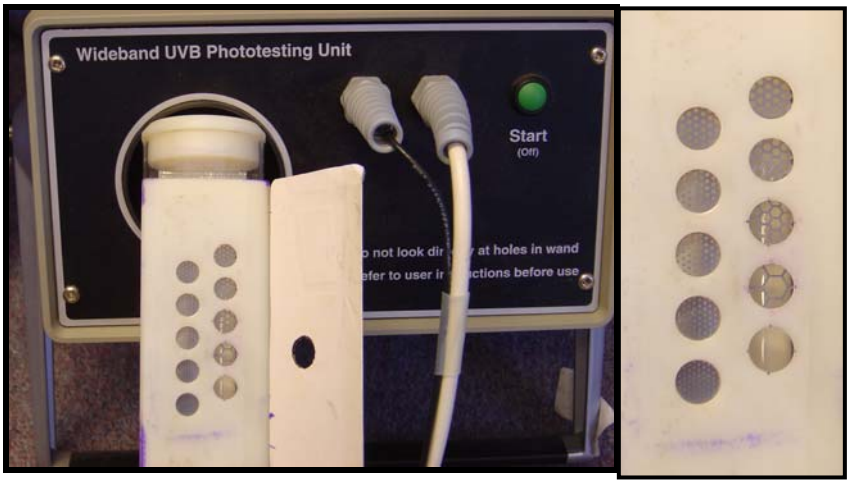
Munsell colour space notations (HVC) were converted to CIE L*, a* and b* colour space representations using Munsell Conversion Software version 8.0.1 (WallkillColor.com, http://livingstonmanor.net/Munsell/index2.htm) for quantitative analysis of eye colour.

2.2.6. UVR source and irradiation

A custom made UVB radiation source (Photo 2.2.6.A) by Emeritus Professor Brian Diffey (Regional Medical Physics Department, Newcastle, UK) was used for the UV irradiation experiments.

Photo 2.2.6. Custom made broadband UVB radiation source

A B

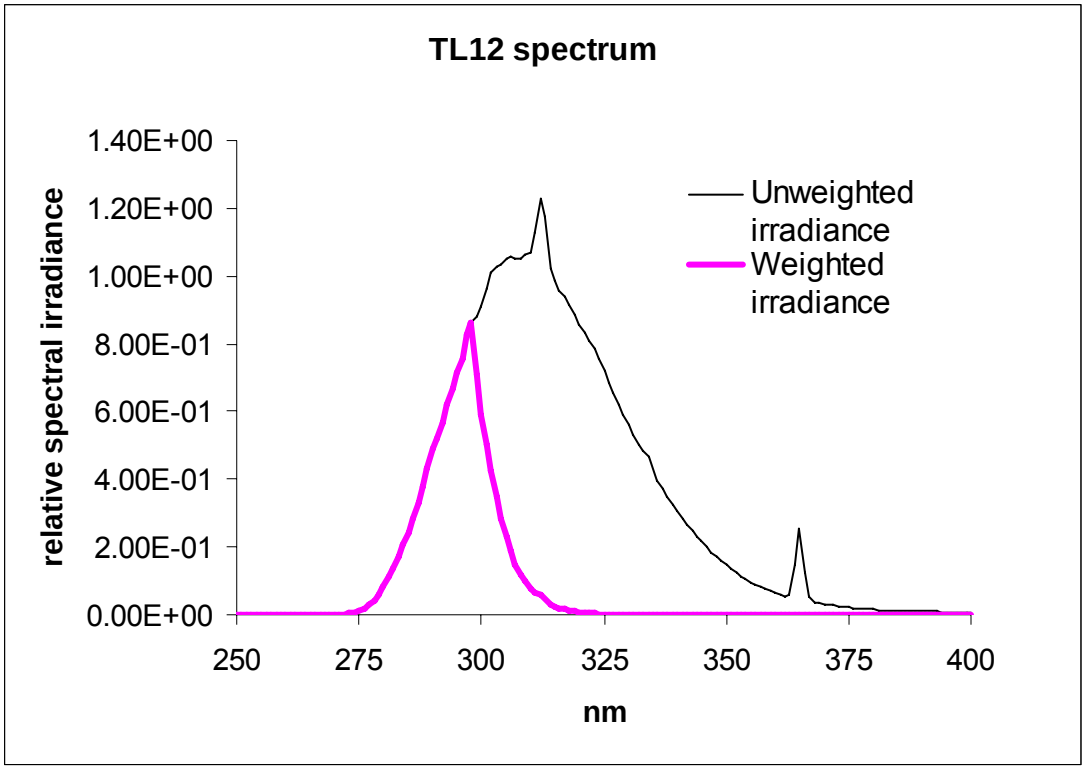


This consisted of a broadband UVB lamp (Philips PLS 9w/12) housed within an enclosed unit, with 10 apertures of 9mm diameter anterior to the UVB lamp to enable 10 doses to be delivered simultaneously. All 10 apertures were within an area of 7cm×3cm (Photo 2.2.6.B). The maximum dose aperture consisted of no metal foil filter, the other 9 dose apertures were backed by perforated metal foil with hole grids of differing but increasing sizes to allow for the 9 increasing dose aperture ranges. The UVB lamp casing is made of transparent plastic that is opaque to UV. The unit also consisted of a digital photodiode that switched the lamp off automatically when the appropriate doses at the apertures were delivered.

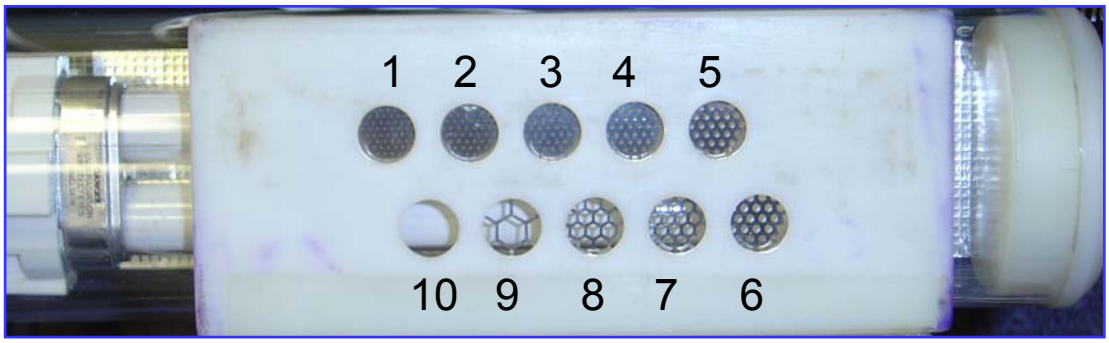
The relative spectral irradiance was plotted for the TL12 UVB lamp in Figure 2.2.6. (Medical Physics, Royal Infirmary of Edinburgh; Philips, UK):

Unweighted irradiance was the absolute spectral irradiance. Weighted irradiance referred to the erythemally-weighted irradiance weighted for biological erythemal effect i.e. distribution of effective radiance with standard erythemal doses (SEDs) for the individuals. 1 SED is equivalent to an erythemal effective radiant exposure of 100 J/m^2 (Diffey *et al.*, 1997).

Figure 2.2.6. Spectral irradiance of TL12 UVB lamp.



All lamps and doses used were calibrated to standards traceable to national and European standards.



UVB Aperture	UVB dose used (Standard erythemal doses SEDs)
1	**1.0**
2	**1.3**
3	**1.6**
4	**2.0**
5	**2.6**
6	**3.2**
7	**4.1**
8	**5.2**
9	**6.5**
10	**8.2**

UVB doses used (SEDs)	
SEDs	**mJ/cm^2**
1.0	38
1.3	47
1.6	60
2.0	75
2.6	95
3.2	119
4.1	150
5.2	189
6.5	238
8.2	300

The 10 increasing increments of UVB doses were in a multiple of $\sqrt[3]{2}$. The equivalent SEDs were shown.

The mid back and buttock skin were irradiated with UVB exposure to graded doses 0, 1, 1.3, 1.6, 2, 2.6, 3.2, 4.1, 5.2, 6.5 and 8 SEDs (Diffey *et al.*, 1997) on one occasion affecting 10 small areas of skin less than 1cm^2 in each (diameter 9mm). Volunteers' end responses were measured after 24 hours and 1-week post irradiation using blood flux (Moor contact Doppler velocimetry), erythema (Diastron erythema meter), melanin index (degree of melanin developed facultatively), L* a* b* colorimetry and spectrophotometry. At 1-week post irradiation, a paper card template with 10 holes corresponding to the 10 apertures of the custom made UVB source was positioned over the site of previous irradiation. The superimposed paper card template holes with the resultant reaction(s) (erythema and/or tanning) also served as a further confirmation of the site of previous irradiation. The peripheries of the apertures were then ink marked. Care was taken in marking the sites of irradition as this is a potential pitfall.

It is important to be able to assess and measure tanning in the absence of blood. If the blood flux was still elevated 1-week post UVR, noradrenaline iontophoresis (Oh *et al.*, 2004) (Photo 6a) was performed to reduce the amount of blood flux back to basal control level before melanin index and colorimetry spectrophotometry were obtained.

For the purposes of ease of description, "Dose 1" was designated as 95mJ/cm^2 which was equivalent to 2.6 SEDs (~ 1 MED) for average Northern European. This dose generates just perceptible erythema in an average normal Northern European (~1 MED).

	UVB dose (mJ/cm^2)	SEDs	MEDs equivalent
Dose 1	95	2.6	1
Dose 2	189	5.2	2
Dose 3	300	8.2	3-4

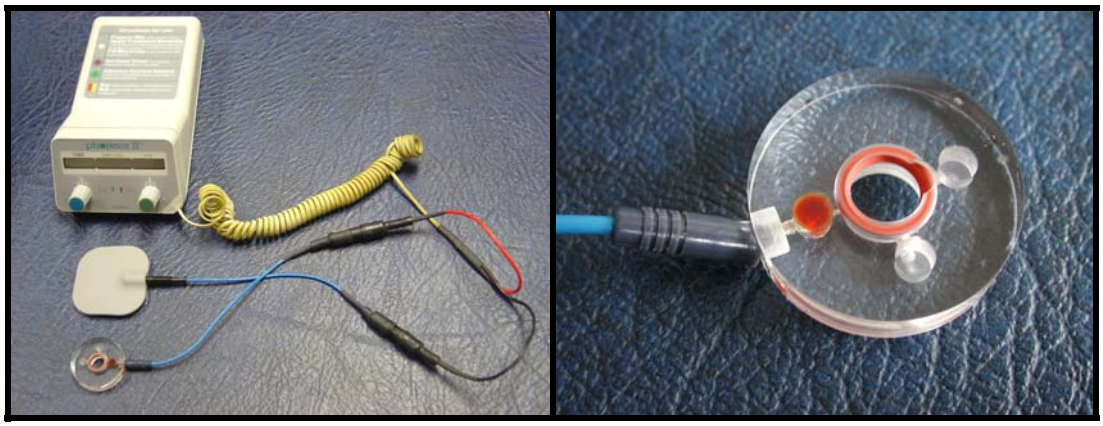
"Dose 2" was designated as 189mJ/cm^2 which was equivalent to 5.2 SEDs (~ 2-3 MEDs). "Dose 3" was designated as 300mJ/cm^2 which was equivalent to 8.2 SEDs (~ 3-4 MEDs).

2.2.7. Noradrenaline iontophoresis

A key problem in measuring pigmentary change after UV irradiation is the presence of erythema caused by the UV irradiation. This erythema in turn poses a problem in the measurement of induced pigment, which may inadvertently measure the erythema as well as the induced pigment. In order to overcome the contributory effect of erythema to pigment / skin colour measurements after UVB irradiation, I used the method of Noradrenaline iontophoresis after UVB irradiation to remove blood and the effect of vasodilatation as the gold standard before reflectance measurements.

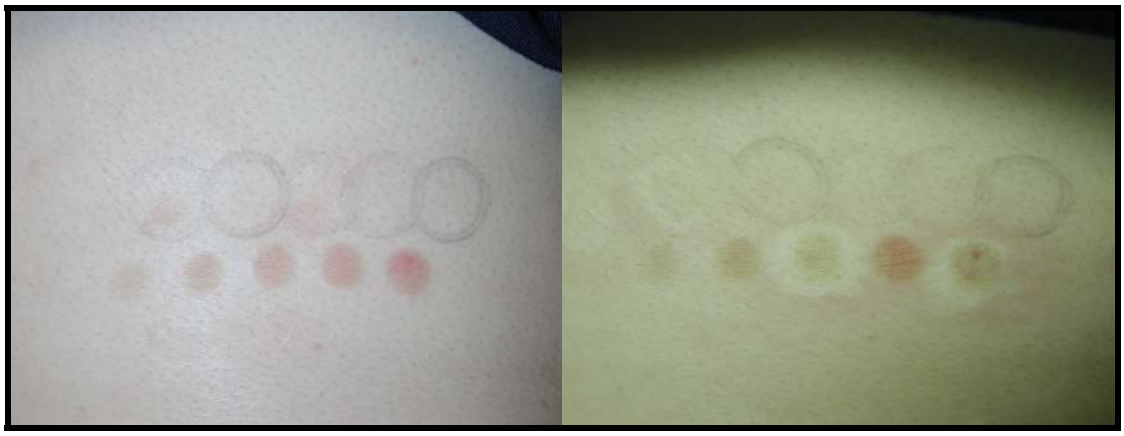
Noradrenaline iontophoresis was performed using Phoresor II Auto unit model PM700 (Iomed, Salt Lake City, Utah, USA) (Oh *et al.*, 2004) (Photo 2.2.7.a) which involved the passage of a small electric current (0.2mA) from a 9V battery for 5 minutes using the drug Noradrenaline 1:1000 (0.1%) (Abbott Laboratories Ltd., UK) applied to a total area of $4cm^2$ via a well of 9mm diameter to make the skin blanch to areas that have gone red from UVR exposure (see Photo 2.2.7.c).

Photo 2.2.7.a. Noradrenaline iontophoresis



Photos 2.2.7.b, 2.2.7.c Before and after noradrenaline iontophoresis

b) Pre noradrenaline **c) Post noradrenaline**



Skin colour was measured after iontophoresis of noradrenaline to remove blood and then skin pigment was measured using tristimulus L* a* b* colorimetry and spectrophotometer. Noradrenaline was driven by the current during iontophoresis and had a potent vasoconstrictor effect. The dosage used was optimized such that any

increase in UVB-induced flux was reduced back to basal blood flow. The method was further developed.

2.2.8. Pressure colorimetry versus Noradrenaline iontophoresis study

Another technique was tried to circumvent the problem posed by erythema after UV induced pigment change. The possibility to study pigmentary changes in the absence of blood flow was to use a technique that utilised pressure – if sufficient pressure was employed via a transparent cover, the pressure exerted onto the skin would exclude and expel blood from blood vessels temporarily, blanching the skin. A tailor made transparent cover (Minolta Co., Ltd, Osaka, Japan) (Photo 2.2.8) was obtained and fitted to the Minolta spectrophotometer CM-2600d (Minolta Co., Ltd, Osaka, Japan).

Photo 2.2.8. Tailor made transparent cover (A) fitted to the Minolta spectrophotometer CM-2600d (B).

A B

A series of preliminary experiments (n=9) were performed to study this technique. L* a* b* colorimetric values and reflectance spectrophotometric values were measured for pressure with cover and post-noradrenaline iontophoresis with cover.

2.2.9. Shriver method

A method of measuring pigmentary phenotype and melanogenic dose response was published - Shriver method (Wagner *et al.*, 2002a). Wagner *et al* concluded that adjusted melanin (AM) index (slope 650:700 nm) is a more accurate measure of melanogenic dose–response (i.e. facultative pigmentary response) than other

traditional indices (e.g. colorimetric L* or melanin index MI), as it allows measurement of induced melanin without the complications of residual erythema. In addition, Wagner *et al* argued that AM is only measuring wavelengths at the red end of the visual spectrum, where haemoglobin does not absorb and that the only chromophore being measured is melanin. The method could be compared with my data and analysed according to this method.

2.2.10. Dwyer method

A method (Dwyer *et al.*, 1998) was previously used to determine the density of cutaneous melanin from skin reflectance measured by a Minolta 508 spectrophotometer. Cutaneous melanin was shown to be estimated by a mathematical difference between 2 measurements of skin reflectance at 400nm and 420nm. Dwyer *et al* argued that this mathematical difference correlated well with Masson Fontana stain. A comparison of this mathematical method with my experimental data analysed will be detailed in the results section in Chapter 5.

2.3. Genetic phenotyping

2.3.1. Collection of blood samples

Venepuncture was performed on all volunteers to obtain a total of 18ml venous blood sample and placed in two 9ml Sarstedt Monovette plastic tubes containing ethylenediamine tetra-acetic acid (EDTA). These were frozen at -80°C and stored until required for DNA extraction.

2.3.2. General reagents used in molecular biology procedures

All chemicals were analytical grade and were supplied by Sigma, Promega, Gibco BRL, BDH, Fisher Scientific, Flowgen, and Boehringer Mannheim. Nucleic acid manipulations were done in 1.5ml centrifuge tubes unless otherwise stated. General solutions were prepared by Human Genetics Unit (HGU) technical staff and autoclaved and stored at room temperature.

Tris.HCl

Tris base (tris[hydroxymethyl]aminomethane) was dissolved in sterile water. HCl was used to adjust the pH to the required value.

EDTA

EDTA (ethyldiaminetetra-acetic acid di-sodium salt) was dissolved in sterile distilled water. The solution was adjusted to pH 8.0 by adding solid NaOH.

TE buffer

10mM Tris.HCl (pH 7.5); 1mM EDTA.

Tris-Borate-EDTA (TBE) buffer, 20× stock

Tris base	216g
Boric acid	110g
0.5M EDTA	80mM

Distilled water was added to a final volume of 1 litre. Stock was diluted to 1× with distilled water.

TAE 50× stock

Tris base	242g
Glacial acetic acid	57.1ml
0.5M EDTA	100ml

Distilled water was added to a final volume of 1 litre. Stock was diluted to 1× with distilled water.

2.3.3. DNA extraction

DNA was extracted using Nucleon Genomic DNA extraction kit (Tepnel Life Sciences PLC, Manchester, UK). Collected whole blood were fully thawed, then lysed, deproteinised with sodium perchlorate, DNA extracted with chloroform, DNA precipitated with ethanol, washed, air-dried for 30 seconds and re-dissolved in 1ml Tris-EDTA and rotary mixed for 24 hours before storage at 4°C.

2.3.4. Polymerase Chain Reaction (PCR)

DNA molecules were amplified by PCR.

dNTPs

Deoxyribonucleotide triphosphate (dNTPs) were purchased as stocks of 100mM. Working stocks of 10mM were made by mixing 10ml of each of the dNTPs (dATP, dCTP, dGTP, dTTP) with 60ml dH_2O to a final volume of 100ml. Stocks were

stored at –20°C. dNTPs were used in PCR reactions at a final concentration of 0.2mM.

Primers

Primers were initially designed using Primer3 (http://fokker.wi.mit.edu/primer3/input.htm). Primers were purchased as lyophilised desalted compounds (Invitrogen). Stocks were made up to 1mg/ml using sterile distilled H_2O. Working stocks were diluted to 1mg/ml and stored at 4°C. Primers were used in PCR reactions at a final concentration of 2ng/ml (1:50 dilution). Primers were assumed to be at the stated concentration without confirmation by spectrophotometry.

MC1R primers

The forward primer (401F) used was 5'-GAA CTA AGC AGG ACA CCT GGA GG-3' and the reverse primer (1492R) used was 5'-GGA CCA GGG AGG TAA GGA ACT GC-3'.

PCR conditions were 94°C 4min, then 35 cycles of 94°C 30s, 55°C 30s, 72°C 2min; followed by 72°C 10min.

POMC primers

Exon 1 forward primer used was 5'-CCG GGA AGG TCA AAG TCC-3' and the reverse primer used was 5'-GCG CAG AAA GTT TGT C GA G-3'.

Exon 2 forward primer used was 5'-TGT TGT TAA TGT TGG CTC AAG G-3' and the reverse primer used was 5'-AGC TCC AGT CCC ATC TAA TGT C-3'.

Exon 3 forward primer used was 5'-CTG GAG TTC AAG AGG GAG CTG-3' and the reverse primer used was 5'-GAA GTG CTC CAT CCT GTA GGG-3'.

PCR conditions were 94°C 4min, then 35 cycles of 94°C 30s, 60°C 30s, 68°C 1min; followed by 72°C 10min.

Taq polymerase, PCR buffer, and Mg^{2+}

These reagents were purchased (Applied Biosystems). Ampli*Taq* (5U/ml) was used at 0.2ml per 25ml reaction. PCR buffer was a 10× stock and therefore diluted 1:10

for reactions. Mg^{2+} was used at a final concentration of 1.5mM. Routine PCRs were done in a Hybaid Omnigene thermocycler using Abgene thin walled thermo tubes.

PCR Recipe for a 25µl reaction:

2.5µl buffer	0.5µl dNTP	0.5µl forward primer
0.5µl reverse primer	0.1µl *Taq* polymerase	
0.5µl DNA	19.65µl water	0.75µl Mg^{2+}

Optiprime protocol

5µl buffer of each of 12 Optiprime buffer

45µl reaction mix

12-reaction mix Recipe:

12.5µl 50× Mastermix	12.5µl dNTP	2.5µl forward primer
2.5µl reverse primer	3µl *Taq* polymerase 30U	
6.25µl DNA	525.75µl water	

2.3.5. Polyacrylamide Gel Electrophoresis (PAGE)

Agarose gel loading buffer

Loading buffer was prepared as a 10× stock and stored at room temperature.

	Final concentration
Ficoll	20%
Orange G (Sigma)	1%
EDTA	100mM

This was made to required volume with distilled water.

For preparing gels, the required amount of agarose (High pure, BioGene) was dissolved in either 1× TBE or TAE by heating. Molten agarose was cooled and ethidium bromide added to a final concentration of 0.5µg/ml agarose. The DNA markers used were 100bp marker (Promega) and 1kb marker (Invitrogen) depending on the expected size of fragments. DNA size markers were run alongside the samples to estimate the size and amount of DNA in the samples. The gel was placed in a gel

tank with 1×TBE buffer and a current applied. After adequate migration as indicated by the loading buffer, the gel was placed in a UV-transilluminator. Samples were run in 1× loading buffer.

2.3.6. Determining concentrations of DNA

The intensity of DNA was visualized and estimated against known DNA concentration on agarose gel by electrophoresis, or by measuring the absorbance (optical density, OD) in a spectrophotometer (GeneQuant) at a wavelength of 260nm (A_{260}). A_{260}/ A_{280} ratio of 1.7-1.9 indicates DNA purity.

2.3.7 Genotyping and sequencing

Volunteers were genotyped to identify *MC1R* (OMIM 155555) status. The sample of venous blood (10-20ml) was used for genotyping. Single Nucleotide Polymorphisms (SNPs) were initially identified using existing bioinformatic resources (e.g. the SNP Consortium (www.snp.cshl.org) and the International HapMap Project (www.hapmap.org). These were subsequently detected in the sample populations using fluorescent primer extension on ABI 310 or 3100 electrophoresis systems at Medical Research Council Human Genetics Unit. The primer extension assay with fluorescence polarization (FP) detection is an assay for SNP genotyping. Fluorescence polarization occurs when a fluorescent dye is excited by plane-polarized light and is detected if the fluorescent dye is part of a large molecule. In assays where small fluorescent molecules are turned into large fluorescent products, FP provides a simple way to determine if the reaction has occurred without the need for separation or purification of the reaction mixture. In the primer extension assay, DNA polymerase incorporates the complementary, allelic nucleotide onto the SNP primer designed to anneal to the target DNA one base upstream of the polymorphic site. When a fluorescently labeled nucleotide is incorporated, high FP is observed for that dye and the genotype of the DNA sample is determined (Kwok, 2004).

MC1R and 34 other genes thought to be important in skin inflammation and susceptibility to ultraviolet radiation and skin cancer were sequenced or genotyped using automated technology.

2.3.8. *MC1R* and *POMC* Sequencing

PCR primers used for gene of interest were used for DNA sequencing. Sequencing primers were diluted to a concentration of 50μg/μl or 20μM and submitted to the HGU Technical service together with the PCR products.

The dye terminators used was BigDye (PerkinElmer, UK), which consisted of DNA polymerase, dNTPs and ddNTPs-Dye terminators. Successive cycles of amplification resulted in the incorporation of one of the 4 ddNTPs, each tagged with a different fluorescent dye in each extension product.

The sequencing reaction was as follows:

Sequencing primer	5pmol
DNA template	10ng
Mg^{2+}	0.4mM
BigDye terminator	0.5ul
DdH2O	up to 20ul

The sequencing programme was as follows: [30s at 96°C, then 15s at 55°C, followed by 4min at 60°C] × 25 cycles. Sequencing products were ethanol precipitated and separated with an ABI3100 by the HGU Technical service to discriminate ssDNA fragments of different length. Sequencing traces were analysed using Sequencher software version 4.7 (Gene Codes Corporation, USA) and Mutation Surveyor software version 2.61 (SoftGenetics LLC, USA).

The candidate gene approach and delineation and choice and the final selection of candidate gene loci and SNPs will be discussed in detail in Chapter 7.

2.3.9. Illumina SNP genotyping

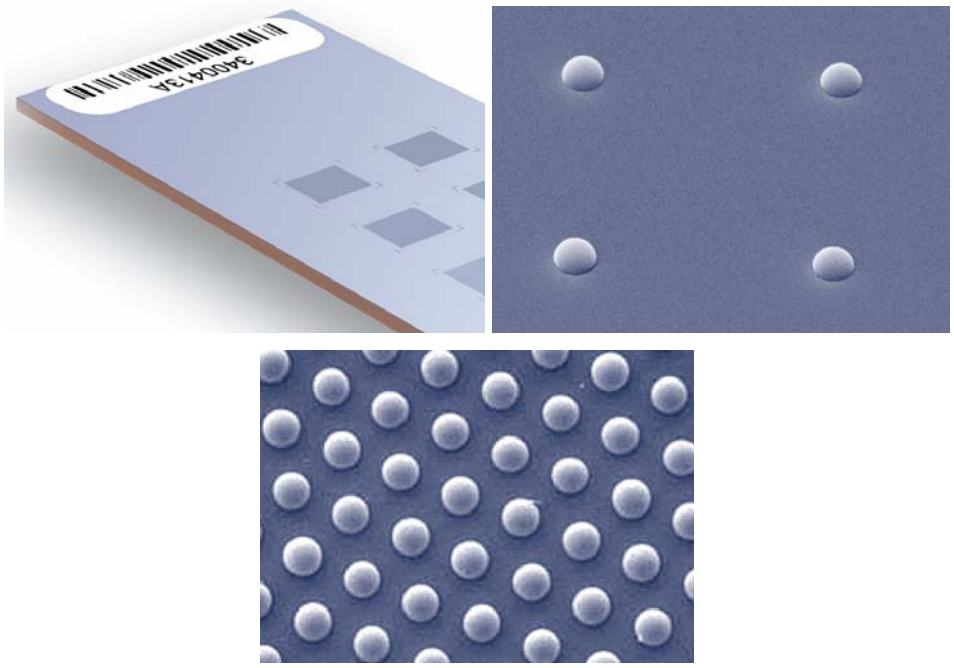
Illumina GoldenGate microarray assay systems (Illumina Inc, San Diego, California, USA) (Fan *et al.*, 2003; Steemers and Gunderson, 2005) was a novel highly multiplexed assay used for genotyping in this study. This was performed at the Wellcome Trust Clinical Research Facility Genetics Core (Edinburgh, UK).

A total of 33 genes were haplotype tagged and genotyped in this population using Illumina GoldenGate SNP genotyping technology.

The basis of Illumina GoldenGate Assay Genotyping is as follows:

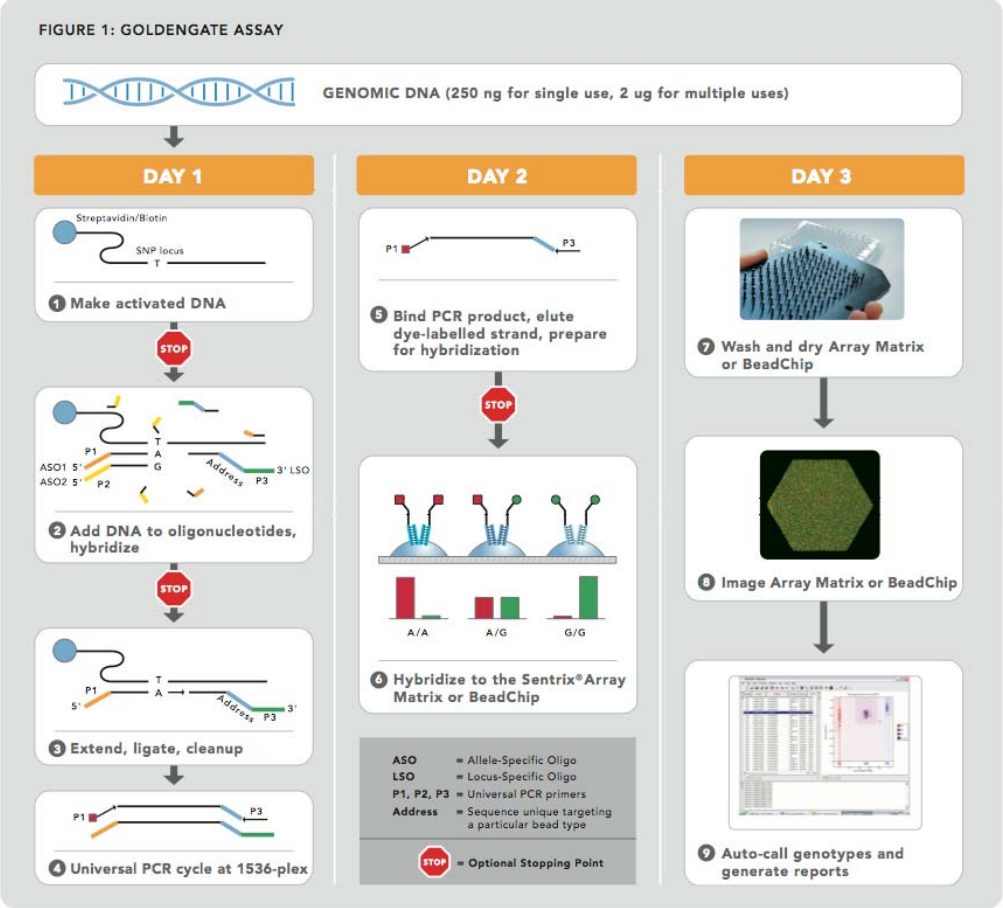
This assay utilises the BeadArray technology, where targeted regions of DNA are immobilized on beads randomly arranged into arrays, and the SNPs visualized through fluorescent tags, which differentiate among alleles.

Figure 2.3.9.a. The Sentrix BeadChip (left) features eight array clusters, each with 50,000 beads and 20-micron spacing between beads (right)



Source: www.illumina.com

Figure 2.3.9.b. Illumina GoldenGate Genotyping Assay



Source: www.illumina.com

DNA sample used is 'activated' for binding to paramagnetic particles by binding to streptavidin and biotin. Next, assay oligonucleotides, buffer and paramagnetic particles are combined with the 'activated' DNA in the hybridization step. 3 oligonucleotides were designed for each SNP locus. Two oligos were allele-specific to the SNP i.e. Allele-Specific Oligo (ASO). The third oligo hybridizes several bases downstream from the SNP site is the Locus-Specific Oligo (LSO). All three oligonucleotide sequences contain regions of genomic complementarity and universal PCR primer sites. The LSO also contains a unique address sequence that targets a particular bead type. The 384 SNPs were tested (in fact up to 1,536 SNPs may be tested simultaneously) using GoldenGate technology. Following hybridization, several wash steps are performed, reducing noise by removing excess and mis-hybridized oligonucleotides. Extension of the appropriate ASO and ligation of the extended product to the LSO joins information about the genotype present at

the SNP site to the address sequence on the LSO. These joined, full length products provide a template for PCR using universal PCR primers. The single-stranded, dye-labeled DNAs are hybridized to their complement bead type through their unique address sequences. Hybridization of the GoldenGate Assay products onto the BeadChip allows for the separation of the assay products in solution, onto a solid surface for individual SNP genotype readout. After hybridization, the BeadArray Reader (BeadStation 500GX) was used to analyze fluorescence signal on the BeadChip, which is in turn analyzed using software for automated genotype clustering and calling. Average call rates were >99%. This translates to a particular allele. The genotypic data were related to phenotypic measurements to look for associations.

Figure 2.3.9.c. BeadStation 500GX Genotyping system

Source: www.illumina.com

Prior to running the custom GoldenGate Genotyping assay, Illumina Assay Design Tool was used to refine and eliminate cross hybridisation and improve success rate. The SNP selection design began from RS list, Sequence list, Regions (by coordinate), or Gene List. Using the tool, full annotation as part of standard results including design score, design rank, minor allele frequency (MAF) and validation status were available for screening. Information from the most up to date sources for both genome build and dbSNP version was used. MAF, validation status* (GoldenGate validation, two-hit validation status, non-validated) and SNP scores were obtained from Illumina. Design, failure codes were also obtained and considered before eliminating down to the final 384 SNPs from 33 genes.

***GoldenGate validation status:** SNP has been previously designed and successfully generated polymorphic results on the Illumina platform. Designed oligonucleotides have 100% sequence match to those previously designed.

***Two-hit validation status:** Both alleles of the SNP have been seen in two independent methods and populations

***Non-validated:** SNP seen in only one method or population. Even if it has a high design score, there is still an increased chance that it is monomorphic

2.3.10 Hair melanin assay

Hair melanins in mice have previously been determined chemically (Ozeki *et al.*, 1995) and used in a previous study (Healy *et al.*, 2001). An attempt to quantify the amount of melanins in human hair was made using an adaptation of this method.

2.3.10.1 Total Melanin (TM) content

1-1.5mg of human hair was weighed in 2ml screw top tube. 1ml of Soluene mix (100ul sterile water + 900ul Soluene 350) per mg of hair was added. This was then heated for 30 minutes in a 90°C water bath and then vortexed to sediment any precipitants. This released melanin into the supernatant. This was followed by a further 15 minutes of heating in water bath, vortexed and allowed to cool to room temperature. This was then centrifuged for 10 minutes at 13000rpm in microfuge. Absorbances were read with an Ultraspec 2000 UV Visible Spectrophotometer (Pharmacia Biotech, UK) at 500nm against a standard blank of 9:1 Soluene 350:water that also went through the same heating procedure. Quartz cuvettes were used. The mean absorbance was equal to the total melanin (TM) content.

2.3.10.2 Alkaline Soluble Melanin (ASM) content

Alkaline soluble melanin (ASM) content was determined as follows. 2mg of hair was weighed in 2ml screw top tube. 1ml of 6.4M Urea/0.8M NaOH mix was added to every 2mg of hair. The mixture was shaken horizontally for 30 minutes in a 37°C incubator. After vortexing, the mixture was centrifuged for 10 minutes at 13000rpm in microfuge. Absorbances were read with an Ultraspec 2000 UV Visible Spectrophotometer (Pharmacia Biotech, UK) at 400nm against a standard blank of 6.4M Urea/0.8M NaOH. Results were expressed as a ratio of ASM to TM.

The human hair melanin assay was found to give inconsistent results and not very useful and it was not pursued further. The results will be presented in Appendix 5.

2.4 Data analysis and statistics

Volunteers were anonymised and deidentified by allocating a unique number so that their confidentiality was maintained. Data were captured and processed using

Microsoft Excel (version 10.6829.6830 SP3 2002, Microsoft Corporation) and pasted to Minitab (version 14 Minitab Ltd., Coventry, UK) and PLINK version 0.99r, Shaun Purcell, pngu.mgh.harvard.edu/purcell/plink (Purcell 2007). Graphs were drawn using Microsoft Excel and Minitab. Statistical analyses were performed on Minitab version 14 and PLINK and described in relevant sections in the thesis. Fisher's Exact test was used for comparison of contingency tables. The Fisher-Freeman-Halton Test was used for the analysis of the r × c contingency tables using StatsDirect (version 2.6.9 StatsDirect Ltd., www.statsdirect.com, Cambridge, UK). Non-parametric tests were used if distribution was not normal. Mann Whitney U test was used to compare medians. Analysis of variance was used when results were normally distributed and parametric. Kruskal-Wallis test was used to perform non-parametric ANOVA. A large number of data has to be presented and a compromise was made between visibility and readability of the graphs plotted in data presentation.

Chapter 3 Static Phenotypes

3.1. Introduction

The data set comprised 159 individuals who participated in this study (Figure 3.1). Demographic and background data were recorded from a volunteer questionnaire (Appendix 3). Phenotypic variables were measured at 22 anatomical body skin sites (11 on each side) using the following measures: baseline tristimulus L* a* b* colorimetry and baseline spectrophotometric reflectance data. Other phenotypic characteristics listed in section 3.1.1 were also measured and recorded.

3.1.1. List of variables

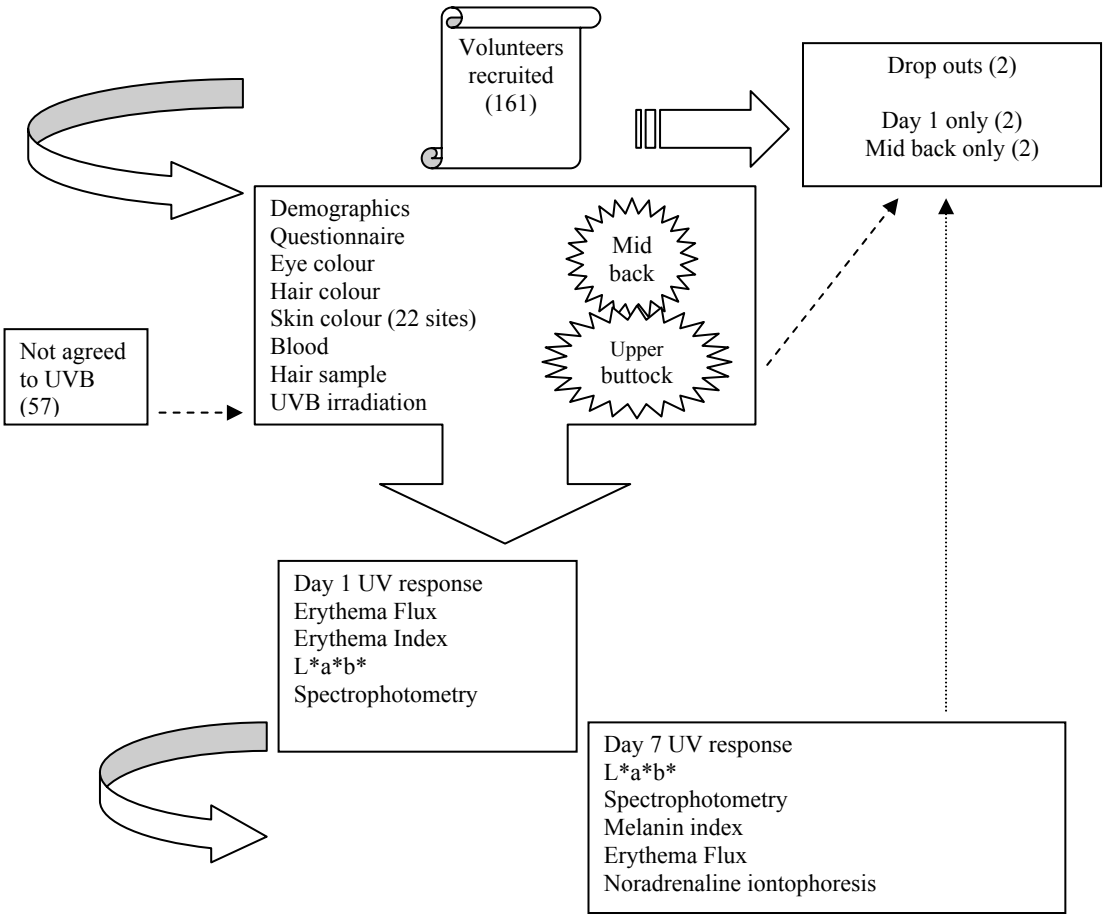
Individuals were grouped by age, sex, hair colour groups, eye colour groups, presence of freckling (yes or no), number of freckling sites (0-7), ethnicity, Fitzpatrick skin type, blonde hair group, presence of hair dye (yes or no), hair colour L* a* b*, Munsell eye colour and eye colour L* a* b*. These will be considered in turn.

3.2. Results

3.2.1. Completeness of data

57 individuals (14 males and 43 females) did not wish to participate in the UVB part of the study (Figure 3.1). 2 individuals only returned once for day 1 erythemal measurements and did not attend the 1-week tanning measurements. Tanning data from the back was available from 2 individuals who did not wish to expose the buttock for tanning experiments. Erythemal data will be discussed in Chapter 4. Tanning data will be discussed in Chapter 5.

Figure 3.1. Study flowchart for volunteers



3.2.2. Volunteer phenotypic characteristics

3.2.3. Age

Volunteers were between the ages of 18-40. The median ages for males and females were similar at 23 and 24 respectively.

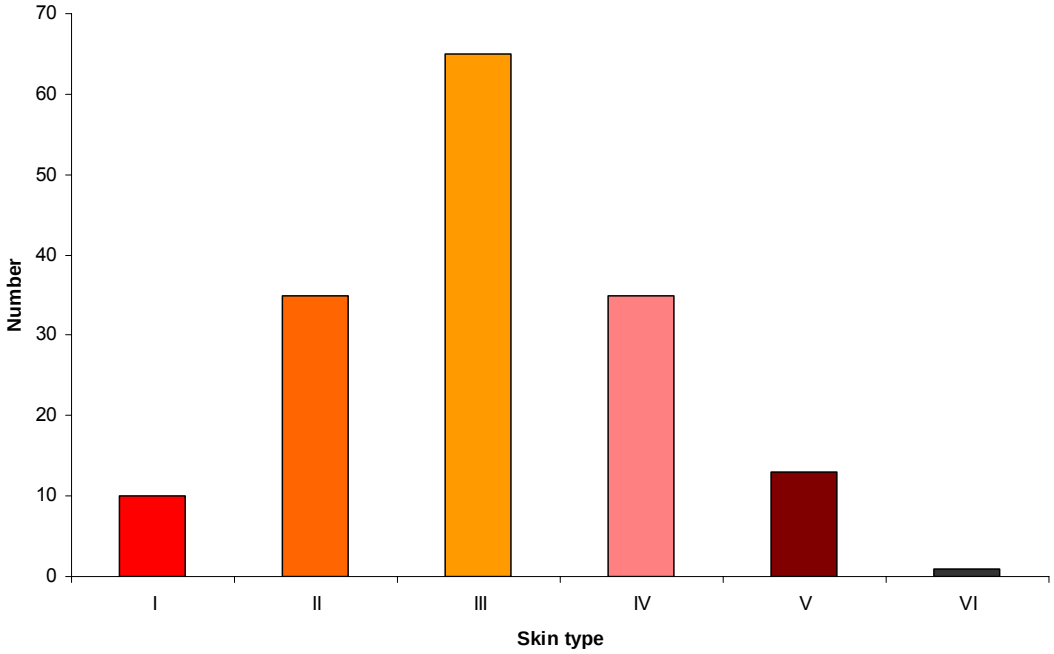
3.2.4. Sex

The 159 volunteers were made up of 33 males (21%) and 126 females (79%). Two volunteers defaulted to attend the study.

3.2.5. Fitzpatrick Skin types (I - VI)

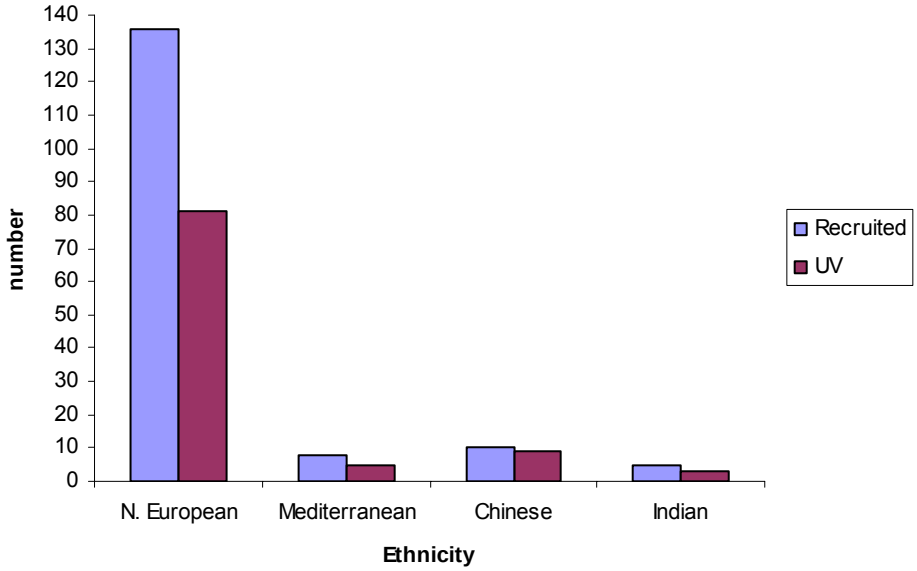
The participants comprised of 10 skin type I, 35 skin type II, 65 skin type III, 35 skin type IV, 13 skin type V and 1 skin type VI.

Figure 3.2.5. Fitzpatrick skin type distribution



3.2.6. Ethnicity

Figure 3.2.6. Ethinicity of volunteers



	N. European	Mediterranean	Chinese	Indian
Recruited	136	8	10	5
UV	81	5	9	3

Chinese and Indians were grouped together as Asians for subsequent analysis, on the basis of their Asian origin, darker skin colour and lower rate of skin cancer.

3.2.7. Freckling

Freckling was defined as small, less than 0.5cm in diameter, well defined light brown, golden brown to brown macules of at least 10 in number. The freckling sites investigated include the face, shoulder, chest, back, abdomen, arm and leg. Freckles were present in 80 out of 159 volunteers on 1-7 of these freckling sites. Figure 3.2.7.1 shows the distribution of individuals with freckling. When present, the commonest sites of freckling were face and arms, followed by the shoulders, chest, back, legs and abdomen.

freckles	80
no freckles	79

Figure 3.2.7.1. Distribution of freckling

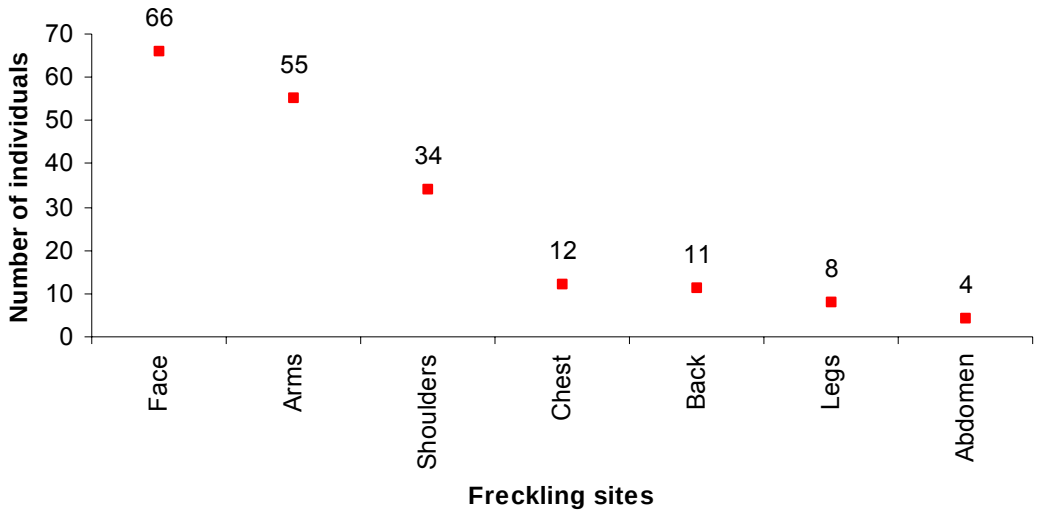
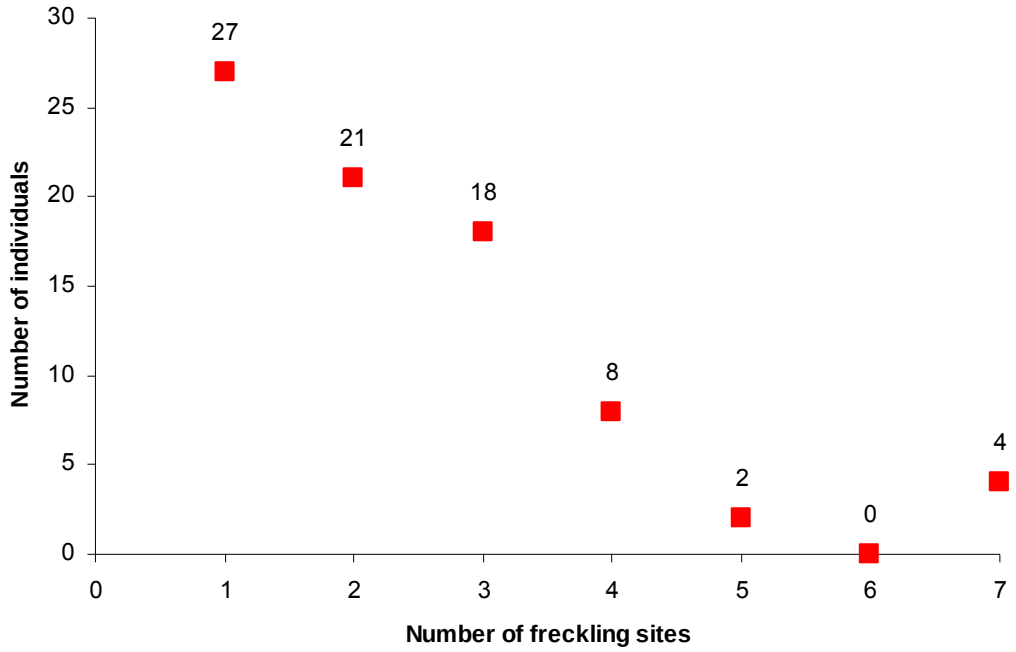


Figure 3.2.7.2 shows the number of freckling sites individuals had. 27 out of 80 people with freckles had the commonest number of freckling sites (one). 4 individuals had the maximum number of freckling sites (seven).

Figure 3.2.7.2. Number of freckling sites



3.2.8. Preliminary studies of hair, skin and eye colour

Prior to the initiation of the research study formally, preliminary studies were performed with regards to skin colour, hair colour and eye colour measurements.

3.2.9. Hair colour variation study

There are 3 types of variability – of the sample, the population and the estimate (Grafen, 2002). First, variability of the sample i.e. how variable the sample is can be estimated by the sample mean and the variance by the standard deviation / spread of the data. Second, variability of the population i.e. how variable the population is can be estimated by the population variance of the sample or in other words, deviation from the true population mean (SEM). Accuracy of the estimate of population variability, quantifies the confidence for the estimate of the population mean. Third, variability of the estimate – obtain the mean of the estimate or, in the case of this experiment, measurement. This gives an idea of how accurate the estimate is.

The objective of the hair colour variation study was to investigate whether there was a day-to-day variation on hair colour measurements using objective means of measurement (Minolta spectrophotometer CM-2600d). i.e. Do hair colour

measurements change from day to day (systematic variation)? What about hair at different sites on the scalp in the same person?

Six scalp hair sites were measured to obtain L* a* b* tristimulus colorimetry and spectrophotometric reflectance data:

Left and right frontal (8cm superiorly from supraorbital ridge)

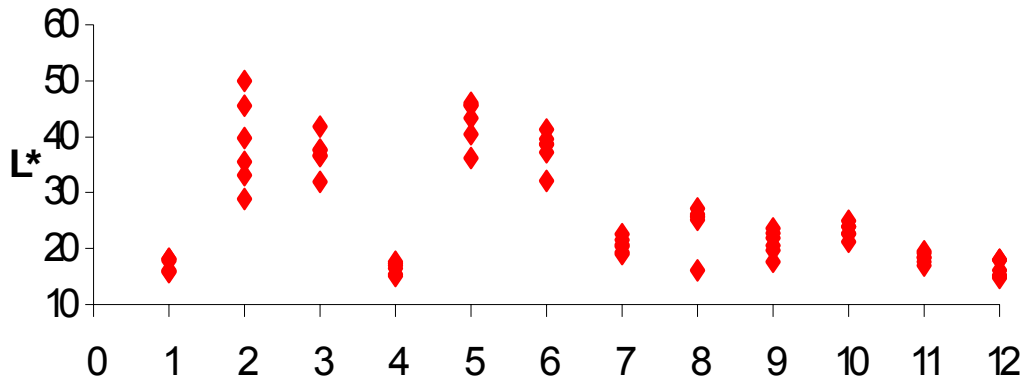
Left and right temple (8cm laterally from supraorbital ridge)

Left and right occipital (5cm laterally from occiput)

Triplicate measures were obtained for each site. Data from "specular component included" setting were compared against "specular component excluded". Advice was obtained (personal communication, Emeritus Professor B. L. Diffey, Regional Medical Physics Department, Newcastle, UK) and it was felt that "specular component excluded" was the more appropriate for measuring skin and hair colours as it excludes the specular reflectance from the measurement and only the diffuse reflectance is measured. This equates to a colour assessment that correlates to the way an observer sees the colour of an object. Data were acquired for three volunteers for three different days within an immediate two-week period (total of 72 datasets per person per day).

Figure 3.2.9 shows the range of L* for 12 individuals in the preliminary hair colour study.

Figure 3.2.9. Range of hair colour L* within individuals (preliminary study)



Y-axis shows hair colour L*. X-axis shows the volunteer number (n=12).

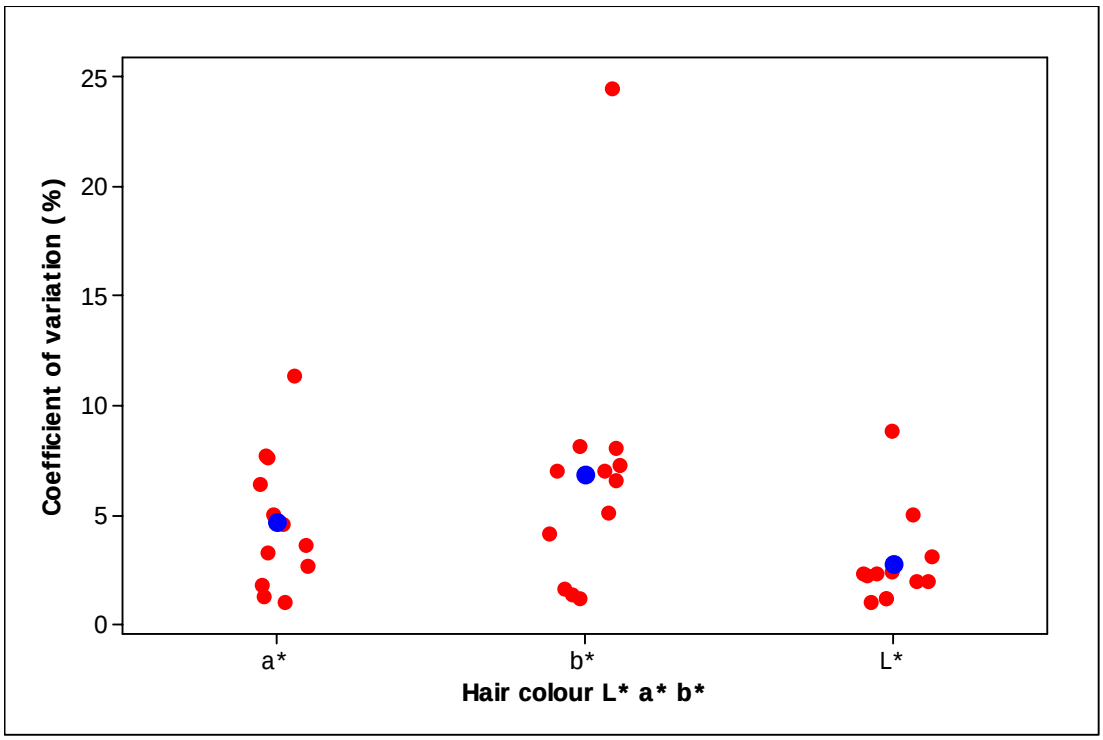
The coefficient of variation (SD/mean) was calculated for hair colour. Hair colour variation was estimated using the coefficient of variation, which was <9% for 12

individuals measured on 3 different days within 2 weeks on the scalp (data not shown). Other sites showed similar coefficient of variation. One-way ANOVA for measured L*, a* and b* values on the scalp on 3 different days showed no significant difference between the means (P=0.975, 0.999, 0.996). There was no significant day-to-day variation in the hair measurements of L*, a* and b* by reflectance.

3.2.10. Hair colour reproducibility

The variability of the estimate on hair colour measurements using a spectrophotometer was investigated. The reproducibility of hair colour measurements was estimated using coefficient of variation.

Figure 3.2.10. Hair colour reproducibility (preliminary study)



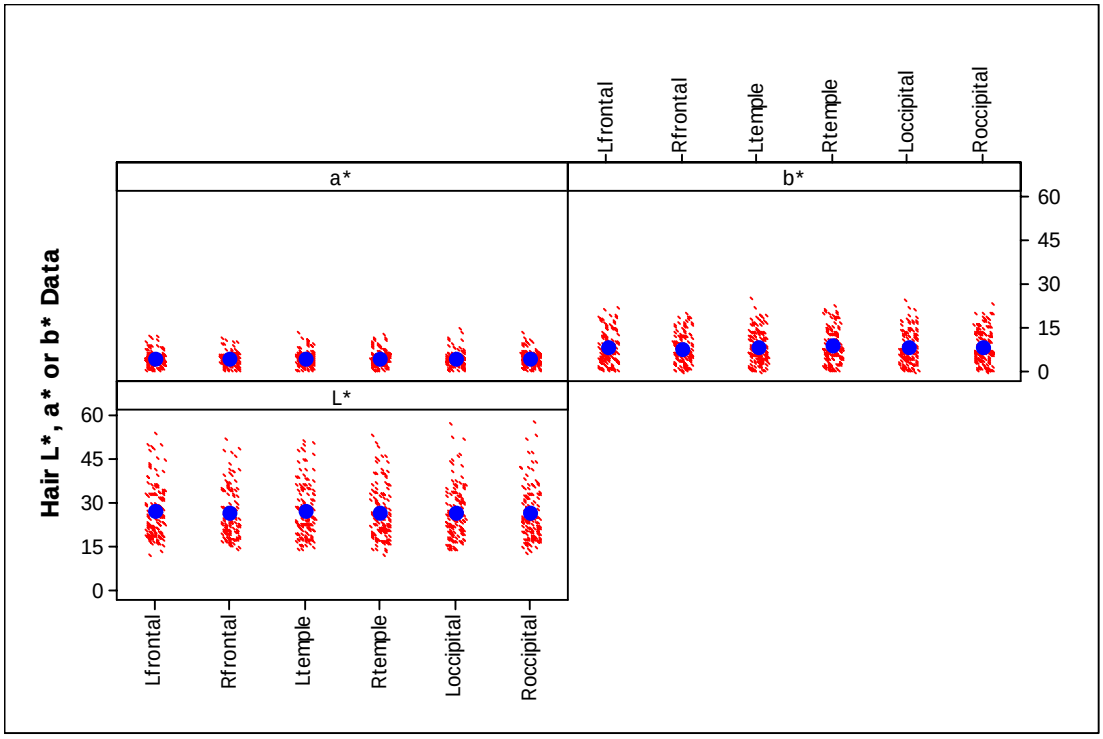
This is an individual value plot of hair colour reproducibility. Y-axis shows the coefficient of variation (%). X-axis shows hair colour L* a* b*. Blue marker denotes the mean value.

Hair colour reproducibility was estimated using the coefficient of variation, which was usually <11% for 12 individuals, except 1 individual's b* value which was very small in number. This meant that a small change resulted in a larger change in percentage of coefficient of variation.

3.2.11 Hair colour results – by site

Are there significant variations in hair colour by site? Figures 3.2.11.1 showed hair colour L*, a* and b* by 6 scalp hair sites. Blue marker denotes the mean value.

Figure 3.2.11.1. Hair colour L*, a* and b* (6 sites)



One-way ANOVA for hair colour L*, a* and b* at the 6 scalp sites showed no significant differences (P=0.95, P=0.33, P=0.61).

Figure 3.2.11.2. Hair colour L*, a*, b* trends for 3 individuals (6 sites)

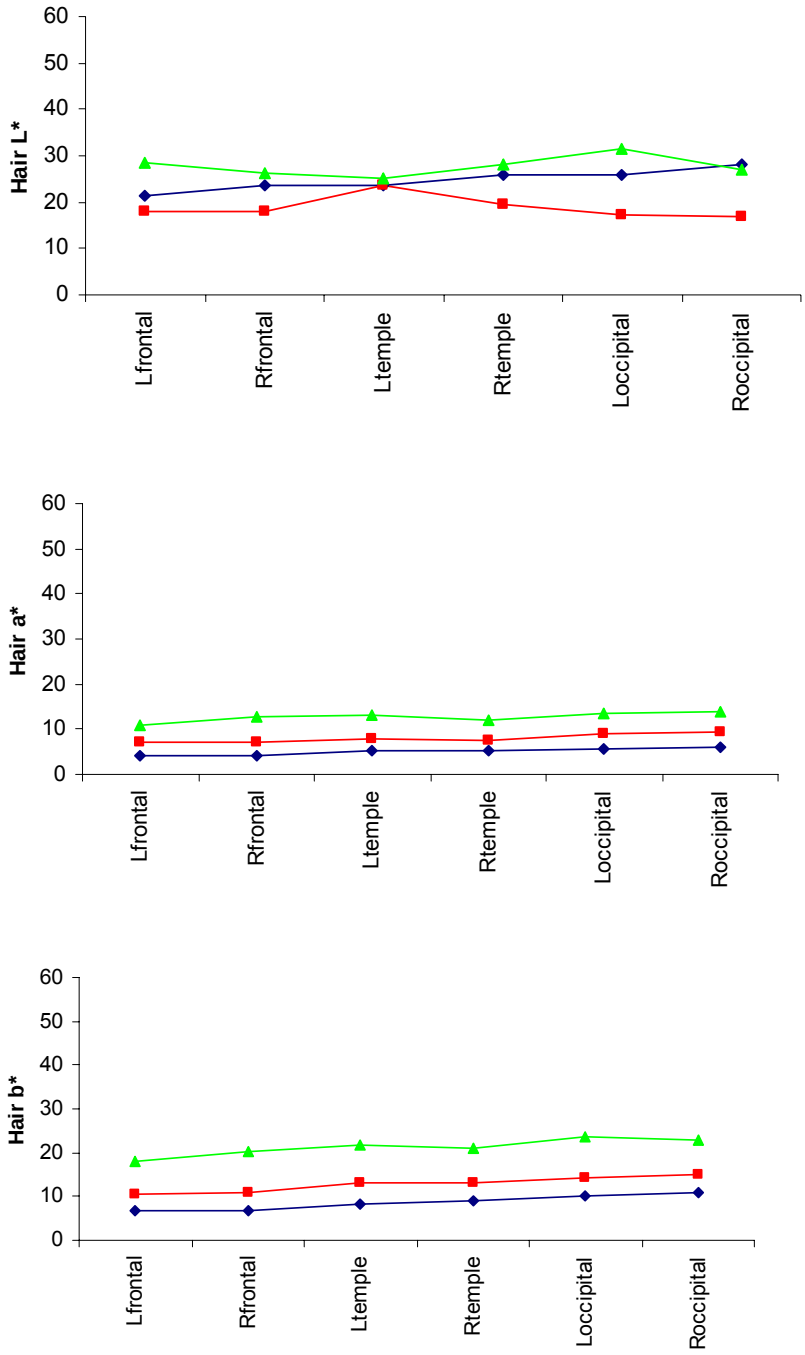


Figure 3.2.11.2 shows hair colour L*, a* and b* variance at 6 scalp sites for 3 representative individuals (green triangles, red squares and blue circles).

3.2.12. Hair colour results

Measurements took place between November 2003 and April 2005.

There were 133 individuals with natural hair colour. 86 individuals have dark hair, 27 individuals have fair hair, out of which 19 individuals have blonde hair and 20 volunteers have red hair.

Figure 3.2.12. shows the range of hair colour for 133 volunteers (dyed excluded) by L*, a*, b* colorimetry. Blue marker denotes the mean value

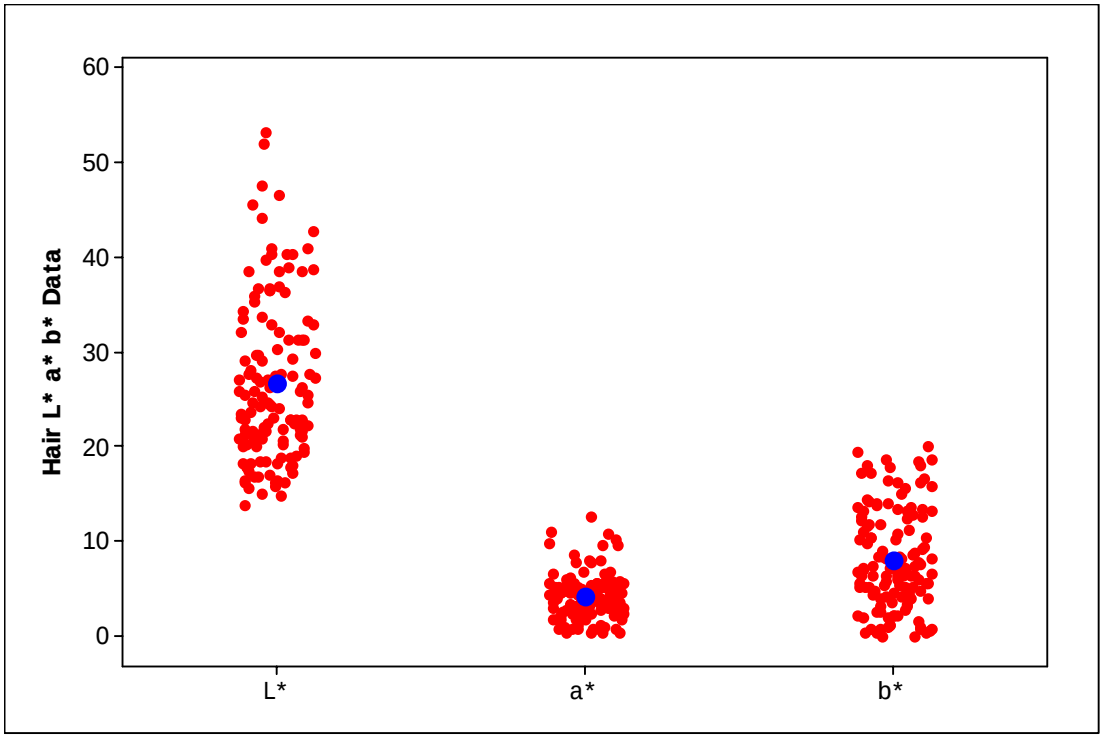


Table 3.2.12. Hair colour ranges by L* a* and b* (n=133, dyed excluded)

Mean L*	SEM	Min L*	Max L*	Mean a*	SEM	Min a*	Max a*	Mean b*	SEM	Min b*	Max b*
26.84	0.74	13.92	53.20	4.29	0.20	0.40	12.7	8.18	0.45	0.05	20.2

Results shown are mean ± standard error of the mean. Hair L* = 26.84 ± 0.74, a* = 4.29 ± 0.2 and b* = 8.18 ± 0.45. Readings were averaged to provide a mean value for hair colorimetry values.

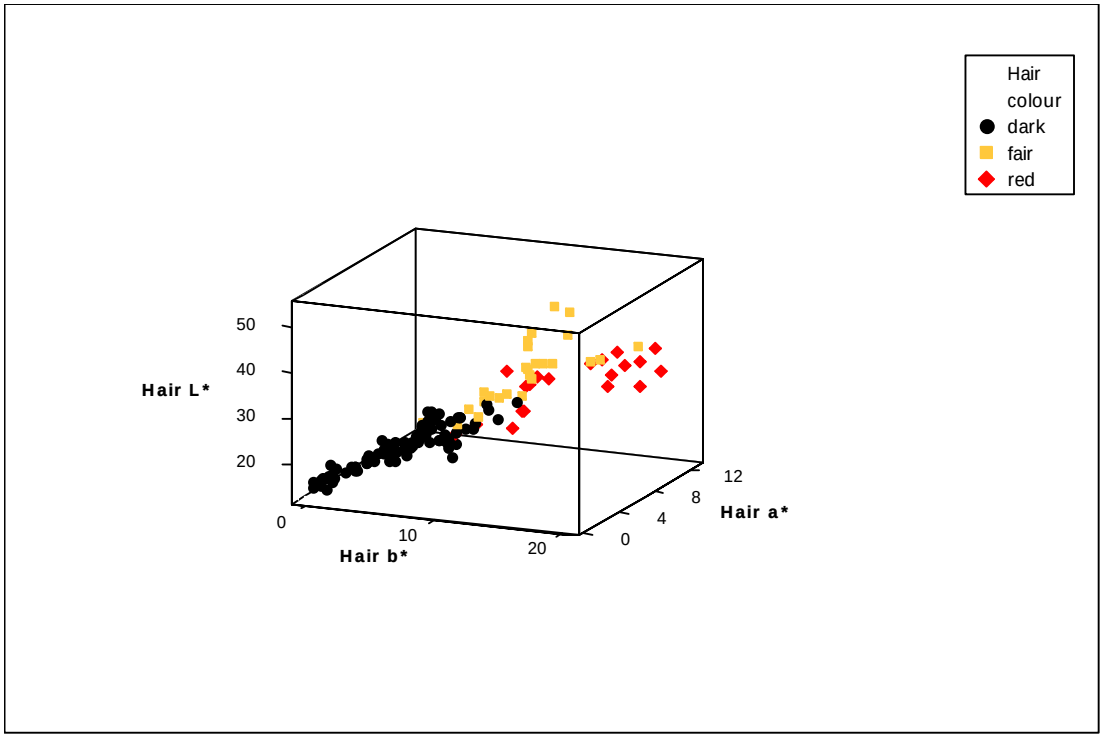
The range of hair colour L* was 13.92 (darkest) to 53.2 (lightest). Dark hair volunteers had a mean L*, a*, b* of 21.92 ± 0.45, 3.13 ± 0.15, 5.05 ± 0.31 respectively. Fair hair volunteers had a mean L*, a*, b* of 34.33 ± 2.13, 5.23 ± 0.41, 12.47 ± 1.35 respectively. Blonde hair volunteers had a mean L*, a*, b* of 39.67 ±

1.66, 5.26 ± 0.17, 13.62 ± 0.63 respectively. Red hair volunteers had a mean L*, a*, b* of 32.83 ± 1.21, 8.00 ± 0.51, 14.76 ± 0.78 respectively.

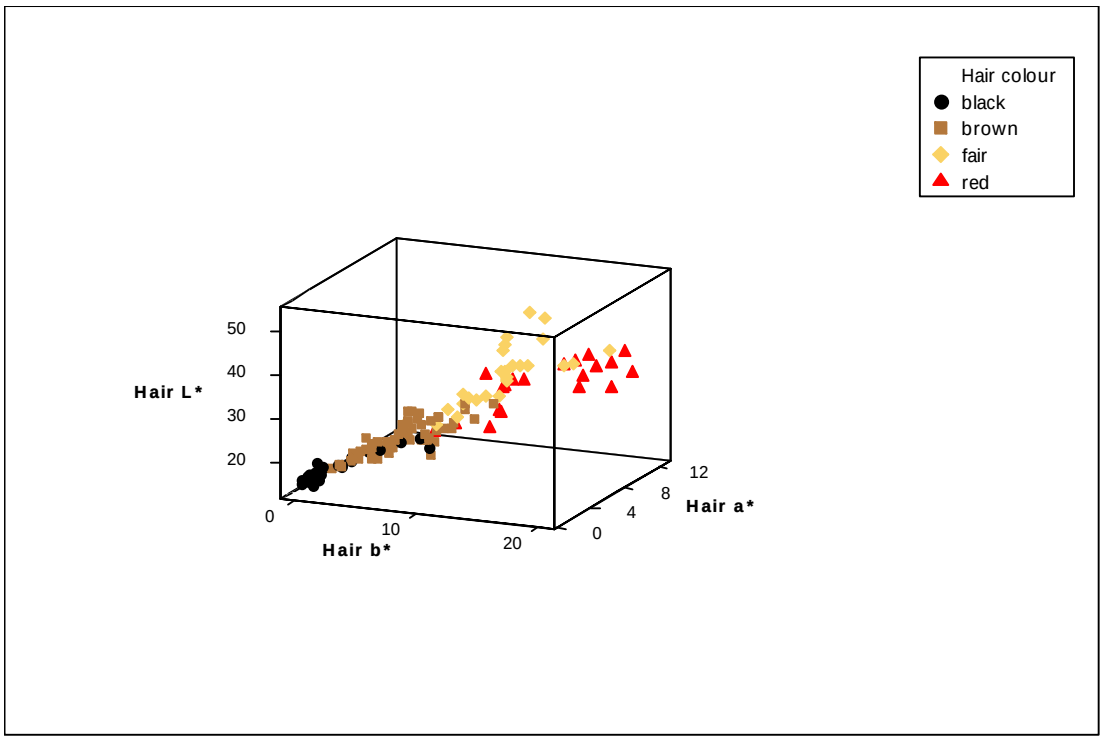
3.2.13. Hair colour groups

Hair colour was divided into 3 groups – Dark, fair and red. Dark hair colour was further subdivided into black and brown. Blonde hair colour will be discussed in Chapter 8.

Figure 3.2.13. 3D scatter plot of hair colour groups



From the above 3D scatter plot, there was clear clustering of the hair colour groups. Dark hair colour group was further subdivided into brown and black hair colour and 3D scatter plot redrawn.



This showed clear clustering of the 4 hair colour groups.

3.2.14. Hair dye

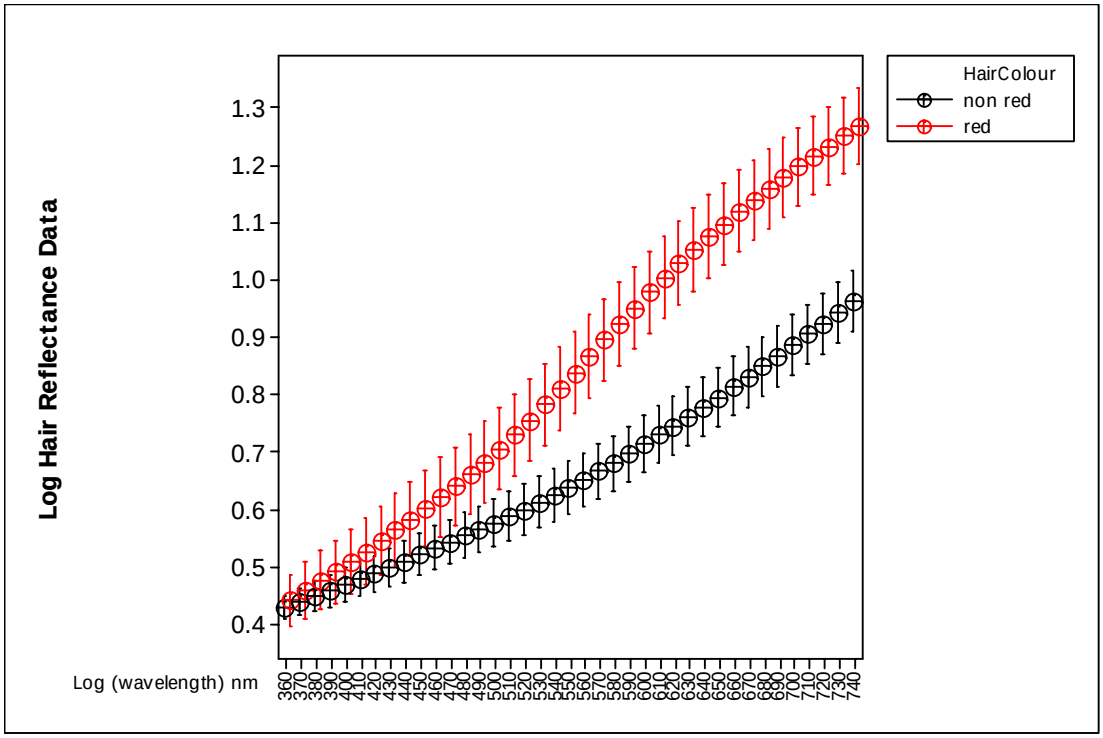
26 volunteers had highlighted their hair between 1 month to 2 years prior to the study. These have been excluded in the analysis. The remaining 133 volunteers who had natural hair colour were included in the analysis.

Hair Dye	No.
dyed	26
no dye	133

3.2.15. Hair colour spectrophotometry and reported / observed hair colour

Next the objective quantitatively measured hair spectrophotometric reflectance was compared to the categorical reported / observed hair colour from volunteers. Initially a log reflectance graph of red versus non-red individuals was plotted.

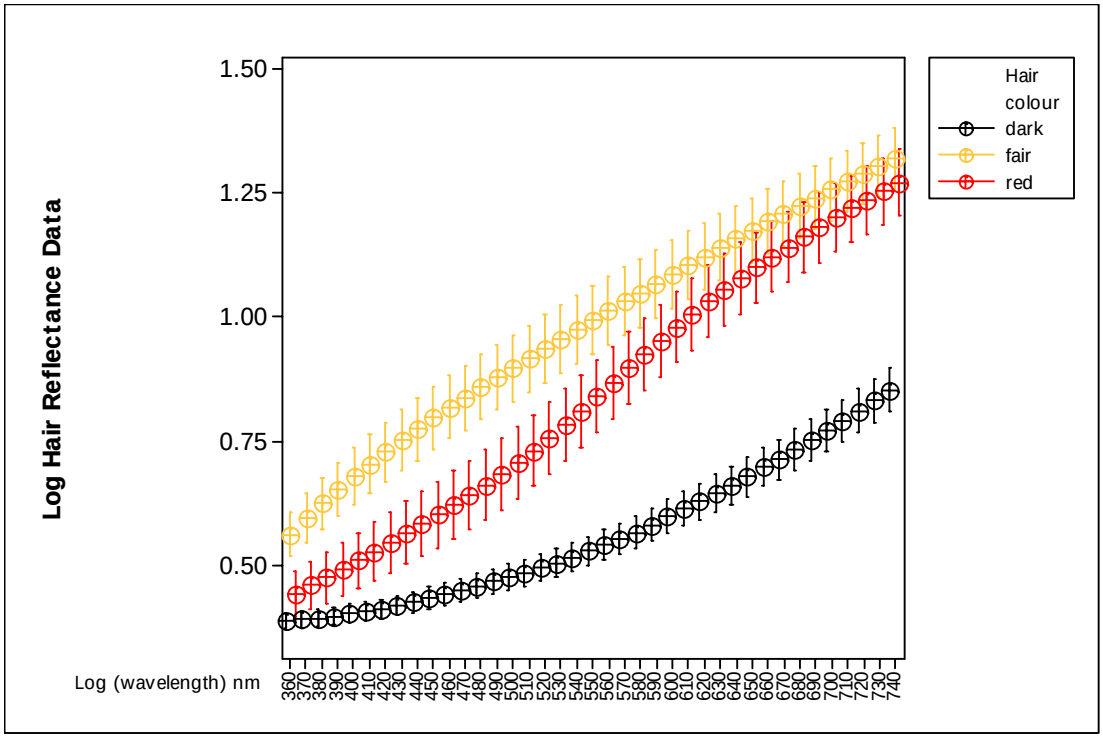
Figure 3.2.15.1. Hair colour spectrophotometric reflectance and red hair



This is an interval plot of log hair spectrophotometric reflectance ± SEM (95% CI for the mean). Y-axis shows the log spectrophotometric reflectance data. X-axis shows the 10nm increments of 360-740nm wavelengths. Red hair (red), non-red hair (black).

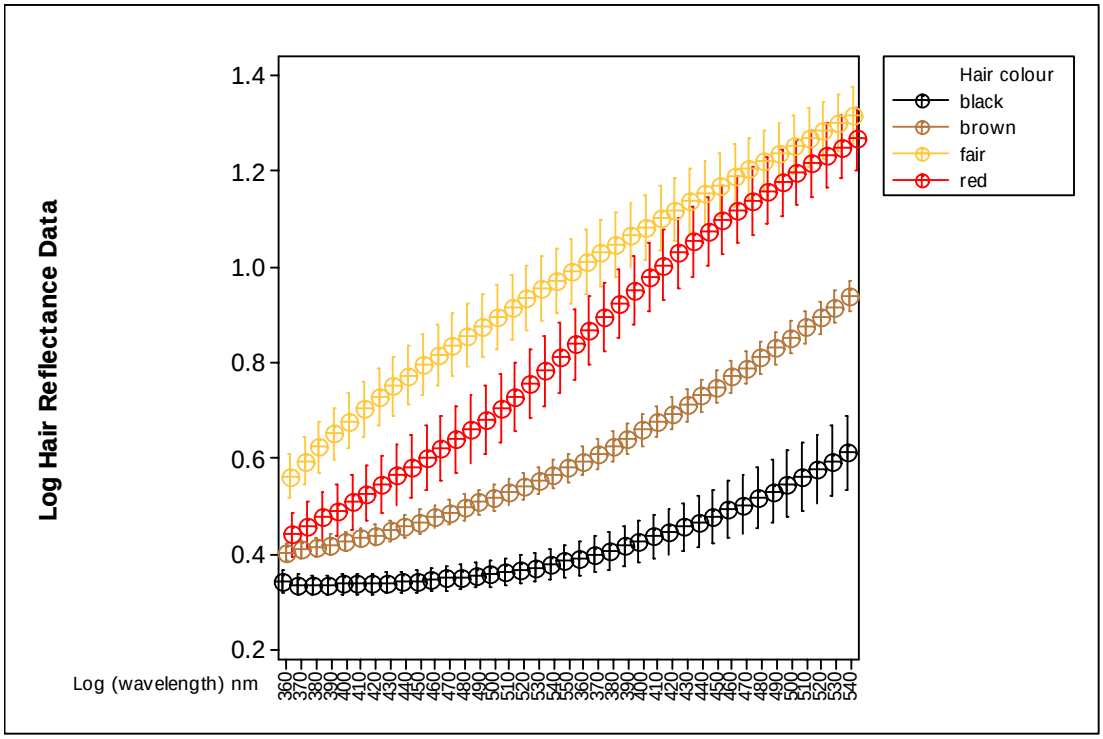
There was a kink in the graph at around log(600nm) for the red haired individuals. Were the reds more special in terms of the reflectance? Next the reported hair colour of individuals were categorised into dark (brown, black), fair (fair, blonde) and red. The measured hair reflectance was plotted according to their reported hair colour groups (dark, fair and red).

Figure 3.2.15.2. Hair colour spectrophotometric reflectance and hair groups



What if dark hair colour was subdivided into brown and black categories?

Figure 3.2.15.3. Hair colour spectrophotometric reflectance and hair groups



The hair colour groups were clearly distinguishable spectrophotometrically. Principle component analysis technique (personal communication, Professor J. L. Rees) may be useful in analysing the data.

3.2.16. Skin colour variation study

The objective of this preliminary study was to investigate whether there was a day-to-day variation on skin colour measurements at various body sites using objective means of measurement i.e. do skin measurements change from day to day?

12 body sites were measured to obtain L* a* b* tristimulus colorimetry and spectrophotometric data:

Left and right forehead (2cm superiorly from supraorbital ridge)

Left and right temple (3cm laterally from supraorbital ridge)

Left and right inner arm (10cm superiorly from medial epicondyle)

Left and right dorsal hand (3cm proximally from 3rd proximal interphalangeal joint)

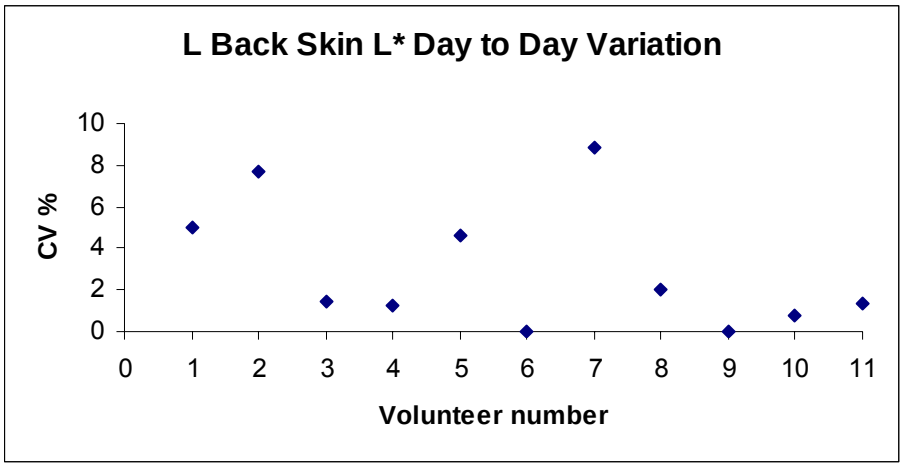
Left and right lower back (8cm superolaterally from coccyx)

Left and right buttock (8cm inferomedially from posterior superior iliac spine)

Triplicate measures were obtained for each site (total of 72 datasets per person per day). Data were acquired for 11 volunteers for 3 different days within immediate 2 weeks.

3.2.17. Skin colour day to day variation study results

Figure 3.2.17. Skin colour day to day variation results

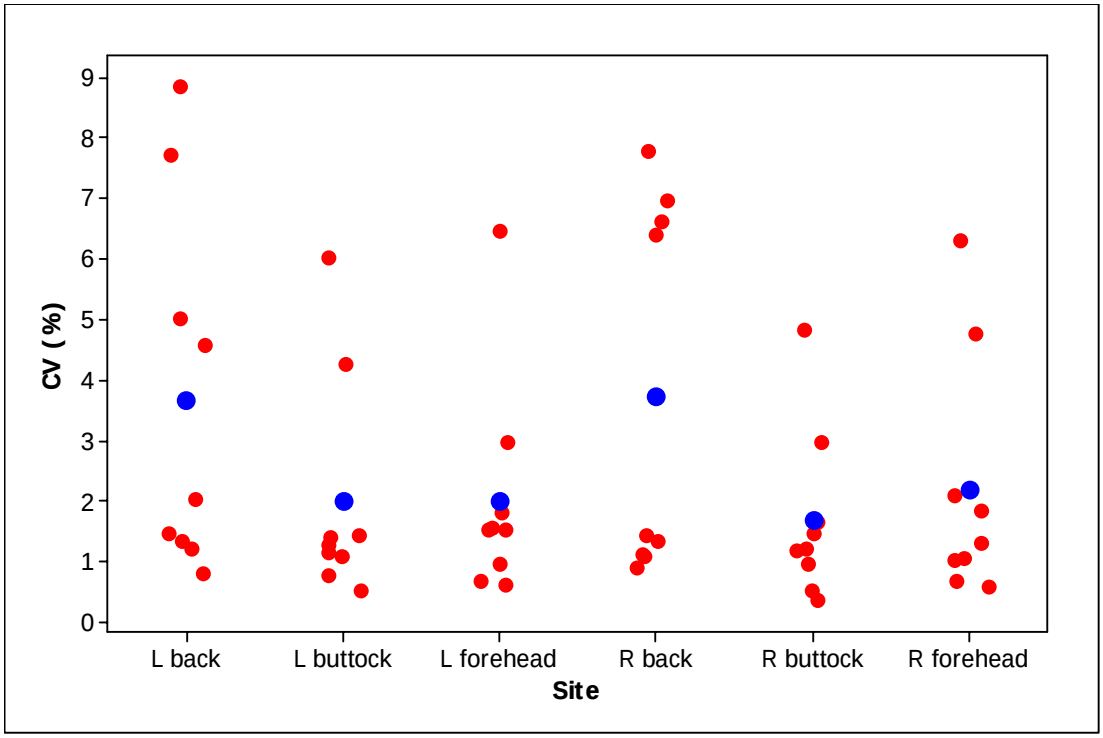


Coefficient of variation was <9% for 11 individuals measured on 3 different days within 2 weeks at left back. Other sites showed similar coefficient of variation.

One-way ANOVA for measured L* value at Left back on 3 different days showed no significant difference between the means (P=0.93). There was no significant day-to-day variation in the skin measurements of L*, a* and b* by reflectance spectrophotometry.

3.2.18. Skin colour reproducibility

Figure 3.2.18. Skin colour reproducibility



Blue marker = mean

Random sites chosen from both photo protected and photo exposed skin sites. Average coefficient of variation was <4%.

3.2.19. Skin colour results

The plotting of some of the variables of particular interest, such as L* a* b* characteristics show that the distributions were not always normal. In particular, some of the distributions showed a negative skew. Attempts to correct this, using reflection together with adding a constant and taking logs, were not successful. Because the characterisation of the initial data set was meant to confirm previous

work, it was therefore felt more appropriate to use non-parametric methods. Therefore, throughout this chapter, medians were usually quoted rather than means (although in most cases the means and medians were very close) and comparisons between groups were made using the non-parametric tests such as the Kruskal-Wallis analysis of variance or the Wilcoxon signed rank test.

For most of this chapter the L* score of the L* a* b* reading was used as a phenotypic marker of skin colour. Differences in the other measures of the L* a* b* score were seen but the L* score has been used commonly in previous work (Han *et al.*, 2006; Oh *et al.*, 2004; Wei *et al.*, 2007). As expected, median L* scores varied between body site and this body site variation was maintained by sex and by ethnic group. There did not appear to be any major interactions between these variables. Tables of the relevant median were shown (Tables 3.2.19.1-4).

Table 3.2.19.1. Median skin L* score for left versus right side

Left	Right
65.76	65.64

Table 3.2.19.2. Median skin L* score for 11 anatomical skin sites

Abdomen	68.20
Back	68.42
Buttock	71.39
Calf	65.33
Cheek	63.62
Chest	66.76
Forearm	62.09
Forehead	62.31
Hand	63.03
Thigh	66.01
Upper inner arm	69.15

Table 3.2.19.3. Median skin L* score for 11 anatomical skin sites by sex

	F	M
Abdomen	68.72	65.91
Back	68.74	65.92
Buttock	71.50	70.97
Calf	65.94	61.97
Cheek	64.24	61.17
Chest	67.17	64.70

Forearm	62.51	59.08
Forehead	63.02	59.79
Hand	63.61	60.59
Thigh	66.81	64.24
Upper inner arm	69.04	69.46

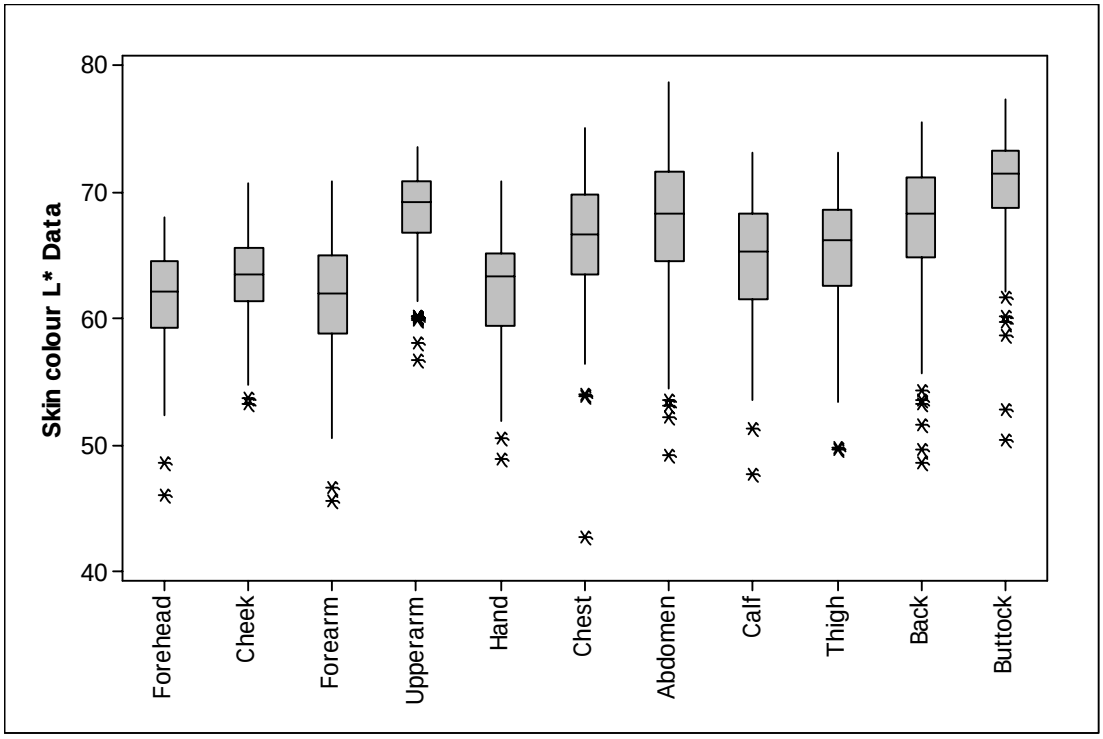
Females have lighter skin colour (i.e. higher L*) than males at all 11 anatomical skin sites. This is consistent with previous literature (Jablonski and Chaplin, 2000; Robins, 1991). One possible reason for this is that females require more calcium during pregnancy and lactation, therefore the lighter skin colour permits more UV light to penetrate the skin for vitamin D synthesis (Jablonski and Chaplin, 2000).

Table 3.2.19.4. Median skin L* score for 11 anatomical skin sites by ethnicity

	Asian	Mediterranean	Northern European
Abdomen	61.37	66.21	68.86
Back	62.28	66.20	69.16
Buttock	66.25	71.59	71.68
Calf	62.88	64.94	65.59
Cheek	59.88	62.55	63.96
Chest	60.97	65.70	67.19
Forearm	57.71	61.30	62.32
Forehead	54.67	59.68	62.92
Hand	56.40	62.22	63.56
Thigh	61.06	64.41	66.78
Upper inner arm	65.62	69.06	69.41

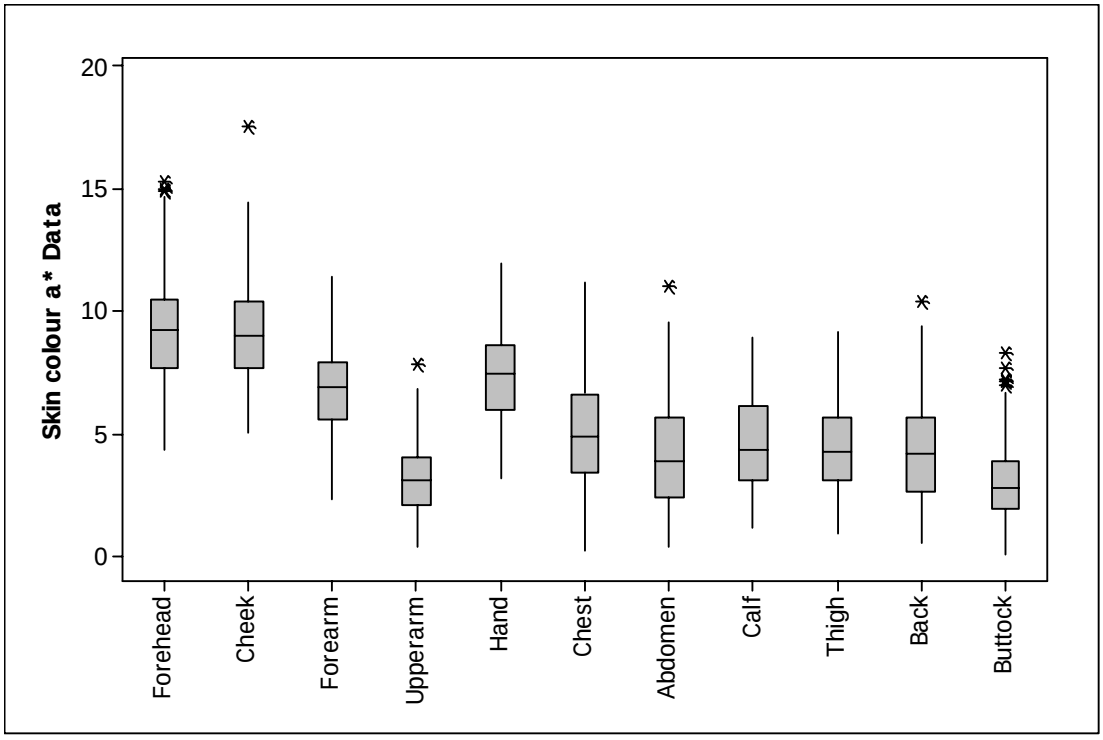
3.2.20. Skin colour range and site

Figure 3.2.20.a. Skin colour L* 11 anatomical sites n=159



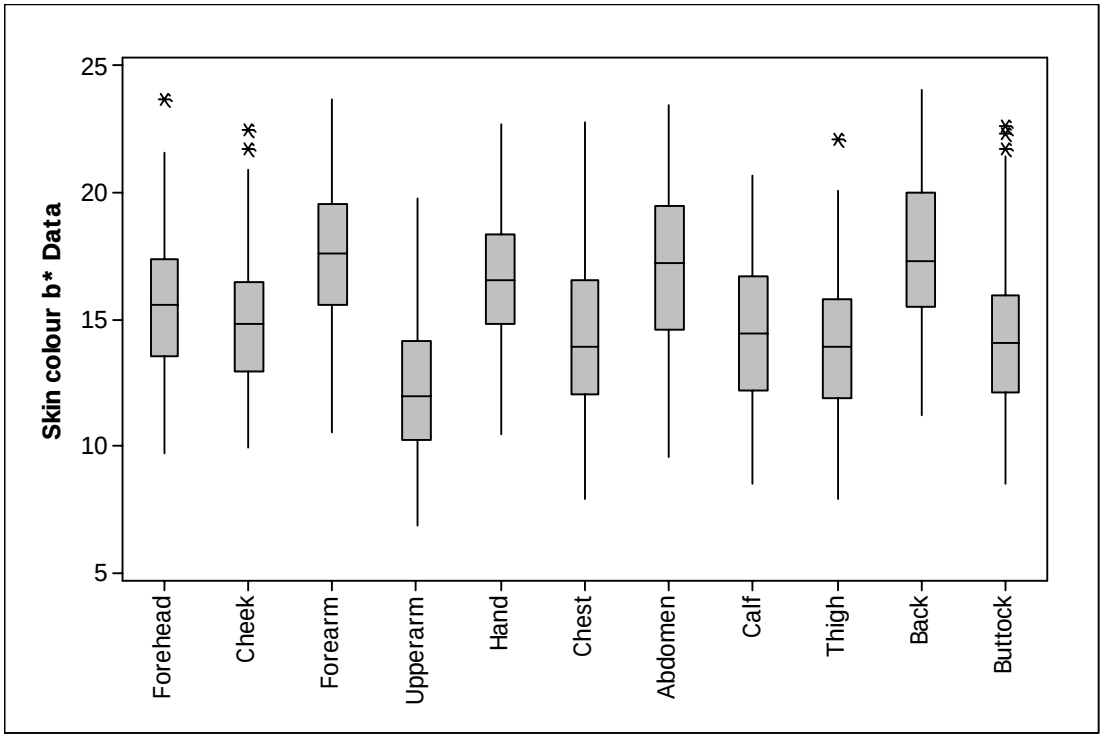
This is a boxplot of the skin colour L*. Median ± interquartile range and outliers.

Figure 3.2.20.b. Skin colour a* 11 anatomical sites n=159



This is a boxplot of the skin colour a*. Median ± interquartile range and outliers.

Figure 3.2.20.c. Skin colour b* 11 anatomical sites n=159



This is a boxplot of the skin colour b*. Median ± interquartile range and outliers.

Table 3.2.20. show the average skin colour L* (mean ± SEM) for 11 sites

Range of skin darkness – lightest L* value of 78.7; darkest L* value of 42.8.

The lightest skin areas by rank include buttock (L* = 70.6 ± 0.3), inner upper arm (L* = 68.6 ± 0.3), abdomen (L* = 67.4 ± 0.4). and back (L* = 67.3 ± 0.4). The sites are ranked by decreasing L* values i.e. increasing darkness:

Site	Mean L*	SEM
Buttock	70.59	0.33
Inner arm	68.62	0.26
Abdomen	67.41	0.44
Back	67.34	0.42
Chest	66.28	0.38
Thigh	65.3	0.38
Calf	64.55	0.38

Cheek	63.42	0.33
Hand	62.27	0.35
Forehead	61.77	0.32
Forearm	61.5	0.37

Colorimetric data a*, b* and spectrophotometric data from 22 skin areas were obtained and analysed.

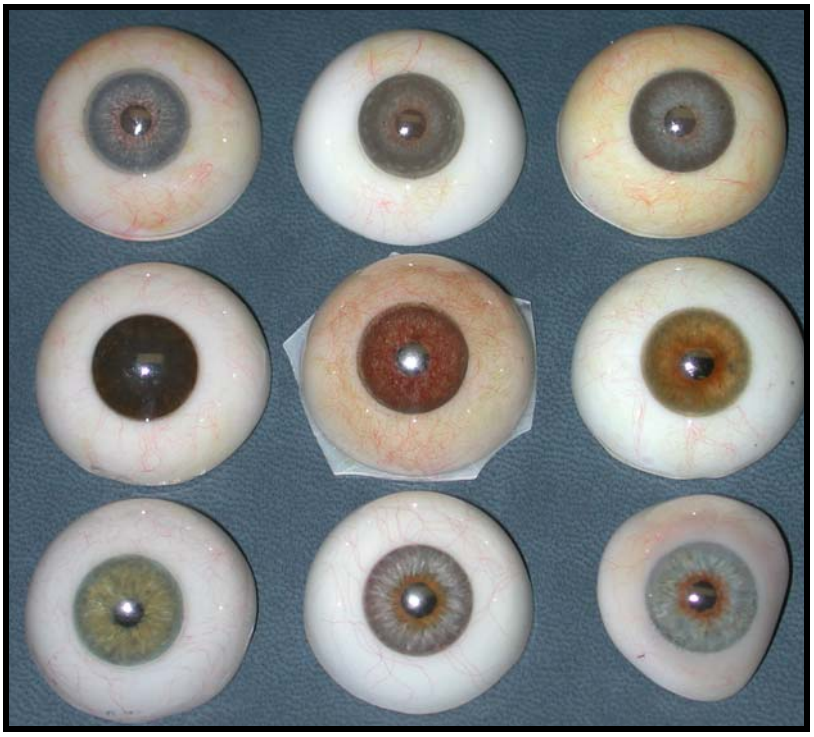
3.2.21. Eye colour preliminary study

The range of eye colours was first ascertained by going through around 1000 prosthetic eyes at the ophthalmology department. These were the prosthetic eyes used to match patients' remaining eye and used as eye prosthesis. The colour of the iris were matched using Munsell colour atlas with over 1500 colour plates under standardised daylight illumination (custom-made) fitted with two (2 feet) Philips F20W/54 Daylight fluorescent tubes (colour temperature 6500K). The observer, having spectacles removed, performed eye matching such that any variation in iris colours were blended to one uniform colour due to myopic vision.

3.2.22. Eye colour preliminary study results

In the preliminary eye colour study, the categories of eye colour have been selected and narrowed down by looking at around 1000 prosthetic eyes at the ophthalmology department, resulting in 9 typical prosthetic eyes that resemble real eyes colour ranges. These are as shown in Photo 3.2.22.1.

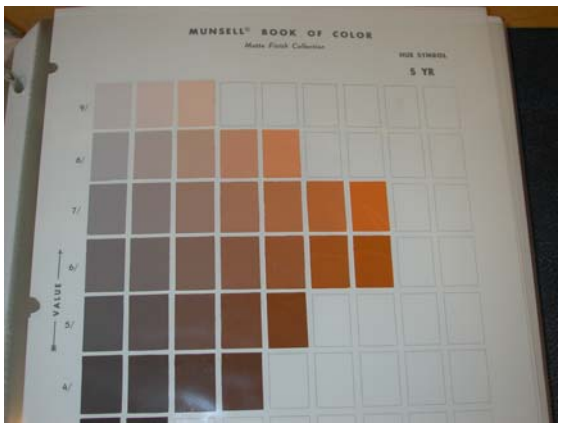
Photo 3.2.22.1. Typical prosthetic eyes resembling real eye colour ranges

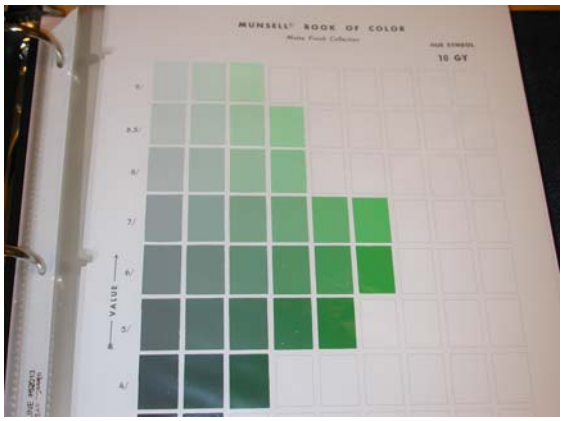


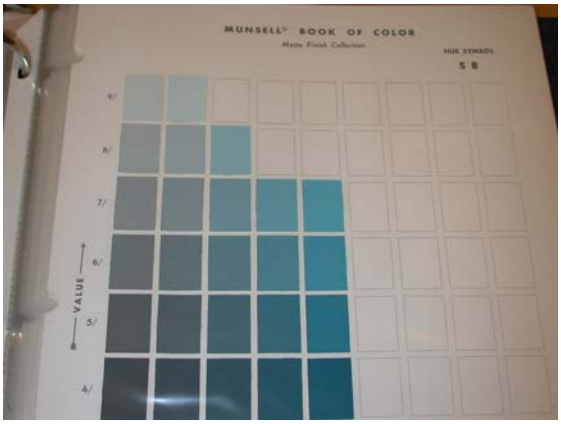
A photo showing 9 representative eye colours from prosthetic eyes.

The Munsell system of colour notation identifies colour in terms of three attributes: hue (H), value (V) and chroma (C). Hue notation of a colour indicates its relation to a visually equally spaced scale of 100 hues. Value notation indicates the lightness or darkness of a colour in relation to a neutral gray scale, which extends from absolute black to absolute white. The value symbol 0/ is used for absolute black, 10/ is absolute white. Chroma notation indicates the degree of saturation or degree of departure of a given hue from a neutral gray of the same value. The complete Munsell notation for a chromatic colour is represented symbolically: H V / C. Figure 12 shows typical colour plates from the Munsell colour atlas.

Photo 3.2.22.2. Munsell colour atlas







3.2.23. Eye colour – quantitative Munsell notation

The volunteers' matched eye colours were detailed in Table 3.2.23.

Table 3.2.23. Eye colours of volunteers by Munsell notation

5G 7/2	10B 7/6	10YR 6/8	5B 7/6	10Y 8.5/8	10B 7/4	10YR 6/8	2.5B 8/4
10BG 8/2	2.5B 9/2	10BG 8/2	10YR 6/8	2.5Y 7/10	5YR 5/8	10B 8/4	10YR 5/4
5B 9/2	5Y 7/8	10YR 6/8	5GY 8/4	10B 7/4	10YR 6/10	5Y 7/6	10B 7/6
10B 7/4	5BG 8/2	10B 6/4	10Y 8.5/8	10B 8/2	10B 7/4	10B 6/4	5B 8/4
10B 7/4	10YR 7/6	7.5YR 4/4	10B 6/6	7.5YR 4/8	5YR 5/1	10YR 6/6	5GY 8/2
10YR 6/4	5YR 5/6	5GY 8.5/2	10Y 8.5/8	10Y 8/6	10YR 7/8	10GY 7/4	5GY 8/2
10B 7/4	10B 8/4	5B 7/4	10BG 7/4	10BG 7/2	7.5Y 8.5/8	10BG 7/4	10B 7/6
10YR 7/4	5Y 8/4	5GY 8.5/2	10YR 7/10	7.5Y 8.5/6	5B 9/2	10YR 6/8	5B 9/2
10YR 5/6	10YR 5/6	10YR 6/4	2.5Y 8/8	10B 9/2	5B 7/4	10B 6/6	10B 7/6
5YR 3/2	10Y 9/6	2.5Y 7/10	5Y 8/8	10B 7/4	5B 7/4	5Y 6/6	10Y 8/4
5Y 7/6	5B 7/4	10Y 8/8	10B 7/4	10YR 5/6	7.5B 9/2	10B 7/4	10B 6/6
10YR 7/8	5Y 7/8	10B 6/4	10B 6/4	10Y 8.5/6	10B 6/6	10YR 5/8	10B 8/4
5Y 7/6	10YR 5/8	10BG 7/4	5B 7/4	2.5Y 7/8	2.5Y 7/8	7.5B 7/4	5B 8/4
10YR 6/10	10YR 5/6	10YR 6/8	10YR 6/8	5GY 8/4	10BG 7/4	10B 6/6	5Y 7/8
5GY 8/4	10YR 5/4	5B 7/4	10YR 6/6	5GY 8.5/4	5Y 6/6	10YR 6/8	7.5YR 6/4
5B 9/2	10B 8/4	2.5Y 7/10	10YR 5/8	10BG 8/2	5B 7/4	10B 8/4	10YR 6/6

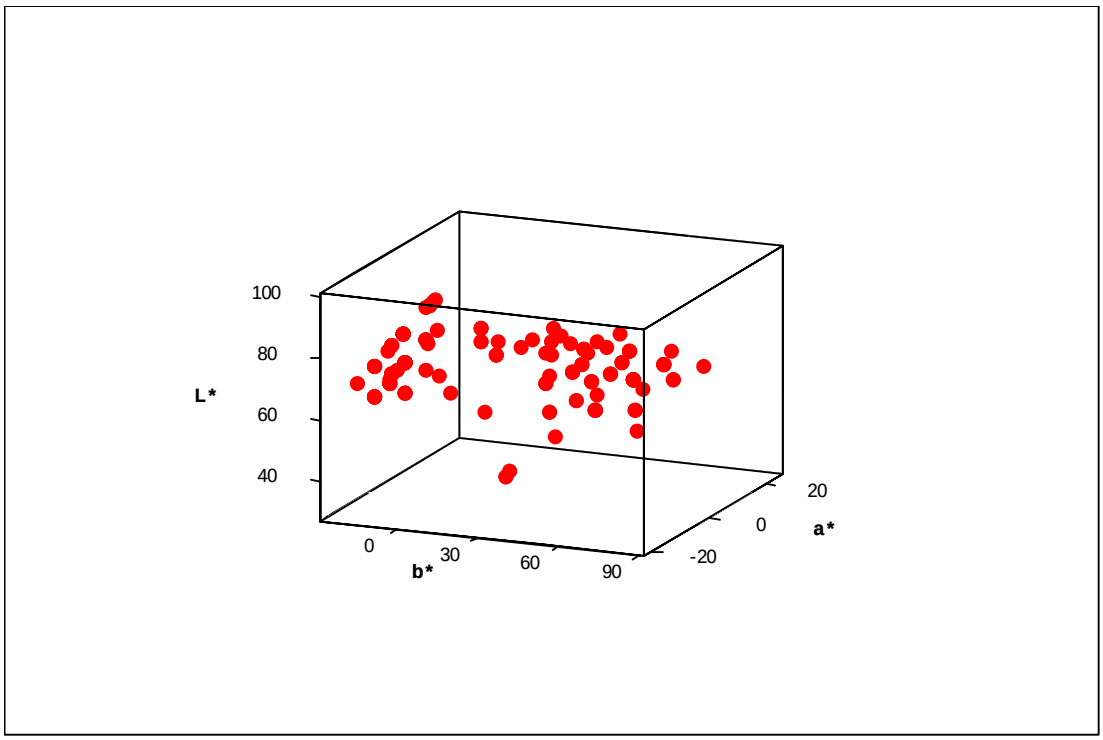
5Y 7/6	**10B 7/6**	**10YR 3/2**	**10B 8/4**	**10B 8/4**	**10B 6/6**	**2.5B 7/4**
2.5Y 7/12	**10B 7/4**	**10YR 3/2**	**5B 7/4**	**10B 8/4**	**10BG 8/2**	**10B 8/4**
2.5Y 7/10	**10YR 7/8**	**10Y 8/8**	**10YR 5/6**	**5Y 8/6**	**10YR 5/6**	**10B 7/4**

These show the matched eye colour and denoted in Munsell H V/C annotation where H=hue, V=value, C=chroma (n=149).

Each of these annotations correspond to a colour plate with specific L* a* b* colorimetric values. These Munsell colour space notations (HVC) were converted to CIE L*, a* and b* colour space representations using Munsell Conversion Software version 8.0.1 (WallkillColor.com, http://livingstonmanor.net/Munsell/index2.htm) for quantitative analysis of eye colour.

3.2.24. Eye colour – quantitative L* a* b*

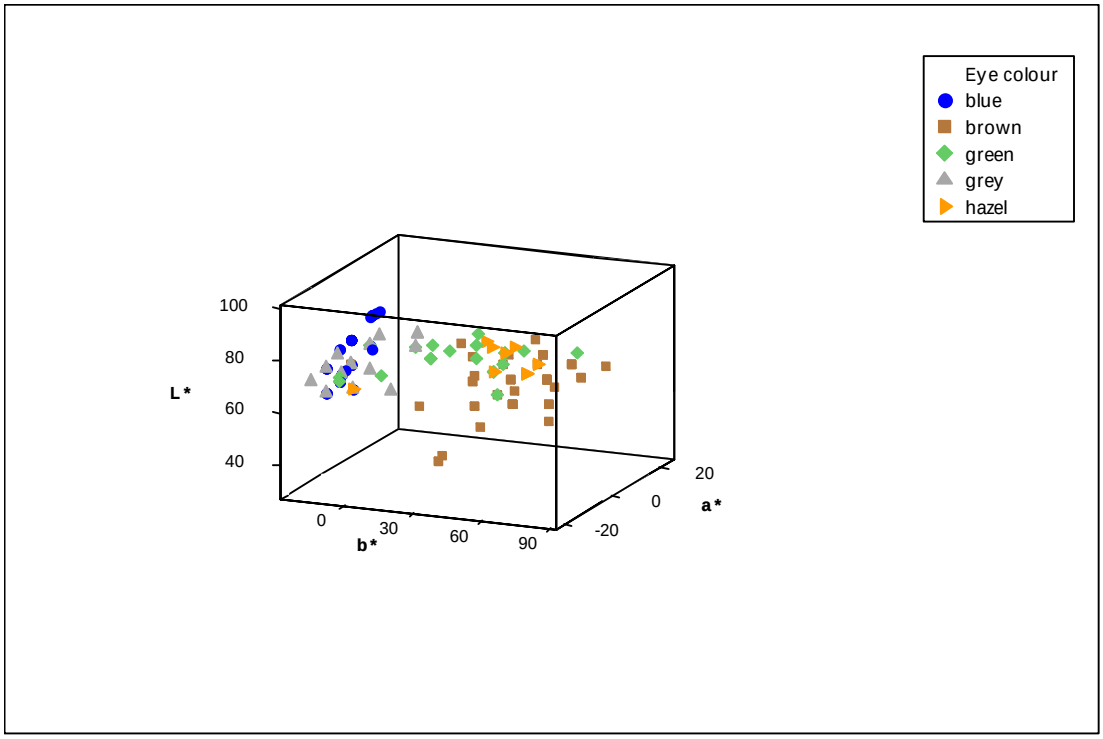
Figure 3.2.24. Eye colour distribution – quantitative L* a* b*



This is a scatterplot of the distribution of eye colours converted from Munsell HVC notation to L* a* and b* values (n=149).

3.2.25. Eye colour by eye colour groups (categorical)

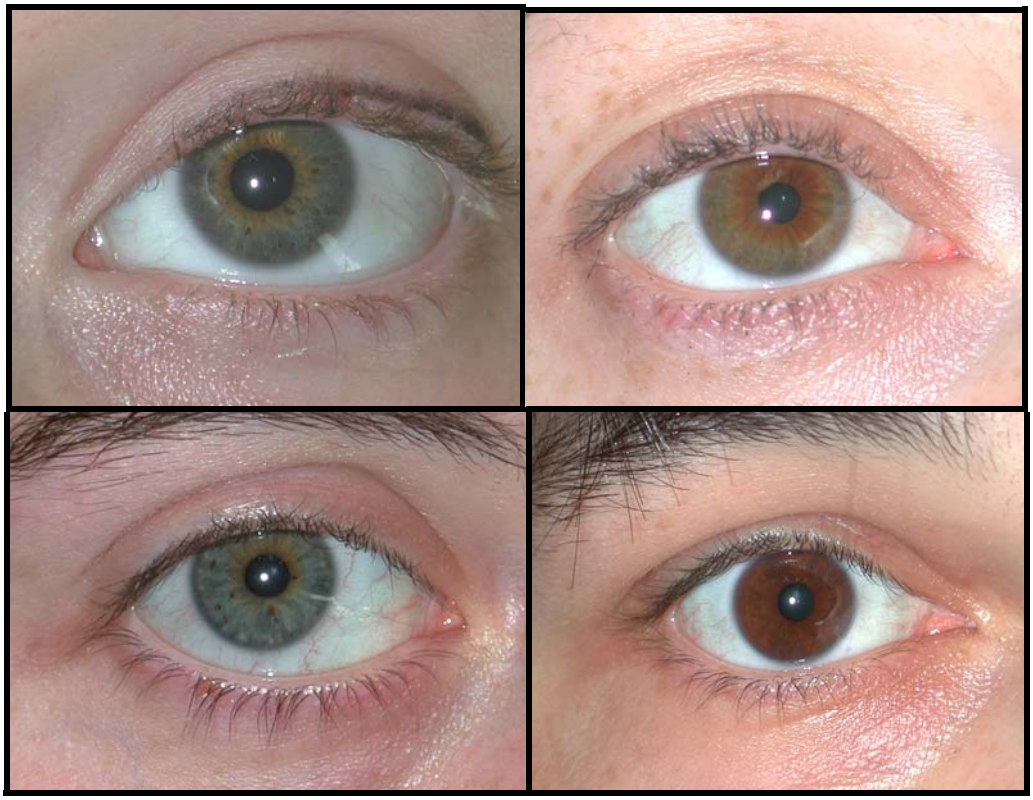
Figure 3.2.25. Eye colour (quantitative L* a* b*) by eye colour groups (categorical)



This is a scatterplot of the distribution of eye colours converted from Munsell HVC notation to quantitative L* a* and b* values by eye colour groups (blue, brown, green, grey, hazel) (n=149).

As demonstrated in the above figure, there was clear clustering for the eye colour groups. The Munsell colour system and the subsequent converted L* a* b* data for eye colour suggested that this might be a good and more appropriate method to consider and better way of performing comparisons in future eye colour genotype studies.

Photo 3.2.25. Iris photos from 4 individuals using Munsell colour matched method



These are iris photos from 4 individuals showing typical examples of Munsell colour matches of **5Y 7/8** (top left panel), **2.5Y 7/10** (top right panel), **10BG 8/2** (bottom left panel) and **10YR 5/8** (bottom right panel).

3.3. Analysis of key phenotypic variables by co-factors such as sex, freckling etc.

Because there was little evidence of interaction between body site in different groups, and for simplicity, the L* a* b* readings at different body sites were averaged and used as a summary variable in the following section. The alternative to this would have been to use a multi level model (e.g. split plot analysis of variance) which given the distribution of the data would have been difficult.

3.3.1. Skin colour L* a* b* and age

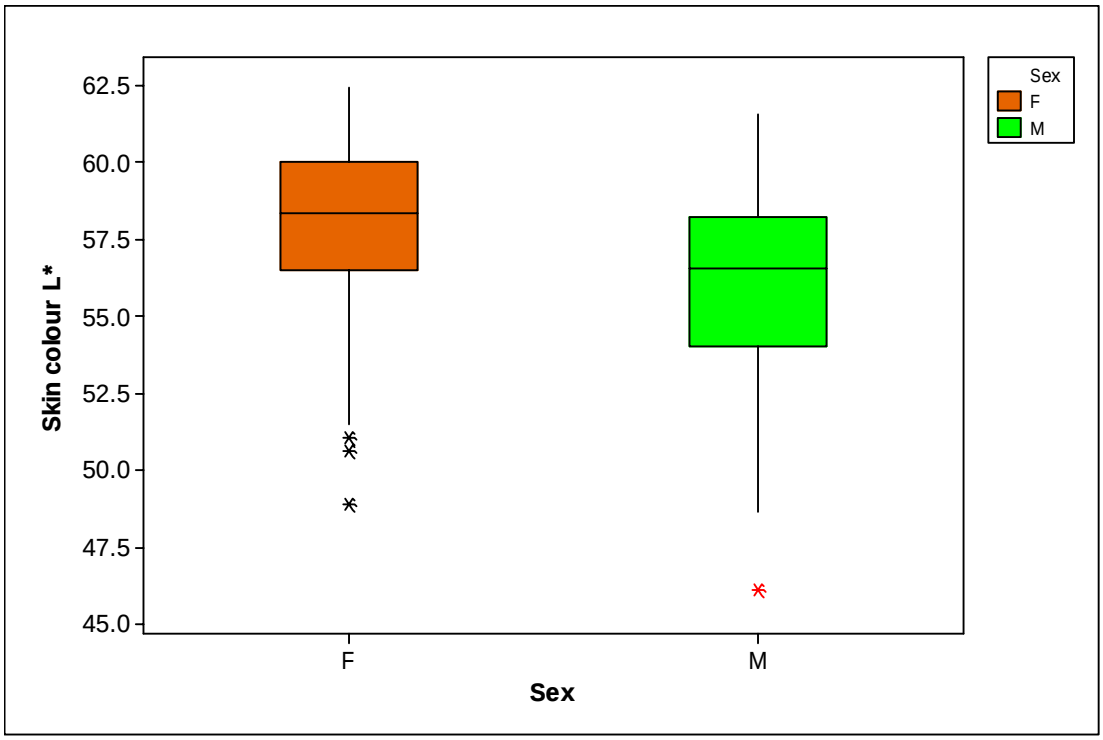
Plotting of age by L* a* b* score showed no relation. This was not surprising given the narrow range of ages studied and it will not be considered further. The Spearman's rank correlation coefficient $\rho = -0.055$ (P=0.4894) for skin colour L* and age.

A number of expected correlations between individual variables were found and the chief ones of these are shown in box plots.

3.3.2. Skin colour and sex

Is there a skin colour difference between males and females? The skin colour L* a* and b* were compared in males and females.

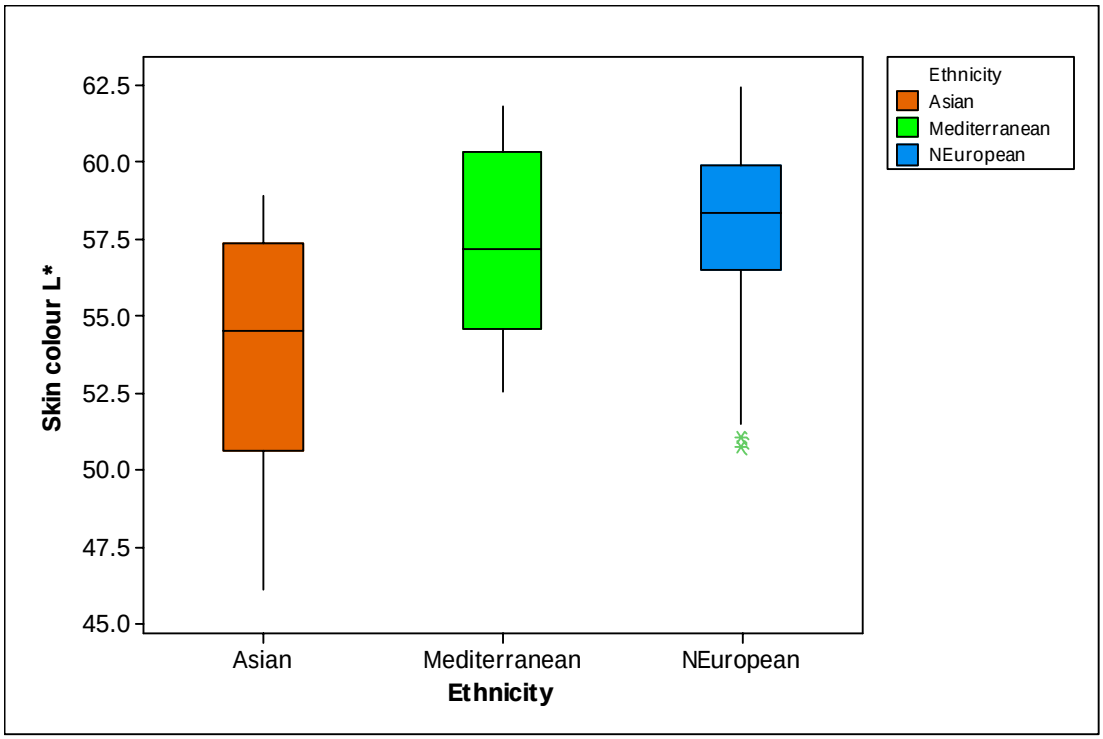
Figure 3.3.2. Mean skin colour L* score by sex



As shown in the above boxplot, females were lighter in skin colour (larger L* score) than males. A Kruskal-Wallis rank sum test showed significant difference between females and males (P=0.0002821) for skin L*.

3.3.3. Skin colour and ethnicity

Figure 3.3.3.a. Mean skin colour L* score by ethnic group



There was considerable overlap of skin colour between ethnic groups even for the same anatomical site. Skin colour trend was as expected. Asians were darkest, followed by Mediterraneans and Northern Europeans. Ethnicity was associated with / explained skin colour darkness/lightness. Kruskal-Wallis rank sum test showed significant difference between ethnic groups for skin colour L* ($P=1.413\times10^{-5}$).

Figure 3.3.3.b. 3D scatter plot of back skin colour L* a* b* by ethnicity

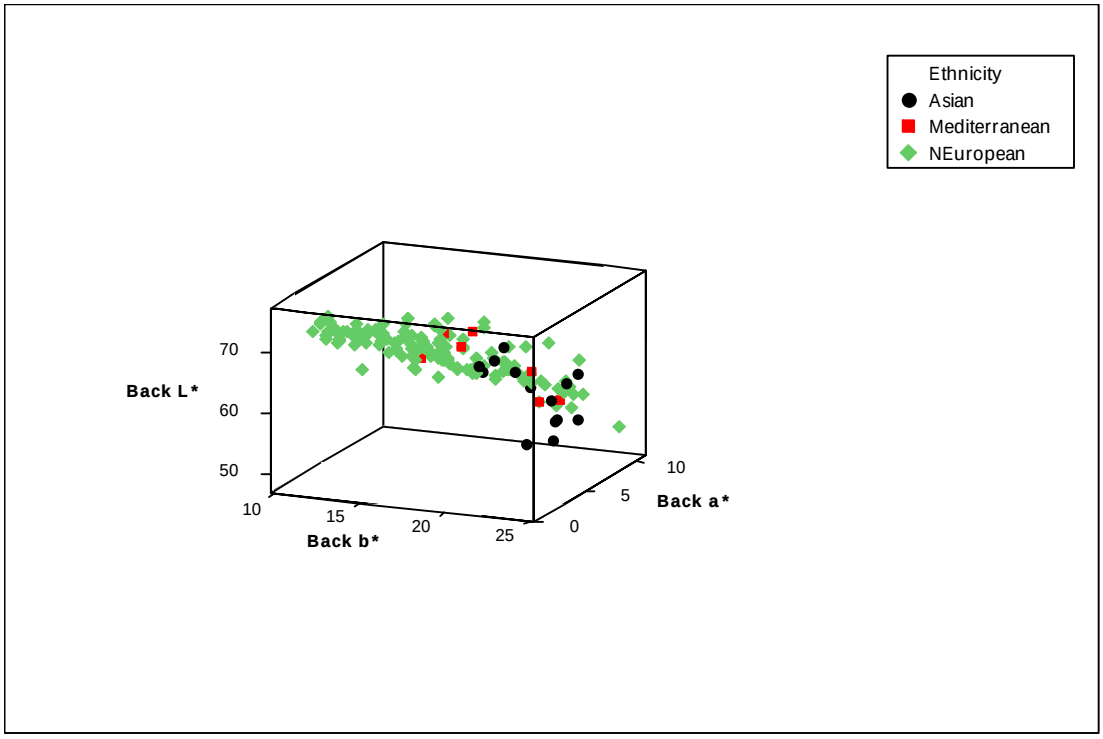
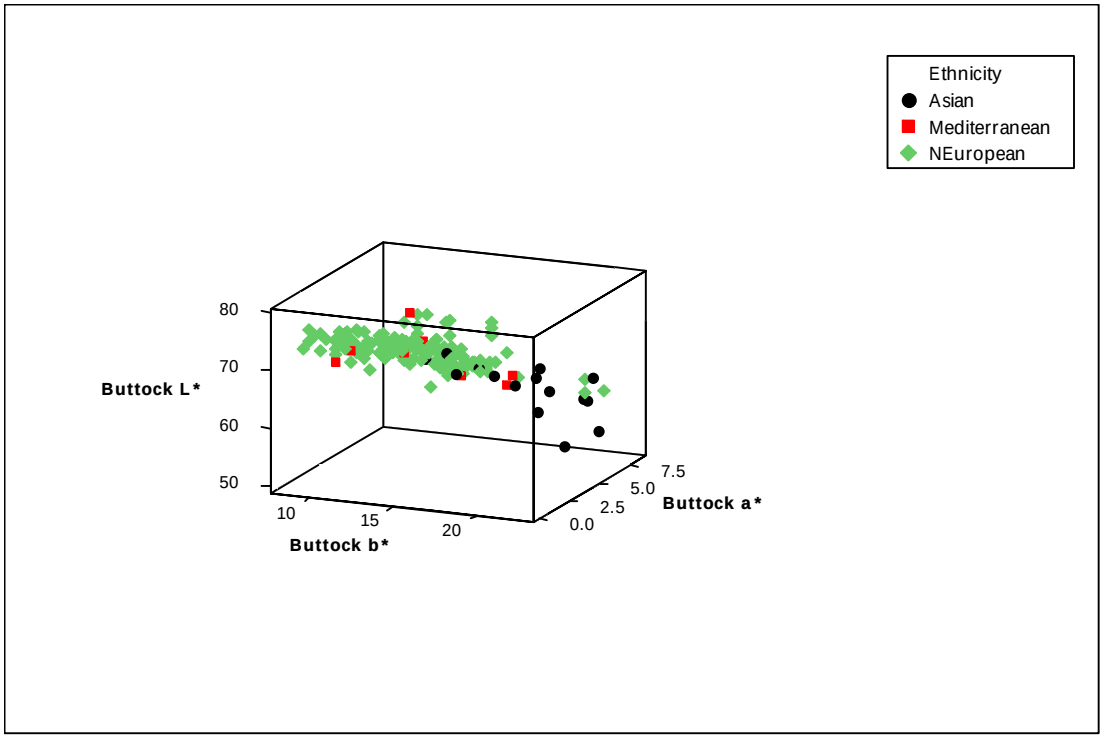


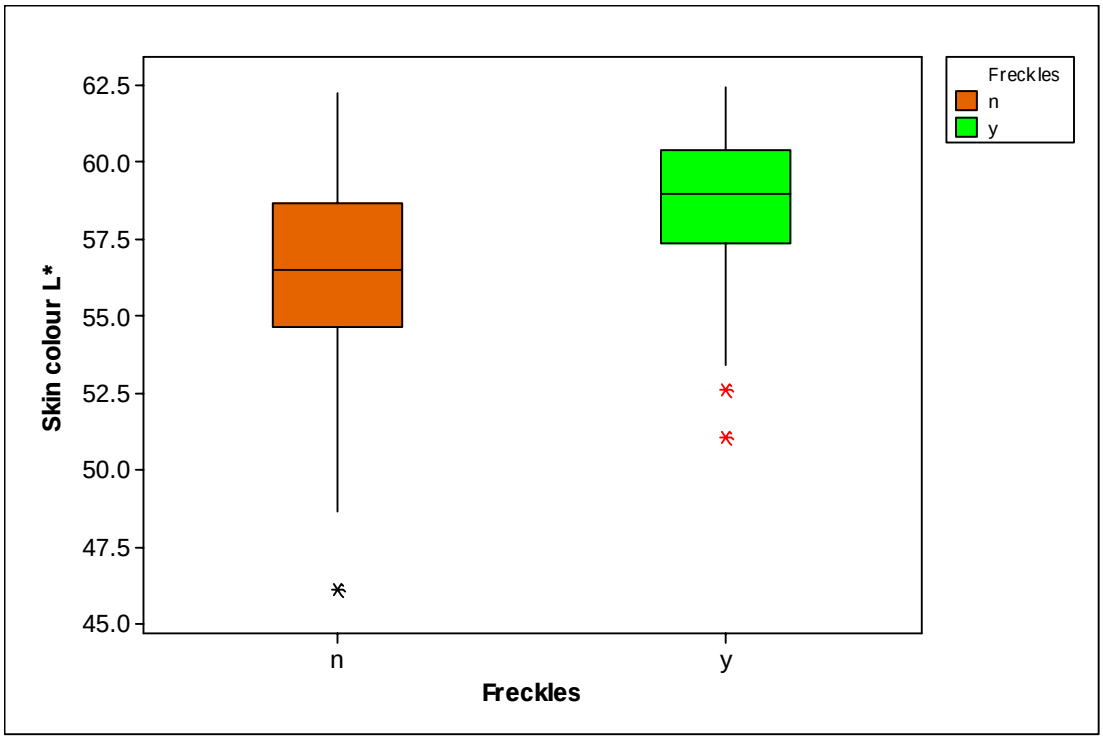
Figure 3.3.3.c. 3D scatter plot of buttock skin colour L* a* b* by ethnicity



There appeared to be clustering for ethnic groups.

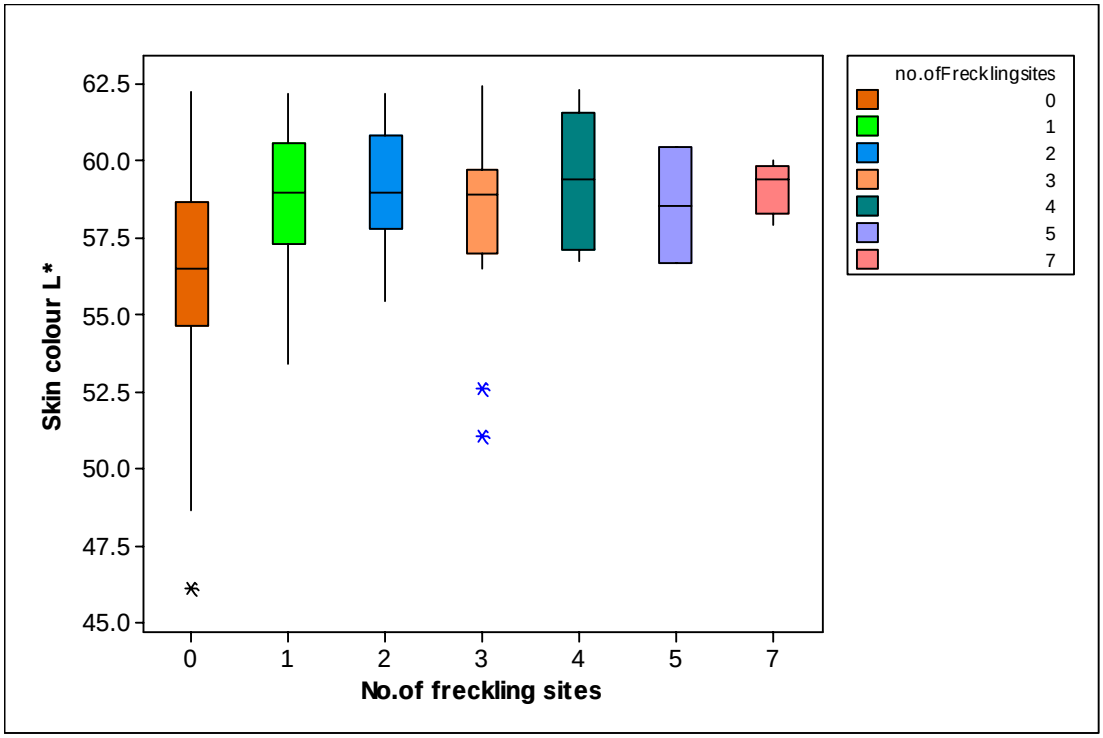
3.3.4. Skin colour and freckling

Figure 3.3.4.1. Mean skin colour L* score by presence of freckles



As expected, individuals with freckles have lighter skin colour. Kruskal-Wallis rank sum test showed significant difference between those with and without freckles ($P=7.603\times x10^{-9}$). Those with freckles have a mean L* of 58.82 ± 0.24 and those without a mean of 56.32 ± 0.35.

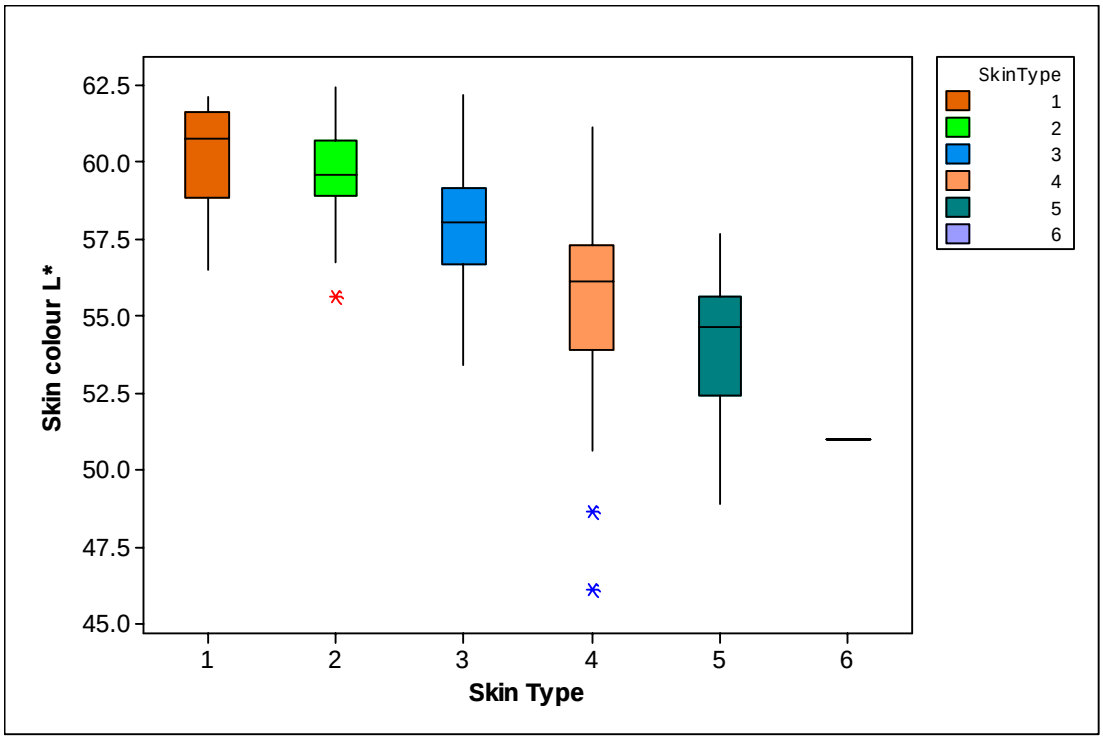
Figure 3.3.4.2. Mean skin colour L* score by number of freckling sites



Those with freckles were lighter. Those without freckles were darker. The number of freckling sites (1-7) did not affect skin colour L* beyond the presence of freckles.

3.3.5. Skin colour and skin type

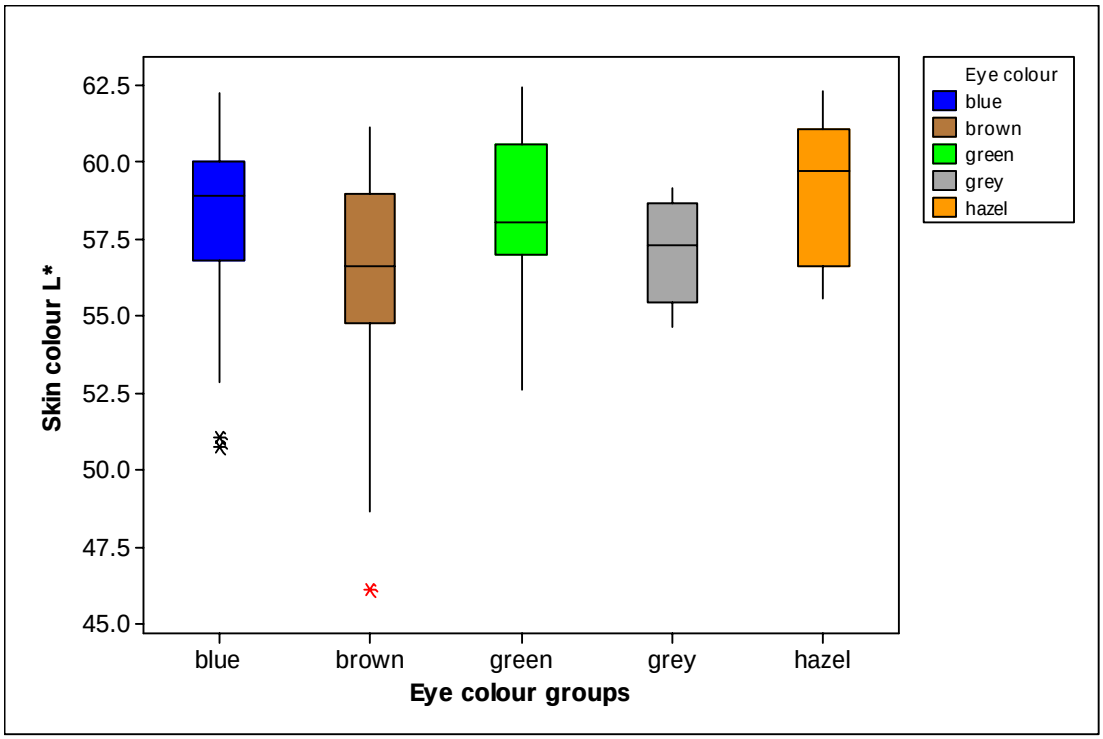
Figure 3.3.5. Mean skin colour L* score by skin type



Skin colour L* was associated with skin type. As expected, individuals with skin type 1 were lighter in skin colour. As one go from skin type 1 to 6, skin colour of individuals become darker. Kruskal-Wallis rank sum test showed significant difference between skin type and skin colour L* ($P=1.685\times10^{-14}$).

3.3.6. Skin colour and eye colour group

Figure 3.3.6. Mean skin colour L* score by eye colour group

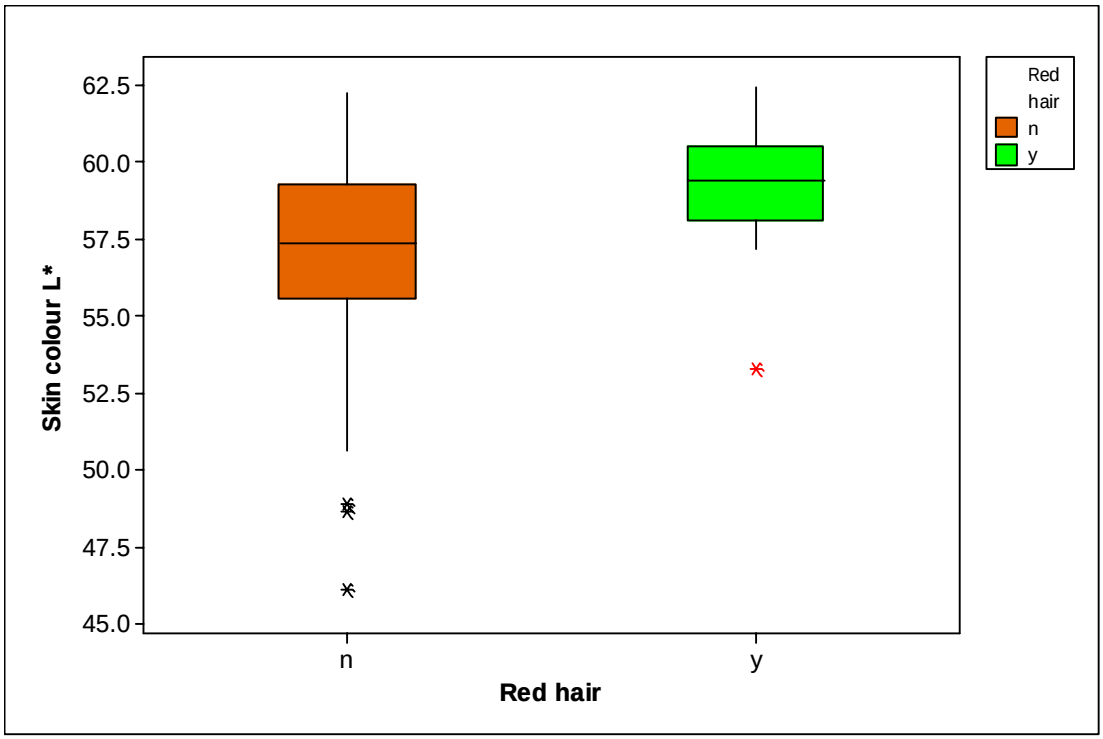


Skin colour L* a* b* were associated with eye colour. Individuals with blue and hazel eye colours had lighter skin L*. Kruskal-Wallis rank sum test showed significant difference with skin colour L* (P=0.0006244), a* (P=0.0008543) and b* ($P=1.369\times10^{-5}$) with eye colour.

Similar relations were found with other measures of skin colour such as mean a* and mean b* with eye colour, and some of the other variables (data not shown).

3.3.7. Skin colour and presence of red hair

Figure 3.3.7. Mean skin colour L* score by presence of red hair



Individuals with red hair have lighter skin colour. Kruskal-Wallis rank sum test showed significant difference in skin colour with presence of red hair (P=0.0003231).

3.3.8. Skin colour and hair colour

There was a subset of data (n=26) where individuals who have dyed their hair were excluded. There were marked correlations between skin colour L* a* b* readings and hair colour L* a* b* readings. A table summarising some of these correlations is shown (Table 3.3.8.). Lighter skin individuals have lighter hair colour also.

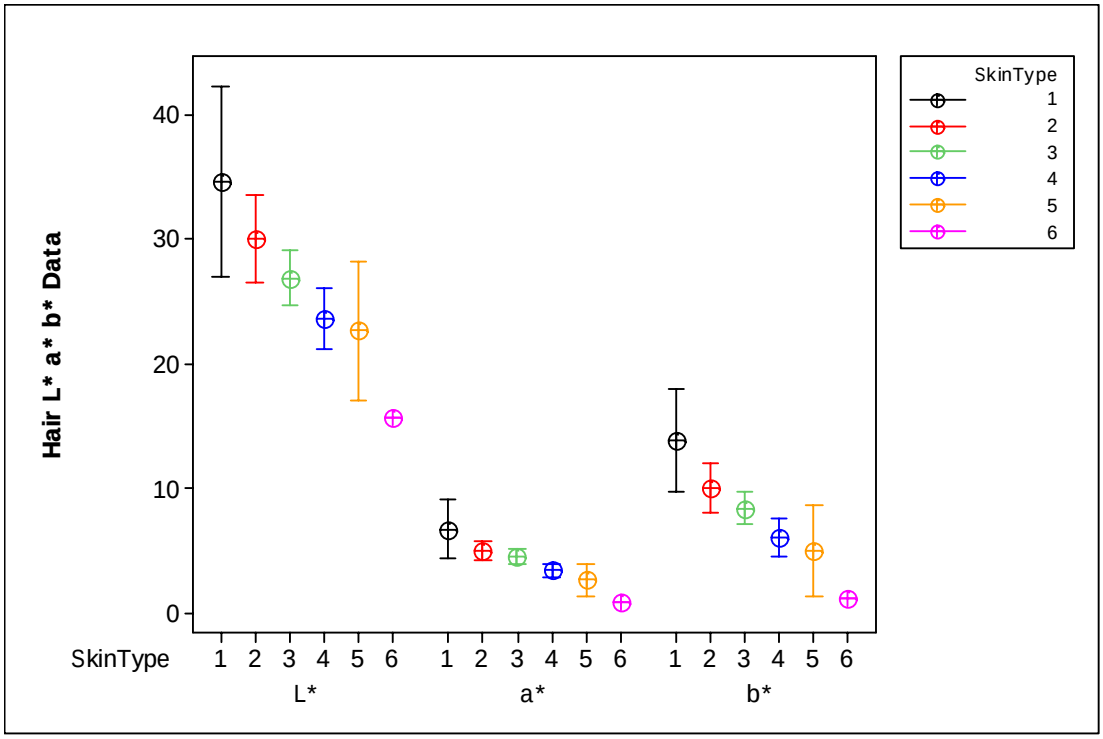
Table 3.3.8. Correlations between skin colour and hair colour

Correlations		Spearman's rank correlation coefficient rho	P
Mean skin L*	Hair L*	0.339	7.188×10^{-5}
Mean skin L*	Hair a*	0.370	1.15×10^{-5}
Mean skin L*	Hair b*	0.360	2.397×10^{-5}

3.3.9. Hair colour and skin type

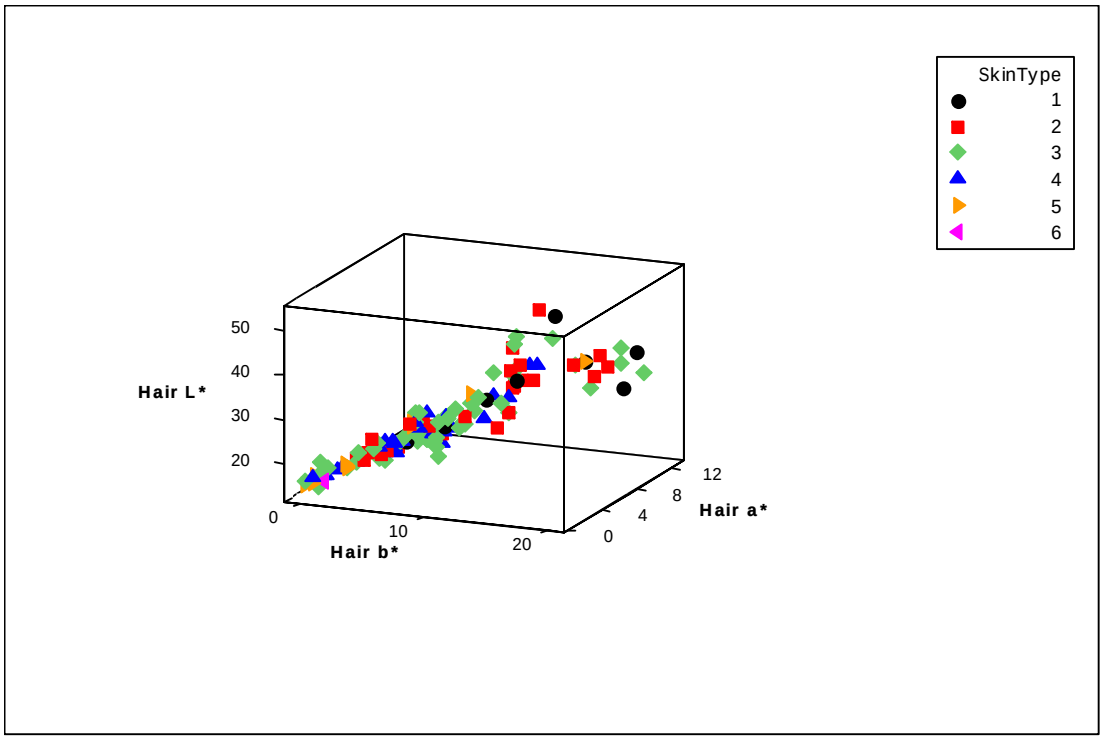
As could be expected, hair L* a* b* readings were also related to skin type.

Figure 3.3.9.a. Hair colour and skin type



Individuals with Skin type 1 had lighter hair L* a* b*. Kruskal-Wallis rank sum test showed significant difference with hair L* (P=0.00124), a* (P=0.0004855) and b* (P=0.0003409) with skin type.

Figure 3.3.9.b. 3D scatter plot of hair colour L* a* and b* with Fitzpatrick skin type



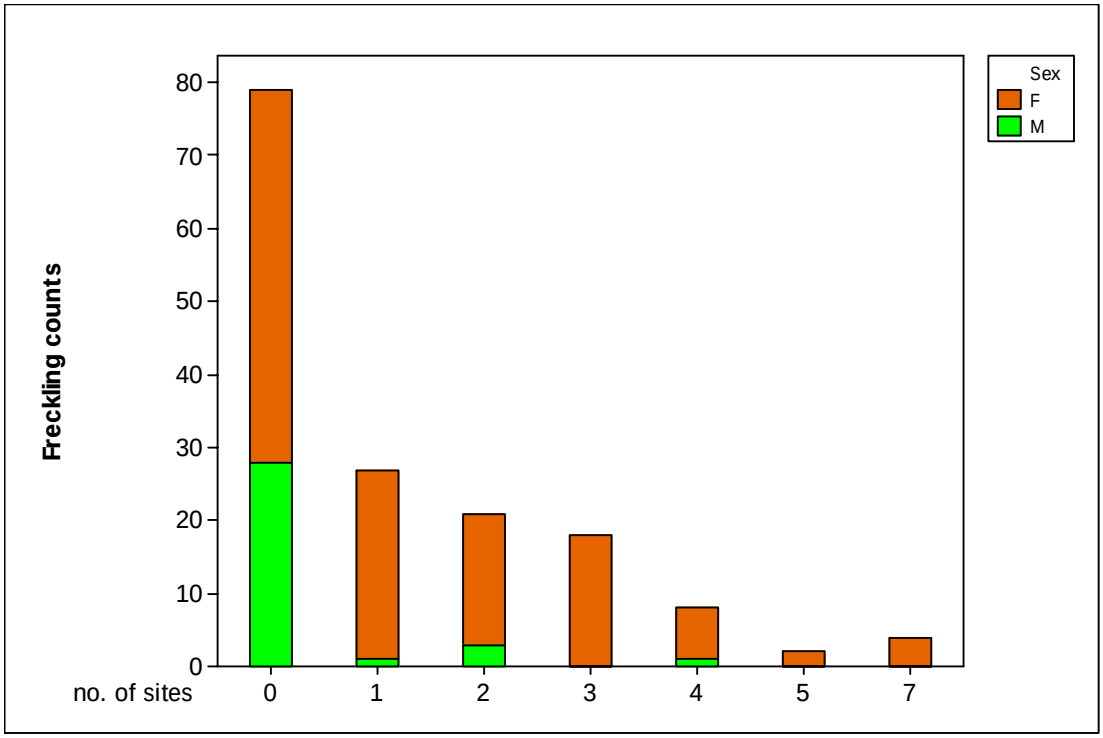
This is a 3D scatter plot of hair colour L* a* and b* with skin type (1-6).

3.3.10. Interactions between other phenotypic variables

3.3.11. Freckling and sex

Do males or females have more freckles? The number of freckling sites with sex for the Edinburgh population was plotted.

Figure 3.3.11.a. Freckling (number of sites) and sex



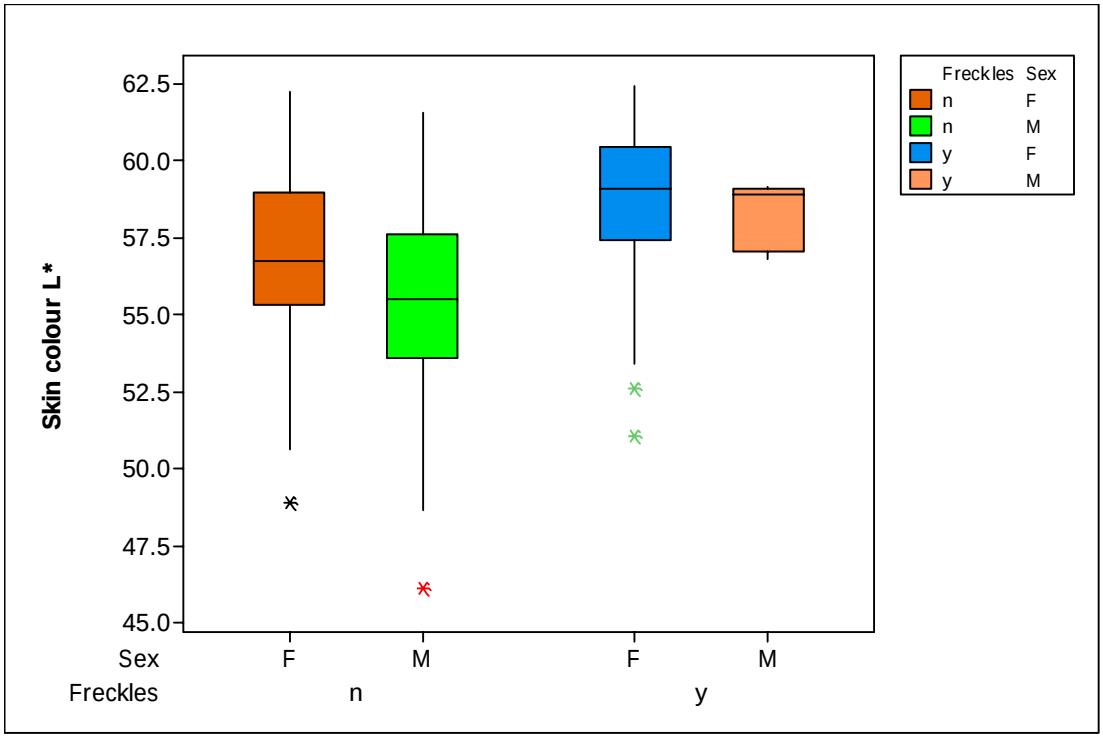
It seemed that females have more freckling sites than males. A Mann-Whitney Test was performed which compared the 2 medians for males and females and showed that females have significantly more freckling sites than males ($P<0.05$).

Table 3.3.11.b. The presence of freckling and sex

	F	M	All
No freckles	51	28	79
Freckles	75	5	80
All	126	33	159

There were more females (94%) with freckles than males (6%). Is freckling and sex related to paler skin in females?

Figure 3.3.11.b. Skin colour L*, presence of freckling and sex



This showed that freckling is the major determinant of paler skin colour, rather than sex. It is also possible that freckling could be related to previous sun exposure and differences in behavioural patterns in males and females may contribute to the difference between sex.

3.3.12. Freckling and age

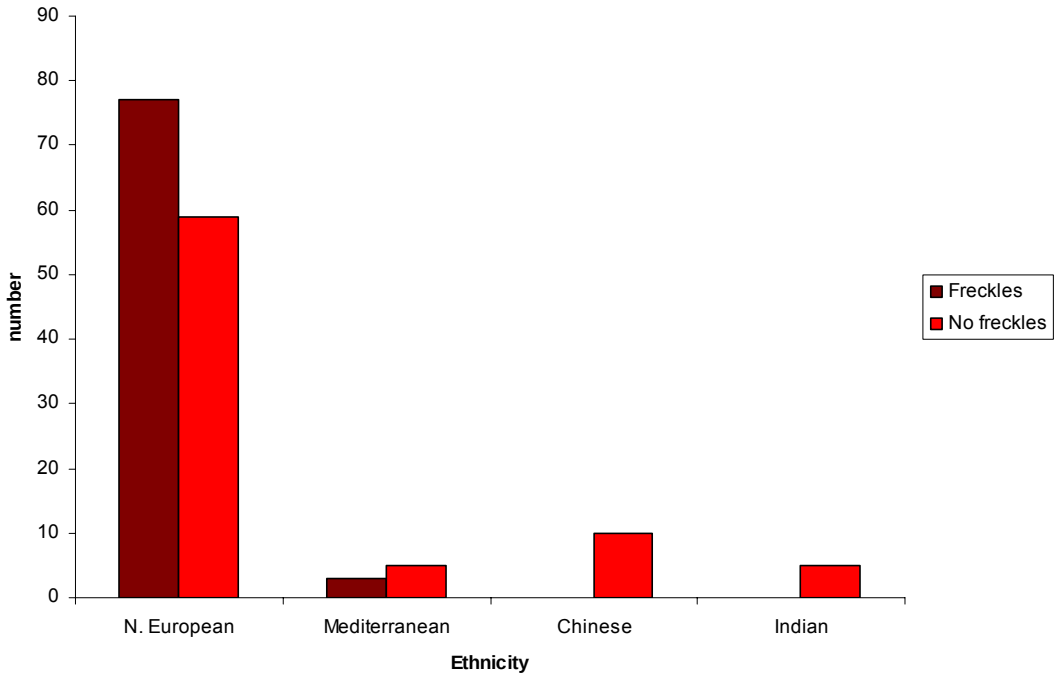
Log age was plotted with the number of freckling sites and showed no obvious trend and no evidence of large difference. Low sample numbers for some of the freckling sites may be a limitation.

A Mann-Whitney Test was performed which compared the 2 medians for with or without freckles and showed no significant difference between the groups (P=0.78).

Individuals with or without freckles do not differ significantly with age.

3.3.13. Freckling and ethnicity

Figure 3.3.13. Presence of freckling and ethnicity

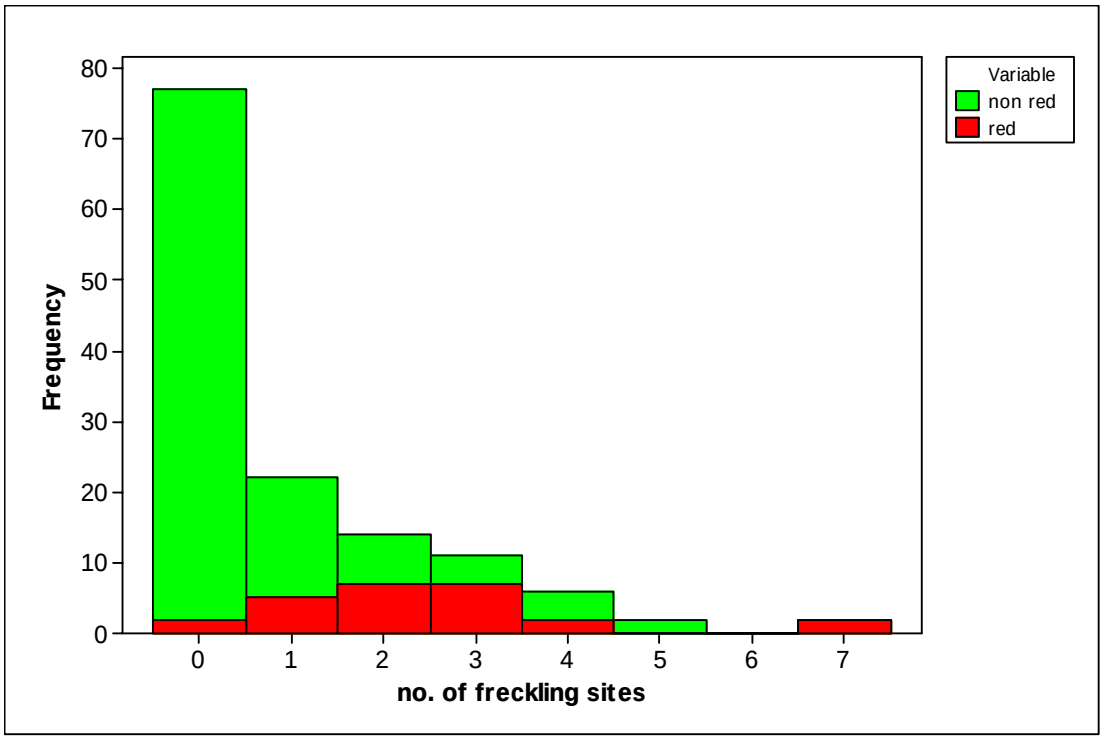


	N. European	Mediterranean	Chinese	Indian
Freckles	77	3	0	0
No freckles	59	5	10	5

Northern Europeans tend to have more freckles than non-Northern Europeans.

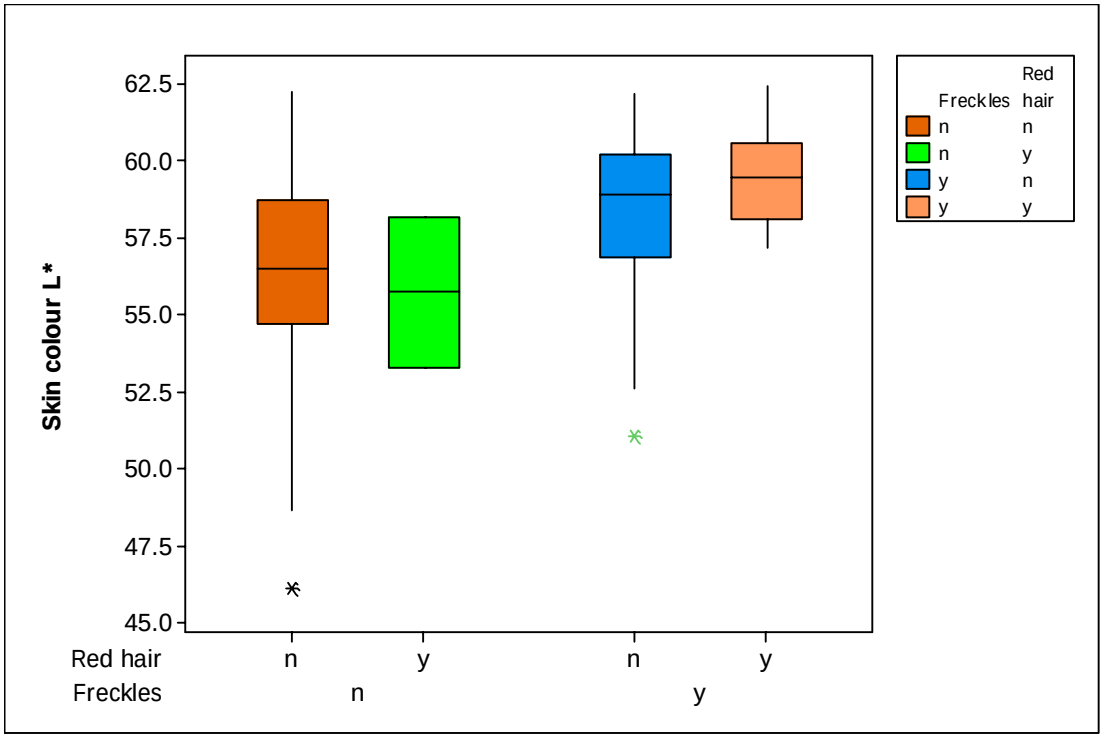
3.3.14. Freckling and red hair colour

Figure 3.3.14.1. Freckling and red hair



Non-red hair individuals have fewer or no freckles whereas red hair individuals have more freckles. A Mann-Whitney Test was performed which compared the 2 medians for reds and non-reds and showed that the number of freckling sites was significantly different for reds and non-reds ($P<0.05$).

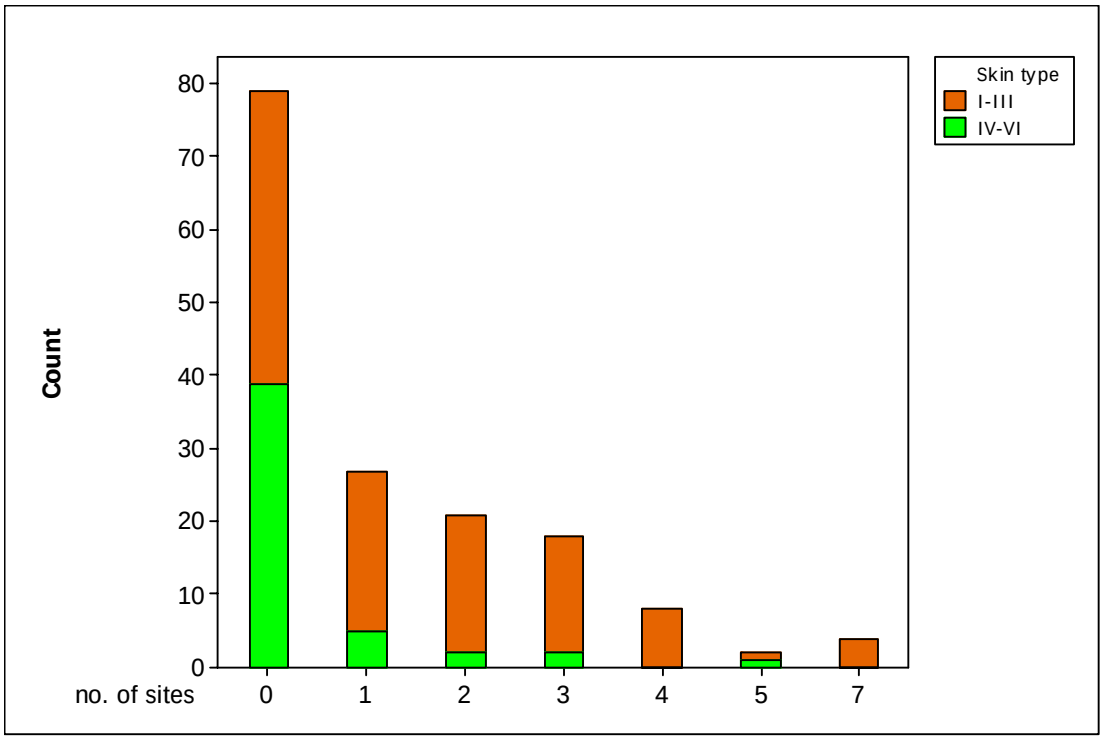
Figure 3.3.14.2. Freckling, red hair and skin colour L*



The association of red hair and freckling is due to pale skin colour. Individuals with red hair and freckles have the lightest skin colour.

3.3.15. Freckling and skin type

Figure 3.3.15. Number of freckling sites and skin type

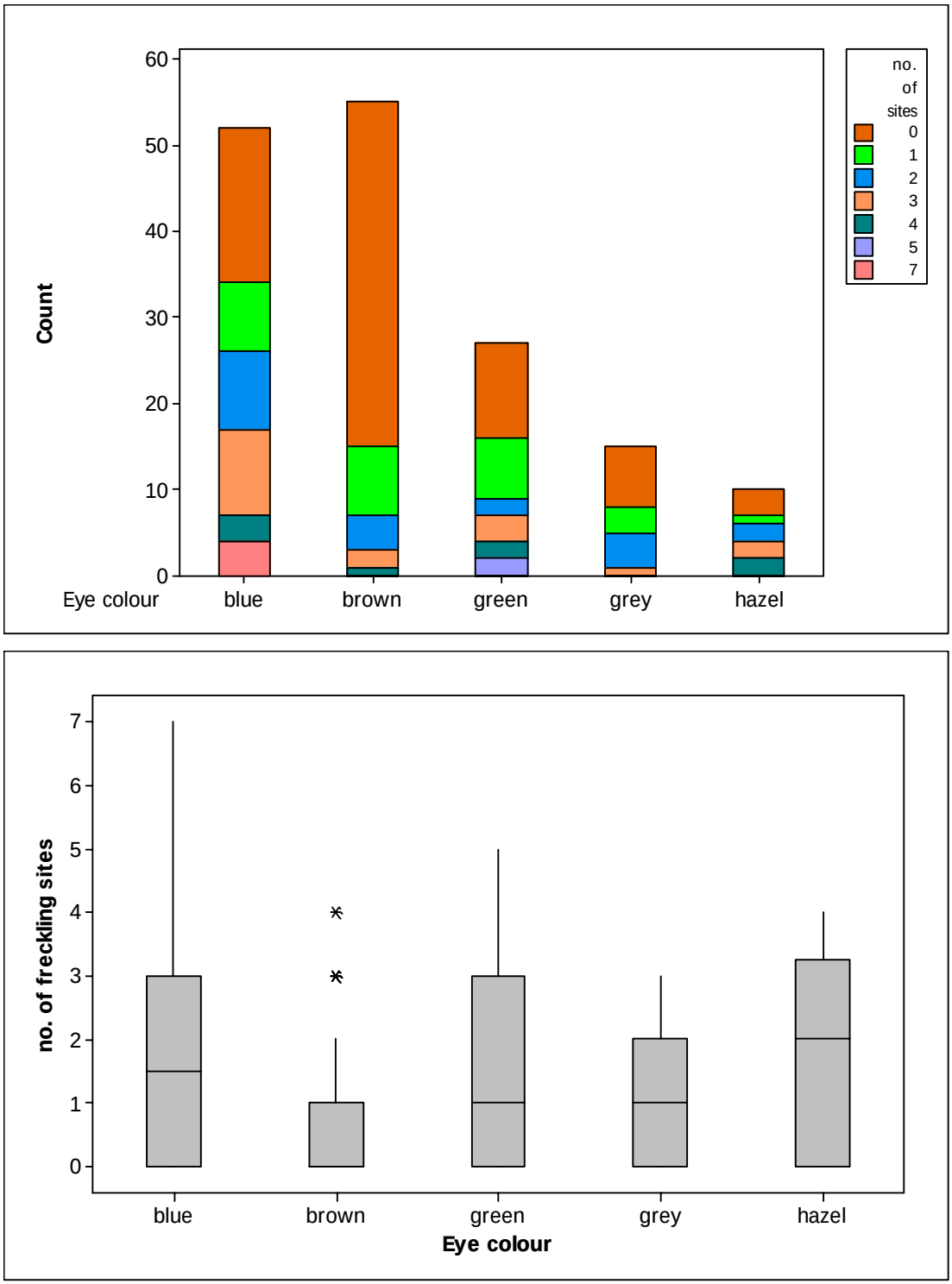


The number of freckling sites was plotted against skin type. Skin type was categorised into skin type (IV-VI) or (I-III).

As expected skin types I-III have more freckling sites than skin types IV-VI. A Mann-Whitney Test was performed which compared the 2 medians for skin types I-III and skin types IV-VI and showed that the groups were significantly different ($P<0.05$).

3.3.16. Freckling and eye colour

Figure 3.3.16. Number of freckling sites and eye colour groups

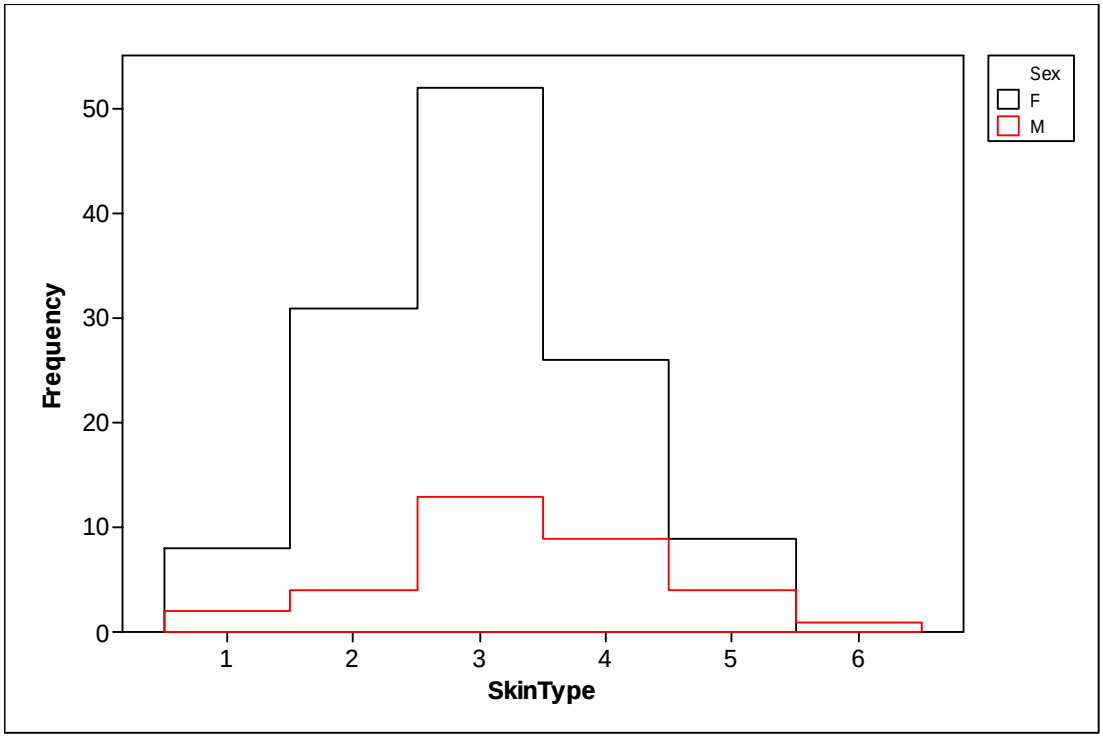


Kruskal-Wallis Test was performed which compared the medians for eye colour groups and number of freckling sites and showed that the groups were significantly different ($P<0.05$). Blue and hazel eye colour individuals have more freckling sites.

3.3.17. Fitzpatrick skin type (I - VI) and sex

Is there a sex difference in Fitzpatrick skin types?

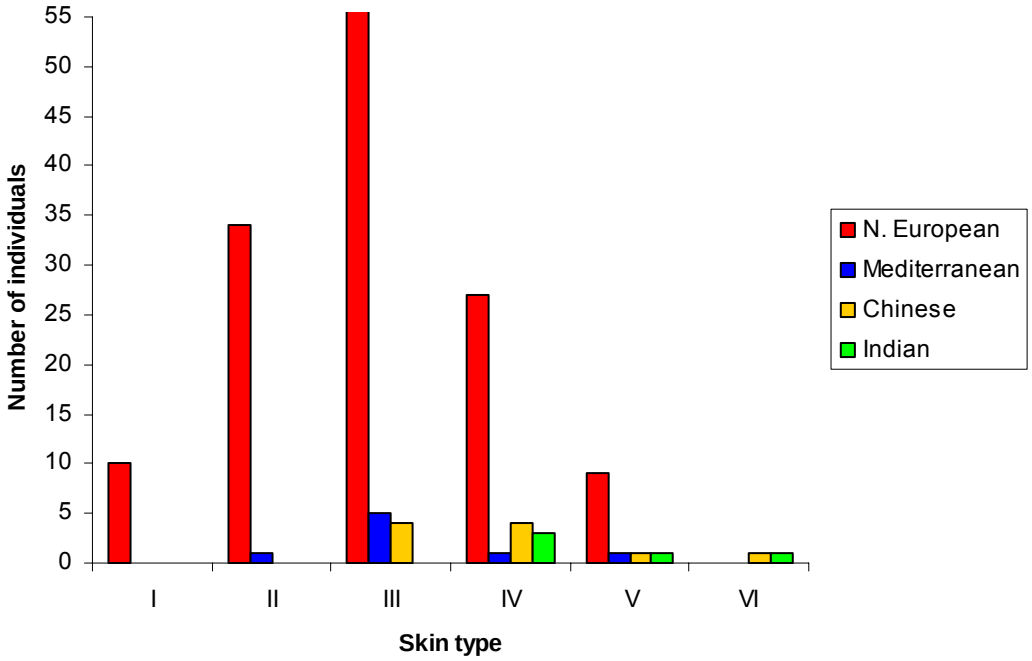
Figure 3.3.17. Fitzpatrick skin type (I - VI) and sex



One-way ANOVA of Fitzpatrick skin type versus sex showed no significant difference between males and females (P=0.056).

3.3.18. Ethnicity and skin type

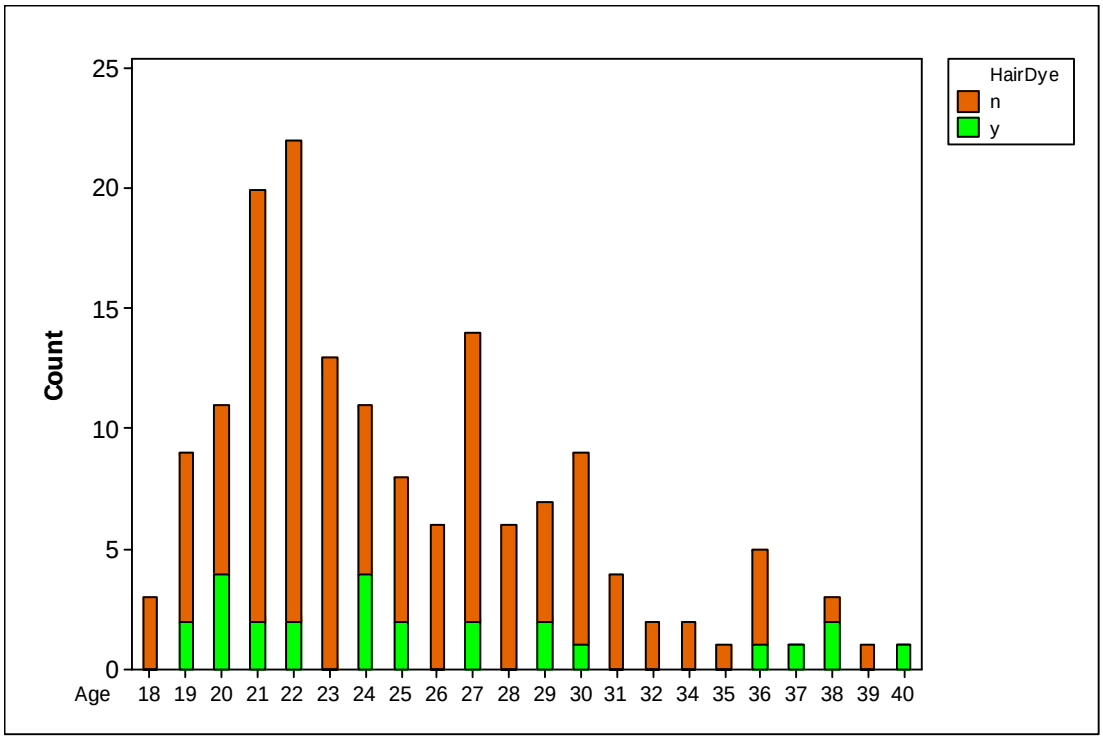
Figure 3.3.18. Ethnicity and skin type



	N. European	Mediterranean	Chinese	Indian
I	10	0	0	0
II	34	1	0	0
III	56	5	4	0
IV	27	1	4	3
V	9	1	1	1
VI	0	0	1	1

3.3.19. Hair dye and age

Figure 3.3.19. Hair dye and age



Individuals who dyed their hair tend to be younger (19-30), however several individuals aged 36-38 and 40 also dyed their hair.

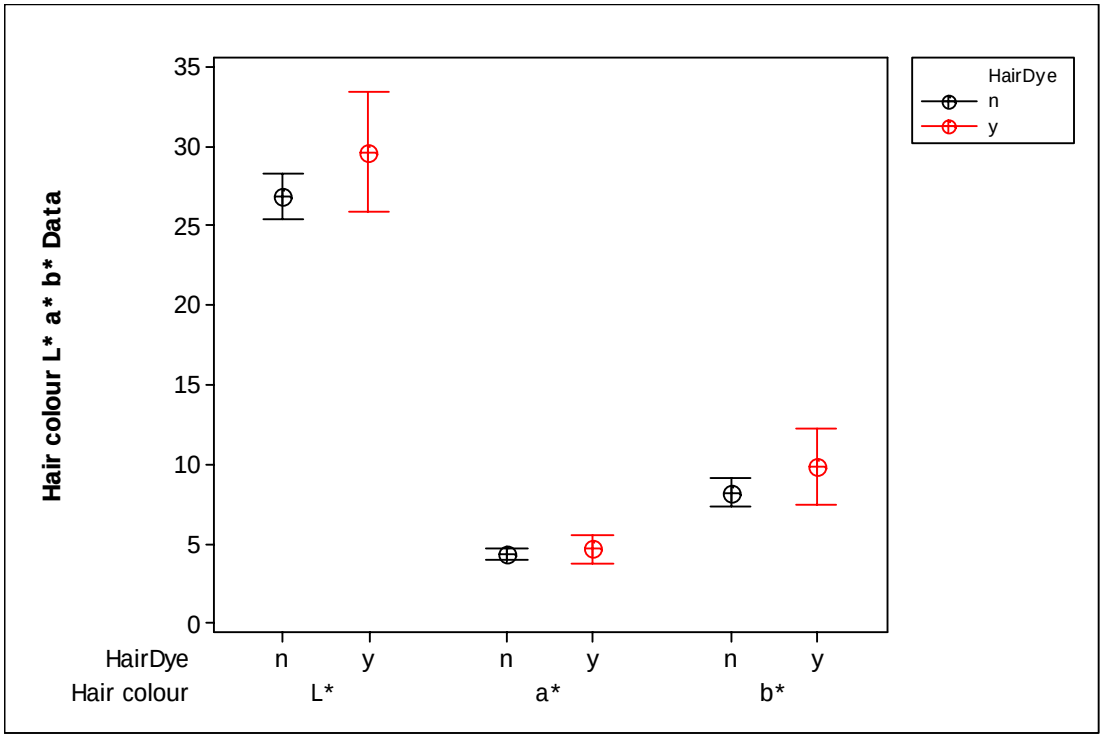
3.3.20. Hair dye and Sex

The dyed individuals consisted of 25 females and 1 male.

3.3.21. Hair colour L* a* b* and hair dye

Reflectance data was obtained for individuals who were dyed or not.

Figure 3.3.21. Hair colour L* a* b* and hair dye

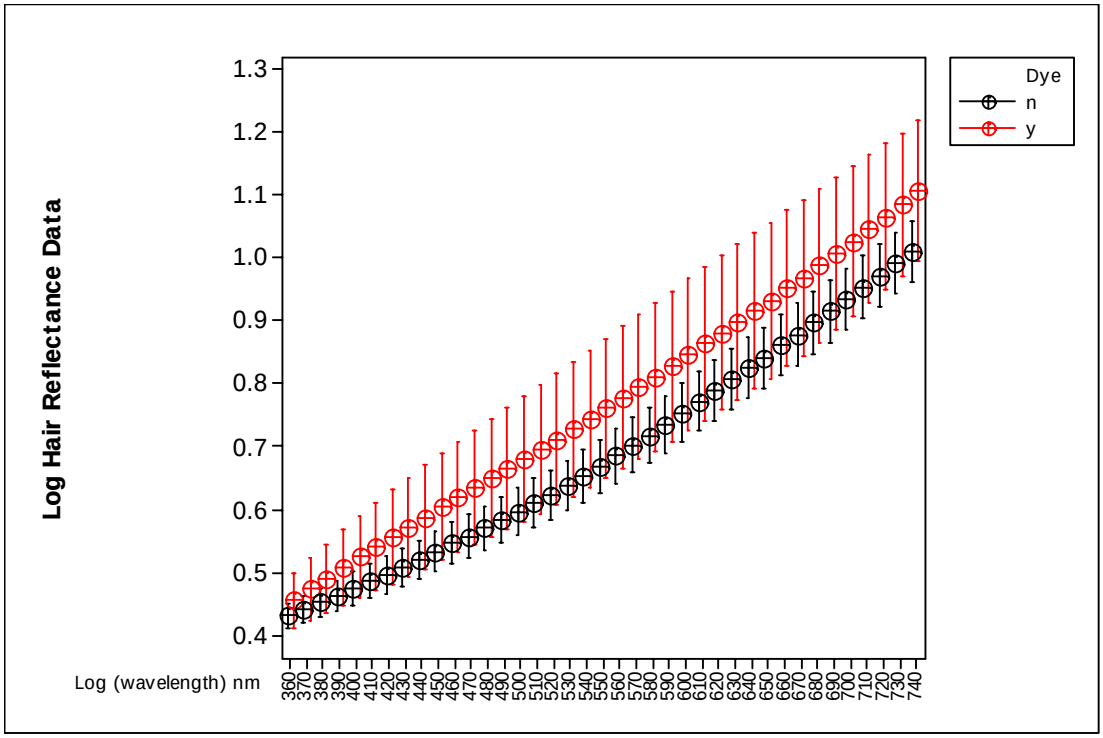


Hair dye appeared to lighten the colour of the hair. This may be due to the choice of hair dye – perhaps individuals liked to dye their hair lighter.

3.3.22. Hair colour spectrophotometric reflectance and hair dye

Spectrophotometric data by 10nm increment of visible colour spectrum wavelength between 360-740nm were obtained.

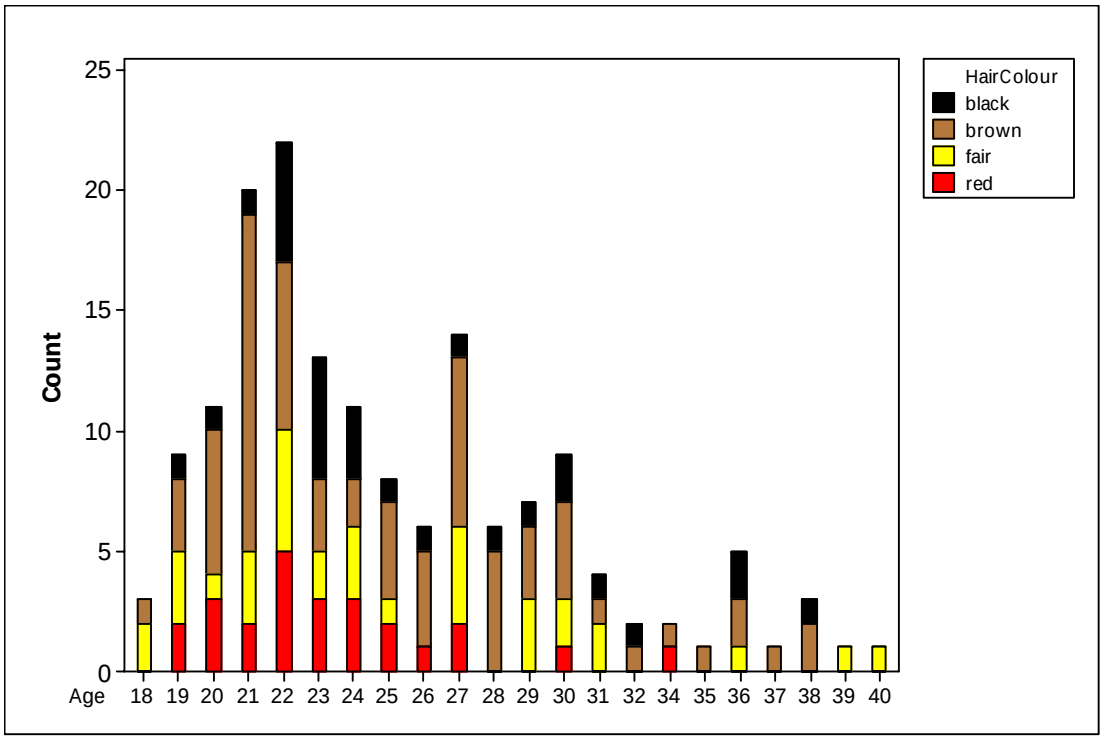
Figure 3.3.22. Hair colour spectrophotometric reflectance and hair dye



This confirmed the expected observation that individuals who dyed their hair tend to dye them lighter. This also confirmed that hair spectrophotometry as a valid measure. Human scalp hair grows at about 0.35mm (Myers and Hamilton, 1951) to 0.5mm/day (Robertson, 1999). Despite taking hair growth rate into account and that hair colour measurement of dyed hair was taken closer to the hair root when volunteers' hair were dyed, this highlighted the need to exclude dyed hair in any analysis.

3.3.23. Hair colour and age

Figure 3.3.23. Hair colour and age

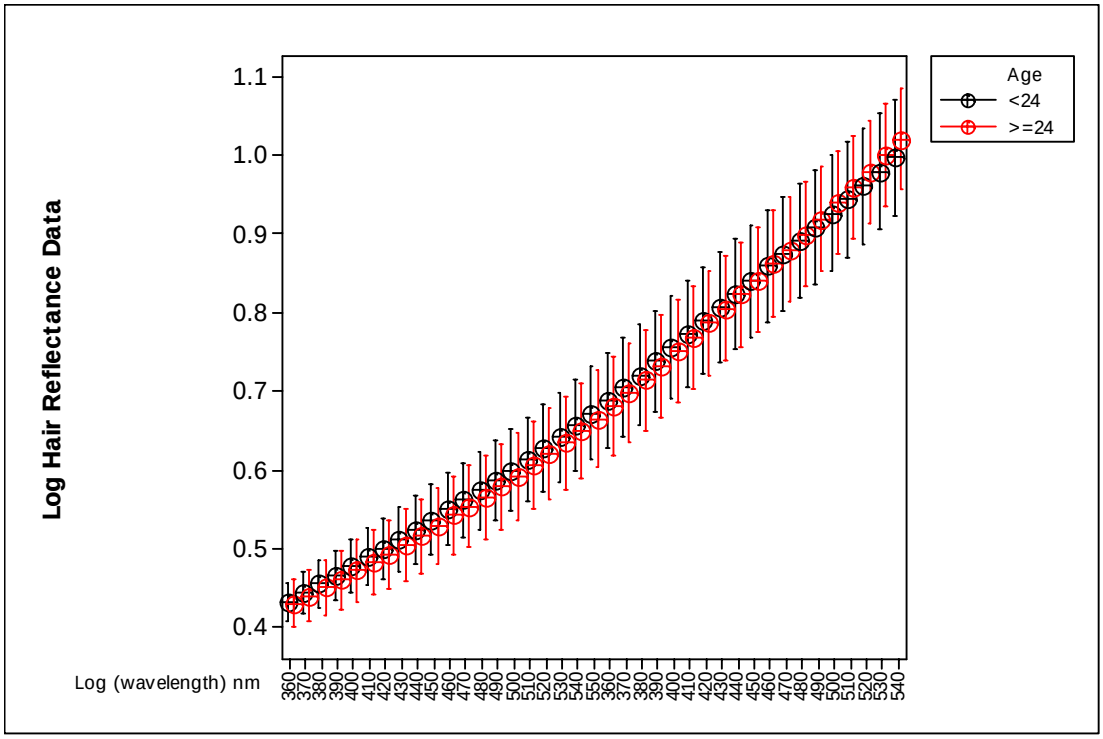


There was no relation for hair colour and age.

3.3.24. Hair colour spectrophotometric reflectance with age

Hair reflectance was plotted for age<24 and age>24.

Figure 3.3.24. Hair colour spectrophotometric reflectance with age



There was no relation for hair spectrophotometric reflectance and age.

3.3.25.1. Hair colour and sex

Figure 3.3.25.1. Hair colour and sex

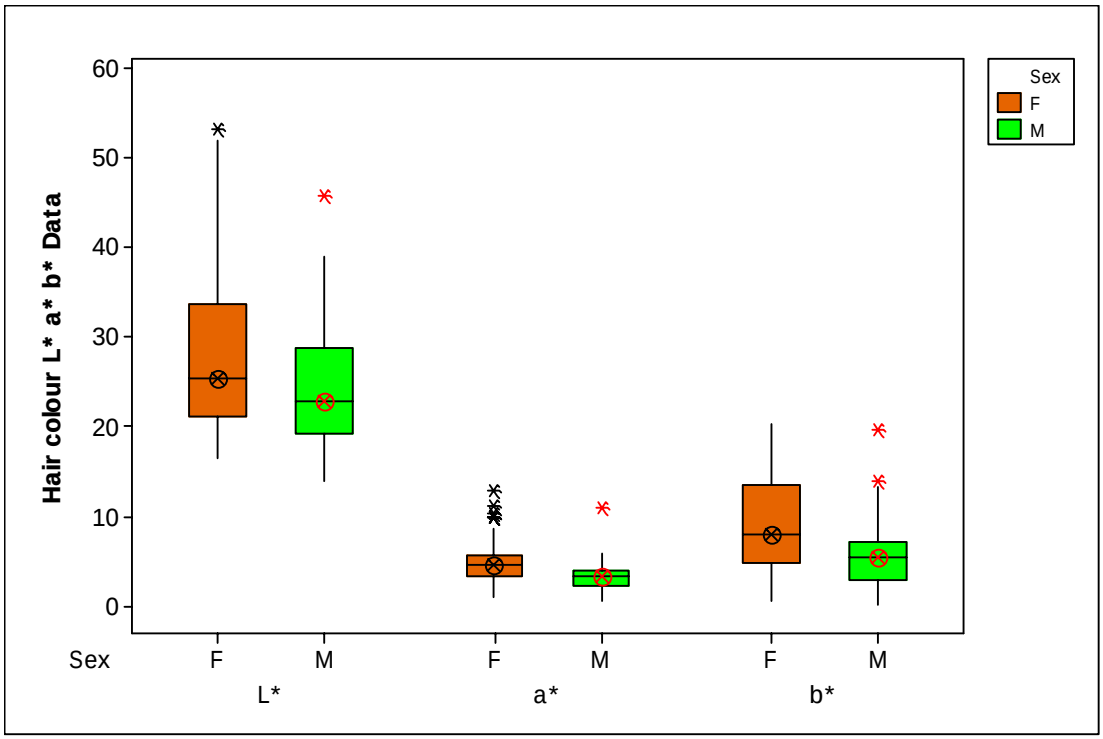
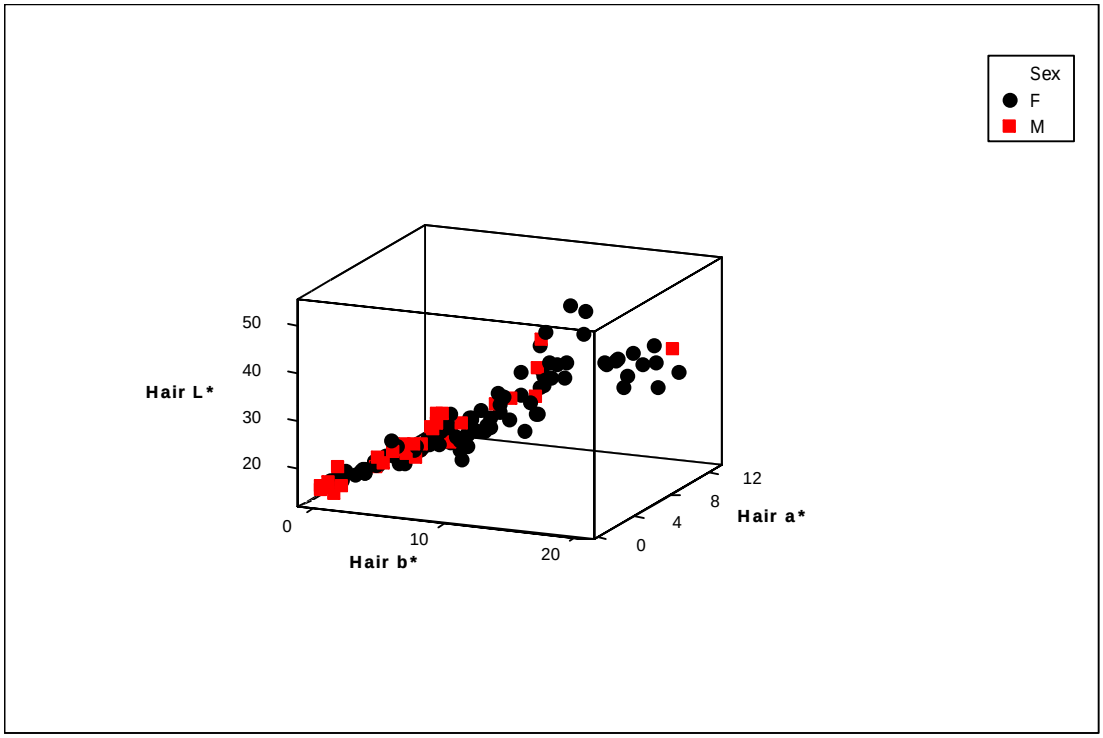


Table 3.3.25. Mean, min and max hair colour L* a* b* values by sex (n=133)

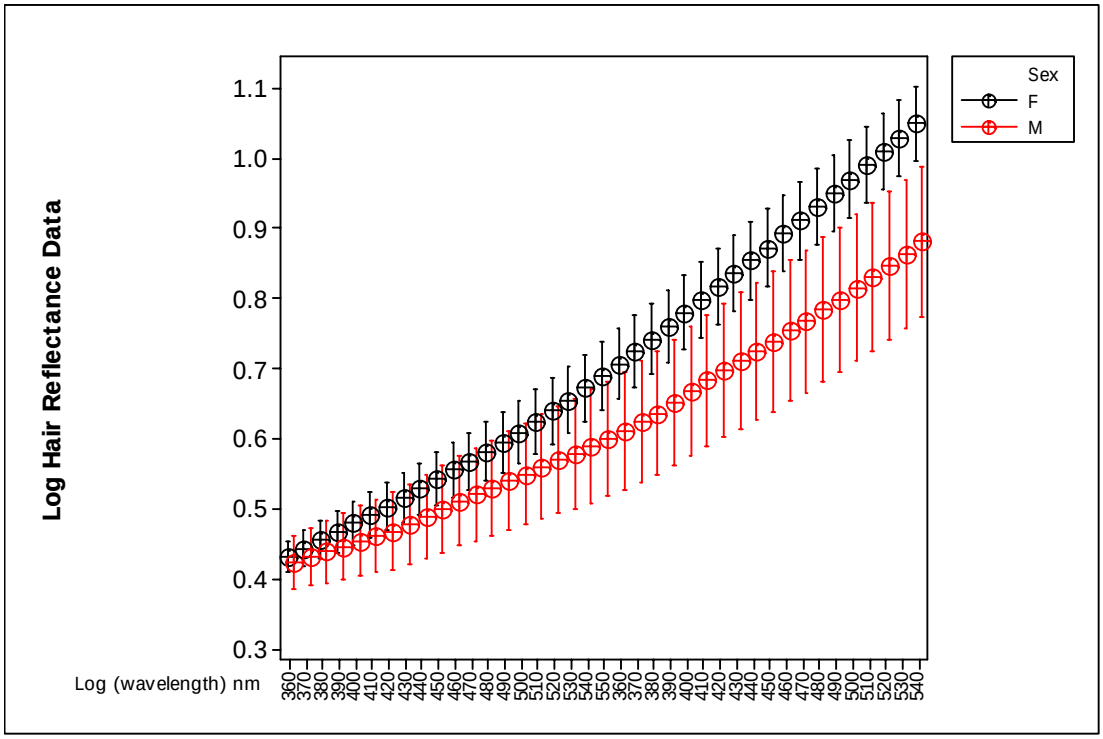
Sex	Mean L*	SEM	Min L*	Max L*	Mean a*	SEM	Min a*	Max a*	Mean b*	SEM	Min b*	Max b*
M	24.41	1.34	13.92	45.70	3.14	0.36	0.40	10.82	5.93	0.80	0.05	19.51
F	27.61	0.87	16.38	53.20	4.66	0.23	0.82	12.74	8.90	0.52	0.51	20.20

Figure 3.3.25.2. 3D scatter plot for hair colour L* a* b* by sex



3.3.26. Hair spectrophotometric reflectance and Sex

Figure 3.3.26. Hair spectrophotometric reflectance and Sex

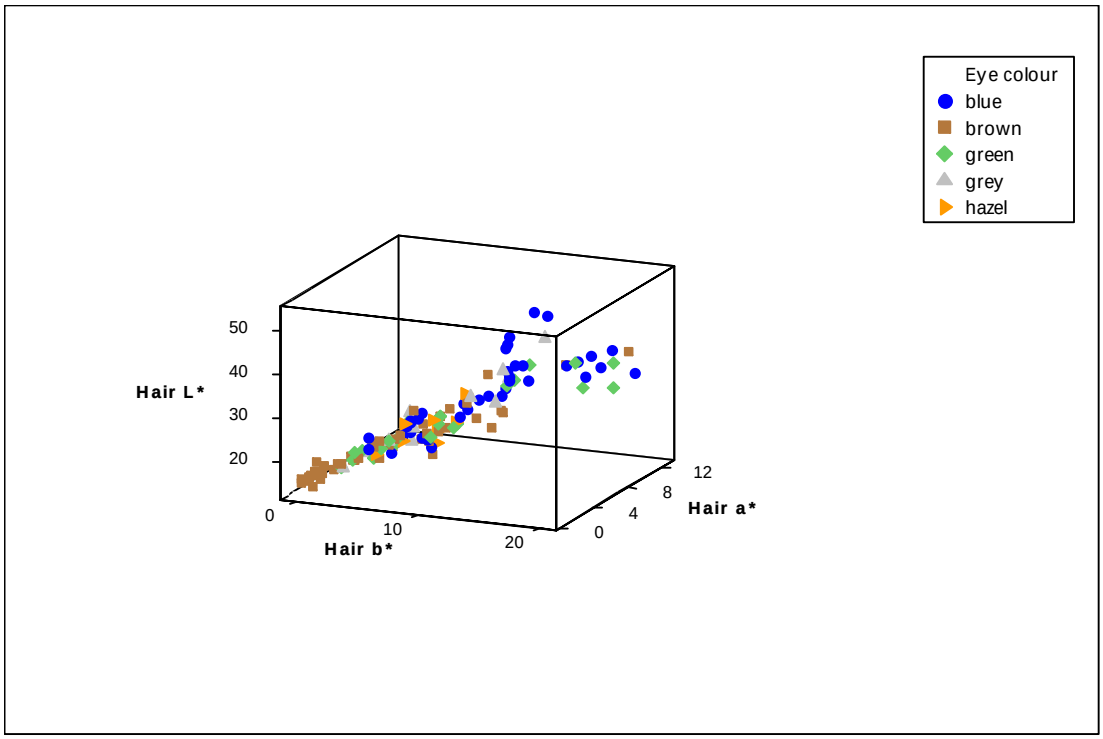


This is an interval plot of log hair spectrophotometric reflectance ± SEM (95% CI for the mean) for males (red) and females (black) n=133. Y-axis shows the log hair spectrophotometric reflectance. X-axis shows the 10nm increments of 360-740nm wavelength.

This showed that females have generally lighter hair colour. Two-sample t-test showed significant differences from log(570nm)-log(740nm) ($P<0.05$).

3.3.27. Distribution of hair colour L* a* b* with different eye colour groups

Figure 3.3.27. 3D scatter plot of hair colour L* a* b* with different eye colour groups



This is a 3D scatter plot of hair colour L* a* b* and eye colour groups.

3.3.28. Distribution of hair colour L* a* b* with presence of freckling

Figure 3.3.28.1. Hair colour L* a* b* with presence of freckling

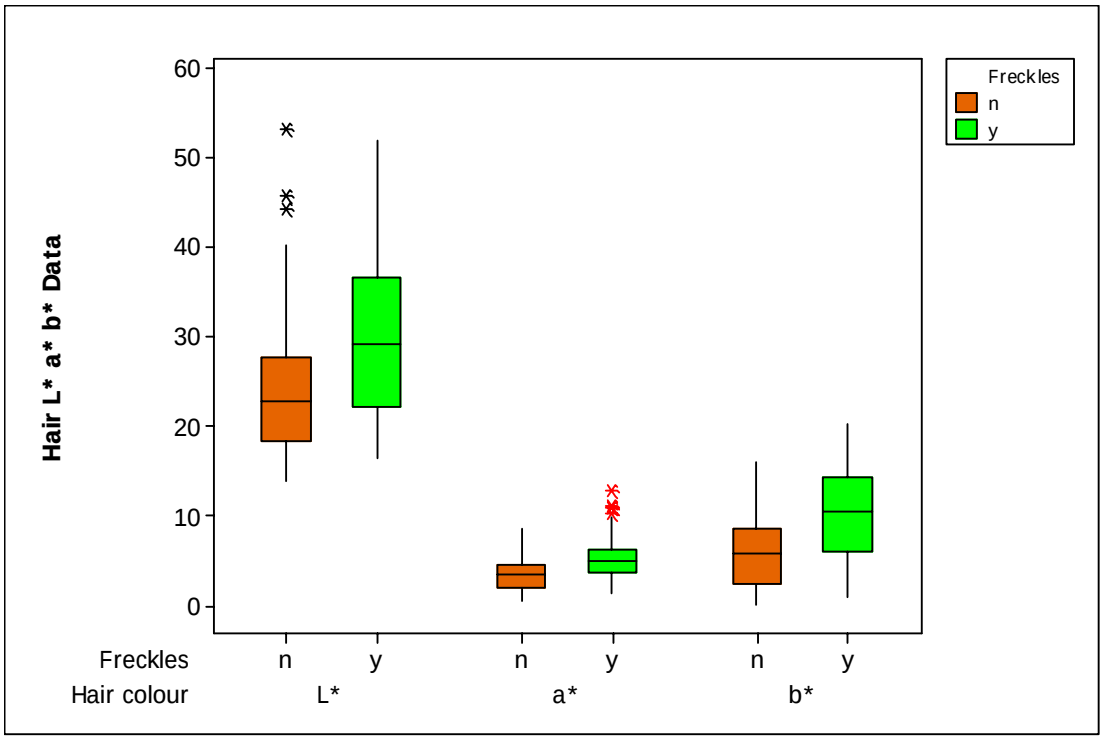
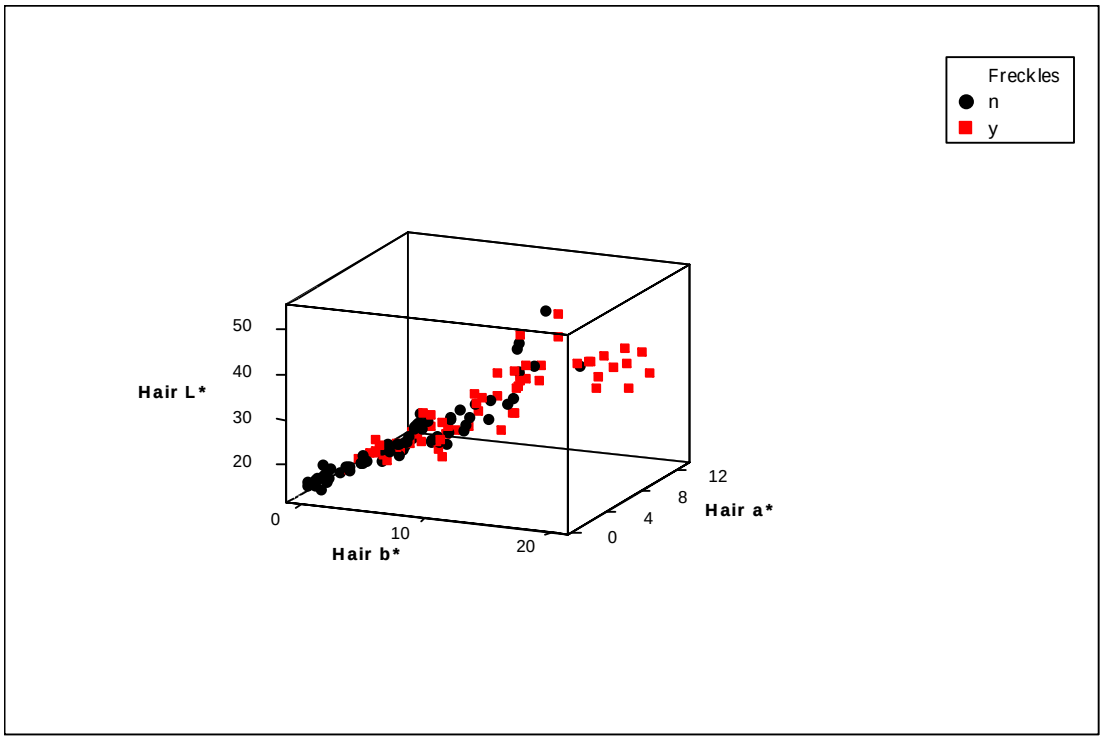
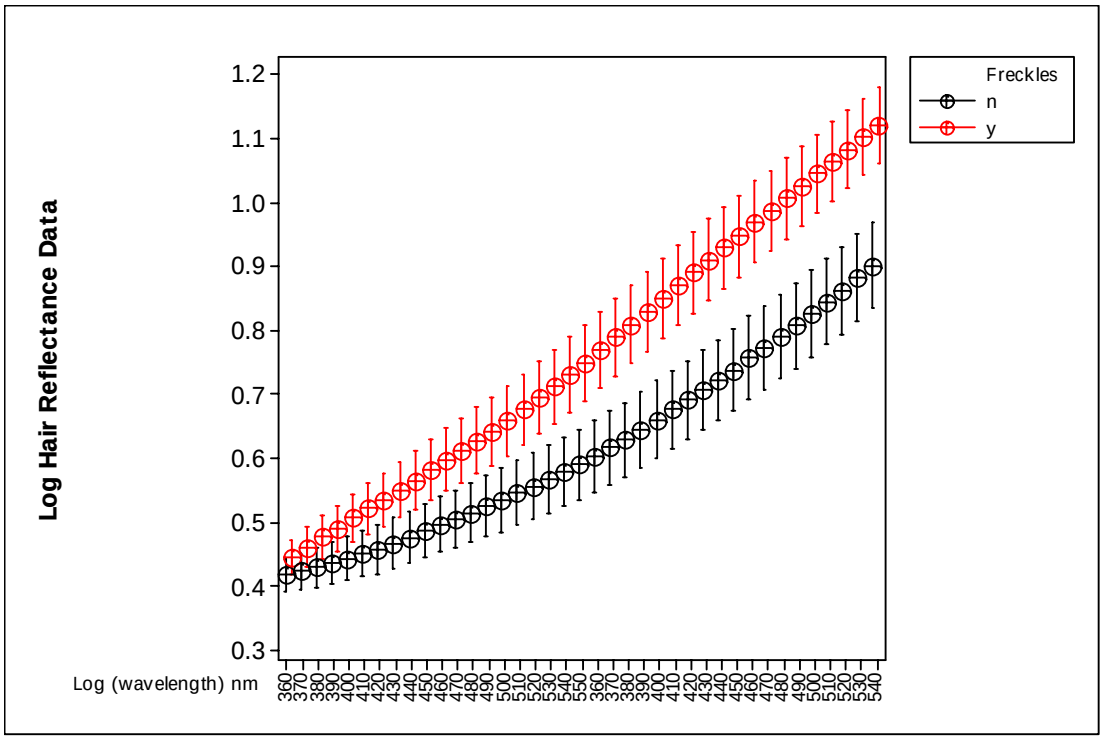


Figure 3.3.28.2. 3D scatter plot of hair colour L* a* b* and freckling



3.3.29. Hair colour spectrophotometric reflectance with freckling

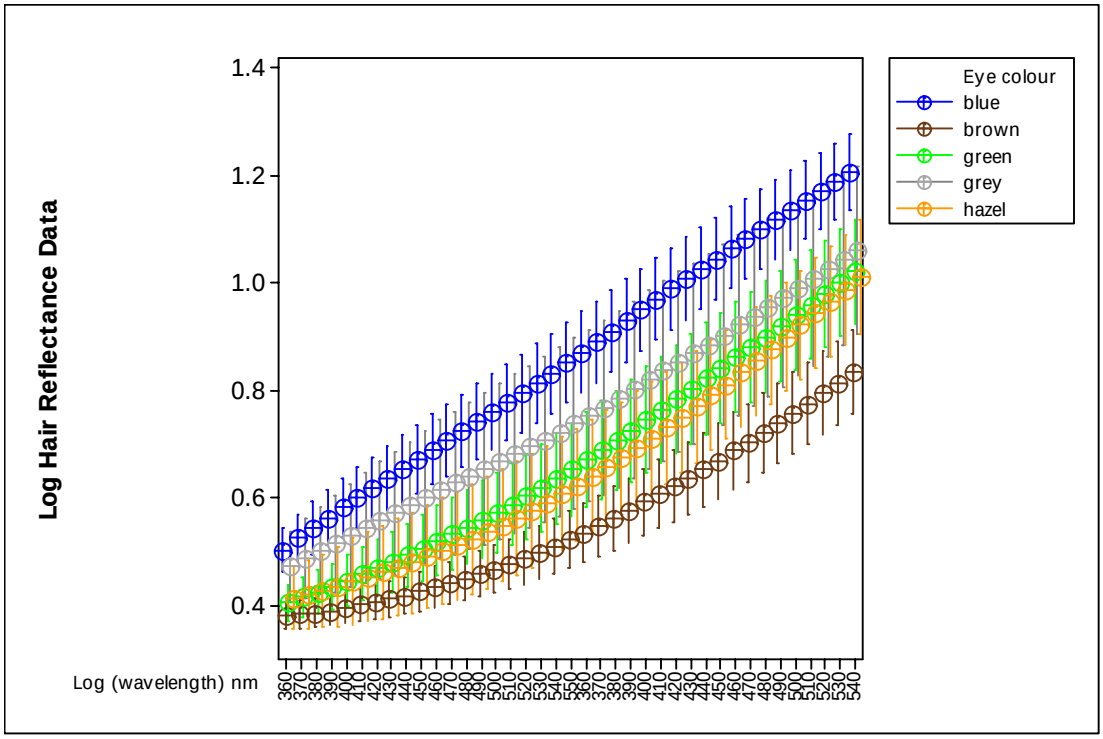
Figure 3.3.29. Hair colour spectrophotometric reflectance with freckling



Next the effect of freckling on hair colour was analysed. This figure showed the log hair reflectance data of 133 individuals with freckling. The error bars present 95% confidence interval for the mean. This showed that individuals with freckles have generally lighter hair colour. Two sample t-test showed significant differences from log(570nm)-log(740nm) ($P<0.05$).

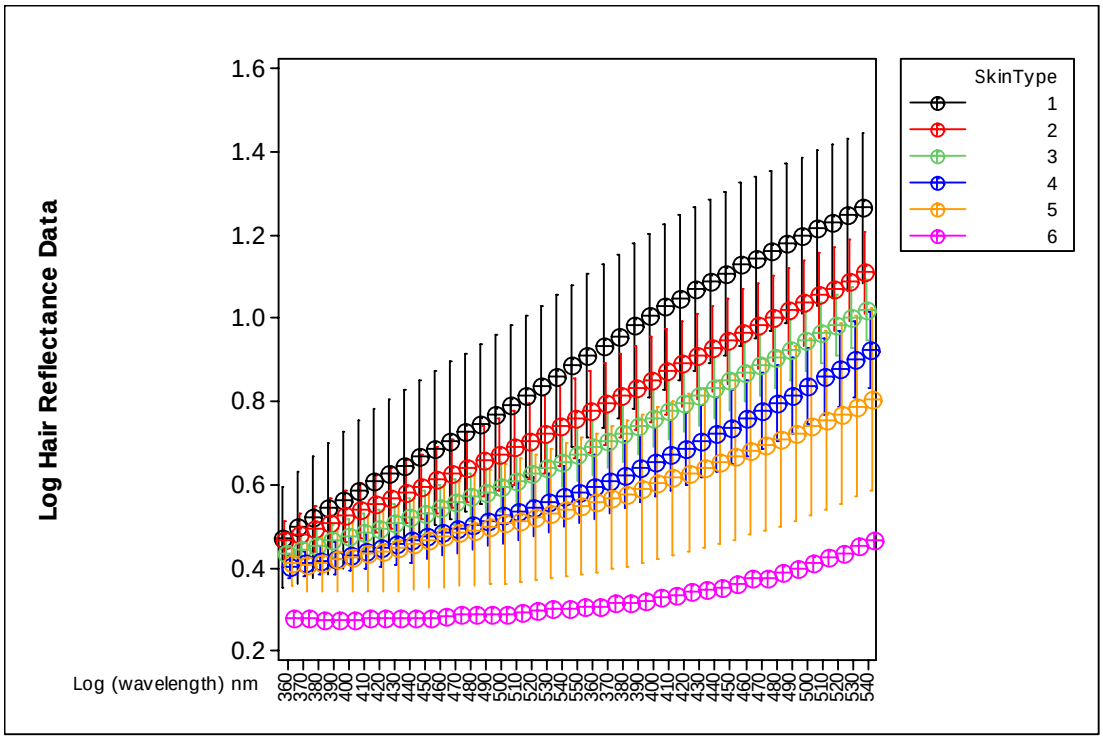
3.3.30. Hair colour spectrophotometric reflectance and eye colour

Figure 3.3.30. Hair colour spectrophotometric reflectance and eye colour groups



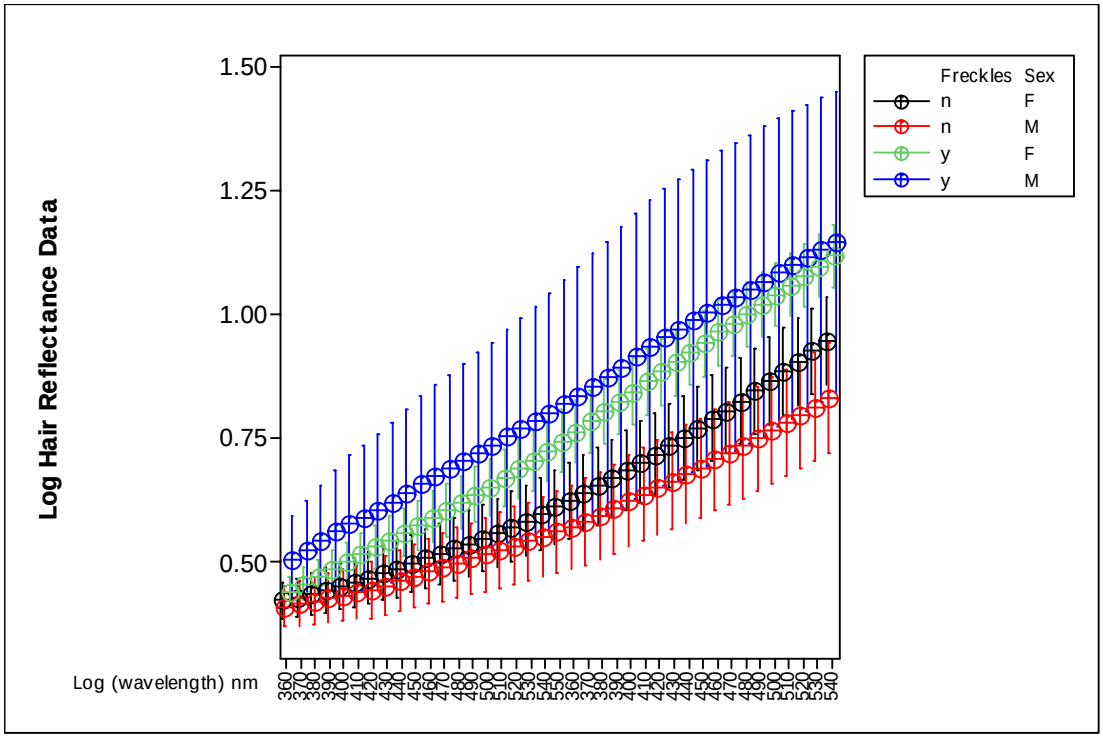
3.3.31. Hair colour spectrophotometric reflectance and skin type

Figure 3.3.31. Hair colour spectrophotometric reflectance and skin type



3.3.32. Hair spectrophotometric reflectance with sex and freckling

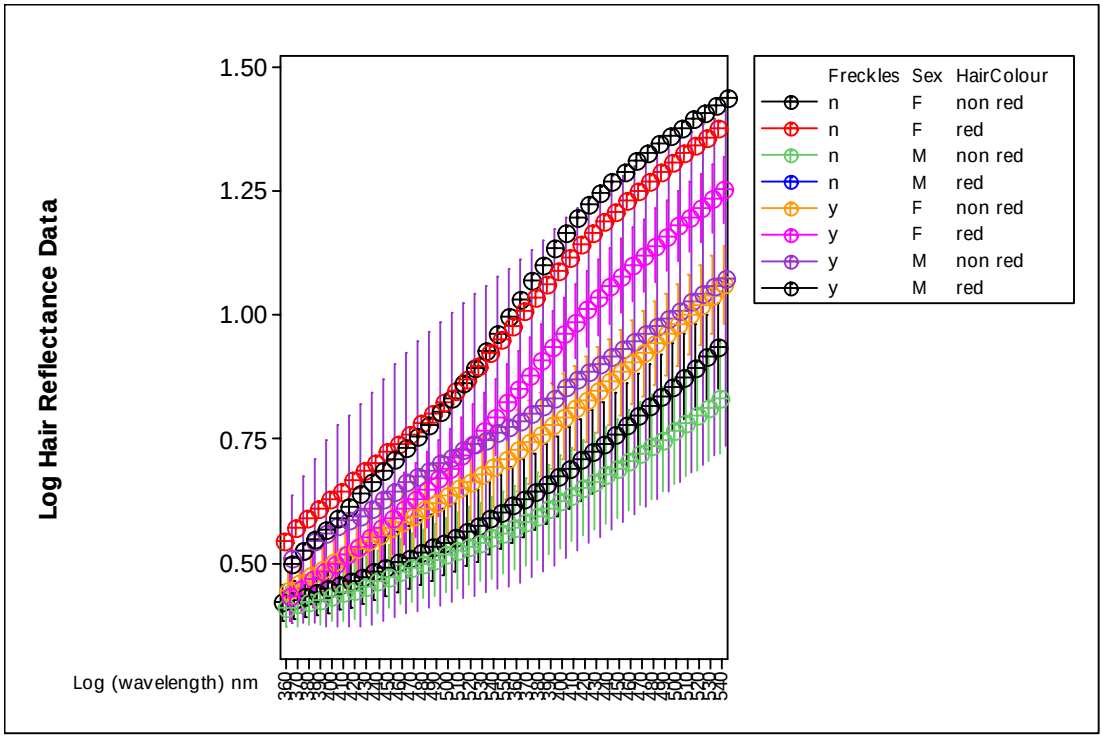
Figure 3.3.32. Hair spectrophotometric reflectance with sex and freckling



Next the effect of sex and freckling on hair colour was analysed. This figure showed the log hair reflectance data of 133 individuals. The error bars present 95% confidence interval for the mean. Sex difference was lost / lessened when the effect of freckling was taken into account. Without freckles, females were lighter than males. Females with and without freckles showed reflectance at wavelength log(550nm) of 0.74±0.03 and 0.61±0.04 respectively. Males with or without freckles showed reflectance at wavelength log(550nm) of 0.82±0.09 and 0.56±0.04 respectively. Similarly at wavelength log(740nm), females with and without freckles showed reflectance of 1.12±0.03 and 0.95±0.04 respectively. Males with or without freckles showed reflectance at wavelength log(740nm) of 1.15±0.11 and 0.83±0.05 respectively.

3.3.33. Hair spectrophotometric reflectance with reported hair colour, sex and freckling

Figure 3.3.33. Hair spectrophotometric reflectance with reported hair colour, sex and freckling

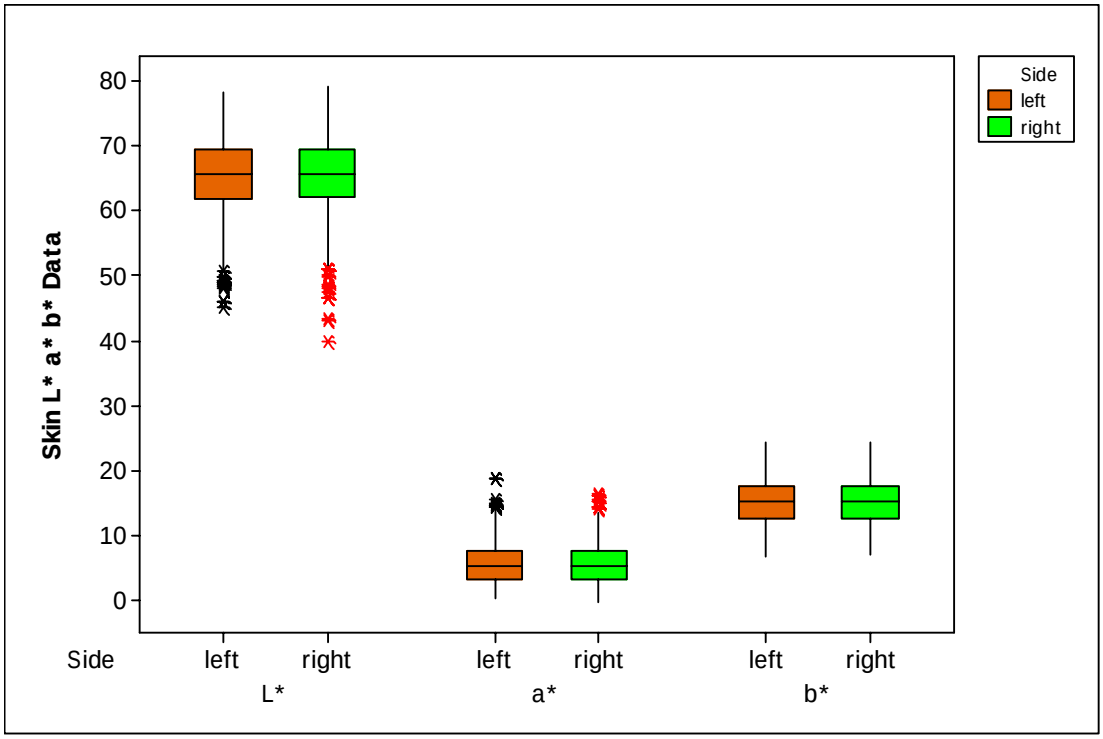


As expected, individuals with the highest hair reflectance (lightest) were not red females with freckles, but red males with freckles.

The darker individuals were non-red males without freckles, followed by non-red females without freckles, non-red females with freckles, non-red males with freckles, red females with freckles, red females without freckles, red males with freckles. There were no red males without freckles.

3.3.34. Skin colour and sidedness

Skin colour is assumed to be the same in an individual between the left and the right side. Is there a difference between left and right skin sites?



There was no significant difference between left and right measured skin L*, a* or b* as tested by two sample t-test (P=0.79, P=0.90, P=0.62).

3.3.35. Inter-relationship between skin colour, sex and site

The effect of skin colour of various sites is different in males and females.

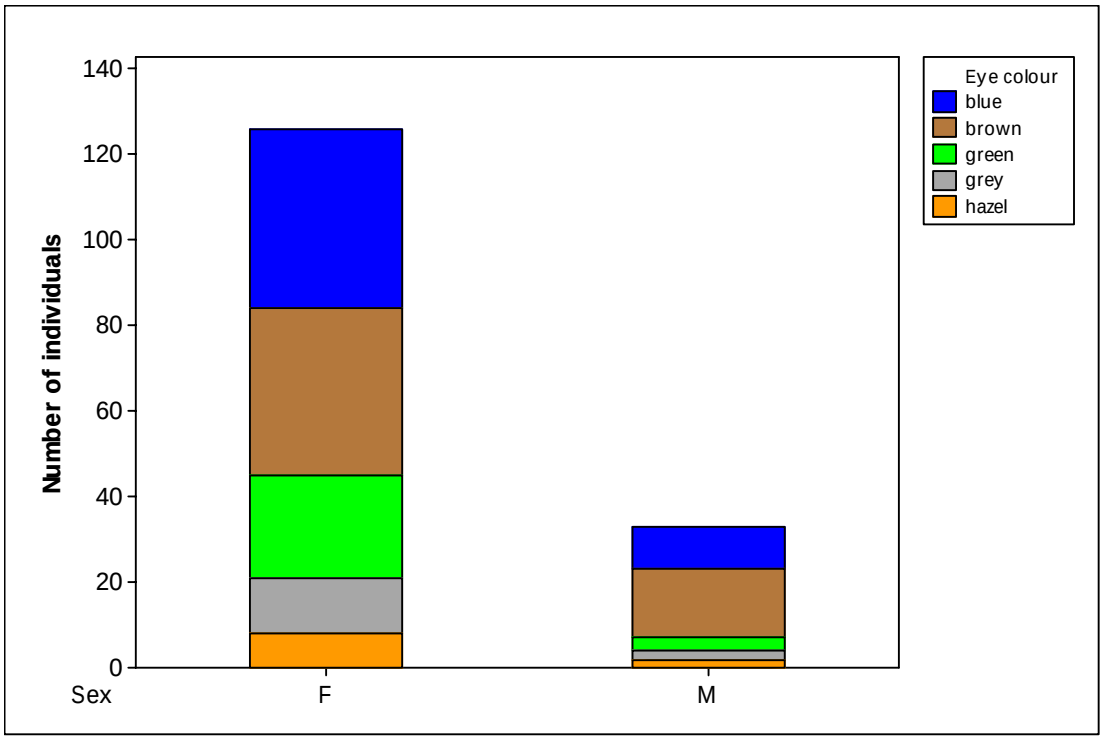
The relationship between skin colour L*, sex and site were fitted to a General Linear Model (GLM). This showed an R-squared value of 32.31%. Skin colour a*, sex and site showed an R-squared value of 53.38%. Skin colour b*, sex and site showed an

R-squared value of 22.19%. Inclusion of left right sidedness did not result in any increase in R-squared value.

3.3.36. Eye colour and sex

Comparison of eye colour in males and females

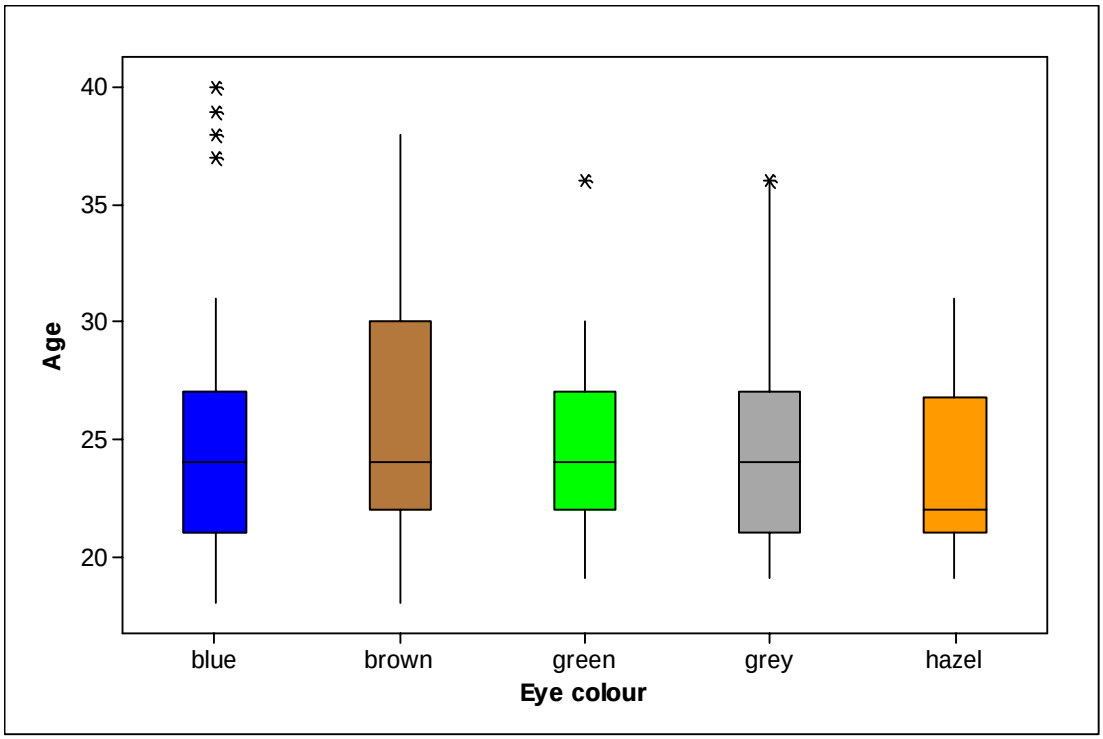
Figure 3.3.36. Eye colour and sex



Fisher's Exact Test for eye colour and sex did not show any significant difference between males and females (P=0.40). Males and females do not differ significantly in eye colour.

3.3.37. Eye colour and age

Figure 3.3.37. Eye colour and age



One-way ANOVA was performed which showed that the 5 eye colour groups did not differ by age (P=0.525).

The following were plotted for illustration purposes only.

3.3.38. Distribution of eye colour with red hair colour

Figure 3.3.38.1. Distribution of eye colour with red hair

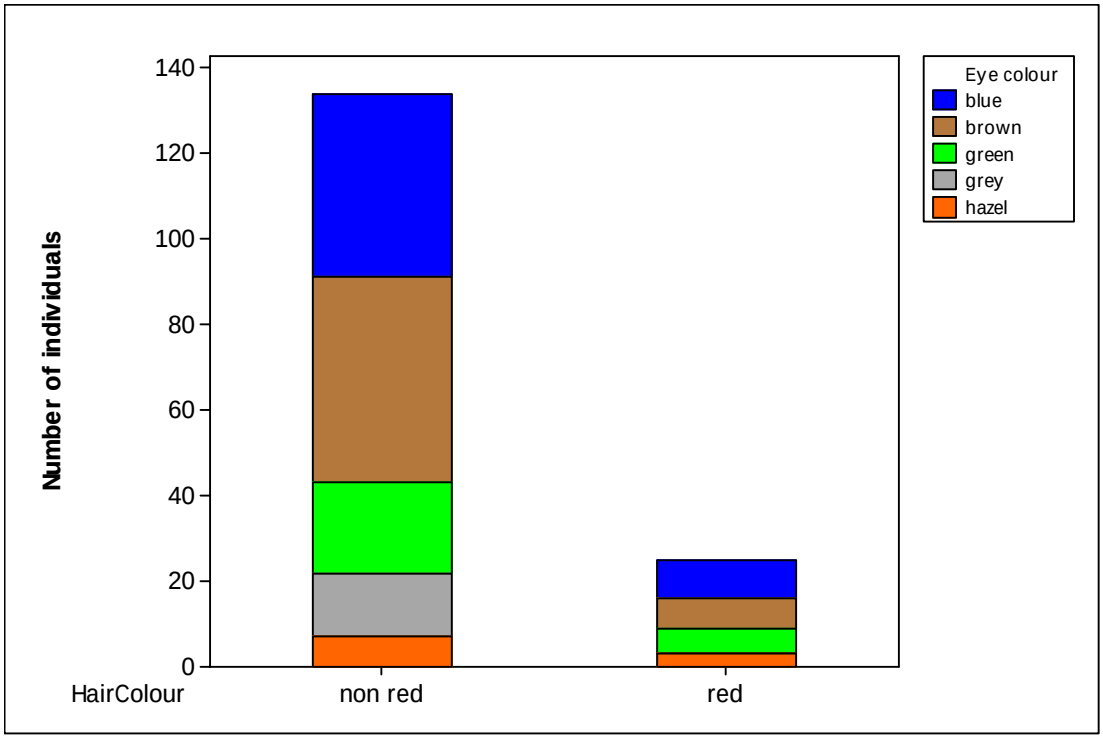


Figure 3.3.38.2. 3D scatter plot of quantitative Munsell L* a* b* eye colour with red hair

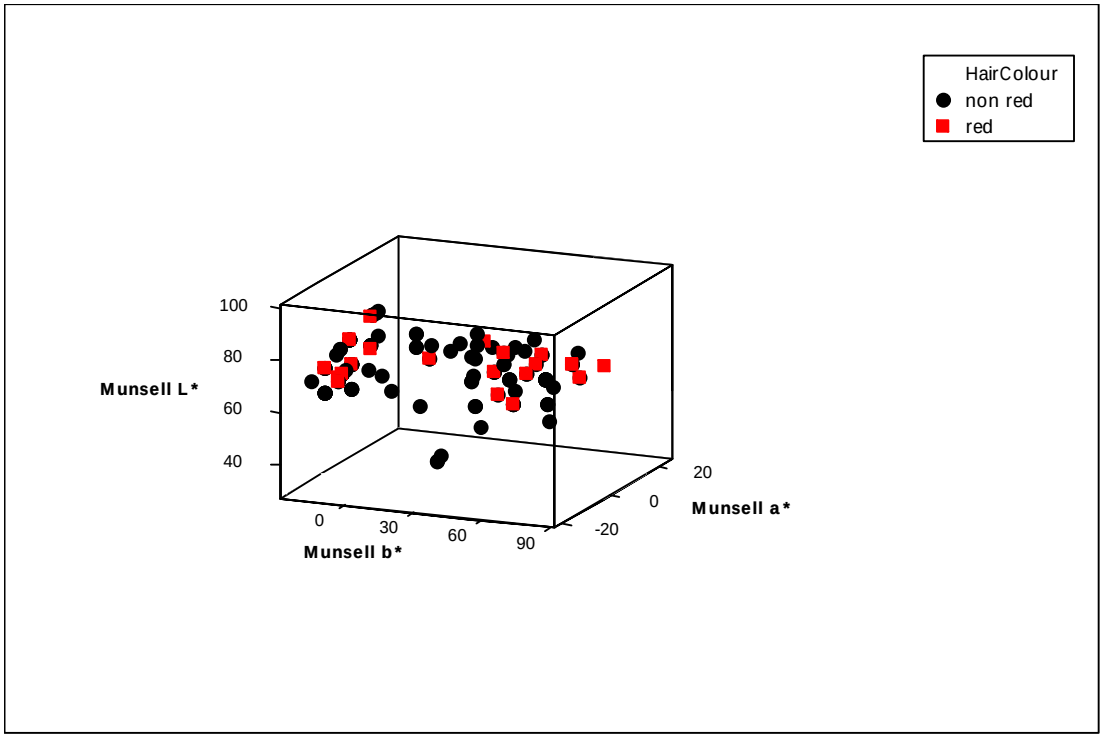
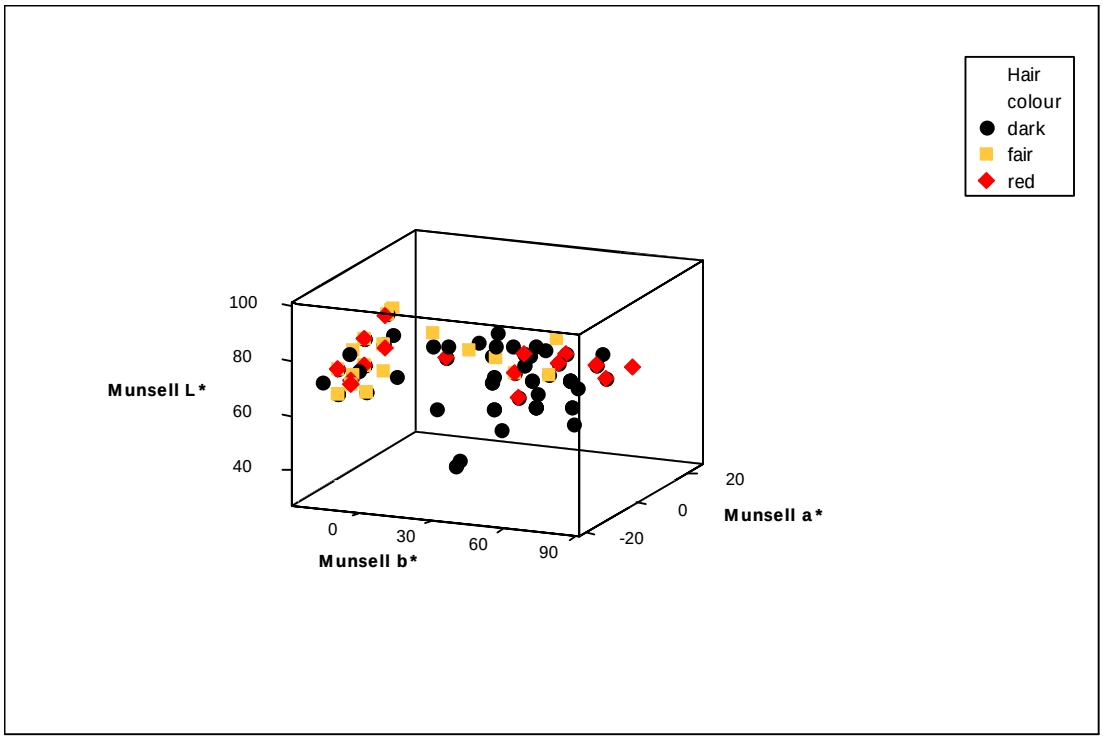
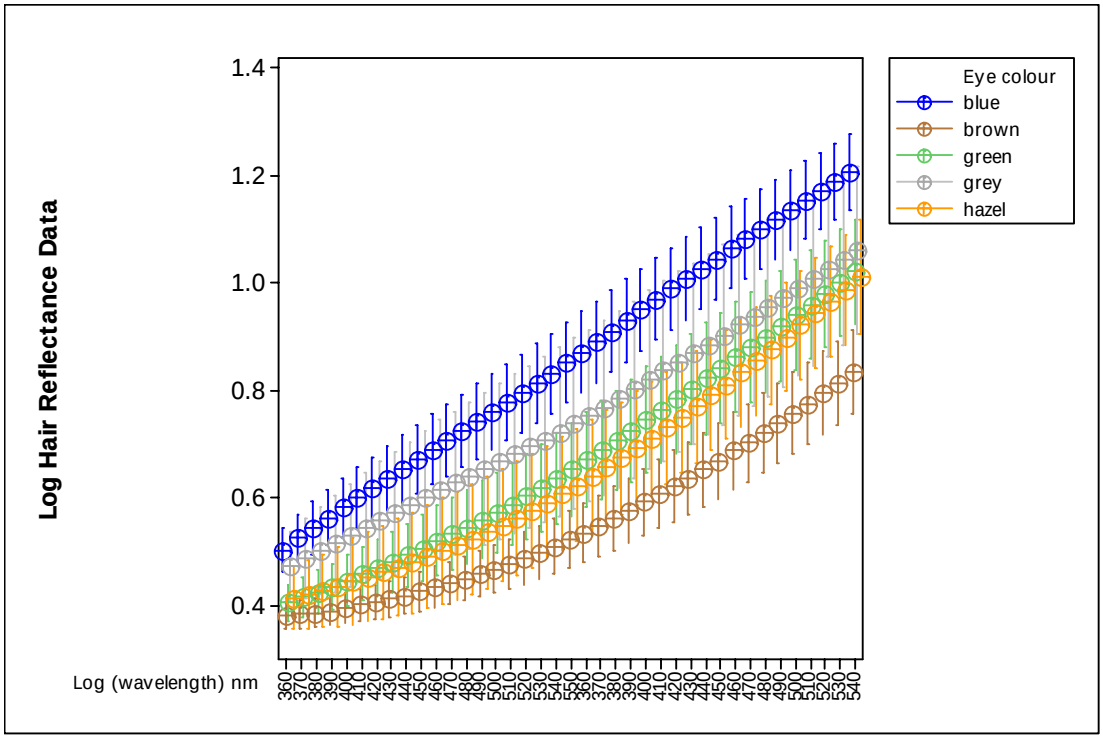


Figure 3.3.38.3. 3D scatter plot of quantitative Munsell L* a* b* eye colour with hair colour



3.3.39. Distribution of eye colour with different hair colour

Figure 3.3.39. Distribution of eye colour with hair colour spectrophotometric reflectance



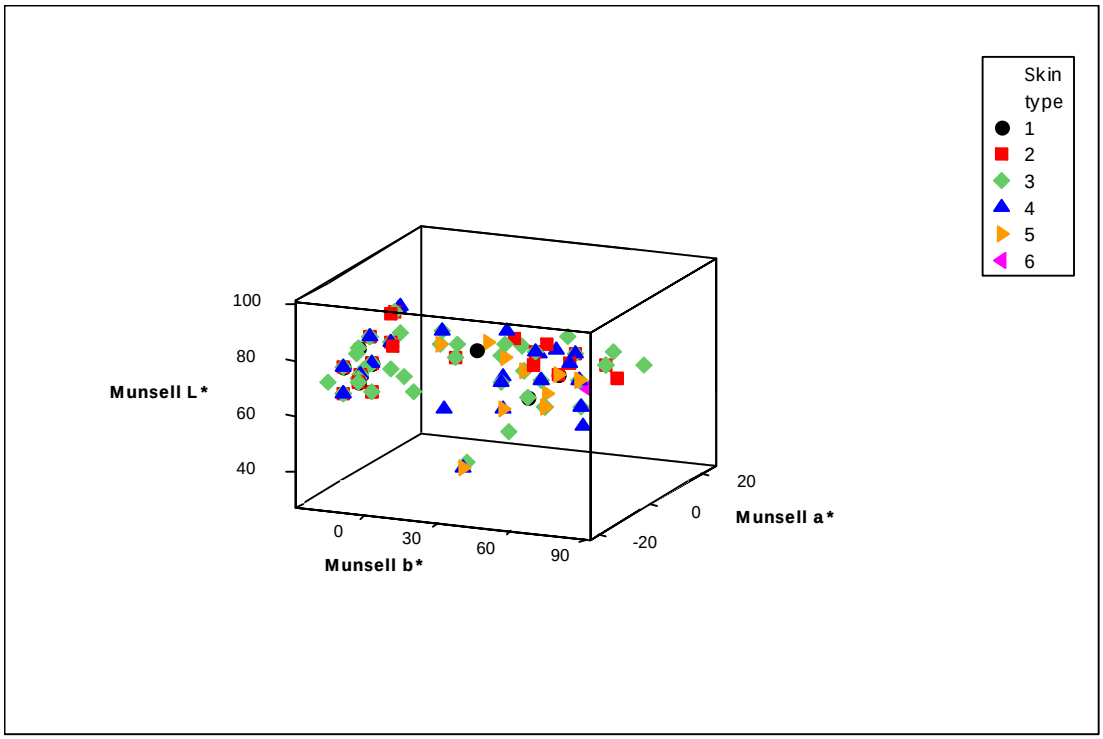
This is an interval plot of the log hair Spectrophotometric reflectance ± SEM (95% CI for the mean) with eye colour. Y-axis shows the log spectrophotometric reflectance data. X-axis shows the 10nm increments of 360-740nm wavelengths.

Blue eye colour individuals have lightest hair colour. Grey, green and hazel eye colours have the intermediate hair colour. Brown eye colour individuals have the darkest hair colour.

3 wavelengths were chosen (400nm, 550nm and 740nm) based on visible light spectrum of violet, green and red respectively. One-way ANOVA was performed at each wavelength for the 5 eye colour groups which showed significant difference ($P<0.05$).

3.3.40. Distribution of eye colour with skin type

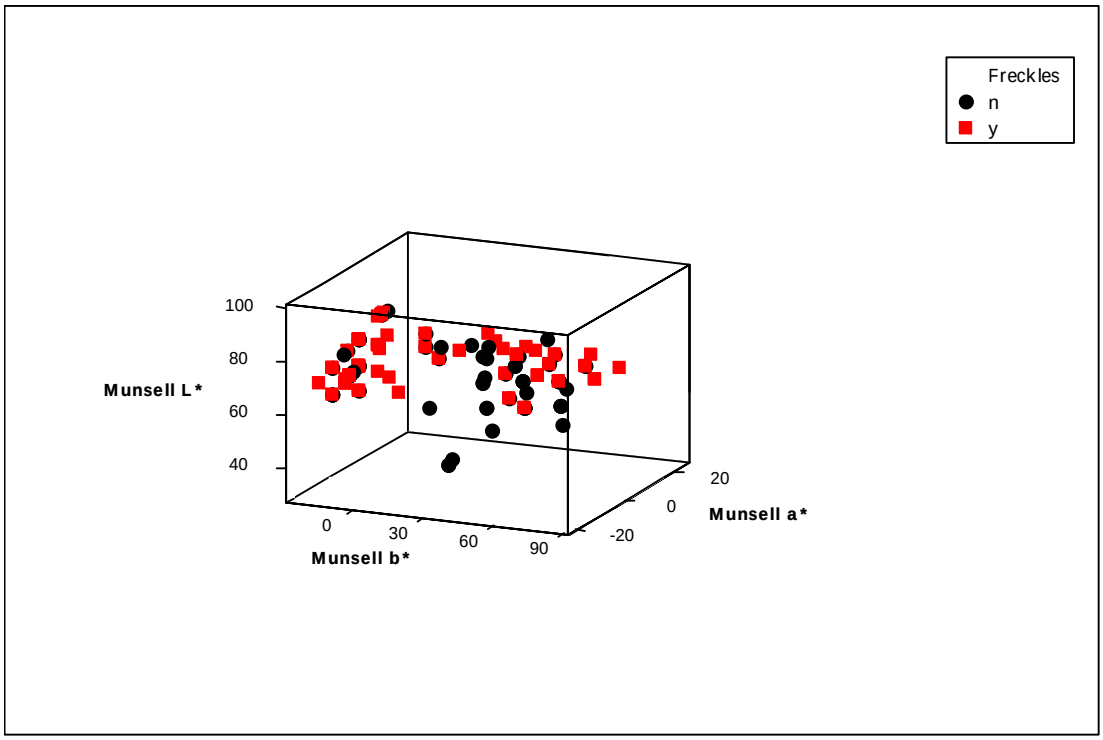
Figure 3.3.40. 3D scatter plot of eye colour with skin type



3.3.41. Distribution of eye colour with freckles

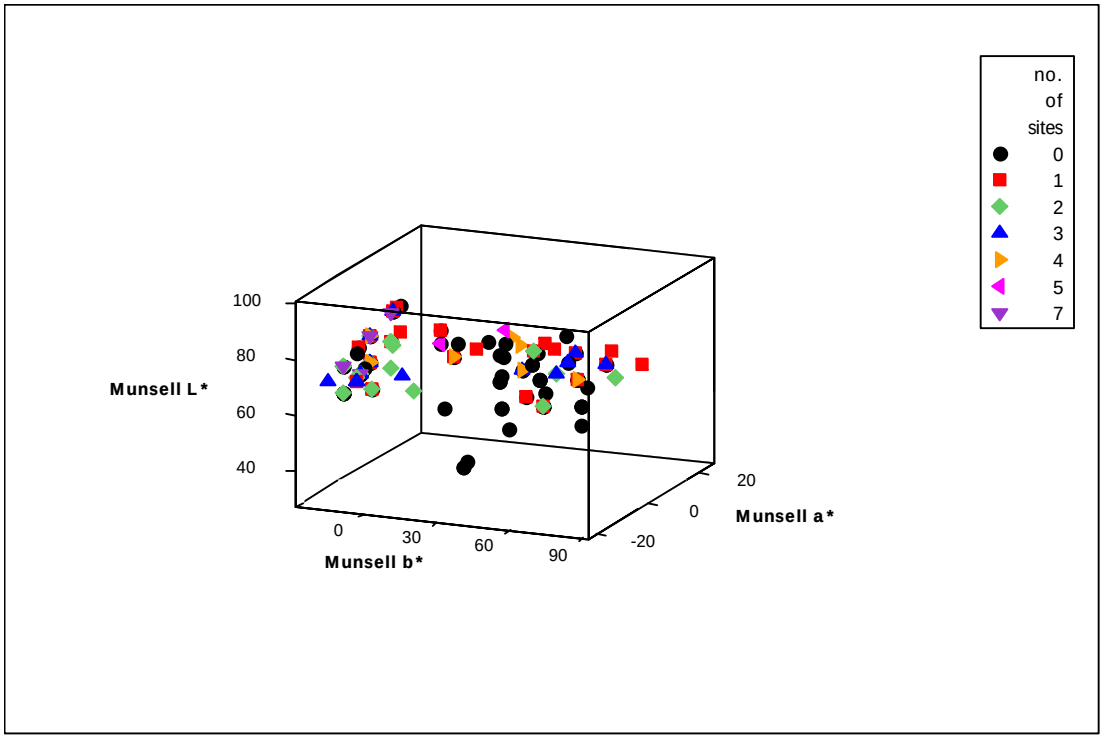
Presence of freckling

Figure 3.3.41.1. 3D scatter plot of quantitative Munsell L* a* b* eye colour with freckles



Number of freckling sites

Figure 3.3.41.2. 3D scatter plot of quantitative Munsell L* a* b* eye colour with number of freckling sites



3.4. Discussion

The static phenotypic variables of the 159 individuals in this study were presented in detail in this chapter. The key findings were summarised as follows.

This study sample consisted of younger individuals (mean age 25, median age 24), predominantly Northern European in origin. The higher proportion of females took part in the study probably reflecting females were more likely to agree to participate in volunteer studies. This was consistent with previous studies (Wagner *et al.*, 2002a; Wagner *et al.*, 2002b). The lesser number of skin type 5 and 6 in the study were due to the predominantly Northern European in origin of the study population and sample.

Hair and skin spectrophotometric reflectance data were new measures and did distinguish between groups.

Interestingly, females' skin colour is lighter than males. This is consistent with previous literature (Jablonski and Chaplin, 2000; Robins, 1991). One possible reason for this is that females require more calcium during pregnancy and lactation, therefore the lighter skin colour permits more UV light to penetrate the skin for vitamin D synthesis (Jablonski and Chaplin, 2000).

Other inter-relationships with phenotypic variables were as expected. E.g. those with freckles have lighter skin colour. Ethnicity was related to skin colour. The higher the skin type e.g. 6, the darker the skin colour (smaller L*). Eye colour was associated with skin colour. Red heads have lighter skin colour. Skin colour and hair colour were related. Hair colour and skin type were associated. Lighter hair coloured individuals have lighter skin colour. People who dyed their hair chose to dye their hair lighter in colour. The results from the static phenotypes were as expected.

I attempted to develop the methodology of quantitatively measuring eye colour further and showed that quantitative Munsell L* a* b* was useful and showed clustering of eye colour groups. This newer quantitative method could be used as a phenotypic measure in future studies.

Chapter 4 Induced Phenotype - Determinants of Erythema

4.1. Introduction

In this chapter, the erythemal responses of a subset of 98 individuals who have undergone UVB irradiation will be discussed. The determinants of erythema will also be presented. Erythema was measured using 2 assays:

i) change in erythemal index (with baseline erythemal index subtracted) and

ii) change in erythemal flux (with baseline erythemal flux subtracted).

The main measure of erythema data, erythemal index will be presented in section 4.2.1. Erythemal flux data will be presented in section 4.2.2. The primary analysis of erythema data was performed on erythemal index.

98 volunteers out of the initial cohort of 159 went on to have UVB irradiation. 57 volunteers did not undergo UVB irradiation. Volunteers who did not undergo UVB irradiation were excluded from analysis for this part of the study. The 98 volunteers were made up of 19 males (19%) and 79 females (81%). Two individuals returned for 24 hour measurements only and then defaulted. Two individuals did not wish to have their buttock irradiated and only had their back irradiated with UVB.

Table 4.1. Number of UVB irradiated individuals and sex

M	19	19%
F	79	81%
Total	98	

M denotes males, F denotes females

Erythema was chosen as an endpoint to measure experimentally induced, cutaneous phenotypic response to UVB irradiation.

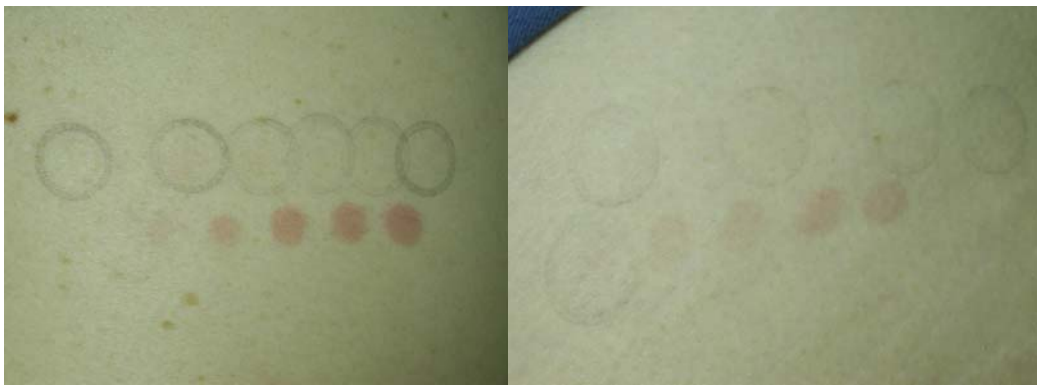
4.2. Erythemal response to UVB radiation

4.2.1. Determinance of erythemal sensitivity measured as the erythema index

Representative erythemal responses for volunteers to UVB were shown in Photo 4.2.1.

Photo 4.2.1. Visible erythema on the back and buttock 24-hour post UVB irradiation

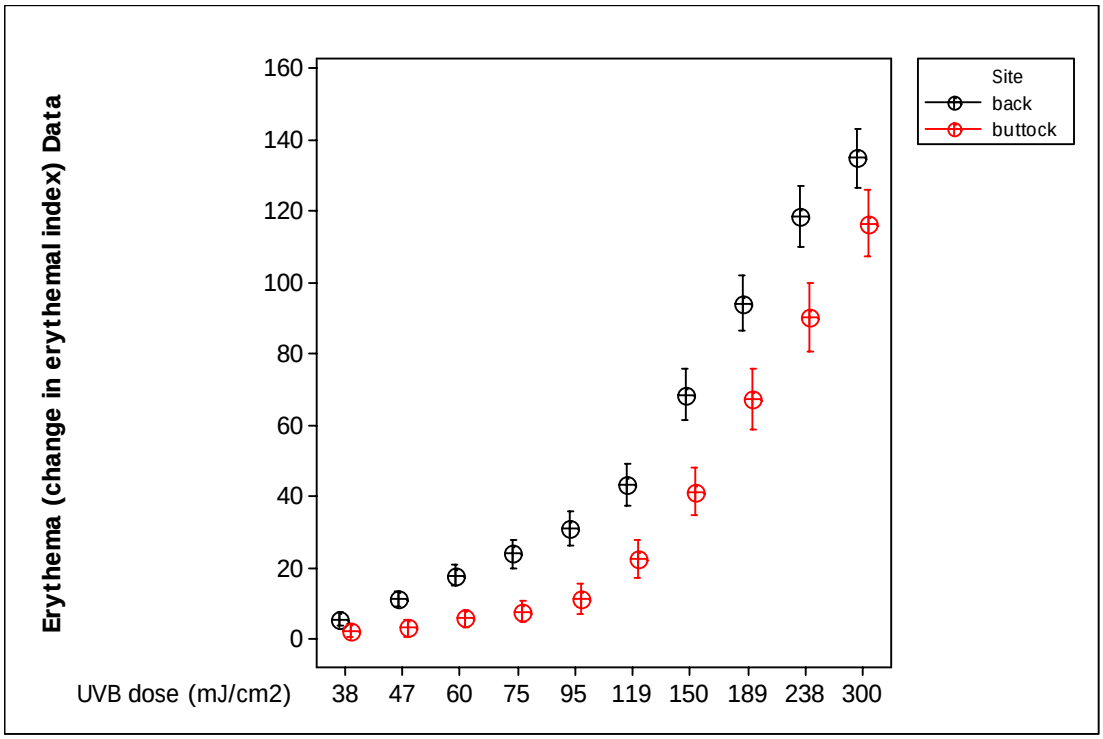
a) **b)**



Erythemal responses of a) back and b) buttock were shown. Ink markings were made on the skin following a cardboard template of where the 10 UVB doses were irradiated by the custom made UVB source.

The erythemal dose responses for back and buttock are shown in Figure 4.2.1.1.

Figure 4.2.1.1. Erythemal dose responses (EI) over back and buttock



This is an interval plot of EI ± SEM (95% CI for the mean) for the back (black) and buttock (red). Y-axis shows the change in erythemal index (with baseline erythemal index subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

The back responded to UVB more by becoming more erythematous than the buttock. Two sample t-test comparing UVB-induced back and buttock erythemal index showed significant difference for all 10 doses ($P<0.05$).

Because the data set is large, and because multiple readings were taken on the individual, then the problem of pseudo replication arises. Mixed effects model could be used to analyse these balanced and unbalanced grouped data which have arisen from repeated measures data by fitting these data into mixed effects model software. Rather than fit a complex mixed effects model, it was felt appropriate to inspect the curves and then choose representative doses.

For the majority of subjects, only 4 or 5 erythemal areas were visible (see Photo 4.2.1). Therefore, the top 5 doses were subsetted from the main data set and these form the basis for the following analyses.

Separate analyses were performed on the buttock and the back. Initially, dose responses for these top 5 doses for each individual were inspected. In the majority of these instances there was no convincing evidence of plateauing of the response when erythema, measured as the erythemal index, was plotted against the logarithm of the dose. It was, therefore, decided that analysis of the top dose (300mJ/cm^2) was appropriate to be examined. However, since the back is known to be more sensitive than the buttock, both doses (238mJ/cm^2 and 300mJ/cm^2) for both sites were looked at. The results are, however, similar and the results are presented for the highest dose.

Histograms of erythema show that the data appeared normally distributed and, therefore, parametric statistics were used. Initially the buttock responses were examined, followed by the back. Broadly, the results are remarkably similar in terms of factors that are thought to be important.

4.2.1.1. Erythema and age

Surprisingly, correlation tests and linear regression showed a positive effect of age, with an increasing erythemal dose with age. However, inspection of raw data showed that an individual age 40 was markedly influencing the regression line. When this

individual was excluded there was no relation with age (P=0.1276). Age is therefore not considered further.

4.2.1.2. Erythemal index results for buttock

4.2.1.2.1. Erythema and sex

There were no differences in erythemal sensitivity between the sexes with a mean of 133 in the females and 141 in the males (P=0.5459, two sample t-test).

4.2.1.2.2. Erythema and red hair

The presence or otherwise of red hair also had no effect on mean erythema (132) in those without red hair, compared with 149 in those with red hair (P=0.156, two sample t-test).

4.2.1.2.3. Erythema and blonde hair

Similarly, blonde hair had no effect with a mean of 134 in those without blonde hair and 140 in those with blonde hair (P=0.5889, two sample t-test).

4.2.1.2.4. Erythema and presence of freckles

The presence or otherwise of freckles has a striking effect on presence of erythema. In those without freckles, mean erythema was 121 whereas in those with freckles it was 147 (P=0.002018, two sample t-test).

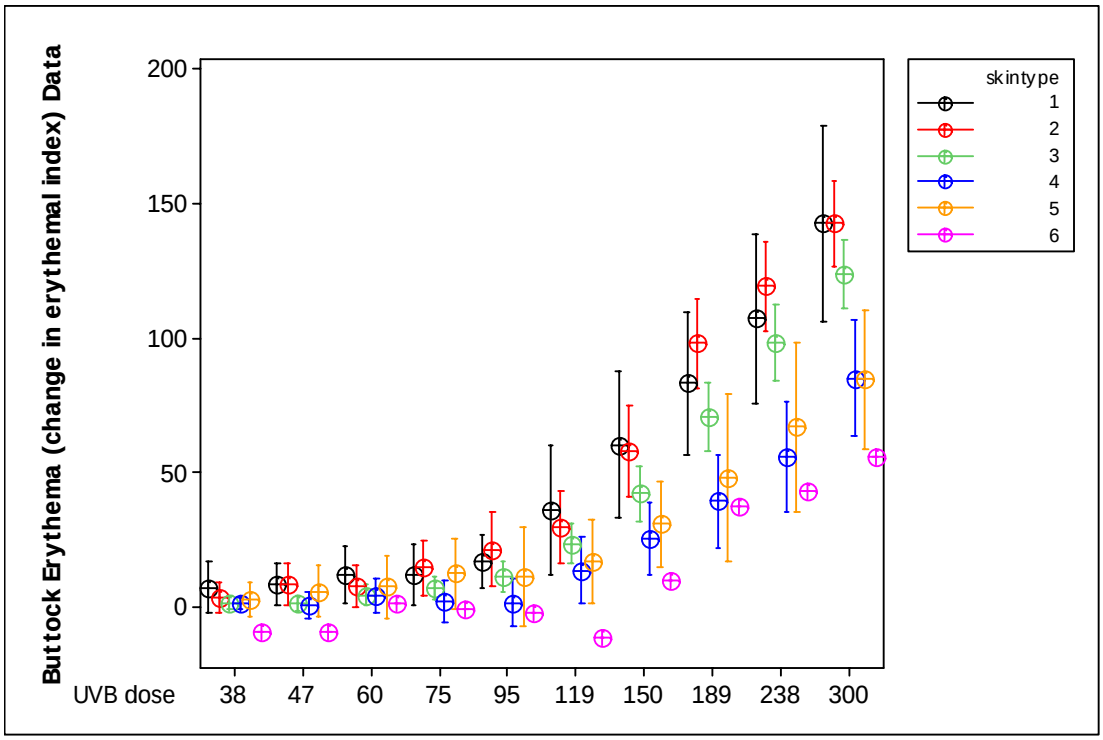
4.2.1.2.5. Erythema and ethnicity

Ethnic groups, when tested in an analysis of variance, also showed a marked effect ($P=2.6\times10^{-5}$). Tukey's HSD test was then applied. Highly significant differences were noted between Northern Europeans and Asians (P=0.0004467) and Northern Europeans and Mediterraneans (P=0.0051447) with mean responses of 98 in the Asians, 93 in the Mediterraneans and 143 in the Northern Europeans.

4.2.1.2.6. Erythema and skin type

Skin type was not dealt with as a factor, but treated as an equally spaced continuous variable. Regression showed, as expected, a marked effect of skin type with an adjusted R^2 of 0.2066 ($P=1.54\times10^{-6}$).

Figure 4.2.1.2.6. Erythemal dose responses (EI) over buttock and skin type



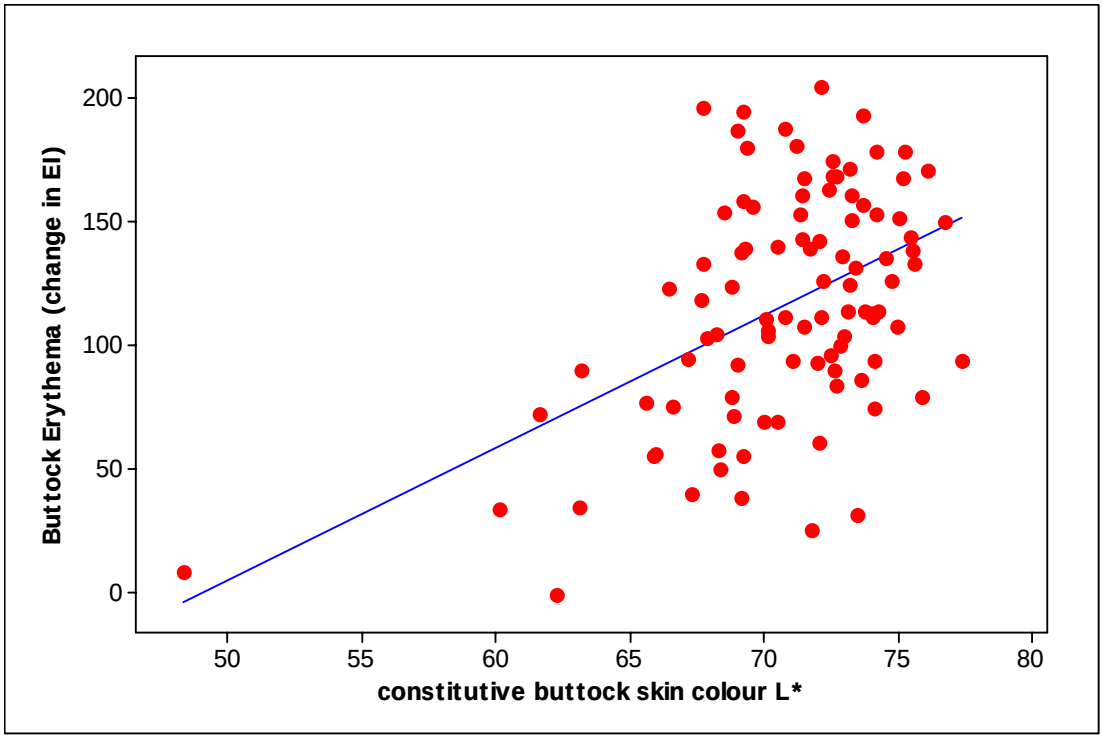
This is an interval plot of EI ± SEM (95% CI for the mean) for the buttock by skin type 1-6. Y-axis shows the change in erythemal index (with baseline erythemal index subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

4.2.1.2.7. Erythema and constitutive skin colour

Correlations were observed between erythema on the buttock and the L* score of the L* a* b* reading on the buttock. Regression of erythema on L* scale was highly significant ($P=1.29\times10^{-6}$), with an adjusted R^2 of 0.209.

Similarly, correlations were also observed between constitutive buttock skin colour and erythema. The P-value of 1.29×10^{-6} and a correlation of 0.47 between erythema and the L* score of buttock skin; and of P-value of 0.003168 and correlation coefficient of -0.30 of the a* reading of the L* a* b* score; of the b* score of buttock skin of $P=2.039\times10^{-5}$ and correlation of -0.42 were observed.

Figure 4.2.1.2.7. Erythema responses (erythemal index EI) and constitutive skin colour over buttock



This is a scatter plot of buttock erythema (EI) and constitutive buttock skin colour L*. Y-axis shows the change in erythemal index (with baseline erythemal index subtracted). X-axis shows the constitutive buttock skin colour in L* scale. Pearson correlation = 0.47. Adjusted R^2 = 0.209 ($P=1.29\times10^{-6}$).

Inspection of the raw data showed that an Asian (Indian) individual V160 with EI=8, L*=48 was possibly an outlier. If this individual was excluded from the analysis, similar correlations were still observed between constitutive buttock skin colour and erythema with Pearson correlation = 0.42, and adjusted R^2 = 0.178 ($P<0.0001$). However there was no particular reason to exclude this Asian since this population sample included Asians.

4.2.1.2.8. Erythema and hair colour

Correlations were also observed between hair colour and erythema. The P-value of 0.01714 and a correlation of 0.24 between erythema and the L* score of hair; and of P-value of 0.000353 and correlation coefficient of 0.35 of the a* reading of the L* a*

b* score; of the b* score of hair of P=0.004311 and correlation of 0.29 were observed.

4.2.1.2.9. Model to explain erythemal sensitivity

Linear regression was then used with multiple variables to explain erythemal sensitivity. Once skin colour at the particular site (buttock) was factored in, freckles were no longer significant. However, skin type remained significant (whichever way the regression was ordered) but once the skin type had been factored in, ethnic factor had no influence. The regression with skin with buttock L* and skin type is shown below.

Formula = Erythema buttock ~ buttock L* + skin type

	Estimate	SE	t value	P r(>t)
(Intercept)	-51.15	80.95	-0.63	0.52901
Buttock L*	3.16	1.04	3.05	0.00294
Skin type	-12.45	4.16	-2.99	0.00353

F-statistic 18.93 on 2 and 95 degrees of freedom, $P=1.205\times10^{-7}$

This regression explains about 27% of the variation as reflected by the adjusted R^2.

4.2.1.3. Erythemal index results for back

4.2.1.3.1. Erythema and sex

There was no difference in the erythema index between sex (P=0.5459, two sample t-test). Female mean 133, male mean 141.

4.2.1.3.2. Erythema and red hair

There were no differences between those with or without red hair (P=0.156, two sample t-test), mean in those without red hair 132 and in those with red hair 149.

4.2.1.3.3. Erythema and blonde hair

There were no differences between those with or without blonde hair with respect to erythemal index on the back, mean in those without blonde hair 134 and in those with blonde hair 140 (P=0.5889, two sample t-test).

4.2.1.3.4. Erythema and presence of freckles

As with the results for the buttock, freckles was highly significant (P=0.002018, two sample t-test) with the mean in those without freckles of 122 compared with 147 in those with freckles.

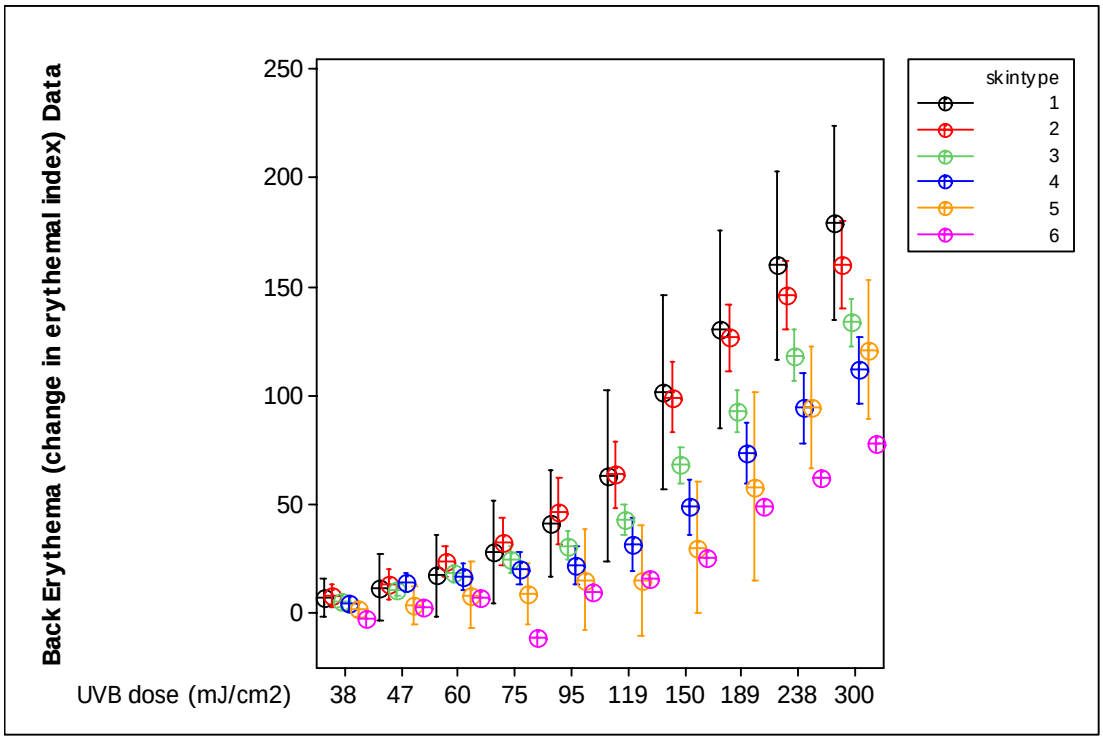
4.2.1.3.5. Erythema and ethnicity

An analysis of variance showed that ethnic factor was highly significant ($P=2.6\times10^{-5}$) and Tukey's test showed highly significant differences between Northern Europeans and Asians (P=0.0004467) and Northern Europeans and Mediterraneans (P=0.0051447). The direction of the differences was as for the responses on the buttock.

4.2.1.3.6. Erythema and skin type

Skin type treated as a continuous variable was also highly significant, with an adjusted R^2 of 0.21 ($P=1.54\times10^{-6}$).

Figure 4.2.13.6. Erythema dose responses (EI) over back and skin type

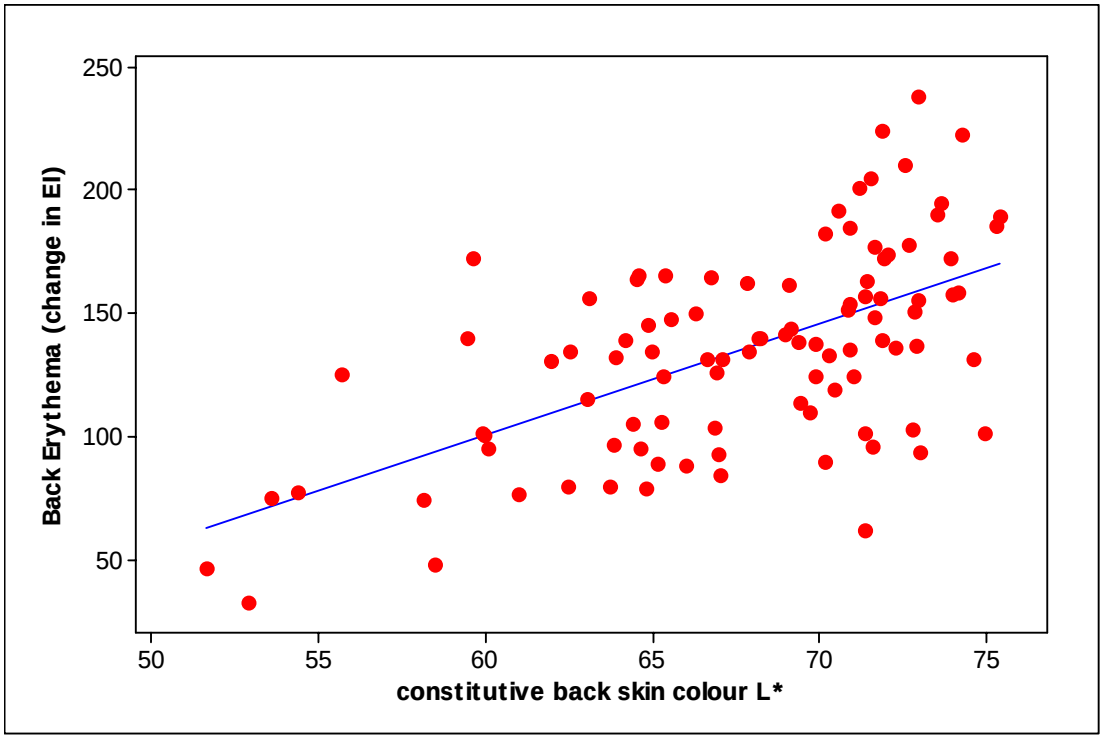


This is an interval plot of EI ± SEM (95% CI for the mean) for the back by skin type (1-6). Y-axis shows the change in erythemal index (with baseline erythemal index subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

4.2.1.3.7. Erythema and constitutive back skin colour

As for results on the buttock, there were highly significant correlations between erythema index and the L*, a* and b* scores on the L* a* b* measure on the back. The P-value of 1.49×10^{-10} and a correlation of 0.59 between erythema and the L* score of back skin; and of P-value of 3.42×10^{-8} and correlation coefficient of -0.52 of the a* reading of the L* a* b* score on back; of the b* score of back skin of P<0.0001 and correlation of -0.504 were observed. Constitutive back skin colour L* predicts erythema index over the back.

Figure 4.2.1.3.7. Erythema responses (erythemal index EI) and constitutive skin colour over back



This is a scatter plot of back erythema (EI) and constitutive back skin colour L* (n=98). Y-axis shows the change in erythemal index (with baseline erythemal index subtracted). X-axis shows the constitutive back skin colour in L* scale. Pearson correlation = 0.59. Adjusted R^2 = 0.34 (P=1.49×10^{-10}).

4.2.1.3.8. Model to explain erythemal sensitivity - back

As for the buttock, a linear model was fitted with freckles not being significant once back and skin colour had been taken into account. Unlike the buttock, with skin type included the R^2 failed to achieve formal significance with a P-value of 0.161. This difference could be explained by the difference in body site – the back may have undergone acclimatisation to solar exposure and therefore have taken into account the effect of skin type.

The results for the linear regression of erythemal index of the back on back L* are shown.

Formula = Erythema back ~ back L*

	Estimate	SE	t value	P r(>t)
(Intercept)	-164.71	41.90	-3.93	0.00016
Back L*	4.45	0.62	7.18	1.49×10^{-10}

F-statistic = 51.53 on 1 and 96 degrees of freedom, $P=1.49\times10^{-10}$

This regression explains about 34% of the variation as reflected by the adjusted R^2.

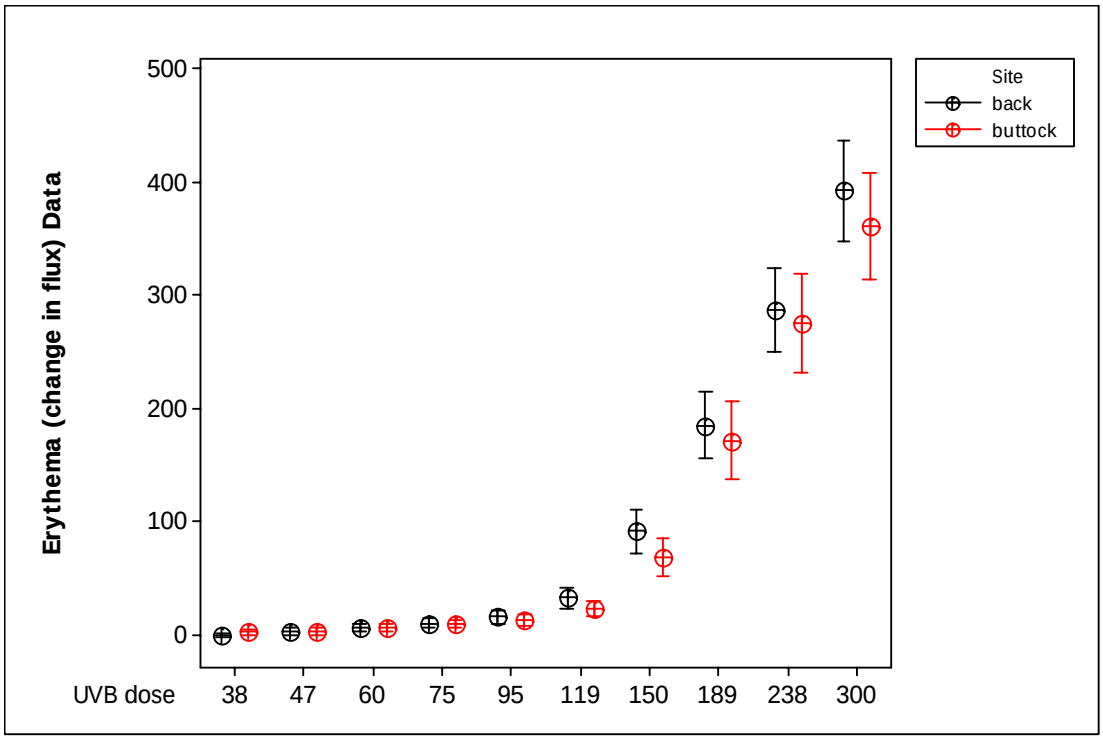
The findings of erythema at the second highest UVB dose 238mJ/cm^2 were in keeping with those at 300mJ/cm^2.

4.2.2. Erythema flux results

Erythema flux was the second assay used to measure erythema. For ease of clarity and conciseness, important points will be presented and highlighted in terms of erythemal flux in this section. The measure of erythema flux (via the contact Doppler probe) is very sensitive to pressure and touch. Er ythema flux has more variability (coefficient of variability (SD/mean) of up to 10%) than Erthemal Index (EI) (coefficient of variation of up to 5%). Despite these potential limitations, broadly similar results were found.

4.2.2. Erythema dose response (flux) by site

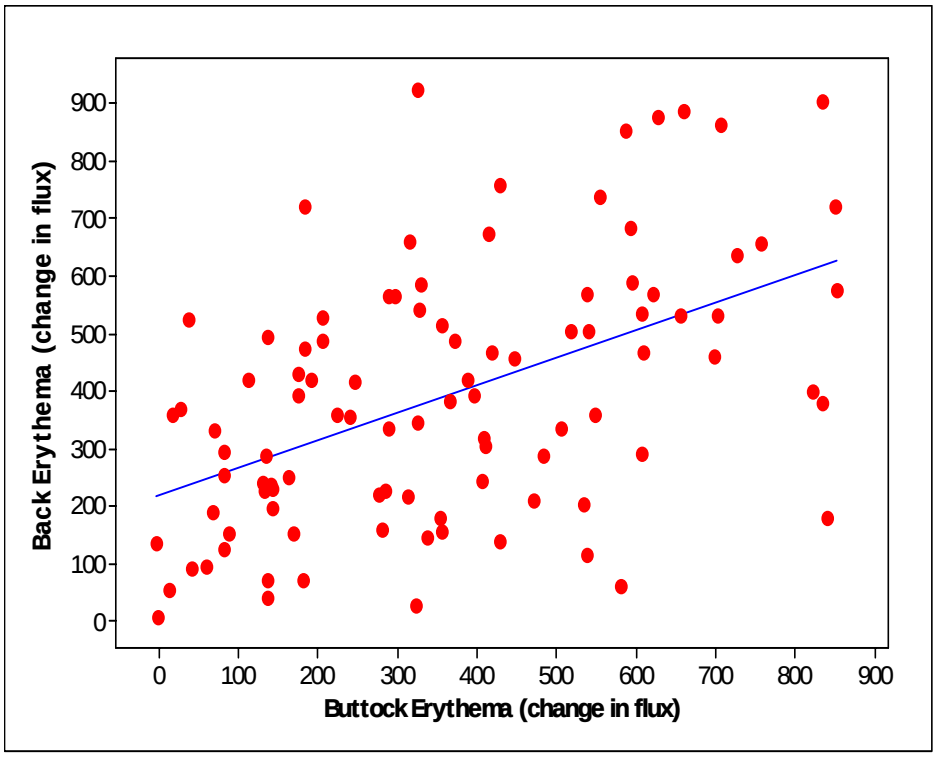
Figure 4.2.2. Erythema dose response (flux) on back and buttock



This is an interval plot of erythemal flux ± SEM (95% CI for the mean) for the back (black) and buttock (red) by UVB doses. Y-axis shows the change in erythema flux (with baseline erythema flux subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

4.2.3. Does back erythema predicts buttock erythema?

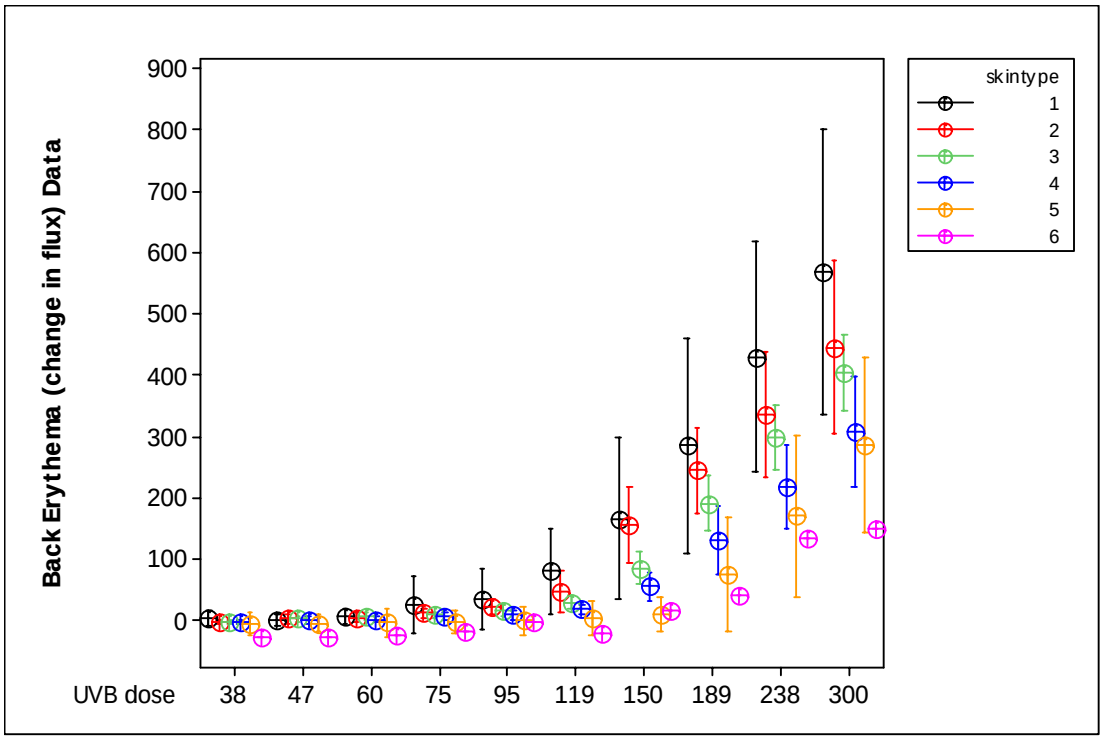
Figure 4.2.3. Back Erythema and Buttock Erythema (flux)



This is a scatter plot of back erythema (flux) and buttock erythema (flux). Y-axis shows the change in erythemal flux (with baseline erythemal flux subtracted) over back. X-axis shows the change in erythemal flux (with baseline erythemal flux subtracted) over butttock. Pearson correlation = 0.498. Adjusted R^2 = 0.248 (P<0.0001).

4.2.4. Erythema Flux and Skin Type (Back)

Figure 4.2.4. Erythemal dose responses (Flux) over back and skin type



This is an interval plot of erythemal flux ± SEM (95% CI for the mean) for the back by skin type 1-6. Y-axis shows the change in erythemal flux (with baseline erythemal flux subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

It is clear from the above graph that the means of erythemal flux move with skin type. The means of erythemal flux reduce as you increase from skin type 1 to 6.

The degree of UVB-induced erythema over back was greater than that of the buttock. The mean erythema flux over back for skin type I at 8.2 SEDs (300mJ/cm^2) = 571 ± 95.

Europeans skin types 1-2 are roughly twice more sensitive than non-Europeans skin types 4-5 (Average flux 600:300).

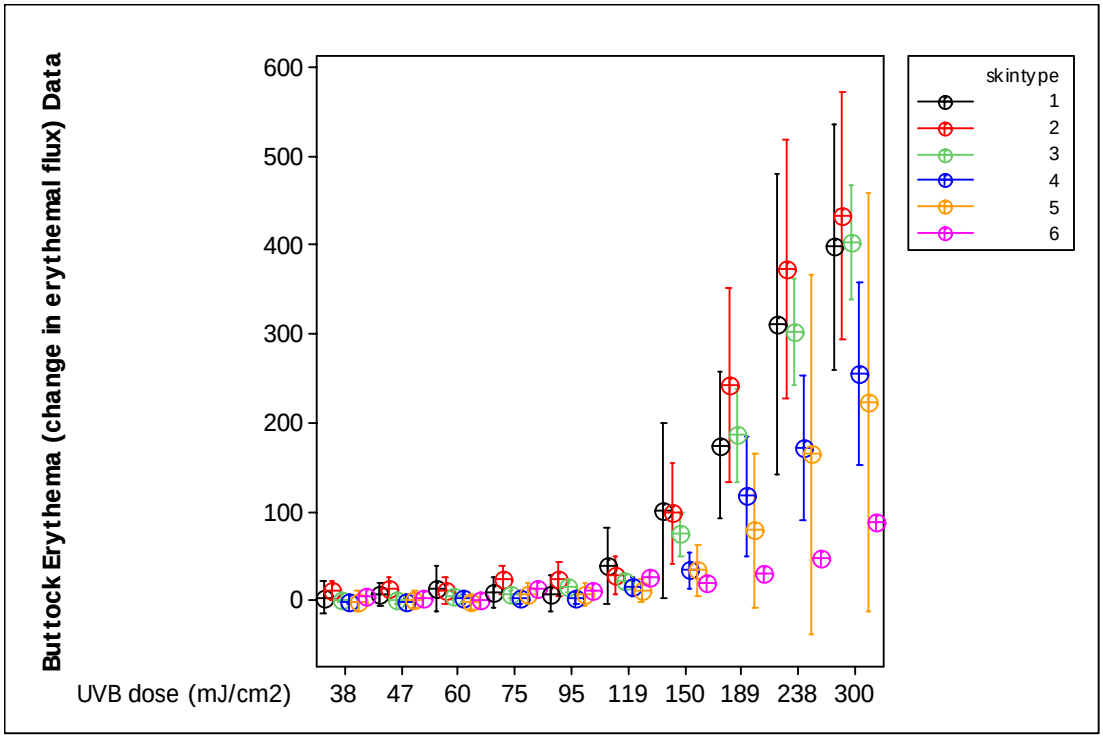
Table 4.2.4. Mean erythemal flux at 8.2 SEDs (300mJ/cm^2) i.e. dose 3

Skin type	Mean erythemal flux (back)	SEM (back)	Mean erythemal flux (buttock)	SEM (buttock)	n
I	571	95	399	57	7
II	447	66	434	66	16
III	405	31	405	32	46
IV	308	43	257	50	22
V	287	56	224	92	6
VI	151	-	89	-	1

Skin type and erythema flux summary – The erythemal response of the back to UVB irradiation was greater than that of the buttock. This trend was similar across all skin types.

4.2.5. Erythema Flux and Skin Type (Buttock)

Figure 4.2.5. Erythemal dose responses (Flux) over buttock and skin type

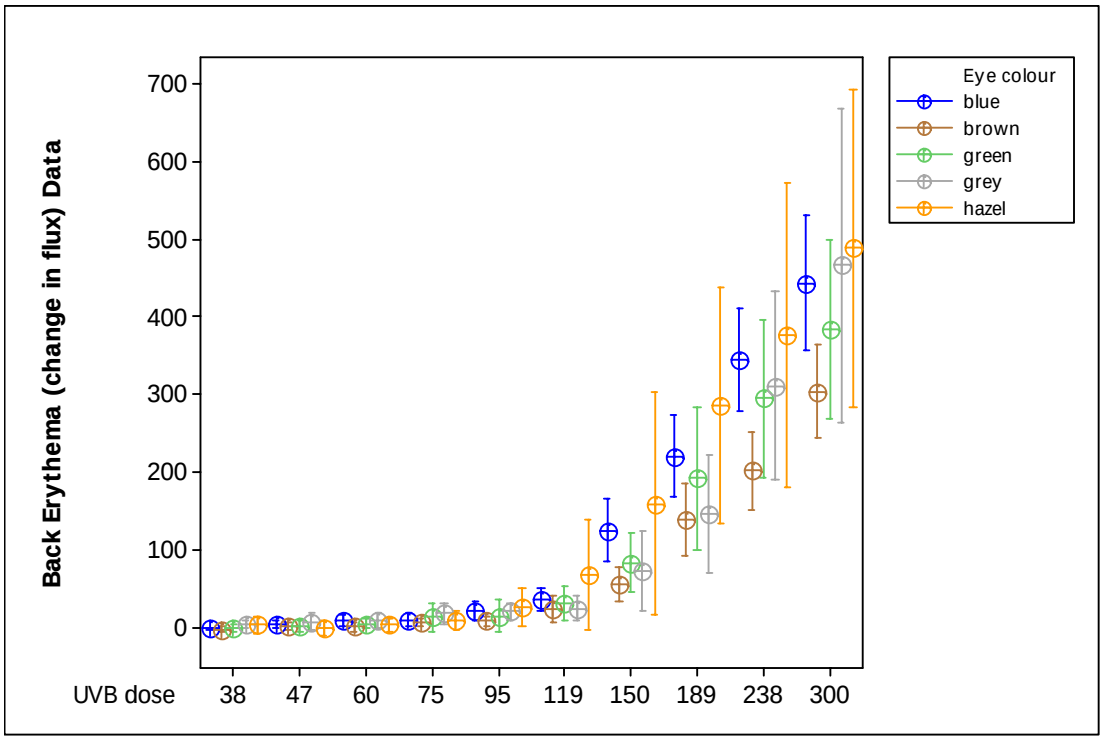


This is an interval plot of erythemal flux ± SEM (95% CI for the mean) for the buttock by skin type 1-6. Y-axis shows the change in erythemal flux (with baseline erythemal flux subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

The degree of UVB-induced erythema over buttock was less than that of the back. Mean erythema flux over buttock for skin type I at 8.2 SEDs (300mJ/cm^2) = 399±57.

4.2.6. Erythema Flux and eye colour

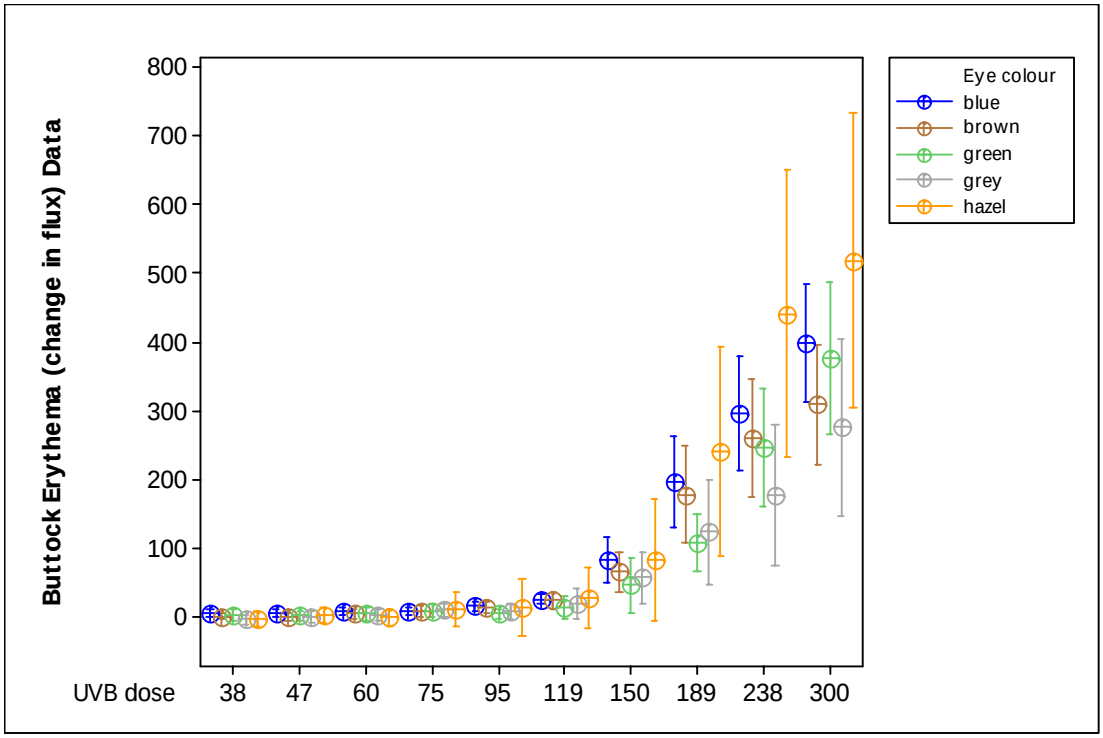
Figure 4.2.6.1. Erythema Flux (back) and eye colour



One-way ANOVA showed no significant difference between eye colour groups and erythema flux over the back (P=0.0559). Although this did not reach formal significance, individuals with blue eye colour appear to develop more erythema (than brown or green). This is in keeping with other studies which reported association of blue eye colour characteristics and skin cancers e.g. blue eye colour has a RR of 1.55 times that of brown eye colour in developing melanoma (Bliss *et al.*, 1995). This is important even if the result was not formally significant. A larger sample size may demonstrate a significant result.

However, some studies did not find any association of eye colour with erythema or burning. Azizi *et al* found no effect of eye colour on minimal erythemal dose (MED) (Azizi *et al.*, 1988).

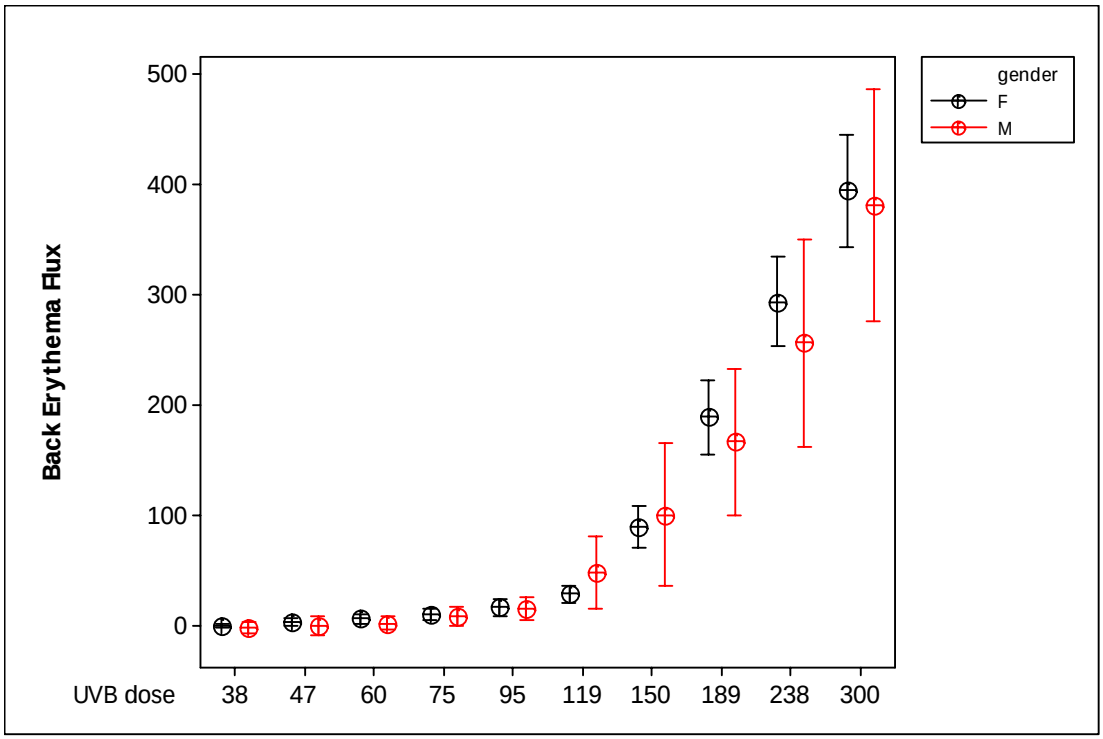
Figure 4.2.6.2. Erythema Flux (buttock) and eye colour



One-way ANOVA showed no significant difference between eye colour groups and erythema flux over the buttock (P=0.133).

4.2.7. Erythema Flux and sex

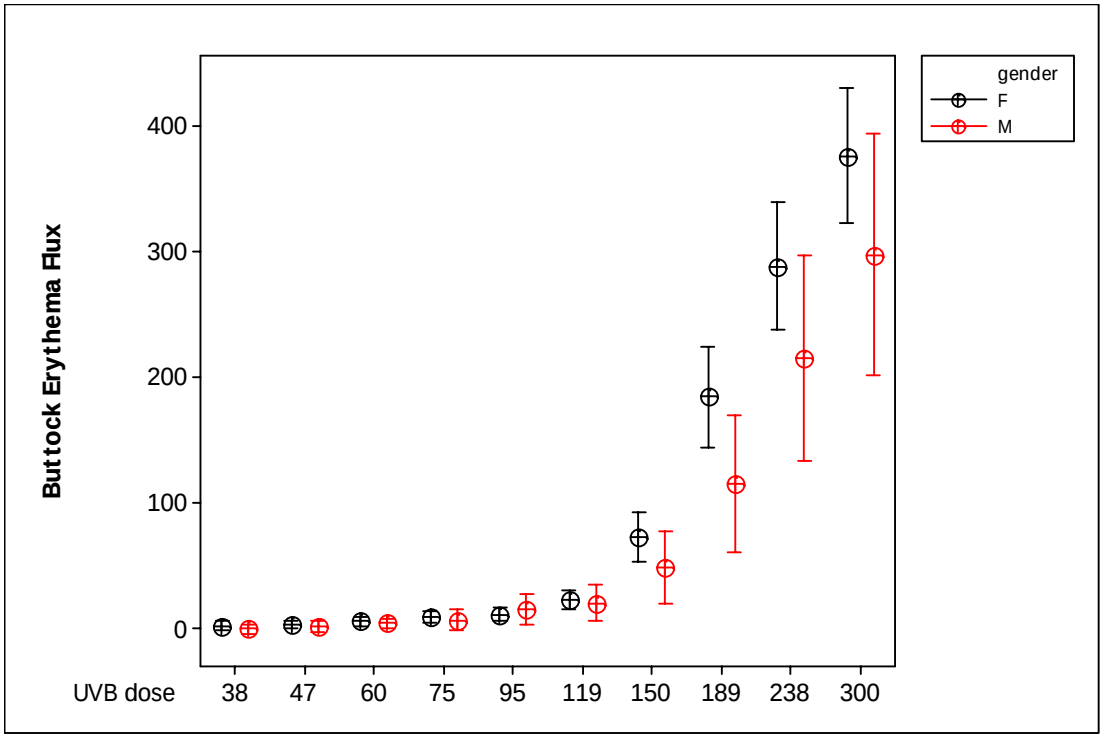
Figure 4.2.7.1. Erythema Flux (back) and sex



This is an interval plot of erythemal flux ± SEM (95% CI for the mean) for the back by sex, Y-axis shows the change in erythemal flux (with baseline erythemal flux subtracted). Females (black), males (red). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

Two sample t-test comparing males and females for back erythemal flux at UVB Doses 1-3 showed no significant difference (P=0.86, P=0.53, P=0.81).

Figure 4.2.7.2. Erythema Flux (buttock) and sex



This is an interval plot of erythemal flux ± SEM (95% CI for the mean) for the buttock by sex, Y-axis shows the change in erythemal flux (with baseline erythemal flux subtracted). Females (black), males (red). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

Two sample t-test comparing males and females for buttock erythemal flux at UVB doses 189mJ/cm^2 (dose 2) showed significant difference (P=0.04). Other doses 95, 150, 238, 300mJ/cm^2 were not significant (P=0.50, P=0.18, P=0.12, P=0.15). Females showed a higher erythemal response (flux) than males at 189mJ/cm^2 (dose 2).

4.3. Discussion

Erythema was used as an endpoint to measure UVB-induced responses. Erythemal index (EI) was chosen to be the primary measure for erythemal responses. As mentioned, the primary analysis of erythema data was performed on erythemal index. Erythema flux measurements have a higher degree of variability (coefficient of variability of up to 10%) and also sensitive to pressure and touch. Nevertheless, similarly results were obtained with both assays of erythema. Erythema flux was used to confirm no increase in blood flow after noradrenaline iontophoresis was performed.

There was a dose dependent increase in erythema with increasing UVB radiation over both back and the buttock at 24 hours. The back developed more erythema than the buttock. This is in keeping with previous findings (Rhodes and Friedmann, 1992; Waterston *et al.*, 2004).

The major determinants of erythema for this study population were constitutive buttock skin colour L* and skin type. Constitutive buttock skin colour does predict erythema index of buttock, accounting for about 21% of the variation (R^2 of 0.209, $P=1.29\times10^{-6}$).

This is in keeping with Ha *et al* that pigmentary differences between people are a major determinant of erythemal responses (Ha *et al.*, 2003).

It is important to note that skin type is actually just as good as constitutive skin colour L* in predicting erythema, accounting for about 21% of the variation (R^2 of 0.2066, $P=1.54\times10^{-6}$).

Other factors e.g. freckling may already be a marker of lighter constitutive skin colour and the propensity to burn or go red (Bliss *et al.*, 1995; Palmer *et al.*, 2000; Valverde *et al.*, 1995) and therefore is, as expectedly, related to erythema.

Ethnicity and hair colour are factors that are related to constitutive skin colour. Different ethnic groups have different ranges of constitutive skin colours. Overlap in skin colour range has been taken into account when analysing the results quantitatively. Lighter hair colour individuals tend to be lighter skin coloured as well. This in turn may explain their erythemal responses.

For the back, the inclusion of skin type in the erythema model still failed to achieve formal significance. This may be due to the fact that at the photoexposed back, the

ability of the constitutive back skin to develop erythema has already been "factored" in, and Fitzpatrick skin type did not add additional predictive value. Whereas at the normally photoprotected buttock, skin type does add additional predictive value, according to the model.

The erythema results presented were in agreement with Wagner *et al* (Wagner *et al.*, 2002a). This study showed that buttock erythema was related to constitutive buttock skin colour L* and skin type and that back erythema was related to constitutive back skin colour L*. Wagner argued that there was a very strong inverse relationship between constitutive pigmentation and erythemal dose response (EDR) in their population i.e. people with higher constitutive pigmentation levels have lower burning responses. They used different assays to measure constitutive skin colour (adjusted melanin index AMI - which is the slope of 650: 700nm calculated from spectrophotometer apparent absorbance), erythema (slope of change in a*) and facultative pigmentation (adjusted melanin index AMI) (Wagner *et al.*, 2002b).

In addition their methodology did not remove erythema before using L* as a measure of tanning. Wagner showed that skin L* and baseline erythema were correlated (R^2 = 17.6%, P<0.001) (Wagner *et al.*, 2002b) and a* correlated with adjusted slope of change in a* (not EI or flux).

The variation explained by this study is higher than that of Wagner *et al*. R^2 = 27% (buttock) to 34% (back); $P=1.205\times10^{-7}$ (buttock), $P=1.49\times10^{-10}$ (back). This difference in variance explainable may be due to difference in methodology and / or the site of UVB irradiation. Their population of study is different in terms of bigger sample size, broader population with more Asians. On the contrary this population of study consisted of a homogenous population mostly of Caucasian Northern Europeans and smaller number of reds and smaller sample size. This study methodology used direct measures of erythema (erythemal index and erythemal flux) whereas Wagner *et al* used derived equations for erythemal responses.

One could arrive at different answers depending on what population is being studied. Another difference is that their constitutive skin colour choice of skin site was upper medial arm.

Wagner *et al* found constitutive pigmentation in females was slightly lower than in males in all three samples, but the difference was not significant. While no

differences were observed in melanogenic dose response (MDR) between sexes, males had a higher EDR than females (i.e. steeper slope of a*) regardless of population or constitutive pigmentation level, and this sex difference was significant in European Americans and Hispanics. However in their study, the site of UV irradiation was on medial aspect of arms. This study used the mid back and the buttock. Previous literature reported sex difference (Wagner *et al.*, 2002b). I found females to have a higher erythemal dose response in terms of erythemal flux (P=0.04 at dose 2). This could have arisen by chance 4 out of 100 times. The study populations were different in the 2 studies, with different methodology also – these could all explain the different findings. The importance of differences in study methodology and the study population should be emphasized.

There were no other previous reports on sex differences in cutaneous erythemal responses. Wagner *et al* suggested that perhaps hormonal or physiological causes may be plausible explanations e.g. Mercurio *et al* showed that oestrogen increases water holding capacity of stratum corneum (Mercurio, 1998).

Previous studies did not find an effect of age on erythema (Cox *et al.*, 1992; Lim *et al.*, 2008). This is not surprising given this study has a limited age range of 18-40.

Given the narrow age range, it was not surprising that no significant effect of age on tanning was found in my study.

It is also worthy to note that the previous literature was confusing on minimal erythemal dose (MED) and skin type, with different conclusions drawn from different studies:

1) no correlation (Chung *et al.*, 1994; Tejasvi *et al.*, 2007; Westerhof *et al.*, 1990)

2) poor or weak (Rampen *et al.*, 1988; Youn *et al.*, 1997)

3) unreliable (Park *et al.*, 1998)

4) inconclusive (Stanford *et al.*, 1996)

5) correlate (Li and Chu, 2007; Liu *et al.*, 2006b; Wee *et al.*, 1997)

MED determination is very subjective method, and dependent on the operator, skin colour, lighting (Diffey and Robson, 1992) and environmental temperature (Wagner *et al.*, 2002a). This study's skin type data were objective and comprised of quantitative measurements of erythema. This is in keeping with (Li and Chu, 2007; Liu *et al.*, 2006b; Wee *et al.*, 1997) despite the shortcomings recognised with skin

type. One important point to note is the way the skin type was obtained in this study by reading the classification criteria to the volunteers and they make their choice. It is important to standardise the criteria of skin type and how the questionnaires were administered.

In summary, erythemal responses can be predicted simply from constitutive skin colour L* and/or skin type. It is interesting to note that a recent paper by Pershing *et al* (Pershing *et al.*, 2008) argued that reflectance spectrophotometer of upper volar arm provides a rapid non-invasive and reliable method to objectively determine 6 skin types using only skin colour at the upper volar arm. Pershing *et al* did not have any erythema or tanning measures though and the site of constitutive skin colour assessment was different to this study.

The relationship between erythema and *MC1R* genotype will be discussed in Chapter 6.

Chapter 5 Tanning responses – Determinants of Tanning

5.1. Introduction

Individuals vary in their sensitivity to the sun. The facultative pigmentary response (tanning) also varies. The aim of this part of the study was to irradiate a subset of 98 individuals with UVB radiation and objectively measure their tanning responses to investigate the determinants of tanning.

The results are presented under several headings. First the results from the objective measure of tanning (5.2.1), then the correlation between experimentally induced tanning and co-factors including erythema in section 5.2.2, and finally the discussion is in section 5.3.

5.2. Results

5.2.1. Objective measures of tanning responses

The facultative pigmentary (tanning) responses were measured objectively using 3 assays:

a) Tanning by change in L*, a* and b* values. L* was used as the chief measure of tanning. This will be discussed later.

b) Tanning by change in melanin index (MI). The limitations with this assay will be discussed later.

c) Tanning by change in spectrophotometry 360-740nm. An attempt to analyse the results from this assay proved difficult.

3 other tanning assays were developed while undertaking this study:

d) Tanning by pressure spectrophotometry, the methodology discussed in Chapter 2.

e) Tanning by change in 'Dwyer calculation method' 420-400nm from baseline. The tanning data was analysed by following the Dwyer principles (Dwyer *et al.*, 1998).

f) Tanning by the "Shriver method" (Wagner *et al.*, 2002a). The analysis of the data also proved complicated.

It was therefore decided that noradrenaline iontophoresis was used instead and tanning represented by change in L* score.

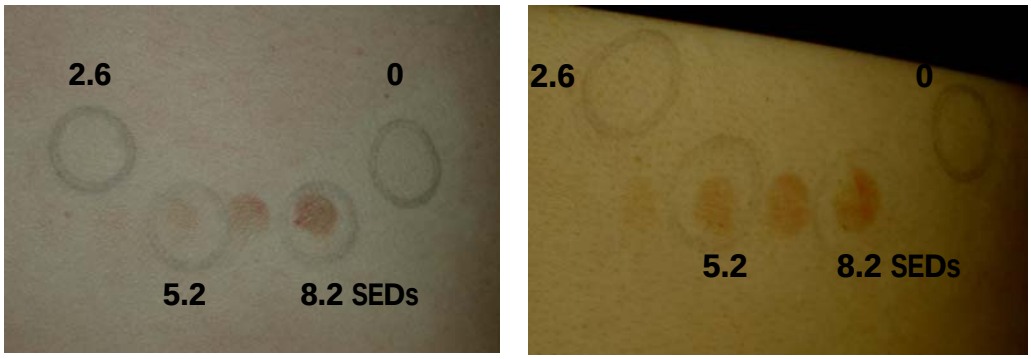
Two anatomical sites were chosen for the tanning experiments: back (photoexposed) and buttock (photoprotected). UVB-induced facultative pigmentary responses (tanning) were recorded. The time point of maximal tanning at 7 day was previously determined (Oh *et al.*, 2004) and used. All tanning assay results presented in this thesis referred to the same time point of 7 day to measure tanning.

Representative tanning responses for volunteers to UVB were shown in Photo 5.2.1.

Photo 5.2.1. Tanning responses on the back and buttock 7 days post UVB irradiation Post-noradrenaline iontophoresis.

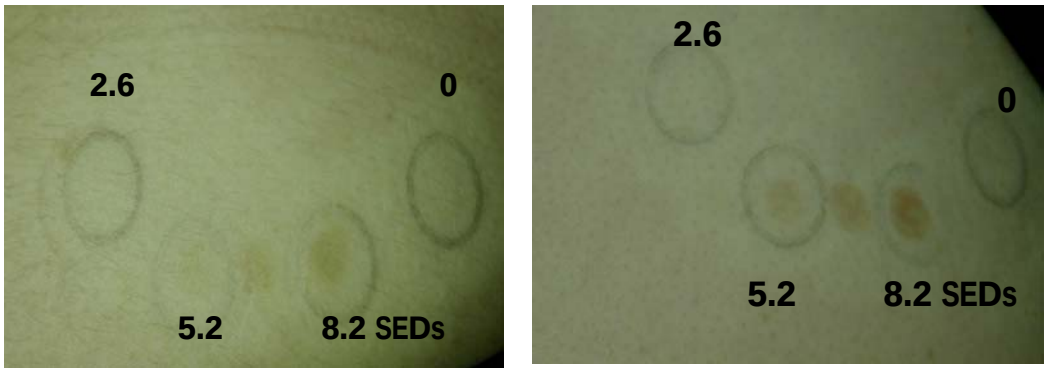
a)

Back:



b)

Buttock:

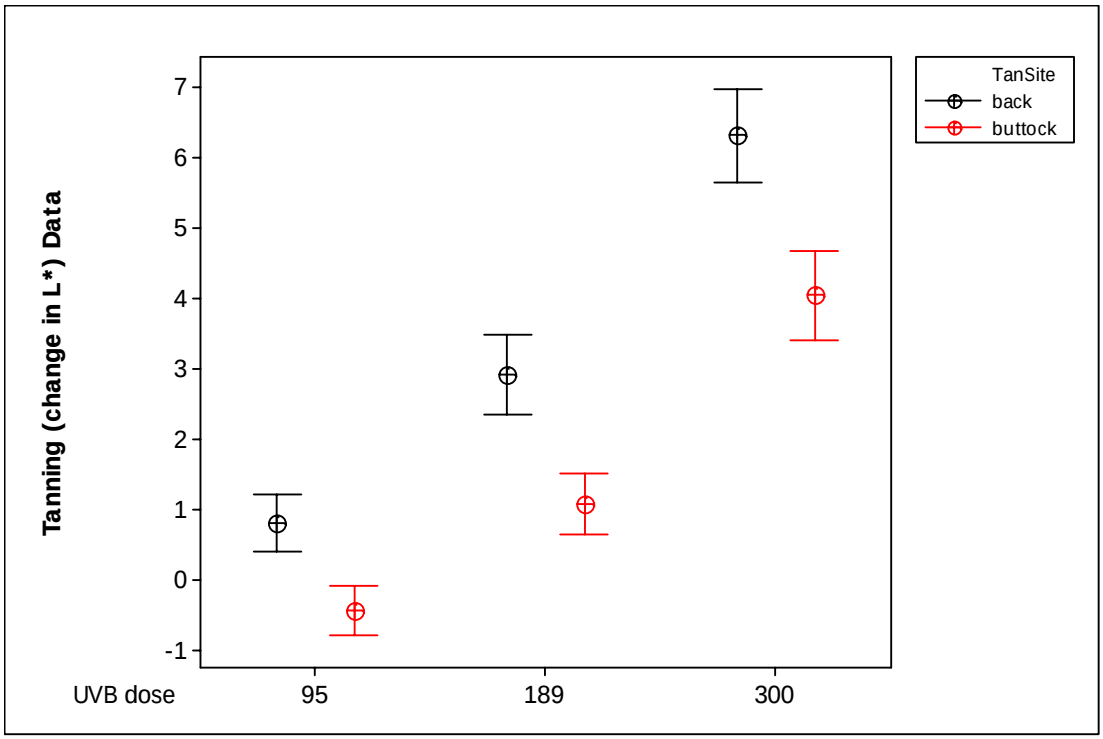


Tanning responses of a) back and b) buttock were shown. Ink markings were made on the skin following a cardboard template of where the UVB doses were irradiated by the custom made UVB source. 3 UVB doses (doses 1-3) i.e. 2.6, 5.2 and 8.2 SEDs were measured. These were post noradrenaline iontophoresis.

5.2.1.a. Tanning by change in L*, a* and b*

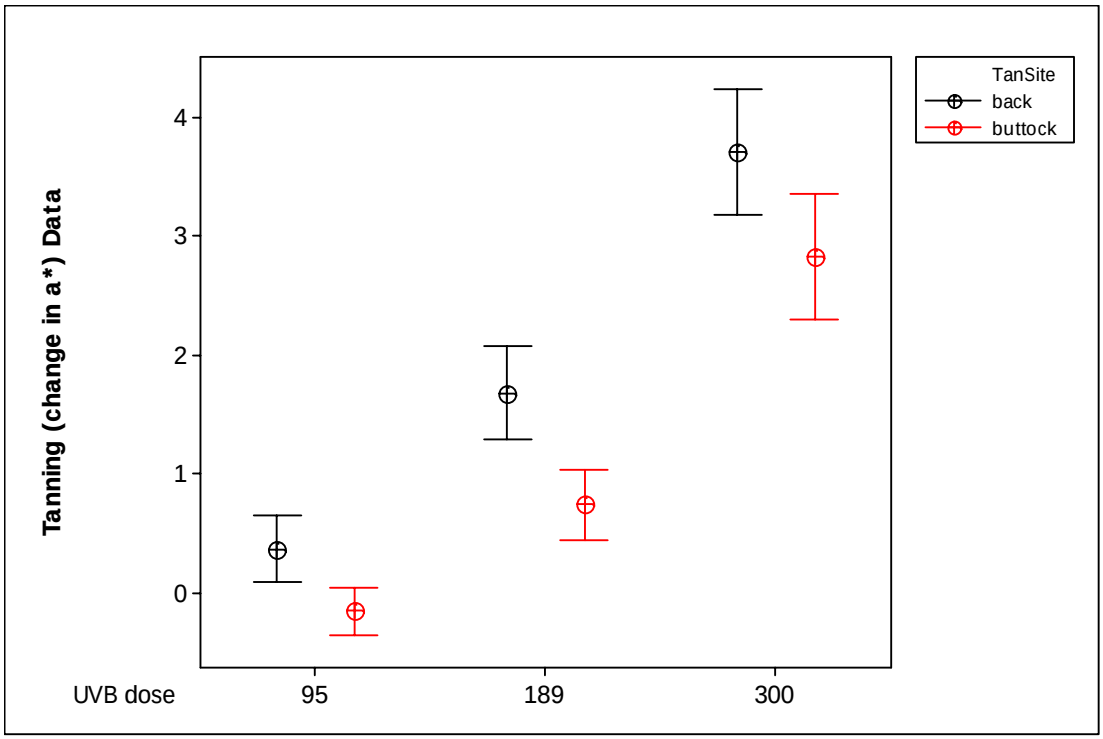
Quantitative measurements in terms of the change in L*, a* and b* were chosen as the main assay endpoint to measure tanning.

Figure 5.2.1.a.1. Tanning responses in terms of change in L* to UVB doses 1-3 on the back and buttock



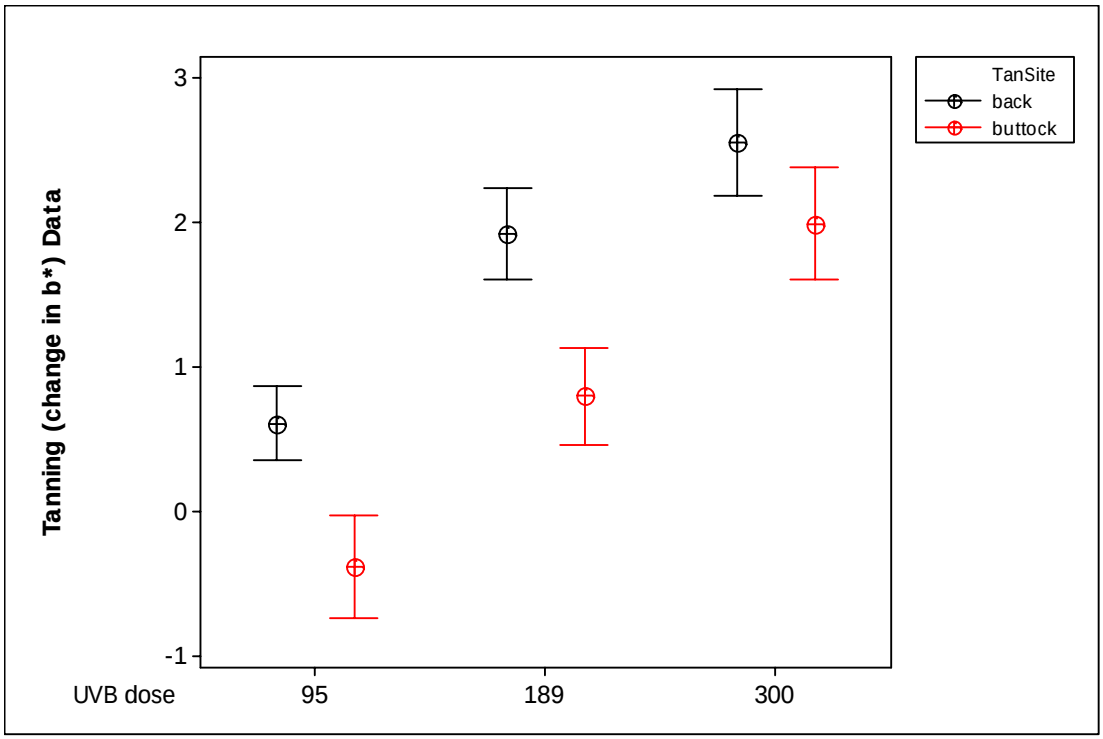
This is an interval plot of change in L* ± SEM (95% CI for the mean) for the back (black) and buttock (red) in 98 UVB-irradiated individuals at 7 days. Y-axis shows the change in L* (with baseline L* subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

Figure 5.2.1.a.2. Tanning responses in terms of change in a* to UVB doses 1-3 on the back and buttock



This is an interval plot of change in a* ± SEM (95% CI for the mean) for the back (black) and buttock (red) in 98 UVB-irradiated individuals at 7 days. Y-axis shows the change in a* (with baseline a* subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

Figure 5.2.1.a.3. Tanning responses in terms of change in b* to UVB doses 1-3 on the back and buttock



This is an interval plot of change in b* ± SEM (95% CI for the mean) for the back (black) and buttock (red) in 98 UVB-irradiated individuals at 7 days. Y-axis shows the change in b* (with baseline b* subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

From the graphs one can see a dose dependent increase in tanning for back and buttock. The increase in tanning at the back appeared greater than that of the buttock. Two sample t test comparing tanning at back vs buttock at dose 1 ($P<0.05$), dose 2 ($P<0.05$) and dose 3 ($P<0.05$) showed significant difference for change in L* score. Similarly significant results comparing tanning between back and buttock for a* and b* were shown.

What about between different UVB doses? Significant differences for tanning L* between back dose 1 and 2, dose 2 and 3, dose 1 and 3 ($P<0.05$, $P<0.05$, $P<0.05$). Similarly significant results comparing tanning between doses of back for a* ($P<0.05$, $P<0.05$, $P<0.05$) and b* ($P<0.05$, $P<0.05$, $P<0.05$) were shown. As for the buttock, there were significant differences for L* between buttock at dose 1 and 2 ($P<0.05$), dose 2 and 3 ($P<0.05$), dose 1 and 3 ($P<0.05$). Similarly significant results

comparing tanning between doses of buttock for a* ($P<0.05$, $P<0.05$, $P<0.05$) and b* ($P<0.05$, $P<0.05$, $P<0.05$) were shown.

Table 5.2.1. Mean tanning in terms of change in L* a* b* with SEM with UVB doses 1, 2 and 3 at 7 days

Tanning site	Dose	Mean tanning (change in L*)	SEM (L*)	Mean tanning (change in a*)	SEM (a*)	Mean tanning (change in b*)	SEM (b*)
Back	1	0.82	0.21	0.37	0.14	0.61	0.13
Buttock	1	-0.43	0.18	-0.16	0.10	-0.38	0.18
Back	2	2.92	0.28	1.68	0.20	1.93	0.16
Buttock	2	1.09	0.22	0.74	0.15	0.80	0.17
Back	3	6.31	0.33	3.70	0.27	2.56	0.19
Buttock	3	4.05	0.32	2.82	0.26	2.00	0.19

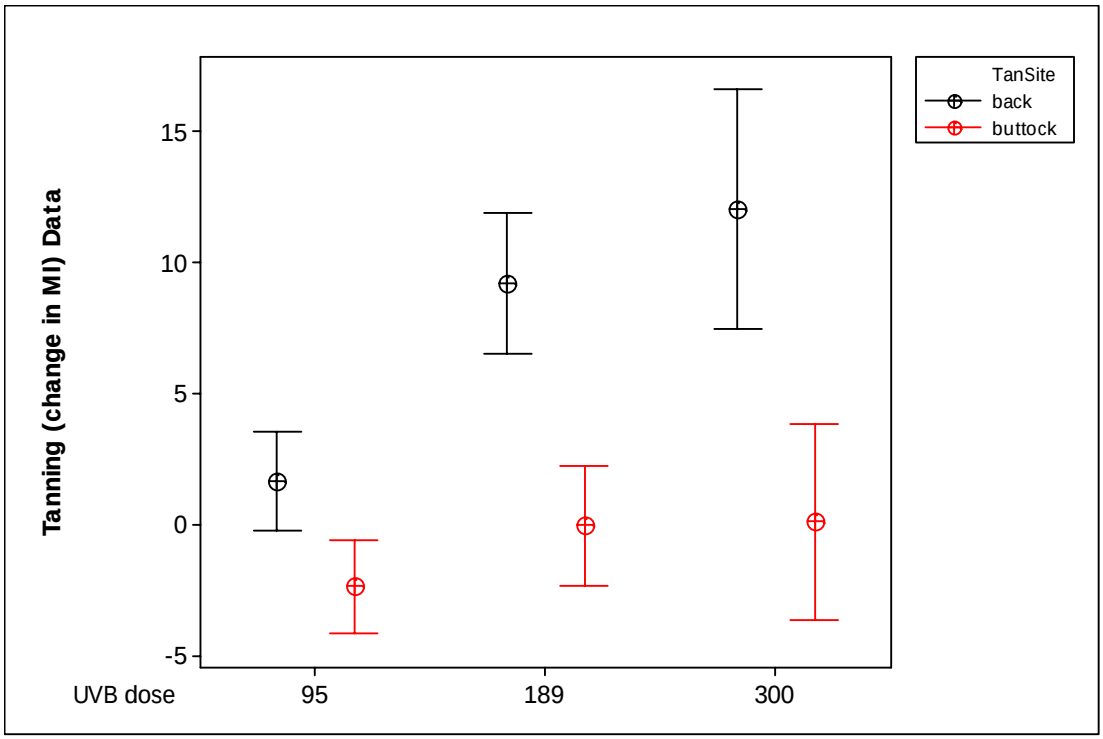
n=98

The back tans significantly more than the buttock with a mean increase of 6.3 ± 0.3 L* units for the back and 4.1 ± 0.3 L* units for buttock ($P<0.05$) in response to dose 3 (8.2 SEDs) of UVB. There was a dose-dependent increase in tanning for both the back and the buttock. There was a dose dependent UVB-induced increase in L*, a* and b*.

The average gradient of tanning response for the back L* (0.98) was higher than that for the buttock L* (0.80).

5.2.1.b. Tanning by change in Melanin Index at 7 days – back and buttock

Figure 5.2.1.b. Tanning (change in Melanin Index) – back and buttock



This is an interval plot of change in MI ± SEM (95% CI for the mean) for the back (black) and buttock (red) in 98 UVB-irradiated individuals at 7 days. Y-axis shows the change in MI (with baseline MI subtracted). X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale.

Similarly the back tans more than the buttock in terms of change in MI. Two sample t test showed significant difference between back and buttock at dose 1, 2 and 3 ($P<0.05$, $P<0.05$, $P<0.05$). Significant difference between back dose 1 and 2 ($P<0.05$), dose 1 and 3 ($P<0.05$). But no significant difference between back dose 2 and 3 ($P=0.29$). There were no significant difference between buttock at dose 1 and 2 ($P=0.12$), dose 2 and 3 ($P=0.94$), dose 1 and 3 ($P=0.24$). There was no further dose dependence for the 2 higher doses at the back and no dose dependence for the buttock.

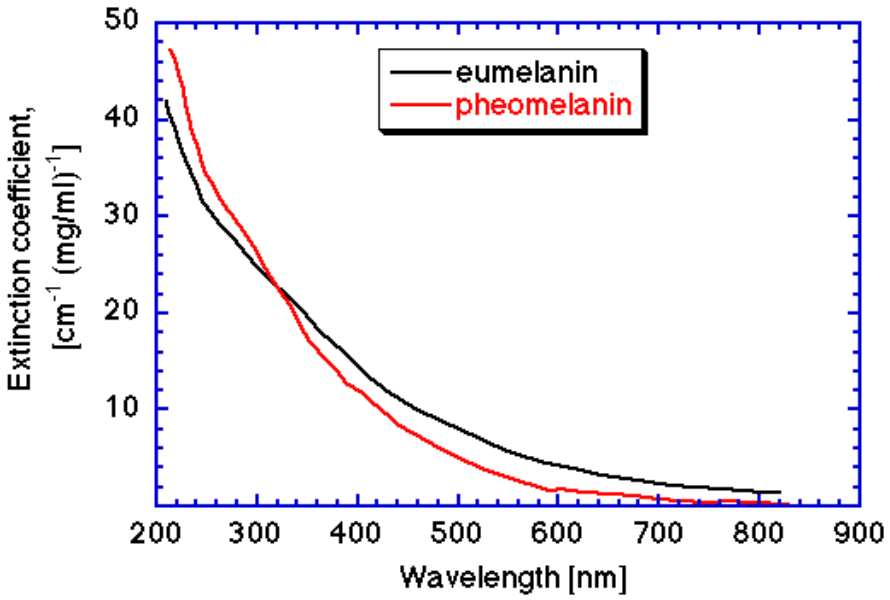
Tanning by MI data (Figure 5.2.1.b.) did not show an as "neat" dose response as compared to tanning by change in L* data (Figure 5.2.1.a.1) in the absence of blood. There was no obvious explanation for this. Possible explanation of this discrepancy could be the rationale of the measurement of MI by the erythema meter in that it compares blood and melanin at 632 nm and then use a reference wavelength of

905nm. MI measurement is a function of the remittance at 632nm (for melanin absorption) and 905nm, the reference signal (Diffey *et al.*, 1984; Feather *et al.*, 1988). An increase in pigmentation should result in a more positive melanin index, but not always the case as seen in the tanning response over the buttock. Melanin index measurements are independent of blood flow, however even after noradrenaline iontophoresis, the melanin index data still did not give a neat dose response. As L* a* b* scores are more widely used and standardised, it would seem preferable that L* a* b* should be used chiefly.

5.2.1.c. Tanning by reflectance spectrophotometry 360-740nm

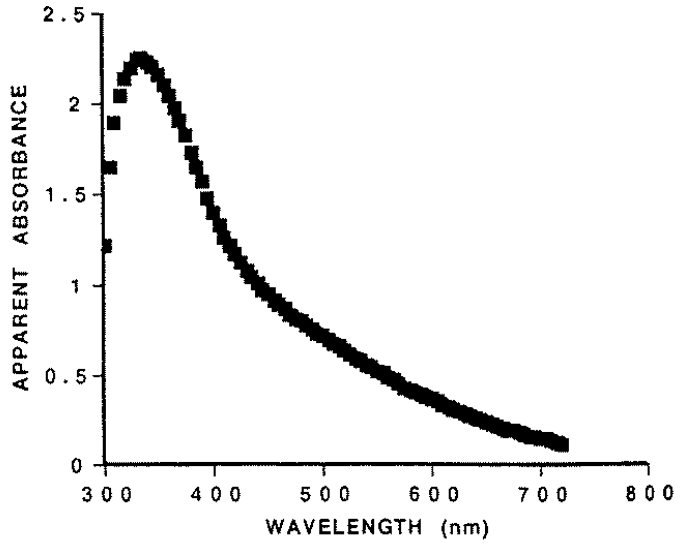
The third assay measure of tanning was using reflectance spectrophotometry. In a physiological setting, reflectance was due to blood (haemoglobin) and pigment (melanin) normally in the skin. The pigment melanin absorbs maximally at 335nm wavelength, and absorbs almost none at longer than 700nm (Kollias, 1995). Note the type of melanin was human melanin. Reflectance is inversely proportional to absorbance.

Figure 5.2.1.c.1. Spectral absorption of melanins



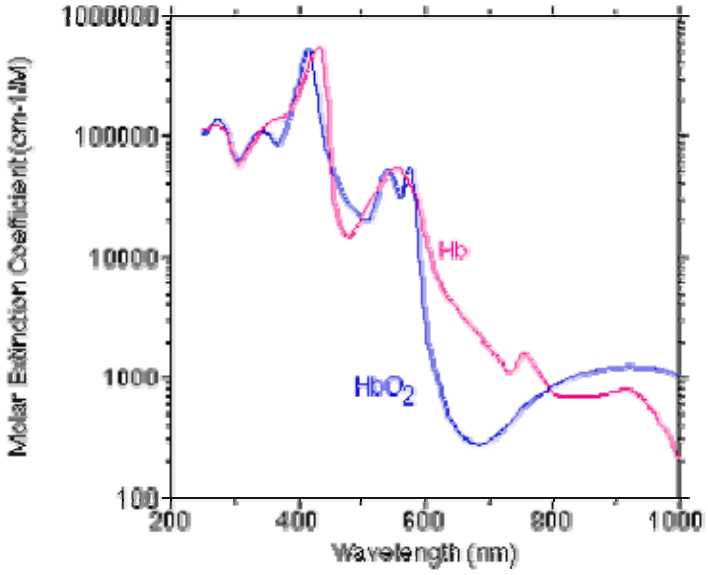
These are spectral absorption graphs for eumelanin and phaeomelanin. Y-axis, relative absorption. X-axis, wavelength (nm). Note these are synthetic melanins eumelanin (using DHI monomer) and phaeomelanin. Source: (Sarna, 1988).

Figure 5.2.1.c.2. Spectral absorption of melanin



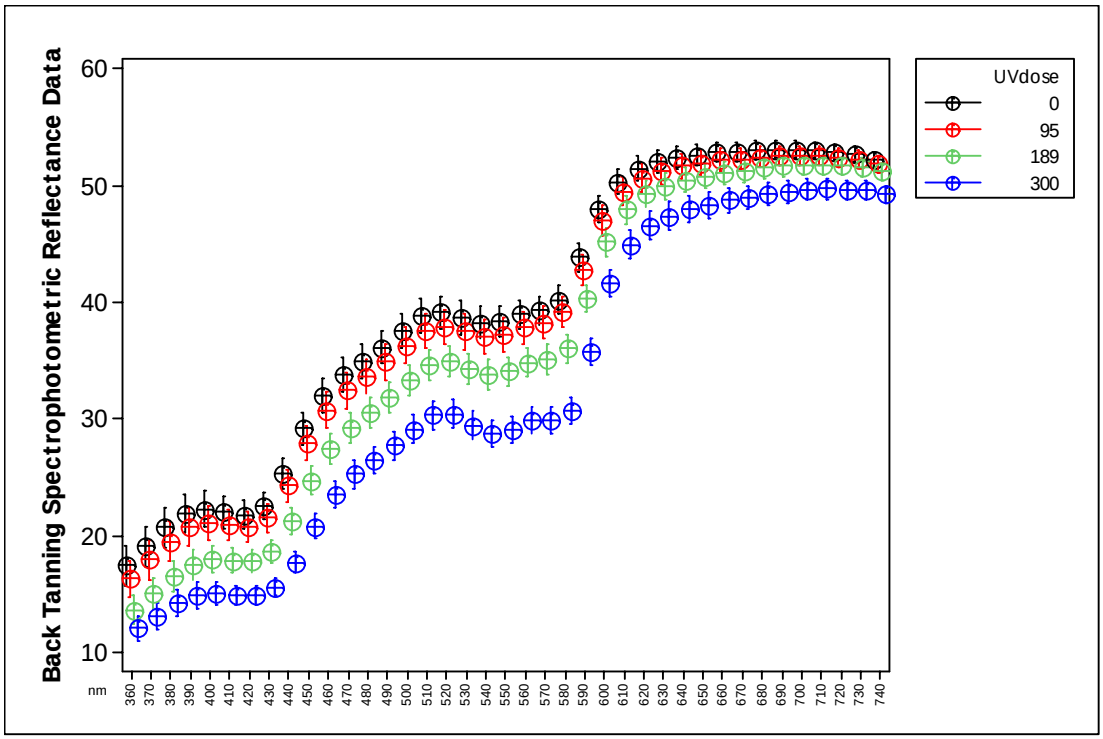
This is a spectral absorption graph for human melanin. Y-axis shows the apparent absorbance. X-axis is wavelength (nm). Source: (Kollias, 1995).

Figure 5.2.1.c.3. Spectral absorption of haemoglobin



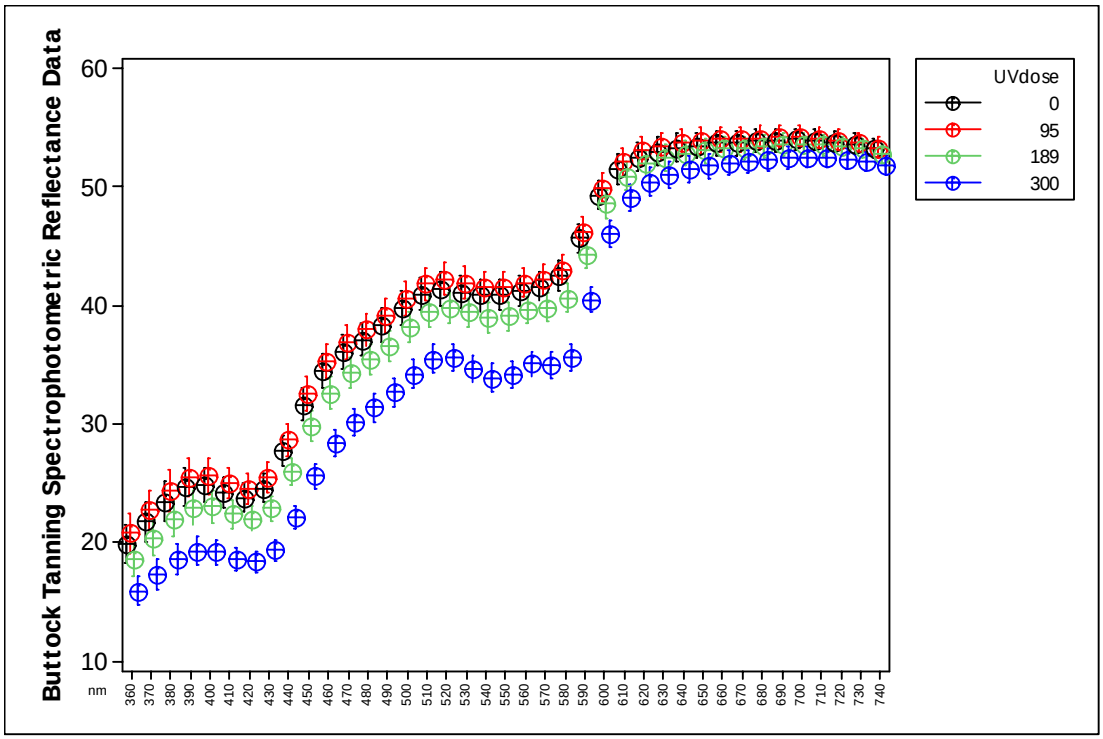
These are spectral absorption graphs for oxyhaemoglobin and deoxyhaemoglobin. Y-axis, relative absorption. X-axis, wavelength (nm). Source: http://omlc.ogi.edu/spectra/hemoglobin/summary.html. Data from W. B. Gratzer, Med. Res. Council Labs, Holly Hill, London and N. Kollias, Wellman Laboratories, Harvard Medical School, Boston.

Figure 5.2.1.c.4. Back Tanning results by reflectance spectrophotometry 360-740nm (absolute)



This is an interval plot of tanning by spectrophotometry ± SEM (95% CI for the mean) for the back (n=98). Y-axis shows the absolute spectrophotometric reflectance data (without baseline spectrophotometric reflectance data subtracted). X-axis shows the 10nm increments of 360-740nm wavelengths.

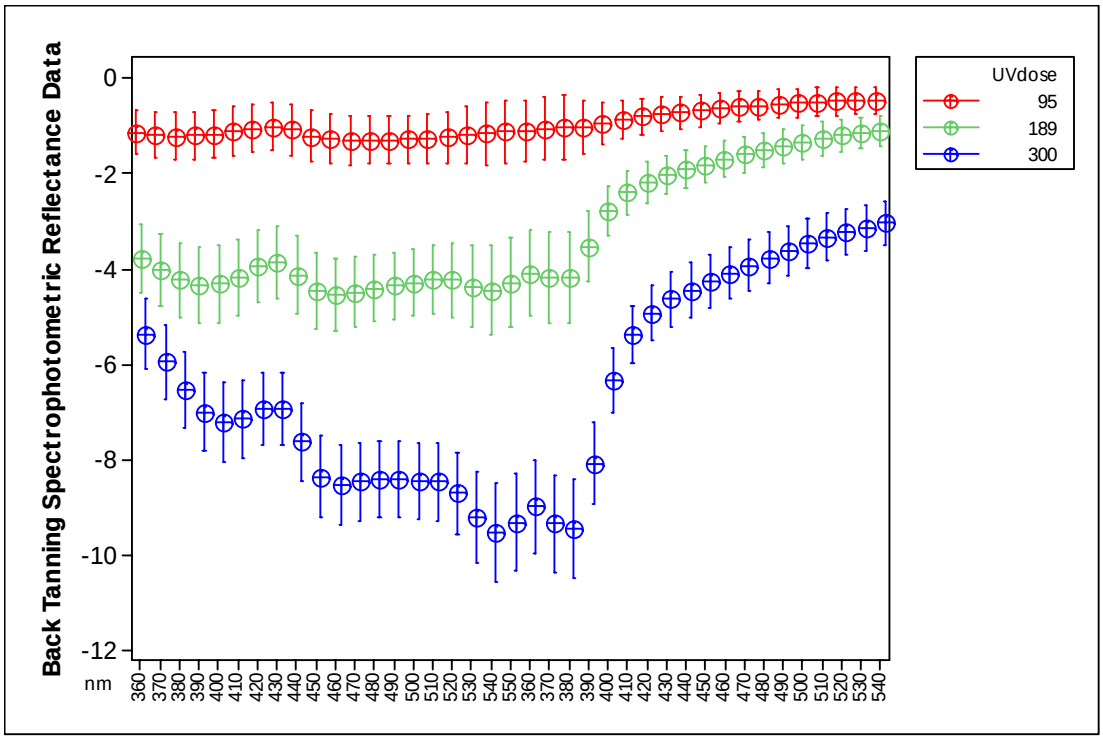
Figure 5.2.1.c.5. Buttock Tanning results by reflectance spectrophotometry 360-740nm (absolute)



This is an interval plot of tanning by spectrophotometry ± SEM (95% CI for the mean) for the buttock (n=98). Y-axis shows the absolute spectrophotometric reflectance data (without baseline spectrophotometric reflectance data subtracted). X-axis shows the 10nm increments of 360-740nm wavelengths.

The above absolute spectrophotometric reflectance graphs were used to plot the following spectrophotometric reflectance graphs with baseline subtracted (n=98).

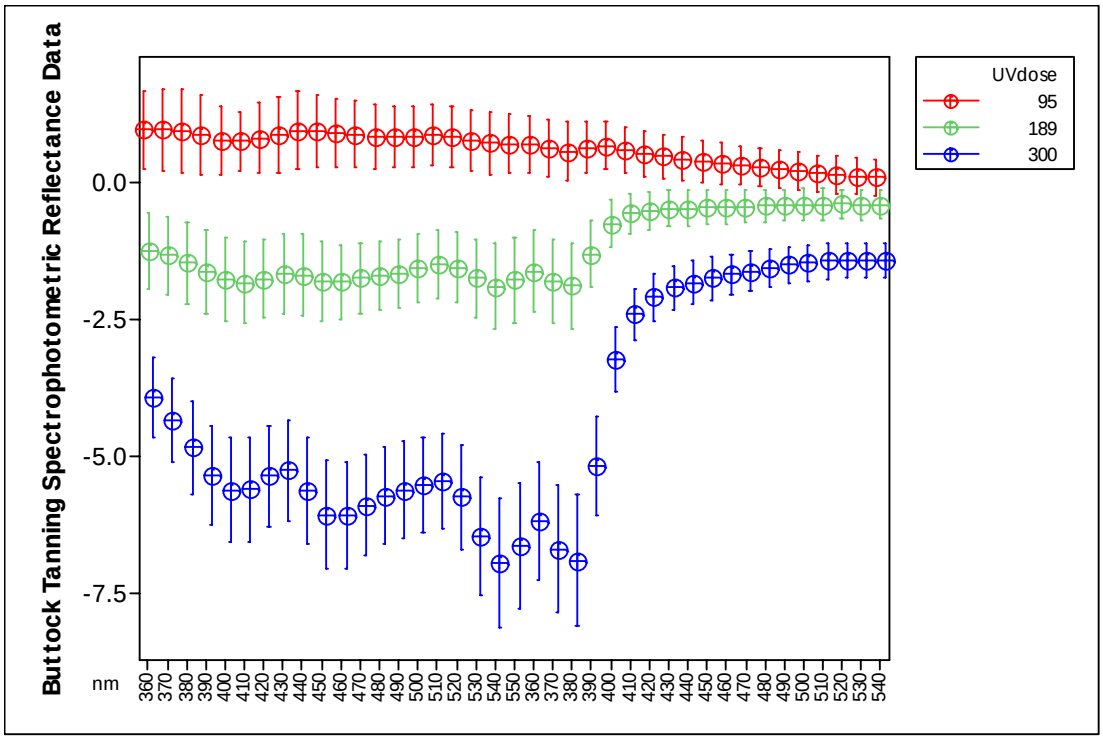
Figure 5.2.1.c.6. Back Tanning results by reflectance spectrophotometry 360-740nm



This is an interval plot of tanning by spectrophotometry ± SEM (95% CI for the mean) for the back. Y-axis shows the change in spectrophotometric reflectance data (with baseline spectrophometric reflectance subtracted). X-axis shows the 10nm increments of 360-740nm wavelengths.

Tanning causes a reduction in reflectance at the back. For Dose 3, there was some reduction in reflectance at the shorter wavelengths 360-400nm (up to -7.2±0.4 units). The reduction in reflectance was greatest at wavelengths 540nm (-9.53±0.5 units) and 580nm (-9.46±0.5 units). The change in reflectance was less at the longer wavelengths 690-740nm (about –3±0.2 units).

Figure 5.2.1.c.7. Buttock Tanning results by reflectance spectrophotometry 360-740nm



This is an interval plot of tanning by spectrophotometry ± SEM (95% CI for the mean) for the buttock. Y-axis shows the change in spectrophotometric reflectance data (with baseline spectrophometric reflectance subtracted). X-axis shows the 10nm increments of 360-740nm wavelengths.

Similarly, tanning causes a reduction in reflectance at the buttock, to a lesser degree than the back. For Dose 3, there was some reduction in reflectance at the shorter wavelengths 360-400nm (up to -5.6±0.5 units). The reduction in reflectance was greatest at wavelengths 540nm (-6.9±0.6 units) and 580nm (-6.9±0.6 units). The change in reflectance was less at the longer wavelengths 690-740nm (about –1.5±0.2 units).

Absolute reflectance was lower at shorter wavelengths and higher at longer wavelengths. As one goes from lower to higher wavelengths (360-740nm), melanin absorbs maximally at 335nm i.e. lowering of reflectance and gradually with decrease in absorption (Figure 5.2.1.c.2.). The reduction in reflectance in the spectrophotometric graphs for back and the buttock are therefore likely due to melanin, this is as expected. At longer wavelength >700nm, absolute reflectance is

the highest i.e. absorption lowest because melanin absorbs closely to nil at the longer wavelengths. The change in reflectance is therefore less.

I expect that there should be no contribution from haemoglobin as the noradrenaline iontophoresis has removed any UVB-induced increase in blood flow (confirmed by contact laser Doppler) and that the subtraction of baseline spectrophotometric reflectance should also have removed the effect of basal blood flow.

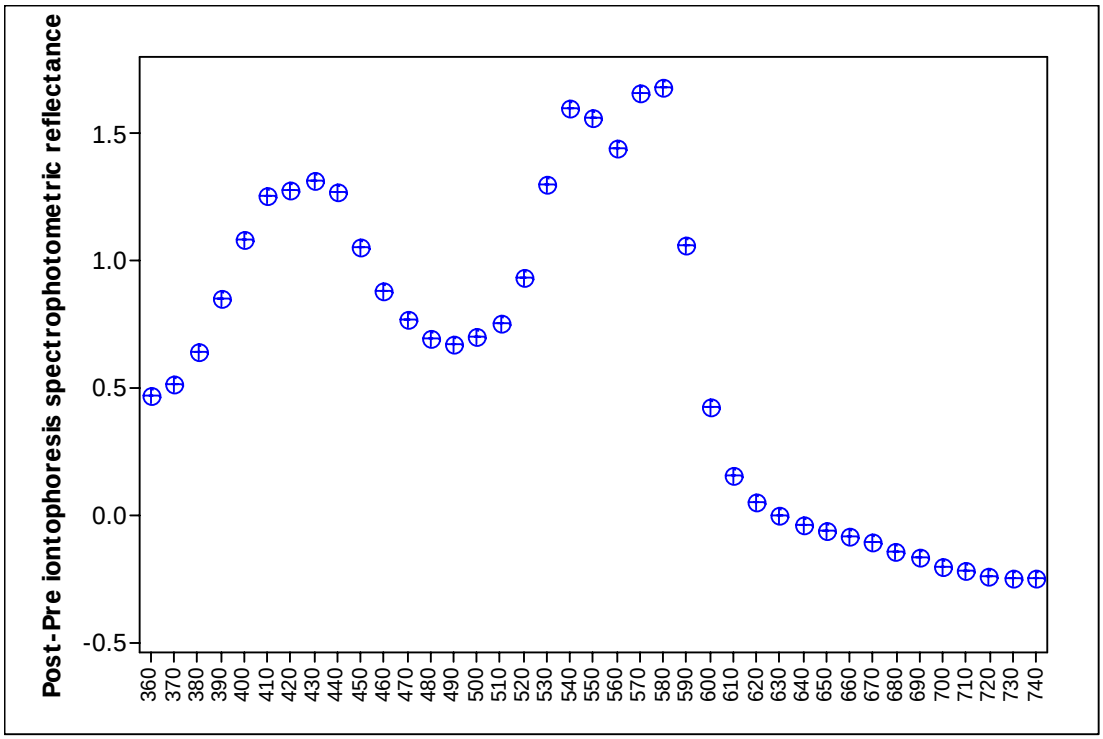
It is possible that other chromophores e.g. bilirubin, β-carotene may have contributed to the change in reflectance, although the assumption is that these are negligible in physiologically normal healthy volunteers.

The skin tanning spectrophotometry data can be difficult to analyse, similar to hair colour spectrophotometry data, despite obvious striking difference between groups. Principle component analysis technique (personal communication, Professor J. L. Rees) may be useful in analysing the data. The analysis is beyond the remit of this thesis. Future work aims to address this.

5.2.1.c.8. Comparison between pre and post noradrenaline iontophoresis absolute spectrophotometric reflectance graphs

Although we confirmed the absence of increased blood flow with a contact Doppler probe, it would be useful to confirm this with our spectrophotometric reflectance measurements. How much difference in reflectance did noradrenaline iontophoresis contribute at each wavelength?

Figure 5.2.1.c.8. Difference in spectral reflectance 360-740nm Post-Pre noradrenaline iontophoresis



This is an interval plot of the difference in spectrophotometric reflectance ± SEM (95% CI for the mean). Y-axis shows the difference (post-pre noradrenaline iontophoresis) in spectrophotometric reflectance data. X-axis shows the 10nm increments of 360-740nm wavelengths (representative from 264 data points).

From the graph, post noradrenaline iontophoresis resulted in greater reflectance. Maximal differences in reflectance occur at 2 peaks: 400-450nm and 520-590nm. These corresponded to the haemoglobin absorption peaks (see Figure 5.2.1.c.3) and is in keeping with blood as expected.

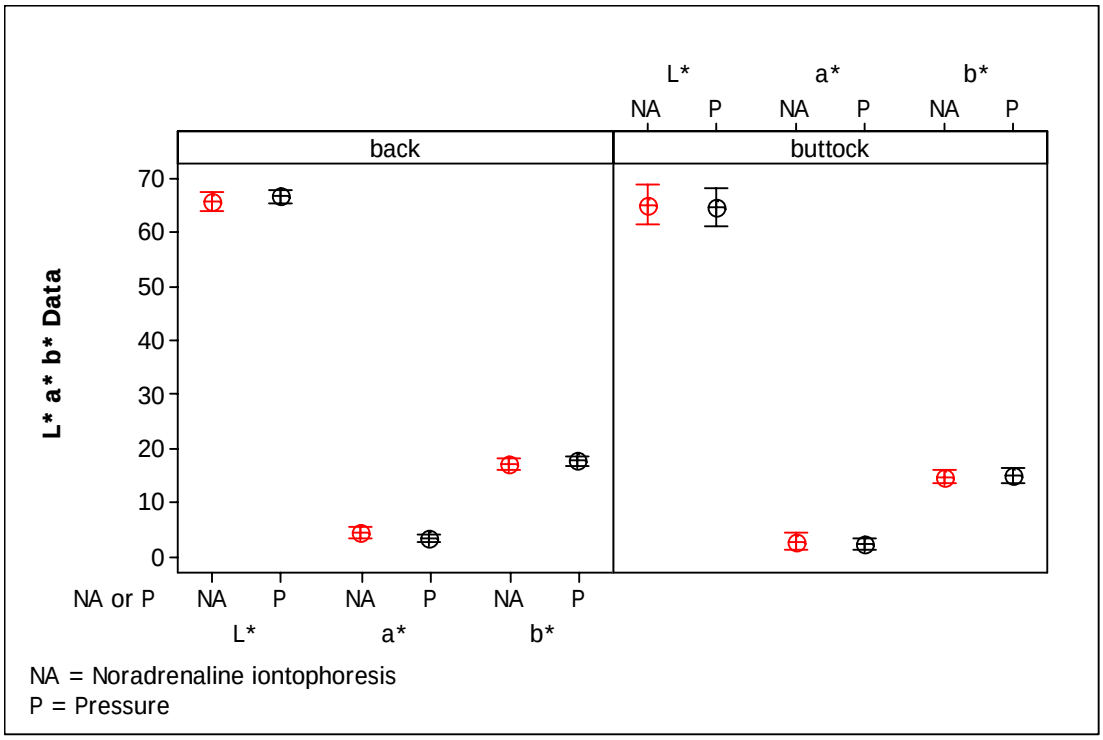
The above spectral reflectance data, together with laser contact Doppler flux data, confirmed that noradrenaline iontophoresis could rid the increase in blood flow post UVB irradiation at 7 days.

The next 3 assays to measure tanning were investigated and developed further in an attempt to measure tanning in the absence of blood after UV irradiation.

5.2.1.d. Pressure colorimetry against Noradrenaline iontophoresis study results

I wondered if pressure exerted on the skin could physically remove the increased blood flow temporarily by using the newly described Pressure colorimetry method in Chapter 2.

Figure 5.2.1.d. Pressure colorimetry versus noradrenaline iontophoresis



This is an interval plot of the absolute value of L* a* b* ± SEM (95% CI for the mean) for the noradrenaline iontophoresis (NA) (red) and pressure colorimetry (P) (black), n=9. Y-axis shows the absolute L* a* b* values. X-axis shows the effect of noradrenaline iontophoresis (red) and pressure colorimetry (black). The panels show data from the back (left) and buttock (right).

Paired t-test comparison between Noradrenaline iontophoresis versus Pressure showed no significant difference for L* (two sided P=0.1827), but significant difference for a* score (two sided P=0.0063) and b* score (two sided P=0.0168). This suggests that pressure has a similar effect on L*, whereas the measurements of a* and b* scores were still different significantly between noradrenaline iontophoresis and Pressure.

It is possible that Pressure exerted could have removed any increased blood flow post-UVB irradiation, however it is not possible at present to confirm whether all the increased blood flux was removed by pressure – a technical limitation means that a contact laser Doppler probe could not be physically fitted onto the spectrophotometer cover.

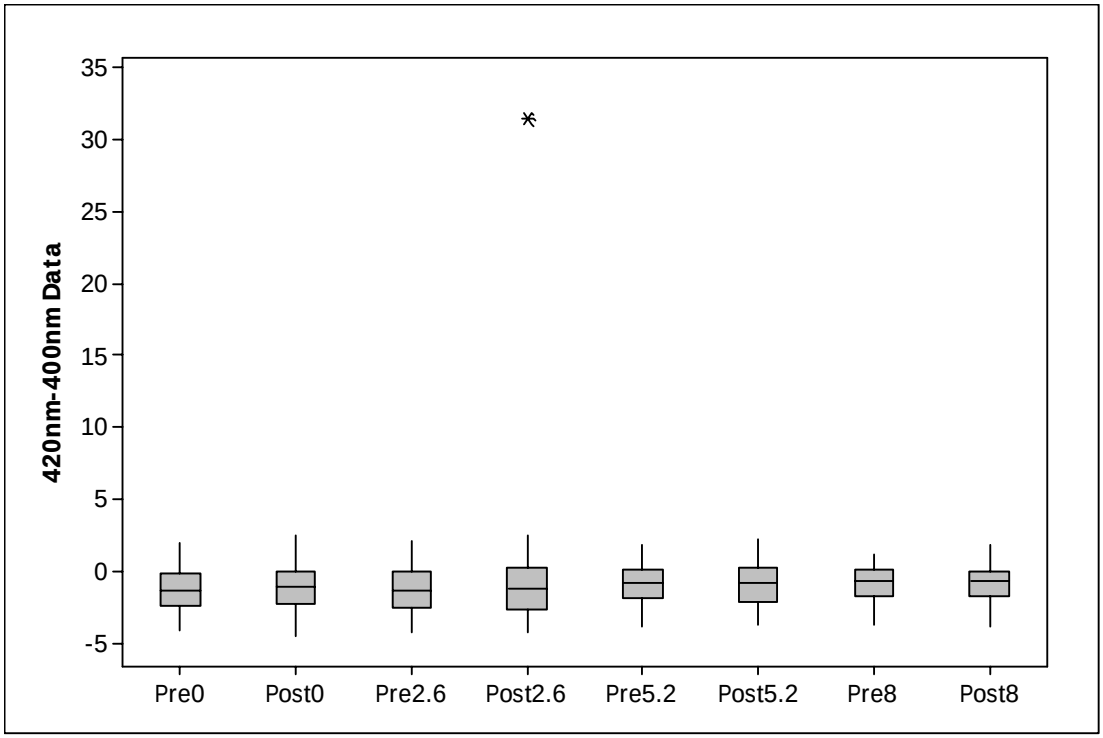
5.2.1.e. Results from the comparison with Dwyer method

An alternative assay method of analysing the day 7 tanning spectrophotometric reflectance data was performed. As was discussed in Methods section in Chapter 2, Dwyer *et al* argued that the difference between reflectance at 420nm and 400nm on skin measurements can be calculated and this difference has been shown to correlate (r=0.68) with cutaneous melanin density as measured by Masson Fontana staining histologically (Dwyer *et al.*, 1998). The difference has been assumed to be void of contribution by blood / haemoglobin since the absorption at these 2 wavelengths should cancel out each other, according to the authors. Hypothetically the difference in the presence and absence of blood should be the same, if blood at 420-400nm is negligible, unless there are contributions from other things.

The facultative pigmentary response to UV radiation in man remains poorly characterised. It is important to measure facultative pigmentation in the absence of changes in blood flow, but this is made difficult because of the spectral overlap of haemoglobin and melanin. The aim is to compare a calculation method of measuring skin colour ('Dwyer method') with a more invasive method that we have recently published (Oh *et al.*, 2004). We use the latter as the gold standard.

Melanin absorbs more light at 400nm>420nm. But haemoglobin absorption is high and similar at both wavelengths. Differences in absorption at 400 and 420nm are therefore said to be due to pigment and not blood according to the Dwyer method.

Figure 5.2.1.e. Dwyer model versus Pre Post NAdr iontophoresis at different UVB irradiated doses



n=9

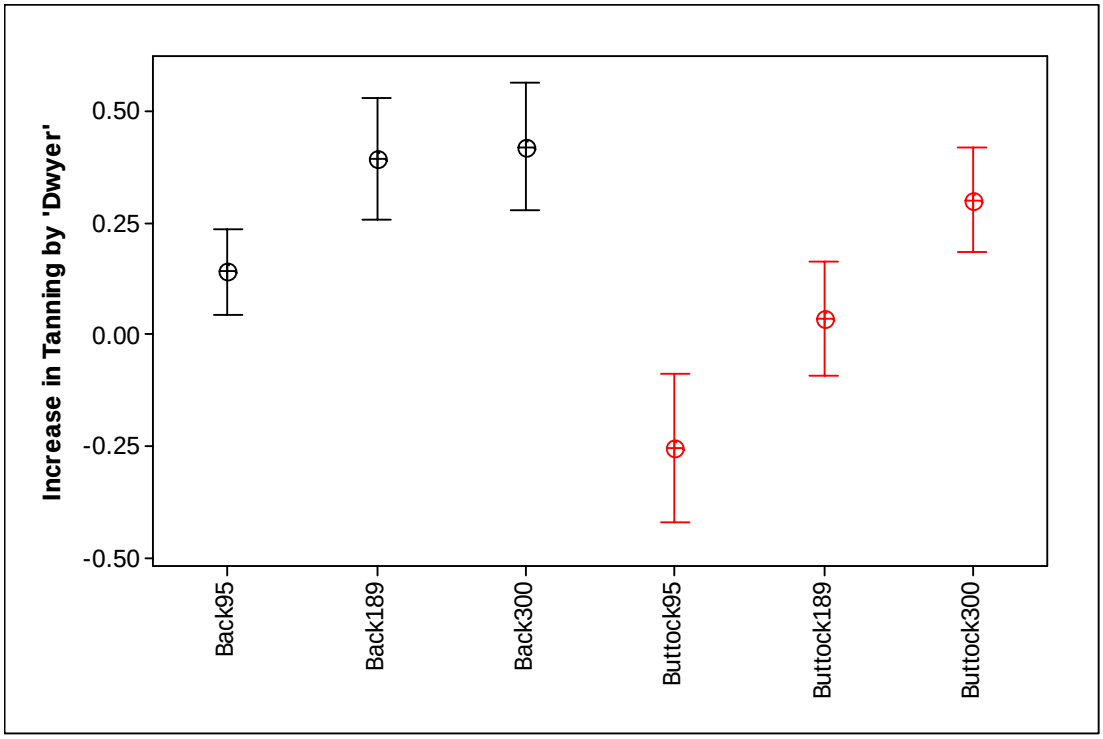
Student's t test was performed to compare the pre and post noradrenaline 420-400nm reflectance data for 0 SEDs (-1.24±0.19, -1.07±0.20) (P=0.55), 2.6 SEDs (-1.31±0.20, -0.67±0.62) (P=0.32), 5.2 SEDs (-0.91±0.19, -0.84±0.18) (P=0.80) and 8.2 SEDs (-0.79±0.15, -0.86±0.17) (P=0.74).

Our data showed no statistically significant difference between the pre and post noradrenaline iontophoresis results, in keeping with the predictions of the Dwyer model. This confirmed that it was uninfluenced by blood (independent of blood flow / flux). The Dwyer model deserves more attention.

5.2.1.f. Results of facultative pigmentation using Dwyer method

Next my results were compared with Dwyer method (Dwyer *et al.*, 1998). If my tanning data (spectrophotometry) was analysed using Dwyer method by subtracting 420nm-400nm, this should correlate with amount of melanin generated by pigmentary response.

Figure 5.2.1.f. Tanning by 'Dwyer' calculation (420-400nm) for n=98 plotted for UVB doses 1-3 on back and buttock (with baseline subtracted i.e. 'increase in tanning by Dwyer')



This is an interval plot of tanning by 'Dwyer' ± SEM (95% CI for the mean) for the back (black) and buttock (red). Y-axis shows the tanning by 'Dwyer' calculation (420-400nm) for 98 individuals. X-axis shows the UVB doses (mJ/cm^2) in a logarithmic scale for the back (black) and buttock (red).

There was a better dose response for the buttock than the back with 'Dwyer' calculation method. There was a good dose response for the buttock although not as marked as the tanning measure of 'change in L*'.

In theory, Dwyer argued that 420-400nm should correlate well with the amount of pigment i.e. melanin. In practice this assay was not useable, in theory ok. Even though subtracting 420-400nm did get rid of blood, there may be other unknown contributory factors. It is possible that for instance other chromophores in skin which may account for this difference (bilirubin, β-carotene). SEM bars overlap. There were 2 different trends between back and buttock.

Therefore the 'Dwyer' calculation method could also rid the increase in blood flow, but did not give a good tanning assay response for the back.

5.2.2. Correlation between experimentally induced tanning and co-factors including erythema

The principle aim of this section was to relate changes in tanning with independent variables that might explain the changes in tanning. Tanning was measured at 3 doses of UVB radiation on the back and on the buttock. Two factors must be kept in mind. A large number of variables were recorded and, second, some of these variables could not be considered independent. For instance, L*, a* and b* values were recorded as a measure of change in skin colour after tanning. Although the analyses could be repeated with all 3 variables, the results presented here are for L* only. The justification for this is simplicity and previous published work from our group showed that L* lends itself to dose dependent measures of tanning and distinguishes between different patient groups (Oh *et al.*, 2004).

The second issue is that because tanning responses were sought at 3 different doses within any one individual, therefore the results to the UVB challenge cannot be considered statistically independent. The most appropriate analytical method would be the use of either a split plot design or a mixed effects model. The split plot design is useful when a particular type of restricted randomization has occurred during the experiment. The split plot design involves two experimental factors A and B. Levels of A are randomly assigned to whole plots i.e. main plots, and levels of B are randomly assigned to split plots i.e. subplots within each whole plot. The subplots are assumed to be nested within the whole plots so that a whole plot consists of a cluster of subplots and a level of A is applied to the entire cluster. The design provides more precise information about B than about A. Mixed effects model could be used to analyse these balanced and unbalanced grouped data which have arisen from repeated measures data by fitting these data into mixed effects model software. These are complex. Inspection of the raw data showed that, for most individuals, evidence of a dose effect was present between tanning and UV dose. For simplicity, and for ease of modelling, results at the highest dose (dose 3 i.e. 300mJ/cm^2) were

used for the analysis. The approach was to look at univariate measures initially before moving on to more complicated analyses.

5.2.3. Tanning Results

The score for tanning on back and buttock appeared reasonably normally distributed at the 300mJ/cm^2 dose. Parametric statistics were therefore used where appropriate.

5.2.3.1. Tanning and sex

Simple t-tests show that there was no relation between tanning on the back or the buttock and sex.

5.2.3.2. Tanning and freckles

There was no relation between tanning on the back and freckles, but conversely there was a highly significant relation between the presence, or otherwise, of freckles and tanning on the buttock (P=0.01) with the mean increase in tanning of 4.75 in those who freckled, compared with 3.27 in those who did not freckle.

5.2.3.3. Tanning and red hair

No difference was seen between those with red and those without red hair in back tanning, but again differences between the red and non-red group were almost significant for buttock tanning (P=0.06, two sample t-test) with the tanning mean of 5.61 in those with red hair and 3.74 in those without red hair.

5.2.3.4. Tanning and blonde hair

Similarly, there was no relation between tanning on the back and the presence of blonde hair or otherwise, but tanning on the buttock was greatest at 4.33 in those without blonde hair, compared with 1.99 in those with blonde hair (P=0.02, two sample t-test).

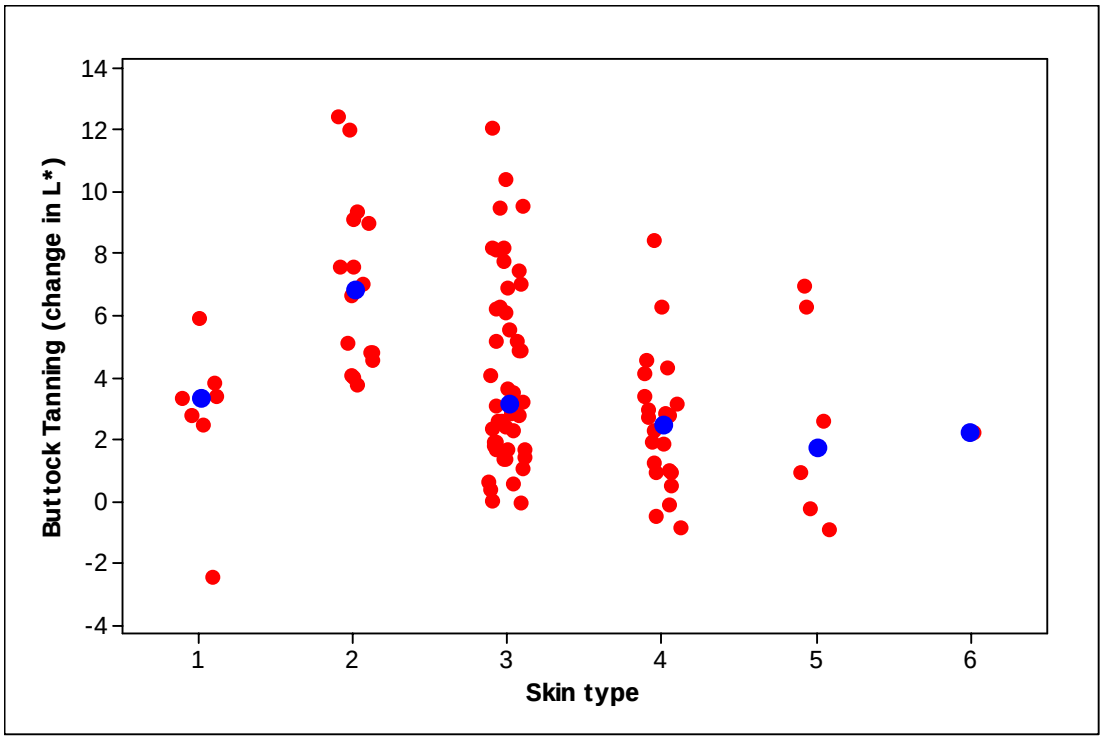
5.2.3.5. Tanning and ethnicity

A simple analysis of variance (ANOVA) failed to show any significant relation between back or buttock tanning and ethnic group. Small numbers in some groups may have limited the power of this test.

5.2.3.6. Tanning and skin type

Whereas simple analysis of variance failed to reveal any relation between skin type treated as a factor and back tanning (P=0.27), there was a highly significant relation (P=0.0001) for skin type and tanning on the buttock.

Figure 5.2.3.6. Buttock Tanning L* at dose 3 (300mJ/cm^2) and skin type



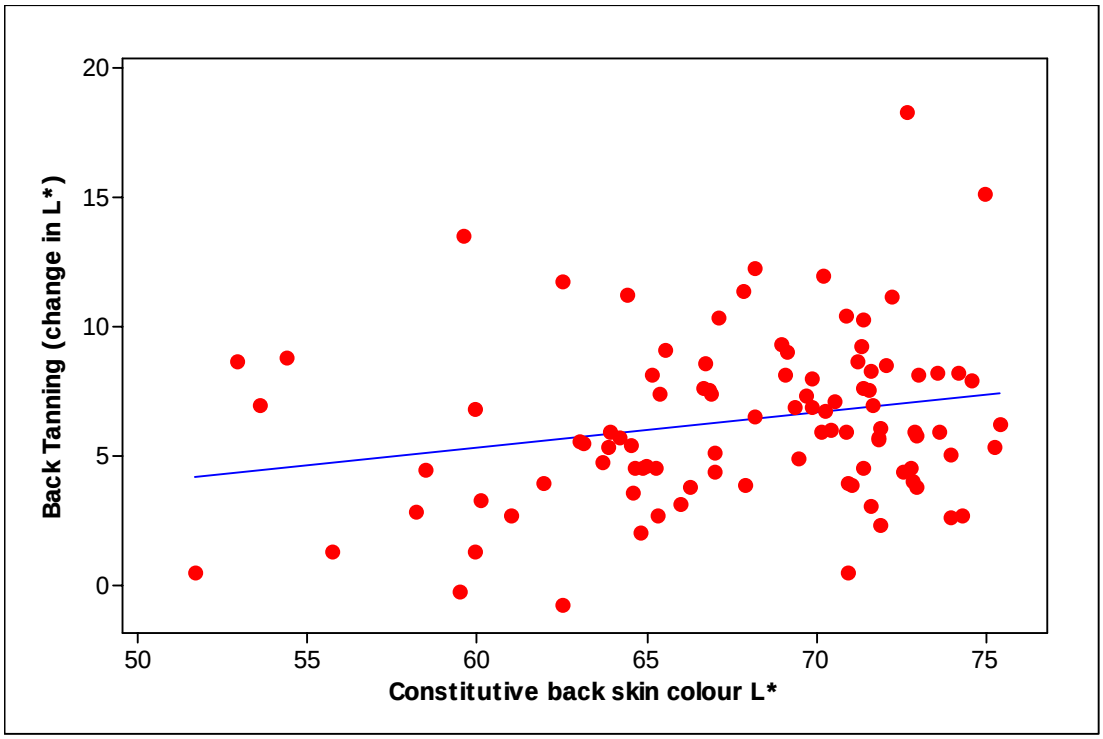
Inspection of an individual value plot with skin type on the x-axis and buttock tanning L* on the y-axis, shows that clear differences between skin type groups 2, 3, 4 and 5 appeared obvious. Comparison of the individual skin types using the Tukey method reveals the following differences: Skin type 1 versus skin type 2 (P=0.016); skin type 3 versus skin type 2, (P=0.01); skin type 4 versus skin type 2, (P=0.00008); skin type 5 versus skin type 2, (P=0.02). The significances of these differences shown on buttock skin, but not back skin, will be returned to later.

5.2.3.7. Tanning and constitutive skin colour

If skin colour was treated as a continuous variable, then there were significant correlations between constitutive skin colour (at a particular site) and the change in L* following UV radiation. Note that the change in L* refers to, in fact, a darkening

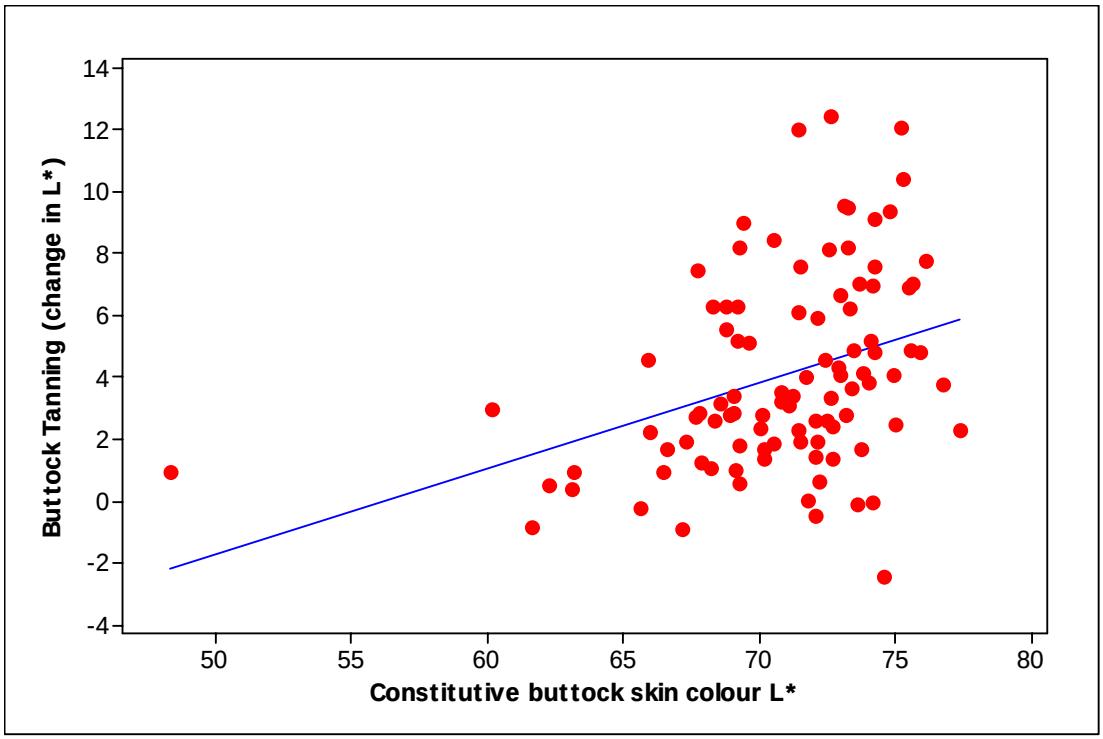
(because small values on the L* scale reflect darker skin) whereas with the basal measure, the more positive it is, the lighter the skin.

Figure 5.2.3.7.1. Tanning responses (change in L*) over back and constitutive back skin colour L*



This is a scatter plot of back tanning (change in L*) and constitutive back skin colour L* at UVB dose 3 (300mJ/cm^2) for 98 individuals. Y-axis shows the back tanning change in L* (with baseline L* subtracted). X-axis shows the constitutive back skin colour in L* scale.

Figure 5.2.3.7.2. Tanning responses (change in L*) over buttock and constitutive buttock skin colour L*



This is a scatter plot of buttock tanning (change in L*) and constitutive buttock skin colour L* at UVB dose 3 (300mJ/cm^2) for 98 individuals. Y-axis shows the buttock tanning change in L* (with baseline L* subtracted). X-axis shows the constitutive buttock skin colour in L* scale.

These results show that the greatest increase in tanning was in those with the lightest skin. i.e. individuals who have a lighter constitutive skin colour tend to tan more.

The relation between back L* and tanning on the back for L* was P=0.03748 with a correlation coefficient of 0.21; between buttock L* and buttock tanning L*, the P-value was equal to 0.0004602 and the correlation coefficient was 0.347. Therefore back constitutive skin colour could explain 4% of the tanning response (R^2 of ~4%). Buttock constitutive skin colour could explain 12% of the tanning response (R^2 ~12%). The diminished amount of tanning in individuals with darker skin could be explained by either i) a limited amount of further tanning in individuals with darker skin or ii) a smaller amount of UVB dose received biologically.

5.2.3.8. Tanning and erythema

It is known from previous work that tanning is related to UV-induced erythema (Parrish *et al.*, 1982). Simple correlations for back skin showed a P value of 0.07 with a correlation coefficient of 0.18 between erythema measured as the erythemal index at 300mJ/cm^2 and the change in L* on the back. For the buttock, the comparable figures were P=1.473×10^{-6} and with a correlation coefficient of 0.46. Again, it is noted that the results between the buttock and the back differ with a clearer relation been seen on the buttock than on the back.

Table 5.2.3.8. Summary of ANOVA

Variable 1	**Variable 2**	**Correlation**	**P**
Constitutive buttock L*	Tanning buttock L*	0.35	0.00046
EI buttock 300	Tanning buttock L*	0.46	1.473×10^{-6}

5.2.3.9. Multivariate Analysis

A simple linear model was fitted with erythema at 300mJ/cm^2 and constitutive buttock skin colour L*. There was no evidence of interaction between erythema and constitutive skin colour and this term was removed.

Linear model formula

= Buttock Tan L* ~ buttock erythemal index 300 * Buttock constitutive L*

Table 5.2.3.9. Summary of Linear Model for tanning

	Estimate	SE	t value	P r(>t)
(Intercept)	-8.10	5.29	-1.53	0.128998
Buttock EI 300	0.03	0.01	3.80	0.000254
Buttock L*	0.13	0.08	1.62	0.108098

F-statistic 11 on 2 and 95 degrees of freedom, P=2.687×10^{-6}

This regression explains about 24% of the variation as reflected by the adjusted R^2.

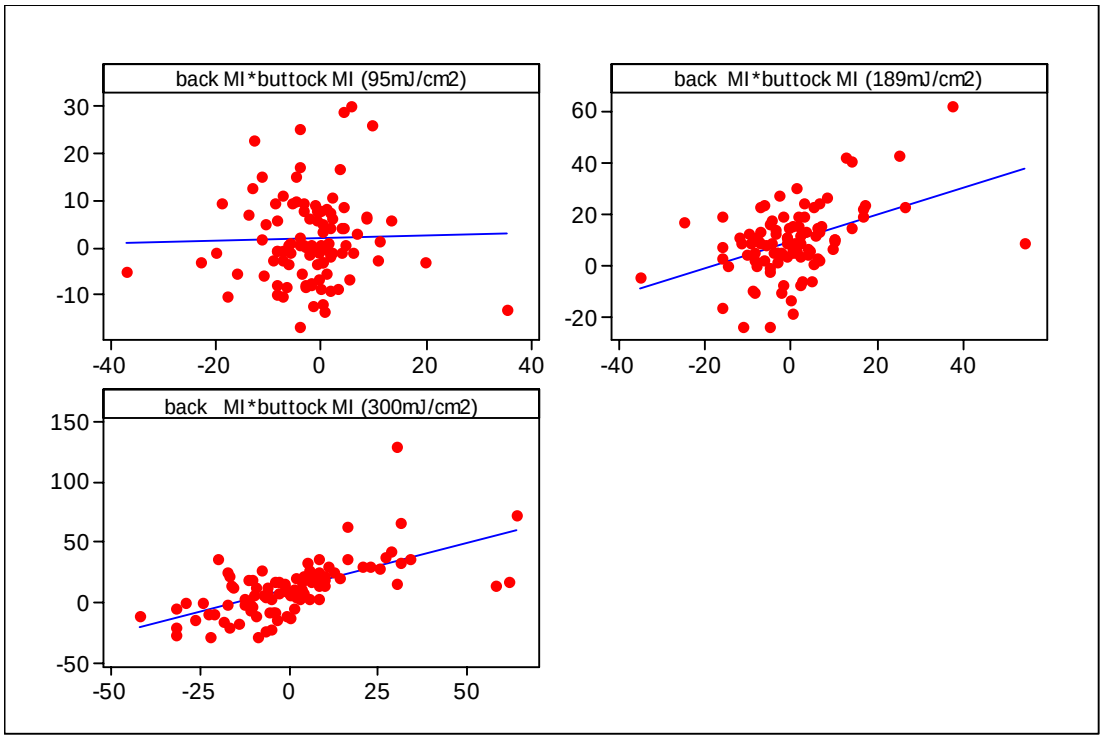
A summary analysis of variance table is shown and it can be seen that the erythemal index (EI) of 300mJ/cm^2 is highly significant of tanning ability at the buttock, but that the constitutive buttock colour is no longer significant (P=0.108098), as seen in the univariate analysis. The R^2 for this model is 0.24 (P=2.687×10^{-6}).

The corresponding model for back skin attempting to relate erythema at 300mJ/cm^2 to constitutive colour does not reveal any significant results (P=0.14). With regards to buttock skin, the addition of neither skin type, nor ethnic group nor the presence, or otherwise, of red hair, nor the presence, or otherwise, of freckles, was significant beyond that accounted for by erythema.

As could be expected, UVB-induced tanning on the back and buttock were correlated (P=0.04, correlation coefficient = 0.28).

5.2.10. Tanning (change in Melanin Index) correlation back and buttock

Figure 5.2.10. Tanning (change in Melanin Index (MI)) correlation back and buttock



This is a scatter plot of back tanning (change in melanin index (MI) and buttock tanning (change in melanin index (MI) at 3 UVB doses (95, 189 and 300mJ/cm^2) for 98 individuals. Y-axis shows the back tanning (change in melanin index (MI) (with baseline MI subtracted).

X-axis shows the buttock tanning (change in melanin index (MI) (with baseline MI subtracted).

Table 5.2.10. Correlation between back tanning by melanin index (MI) and buttock tanning by MI

SEDs	Pearson coefficient of correlation	P	Regression r^2(adj)
Dose 1	0.026	0.797	0.1%
Dose 2	0.455	<0.05	19.9%
Dose 3	0.633	<0.05	39.4%

Similarly for melanin index (MI), UVB-induced tanning on the back and buttock were correlated ($P<0.05$, correlation coefficient = 0.63) for dose 3.

The relationship between tanning and *MC1R* genotype will be discussed in Chapter 6.

5.3. Discussion

There were 6 different assays used to quantitatively measure tanning. Tanning by change in L* in the absence of increased blood flow (by performing noradrenaline iontophoresis) was found to be the optimal measure of tanning in this study. Melanin index also presented some limitations due to the way MI was estimated by the erythema meter. Tanning by change in spectrophotometric reflectance presented some difficulty in analysing the data. The Dwyer calculation method appears to be effective in measuring tanning also, although the dose response curves were less defined. Shriver method was also difficult to analyse.

The aim of this section was to summarise what the determinants of tanning are. Firstly, tanning is dependent on UVB dose. As the UVB dose increases, there is more tanning. Secondly, tanning is also site dependent. The back tans much more than the buttock. Thirdly, the phenotypes freckling and skin type are correlated with tanning (over the buttock P=0.01, P=0.0001). Buttock tanning correlates with freckles and skin type. Skin type is a factor for buttock tanning, not back tanning. Freckle and

back tanning were not significant. It is possible that individuals who tan well, may expose their back more. Fourthly, constitutive skin colour. Back constitutive skin colour correlated with back tanning with a correlation coefficient of 0.21 – R^2 of ~4% i.e. importantly only explained 4% of variation. Buttock constitutive skin colour correlated with buttock tanning R^2 ~12% i.e accounting for 12% of the variation. Finally, erythema is the most important determinant which we will return to disuss.

Ethnicity does not seem to have a large effect on tanning in this population sample. This may be due to the small numbers in some of the ethnic groups which in turn is reflective of this Scottish population.

Red hair status was almost significant (P=0.06) towards tanning when compared to non-reds. Blonde hair status does seem to have less of a tanning effect (P=0.02).

Do people with darker constitutive skin colour tan more? No. In fact, lightest skin tanned the most due to becoming the reddest in response to UVB over the buttock and effectively received a larger effective biological UVB dose. In other words, the individuals with darker constitutive skin colour cannot be compared equivalently to those with lighter skin colour because although they were irradiated with the same mJ/cm^2 of UVB, these were not equal erythemogenic doses of UVB to those individuals with different skin colours.

Different kinetics of tanning to UVB could explain some of the tanning responses. Individuals who have the lowest starting point i.e. lightest skin colour could in theory generate the most magnitude of tanning. These paler individuals could increase blood flow (i.e. erythema) the most, in response to UVB irradiation and therefore produce more melanin. For those individuals who showed an increased in erythema (blood flow) at 7 days, it is possibe that they may have the capacity to tan further and this could not be excluded.

Erythema matters most and explains much of the variation in tanning. Buttock erythema correlates with buttock tanning (r=0.46). One goes brown proportional to redness. Buttock tanning correlates with buttock constitutive skin colour. Constitutive skin colour is also important in explaining some of the variation in

tanning. When erythema is put into a simple linear model, all these other factors are taken into account by erythema. Although other co-factors like skin type (P=0.0001), freckles (P=0.01) were associated with buttock tanning – all these other factors ultimately determine an individual's propensity to go red with UVB. Once erythema was put into the equation, all other factors are out. Since erythema is ultimately dependent on all these factors. How red you go is a reflection of these other factors.

In explaining the determinants of tanning, erythema (with EI chosen as the assay measure) is the most important. Adding ethnicity has no effect, probably because ethnicity tells you how red you go and reflect the degree of redness you go after UV exposure. Skin type explains tanning. This is in keeping with (Pershing *et al.*, 2008). Some skin type e.g. type 2 matters and develops redness with UV exposure. There is no additional information beyond what could be explained by erythema, beyond skin type. Thus Fitzpatrick skin type, as a continuous variable, provided no further information beyond what erythema did.

Buttock is a better site to measure for tanning responses since it is photoprotected and naïve to UV. One limitation encountered in this study was that some individuals (2) did not want to expose their buttocks to be irradiated.

Erythema is a useful and good phenotypic measure. The best correlation found was between buttock erythema and buttock tanning (r=0.46).

To date, no previous study has ever measured tanning in this detail and quantitatively. This is the first study of tanning in this detail, despite a large quantitative dataset; it was disappointing that these other factors did not contribute much towards the determination of tanning responses.

Tanning data was normally distributed. Parametric methods were used. The analysis of tanning data was focussed on change in L* score as the measure of tanning (in the absence of increased blood flow). Perhaps other alternative measures e.g. a* score or b* score could be used too. Indeed, L* score correlates with a* too. Alaluf *et al* suggested that melanin contributes to b* score in lighter skin but as melanin increases in darker skin, b* reaches a maximum and then decreases, possibly due to the optical masking of yellow light by high concentrations of melanin (Alaluf *et al.*, 2002).

However, we have previously established a valid, proven and reproducible method using L* score (Oh *et al.*, 2004).

The tanning data analysis was performed for dose 3, the maximum UVB dose, as I was expecting the largest effect and that any effect would be easiest to spot. In order to ensure that the effects found were not supraphysiological, I also looked at tanning at another dose (dose 2, 189mJ/cm^2). As expected, at dose 189mJ/cm^2, EI buttock correlated with buttock tanning 0.31 (P=0.0018) also. Erythema is still the only real predictor; other factors were not very strong. At 189mJ/cm^2, comparisons not as impressive i.e. when corrected for multiple testing.

In summary, the chief determinant of tanning is erythema, which in turn is dependent on the dose of UV irradiated and received by the skin biologically. Constitutive skin colour comes into play and determines how much UV is received into the skin, together with other co-factors e.g. ethnicity, hair colour, which in turn determines the propensity to develop erythema. It is these factors that make up a person and determines whether that person burns or not.

Hence the model of tanning:

Linear model formula = Buttock Tan L* ~ buttock erythemal index 300 * Buttock constitutive L*

The key finding is that the only real predictor of tanning using photoprotected buttock is erythema (R^2=0.24, P=2.96×10^{-6}). My tanning results are in keeping with Wagner (Wagner *et al.*, 2002b) – positive correlation between tanning and burning response in European Americans i.e. Tanning correlates with Erythema, despite difference between the 2 studies with regards to the irradiation sites. Wagner *et al* used medial arm whereas I used mid back and buttock.

Erythema accounts for 24% of variation in tanning response over the buttock. What is left? Tanning is not a perfect assay. There is noise in the system. Other unknown and not yet identified factors may be accountable for the rest of the variation. Non-pigmentary photoadaptation may account for the difference in facultative pigmentary response e.g. skin hyperplasia or thickening.

Further experiments

The findings from this study raised the point that erythema is the key determinant for tanning. What if normalised for erythema? What if normalised for skin colour?

Asians received lower erythemogenic UV dose. If they are normalised for erythema, do they tan more? A dose response could be performed to investigate this.

Another additional experiment to perform is to compare 'matched colour' individuals between different ethnicity, and to see if they tan to the same extent or to a different extent? Is it possible to measure redistribution in skin if there is no great change in the overall melanin amount? Are Asians not very different to Europeans when skin colour has been accounted for? If we recruit a sample of Asians, Japanese and Europeans and measure their tanning responses if normalised for skin colour, would there be any difference? Do Italians and Asians with the same skin colour tan the same degree?

Another further experiment is to look at population difference using the assays performed in this study and perform in a different population. It is often difficult to compare results between different studies as other studies use different study criteria, different assays, different UV lamps and that factors are not constant. This can be exemplified by the Wagner study (Wagner *et al.*, 2002b) and my study.

Chapter 6 *MC1R* genotype

6.1 Introduction

In this chapter I discuss the findings of *MC1R* genotype in this study. Next the relationship between *MC1R* and various phenotypic characteristics will be presented.

6.2. Results

The results will be discussed as follows: 1) *MC1R* genotype. 2) Relationship between *MC1R* sequence variants and phenotypic characteristics including red hair

6.2.1 Summary of *MC1R* sequence variants detected

Position	Sequence variant		Nucleotide	*MC1R* Status	Volunteer
29	29insA	29insA	c.86_87insA	R	
51*	Val51Ala	V51A	c.152T>C GTG-GCG	Novel	V157
60	Val60Leu	V60L	c.178G>T GTG-TTG	r	
63*	Ile63Met	I63M	c.189C>G ATC-ATG	Novel	V40
84	Asp84Glu	D84E	c.252C>A GAC-GAA	R	
92	Val92Met	V92M	c.274G>A GTG-ATG	r	
111	Ala111Val	A111V	c.332C>T GCG-GTG		
131*	Ser131Asn	S131N	c.392G>A AGC-AAC	Novel	V120
142	Arg142His	R142H	c.425G>A, CGC-CAC		
151	Arg151Cys	R151C	c.451C>T, CGC-TGC	R	
155	Ile155Thr	I155T	c.464T>C ATC-ACC		
160	Arg160Trp	R160W	c.478C>T CGG-TGG	R	
163	Arg163Gln	R163Q	c.488G>A CGA-CAA	r	
212*	Ala212Ser	A212S	c.634G>T GCC-TCC	Novel	V101,V129
213	Arg213Trp	R213W	c.637C>T CGG-TGG		
230	Pro230Leu	P230L	c.689C>T CCG-CTG		
233	Gln233Gln	Q233Q	c.699G>A, CAG-CAA		
279	Asn279Lys	N279K	c.837C>A, AAC-AAA		

294	Asp294His	D294H	c.880G>C GAC-CAC	R	
314	Thr314Thr	T314T	c.942A>G ACA-ACG		

This is a table summarizing the *MC1R* sequence variants detected in this study. The *MC1R* sequence was based on GenBank NM_002386.2 and nucleotide+1 is defined as the A of the ATG translation initiation codon. (*) means 'new' variants. *MC1R* status according to Duffy (Duffy *et al.*, 2004). R = high penetrance , r = low penetrance.

MC1R was sequenced successfully from 156 individuals. The DNA from 3 individuals (V35, V71 and V85) failed *MC1R* sequencing Of the remaining 156, a total of 20 *MC1R* variants were detected. 17 *MC1R* sequence variants were found in the subset of UVB irradiated individuals. These are as follows:

All individuals (156 sequences)	**UVB-irradiated individuals (98 sequences)**
29insA	29insA
V51A	V51A
V60L	V60L
I63M	I63M
D84E	D84E
V92M	V92M
A111V	
S131N	S131N
R142H	R142H
R151C	R151C
I155T	I155T
R160W	R160W
R163Q	R163Q
A212S	A212S
R213W	
P230L	P230L
Q233Q	
N279K	N279K
D294H	D294H
T314T	T314T

Therefore in the UVB subset of individuals, *MC1R* sequence variants A111V, R213W, Q233Q were not included.

4 novel *MC1R* sequence variants were found in this population – V51A, I63M, S131N and A212S. These were added to Table 1 in Chapter 1.

MC1R variants	Allelic frequency in current study
WT	0.42
V60L	0.12
V92M+T314T	0.090
R151C	0.083
R160W	0.080
R163Q	0.074
D294H	0.032
I155T+T314T	0.013
T314T	0.013
D84E	0.0096
I155T	0.0096
R142H	0.0064
I63M	0.0032
A111V	0.0032
S131N	0.0032
R213W	0.0032
P230L	0.0032
N279K	0.0032
Q233Q	0
29insA	0.0064

This table shows the allelic frequencies of *MC1R* allelic variants detected.

V60L was the commonest sequence variant detected in this population with a frequency of 12%. The red hair allelic variants detected (D84E, R142H, R151C, R160W, D294H, 29insA) were at a frequency of 1.0, 0.6, 8.3, 8.0, 3.2 and 0.6% respectively.

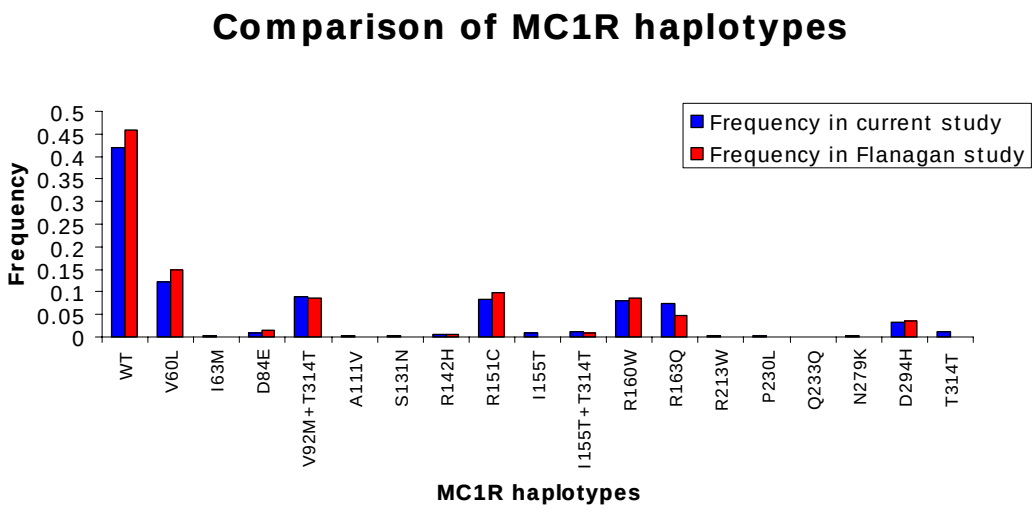
Focusing on the major *MC1R* allelic variants:

	1 allele	2 alleles
V60L	35	3
D84E	3	0
V92M+T314T	28	0
V92M	3	0
R142H	2	0
R151C	25	1
R160W	24	1
R163Q	20	3
D294H	10	0
29insA	2	0

This table shows the number of *MC1R* allelic variants detected: 1 allele or 2 alleles.

6.2.2 Allelic frequency comparison of *MC1R* studies

A comparison with the Flanagan study (Flanagan *et al.*, 2000) of the *MC1R* allelic frequencies was performed. This was done in order to confirm that the allelic frequencies in both studies were similar.



On inspection of the above figure, the frequencies appeared remarkably similar between the studies.

One important point to make is that *MC1R* is so polymorphic, what is referred to as wildtype ("WT") is the consensus sequence from Africa (Harding *et al.*, 2000). I will refer to and quote the consensus sequence as wildtype (WT) (Appendix 7).

MC1R allelic variants were ranked and coded according to Duffy (Duffy *et al.*, 2004) into homozygous (R/R) or heterozygous (R/r or R/-) for high penetrance 29insA, D84E, R142H, R151C, R160W and D294H. Low penetrance alleles 60 and 92 were classed as r. Other variants e.g. 51, 60, 63, 92, 111, 131, 155, 163, 212, 213, 230, 233, 279 and 314 were classed as wildtype (WT).

Of the 156 successfully sequenced, 13 were homozygous (Hm), 41 heterozygous (Ht) and 102 wildtype (WT)/pseudo-WT.

An alternate way of classifying *MC1R* genotype R r status into 5 groups was also performed. RR, Rr, rr, r/-, -/-. V60L was classed as r.

***MC1R* R r status**	**Category**
R R	1
R r	2
R – or r r	3
r –	4
– –	5

6.2.3. Relationship between *MC1R* genotype and phenotypic characteristics

6.2.3.1. *MC1R* and age

Firstly, age was looked at to see whether there was any association with *MC1R* genotype. There was no effect of age on *MC1R* genotype as tested by Kruskal-Wallis test (P=0.275).

6.2.3.2. *MC1R* genotype and sex

Next, sex was looked at to see whether there was any association with *MC1R* genotype. Females are referred as F. Males are referred as M.

Figure 6.2.3.2. Sex distribution for *MC1R* genotype status

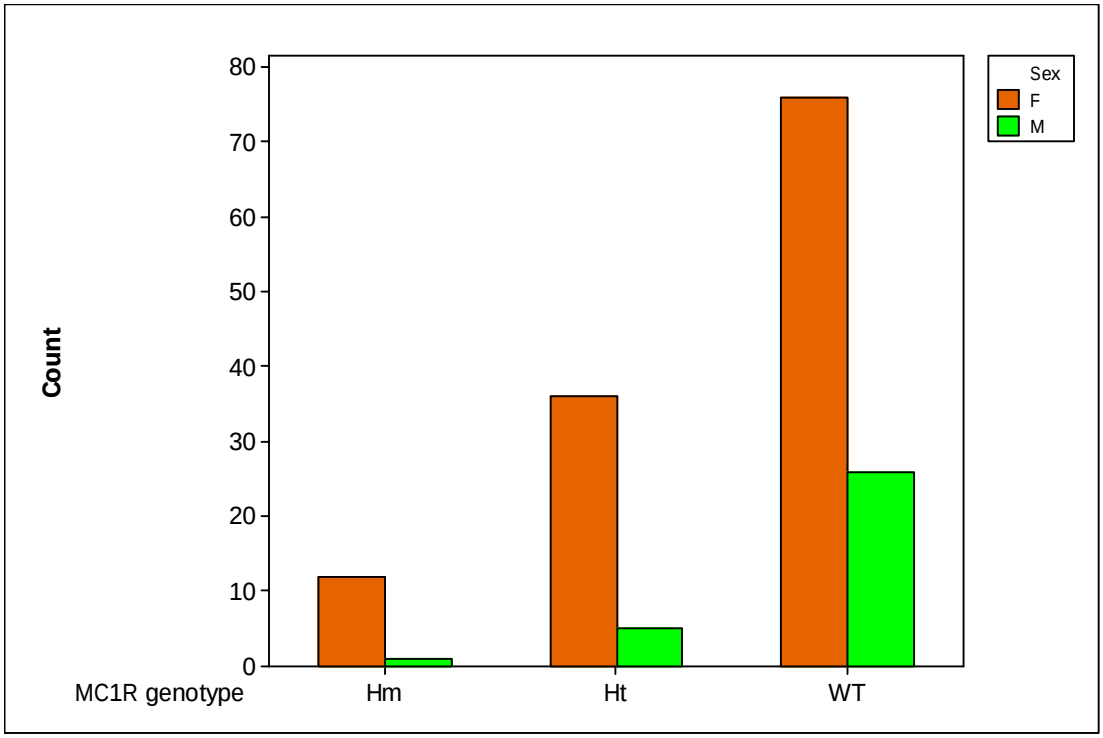


Table 6.2.3.2.a. *MC1R* genotype (Hm/Ht/WT) and sex

	Hm	Ht	WT	Missing	All
F	12	36	76	2	126
M	1	5	26	1	33
All	13	41	102	3	159

There was no effect of sex on *MC1R* genotype Hm/Ht/WT (P=0.124).

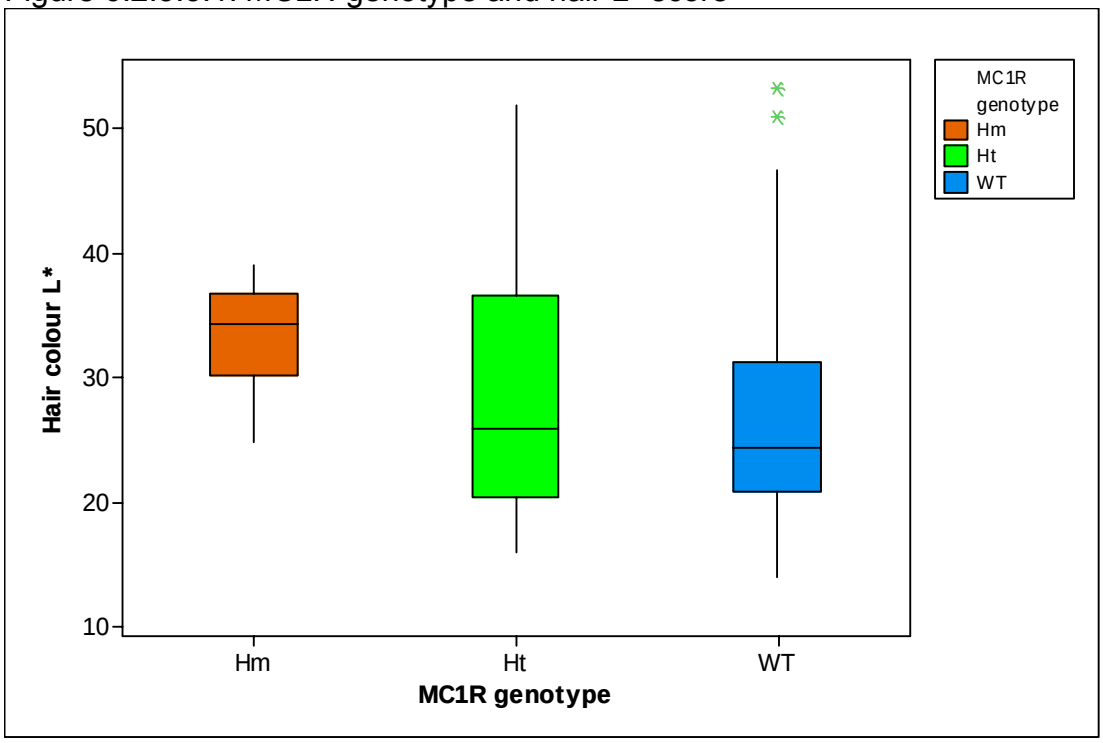
Table 6.2.3.2.b. *MC1R* genotype (Rr 1-5) and sex

MC1R Rr	1	2	3	4	5	Missing	All
F	12	5	34	21	52	2	126
M	1	0	6	6	19	1	33
All	13	5	40	27	71	3	159

There was no effect of sex on *MC1R* genotype Rr1-5 (P=0.3671).

6.2.3.3. *MC1R* and hair colour L* a* b*

Figure 6.2.3.3.1. *MC1R* genotype and hair L* score

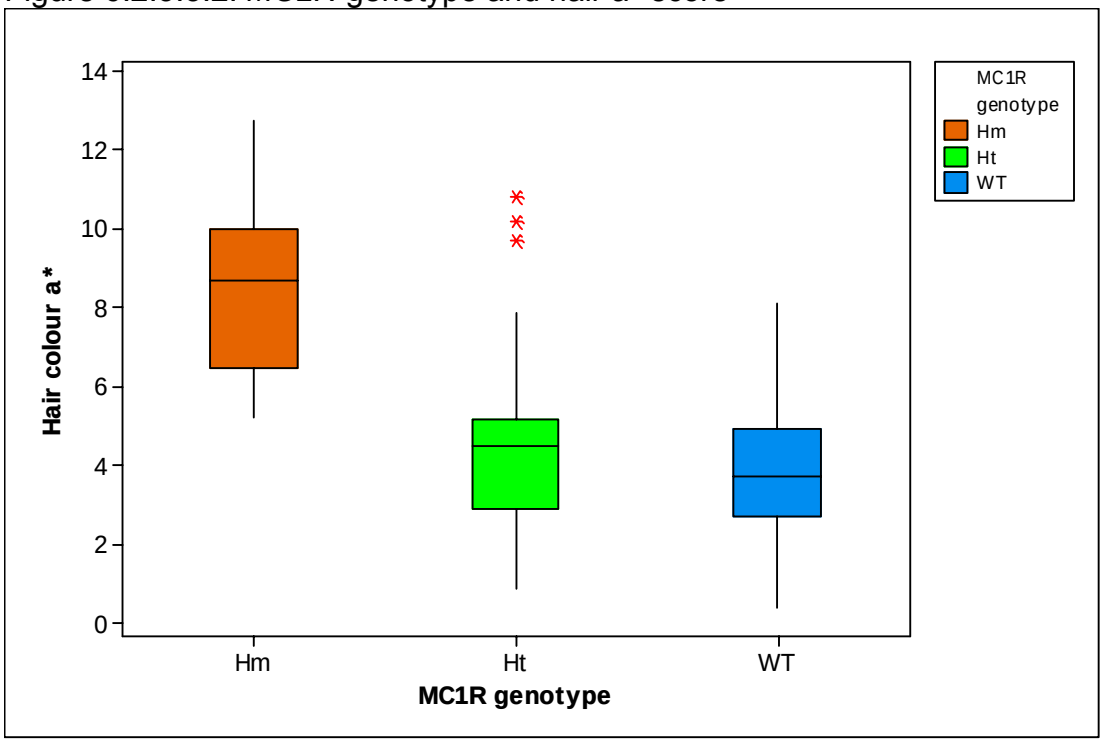


The relationship between *MC1R* genotype and hair L* score. Higher L* values indicate lighter hair colour. Hm, homozygous for *MC1R*; Ht, heterozygous for *MC1R*; WT, wildtype or pseudo-WT.

Individuals homozygous for *MC1R* genotype have lighter hair colour i.e. greater L* score (P=0.0172). Pairwise comparison Hm vs WT (P=0.0183). Hm vs Ht was not significant (P=0.1915). Ht vs WT was not significant (P=0.3586).

There was no dosage effect for this normal population. Failure to demonstrate a dosage effect does not necessary mean a lack of such dosage effect. Small numbers and thus inadequate power may explain this. Previous study showed a dosage effect from a non-random population selected for red hair (Naysmith *et al.*, 2004). If my study sample was larger from the normal population, such a dosage effect may be demonstrable. In the normal population, heterozygous *MC1R* status does not appear to make a difference to hair colour L*. Homozygous for *MC1R* does contribute to lighter hair colour L*.

Figure 6.2.3.3.2. *MC1R* genotype and hair a* score

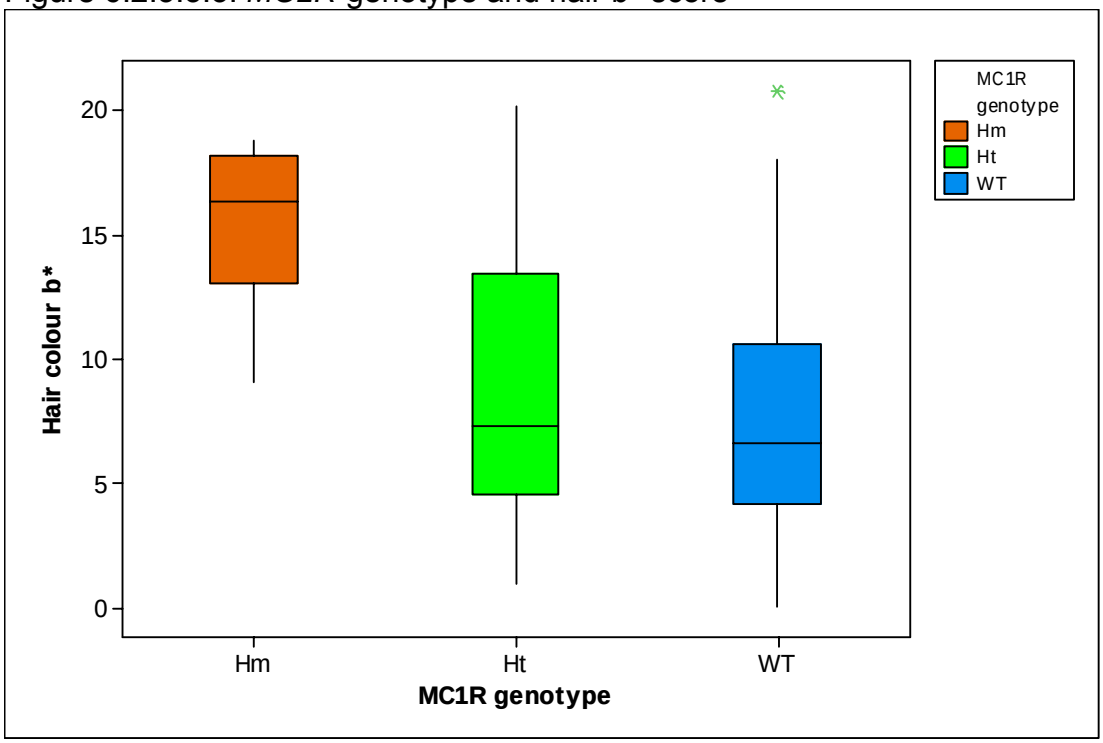


The relationship between *MC1R* genotype and the mean of hair a* score. Higher a* values indicate greater shades of red in hair colour. Hm, homozygous for *MC1R*; Ht, heterozygous for *MC1R*; WT, wildtype or pseudo-WT.

Individuals homozygous for *MC1R* genotype has higher hair a* value i.e. redder (P<0.0001). Pairwise comparison Hm vs WT (P<0.0001), Hm vs Ht (P<0.0001). Ht vs WT not significant (P=0.0877).

The hair a* scale seems to be a good measure of red hair colour but may not be as good a measure for other colour characteristics of hair, if investigating for future studies. Similarly, heterozygous effect is small; there was no dosage effect. However the proportion of reds in this study was smaller than in the previous study. Ht appeared to act as a recessive model with WT.

Figure 6.2.3.3.3. *MC1R* genotype and hair b* score



The relationship between *MC1R* genotype and hair b* score. Higher b* values indicate greater shades of yellow in hair colour. Hm, homozygous for *MC1R*; Ht, heterozygous for *MC1R*; WT, wildtype or pseudo-WT.

Individuals who are homozygous for *MC1R* genotype has higher hair b* value i.e. more yellow (P<0.0001). Pairwise comparison Hm vs WT (P<0.0001), Hm vs Ht (P=0.0001). Ht vs WT not significant (P=0.2987).

Table 6.2.3.3. ANOVA comparisons for relationship between *MC1R* genotype and hair colour L* a* b*

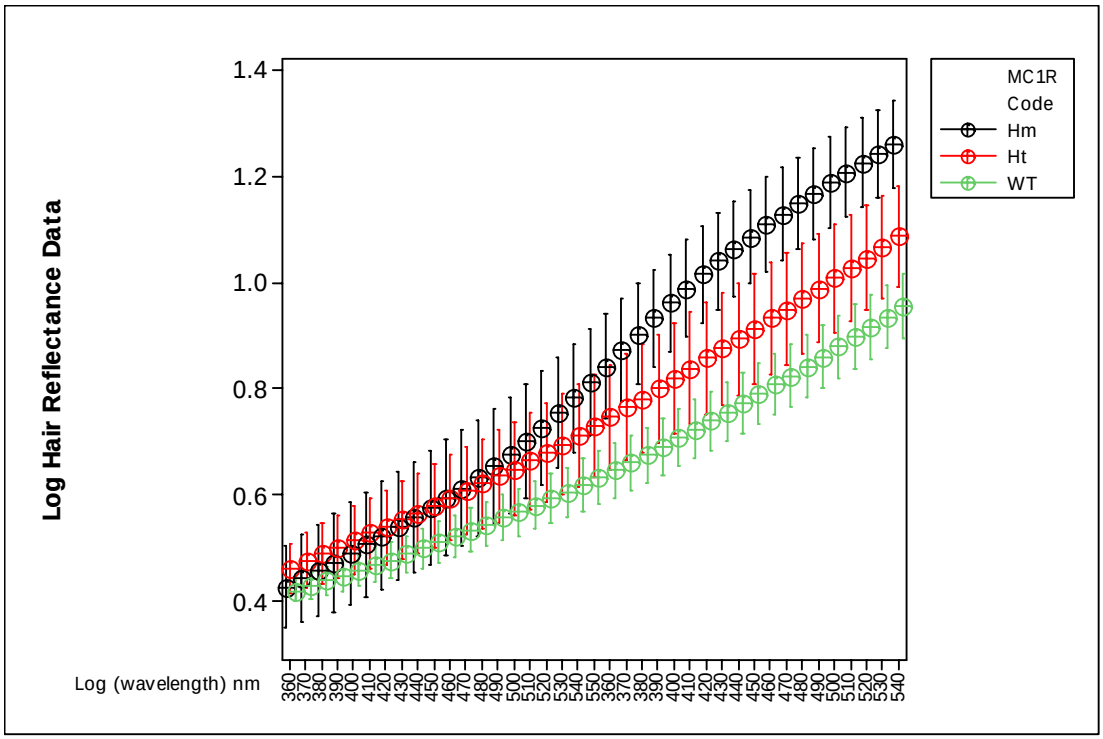
	Hair L*		**Hair a***		**Hair b***	
	Mean	SEM	Mean	SEM	Mean	SEM
Hm (n=13)	33.15	1.26	8.60	0.61	15.47	0.88
Ht (n=41)	28.40	1.53	4.51	0.36	8.83	0.87
WT (n=102)	26.23	0.83	3.76	0.17	7.48	0.47
	P value		P value		P value	
Hm-WT	0.0183		<0.0001		<0.0001	
Hm-Ht	0.1915		<0.0001		0.0001	
Ht-WT	0.3586		0.0877		0.2987	

Summary of ANOVA comparisons for *MC1R* genotype and hair colour L* a* b* values in 156 individuals. Pairwise estimates, SEM, and P values are shown for Hm-WT, Hm-Ht and Ht-WT comparisons for hair colour L* a* b* readings.

6.2.3.3.4. *MC1R* genotype (Hm/Ht/WT) and hair spectrophotometric reflectance

Does *MC1R* have any effect on hair colour?

Figure 6.2.3.3.4. *MC1R* genotype (Hm/Ht/WT) and hair spectrophotometric reflectance



This is an interval plot of hair spectrophotometric reflectance ± SEM (95% CI for the mean) by *MC1R* genotype Hm/Ht/WT Hm (black), Ht (red), WT (green) (n=130). Y-axis shows the log spectrophotometric reflectance data. X-axis shows the 10nm increments of 360-740nm wavelengths.

There was a clear heterozygote effect of *MC1R* on hair reflectance by spectrophotometry. One-way ANOVA at log (740nm) showed significant difference for hair spectrophotometry at 740nm (P=0.0009). Tukey-Kramer multiple comparisons showed pairwise comparison Hm vs WT (P=0.0027), Ht vs WT (P=0.0452). Hm vs Ht was not significant (P=0.2987). The results from the figure suggest a dosage effect although Hm vs Ht was not significant. With larger sample size, this might have showed significant changes. While there were variations in the results, overall this is compatible with a dosage effect.

The spectrophotometry method may be a more powerful means to demonstrate dosage effect. The spectrophotometry data can be difficult to analyse, despite obvious striking difference between groups. Other groups (Australia, France) are beginning to try using principle component analysis technique (personal

communication, Professor J. L. Rees) to analyse. The analysis is beyond the remit of this thesis. Future work would aim to address this.

6.2.3.3.5. Hair reflectance with *MC1R* genotype (R r 1-5)

This classification separated the heterozygous for *MC1R* group into 3 groups (R r; R-/rr; r-) i.e. 2, 3, 4 of the classification. Homozygous for *MC1R* was classified as group 1. Wildtype was classified the same as group 5.

The division of hair reflectance spectrophotometry data into 5 *MC1R* R r 1-5 groups meant very few people in many subgroups. This in turn meant a loss of power and was therefore not performed.

6.2.3.4. Hair colour group with *MC1R* genotype (Hm/Ht/WT)

Figure 6.2.3.4. Hair colour with *MC1R* genotype (Hm/Ht/WT)

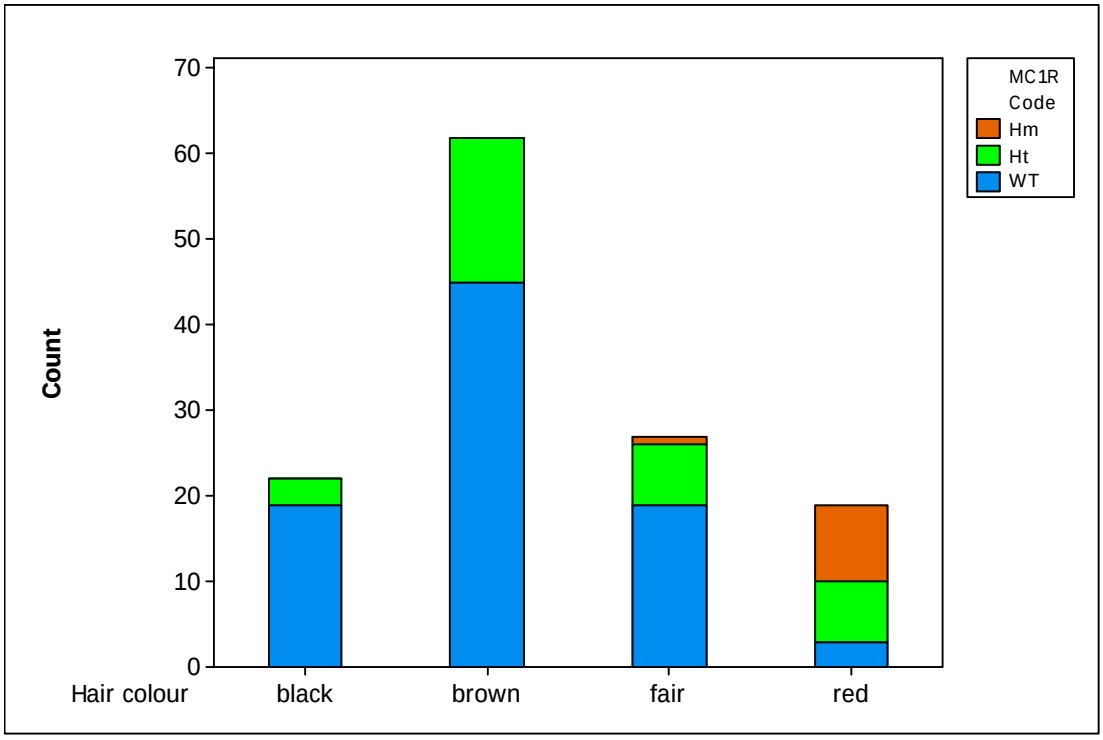


Table 6.2.3.4. Relationship between *MC1R* genotype and hair colour (categorical)

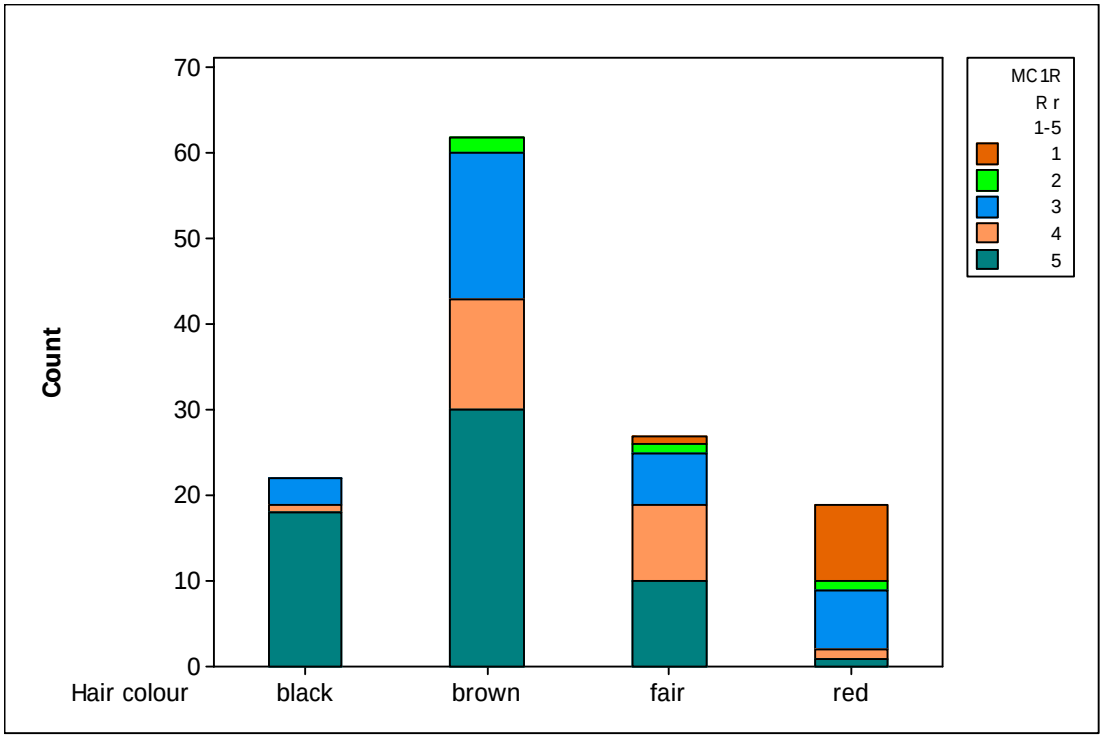
	Hm	Ht	WT	Missing	All
black	0	4	23	1	28
brown	0	20	51	1	72
fair	1	9	24	0	34
red	12	8	4	1	25
All	13	41	102	3	159

Hair colour was classified categorically.

There was a significant association of hair colour with *MC1R* genotype (P<0.0001). Red hair individuals were more likely to be homozygous or heterozygous for *MC1R*. There was an obvious overrepresentation of Hm with red hair and also more Ht with red hair, therefore suggesting a heterozygous effect.

6.2.3.4.2. Hair colour with *MC1R* genotype (R r 1-5)

Figure 6.2.3.4.2. Hair colour with *MC1R* genotype (R r 1-5)

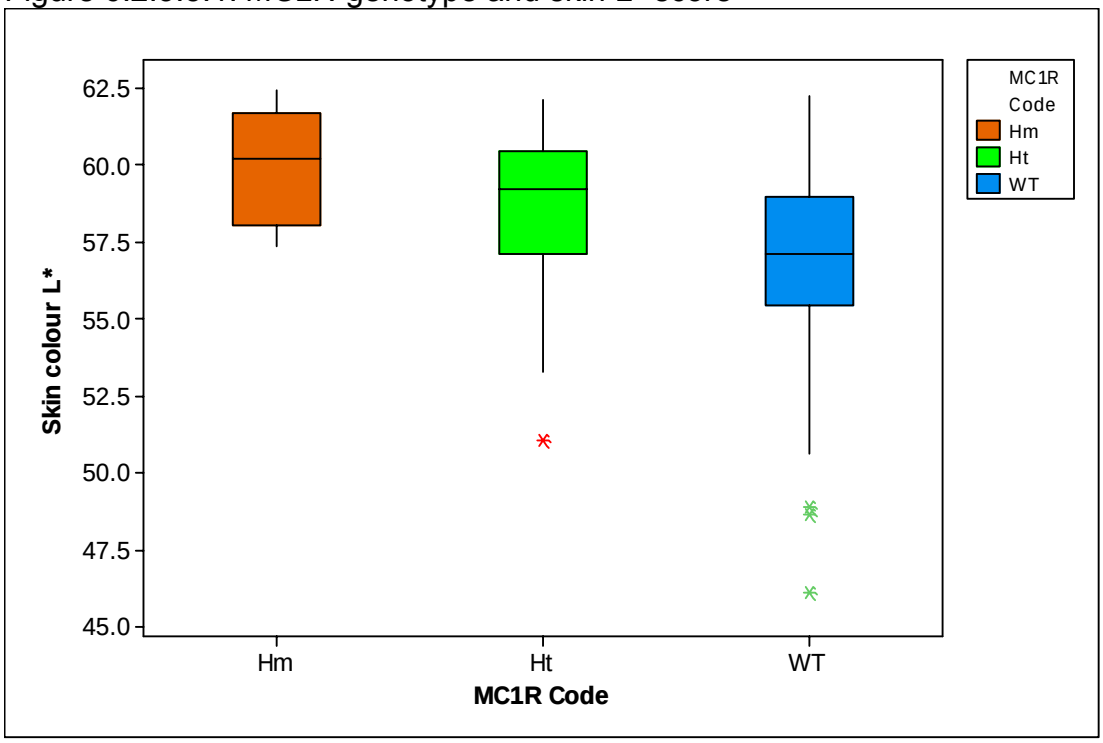


This figure is for illustration only. Homozygosity for *MC1R* accounted for a large proportion of red hair.

6.2.3.5. Skin colour reflectance with *MC1R* genotype (Hm, Ht, WT)

Does *MC1R* have any effect on skin colour?

Figure 6.2.3.5.1. *MC1R* genotype and skin L* score

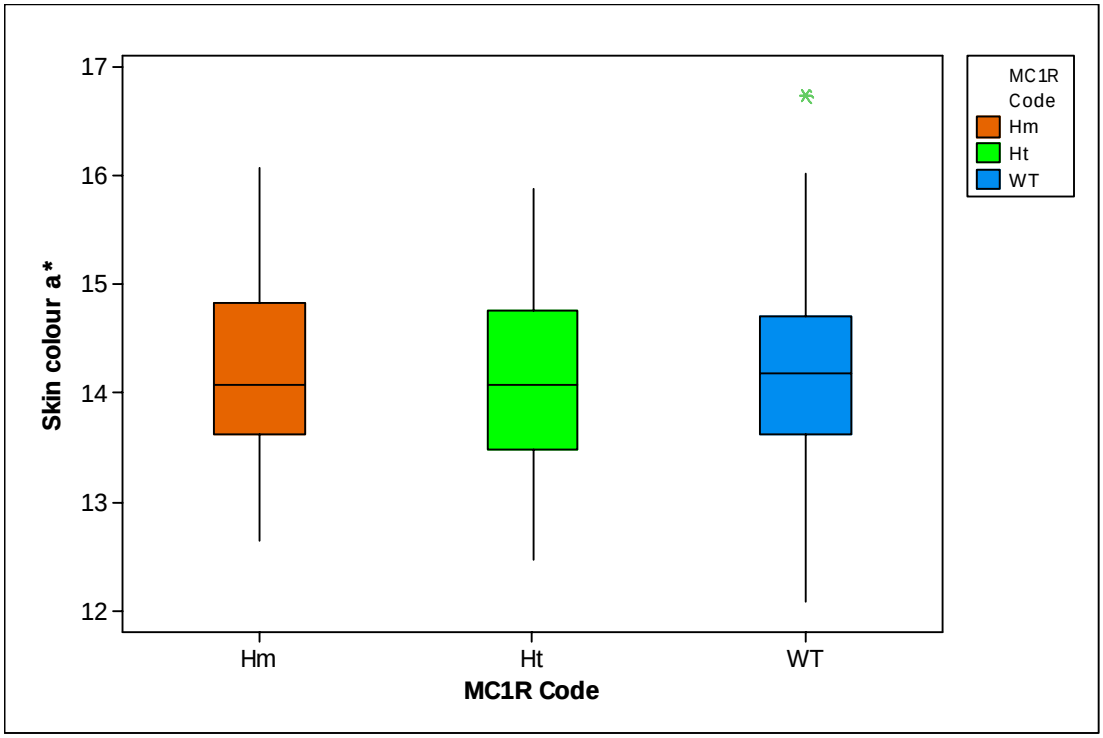


The relationship between *MC1R* genotype and the mean of skin L* score. Higher L* values indicate lighter skin colour. Hm, homozygous for *MC1R*; Ht, heterozygous for *MC1R*; WT, wildtype or pseudo-WT. n=156

Individuals homozygous or heterozygous for *MC1R* genotype has lighter skin colour i.e. greater L* score ($P<0.0001$). Pairwise comparison Hm vs WT ($P=0.001$). Ht vs WT significantly different ($P=0.0019$). Hm vs Ht not significant ($P=0.3609$).

This is compatible with a dominant effect rather than a dosage effect.

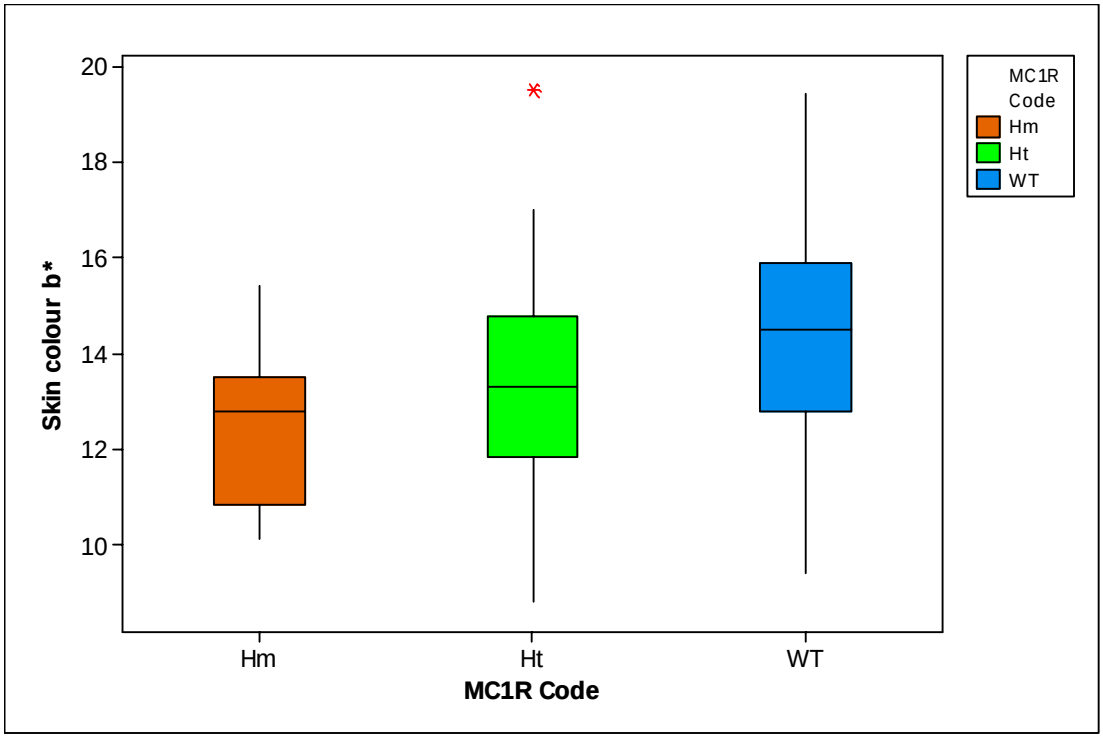
Figure 6.2.3.5.2. *MC1R* genotype and skin a* score



The relationship between *MC1R* genotype and the mean of skin a* score. Higher a* values indicate greater redness in skin colour. Hm, homozygous for *MC1R*; Ht, heterozygous for *MC1R*; WT, wildtype or pseudo-WT.

There was no significantly association with skin a* value with *MC1R* genotype status (P=0.6562). Skin a* appears not to be as useful a scale to use. Colorimetric a* scale may not be as informative. This is in keeping with the findings of Wagner *et al* (Wagner *et al.*, 2002a).

Figure 6.2.3.5.3. *MC1R* genotype and skin b* score



The relationship between *MC1R* genotype and the mean of skin b* score. Higher b* values indicate greater shades of yellow in skin colour. Hm, homozygous for *MC1R*; Ht, heterozygous for *MC1R*; WT, wildtype or pseudo-WT.

Individuals who are wildtype for the *MC1R* genotype have higher skin b* values i.e. more yellow (P=0.0016). Pairwise comparison Hm vs WT (P=0.0075), Ht vs WT (P=0.0313). Hm vs Ht not significant (P=0.3738).

There is an effect, but what this effect represents in terms of actual clinical difference is not known. Colour is subjective and dependent on illumination. Nevertheless this is compatible with a recessive model.

Table 6.2.3.5. ANOVA comparisons for relationship between *MC1R* genotype and skin colour L* a* b*

	Skin L* Mean	SEM	Skin a* Mean	SEM	Skin b* Mean	SEM
Hm (n=13)	59.88	0.51	14.20	0.25	12.38	0.44
Ht (n=41)	58.67	0.38	14.08	0.14	13.33	0.36
WT (n=102)	56.89	0.30	14.22	0.08	14.38	0.23

	P value	P value	P value
Hm-WT	0.001	0.9971	0.0075
Hm-Ht	0.3609	0.8883	0.3738
Ht-WT	0.0019	0.632	0.0313

Summary of ANOVA comparisons for *MC1R* genotype and average skin colour L* a* b* values in 156 individuals. Pairwise estimates, SEM, and P values are shown for Hm-WT, Hm-Ht and Ht-WT comparisons for averaged skin colour L* a* b* readings.

6.2.3.5.4. Skin colour reflectance with *MC1R* genotype (R r 1-5)

As discussed before, the division into 5 groups meant a loss of power in testing and was not performed.

6.2.3.6. *MC1R* genotype (Hm/Ht/WT) and eye colour groups

Figure 6.2.3.6. *MC1R* genotype and eye colour

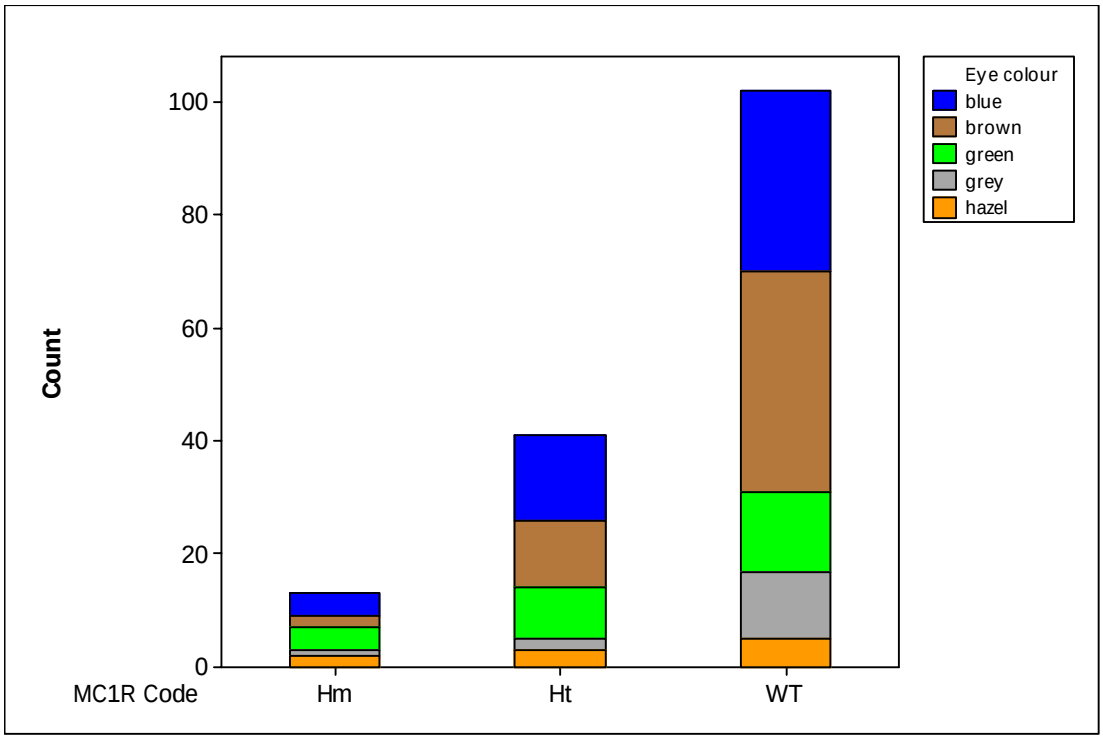


Table 6.2.3.6. Relationship between *MC1R* genotype and eye colour groups

	Hm	Ht	WT	Missing	All
blue	4	15	32	1	52
brown	2	12	39	2	55
green	4	9	14	0	27
grey	1	2	12	0	15
hazel	2	3	5	0	10
All	13	41	102	3	159

A contingency table of relationship between *MC1R* genotype and eye colour classified categorically into 5 different groups.

There was no significant association with a particular eye colour with *MC1R* genotype (P=0.315) in this population sample, even with Hm and Ht combined (P=0.1751). The numbers presented and tested were small. Nevertheless blue eye colour accounted for the highest percentage of eye colour - 19 (35%) out of 54 *MC1R* genotypes (homozygous and heterozygous combined). Eye colour was not significantly associated with *MC1R* SNP 'R' or 'r' when analysed together with other SNPs of candidate genes. The effect of *MC1R* on eye colour was not big. This may be due to the way of classification could be subjective.

6.2.3.7. *MC1R* genotype (Hm RR, Hetero (Ht), WT) and skin type

Does *MC1R* have any effect on skin type?

Figure 6.2.3.7. *MC1R* genotype (Hm RR, Hetero (Ht), WT) and skin type

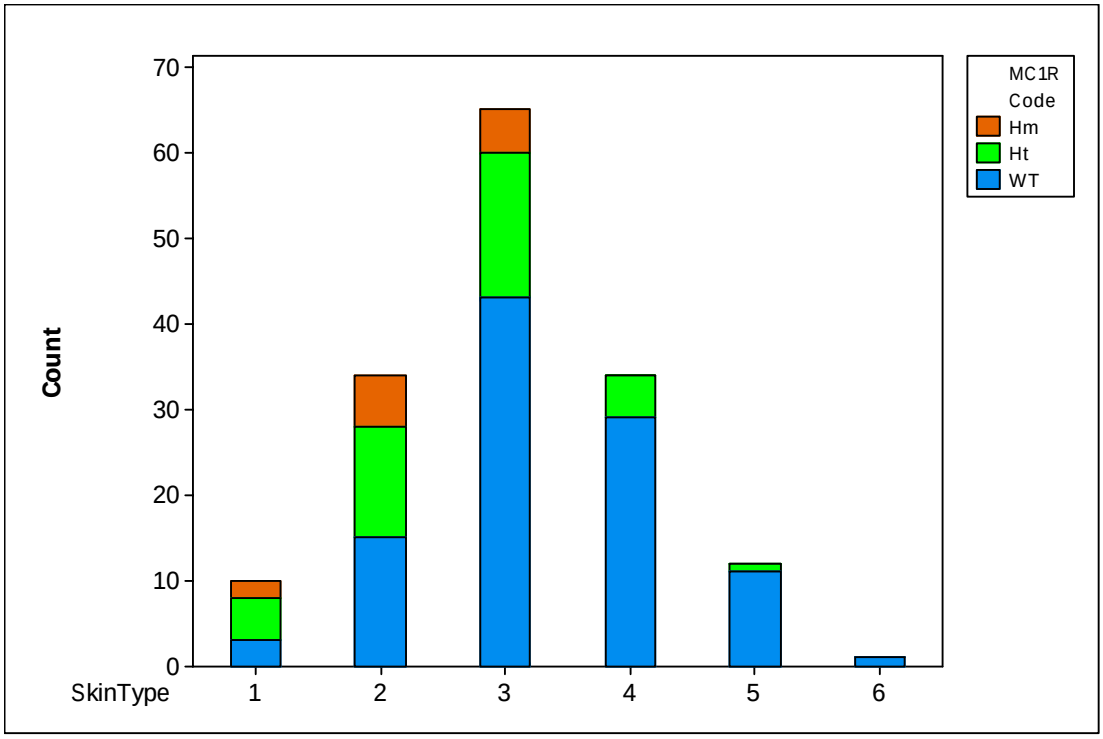


Table 6.2.3.7. Relationship between *MC1R* genotype and Fitzpatrick skin type

	Hm	Ht	WT	Missing	All
1	2	5	3	0	10
2	6	13	15	1	35
3	5	17	43	0	65
4	0	5	29	1	35
5	0	1	11	1	13
6	0	0	1	0	1
All	13	41	102	3	159

A contingency table of relationship between *MC1R* genotype and skin type in terms of Fitzpatrick classification.

MC1R genotype has a significant effect on skin type (P=0.002203), P=0.0002 with homozygous and heterozygous combined.

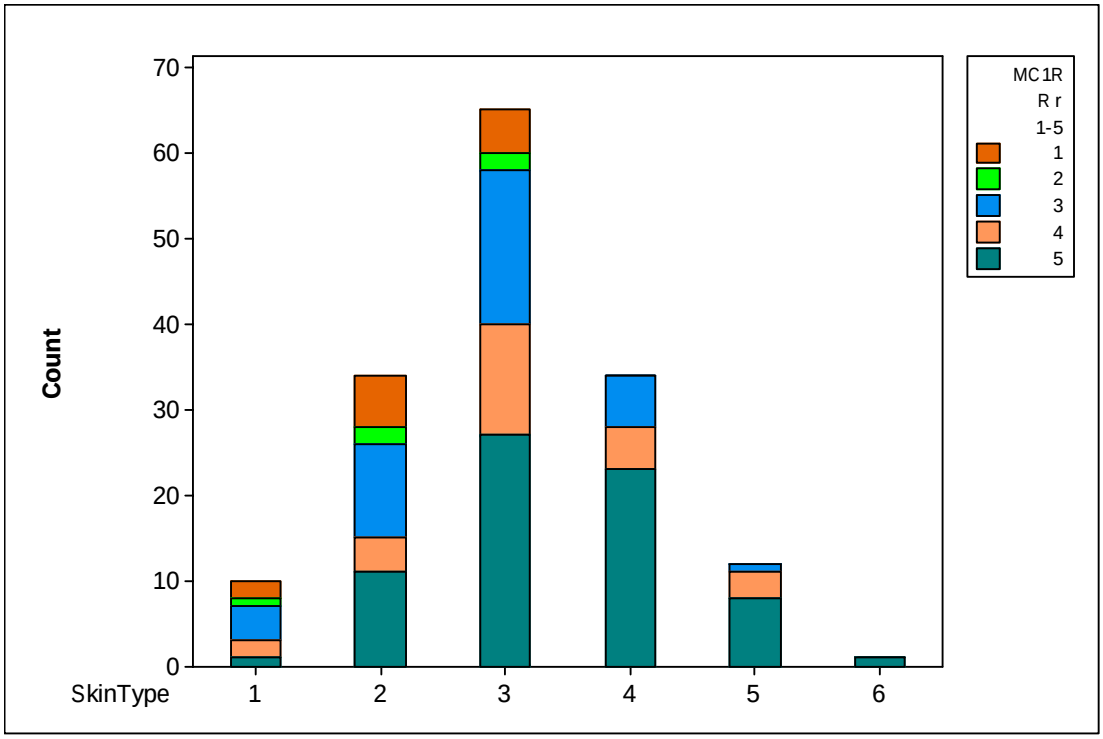
Testing skin type 1-3 vs skin type 4-6:

	Hm	Ht	WT
Skin type 1-3	13	35	61
Skin type 4-6	0	6	41

MC1R genotype has a significant effect on skin type 1-3 and 4-6 (P=0.0002).

6.2.3.7.2. *MC1R* genotype (R r 1-5) and skin type

Figure 6.2.3.7.2. *MC1R* genotype (R r 1-5) and skin type



For illustrative purpose only. n=156.

Skin type 1-3 comprises of individuals with *MC1R* group 1 (RR) genotypes.

6.2.3.8. *MC1R* genotype (Hm/Ht/WT) and freckles

Does *MC1R* have any effect on freckles (yes / no)?

Figure 6.2.3.8. *MC1R* genotype (Hm/Ht/WT) and freckles

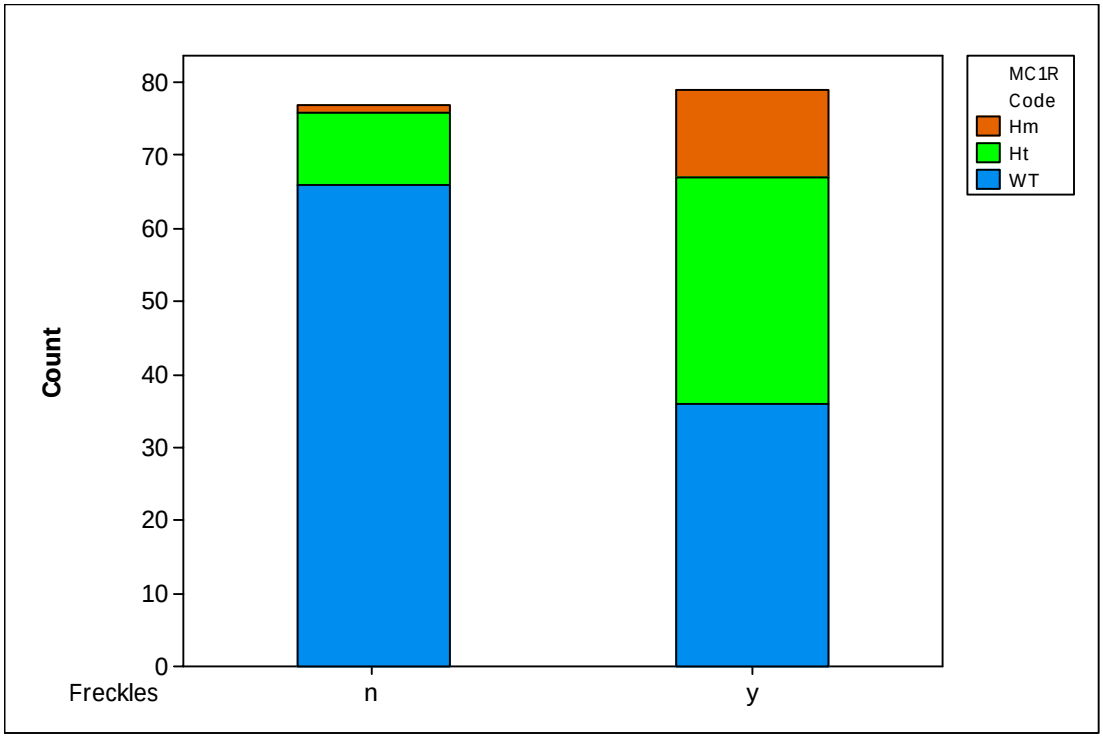


Table 6.2.3.8. Relationship between *MC1R* genotype and freckles

	Hm	Ht	WT	Missing	All
No freckles	1	10	66	2	79
Freckles	12	31	36	1	80
All	13	41	102	3	159

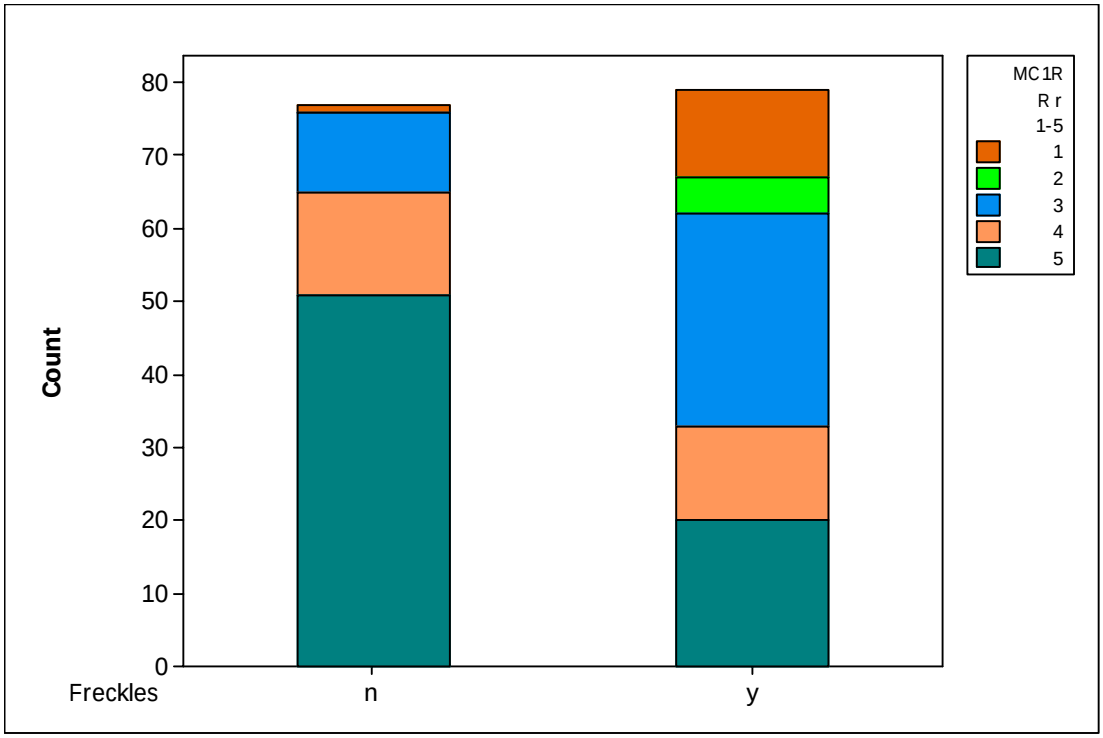
The presence of freckles was classified categorically.

There was a significant association of *MC1R* genotype and the presence of freckles (P<0.0001). There was an obvious overrepresentation of Hm with freckles and also more Ht with freckles. *MC1R* appeared to have a dosage effect on freckling. Only 35% WT had freckles, as compared to 76% Ht had freckles and 92% Hm had freckles. Freckling is a remarkably good measure of phenotype.

6.2.3.8.2. *MC1R* genotype (R r 1-5) and freckles

Does *MC1R* have any effect on freckles?

Figure 6.2.3.8.2. *MC1R* genotype Rr 1-5 and the presence of freckles



6.2.3.8.3. *MC1R* genotype (Hm/Ht/WT) and number of freckling sites

Does *MC1R* have any effect on freckling sites?

MC1R and number of freckling sites

Figure 6.2.3.8.3. *MC1R* genotype (Hm/Ht/WT) and number of freckling sites

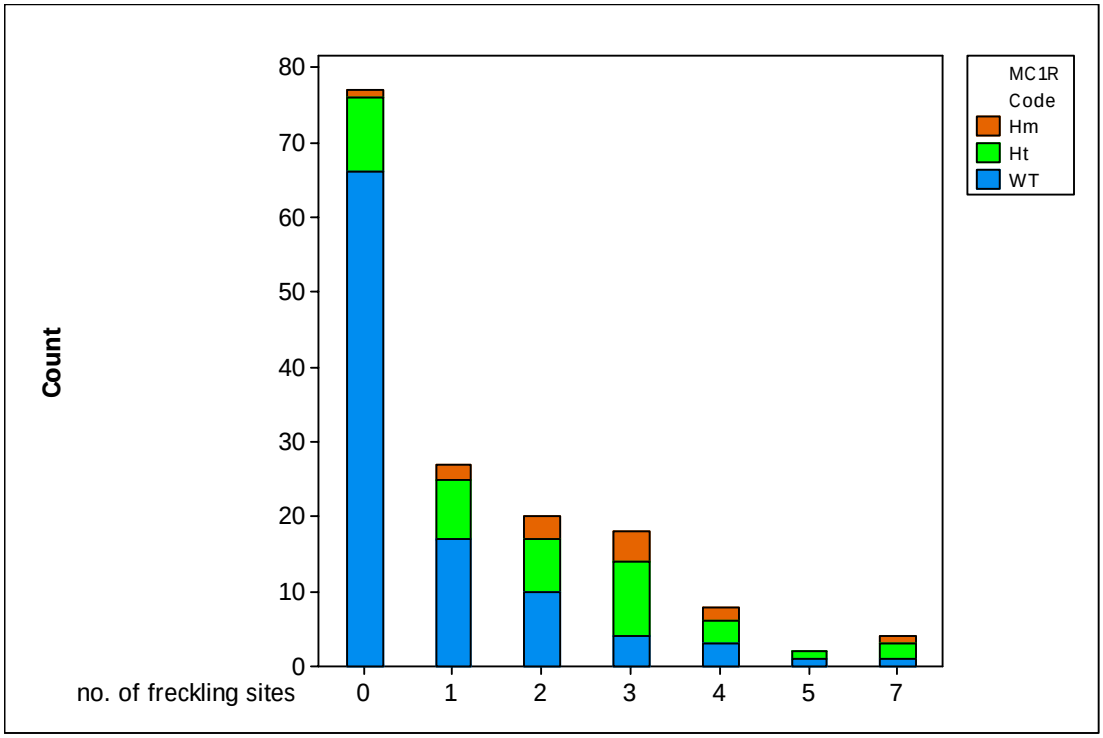


Table 6.2.3.8.3. Relationship between *MC1R* genotype and number of freckling sites

	Hm	Ht	WT	Missing	All
0	1	10	66	2	79
1	2	8	17	0	27
2	3	7	10	1	21
3	4	10	4	0	18
4	2	3	3	0	8
5	0	1	1	0	2
7	1	2	1	0	4
All	13	41	102	3	159

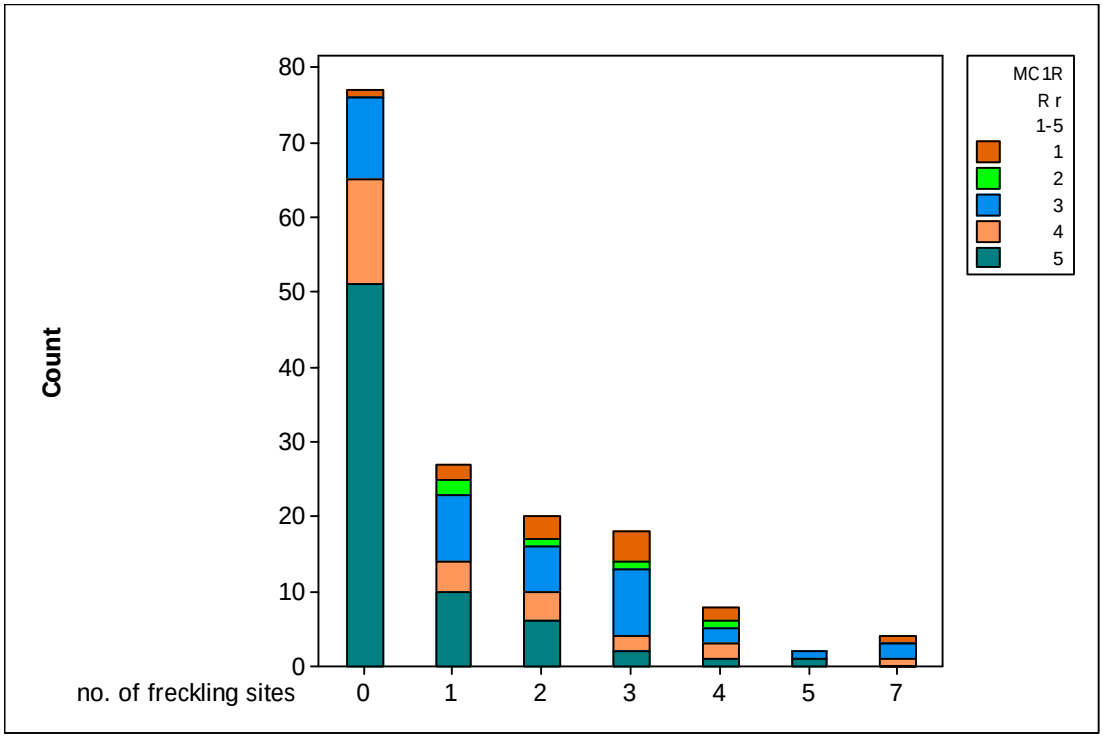
The number of freckling sites was presented in absolute numbers.

There was a significant association between *MC1R* genotype and number of freckling sites ($P=9.598x10^{-7}$), $P<0.0001$ with homozygous and heterozygous combined. Homozygous and heterozygous for *MC1R* accounts for higher number of freckling sites. Wildtype for *MC1R* tends to have no freckling sites.

6.2.3.8.4. *MC1R* genotype (R r 1-5) and number of freckling sites

Does *MC1R* has any effect on freckling sites?

Figure 6.2.3.8.4. *MC1R* genotype (R r 1-5) and number of freckling sites



For illustrative purpose only. n=156.

6.2.3.9. *MC1R* genotype (Hm/Ht/WT) and ethnicity

Figure 6.2.3.9. *MC1R* genotype (Hm/Ht/WT) and ethnicity

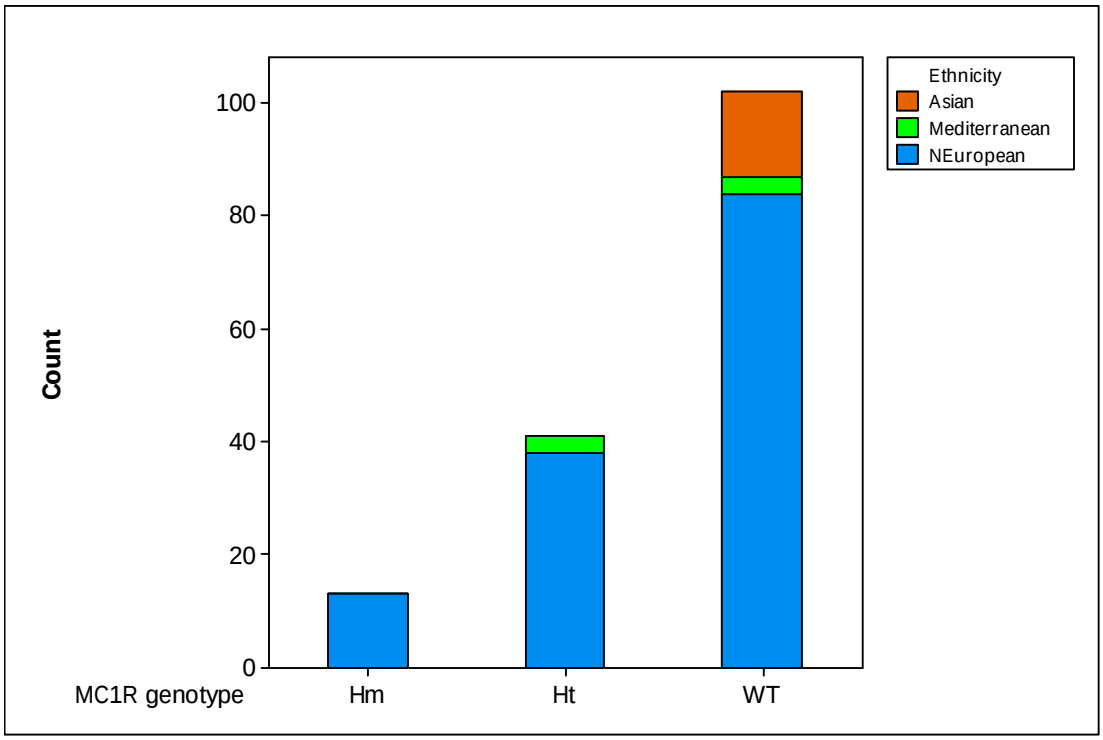


Table 6.2.3.9. Relationship between *MC1R* genotype and ethnicity

	Hm	Ht	WT	Missing	All
Asian	0	0	15	0	15
Mediterranean	0	3	3	2	8
NEuropean	13	38	84	1	136
All	13	41	102	3	159

Ethnicity was classified categorically. Data was presented in numbers.

There was a significant association between *MC1R* genotype and ethnicity (P=0.02). Individuals from Northern European descent were more likely to be Hm or Ht for *MC1R* genotype than Mediterranean or Asian.

6.2.3.10. Red hair and *MC1R* sequence variants

Table 6.2.3.10 Red hair and *MC1R* genotype Hm/Ht/WT

	Hm	Ht	WT	Missing	All
n	1	33	98	2	134
y	12	8	4	1	25
All	13	41	102	3	159

There was a significant association between red hair status and *MC1R* genotype Hm Ht (P<0.0001).

	Hm	Ht	WT
Red hair	12	8	4
Non-red hair	1	33	98

ORs for red hair Hm vs WT = 245.74 (95%CI 27.1-12665.3) P<0.0001

ORs for red hair Ht vs WT = 5.85 (95%CI 1.46-28.32) P=0.005

D84E	Hm	Ht	WT
Red hair	0	1	4
Non-red hair	0	2	98

ORs for red hair Ht vs WT (D84E) = 12.25 (95%CI 0.2-268.4) P=0.1374

R151C	Hm	Ht	WT
Red hair	1	13	4
Non-red hair	0	11	98

ORs for red hair Hm vs WT (R151C) = infinity (95%CI 0.5-infinity) P=0.0485

ORs for red hair Ht vs WT (R151C) = 28.95 (95%CI 7.03-136.81) P<0.0001

R160W	Hm	Ht	WT
Red hair	1	10	4
Non-red hair	0	13	98

ORs for red hair Hm vs WT (R160W) = infinity (95%CI 0.5-infinity) P=0.0485

ORs for red hair Ht vs WT (R160W) = 18.85 (95%CI 4.45-90.91) P<0.0001

D294H	Hm	Ht	WT
Red hair	0	3	4
Non-red hair	0	7	98

ORs for red hair Ht vs WT (D294H) = 10.5 (95%CI 1.23-73.88) P=0.0151

29insA	Hm	Ht	WT
Red hair	0	1	4
Non-red hair	0	1	98

ORs for red hair Ht vs WT (29insA) = 24.5 (95%CI 0.25-1940.25) P=0.0943

One would expect this to be loss of function allele to be significant, but due to small number (1 out of 2 individuals who has red hair) there is little chance to expect this to be significant.

V60L	Hm	Ht	WT
Red hair	0	2	4
Non-red hair	3	33	98

ORs for red hair Hm vs WT (V60L) = 0 (95%CI 0-74.37) P=0.889

ORs for red hair Ht vs WT (V60L) = 1.48 (95%CI 0.13-10.88) P=0.4814

V92M	Hm	Ht	WT
Red hair	0	1	4
Non-red hair	1	30	98

ORs for red hair Hm vs WT (V92M) = 0 (95%CI 0-965.25) P=0.9612

ORs for red hair Ht vs WT (V92M) = 0.82 (95%CI 0.016-8.69) P=0.669

R163Q	Hm	Ht	WT
Red hair	0	0	4
Non-red hair	3	20	98

ORs for red hair Hm vs WT (R163Q) = 0 (95%CI 0-74.37) P=0.889

ORs for red hair Ht vs WT (R163Q) = 0 (95%CI 0-7.93) P=0.4838

R142H	Hm	Ht	WT
Red hair	0	1	4
Non-red hair	0	1	98

ORs for red hair Ht vs WT (R142H) = 24.5 (95%CI 0.25-1940.25) P=0.0943

6.2.3.10. Summary of results presented in tables

V60L variant was the commonest *MC1R* sequence variant found in this population, occurring in 38/156 (24%) (allelic frequency 12%). 1 individual heterozygous for V60L with a wildtype allele had red hair. The other individual was a compound heterozygous R151C with V60L (151/60).

V92M variant was also quite frequent in this population, occurring in 31/156 (20%) (allelic frequency 9%). There was no significant association between V92M and red hair.

ORs for red hair V92M = 0.1 (95%CI 0.0-1.0) P=0.0483. Only 1 individual with V92M had red hair, coupled with a wildtype allele (92/WT).

None of the individuals heterozygous for R163Q variant had red hair. The 20 individuals comprised 12 black, 5 brown and 3 fair hair.

1 individual compound heterozygous for D84E and R151C (84/151) had red hair. The other 2 individuals were brown (84/WT) and fair hair (84/WT).

Only 2 individuals had R142H variant. One was compound heterozygous for R151C (142/151) and had red hair. The other had brown hair and was heterozygous for R142H (142/WT).

14 out of 24 red hair individuals sequenced were compound heterozygous (8 with other red hair alleles: 5 with R151C, 1 with D84E, 1 with R142H, 1 with D294H) or heterozygous for R151C (1 with V60L, 1 with 131, 1 with 279, 2 WT). 1 individual was homozygous for R151C. 11 other individuals heterozygous for R151C were non-reds. 5 were fair hair and 6 had brown hair. 1 fair hair individual was compound heterozygous for R160W (151/160). 4 other fair hair were heterozygous for R151C (1 with V60L, 1 with V92M, 2 WT). The brown hair individuals were heterozygous for R151C (1 with 60, 2 with 92, 3 WT).

11 out of 24 red hair individuals sequenced were compound heterozygous (7 with other red hair alleles: 5 with R151C, 2 with D294H) or heterozygous for R160W (3WT). 1 individual was homozygous for R160W. 13 other individuals heterozygous

for R160W were non-reds. 3 were fair hair, 9 were brown and 1 had black hair. 1 of the 3 fair hair individuals was compound heterozygous for R160W with R151C. The other 2 were with V60L or WT. The 9 brown hair individuals were heterozygous for R160W (2 with V92M, 1 with R163Q, 1 with 212,230 and 5 WT). The black hair individual was heterozygous for R160W with WT.

10 individuals were heterozygous for D294H. 3 had red hair were compound heterozygous for D294H with R151C (1 individual) or R160W (2 individuals). 3 other individuals had brown hair. 3 had black hair and 1 had fair hair. 6 of these individuals were heterozygous for D294H (294/WT) except 1 brown hair individual who was heterozygous for V60L (294/60).

Only 2 individuals had 29insA variant. One had red hair and the other fair hair.

Table 6.2.3.10.2. Red hair and *MC1R* genotype R r 1-5

	1	2	3	4	5	Missing	All
n	1	4	32	26	69	2	134
y	12	1	8	1	2	1	25
All	13	5	40	27	71	3	159

There was a significant association between red hair status and *MC1R* genotype classified by Rr status ($P<0.0001$).

To assess the penetrance of *MC1R* red hair allelic variants (84, 142, 151,160, 294 and 29insA) in this study, the proportion of individuals with 2 variants that have red hair were looked at.

24 individuals out of 156 sequenced have red hair. 13 individuals were homozygous or compound heterozygous for 2 of these variants. 12 out of 13 of these had red hair. The individual that did not have red hair had *MC1R* variant of 151/160 (V81) and blonde hair. 5 out of 24 were heterozygous for 1 of the *MC1R* variants and only 1 out of 5 had red hair. Only 8 out of 40 had red hair with R– or rr alleles. 3 out of 98 who were wildtype for *MC1R* variants had red hair.

Most instances when 84, 142, 151 and 160 are present, individuals have red hair. 294 appeared to be a weaker red allele, giving strawberry blonde. This was in keeping with findings from previous studies. 160 and 294 together also gave a less red shade

i.e. auburn, suggesting perhaps 160 appeared to be weaker in exerting its red hair effect than 151. Although number of individuals involved was small.

Volunteer number	Red hair shade	MC1R genotype	MC1R classification (R r)
V6	strawberry blonde	160/294	RR
V25	strawberry blonde	151/294	RR
V32	red	151/160	RR
V66	red	151/160	RR
V73	red	151/160	RR
V87	red	84/151	RR
V96	red	151/151	RR
V106	red	151/160	RR
V121	red	142/151	RR
V30	auburn	160/294	RR
V43	auburn	151/160	RR
V108	auburn	160/160	RR
V50	red	151/60	R-
V33	strawberry blonde	29insA/314	R-
V72	strawberry blonde	151/WT	R-
V136	red	160/WT	R-
V126	auburn strawberry blonde	151/WT	R-
V110	auburn	160/155+314	R-
V120	auburn	151/131	R-
V133	auburn	151/279	R-
V143	auburn	160/WT	R-
V31	auburn	60/WT	r-
V47	red	92+314/WT	--
V154	auburn	WT/WT	--
V35	red	?	?

All individuals, except one, harbouring two RR allelic variants have red hair (12).

This 1 individual (V81) was Hm for RR or a compound heterozygote (151/160) without red hair – with natural undyed blonde fair hair, blue grey eyes and of skin type 2. This was contrary to Smith *et al*, where no individuals harbouring two RR allelic variants did not have red hair (Smith *et al.*, 1998).

The reason for this is unclear – unless there was incorrect classification of hair colour. This individual definitely had blonde fair hair colour and not red hair. I looked back through my experimental records and confirmed that there were no mistakes in the classification of the hair colour. Similarly, I went back to the *MC1R* sequencing data and confirmed that there was no mix up of samples.

The possibility of interaction of *MC1R* with other genes e.g. *ASIP* could be possible, contributing to the less red hair colour.

Was there any Ht without red hair? Yes, 33 individuals had R/r or R/WT – among these 4 had black hair, 20 had brown and 9 had fair hair. *MC1R* could possibly interact with *ASIP*. ASIP blocked the binding of α-MSH to the MC1R and inhibited the effects of α-MSH on human melanocytes (Suzuki *et al.*, 1997). There is evidence that agouti signaling protein (*ASIP*) gene (8818G allele) is associated with darker skin color in African Americans (Bonilla *et al.*, 2005).

8 red haired individuals have one allelic variant R only. 4 red hair individuals had 60r/WT, 92r/WT, 151R/60r or WT/WT. For the WT/WT individual, could it be due to a mutation outside the *MC1R* gene e.g. a promoter?

6.2.3.11. *MC1R* genotype and blonde hair

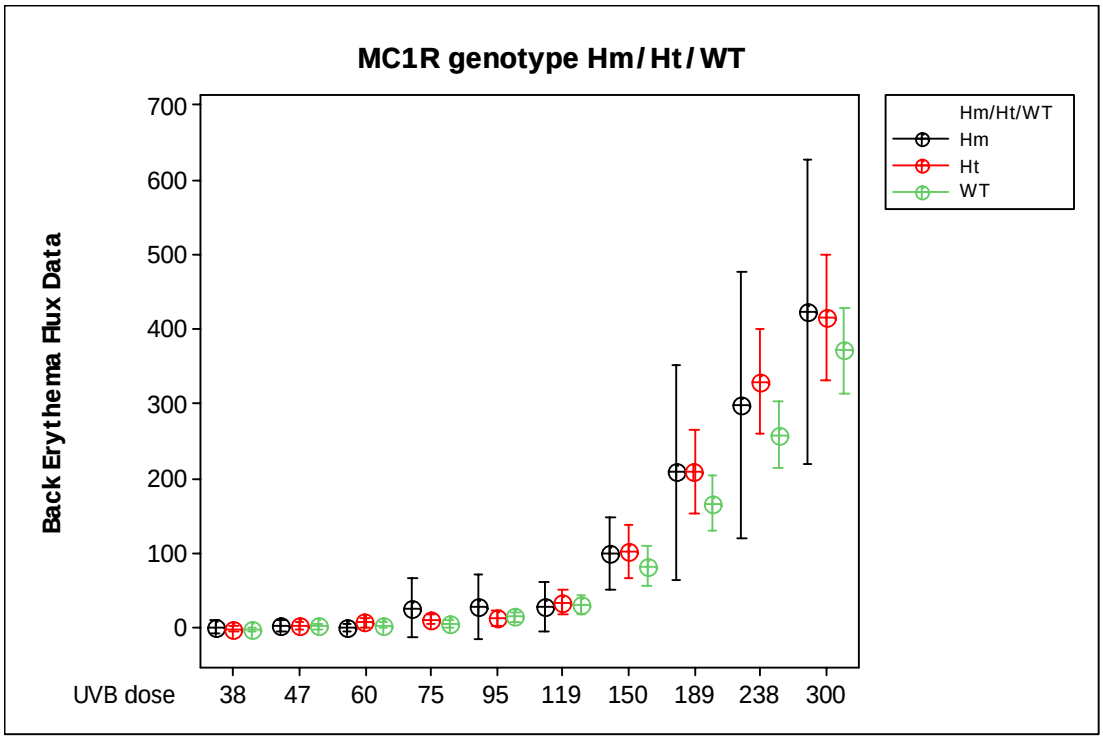
Table 6.2.3.11. Blonde hair and *MC1R*

	Hm	Ht	WT	Missing	All
n	12	36	89	3	140
y	1	5	13	0	19
All	13	41	102	3	159

There was no significant association between blonde hair status and *MC1R* genotype (P>0.9999).

6.2.3.12. Erythemal flux and *MC1R* genotype Hm/Ht/WT

Figure 6.2.3.12.1. Back erythemal flux and *MC1R* genotype Hm/Ht/WT



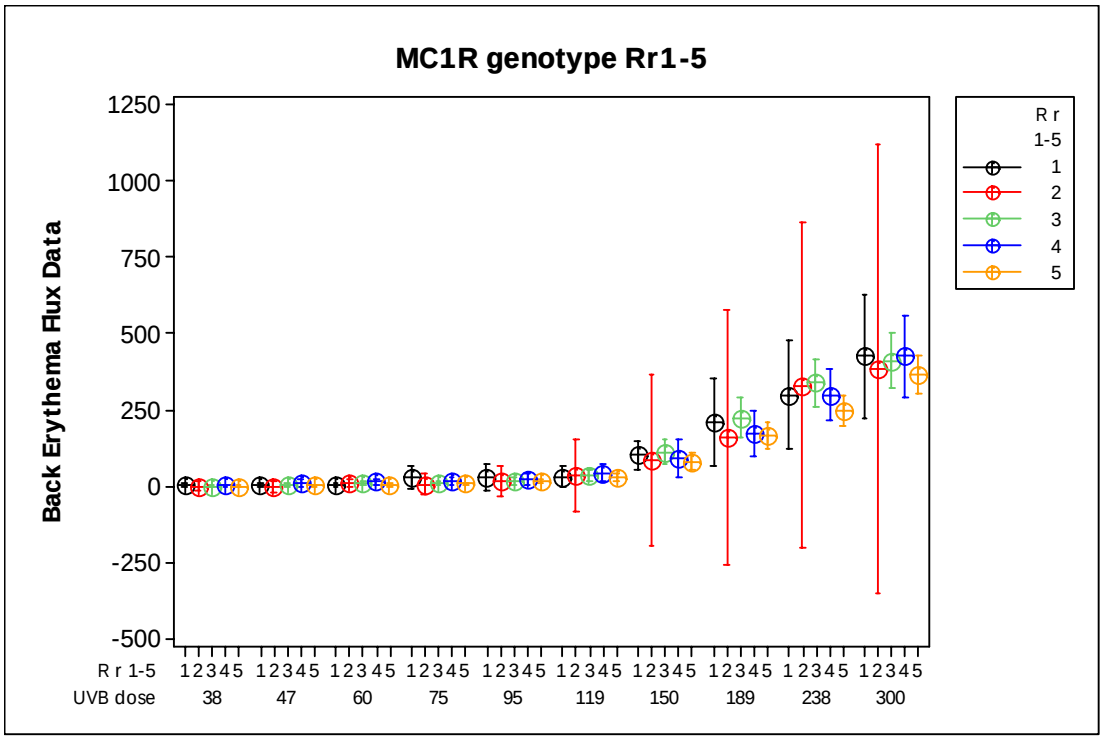
This shows back erythema flux and *MC1R* genotype (Hm/Ht/WT). Y-axis shows back erythema flux ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).

MC1R status did not make much difference to the erythemal flux for back.

Do genes affect blood flow phenotype? No, *MC1R* did not contribute to back erythemal flux. One-way ANOVA for back erythema flux at dose 3 (300mJ/cm^2) showed no significant difference (P=0.613).

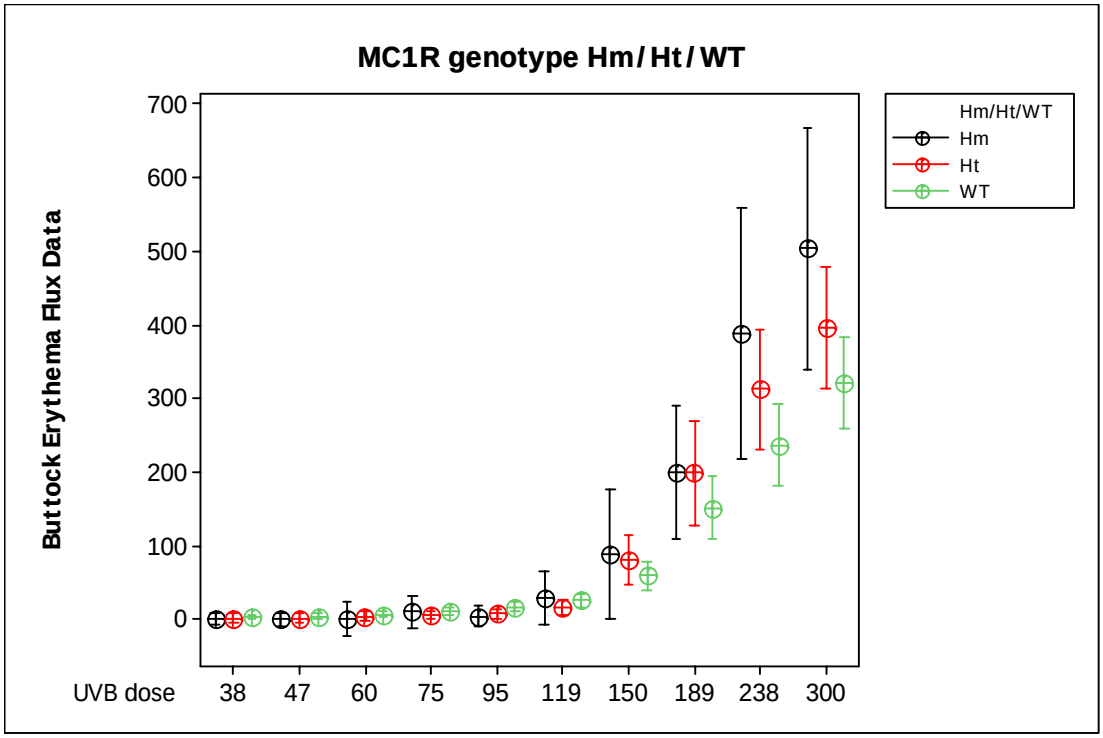
6.2.3.12.2. Back erythemal flux and *MC1R* genotype Rr1-5

Figure 6.2.3.12.2. Back erythemal flux and *MC1R* genotype Rr1-5



This shows back erythema flux and *MC1R* genotype (Rr1-5). Y-axis shows back erythema flux ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).

Figure 6.2.3.12.3. Buttock erythemal flux and *MC1R* genotype Hm/Ht/WT

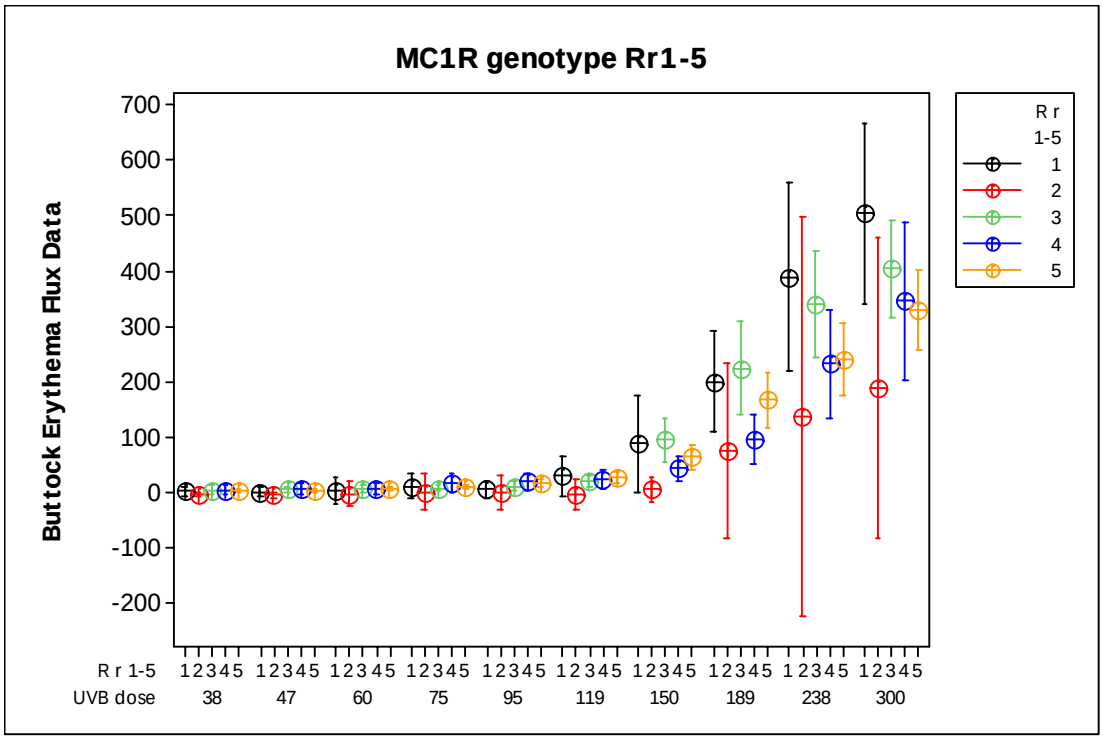


This shows buttock erythema flux and *MC1R* genotype (Hm/Ht/WT). Y-axis shows buttock erythema flux ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).

MC1R homozygosity status contributed to the erythemal flux for buttock. One-way ANOVA for buttock erythema flux at dose 3 (300mJ/cm^2) showed no significant difference (P=0.071). Do genes affect blood flow phenotype? No, *MC1R* did not contribute significantly to buttock erythemal flux.

6.2.3.12.4. Buttock erythemal flux and *MC1R* genotype Rr1-5

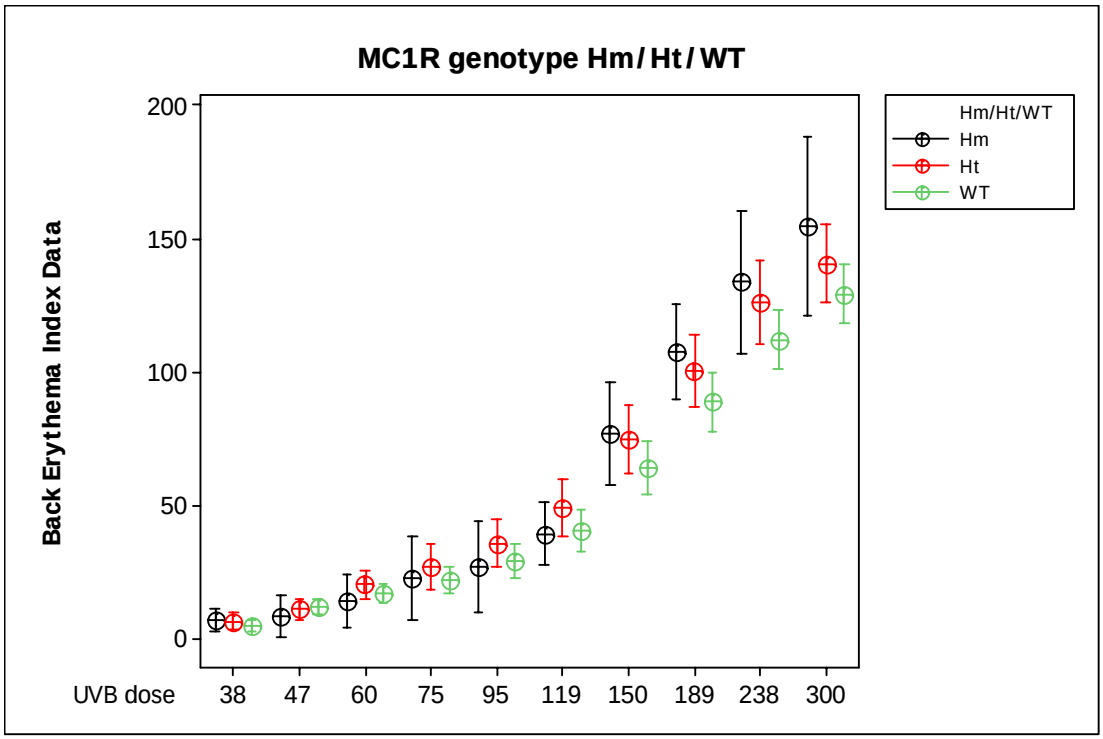
Figure 6.2.3.12.4. Buttock erythemal flux and *MC1R* genotype Rr1-5



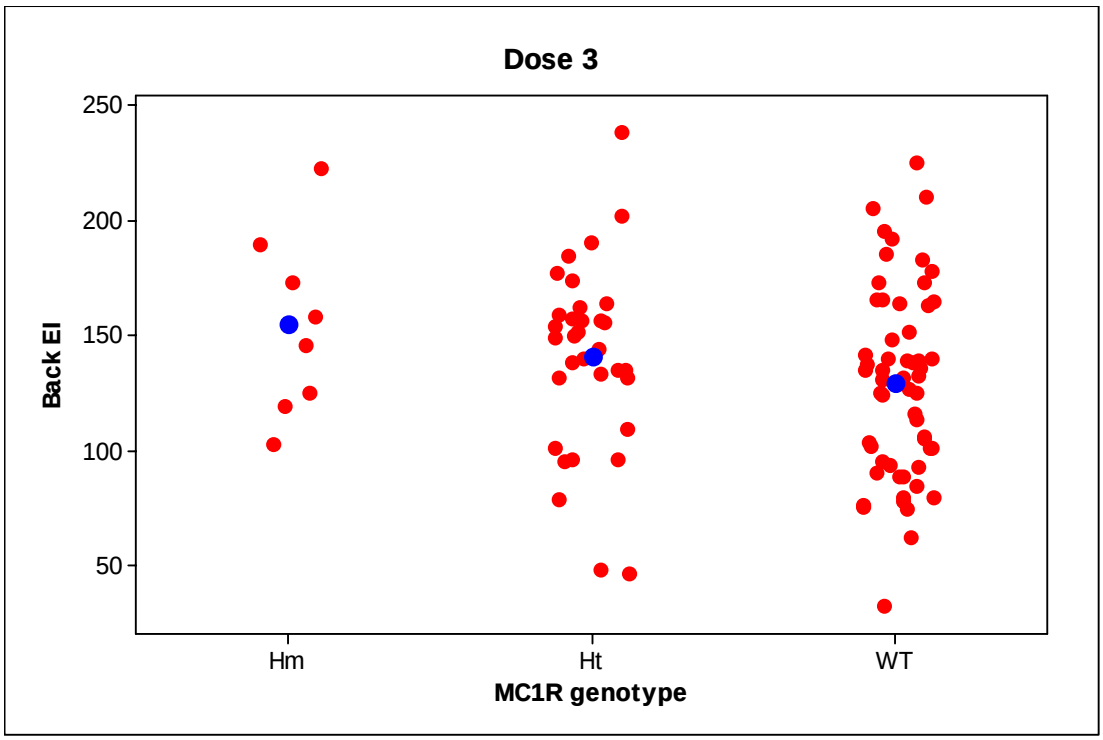
This shows buttock erythema flux and *MC1R* genotype (Rr1-5). Y-axis shows buttock erythema flux ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).

6.2.3.12.5. Erythemal Index (EI) and *MC1R* genotype Hm/Ht/WT (back)

Figure 6.2.3.12.5. Back EI and *MC1R* genotype Hm/Ht/WT



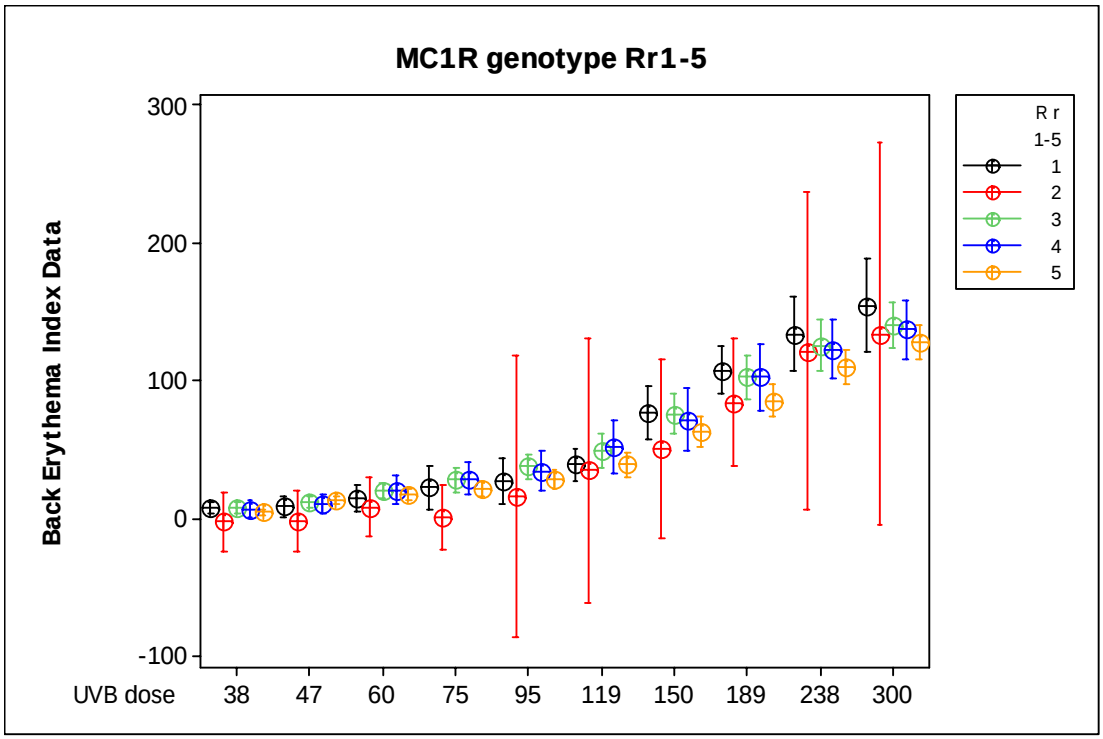
This shows back erythema index and *MC1R* genotype (Hm/Ht/WT). Y-axis shows back erythema index ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).



One-way ANOVA for back erythema EI at dose 3 vs *MC1R* Genotype Hm/Ht/WT showed no significant difference (P=0.169).

6.2.3.12.6. Back Erythema Index and *MC1R* genotype Rr1-5

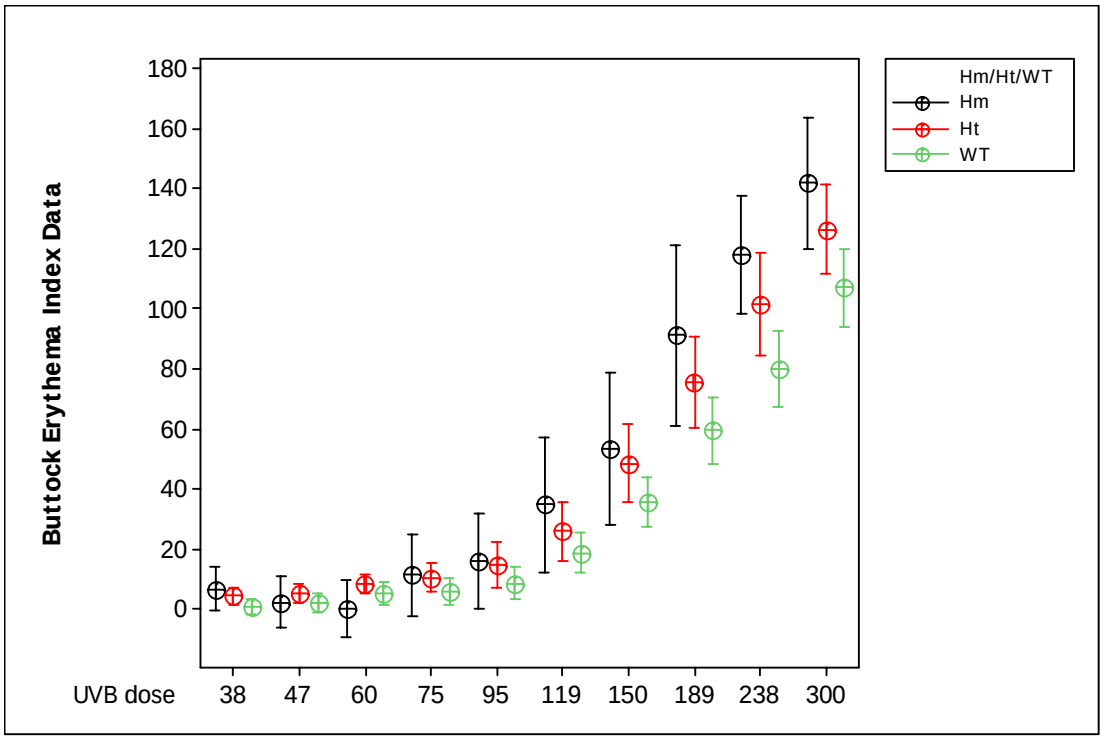
Figure 6.2.3.12.6. Back Erythema Index and *MC1R* genotype Rr1-5



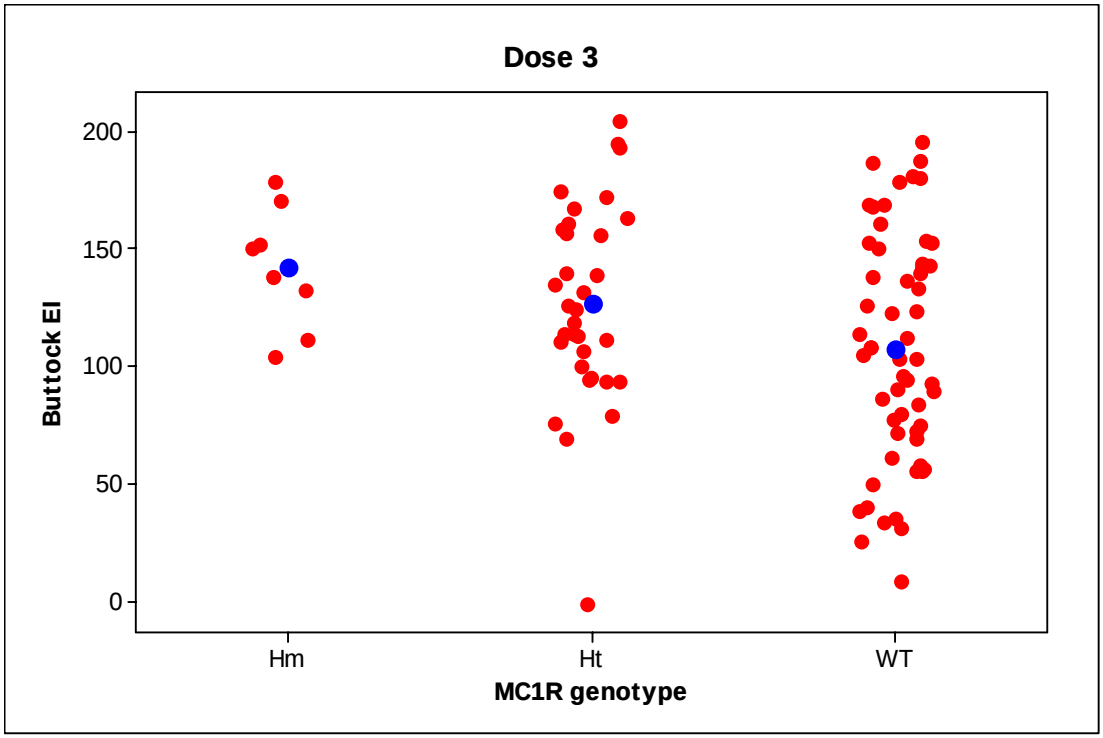
This shows back erythema index and *MC1R* genotype (Rr1-5). Y-axis shows back erythema index ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).

6.2.3.12.7. Buttock Erythema Index and *MC1R* genotype Hm/Ht/WT

Figure 6.2.3.12.7. Buttock Erythema Index and *MC1R* genotype Hm/Ht/WT

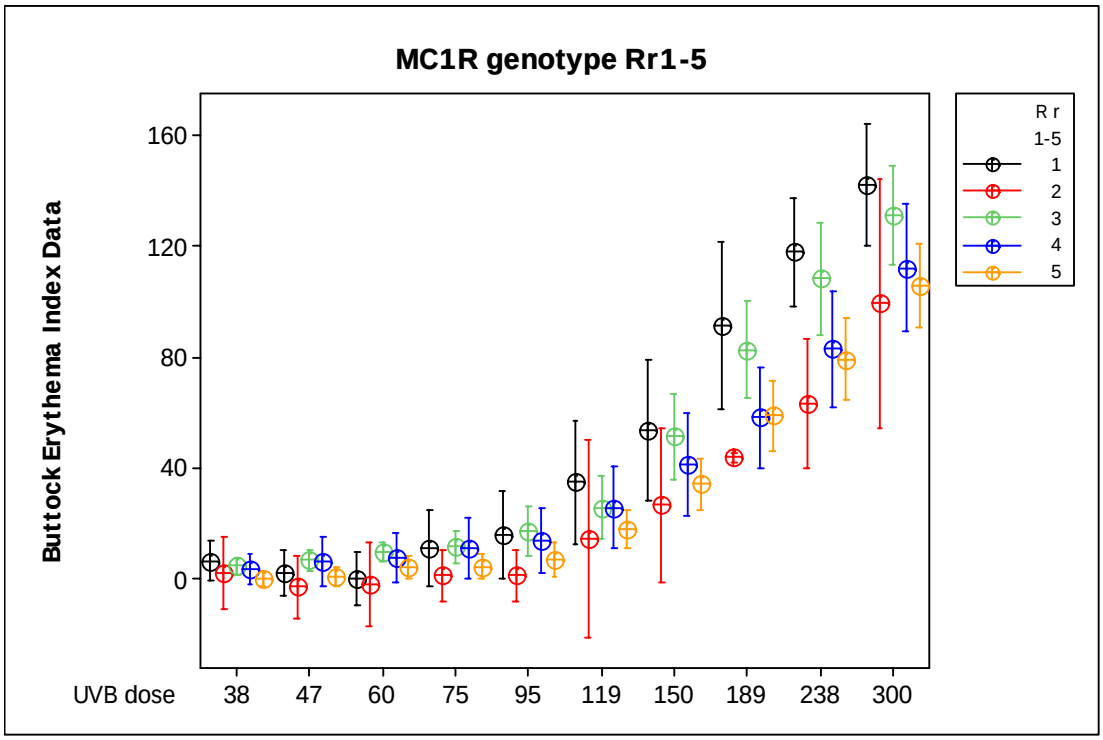


This shows buttock erythema index and *MC1R* genotype Hm/Ht/WT. Y-axis shows buttock erythema index ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).

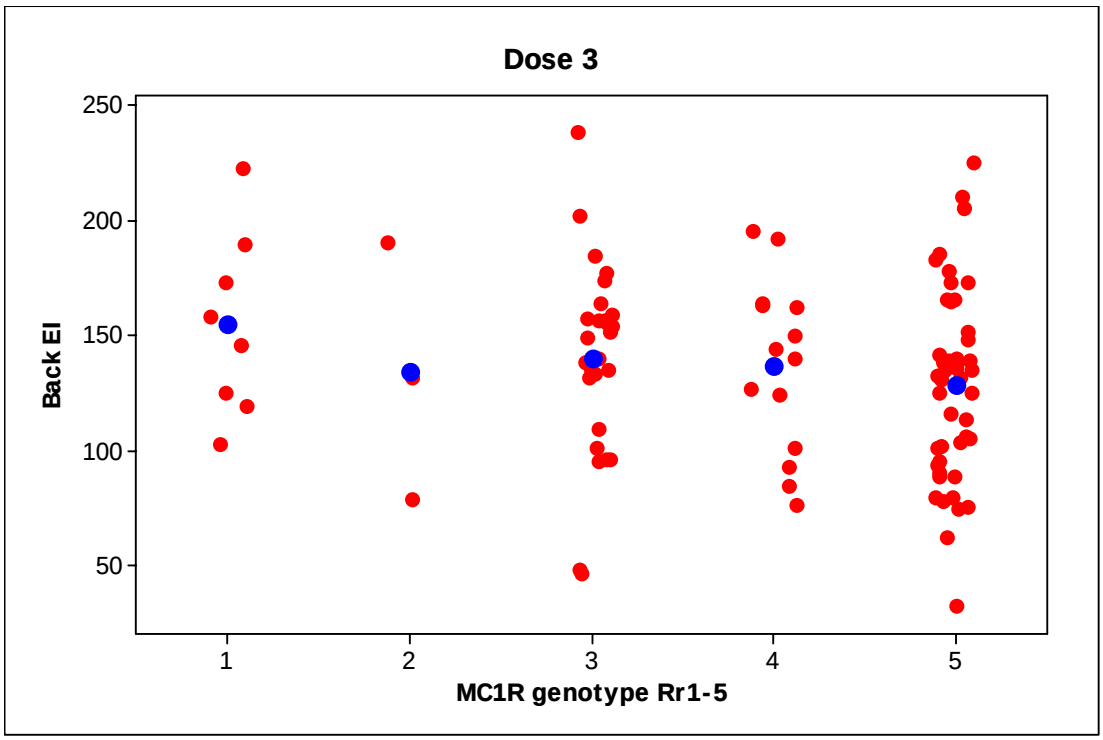


One-way ANOVA for buttock erythema EI at dose 3 vs *MC1R* genotype Hm/Ht/WT showed no significant difference (P=0.091).

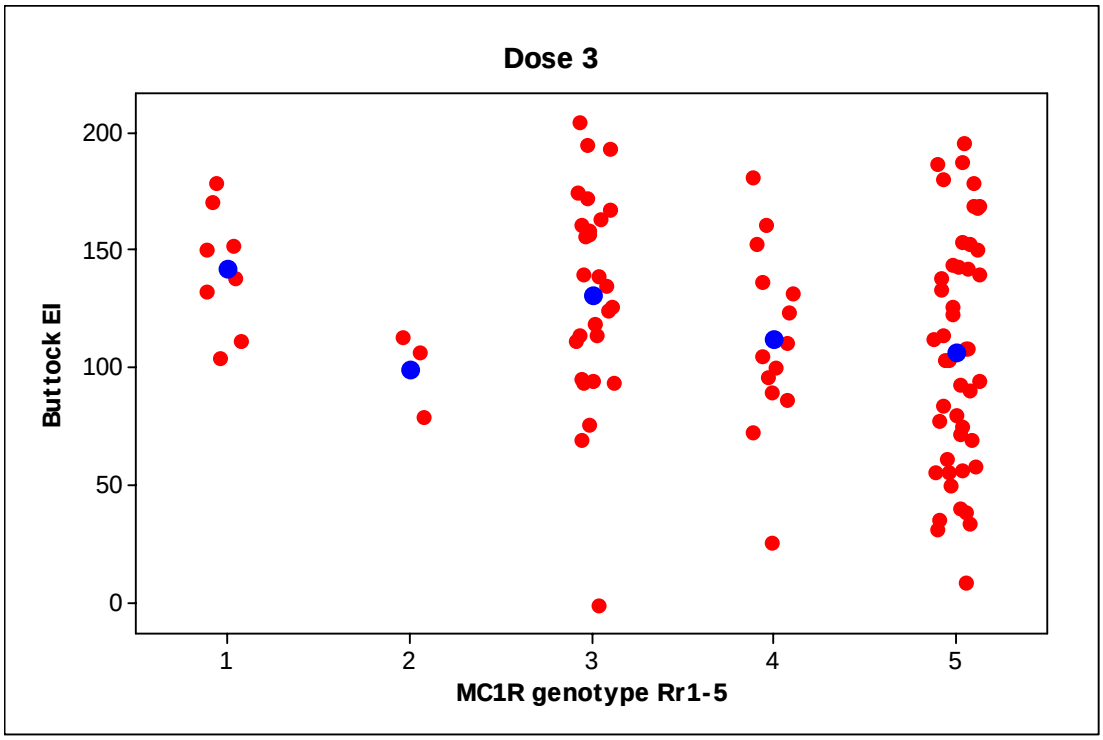
Figure 6.2.3.12.8. Buttock Erythema Index and *MC1R* genotype Rr 1-5



This shows buttock erythema index and *MC1R* genotype (Rr1-5). Y-axis shows buttock erythema index ± SEM (95% CI for the mean). X-axis shows the UVB doses (mJ/cm^2).



One-way ANOVA for back erythema EI at dose 3 vs *MC1R* genotype R r 1-5 showed no significant difference (P=0.169).



One-way ANOVA for buttock erythema EI at dose 3 vs *MC1R* genotype R r 1-5 showed just significant difference (P=0.0408). Tukey-Kramer multiple pairwise comparisons between Hm, Ht or WT showed no significant differences.

Table 6.2.3.12. ANOVA comparisons for relationship between *MC1R* genotype and erythema

	Back Erythema EI Mean SEM		Buttock Erythema EI Mean SEM	
Hm (n=8)	155	14	142	9
Ht (n=33)	141	7	127	7
WT (n=57)	129	5	107	6
	P value		P value	
Hm-WT	0.2328		0.109	
Hm-Ht	0.6673		0.6676	
Ht-WT	0.4052		0.1255	

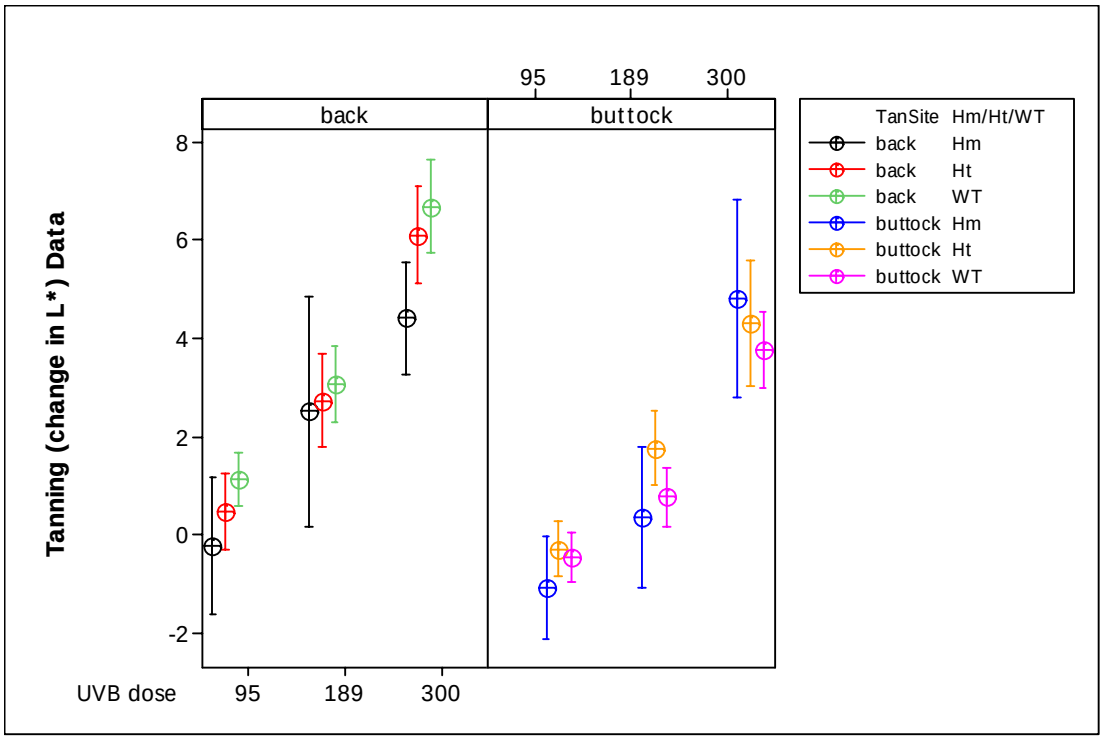
Summary of ANOVA comparisons for *MC1R* genotype and erythema in terms of change in erythemal index (EI) 24 hours after UVB irradiation (dose 3) in 98 individuals. Pairwise estimates, SEM, and P values are shown for Hm-WT, Hm-Ht and Ht-WT comparisons for averaged change in EI readings.

6.2.3.13. *MC1R* genotype and tanning

Does *MC1R* have an effect on tanning?

This initial figure was plotted:

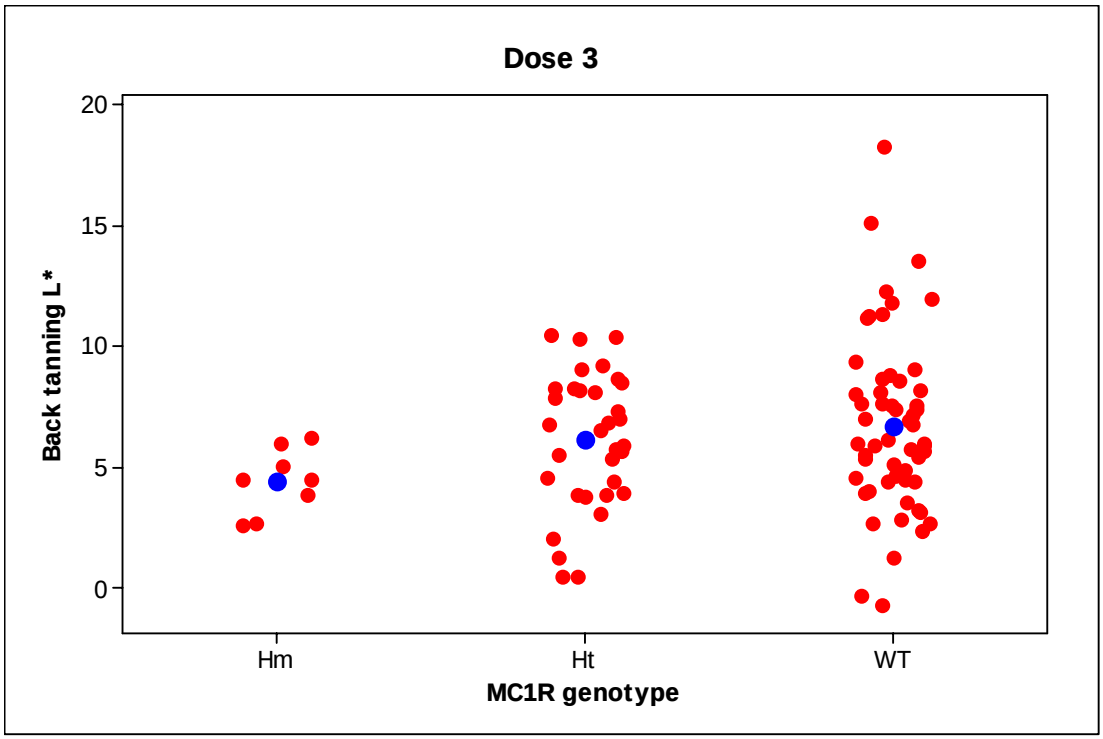
Figure 6.2.3.13.1. *MC1R* genotype (Hm/Ht/WT) and tanning by change in L* (back and buttock) – 3 UVB doses



This shows tanning on back and buttock and *MC1R* genotype (Hm/Ht/WT). Y-axis shows back or buttock tanning (change in L*) ± SEM (95% CI for the mean). X-axis shows the 3 UVB doses (mJ/cm^2): 95 (dose 1), 189 (dose 2) and 300 (dose 3).

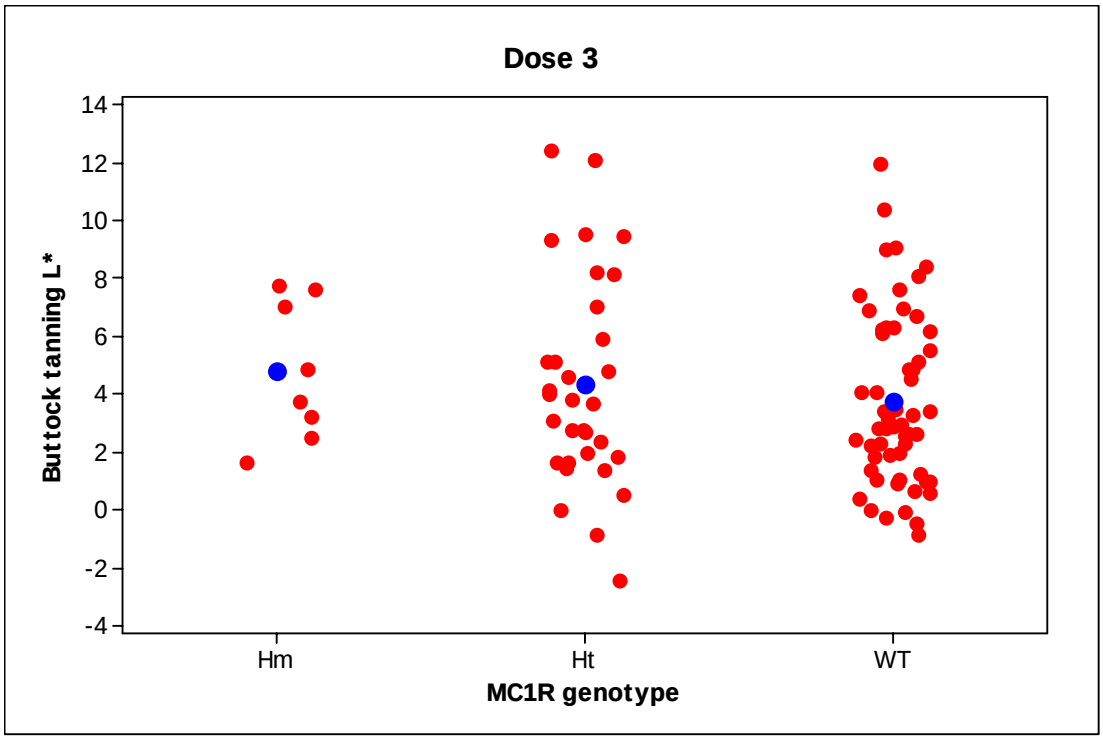
Then dose 3 and L* were looked at more closely:

Figure 6.2.3.13.2. *MC1R* genotype Hm/Ht/WT and back tanning (change in L*)



One-way ANOVA for back tanning L* at dose 3 vs *MC1R* genotype Hm/Ht/WT showed no significant difference (P=0.1627). Individuals who were homozygous for *MC1R* appear to tan less over their back than those who were heterozygous or wildtype (see Table 6.2.3.13). The difference was not statistically significant.

Figure 6.2.3.13.3. *MC1R* genotype Hm/Ht/WT and buttock tanning (change in L*)



MC1R genotype has no significant effect of tanning / facultative pigmentation on buttock as measured by the change in L* (P=0.57). One-way ANOVA for buttock tanning L* at dose 3 vs *MC1R* Genotype Hm/Ht/WT showed no significant difference (P=0.5699).

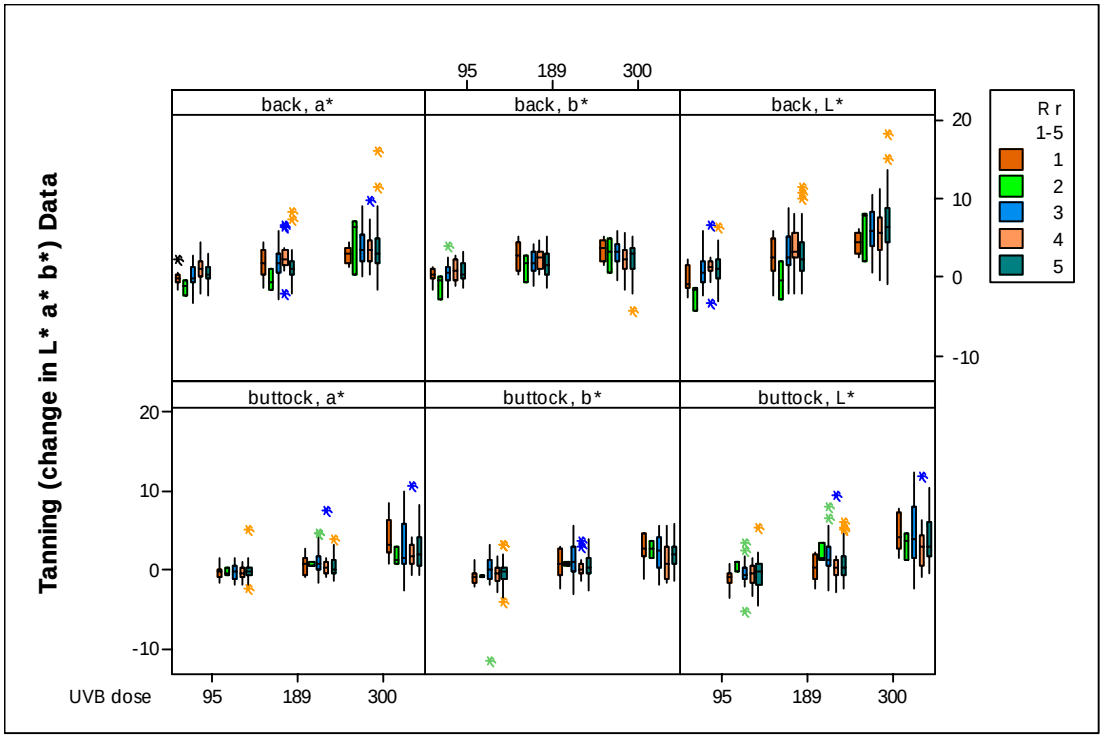
Table 6.2.3.13. ANOVA comparisons for relationship between *MC1R* genotype and tanning

	Back Tanning L* Mean SEM		Buttock Tanning L* Mean SEM	
Hm (n=8)	4.42	0.48	4.81	0.85
Ht (n=33)	6.11	0.49	4.33	0.62
WT (n=57)	6.70	0.48	3.78	0.40
	P value		P value	
Hm-WT	0.1526		0.665	
Hm-Ht	0.3822		0.9206	
Ht-WT	0.683		0.7094	

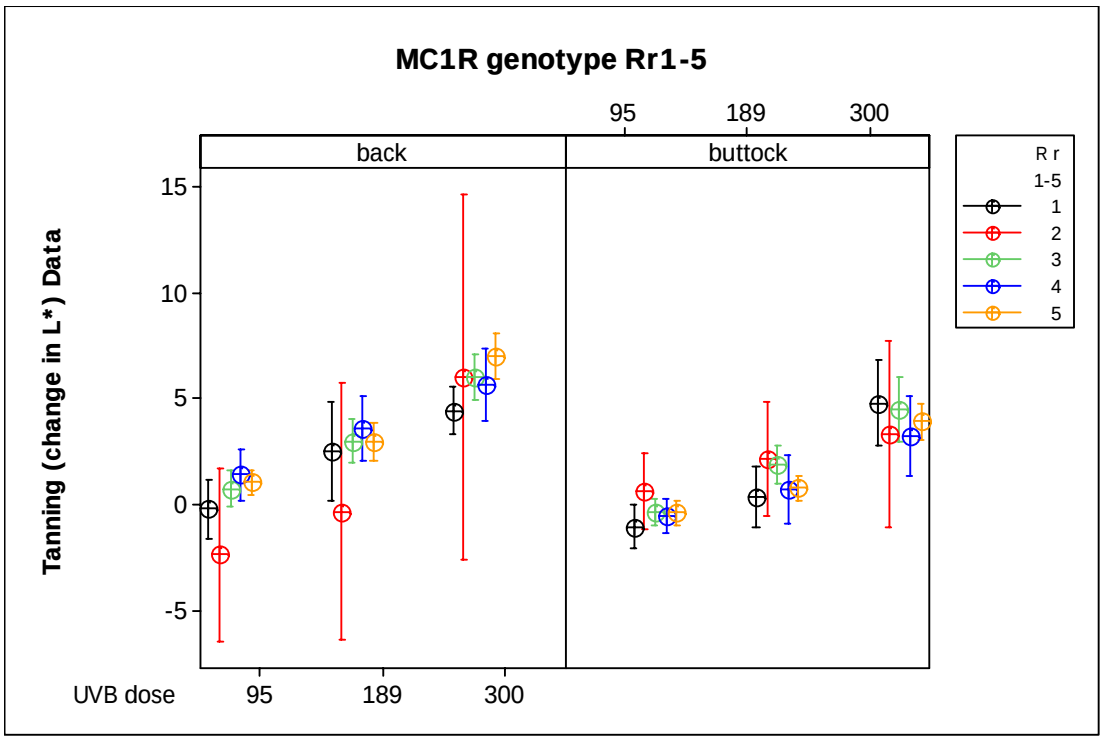
Summary of ANOVA comparisons for *MC1R* genotype and tanning in terms of change in skin colour L* values 7 days after UVB irradiation (dose 3) in 98 individuals. Pairwise estimates, SEM, and P values are shown for Hm-WT, Hm-Ht and Ht-WT comparisons for averaged change in L* readings.

Similar results were found with *MC1R* genotype (R r 1-5) and also with MI.
The following graphs were plotted as illustration.

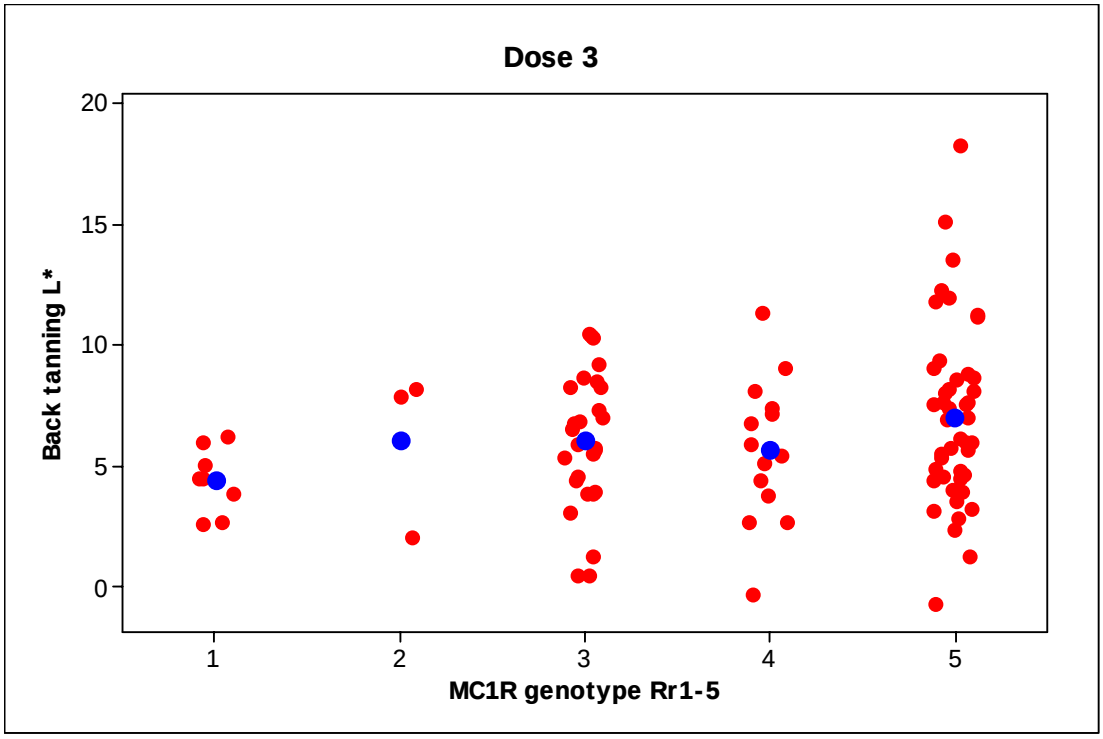
Figure 6.2.3.13.4. *MC1R* genotype (R r 1-5) and tanning by change in L* a* b* (back and buttock)



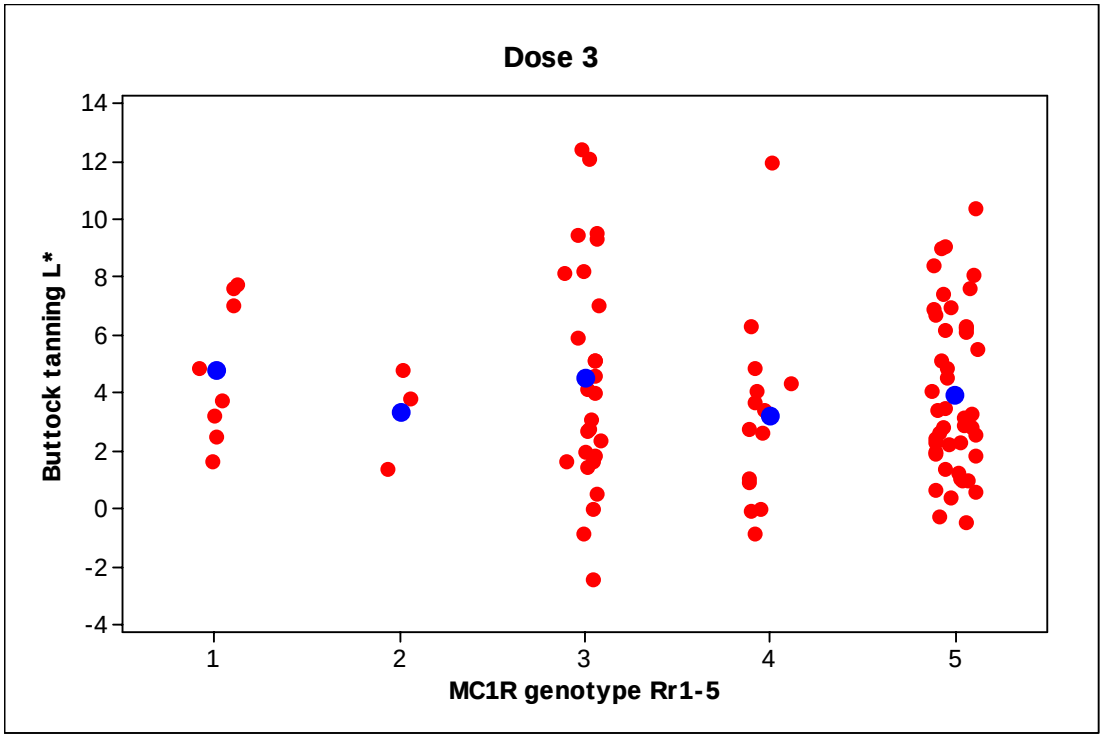
This shows tanning on back and buttock and *MC1R* genotype (R r 1-5). Y-axis shows back or buttock tanning (change in L*, a* or b*) ± SEM (95% CI for the mean). X-axis shows the 3 UVB doses (mJ/cm^2): 95, 189 and 300; L*, a* or b* and *MC1R* genotype (R r 1-5).



This shows tanning on back and buttock and *MC1R* genotype (R r 1-5). Y-axis shows back or buttock tanning (change in L*) ± SEM (95% CI for the mean). X-axis shows the 3 UVB doses (mJ/cm^2): 95, 189 and 300; L*, a* or b* and *MC1R* genotype (R r 1-5).



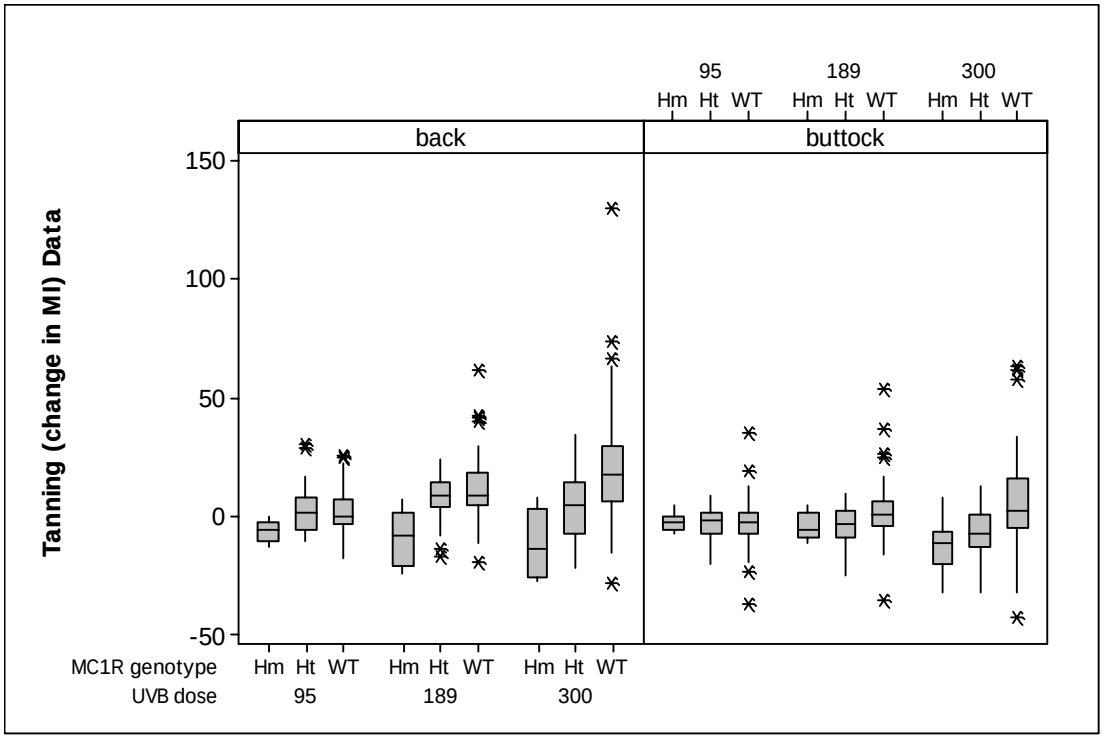
One-way ANOVA for back tanning L* at dose 3 vs *MC1R* genotype R r 1-5 showed no significant difference (P=0.223).



One-way ANOVA for buttock tanning L* at dose 3 vs *MC1R* genotype R r 1-5 showed no significant difference (P=0.57).

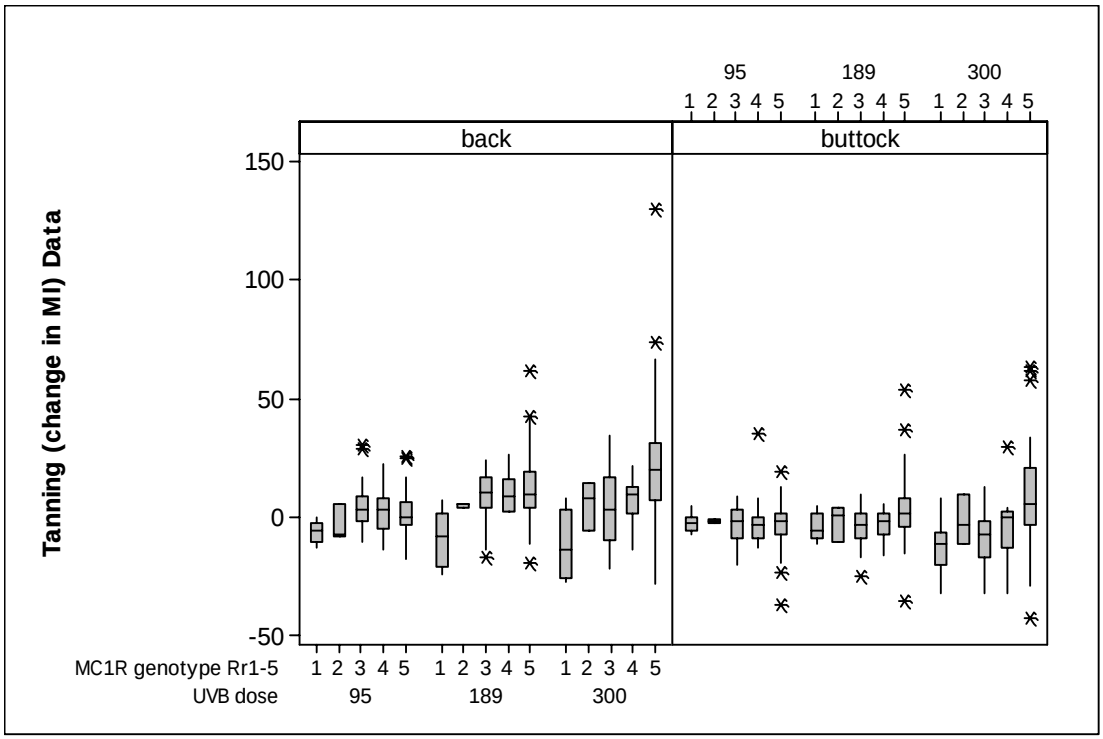
6.2.3.14. *MC1R* genotype (Hm/Ht/WT) and tanning by MI (back and buttock)

Figure 6.2.3.14. *MC1R* genotype (Hm/Ht/WT) and tanning by MI (back and buttock)



This boxplot shows tanning on back and buttock and *MC1R* genotype (Hm/Ht/WT). Y-axis shows back or buttock tanning (change in MI) ± SEM (95% CI for the mean). X-axis shows the 3 UVB doses (mJ/cm^2): 95, 189 and 300; and *MC1R* genotype (Hm/Ht/WT). Centre bar = median.

Figure 6.2.3.15. *MC1R* genotype (Rr1-5) and tanning by MI (back and buttock)



This shows tanning on back and buttock and *MC1R* genotype (R r 1-5). Y-axis shows back or buttock tanning (change in MI) ± SEM (95% CI for the mean). X-axis shows the 3 UVB doses (mJ/cm^2): 95, 189 and 300; and *MC1R* genotype (R r 1-5). Centre bar = median.

6.2.4. Correlation of *MC1R* genotype with phenotypic characteristics

6.2.4.1. *MC1R* sequence variants and hair colour

In the irradiated group, 16 individuals (16 out of 98, 16%) had red hair. One individual had no known *MC1R* sequence variant associated with her red hair.

Volunteer number	Hair colour	MC1R genotype	R r classification	
V32	red	151/160	RR	Hm
V96	red	151/151	RR	Hm
V106	red	151/160	RR	Hm
V121	red	142/151	RR	Hm
V6	strawberry blonde	160/294	RR	Hm
V25	strawberry blonde	151/294	RR	Hm
V143	auburn	160/160	RR	Hm
V30	auburn	160/294	RR	Hm
V33	strawberry blonde	29insA/WT	R-	Ht
V110	auburn	160/WT	R-	Ht
V120	auburn	151/WT	R-	Ht
V126	auburn	151/WT	R-	Ht
V133	auburn	151/WT	R-	Ht
V136	red	160/WT	R-	Ht
V31	auburn	60/WT	r-	Ht
V154	auburn	WT/WT	--	WT

6.2.4.2. Penetrance of *MC1R* sequence variants for red hair

It appeared that the R160W sequence variant might be less potent for red hair. 2 individuals with 60/WT and WT/WT alleles have auburn hair.

6.3. Discussion

The *MC1R* sequence variants found in the 159 individuals in the population were in keeping with previous studies. Four novel *MC1R* sequence variants V51A, I63M, S131N and A212S were found. The *MC1R* allelic frequency of this study was comparable to a previous study (Flanagan *et al.*, 2000). *MC1R* homozygosity was associated with lighter hair colour (higher L* value), also higher a* and b* values.

MC1R on hair spectrophotometric reflectance revealed a dominant effect for Hm and Ht on WT. However, the results from the figure suggest a dosage effect although Hm vs Ht was not significant. With larger sample size, this might have showed

significant changes. While there were variations in the results, overall this is compatible with a dosage effect. This spectrophotometric method may be more powerful to demonstrate dosage effect than L* a* b*. The effect of *MC1R* genotype on red hair colour group was consistent with a dosage or heterozygous effect.

MC1R homozygosity and heterozygosity was associated with lighter skin colour (higher L* value) and was compatible with a dominant effect.

There were some inconsistencies with the above *MC1R* results when compared with previous work (Flanagan *et al.*, 2001; Naysmith *et al.*, 2004). Some findings e.g. hair spectrophotometry, skin L* are compatible with a dominant effect (i.e. Ht with Hm vs WT). In other findings e.g. hair a* were compatible with a recessive effect (i.e. Ht with WT vs Hm). In other word, Ht was either like Hm or WT, dependent on a particular trait.

The most likely explanation for this is due to the power of testing and that this was an unselected group rather than a population that had been enriched for *MC1R* variants (Naysmith *et al.*, 2004). Sample size, different population of study and variability may play a role. A larger sample size may answer any future questions.

The effect of *MC1R* on eye colour was small. However *MC1R* has an effect on skin type (P=0.0002). One cautionary point to highlight was that the way I administered the questionnaire to obtain skin type data, as this is subjective measure, and different to others. Rampen *et al* argued that skin type was not reproducible (Rampen *et al.*, 1988).

MC1R homozygosity and heterozygosity were significantly associated with the presence of freckling (P<0.0001) and the number of freckling sites (P<0.0001). There appeared to be a dosage effect on freckling. This was expected, as *MC1R* is associated with individuals with red hair (P<0.0001) and fairer skin (P<0.0001).

These results were similar with the findings from previous studies (Bastiaens *et al.*, 2001a; Duffy *et al.*, 2004; Flanagan *et al.*, 2000; Smith *et al.*, 1998).

I confirmed the high ORs of having red hair if an individual was Hm for *MC1R* vs WT = 245.74 (95%CI 27.1-12665.3) P<0.0001 and the ORs of having red hair if the individual was Ht for *MC1R* vs WT = 5.85 (95%CI 1.46-28.32) P=0.005. I also showed the ORs for red hair for having different red hair *MC1R* sequence variants.

These were consistent with previous findings from Sturm *et al* that D84E, R151C, R160W, and D294H, are strongly associated with red hair and fair skin with multinomial regression analysis showing odds ratios of 63, 118, 50, and 94, respectively. An additional three low-penetrance alleles V60L, V92M, and R163Q have odds ratios 6, 5, and 2 relative to the wild-type allele (Sturm *et al.*, 2003). D84E and D294H have previously been shown to have lower cAMP response and slightly impaired binding ability (Ringholm *et al.*, 2004). R142H has also been shown to not signal very well functionally (Schioth *et al.*, 1999). The number of individuals with this *MC1R* variant was small. Despite this, the results were in keeping with what was expected.

The effect of *MC1R* on hair colour was larger than on skin colour. *MC1R* has no effect on back or buttock erythema index, but buttock EI was approaching significance Hm/Ht/WT (P=0.091), Rr1-5 (P=0.041). Similarly, the effect of *MC1R* on erythema flux was not significant for the back (P=0.613, ANOVA). However it was approaching significance for the buttock (P=0.071, ANOVA). Individuals with paler skin do develop more erythema (Chapter 4.2.1.2.7, $P=1.29\times10^{-6}$). Although the effect of *MC1R* on erythema was not statistically significant, the findings are in keeping with Flanagan *et al* (Flanagan *et al.*, 2001) that the gradient of the dose response curve was steeper for reds, but a subtler effect.

MC1R has no significant effect for back or buttock tanning (P>0.05). *MC1R* did not have a large effect on single UVB dose-induced tanning. *MC1R* does not have an effect/less effect on single dose erythema, but determines repeated UVB-induced tanning (Healy *et al.*, 2000). Repeated UVB doses could be used in further experiments to investigate the effect, but there may be more confounding variables.

When this study was first designed, the belief was that objective scoring of phenotypes may be better in elucidating the relationship between genotypes e.g. *MC1R* and erythema and facultative pigmentary responses. The results presented here showed that the effect of *MC1R* on skin phenotype is small. There was a trend but it was not formally significant. I confirmed that hair colour is associated with *MC1R*, in keeping with previous studies (Duffy *et al.*, 2004; Naysmith *et al.*, 2004). Skin colour measurements are not as informative as hair with respect to *MC1R*.

Noise in the system, measurement errors were possible explanation for the different results.

In fact skin type or freckling alone or in combination is better than other quantitative measures.

However, previous studies compared extremes of population with particular characteristics e.g. reds vs non-reds to demonstrate difference by using quantitative assays. In a relatively homogenous unselected population, these assays did show a trend quantitatively, yet not statistically significant.

There was no strong effect of *MC1R* on skin on its own. Although technically possible yet unlikely given the weakness of effect of *MC1R* on skin, this was not added into a linear regression equation as this is unlikely to add to phenotype. This is in keeping with other studies (Dwyer *et al.*, 2004) that genotype information did not add to phenotype.

Chapter 7 Other Genotypes

7.1. Introduction

In this chapter I will present genotype data from 34 other candidate loci other than *MC1R* from this study. I will discuss the rationale of candidate gene approach and the selection of relevant SNPs in the candidate genes. Genotype results will be presented in section 7.2 and discussed in section 7.3.

7.1.1. Candidate gene approach

MC1R has been shown to be a key determinant of pigmentary phenotype (Box *et al.*, 1997; Flanagan *et al.*, 2000; Healy *et al.*, 2000; Schioth *et al.*, 1999). Recently a number of other genes have been implicated in human pigmentation (Lamason *et al.*, 2005; Norton *et al.*, 2007; Sulem *et al.*, 2007). A candidate gene approach that can test the effects of these genetic variants of potentially implicated loci in human pigmentation would be useful. The genotype information obtained could be correlated with phenotypic traits.

7.1.2. Candidate gene selection

The choice of suitable candidate genes relied upon a detailed literature search of genes implicated in human pigmentation. Genes encoding different enzymes involved in the *MC1R* signalling pathway and downstream of *MC1R* were also chosen. This resulted in 34 other candidate loci selected.

It was not possible to sequence all the 35 genes, due to time and financial constraints. Two of the genes *MC1R* and *POMC* were sequenced whole. For the other 33 candidate genes, suitable polymorphisms needed to be selected for testing which would represent the whole gene of interest (i.e. tagging SNPs). The validity of this approach using tagging SNPs has been evaluated previously (Montpetit *et al.*, 2006; Tenesa and Dunlop, 2006).

7.1.3. List of candidate genes

There were a total of 34 other candidate genes chosen on the basis of genes that were reported to play a role in pigmentation and from the melanin biosynthesis and metabolism pathway.

The candidate genes were *ASIP, BLOC1S3, CYP1A2, CYP2C8, CYP4B1, DCT/TYRP2, DTNBP1, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC8, GNAS, GPR143, HPS1, HPS3, HPS4, HPS5, HPS6, KIT, KITLG, MATP, MITF, MYO5A, OCA2, PRKAR1A, SLC24A5, SOX10, TP53, TYR, TYRP1* and *POMC* (Table 7.1.3.).

Table 7.1.3. List of 34 Candidate Genes

Gene	Name	OMIM	Chromosome
ASIP	Agouti Signaling Protein	600201	20q11.2
BLOC1S3	Biogenesis of Lysosome-related Organelles Complex 1, Subunit 3	609762	19q13
CYP1A2	Cytochrome P450, Subfamily I, Polypeptide 2	124060	15q22-qter
CYP2C8	Cytochrome P450, Subfamily IIC, Polypeptide 8	601129	10q23.3
CYP4B1	Cytochrome P450, Subfamily IVB, Member 1	124075	1p34-p12
DCT / TYRP2	Dopachrome Tautomerase / Tyrosinase-Related Protein 2	191275	13q31-q32
DTNBP1	Dystrobrevin-Binding Protein 1 / Hermansky-Pudlak Syndrome 7	607145	6p22.3
ERCC1	Excision Repair Cross-Complementing rodent repair deficiency complementation group 1	126380	19q13.2-q13.3
ERCC2	Excision Repair Cross-Complementing rodent repair deficiency complementation group 2 / XPD	126340	19q13.2-q13.3
ERCC3	Excision Repair Cross-Complementing rodent repair deficiency	133510	2q21

	complementation group 3 /XPB		
ERCC4	Excision Repair Cross-Complementing rodent repair deficiency complementation group 4	133520	16p13.3-p13.13
ERCC5	Excision Repair Cross-Complementing rodent repair deficiency complementation group 5 / XPG	133530	13q33
ERCC6	Excision Repair Cross-Complementing rodent repair deficiency complementation group 6	609413	10q11
ERCC8	Excision Repair Cross-Complementing rodent repair deficiency complementation group 8	609412	5q12
GNAS	Guanine Nucleotide-binding protein, Alpha-Stimulating	139330	20q13.2
GPR143	Ocular Albinism Type 1	300500	Xp22.3
HPS1	Hermansky-Pudlak Syndrome 1	604982	10q23.1
HPS3	Hermansky-Pudlak Syndrome 3	606118	3q24
HPS4	Hermansky-Pudlak Syndrome 4	606682	22q11.2-q12.2
HPS5	Hermansky-Pudlak Syndrome 5	607521	11p15-p13
HPS6	Hermansky-Pudlak Syndrome 6	607522	10q24.32
KIT	KIT Oncogene	164920	4q12
KITLG	KIT Ligand	184745	12q22
MATP / *SLC45A2*	Membrane-Associated Transporter Protein / Solute Carrier Family 45, Member 2 / Oculocutaneous Albinism, Type IV	606202	5p13.3
MITF	Microphthalmia-associated Transcription Factor	156845	3p14.1-p12.3
MYO5A	Myosin VA	160777	15q21
OCA2	Oculocutaneous Albinism, Type II / P gene	611409	15q11.2-q12
PRKAR1A	Protein Kinase, cAMP-dependent, Regulatory,	188830	17q23-q24

	Type I, Alpha		
SLC24A5	Solute Carrier Family 24 (Sodium/Potassium/Calcium Exchanger), Member 5 / NCKX5	609802	15q21.1
SOX10	SRY-Box 10	602229	22q13
TP53	Tumour Protein p53	191170	17p13.1
TYR	Tyrosinase	606933	11q14-q21
TYRP1	Tyrosinase-Related Protein 1 / Oculocutaneous Albinism, Type III	115501	9p23
POMC	Proopiomelanocortin	176830	2p23.3

Source: OMIM (www.ncbi.nlm.nih.gov/sites/entrez?db=omim)

7.1.4. Selection of SNPs in candidate gene loci

SNPs from the 33 candidate genes were screened using data from the International HapMap project (www.hapmap.org) (2003; 2005; Couzin, 2002) for tagging SNPs to represent the whole candidate gene, which are in linkage disequilibrium with other tagging SNPs for the candidate gene.

HapMap provides linkage disequilibrium (LD) information on SNPs that can be used for tagging SNP selection. Tagging SNPs are SNPs that are representative of a gene that is in high linkage disequilibrium. By using tagging SNPs, it is therefore not necessary to genotype all the SNPs present in candidate loci of interest.

A haplotype is a haploid genotype. It represents a combination of alleles at multiple linked loci that are transmitted together on the same chromosome or set of SNPs that are statistically associated. These associations and alleles of a haplotype block can identify all polymorphic sites in the region. The programme Haploview was used within HapMap. Other database consulted include NCBI dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP).

The initial number of SNPs from all the 33 candidate loci was around 10000. Due to time and financial contraints, not all SNPs could be typed. A maximum of 384 SNPs could be included and genotyped using the Illumina platform. The process of fine-tuning involved setting screening criteria by frequency in Caucasian population, published SNPs and using the Illumina screening tool to see whether the chosen SNP was suitable for the final microarray chip or interfere with another SNP. A

GoldenGate score was obtained for each SNP from Illumina. This is a validation status score for a SNP that has previously been successfully generated genotype results on the Illumina platform.

A total of 384 haplotype tagging-SNPs were selected from introns and exons in 33 genes from the HapMap database together with those reported in the literature were included. The 384 SNPs were chosen finally to run on Illumina bead array. The Scottish population was genotyped with all 384 SNPs using Illumina technology. The final 384 SNPs selected were submitted to Wellcome Trust Clinical Research Facility Technical Service. The 384 SNPs selected from 33 candidate gene loci for Illumina genotyping are detailed in Appendix 6. The rationale of Illumina genotyping was discussed in Chapter 2.

7.1.5. Genotype analysis software - PLINK

A whole genome association analysis toolset PLINK [version 0.99r, pngu.mgh.harvard.edu/purcell/plink (Purcell *et al.*, 2007) was used to analyse the genotype data obtained from Illumina. PLINK is a user friendly, free, open-source whole genome association analysis toolset that allows for large-scale analyses in a computationally efficient manner and was developed at the Center for Human Genetic Research, Massachusetts General Hospital and the Broad Institute of Harvard and Massachusetts Institute of Technology.

In brief, genotype and phenotype data were coded to form the following files:

--pheno	Phenotype
--ped	PED Pedigree
--map	MAP
--snps	SNP list
--hardy	Hardy-Weinberg disequilibrium tests (exact)

Exact test was performed within the programme PLINK to estimate any departure from Hardy-Weinberg equilibrium (HWE) proportions (Wigginton *et al.*, 2005).

Eye colour was analysed categorically. The phenotypes hair colour L* a* b*, skin colour L* a* b*, Tanning L* at dose 3, Erythema at dose 3 were analysed using quantitative trait association analysis option:

--assoc --perm

which generates results under headings: CHR (Chromosome), SNP (SNP ID), CHISQ (Association chi-square), P (Asymptotic P-value), EMP1 (Empirical P-value adaptive) and NP (Number of permutations performed for this SNP).

--adjust Adjustment for multiple testing: Bonferroni correction, False Discovery Rate (FDR) and Sidk.

7 SNPs failed frequency test (MAF < 0.01), there are 343 SNPs after frequency and genotyping pruning. Corrected significance values (FDR, Sidak etc) were computed. Multiple-test corrected significance values were shown. Adaptive permutation was performed.

7.2. Genotyping results

7.2.1. *POMC* sequence results

Sequencing results were unavailable for 1 individual. 1 published SNP (rs8192605 C-T) within *POMC* exon 2 was found. No other sequence variations or polymorphisms were identified for *POMC* (exons 1-3) genotypes.

7.2.2. Genotypes and hair colour

Table 7.2.2. Genotype associations between SNPs and quantitative measures of hair colour L* and a*

Gene	Chromosome	Position	SNP	Minor Allele	MAF	HWE P-value	Hair L* P-values	Hair a* P-values
HPS3	3	int 5	rs2254913	A	0.37	0.08	0.007	0.04
		int 8	rs6785780	T	0.41	0.86	0.03	0.0003
		int 13	rs7636389	A	0.07	0.43		0.03
		int 15	rs2681092	T	0.38	0.85	0.03	0.02
KIT	4	int 1.1	rs2237034	A	0.11	1		0.04
		int 1.2	rs2237032	G	0.19	0.57		0.04
MATP	5	int 2	rs3756464	T	0.41	0.27	0.03	
DTNBP1	6	int 1	rs1474605	G	0.17	0.51	0.03	
		int 4	rs2619545	C	0.16	0.51	0.03	
		int 6	rs6909929	A	0.43	0.72	0.046	
		exon 10 (S272P)	rs17470454	A	0.05	0.24	0.03	
CYP2C8	10	int 5	rs1934980	C	0.16	0.74		0.02
		int 8	rs11572177	G	0.30	0.41		0.04
HPS1	10	int 10	rs1061135	T	0.49	0.05		0.04
HPS6	10	exon 1	rs3737243	A	0.07	1	0.04	
Tyr	11	int 3	rs12421746	T	0.01	1	0.02	0.048
KITLG	12	int 1.1	rs1492354	A	0.07	1		0.0005
		int 1.2	rs1907702	G	0.20	0.40		0.04
		int 1.3	rs10777129	A	0.08	1	0.03	0.004
OCA2	15	int 1.1	rs7495174	G	0.05	0.24	0.01	
		int 1.2	rs7174027	A	0.06	0.35	0.004	

		int 2.1	rs7179994	G	0.14	1		0.03
		int 2.2	rs1597196	T	0.15	0.74		0.03
		int 2.3	rs12442147	C	0.11	1	0.01	0.04
		int 2.4	rs12324648	A	0.07	1	0.04	0.04
		int 2.5	rs4778232	T	0.19	0.78		0.04
		int 3	rs13329466			0.41		0.02
		int 23.1	rs2311470	C	0.50	1		0.045
		int 23.2	rs6497235	A	0.50	0.30		0.007
		int 23.3	rs6497233	T	0.41	0.47		0.01
		int 23.4	rs17674017	G	0.42	0.28		0.006
		int 23.5	rs1498509	C	0.49	0.86		0.03
		int 23.6	rs11631195	A	0.37	0.85	0.03	0.006
		int 23.7	rs3947367	G	0.29	0.28	0.03	
		int 23.8	rs11637518	G	0.35	1		0.03
		int 23.9	rs989869	C	0.38	0.06	0.04	
		int 23.10	rs1603784	A	0.23	0.13	0.01	
MC1R	16		R	C-fitive	0.21	0.19		0.000001
			Mc1r	C-fitive	0.48	0.62		0.003
			rlit	C-fitive	0.23	1		
PRKAR1A	17	3' UTR	rs6958	C	0.32	0.31		0.047
		int 2	rs2952275	T	0.36	0.24	0.02	
		5' UTR	rs8080306	C	0.27	0.65	0.02	
Gnas	20	int 3	rs2295583	T	0.28	0.37	0.02	
		int 5	rs3730168	A	0.41	0.14	0.03	
		int 6	rs919197	T	0.43	0.77	0.03	
HPS4	22	int 5	rs17401652	T	0.10	1		0.046

This table shows the gene, chromosome, position, SNP, minor allele frequency (MAF), Hardy-Weinberg Equilibrium (HWE), hair L* and Hair a* P-values. Intron (int). Untranslated region (UTR). Reference SNP (rs). Only quantitative hair data is shown, categorical hair data not shown. Associations shown were from Northern European individuals only.

HPS3

5 SNPs (rs2254913, rs6785780, rs7636389, rs2681092 and rs2689230) were associated with hair colour (lighter) in *HPS3* gene. The most significant association is at intron 8 (rs6785780) of *HPS3* with hair L* (P=0.03), with hair a* (P=0.0003). This is still significant after Bonferroni correction. *HPS3* SNP rs6785780 is also significantly associated with red hair (P=0.008101). Intron 5 (rs2254913) of *HPS3* was significantly associated with hair L* (P=0.007) and hair a* (P=0.04). Intron 13 (rs7636389) of *HPS3* was significantly associated with hair a* (P=0.03). Intron 15 (rs2681092) of *HPS3* was significantly associated with hair L* (P=0.03) and hair a* (P=0.02). High linkage disquilibrium (LD) was observed between the 5 tagging SNPs. *HPS3* SNPs rs2254913, rs2681092 and rs2689230 were associated with fair hair (P=0.02168, P=0.03155, P=0.02867).

KITLG

3 SNPs (rs1492354, rs1907702 and rs10777129) in intron 1 of *KITLG* gene were associated with hair colour (lighter). The significant associations were between rs1492354 with hair a* (P=0.0005). This is still significant after Bonferroni correction. Intron 1.2 (rs1907702) of *KITLG* was associated with hair a* (P=0.04). Intron 1.3 (rs10777129) of *KITLG* was associated with hair L* (P=0.03) and hair a* (P=0.004). The LD is high between these markers. MAF were low (7-20%).

OCA2

OCA2 intron 1: both SNPs (rs7495174, rs7174027) in intron 1 were associated with hair colour L* (P=0.01, P=0.004).

3 SNPs (rs7179994, rs1597196 and rs12324648) in intron 2 were associated with hair colour L* (P=0.01-0.04) and hair a* (P=0.03-0.04).

1 SNP (rs13329466) in intron 3 were associated with hair colour a* (P=0.02).

5 SNPs (rs17674017, rs1498509, rs11631195, rs989869 and rs1603784) in intron 23 were associated with hair colour L* (P=0.01-0.04) and hair a* (P=0.006-0.01).

TYR

1 SNP (rs12421746) in intron 3 of *TYR* gene was associated with hair colour L* (P=0.02) and hair a* (P=0.048). *TYR* SNP rs16913107 was associated with dark hair (P=0.02686, OR=7.7) or dark vs fair hair (P=0.03916, OR=9.0).

MATP

1 SNP (rs3756464) in intron 2 of *MATP* gene was associated with hair colour L* (P=0.03). *MATP* SNP (rs16891982) was associated with dark vs fair hair (P=0.008574, OR=27.7). This is in keeping with a known population marker.

KIT

2 SNPs (rs2237034 and rs2237032) in intron 1.1 and 1.2 of *KIT* gene were associated with hair colour a* (P=0.04, P=0.04).

DTNBP1

4 SNPs (rs1474605, rs2619545, rs6909929 and rs17470454) in intron 1, 4, 6, exon10 (S272P) of *DTNBP1* gene were associated with hair colour L* (P=0.03, P=0.03, P=0.046, P=0.03).

CYP2C8

2 SNPs (rs1934980 and rs11572177) in intron 5 and 8 of *CYP2C8* gene were associated with hair colour a* (P=0.02, P=0.04).

HPS1

1 SNP (rs1061135) in intron 10 of *HPS1* gene were associated with hair colour a* (P=0.04).

HPS4

1 SNP (rs17401652) in intron 5 of *HPS4* gene were associated with hair colour a* (P=0.046).

HPS6

1 SNP (rs3737243) in exon 1 of *HPS6* gene were associated with hair colour L* (lighter) (P=0.04).

MC1R

MC1R SNP "R" was associated with hair colour a* (P=0.000001). This is still significant after Bonferroni correction. *MC1R* SNP "Mc1r" i.e. WT was associated with hair colour a* (P=0.003).

PRKAR1A

3 SNPs (rs6958, rs2952275 and rs8080306) in 3' UTR, intron 2, 5' UTR of *PRKAR1A* gene were associated with hair colour. 3' UTR (rs6958) of *PRKAR1A* was associated with hair a* (P=0.047). Intron 2 (rs2952275) and 5' UTR (rs8080306) of *PRKAR1A* were associated with hair a* (P=0.02, P=0.02).

GNAS

3 SNPs (rs2295583, rs3730168 and rs919197) in intron 3, 5, 6 of *GNAS* gene were associated with hair colour L* (P=0.02, P=0.03, P=0.03).

7.2.3. Genotypes and eye colour

Table 7.2.3. Genotype associations between SNPs and eye colour classified in categories

Gene	Chr	SNP	Positon	Min A	MAF	HWE	Colours (quantitative)	Shades light vs dark		Brown vs blue		Brown vs other	
							P-values	P-values	OR	P-values	OR	P-values	OR
		rs7643410	int 1	G	0.08	0.55						0.04	2.9
		rs2254913	int 5	A	0.37	0.08	0.006	0.03	1.7	0.003	2.6	0.01	1.9
		rs2689229	int 5	A	0.46	1	0.01	0.02	1.8	0.004	2.5		
HPS3	3	rs2689230	int 5	G	0.23	1	0.04	0.046	2.2	0.02	2.6	0.04	2.1
		rs2689234	int 6	G	0.49	0.72	0.03			0.009	2.3		
		rs6785780	int 8	T	0.41	0.86	0.03			0.02	2.3		
		rs2681092	int 15	T	0.38	0.85	0.02			0.01	2.3	0.04	0.5
KIT	4	rs17084733	3' UTR	A	0.11	1				0.02	4.3	0.04	3.6
DTNBP1	6	rs9476886	int 1	T	0.28	0.12				0.04	2.4		
ERCC8	5	rs4647128	int 10	G	0.03	1	0.04	0.02	6.1				
		rs4235483	int 9	A	0.44	0.59		0.04	1.7				
ERCC6	10	rs4253231	3'UTR	C	0.09	1	0.007	0.003	3.6	0.08	2.8	0.01	3.0
CYP2C8	10	rs11572177	int 8	G	0.30	0.41						0.04	1.9
DCT	13	rs9584233	int 6	T	0.11	1				0.03	3.4	0.03	2.4
		rs7495174(a)	int 1.1	G	0.05	0.24	0.001*	0.006	6.3	0.003	NA	0.009	5.9
		rs7174027	int 1.2	A	0.06	0.35	0.009		3.1	0.003	NA	0.03	3.3
		rs7179994(a)	int 2.1	G	0.14	1	0.03			0.03	3.1	0.04	2.3
		rs1597196(a)	int 2.2	T	0.15	0.74	0.008	0.03	2.0	0.008	3.6	0.02	2.4
		rs1470608	int 2.3	A	0.10	1						0.046	2.3
		rs12324648	int 2.4	A	0.07	1	0.006	0.04	2.9	0.009	5.2	0.004	4.1
		rs3794604(a)	int 4	A	0.08	0.55	0.03		2.5	0.04	3.8	0.006	3.7
		rs746861	int 6	C	0.45	0.37		0.02	1.8				
		rs7176632	int 16	T	0.18	0.76	0.0003	0.0008	2.9	0.0008	5.0	0.01	2.3
OCA2	15	rs7173419	int 18.1	T	0.27	0.37	0.01	0.02	2.1	0.046	2.2		
		rs2594938	int 18.2	C	0.26	0.50	0.01	0.02	2.0	0.02	2.3		
		rs1562592	int 18.3	T	0.17	0.36	5.21E-05*	4.12E-05*	3.7	0.0009	4.2	0.0009	2.9
		rs1448490	int 18.4	A	0.16	0.09	0.03	0.02	2.8	0.009	7.0	0.002	8.6
		rs17674017	int 23.1	G	0.42	0.28	0.008	0.04	1.9	0.03	2.2		
		rs1498509	int 23.2	C	0.49	0.86	0.03			0.04	2.0		
		rs11631195	int 23.3	A	0.37	0.85	0.04			0.04	2.0		
		rs989869	int 23.4	C	0.38	0.06	0.02						
		rs1603784	int 23.5	A	0.23	0.13	0.01			0.03	2.6		
		rs11074304	int 23.6	A	0.42	0.006	0.04			0.04	2.2		
		rs11074317	int ?	A	0.17	0.76				0.02	3.5	0.002	0.2
GNAS	20	rs4810147	int 1	A	0.49	0.86	0.02			0.004	2.6	0.04	1.8

This table shows the gene, chromosome (Chr), position, SNP, minor allele (Min A), minor allele frequency (MAF), Hardy-Weinberg Equilibrium (HWE), eye colour groups. Intron (int).

Untranslated region (UTR), reference SNP (rs), odds ratio (OR). Eye colour was classified according to Frudakis *et al* (Frudakis *et al.*, 2003) into 1) colours (quantitative) - all four colours blue/green/hazel/brown 2) shades light (blue/green) vs dark (hazel/brown) 3) brown vs blue 4) brown vs other. Associations shown were from Northern European individuals only.

HPS3

7 SNPs of *HPS3* are associated with eye colour. SNPs (rs2254913, rs2689229, rs2689230, rs2689234, rs6785780, rs2681092) were significantly associated with quantitative eye colours (P=0.006-0.04), brown vs blue (P=0.003-0.02, OR=2.3-2.6) and brown vs other (P=0.01-0.04, OR=1.9-2.1). *HPS3* SNP rs7643410 was significantly associated with brown vs other (P=0.04, OR=2.9).

OCA2

OCA2 was associated with eye colour. Several tagging SNPs in *OCA2* (introns 1, 2, 4, 6, 16, 18, 23) were associated strongly with eye colour. *OCA2* SNP rs1448490 showed a strong association for brown vs blue (P=0.009, OR=7.0), brown vs other (P=0.002, OR=8.6). The association of hair colour in *OCA2* and eye colour ORs in intron 1 have been previously reported (Duffy *et al.*, 2007). These significant associations are in agreement with others, with the same markers found and associated with eye colour and also hair colours.

GNAS

Intron 1 (rs4810147) was associated with quantitative (P=0.01732), brown vs blue (P=0.004438) and brown vs other (P=0.04221).

7.2.4. Genotypes and skin colour

22 SNPs from 13 candidate genes were associated with skin colour.

HPS3

HPS3 SNP rs2689230 was associated with constitutive back skin colour L* (P=0.02136).

HPS5

HPS5 SNP rs2049129 was associated with constitutive back skin colour L* ($P=2.3\times10^{-5}$, $R^2=0.2481$) and constitutive buttock skin colour L* ($P=0.0007761$, $R^2=0.2519$). *HPS5* SNP rs4757637 was also associated with constitutive back skin colour L* ($P=0.0008896$, $R^2=0.1381$) and constitutive buttock skin colour L* ($P=0.02748$, $R^2=0.0539$).

HPS4

HPS4 SNP rs9613187 was associated with constitutive back skin colour L* ($P=0.01953$).

TYR

TYR SNP rs11018542 was associated with constitutive back skin colour L* ($P=0.00889$, $R^2=0.0874$) and constitutive buttock skin colour L* ($P=0.01989$, $R^2=0.0563$). *TYR* SNP rs16913107 was also associated with constitutive back skin colour L* ($P=0.0499$, $R^2=0.0308$).

TYRP1

TYRP1 SNP rs17346161 was associated with constitutive back skin colour L* ($P=0.04906$, $R^2=0.037$) and constitutive buttock skin colour L* ($P=0.04452$, $R^2=0.039$).

TP53

TP53 SNP rs12951053 was associated with constitutive back skin colour L* ($P=0.00398$, $R^2=0.1077$) and constitutive buttock skin colour L* ($P=0.004667$, $R^2=0.0942$). *TP53* SNP rs2078486 was also associated with constitutive back skin colour L* ($P=0.0107$) and constitutive buttock skin colour L* ($P=0.004304$). *TP53* SNP rs8064946 was associated with constitutive buttock skin colour L* ($P=0.02112$).

GNAS

GNAS SNP rs6092704 was associated with constitutive back skin colour L* (P=0.01948) and constitutive buttock skin colour L* (P=0.0155).

CYP4B1

CYP4B1 SNP rs3766209 was associated with constitutive buttock skin colour L* (P=0.00951, R^2=0.0694). *CYP4B1* SNP rs837401 was associated with constitutive back skin colour L* (P=0.01516, R^2=0.065) and constitutive buttock skin colour L* (P=0.02603, R^2=0.0376).

ERCC3

ERCC3 SNP rs4150506 was associated with constitutive back skin colour L* (P=0.01046, R^2=0.0692) and constitutive buttock skin colour L* (P=0.02892).

MATP

MATP SNP rs3756464 was associated with constitutive back skin colour L* (P=0.003246).

DTNBP1

DTNBP1 SNPs rs9476886, rs875462, rs9396593 were associated with constitutive back skin colour L* (P=0.02388, P=0.02723, P=0.02995).

MYO5A

MY05A SNP rs752865 was associated with constitutive back skin colour L* (P=0.04341). *MY05A* SNPs rs11853114, rs17614119 were associated with constitutive buttock skin colour L* (P=0.03055, P=0.04298).

MC1R

MC1R SNP "R" was associated with constitutive back skin colour L* (P=0.04134). *MC1R* SNP "mc1r" i.e. WT was associated with constitutive buttock skin colour L* (P=0.0286).

7.2.5. Genotypes and erythema

24 SNPs from 12 candidate genes were associated with UVB-induced erythema.

KIT

KIT SNPs rs2237029 and rs13135792 were associated with buttock erythema (P=0.03542, P=0.0499).

ERCC8

ERCC8 SNPs rs7726671 and rs976631 were associated with buttock erythema (P=0.04962, P=0.04262).

TYRP1

TYRP1 SNP rs10809828 was associated with buttock erythema (P=0.02008).

CYP2C8

CYP2C8 SNP rs3752988 was associated with back erythema (P=0.004701). *CYP2C8* SNP rs1891071 was associated with back (P=0.04075) and buttock erythema (P=0.04444).

ERCC6

ERCC6 SNP rs2228528 was associated with buttock erythema (P=0.0234).

HPS5

HPS5 SNP rs10766469 was associated with buttock erythema (P=0.04483).

TYR

TYR SNP rs11018542 was associated with buttock erythema (P=0.01872).

KITLG

KITLG SNP rs1492354 was associated with back erythema (P=0.01899).

DCT

DCT SNPs rs9584233 and rs7990565 were associated with back erythema (P=0.014, P=0.03194).

OCA2

OCA2 SNP rs746861 was associated with back (P=0.007241) and buttock erythema (P=0.01083). *OCA2* SNPs rs2055291, rs1800404, rs2871875, rs2122005 were associated with buttock erythema (P=0.03683, P=0.007465, P=0.01777, P=0.02948).

ERCC2

ERCC2 SNP rs238415 was associated with back erythema (P=0.02982).

HPS4

HPS4 SNPs rs739289, rs722997, rs1894706, rs4822721, rs877593 were associated with back erythema (P=0.01899, P=0.02692, P=0.03377, P=0.03547, P=0.04577).

7.2.6. Genotypes and tanning

24 SNPs from 14 candidate genes were associated with UVB-induced tanning.

HPS3

HPS3 SNP rs2131025 was associated with buttock tanning L* (P=0.04255).

CYP4B1

CYP4B1 SNP rs3766209 was associated with back (P=0.04643, R^2=0.0425) and buttock tanning L* (P=0.03346, R^2=0.0417).

ERCC8

ERCC8 SNPs rs7726671 and rs976631 were associated with back tanning L* (P=0.006213, P=0.02507).

DTNBP1

DTNBP1 SNP rs17470454 was associated with back tanning L* (P=0.01552).

TYRP1

TYRP1 SNP rs17346161 was associated with buttock tanning L* (P=0.04362).

CYP2C8

CYP2C8 SNPs rs11572177, rs11572093 and rs1341164 were associated with back tanning L* (P=0.01122, P=0.03523, P=0.03523).

HPS1

HPS1 SNP rs11592273 was associated with back tanning L* (P=0.04771).

ERCC6

ERCC6 SNP rs4253211 was associated with back tanning L* (P=0.04952).

KITLG

KITLG SNP rs1907702 was associated with buttock tanning L* (P=0.01645).

OCA2

OCA2 SNPs rs2055291, rs1448490, rs11074317, rs17566952, rs1448489 were associated with back tanning (P=0.008855, P=0.01136, P=0.02493, P=0.02512, P=0.04056).

MYO5A

MYO5A SNP rs11853114 was associated with buttock tanning L* (P=0.03725).

MC1R

MC1R SNP "R" was associated with back tanning L* (P=0.03095).

TP53

TP53 SNPs rs12951053 and rs2078486 was associated with back (P=0.001211, P=0.008341) and buttock tanning L* (P=0.03533, P=0.01334). *TP53* SNPs rs8064946 and rs1042522 were associated with back tanning L* (P=0.01363, P=0.04248).

ERCC2

ERCC2 SNP rs1799788 was associated with buttock tanning L* (P=0.002839).

7.3. Discussion

This study utilized a candidate gene approach to select for SNPs to investigate the genotypic association with phenotypes. The findings that *HPS3*, *KITLG* and *OCA2* were significantly associated with aspects of phenotypic variation have plausible explanations.

HPS3

The association of hair colour, eye colour and skin colour to *HPS3* is a novel observation. There are no mis-sense candidate SNPs in *HPS3* at chromosome 3q24. Let us look at possible putative mechanisms for this. *HPS3* is involved in lysosome organelle formation and biogenesis in melanosomes, lysosomes and platelet dense granules (Anikster *et al.*, 2001). Mutations in *HPS3* may cause a resultant change in shape and density of melanosomes secreted from the cell (Suzuki *et al.*, 2001). Clinically, *HPS3* defects could result in patients with hypopigmentation and platelet storage pool deficiency (Huizing *et al.*, 2001). One can postulate that although full clinical manifestation of *HPS3* defect may give rise to tyrosinase positive oculocutaneous albinism associated with a bleeding defect, a storage pool deficiency due to an absence of platelet dense bodies, pigmented reticuloendothelial cells (lysosomal) and ceroid-lipofuscinosis, milder subclinical forms may exist or even physiological variation in *HPS3*. The association of *HPS3* SNPs rs6785780 and rs16861552 with hair colour L* and a* (red-green scale), *HPS3* SNP rs2689230 with eye and constitutive skin colour may suggest that HPS3 protein has a role in normal hair colour, eye colour and skin colour variation. *HPS3* SNP rs6785780 may contribute to red hair status (P=0.008101). There were no previous reports of *HPS3* association with red hair. *HPS3* SNPs rs2254913, rs2681092 and rs2689230 may contribute to fair / blonde hair status (P=0.02168, P=0.03155, P=0.02867).

HPS3 gene contains 17 exons and spans 3,921 basepairs (Anikster *et al.*, 2001).

Animal model 'cocoa' (coa) mouse has a HPS-like mutant phenotype and is homologous to the human *HPS3* gene (Suzuki *et al.*, 2001).

HPS4

The *HPS4* SNP rs9613187 was also significantly associated with constitutive back skin colour L* (P=0.01953).

HPS5

The strong association of *HPS5* to skin colour is a novel observation. *HPS5* was however not associated with tanning, nor hair, nor eye colour. The *HPS5* SNP rs2049129 was associated with constitutive back skin colour L* ($P=2.3\times10^{-5}$, $R^2=0.2481$) and buttock skin colour (P=0.0007761, $R^2=0.2519$) and explains about 25% of the variation. The other *HPS5* SNP rs4757637 explains between 5-14% of the skin colour variation over back (P=0.0008896, $R^2=0.1381$) and buttock (P=0.02748, $R^2=0.0539$).

Perhaps in Northern European population, *HPS3*, *HPS4* and *HPS5* may be novel candidates for pigmentation and may explain a significant proportion of pigmentary characteristics.

Genotype association to hair colour:

Most significant association is found in *HPS3*, *KITLG*, *OCA2* to both hair colour L* and a*. The most significant associations are located in:

1) *HPS3* intron 6 and 8

2) *KITLG* intron 1

3) *OCA2* intron 1 for hair L*

4) *OCA2* intron 23 for hair a*

Sulem *et al* recently reported associations for 6 regions: *SLC24A4*, *KITLG* with hair colour, 2 coding variants in *TYR* with eye colour and freckles, variant on 6p25.3 with freckles, *OCA2* refinement and previous *MC1R* variant (Sulem *et al.*, 2007).

KITLG association is in agreement with Sulem *et al* (Sulem *et al.*, 2007), their candidate SNP is located 350kb upstream of kitlg, but show a 400kb haploblock that is centered on the gene. *KITLG* (mast cell growth factor, stem cell factor or steel factor) is a ligand for *KIT* tyrosine kinase receptor and is involved in the

development and maintenance of the melanocyte lineage in adult skin (Wehrle-Haller, 2003). *KITLG* exerts migratory, proliferative and survival functions in melanocytes expressing KIT receptor.

The association in *OCA2* intron 1 (rs7495174) is in agreement with (Duffy *et al.*, 2007) and Sulem *et al* (Sulem *et al.*, 2007). Sulem *et al* found the most significant SNP in *HERC2* 200kb upstream from *OCA2*.

HPS1

The association of *HPS1* SNP (rs10611135) with hair colour (P=0.04) may suggest its role in normal pigmentary variation. The recent report by Nguyen *et al* suggests that HPS1 may act to regulate melanocytes differentially at follicular and interfollicular sites in mice and may have implications on physiological pigmentary variation in humans (Nguyen and Wei, 2007).

MATP (*SLC45A2*)

The very significant association of *MATP* SNP (rs16891982) with hair colour (P=0.008574, OR=27.7) is noteworthy. This advanced our understanding and showed that *MATP* is associated with hair colour also, not just skin colour. (Stokowski *et al.*, 2007) showed an association between the same SNP rs16891982 (F374L) with skin pigmentation variation in individuals of South Asian descent only. There is a difference in population and hair colour was not investigated in their study.

A different *MATP* SNP (rs3756464) was significantly associated with skin colour (P=0.003246). This may be explained by its transporter function. *MATP* (*SLC45A2*) (OMIM 606202) is a pigmentation gene that is transcriptionally modulated by the melanocyte-specific transcription factor MITF (Du and Fisher, 2002; Newton *et al.*, 2001) and encodes a transporter. Graf *et al* found 2 SNPs (G272K and F374L) associated with normal variation in human pigmentation in different ethnic groups (Caucasians, Asians, African Americans and Australian Aborigines) (Graf *et al.*, 2005) and showed that 2 of the promoter SNPs (rs13289 and rs6867641) out of the 3 were associated with olive skin colour in Caucasians (Graf *et al.*, 2007). The third which was a 3-base pair duplication (1174dupAAT) was not associated. There was evidence of functional significance in an assay.

Genotype association to eye colour:

Eye colour association was performed as categorical data. Most significant association was *OCA2* intron 1 which is in agreement with previously published studies (Duffy *et al.*, 2007) and (Sulem *et al.*, 2007).

The other more significant association was *HPS3* which was found to be associated to eye colour and discussed earlier.

There are possible putative explanations with other significant associations found. The *DTNBP1* / *HPS7* belonged to the HPS group of family and could be involved together in the putative mechanisms with *HPS3* or *HPS5*.

The significant association with *KIT* cannot be easily explained. *KIT* somatic mutations (substituting valine in position 816) are characteristic of sporadic adult mastocytosis (Longley *et al.*, 1999), a mast cell disorder exemplified by hyperpigmented red brown macules, papules and plaques that urticate on rubbing. There is proliferation of melanocytes and melanin production.

One could postulate that the associations of eye colour with *ERCC6*, *ERCC8* could be due to individuals with a certain eye colour have better DNA repair mechanisms than people with other eye colours.

The cytochrome P450 pathway may have a role in the determination of eye colour through *CYP2C8*.

DCT/*TYRP2* is involved in melanin pigment biosynthesis (Jackson *et al.*, 1992) and may act through this to affect eye colour. Kayser *et al* and Sturm *et al* found introns of *HERC2* gene that predicts blue eye colour (Kayser *et al.*, 2008; Sturm *et al.*, 2008). Sturm found that *OCA2* (R419Q) could modify the penetrance of *HERC2* (rs12913832). This study did not include this new candidate. Future studies should include the new candidate loci.

The *GNAS* SNP (rs4810147) that is associated with eye colour is different to the ones associated with hair colour (rs2295583, rs3730168, rs919197). It is possible that *GNAS* has a role in eye and hair colours via its effector Gs cAMP pathway.

It is possible that some associations are spurious.

Genotype association to skin colour:

HPS3, *HPS4* and *HPS5* were discussed already. Different *DTNBP1* SNPs were associated with constitutive (rs9476886, rs875462, rs9396593) and facultative (rs17470454) pigmentation. The plausible explanation will be discussed later.

Two additional *MYO5A* SNPs (rs17614119, rs752865) are associated with skin colour and will be discussed later.

For *OCA2*, in contrast to the significant associations found with eye colour, no significant associations were found with skin colour.

The *TYR* SNPs (rs11018542, rs16913107) were significantly associated with skin colour. Although these are significant, they are new and different to the SNPs reported previously. Stokowski *et al* (Stokowski *et al.*, 2007) and Sulem *et al* (Sulem *et al.*, 2007) showed *TYR* SNP rs1042602 (S192Y) to be associated with skin colour. However Shriver *et al* (Shriver *et al.*, 2003) and Frudakis *et al* (Frudakis *et al.*, 2003) found no such association with skin or eye colour. These may be explained by difference in population.

TYRP1 mutation is known to be the cause of Oculocutaneous Albinism type III (Boissy *et al.*, 1996; Manga *et al.*, 1997).

TP53 is both significantly associated with constitutive and facultative pigmentation. It may play a normal physiological role. *TP53* is normally found in benign skin lesions (Soini *et al.*, 1994). Alternatively it may be via its transcriptional effect on *POMC* to lesser degree or indirect effect via tanning and constitutive skin colour. One could postulate whether p53 has a protective role by contributing to skin colour and subsequently less burning.

GNAS is significantly associated with skin colour. This could play a physiological role in normal pigmentary variation via its Gs-alpha and cAMP pathway. It is known that G-protein mutations (*Gnaq* and *Gna11*) can be associated with dark skin (*Dsk*) in mice (Van Raamsdonk *et al.*, 2004).

CYP4B1 is significantly associated with both constitutive and facultative pigmentation. The cytochrome P450 system may be important candidates for pigmentation.

The association of *ERCC3* with skin colour may suggest that *ERCC3* could exert its protective effect via skin colour or via DNA repair.

Genotype association to erythema:

12 genes are significantly associated with erythema in this study. Both *KIT* and *KITLG* SNPs are significantly associated with erythema. The *KITLG* finding is in keeping with Sulem *et al* (Sulem *et al.*, 2007), although the *KITLG* SNP found was different (rs1492354).

Sulem *et al* found *TYR* to be associated with freckling (Sulem *et al.*, 2007). Since I found freckling to be highly associated with erythema (P=0.002018, discussed in Chapter 4), my finding that *TYR* SNP rs11018542 is associated with erythema is consistent with existing literature and explainable.

As *ERCC* genes are involved in DNA nucleotide excision repair (Troelstra *et al.*, 1992), it is not surprising that *ERCC2*, *ERCC6* and *ERCC8* SNPs are significantly associated with erythema. Individuals with mutations from *ERCC* or having variants of xeroderma pigmentosum may have delayed or prolonged erythemal response (Cripps *et al.*, 1971; Norris *et al.*, 1991).

It is possible to postulate that *CYP2C8* SNP (rs3752988), acting on the cytochrome P450 enzyme system, may affect indirectly the generation of erythemal response.

The 5 *OCA2* SNPs found to be significantly associated with erythema may act via other variables e.g. eye colour, hair colour.

The association of 5 *HPS4* SNPs (rs739289, rs722997, rs1894706, rs4822721, rs877593) and a different *HPS5* SNP (rs10766469) were found to be associated with erythema as compared with 2 other *HPS5* SNPs associated with skin colour. For *KIT, HPS5*, *TYRP1*, *DCT* (*TYRP2*), one could postulate that the function of these genes may affect the erythemal responses indirectly, via other genes or pathways e.g. prostaglandins (Sondergaard *et al.*, 1985), nitric oxide (Deliconstantinos *et al.*, 1996; Deliconstantinos *et al.*, 1995).

Alternatively it is possible that some of the associations could have arisen by chance.

Genotype association to tanning:

The results showed that 14 genes were associated with tanning and therefore are plausible candidate loci for tanning. What does this mean?

The novel group of genes, which are found to be significantly associated with tanning in this study, is the Hermansky Pudlak Syndrome (*HPS*) genes. The significant associations of SNPs in *DTNBP1* (rs17470454, P=0.01552), *HPS1* (rs11592273, P=0.04771) and *HPS3* (rs2131025, P=0.04255) with tanning may be explained by the HPS proteins. Human *DTNBP1* gene mutations are found in individuals with HPS (Li *et al.*, 2003). Dysbindin is a protein that binds to alpha- and beta-dystrobrevins, which are components of the dystrophin-associated protein complex (DPC) (Benson *et al.*, 2001). Dysbindin is also a component of the biogenesis of lysosome-related organelles complex-1 (BLOC1), which regulates trafficking to lysosome-related organelles (Li *et al.*, 2003). Li *et al* showed that BLOC1 is important in producing the HPS phenotype in humans and that dysbindin has a role in the biogenesis of lysosome-related organelles, and highlighted the interactions between components of DPC and BLOC1. This in turn may have effects on melanosome production and ultimately tanning.

Since erythema and tanning are interrelated, as shown and discussed in Chapter 5 and in keeping with Wagner *et al* (Wagner *et al.*, 2002b), some of the candidate genes found significantly associated with erythema may also be relevant to tanning.

Indeed this was the case for 7 genes. SNPs in *KITLG*, *ERCC2*, *ERCC6*, *ERCC8*, *OCA2*, *CYP2C8* and *TYRP1* were similarly associated with both erythema and tanning.

KITLG SNP rs1907702 was associated with buttock tanning L* (P=0.01645).
The significant association of *KITLG* SNP rs1907702 with tanning adds to the findings of Sulem *et al*. *KITLG* exerts migratory, proliferative and survival functions in melanocytes expressing Kit receptor (Wehrle-Haller, 2003). Another indirect piece of evidence is that *KIT* somatic mutations (substituting valine in position 816) are characteristic of sporadic adult mastocytosis (Longley *et al.*, 1999), a disorder of mast cells exemplified by hyperpigmented red brown macules, papules and plaques that urticate on rubbing. There is proliferation of melanocytes and melanin production.

4 *TP53* SNPs (rs12951053, rs2078486, rs8064946 and rs1042522) were significantly associated with tanning (P=0.001211-0.04248). Recently Cui *et al* showed that p53 mediates the upregulation of *POMC* and that *POMC* is a transcriptional target for p53 and therefore tanning (Cui *et al.*, 2007). My findings are in keeping with this. It also highlights the common notion that facultative pigmentation is protective against skin cancer. "Guardian of the genome" p53 plays a central role.

The association of *MYO5A* SNP (rs11853114) with tanning and skin colour raises the possibility that class V myosins may be involved in organelle transport (El-Husseini and Vincent, 1999) and pigment granule transport (Fukuda *et al.*, 2002) as possible explanations for the association.
The association findings of *OCA2* with tanning may be explained by the relationship of the oculocutaneous albinism type 2 (*OCA2*) with skin colour or indirectly via its association with erythema.
CYP4B1 may play a role in facultative pigmentation via its effect on cytochrome P450 pathway.

The association of *TYRP1* SNP (rs17346161) with both tanning and skin colour suggests that oculocutaneous albinism type III (Rufous) may have a role in both constitutive and facultative pigmentation.
The association of *ERCC2*, *ERCC6* and *ERCC8* SNPs may possibly be explained by their action via DNA repair or indirectly via its relation with erythema.
*MC1R.*SNP "R" shows a significant association with tanning (P=0.03095). This is to be expected. There was a tendency for individuals who are homozygous for *MC1R* to tan less (4.4 L* units) as compared with WT (6.7 L* units) (discussed in Chapter 6). However the results did not reach significance (P=0.15).

One could postulate that the function of these candidate genes may affect the facultative pigmentary responses direct or indirectly, via other genes or pathways or through non-pigmentary adaptation e.g. hyperplasia or thickening. As mentioned, an alternative explanation is possibly that some of the associations could have arisen by chance.

One of the limitations of this genetic association study could be false positives from multiple testing. The associations found could be spurious or just by chance. The problem of false positives has been extensively published (Benjamini, 1995; Dudbridge and Koeleman, 2004; Gao *et al.*, 2008; Reiner *et al.*, 2003). Bonferroni's correction for multiple testing was used and a P value of less than 0.05 was chosen as significant.
We are in the process of confirming these positive significant findings in collaboration with another laboratory in Denmark and in that population. Significant findings were also clearly showing up positive with the SNPs tested in this study in the Danish population (unpublished observations, manuscript to be submitted). In conclusion, we found significant associations with SNPs in novel gene *HPS3* and confirmed other associations in *KITLG* and *OCA2*. Further experiments have been planned to confirm other associations and phenotypes.
This European population variation was not explained by *SLC24A5* – there were no significant associations with any pigmentary phenotypes, except hair colour cluster for rs2675347 ($P=7.32\times10^{-6}$, MAF of only 0.00769231). The different finding is due

to a difference in the population under study. Lamason *et al* found the association in African Americans, the proportion of skin colour explained by *SLC24A5* is much larger than *MC1R* (Lamason *et al.*, 2005).
Similarly *ASIP* did not seem to have any effect in this population, probably due to the low frequency in this population and a homogeneous European population, without e.g. African Americans. *ASIP* was not found to be associated with hair, skin or eye colours in this population. It is known that *ASIP* frequency is different in different population (Meziani *et al.*, 2005; Norton *et al.*, 2007). The frequency in this population is too low to detect an association despite the involvement in the production of darker eumelanin and lighter phaeomelanin together with *MC1R*.

Since the completion of this study with the current approach of SNP genotyping using Illumina, a comprehensive approach covering the whole genome (genome wide scan) has been performed by Sulem and others (Gudbjartsson *et al.*, 2008; Han *et al.*, 2008; Stokowski *et al.*, 2007; Sulem *et al.*, 2008; Sulem *et al.*, 2007) and found variants in genes with significant associations with phenotypic traits e.g. *SLC24A4*, *KITLG*, *TYR*, 6p25.3, *OCA2*, *MC1R*, *TPCN2*, *ASIP*.
Further experiments could include the newly found candidate loci in pigmentation.
Using a candidate gene approach I have identified several novel SNPs for hair, eye and skin colour. The findings may suggest physiological roles in normal pigmentary variation and remains to be confirmed in further studies.

Chapter 8 Blondes

8.1. Introduction

The aim of this chapter is to focus on blondes. What is the phenotypic definition of blonde? What is the blonde genotype?

8.2. Results

There were 133 individuals with natural hair colour. 86 individuals have dark hair, 27 individuals have fair hair, out of which 19 individuals have blonde hair and 20 volunteers have red hair. The classification of hair colour was according to careful visual inspection of the natural hair colour of individuals.

8.2.1. Summary of Phenotype

8.2.1.1. Hair colour

Do blonde hair individuals differ from fair hair individuals? The blonde / fair hair individuals were subdivided into 2 groups: blonde (n=19) and fair (n=8) after careful visual inspection of the hair colour of individuals. Individuals with obvious blonde hair colour were classified into blonde hair colour group (n=19). Others with lighter hair colour were classified into fair hair colour group (n=8).

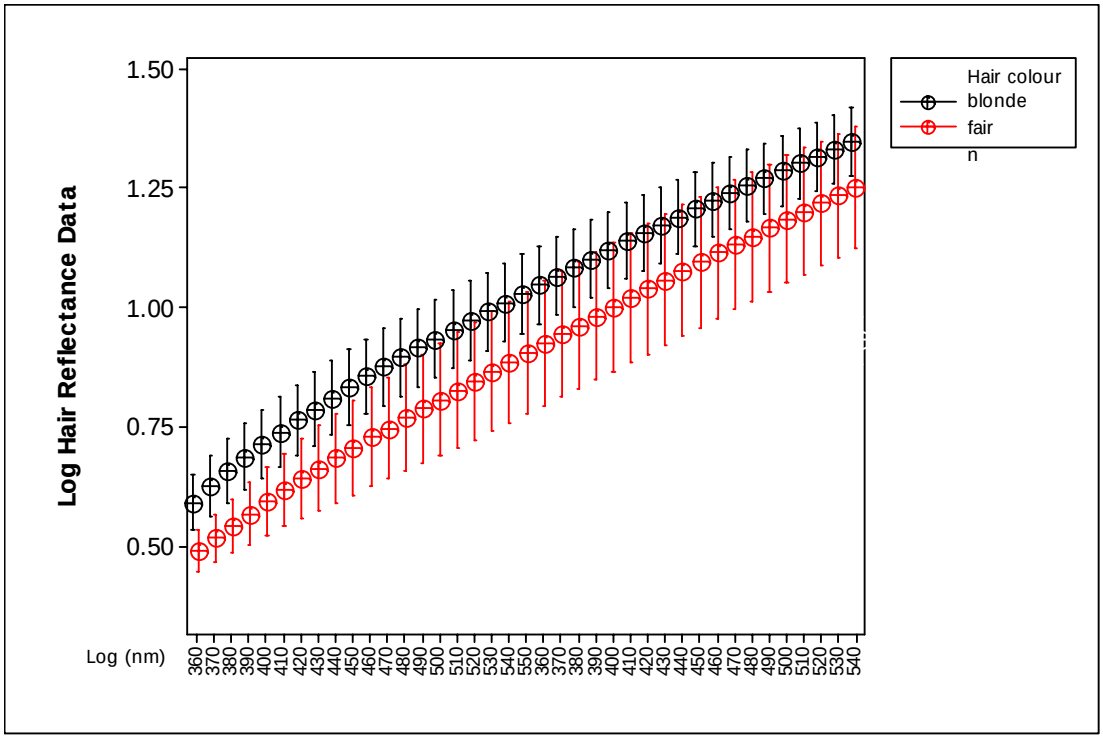
Blonde hair volunteers had a mean L*, a*, b* of 39.67 ± 1.66, 5.26 ± 0.17, 13.62 ± 0.63 respectively.

Fair hair volunteers had a mean L*, a*, b* of 34.33 ± 2.13, 5.23 ± 0.41, 12.47 ± 1.35 respectively.

One-way ANOVA for hair colour L*, a* and b* comparing blonde and fair showed no significant differences (P=0.079, 0.94, 0.385).

8.2.1.2. Hair colour spectrophotometric reflectance

Figure 8.2.1.2. Hair spectrophotometric reflectance of blonde and fair hair individuals



This is an interval plot of log hair spectrophotometric reflectance ± SEM (95% CI for the mean) for blonde and fair hair individuals. Y-axis shows the log hair spectrophotometric reflectance. X-axis shows the 10nm increments of 360-740nm wavelengths.

Two-sample t-test showed significant differences from log(360)nm-log(470)nm ($P<0.05$).

Interestingly, blondes appeared to be distinguishable from fair hair individuals using hair spectrophotometric reflectance data.

8.2.1.3. Skin colour

Blonde hair volunteers had a mean skin L*, a*, b* of 57.16 ± 0.79, 14.60 ± 0.20, 14.37 ± 0.56 respectively.

8.2.1.4. Blonde and eye colour

Table 8.2.1.4. Blonde and eye colour

	N	Y	All
Blue	39	13	52
Brown	55	0	55
Green	24	3	27
Grey	12	3	15
Hazel	10	0	10
All	140	19	159

The majority of individuals have blue eye colour (13), 3 had green eye colour and 3 had grey eye colour.

8.2.1.5. Erythema and blonde hair

Blonde hair had no effect with a mean erythemal index of 134 in those without blonde hair and 140 in those with blonde hair (P=0.5889, two sample t-test).

8.2.1.6. Tanning and blonde hair

There was no relation between tanning on the back and the presence of blonde hair or otherwise, but tanning on the buttock was greatest at 4.33 in those without blonde hair, compared with 1.99 in those with blonde hair (P=0.02, two sample t-test).

8.2.2. Summary of genotype

Please note that "fair hair" in this section is a general term which includes fair and blonde hair individuals.

MC1R

There was no significant association between blonde hair status and *MC1R* genotype (P>0.9999).

HPS3

HPS3 SNPs rs2254913, rs2681092 and rs2689230 were associated with fair hair (P=0.02168, P=0.03155, P=0.02867).

DTNBP1

DTNBP1 SNPs rs6909929, rs9396592, rs17470454 were associated with fair hair (P=0.01388, P=0.03081, P=0.03801).

OCA2

OCA2 SNPs rs7173419 and rs4778137 were associated with fair hair (P=0.01115, P=0.02965).

MYO5A

MY05A SNP rs752864 was associated with fair hair (P=0.04506).

GNAS

GNAS SNPs rs234630, rs3730168 and rs6026561 were associated with fair hair (P=0.01552, P=0.04248, P=0.04283).

8.3. Discussion

The category blonde / fair can be sometimes difficult to distinguish and subjective. There were 8 fair hair individuals, and 19 pure blonde hair individuals in this study. The phenotypic data for blondes as a group was not remarkable.

Blonde hair status does seem to contribute to a lesser tanning effect (P=0.02).

The genotype data suggest that several loci (*HPS3*, *DTNBP1*, *OCA2*, *MYO5A* and *GNAS*) may be candidate genes for blonde / fair hair.

The number of individuals with blonde phenotype was small. Nevertheless, the phenotype and genotype definitions of blondes merit further investigation. Further experiments have been planned in collaboration with a group in Denmark to test a number of my chosen SNPs which were found to be associated with blonde / fair hair to test this in the Danish population.

These also raised further questions to be answered in future studies:

1) Is it possible to map out blonde families in an admixture and attempt to track blonde through generations? Family trees could be constructed from famliy history, siblings' hair colour and kindreds with blonde.
2) What is the mode of inheritance with blonde?
3) Do any blondes have a homozygous *MC1R* mutation? Yes, in my study there was 1 individual (V81). This was discussed in Chapter 6 and was an interesting finding.

Chapter 9 Discussion and Concluding Remarks

9.1. Discussion

This was the first detailed quantitative phenotype-genotype study on erythema and tanning in relation to *MC1R* genotype. The aim of this study is to objectively and quantitatively define pigmentary phenotype of individuals (hair colour, eye colour, skin colour, erythema and tanning) and co-factors (age, sex, ethnicity, freckling and Fitzpatrick skin type) as comprehensively as possible. My study demonstrated the possibility of using quantitative methodology to define phenotypes.

In Chapter 3, I gave a detailed description of the key phenotypic variables. I confirmed previous findings and demonstrated more quantitative results e.g. hair spectral reflectance, skin colour L* with skin type, skin colour L* and hair colour L* a* b*. I showed that the effect of skin colour of various sites is different in males and females.

In Chapters 4 and 5, I presented a full description of the induced phenotype (with UVB), namely erythema and tanning. I confirmed the difference in erythemal sensitivity with site (the back is more sensitive than the buttock), which is in keeping with previous findings (Rhodes and Friedmann, 1992; Waterston *et al.*, 2004).
I showed that the major determinants of erythema for this study population were constitutive buttock skin colour L* and skin type. I used a linear regression model to explain erythemal sensitivity (4.2.1.2.9) and showed that erythema is dependent on constitutive skin colour and skin type. This model explained 27% of the variation in erythema ($P=1.205\times10^{-7}$) for the buttock. In fact, my findings showed that skin type is just as good as constitutive skin colour L* in predicting erythema. Erythema is also associated with other factors e.g. freckling, ethnicity and hair colour. These factors may be the markers of constitutive skin colour already and therefore related to erythema as one might expect. I confirmed that both measures of erythema (erythemal index and erythemal flux) gave similar results.

What are the determinants of tanning? In Chapter 5, I gave a detailed account of using 6 different quantitative measures of tanning. The main determinant of tanning in this study population is erythema. A model which explained tanning (5.2.11) showed that tanning is dependent on erythema and it also explained 24% of the variation in tanning responses ($P=2.687\times10^{-6}$) for the buttock. In fact, constitutive skin colour, skin type, ethnic group, red hair status and freckling were not significant beyond that was accounted for by erythema. Therefore erythema is the key predictor of facultative pigmentation.

What are the genetic determinants that underpin the static phenotype and induced phenotype (erythema and tanning)?

In Chapter 6, I showed that *MC1R* explains the variation in hair colour but the effect of *MC1R* on skin colour is small. This is in keeping with previous publication (Naysmith *et al.*, 2004). *MC1R* is also associated with freckles. My results support previous findings (Flanagan *et al.*, 2000). However, *MC1R* in my study did not significantly account for the differences in erythemal and tanning responses. This is in contrast to the findings of Flanagan *et al* (Flanagan *et al.*, 2001). The reason for this could be due to differences in the selection of study individuals. Flanagan *et al* selectively studied red hair individuals to look for an effect of *MC1R*, whilst my study population consisted of a homogenous unselected population, predominantly Northern Europeans with a small proportion of red hair individuals.

What about other genotypes?

In Chapter 7, I demonstrated the significant genotypic associations with various phenotypes. I presented novel SNPs in *HPS3* (rs2254913, rs6785780, rs7636389, rs2681092 and rs2689230) and *KITLG* (rs1492354, rs1907702 and rs10777129) that were significantly associated with hair colour. Sulem *et al* found a SNP (rs12821256) which is nearest to the gene *KITLG*. My finding of *KITLG* is in accordance with Sulem *et al* (Sulem *et al.*, 2007). Additionally, I found 3 different novel SNPs in *KITLG*.

I also found novel SNPs in *HPS3* (rs2254913, rs2689229, rs2689230, rs2689234, rs6785780, rs2681092, rs7643410) and *GNAS* (rs4810147) that were significantly

associated with eye colour. My findings of *OCA2* confirmed previous findings of its association with eye colour (Duffy *et al.*, 2007).

Skin colour was associated with SNPs of *HPS3*, *HPS4*, *HPS5*, *HPS7*, *TP53*, *TYR*, *MATP* and *MC1R*. The SNPs of *HPS3* (rs2689230), *HPS4* (rs9613187), *HPS5* (rs2049129 and rs4757637), *DTNBP1* / *HPS7* (rs9476886, rs875462 and rs9396593) were novel findings and of special interest as some of these were also significantly associated with hair and eye colour. The novel *HPS5* SNP (rs2049129) accounted for ~25% of the variation in skin colour.

Putative explanation for the *HPS* findings is that *HPS* is involved in lysosome organelle formation. Mutations of *HPS* genes may cause changes in shape and density of melanosomes. This suggests that *HPS* may play a role in hair, eye and skin colour variation.

In terms of erythemal sensitivity, *KITLG*, *TYR*, *ERCC2*, *ERCC6*, *ERCC8*, *OCA2*, *TYR* SNPs showed significant associations with erythema. Novel SNPs were found.

Potential explanations include *ERCC* genes affecting erythema via their DNA nucleotide excision repair function, the effect of *TYR* on erythema through freckling, or through other pathways that affects prostaglandin or nitric oxide production.

Tanning was associated with *DTNBP1* / *HPS7*, *TP53*, *ERCC2*, *ERCC8* and *OCA2*.

The genotypic association of *TP53* to tanning could be explained by p53 mediated upregulation of *POMC* and that *POMC* is a transcriptional target for p53 and therefore tanning. This is consistent with the findings of Cui *et al* (Cui *et al.*, 2007).

9.2. Limitations

I have encountered a number of potential shortcomings during this study. First, during my attempt to further develop quantitative measures of eye colour, I used an adapted method of colour matching to measure eye colour and then converting the Munsell colour into L* a* b* values. However there could be an element of subjectivity of the initial colour matching.

Second, there were limitations with some tanning assays in that they did not provide a good dose response of tanning e.g. Melanin index or "Dwyer method".

Third, it is possible that there are limitations with HapMap. The tagging SNPs may not fully represent the whole candidate gene locus. Another potential problem with

genetic association studies is false positive results from multiple testing. Although a Bonferroni correction was used to correct for this, associations could still have arisen by chance, and "real" associations may have been missed.

Fourth, the sequencing and SNP genotyping experiments failed to provide genotypic data for some individuals. As a result, this reduced the power of this study.

Fifth, while performing statistical analysis, some of the numbers within subcategories were too small or zero which limited the amount of testing that could be done. This may in turn reduce the statistical power. If the number of volunteers taking part in this study was larger, some of the findings may reach statistical significance.

9.3. Development of novel assays to meaure phenotype

During this study I have further developed quantitative methodology for future pigmentary studies (noradrenaline iontophoresis), pressure spectrophotometry, measures of tanning (Dwyer calculation method, pressure spectrophotometry) and indirect quantitative measure of eye phenotype (Munsell L* a* b*).

Recently published studies have not used objective quantitative methodology and still used methods based on self assessment (Han *et al.*, 2008; Sulem *et al.*, 2008). The potential limitations in these studies could be due to problems of misclassification and recall bias. Further use of and development in quantitative methods will allow more precise measurement and definition of phenotypes to be made. This could subsequently lead to a better understanding of the relationship between genotypes, phenotypes and the cutaneous response to UVR in the genes. The differences in methodology also make comparisions of results between different studies difficult.

9.4. Further experiments

This section summarises the further experiments that are planned or could be performed in light of the findings from my quantitative phenotype-genotype study.

First, I have demonstrated freckling, skin type and hair colour L* a* b* as important definitive phenotypic measures for *MC1R*. Therefore in any future study design involving *MC1R*, the inclusion of these phenotypic measures will be sufficient.

Second, for the study of other genotypes, the novel SNPs found in this study could be repeated and tested in another population. A joint study will be undertaken to verify our findings in the Danish population in collaboration with Jonas Mengel-Jorgensen and Niels Morling in Denmark. The following list of SNPs (Table 9.4) has been selected for repeating in the joint study:

Table 9.4. List of SNPs selected to repeat in joint study

Gene	Chr	SNP	Blonde hair	Red hair	L*	a*	b*	Eye
HPS3	3	rs678580	-	√	√	√	√	√
HPS3	3	rs2254913	√	-	√	√	√	√
DTNBP1	6	rs6909929	√	-	√	-	-	-
DTNBP1	6	rs17470454	√	-	√	-	-	-
CYP2C8	10	rs11572177	-	√	-	-	√	√
TYR	11	rs12421746	-	√	√	√	√	-
KITLG	12	rs1492354	-	√	-	√	√	-
KITLG	12	rs10777129	-	√	√	√	√	-
GNAS	20	rs919197	√	-	√	√	√	-
GNAS	20	rs234630	√	-	-	-	-	-
GNAS	20	rs4810147	-	-	-	-	-	√
ERCC6	10	rs4253231	-	-	-	-	-	√
DCT	13	rs9584233	-	√	-	-	-	√

Key: (√) selected to repeat, (-) not selected to repeat.

Third, the findings in this study could be repeated by using buccal swab smear in 100 newly recruited individuals. Any new candidate genes should be included in the new study.

Fourth, the erythemal and tanning dose responses of individuals could be investigated in different people with more varied skin colour.

Fifth, this study could be undertaken in a different population e.g. Asian or other European population to investigate for population differences.

Sixth, as the range of skin colour of individuals is varied even within a population, an experiment could be performed to study the tanning response of individuals of similarly matched degree of skin colour to see whether they respond differently to each other. Other contributory factors could be investigated too.

Seventh, since the completion of this study, new whole genome genotyping technologies have become available: e.g. Infinium® whole-genome genotyping (Illumina), HumanHap300-Duo Genotyping BeadChip. These would allow large scale screening of variation in the human genome. These technologies could be incorporated into the methodology of future studies.

What is the future with genotyping? The ideal study would be to include SNPs from the whole genome as Sulem *et al* and others have performed, using more individuals and carrying out more detailed analysis. The potential limitation with large numbers of individuals involved may mean that only basic phenotypic measures could be analysed. Subsequently, a more detailed quantitative study precisely defining the phenotypes might be necessary to define the contribution or functional significance.

Human pigmentary phenotypes are complex traits. Further experiments in collaboration with other laboratories may help to advance our understanding.

9.5. Concluding remarks

Phenotype-genotype correlations are very dependent on the precise definition of a phenotypic assay. An objective reproducible repeatable phenotypic measure used to define a phenotype may be most advantageous. Sometimes there are several phenotypic measures and assays for a particular phenotype e.g. tanning.

I have quantitatively measured human pigmentary phenotypes including hair colour, skin colour and eye colour and then quantified the cutaneous erythemal and facultative pigmentary response after UVB radiation.

I started out designing a study with quantitative methodology in mind to precisely define phenotypes and then relating these to genotypes. This study demonstrated that it is possible to use a very quantitative, reproducible and relatively economical way to define phenotype and to ascertain genotypes.

Interestingly, it turned out that although phenotypes can be very precisely defined using quantitative methodology, sometimes simple phenotypic measures (e.g. freckling, skin type) may be sufficient to define phenotypes. In the case of *MC1R* for my study population sample, the phenotypic traits of freckling and skin type have already provided sufficient information. Ascertaining their *MC1R* genotypes may not add further useful information above that already provided for by phenotype.

In this study I have developed further quantitative methodology and measures of tanning. I have further developed a quantitative / semi-quantitative method to measure eye colour, which could be used in future phenotype-genotype studies.

I have elucidated the determinants of erythema and tanning in this population that erythema is dependent on skin colour and skin type whereas tanning is dependent on erythema. I have demonstrated the interrelationship between constitutive skin colour, erythema and tanning.

I have correlated the phenotypic data with genotypes in my study population and found novel SNPs in several candidate genes associated with pigmentary traits.

The key novel findings are:

1) Hermansky-Pudlak Syndrome group of genes (e.g. *HPS3*) and *KITLG* with hair colour

2) *HPS3* and *GNAS* with eye colour

3) *HPS3*, *HPS5*, *HPS7* and *GNAS* with skin colour

Further testing could be repeated in another population to determine whether these SNPs are causal for the phenotypic traits or some of which may be false positives. This is already underway.

This study has confirmed previously known associations (e.g. hair colour with *MC1R* and *OCA2*, eye colour with *OCA2*).

These novel findings are interesting and suggest that the relationship between phenotypic traits and normal physiological variation in pigmentation and facultative pigmentation is very complex.

New technological advances and the advent of genome wide scan to screen for phenotypic traits may identify new or novel pigmentation candidate alleles. The causality and functional importance of these newly identified variants may require a precise phenotypic definition of phenotype using a quantitative methodology in order to be able to compare results between studies and among populations. This could advance our understanding in the field of pigmentation (constitutive or facultative) and ultimately cutaneous malignancies.

This study has furthered our understanding of human pigmentation and the cutaneous response to UV radiation.

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Appendix 1

Consent Form for Initial Study

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Dermatology On-Call Number ☎ 44(0)7950 204464
T.Wong@ed.ac.uk
Karen.Muir@ed.ac.uk

Title: Pigmentation and the cutaneous response to ultraviolet radiation
Derm Study Number: Derm/03/JLR/6/TW
Patient Indentification Number for this study:

CONSENT FORM – INITIAL STUDY

Title of Project: Pigmentation and the cutaneous response to ultraviolet radiation

Name of Researcher(s): Dr Terence H Wong

1. I confirm that I have read and understood the information sheet number Derm/03/JLR/6/TW (**version 1.3**) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care of legal rights being affected.

3. I understand that sections of any of my medical notes may be looked at by responsible individuals and the research sponsor, or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. *I understand that this is non-therapeutic research from which I cannot expect to derive any direct benefit.*

5. I agree for notice to be sent to my General Practitioner.

6. Procedures to be performed: measuring skin, hair and eye colour, sample of venous blood or buccal smear swab for genetic analysis **(pigmentation genes e.g. MC1R and other genes important in skin inflammation and susceptibility to ultraviolet radiation and skin cancer)**, and sample of hair, UV irradiation.

7. A separate consent form for substudy has to be completed in order to take part in the substudy.

_____	_____	_____
Name of Patient	Date	Signature
Dr Terence Wong_____	_____	_____
Researcher	Date	Signature

Appendix 2

Consent Form for Substudy

Department of Dermatology
Lauriston Building, Royal Infirmary
Lauriston Place
EDINBURGH EH3 9YW
☎ 44 (0)131 536 2044
☎ 44 (0)131 536 2041
44 (0)131 229 8769
Dermatology Trials Office ☎ 44 (0)131 536 2053
Dermatology On-Call Number ☎ 44(0)7950 204464
T.Wong@ed.ac.uk
Karen.Muir@ed.ac.uk

Title: Pigmentation and the cutaneous response to ultraviolet radiation
Derm Study Number: Derm/03/JLR/6/TW
Patient Indentification Number for this study:

CONSENT FORM – SUBSTUDY

Title of Project: Pigmentation and the cutaneous response to ultraviolet radiation

Name of Researcher(s): Dr Terence H Wong

8. I confirm that I have read and understood the information sheet number Derm/03/JLR/6/TW (version 1.2) for the above study and have had the opportunity to ask questions.

9. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care of legal rights being affected.

10. I understand that sections of any of my medical notes may be looked at by responsible individuals and the research sponsor, or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

11. *I understand that this is non-therapeutic research from which I cannot expect to derive any direct benefit.*

12. I agree for notice to be sent to my General Practitioner.

13. Procedures to be performed: Retention of skin from suction blister for transmission studies and melanin analysis, measuring skin colour after noradrenaline iontophoresis.

14. Two separate consent forms have to be completed in order to take part in the initial and substudy.

_____	_____	_____
Name of Patient	Date	Signature
Dr Terence Wong_____	_____	_____
Researcher	Date	Signature

Appendix 3

Volunteer Questionnaire

Pigmentation and the cutaneous response to ultraviolet radiation

PIN:

Volunteer Demographic details

Name:

Sex:

Date of birth:

Age (18-30):

Address:

Phone number:

GP:

GP Practice address:

Background information

Exclusion criteria:

with photosensitivity	Y / N
on drugs known to interfere with the cutaneous inflammatory response (such as high dose systemic steroids, cyclosporin, methotrexate and MMF)	Y / N
on drugs known to act on the adrenergic pathways (such as Tricyclics, MAOI and betablockers) (substudy only)	Y / N
who are breast feeding (substudy only)	Y / N
who are pregnant (substudy only)	Y / N
attempting to get pregnant before or during the study (substudy only)	Y / N
referred because of acute inflammatory skin disease	Y / N

Skin type:

I	Always burn, never tans?
II	Burns easily, tans with difficulty?
III	Burns moderately, tans moderately?
IV	Burns minimally, tans easily?
V	Rarely burns, tans deeply?
VI	Never burns, tans deeply?

Natural hair colour:
Red / auburn / strawberry blond?
Blond / fair?
Brown / black?

Eye colour:

Past medical history:
Skin disorders?
Pregnant?

Drug history:

Allergies:

Family history:

Sunbed use:	Y / N	How long?
Sunscreen:	Y / N	
Holidays abroad:	Y / N	Where?
Sunny countries:	Y / N	Where?
Sunburn:	Y / N	UK / abroad?
Freckles:	Y / N	
Freckling sites:	face / shoulders / chest / back / abdomen / arms / legs	

Resident country:

Ethnic ancestry:

Place of birth:

Father's place of birth:

Father's hair colour:

Mother's place of birth:

Mother's hair colour:

Appendix 4

Volunteer Information Sheet

Department of Dermatology
Lauriston Building, Royal Infirmary
Lauriston Place
EDINBURGH EH3 9YW
☎ 44 (0)131 536 2044
☎ 44 (0)131 536 2041
44 (0)131 229 8769
Dermatology Trials Office ☎ 44 (0)131 536 2053
Dermatology On-Call Number ☎ 44(0)7950 204464
T.Wong@ed.ac.uk
Karen.Muir@ed.ac.uk

Title: Pigmentation and the cutaneous response to ultraviolet radiation
Dermatology subject project no: Derm/03/JLR/6/TW
Version: 1.3
Date: 08 January 2007

INFORMATION SHEET

1. **Study title**
   Pigmentation and the skin response to ultraviolet radiation (sunlight).

2. **Invitation**
   You have been invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

3. **What is the purpose of the study?**
   People vary considerably in their sensitivity to the effects of sunshine. Understanding these differences is important because sunshine is the main cause of skin cancer, because ultraviolet radiation (the active component of sunlight) is used more and more to treat diseases such as psoriasis and atopic dermatitis, and finally

because there is greater scientific interest in understanding why humans vary so much in terms of hair, eye and skin colour.

4. Why have I been chosen?

We are asking you whether you would be willing to take part in this research project. It is important to emphasise at the outset that this is a research project and is not designed specifically to improve the therapy of any skin problem you have had or will have at some future date, and in the case of volunteers the research will provide no direct benefit to you. We do believe however that the research project is important which is why we are asking people whether they are willing to help us. Medication that may have been prescribed for you may interfere with the tests we are carrying out. If therefore you are receiving cyclosporin or other systemic immunosuppressive drugs, or high dose systemic corticosteroids then unfortunately you may not be eligible for the present study. We will ask what medications, if any, you take.

5. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect receive the standard of care you receive.

6. What will happen to me if I take part?

Initial study

This research study is divided into two parts. The initial study, if you are willing to take part, will involve attending the department on a maximum of eight occasions. We will measure your skin colour, hair colour and eye colour with a non-invasive instrument (modified camera). We would like a small sample of hair (equivalent to the amount of hair in an eyebrow but taken from the scalp) to determine to amount of melanin (pigment) present. In order to interpret the results, we would like to take a small sample (approximately four teaspoons) of venous blood **or buccal cheek smear swab** from you **for genetic analysis (pigmentation genes e.g. MC1R and other genes important in skin inflammation and susceptibility to ultraviolet radiation and skin cancer)**. If relevant, we may ask you to return to do further tests involving shining ultraviolet light (sunlight) onto 10 small areas of skin on your body. We will give you a small amount of a steroid cream just to rub into the skin to speed up any resolution of redness if necessary.

Substudy

If you are willing to take part in our substudy, we will remeasure your skin colour after noradrenaline iontophoresis (in order to make the skin blanch / paler temporarily). We will perform suction blisters to obtain a small sample of skin for further tests in order to determine how transmissible (permeable to light) and to analyse the content of different pigments.

If you are willing to take part in both parts of the study, you will have to complete both consent forms.

7. What do I have to do?

There are no particular lifestyle restrictions for you to take part in this study.

8. What drug or procedure is being tested?

Not applicable

9. What are the alternatives for diagnosis or treatment?

Not applicable

10. What are the side effects of any treatment received when taking part?

Blood taking is associated with discomfort. Application of various doses of ultraviolet radiation is associated with the induction of redness, and swelling in some cases. Such redness and swelling will be associated with itching in some cases. Following application the skin may stay red for up to a few weeks and in some cases it may result in residual pigmentation which will fade over a few months. In order to minimise such irritation it is our normal practice to give the individuals a few grams of medium potency steroid (applied to the affected areas). All of the procedures involved are widely used in dermatological research. The contact numbers for this study are on front of information sheet.

11. What are the possible disadvantages and risks of taking part?

You will not be disadvantaged. The possible risks would be similar to the risk of having any minor skin wound, such as infection, bleeding, reopening of wound; the study will not impose any extra or further risks. The disadvantages of having a sample of venous blood taken from you is similar to any blood-taking procedure you may have had e.g. bruising and discomfort at the site.

12. Pregnancy

There are no problems with you taking part in the preliminary study, you may not be able to take part in the substudy which follows. It is possible that if treatment is given to a pregnant woman it will harm the unborn child. Pregnant woman must not therefore take part in the substudy, neither should women who plan to become pregnant during the study. Any woman who finds that she has become pregnant whilst taking part in the study should immediately tell the research doctor.

13. What are the possible benefits of taking part?

It is important to emphasise as stated earlier that there are no direct benefits of taking part in this research for you. You may derive some satisfaction from helping contribute toward advancement of medical science. You should also understand that there is discomfort involved such as the pain from the taking of blood or suction blisters.

14. What if new information becomes available?

Sometimes during the course of a research project new information becomes available. If this happens your research doctor will tell you about it and discuss

with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form. If any new information would suggest to the research doctor that you should withdraw from the study then he will explain and discuss this with you.

15. What if something goes wrong?

Before conducting any research on humans considerable thought is given to likely benefits and disadvantages. We feel there are no significant risks to you if you agree to take part in this study. The University holds a clinical trial protection insurance policy which provides insurance cover in respect of the legal liability of the University in respect of accidental injury to a research subject arising out of a clinical trial undertaking in the name of the University. The University also holds a no fault insurance clinical trials protection policy which provides compensation to a research subject in respect of accidental injury arising out of a clinical trial undertaking in the name of the University.

16. Confidentiality

If you consent to take part in this research any of your records can be looked at by people from regulatory authorities to check that the studies are being carried out correctly and honestly. Your name, however, will not be disclosed outside the hospital or GP surgery. Any information about you which leaves the hospital/surgery will have your name and address removed so that you cannot be recognised from it. The results of the research study will be published in medical journals available in the public domain.

17. Will my own doctor be told of my participation?

We will inform a patient's own doctor of participation in all our studies. If you do not give us this permission then you cannot take part in this study.

18. What will happen to the results of the research study?

We hope that the results of this study will contribute to improved treatments for patients with skin disease. Such results will be published in the medical literature so that other doctors and patients can benefit from our findings.

19. Funding

This study is funded by the Medical Research Council. Funding for the research is through a contract with The University of Edinburgh. Independent researchers do not benefit financially from your participation.

20. Contact for further information

Dr Terence Wong,
Medical Research Council Clinical Research Fellow,
Room 4.123, Dermatology,
1st Floor, Lauriston Building,
Lauriston Place,
Edinburgh, EH3 9HA.

Telephone 0131 536 2044. Fax 0131 229 8769.
Email T.Wong@ed.ac.uk

Karen Muir
Secretary to Professor Jonathan Rees
Room 4.018, Dermatology,
1st Floor, Lauriston Building,
Lauriston Place,
Edinburgh, EH3 9HA.
Telephone 0131 536 2041. Fax 0131 229 8769.
Email Karen.Muir@ed.ac.uk

Alternatively, Dermatology on-call Registrar 07950 204464 or bleep number #6396.

A copy of this information sheet and consent form is for you to keep.

Appendix 5

Human Hair Melanin Assay Results

Appendix 5. Human Hair Melanin Assay Results

A.5.1. Results

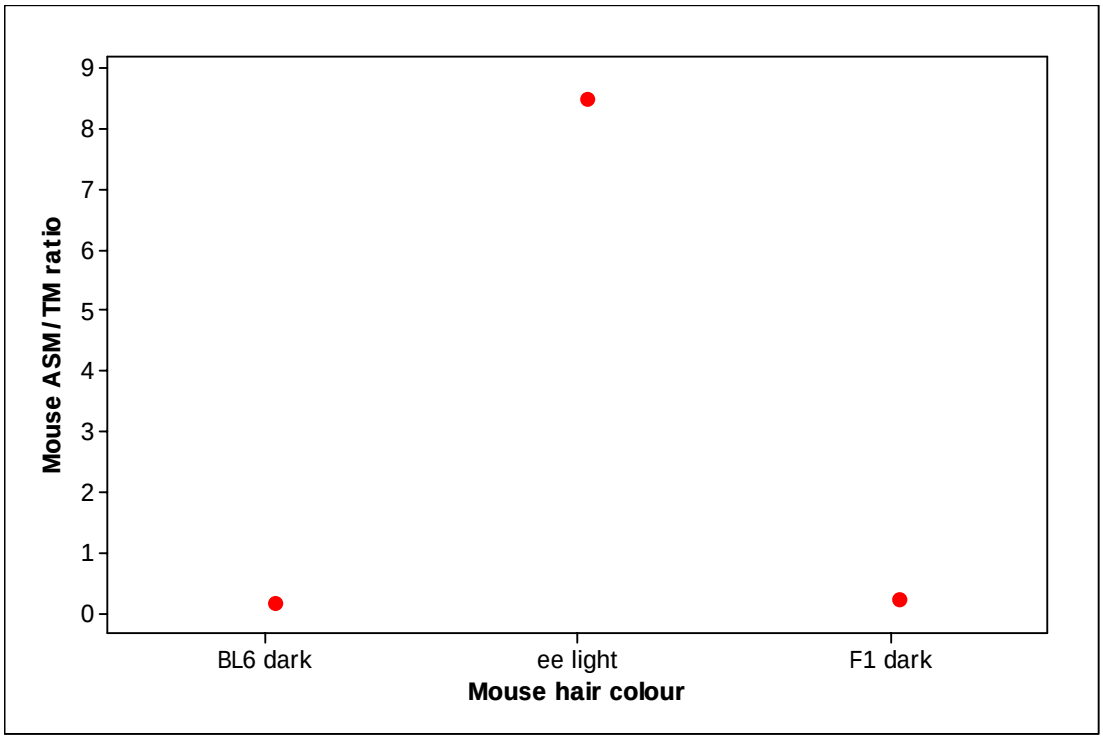
Absorbance at 400nm = Alkaline Soluble Melanin (ASM) ~ Phaeomelanin

Absorbance at 500nm = Total Melanin (TM) ~ Eumelanin

The ratio ASM/TM was calculated.

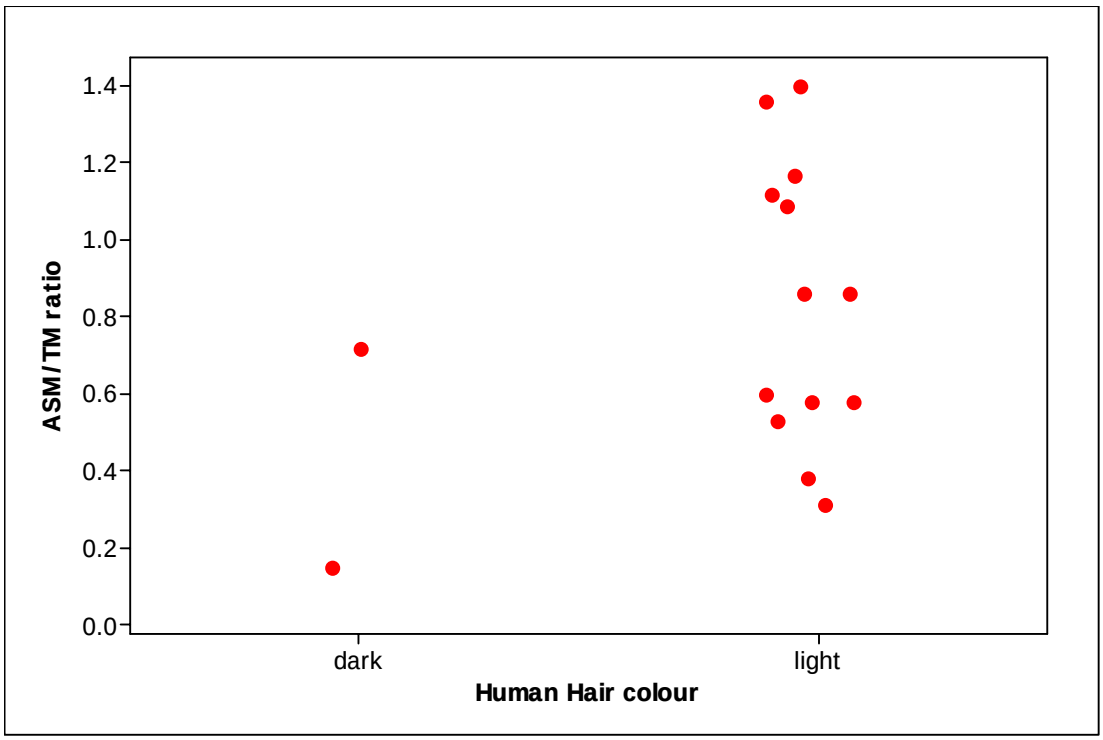
A high ASM/TM value is consistent with higher phaeomelanin content. A low ASM/TM value is consistent with higher eumelanin content.

Figure A.5.1.1. Mouse Hair Melanin Assay Results



The above figure showed a high ASM/TM value with light coloured ee mouse hair, consistent with higher phaeomelanin content. The darker BL6 and F1 mice hair gave a lower ASM/TM value, consistent with higher eumelanin content. This confirmed that the melanin assay worked well with mouse hair.

Figure A.5.1.2. Human Hair Melanin Assay Results



The above figure showed a range of ASM/TM values for light hair coloured individuals. The 2 very dark hair individuals gave varied results. The experiments were repeated by another person in the laboratory and also gave inconsistent results (data not shown). The human hair melanin assay was found to give inconsistent results and not very useful and it was not pursued further.

A.5.2. Discussion

Possible explanations for this may include different amounts of phaeomelanin and eumelanin in human vs mouse hair, technical limitation with the melanin assay e.g. "Alkaline Soluble Melanin" still contained some eumelanin, not just phaeomelanin.

Appendix 6. SNPs selected from 33 candidate gene loci for Illumina genotyping

Gene	SNP_Name	Sequence
ASIP	rs2424984	TGTTCCTGTGCCTGCCACAATGGAGATGAAGGAAGGGGCACCTGGGGTGGCTCTGAGTGGCCCAAGCTCACTTAGGTCGAGGGACCAGGCCCCAC AAGAG[C/T]GTCACAGGCAGATCCCAGTGCCTGCTTGGATTTCCCATTTGCCTTCCCTCAGAGGACACGTTGCTATCAGTGCCTGGCTCCAGGT CAGTAGCCGGGCTAA
ASIP	rs819136	TaatttagcatttctgaaacttaatgaatcacagaactCCTGTCAACAGTAACAAACTTCAGGAAATGCTCCAGAACATATGCAAGTCTGGGATG GACCAGTCCTGTCATGTCAGGGTTG[G/A]GAATGAAGGCTCAGGGGAAAATATGAGGGGCACTGGAGCCTGGCATTGGAGATCTGGTTTGACTT CACCTGATAATAATCATAGACATACTGTGTGGTAGGCActgtgaggtgagtatgg
TYRP1	rs2733832	AGGGGGACAAACCCATTTGTCTCCAAATGATCCTATTTTTGTCCTCCTGCACACCTTCACAGATGCAGTCTTTGATGAATGGCTGAGGAGATACA ATGCTGGTAAGACATTTTCATATGC[T/C]TTTTGCATGCTCAGCTGGGCGGATTGTTTAGATGGCATAGTTATCAGTTCAAGCTGAGCACTCAG CGCATAAAAACACTTTCAAAATAAGGATAGCATAGCTGTAATATCAAGTC
TYRP1	rs2209277	AGATTACTTTTATTACAGTAATGAGTAAACTCAAATTAATAGGTTTAGGAATTAACAAAACCAGCTTTTGTTAAACATGCACTTCTGGGAACTAC ATGGCAATGTTCAAGTTGTATTTAT[T/C]CAGTTATTCACAATGCCGTGGGTGTTAGTAAACTTTTTAGAGGAAATGGAGACTATTCAGTAACC TGGCAAGTCTCTTAAGTGGAGTTTTCTTTCACCTATGAAAGAAAGGAAAG
TYRP1	rs2733831	CCTATTTTTGATCATTTTAATAACTACCTGAATGCACTGATTTGAATATGATTCAATAAAAAAAATCACTATATTTTGGCTAATCTGTAAACTAA GGCCATCAGCACTGATGTTTTAAAA[G/A]ATGTACAGATTGTGTCTTCTTAATGCCCAATCAGAAGACAATTATACTTATTTCCTGACAAAATG TATAAAAAGACGAGTGTCAAGCCACGTTCCATATATATCACAGACAATCTTTAAA
TYRP1	rs11787999	AAATGTAATTAATGATGGTGACCTGCTAATTCATGCTTTTGATAACTGATATCTAGTATGTATATATATATAAACAAAATGACGAGGACAGGGAA TTTAATTATTTGGGTATCACACATG[C/T]AGGTGTTATATATGCCAAATTTTAAAGGTAAAATTTGTGTGAAATGTCACACTTTTATTATTTGT GTGAAATGTCATTTTACATATGGGTTCCATTTTGAAAGTGGTTTGGGAAGGGGGC
TYRP1	rs10809828	TGATGAAATACTAAAACTCCCCTGCTTTTAAACTCTCTTTTTTATTAAGTGGTATATTGGTACTGTATTCAAAGCATTTTCTGTTTTATGTAATT TCTCATCCTGCTGTAGTGAAACTTC[A/G]TATCTTTATTCAGTGTAAAATAAGAATAAAATTTTTTCAGGTTCTCCTTGAATATTGGATGCCTT TAGAACTCAATAATGATAGGaatattaattttattatgtttattaataCGTTGTC
TYRP1	rs2762462	TTCCTCTGTAGAAGTTTAAAACTTAGAATAAATTTGTATTTGTTTGGTATGCTGAATGCATGATCCCACCTTGTGGCAGAGAACTGAAATTGAGG ATCAGGATGTCTGCAGATGCCAAAA[T/C]GTTTATACTCTTTGTGACCAGGCCATTGTATTTTGGCCTGAGAGTTTTCTAGACATTTCCCAATC ACCCTGATGATTGGATACCAAAATAAAACACAGTTTAAATGAGTGAATGC
TYRP1	rs17346161	GCTCTTCTTTGATATCCCAAAAGACTAAAAATTTTATACCCATAATCCACAGTTCTTATTTTTATTACTTAATGGCCCCTCACAAATAGAAATTG TGTTC[C/T]ACTTGAAAAGTTAATGTTGGCTTTTATATCACACTATAGGAAATCTCTTTTAAGTTCATTTCAATGCATTCTTTAAAATACTATT TAAAAGGCCTCTTTC
TYRP1	rs2075508	AATGCCTAAATGACACCTTGTAATAAATTACCAGCTTTGTTAAATAAGGTTTTAACTCCTCTGGGCCCCTCAGACACCGTTGATATACTAACCAG TACCTTATTGTCTGAAGAGAGCTAA[T/C]AGAAATAGACTGTCAGAGAGTAGACCAAACAGAAATGAATAATTGTAAACAGAAGCAGAGAGTAT TAATGTGGTTTCTGTGATCTAGGAAATGTTGCAAGAGCCTTCTTTCTCCC
TYR	rs7112373	CacactttataaatgaggaaactgagattctgataatttatccaattgacaatgttacagagctaacaggtagaaaacatggatCATTAGCAAGG GAGATCATGTCCTCAAGTGTTCCAA[A/G]TAGAAAAGCCAAGTTTTAGATGCAGCTagagaaaagcagttaagagttggactacctgggtttaa

		attctagtactatcacttactagttaaatgacttttgtaaatttaacctgccctt
TYR	rs4396293	AAATCAAAGAAGGTATGCATCTAAAATGCTCTCTATAAAGAGTAGGGAGCATAGTGTGGTAATGAATTAAAGAGTCAGAGAGACCTCGACTGAGA TATGAGTCAGTTAACCACTCAATCA[C/T]GGAGCCTCTGCTCTATATAATGGATATCACATAATTCCACCTTTGTCAGCGTGTTGGAAGTATTT TAAATAACACTCAGGTGATACTGAATAATAAGATGCTACAATAATTATCATTCTC
TYR	rs11018542	AGACTTAATTGTAGGTGATGATAGCAGAAAAGAAAGTATTCAGAGGAAGGTCAGCTGTCTACTCTTGAAATTTTCAAGAAGAAAGGAGAAAATAA AGCATCAAATCATGACTGACAAATG[C/T]TATGGAGTGTTCTTTGTCCCCTCTACAGTAATTTTCTCACCACAGTTAATGAATAAAGGGCACAG ATTTATACCTGCCATTAAGATAATACTATCATCCTCTTGGGTAAAAATAAGAGGA
TYR	rs10830253	AGACTGGATTAGTGAATGGTTAATGATATAACGGTGTTCTATTGATAATCGGAATAGTTACAACATCCTTTCACAGAAAAAGATGGAAAaatcac agctaacatttaattgagtacacac[T/G]atataccaggccctgaatcacttacggatgttatctataaaattcaaacgttccaacaagagggg tattattttcccatttttctgatgaagaaactgaggctttggagtattaggtgta
TYR	rs16913107	AGAAACTAGTTTGAGAATCCAGAAGAGAATGGAGCTGAGTCAGCAGCTGAAGCAAGTTTGGAAAGAAAAACTCAAATTGTAAGCTGATTTAGGTG AAATA[T/C]TGATGAAATTATCATTCTATCATGATAAGAATCAACAGCAGTTGTAAATTGTTTTCTATATGGTAGACATTGTACCAAGGGCTAT ACATACATTGTCTAG
TYR	rs591260	AAAGTTTAAGAAAGAAAGGCAGTTTCGGTGTTTTTTTCTCTCCTTTTCCAAAAAACATTTAATATCATTAAGTATCAGGCACCCTGCTATGAATA ATTCAATGTGCATCCAGGATTGAGA[G/T]ACACTGCATTAGGCAGAAATTGCACTCAAATAAAATGAGCCAGGCCACACTGGCCACAGTAGTCA AGATGAATCTAGAAAATGTCTCCTAACAGACCTCCAGACAATGCTGGGTGTAAAT
TYR	rs12421746	GTCAGGTAAAAAAAGTCAGGAGAAAAAGCTTCAAGATTCACATGATGGGGTATTCTTTTACCAAACCAGGAATCAATTGCTTTGGAGAACGGAAA TAACATTTTTGAAATCCCTTAACAC[C/T]AATGATCCCTGTTCTGTGAGGTGCCTTGATGCTAATCAGACAGTTGCTCATATCAGTTAAATCAT TGAAATGTAATTACCTCTAGTACTCAGTGTTGAGGTATGGAGGTGAGGGAAGCAG
TYR	rs621313	AAGTTGATAATTGTATCAATTTCAGTTCAGGGCTTACTGACTTGACACACTTTTTTTTTTTTCTAGAGAGTATTAAAATTGAACACATTCATGAA GAGAGAAAAAGGAAAAAGTTCTGCT[T/C]GACAATTAATAAGTAATTAGGAAAATAATACTCATACAGTTGTGGATAGCATCTGGTATTACACA TCTCATGTAATTAAAAATTAATGTGTATCTATCTATTAAGATTTCCTGTGGATGT
TYR	rs12807810	GTTTCTGGAGACAGATAGGATTTCAGGACAAGTTTCAAAAATGAGGTGACAGCAGGAACAAAGGTATCTTCTGTTACCCAAAAGGGAAAAAGGAG ACAGGAACAAGGAACAACTTCTTGG[C/T]GTTTAATGGtgtgtttccaattctgagcacagtggctgcctatagtaggtgctcattaagtattg gctgacagactgaTAAATCAATAAATAAAAGAAAACTACCTTCATTGTAAAACTT
TYR	rs17793678	TATTAGACTGTAAACTCTATAAAAATAAAAACTATGTTTATCGTGTTCAGTATGTATTCTTGTATTCTGGTATAATGCCTGACACATAGACATTT TAAAA[C/T]TTCGGTTTGAAAAGCAGCAACAATAAATCAATCATTCTATGAGAGTTCTTTTGATTACATAGTTTGTTAGAAGGTGCTAAGTGCA CAGATAAAGCAGAAA
TYR	rs1827430	GCTTAACATAGGCCATTTTGTACATGGCAACCATGTGAAGAGCAGTAGAATCAGAAGAAGAAAAAAAAAGGTTTTGAGACATGACTCTATCAACT GACTGTAAGGTGACCTGGGAAATTC[A/G]CTCTACATCCCTGAATCTCAGTTTATTCACCTGAAATACTGGGACCAGAACACATTAAAGAATTA TTTAGAATGATACATTAATGAGCCTAGTACAGTGTAACACAGGGTAAACATCCAG
TYR	rs12419351	tctgtaaaatggaatcaataaAATATTAAGCAAGATATTgttcaaaggaaaactttagccaaattaaatttaaaagagtttaattgagcaaagaa caatttatgaaccaggtagcttcct[C/G]agctagagtaggctcagattctctagcacagccacatggtggaagaagattaatgtacagaaaaa gaaaaataacgttaaaaaaatggaagtgaggtacagaaacagtcagattggttat
TYR	rs17191474	CTGTTTACAACTGTTTATATTAATCTGGTATGTATTACAAAGAATCTTGGTTCAACCTGGGCCTTTACATTATTTCTAAGCACATATCACAATGT

		ATTCC[G/A]ACAAATTCCTTGTGCTTTTTCTTTAGCTGTTTTTTTTTTTTTCTTAATAATATTAGGGGCTTTTCTAGATGCAACATCTGTCAGG TCAGTATAGTAGTGA
TYR	rs12421402	gttttgacattgcaaatcctctgaaagagtttcagggacttcaaggggtctgttggccccattttgTTAAGCAATAAAATATTGGGATTGTAGAA TAATTTCTTTTTTAAAAATAAGGGC[A/G]TCTGGGAAGACCTTATTAATTTCAAATACAATTCTGCATTTTCAGGATGAAATGTCAAAGAATGT TGATATCTAAAGGCACATTTGCGAAATCCAAAAAGACATGGTGGCTAACaacgta
TYR	rs4121401	AGTTTAGGTGCAAACCTGAAACAATACATTCCAGGAATGGCCAGCCTGCCTCCCTCCTTAGCTACAGATTAATTACATCTTCTGGAGTGACATTT TGGCTGTATTTTCCAGTGAGGCTTC[C/T]CTTCTACTCTACTCCCCAAGCCTCCCCTTGGCCATCCTGCTAAGAAGACTGTCCTAACCCACATG CCTTCAA
TYR	rs1042602	ATGAAAAATGGATCAACACCCATGTTTAACGACATCAATATTTATGACCTCTTTGTCTGGATGCATTATTATGTGTCAATGGATGCACTGCTTGG GGGAT[C/A]TGAAATCTGGAGAGACATTGATTTTGCCCATGAAGCACCAGCTTTTCTGCCTTGGCATAGACTCTTCTTGTTGCGGTGGGAACAA GAAATCCAGAAGCTG
SOX10	rs139884	ACCAGGTGGTGAGACCGTGGGCAGAGCCACGCCTGGTGGCTTGGAGATCCAGGCGGAGTGTCCACTGGCCACGGCCAGGGCACTGCCCAGCCCAT AGCCGGCTGCTGAGTAGCTGCTCAC[A/G]TGGCCTGGGTGCCCATTGGGCGGCAGGTACTGGTCCAACTCAGCCACATCAAAGGTCTCCATGTT GGACATTACCTCGTGGCTGATCTCACCAATGTCCACGTTGCCGAAGTCGATGTGA
GPR143	rs2521578	TCCCCATGTGAAAGCCCATAACCTGTGTTGCGGCTGGAGCAAGTACTCTAGGATATTTGGGGATTGGAAGAATAATTCTGACATTGGTTCCAAAG GGTCTCACAGATTCTGAAGGAAGAC[G/A]TGTTGCTGGAATtgatgcaggattttattcttggtcactttgcaagctggggacctctggctggt gatgccccgcccaggcctcgctcggccatgctacctgttgcaggagatggcctgc
GPR143	rs5979164	TGGGAGAAATACAGAATCGGGGAAAATACAGGGTATTGTGGGAGATACACGGAATTGTGGGAGAGACAGAGAATTATGGGAGAAACAGAATTGTG GAGAGTAGAGAGGATTGATGGTCAA[A/G]CCAAGAATGGCGGGGTGGGTGTCATATGGAATTGGGGGAGATAAAAGGCATTGTGGGAGATACAG CTACGCTCCTTTCTGGCCATGTTTCTGGGCTCTGAATGGGCCTCACCCCACCTGT
GPR143	rs11796366	AAAGATGGAAGGAACAAGCATAAAAATGAAGTAAGCACTAAACTTCAGGCCAGAGAGACCAAAGTTGATGATGTGATCTACATAAGGAATGTCAA TGATTTTAGAGCGGGGATCTACTGG[A/G]ATTACCCAATATATTCTATAGCCTACATATGATCTCTATTTACTGATATTGGCTTTAAAAGTAAA TTTATTCAATAAATGGTGCTGGRACAACTGGATATCCACATGCAAAATAATGAAG
GPR143	rs5979167	GTCTGAAGGGTATGATGAACAAGTTGAGCTTTATATCCACTGTCTACATAGGGAGAAGCCCATGGCTAAGTTTTTTCTGTATCTGCCATCGTAGT AATCAGGTGCAGCCAATAGGAAAGC[A/G]GCCCTGGTAAGGGGTTTTCCTTGACTTTACGCACAGAAGTAGTATCCGATGCACACTTAACTTAC CCAAATTCACCTCTACTTAGTGAGGGGCTGGGGAGGTATGGCAGGGAGAGTAGGT
OCA2	rs1900758	ACCATAAGGTACGCAAAGCACCTCTGCCGTGGGAGTTGCGGCCAGGTTCTGGCAGGCAGGGGCTCTGCCTGCACTGCCTGGCTCCAGGTTCCATT CTCAGGTGCATGAAAAGGTGGGGGC[A/G]GTTGAGCCCACAGCTCACTGCATTCCAGTCCAGCTCGTGTCTGCTTTGTGTGACTGCAGTACATG CTACAAGCAGTGGGGCCTCAGAAGCTGGTGGCAGAAATGCCTGCAGGAGGTGGAA
OCA2	rs1800414	CTCGTGAAATCTGTCGTGATTCCAGTTGCGTAGGTTATGACACGCTGCAGGAGTCAGAAGGTTGTGCAGAGTAAATGAGCTGTGGTTTCTCTCTT ACAGC[G/A]TAGGATATCTGACGGGATTCTGCTCGCCAAATGCCTGACAGTGTTGGGATTTGTTATCTTCATGTTTTTCCTCAATTCGTTTGTC CCTGGCATTCATCTT
OCA2	rs1800411	ggaaagtgctgggattacaggcgtgagccaccatgcctggGCTGCCATTTCATTTCCCCTTGTTTATTTCCAGGGCCTGGACTTTGCCGGATTCA CTGCACACATGTTCATTGGGATTTG[T/C]CTTGTTCTCCTGGTCTGCTTTCCGCTCCTCAGACTCCTTTACTGGAACAGAAAGCTTTATAACAA GGAACCCAGTGAGATTGTTGGTGAGTACAAGTGCAACCTCATGTAGGCTCAGATT

OCA2	rs1800404	CACAGTGGCAGATATAGACCCCCTCATGTCCACACAGGCTTTCGTGTGTGCTAACTCCCTCGTGCACTGGAACGCGGTAATTTCCTGTGCTTCTT TCCAGATCGTGCACAGAACTCTGGC[A/G]GCCATGCTGGGTTCCCTTGCAGCACTGGCAGCACTGGCTGTGATTGGCGATGTAAGTTGTCACAG TCCCAATCCCTGGCTTACCACTCAGTGGGATGTCAGCTCAAAGATGTTCCAGGAT
OCA2	rs3903042	GGCCACAGCCGGGACATTTGACAACAGCCGCAAGAGTTCTCCAATTTGGAA[C/T]TTCACCAAAAAAATAACAGCCACCGAGTTAGTTCAGGAT TACCACACCCAGGCCCTGGAAGGAGAGCAACAGCCAAGCAACTAGAGGGCCAACCTAGGCAGAAACTAAATTCCAGGCTGC
OCA2	rs7497656	tgccagggctccacttcacaatacagaaacctggcagacaccacatgagccaagtgatcgatgttaacactttcagtaataggacatgccaatgt ttgtaccccaatatgatgcactaga[C/A]aaggcacatcacctctgtgatctcgccaaaacttcatagcctcaaaactgtcatgagaaaacatg agacaaacccaaactgagaaatctctacaaggtaactaactaatacttttcaaaa
OCA2	rs6497235	atcaaattttacattttaagtatttgtaatttattgtatgtcaattatatctcaacaagactgtttaaaaaaaTAAAAAGAGACTATGGCAGAAT ATAGTAAAGTGAGAAAAACCACACC[A/G]AAAGGAATTTATGGGTTCATTCTGAAAAAATCGCTAACAAACCACAAGTACAATTTTCACCCAGG AATTGTGAGAAGAACAGCAAACTGCGGCGTTCTACCCTTGTCTACTATGAAGTGC
OCA2	rs6497233	ATTAGTCAGGTTAAGAAAAGAAGATGAAAATGTCTTTTAAGATGGGAGGTGGGGTCAGAGGGGAGAGCTTGGAGGAAGAGGAAGGGCCAGGCTAT GACCAATCGTCTTGCTTCCTCTTCT[T/C]GGCTCACAGGAGGCTGACCGGAGCTCTTGTGAGCTCGGTTCTGCCTAAGAGAGGTGGGAATAACA AACATCCAATTTTTTGGTGATACTTTTTCCCCAAAGGTACCAACAAATTCTACCG
OCA2	rs6497249	aagttgtccttttggggaaaaaaatgctttagttatttgaatttagtcctttgaatgtctacataaattgtgtaattagcttgtcattctctgca gaaaaacctagttcaatttttatta[C/G]aattgcacttaaatctgtaagtcaatgtgggaagaactgcacactaaacaacactgagtcttctt atctatggacagactatatctcttcattagatctttagatcttttacaaaaattt
OCA2	rs1375166	TGCAGACCTGAAACTCCTCTCCACAGGAAGAGACATCTGGGCAGCCCAGCCTGGGGATATCCCTTCCAGCCCCCAAGGCAGCACCCAGCAGCACT GGAGAAACCAAACAGATCAAAGTAG[C/T]ACTGCAAGATCTCTGAAAATTAAATCTTCATTTGAACAACAGGCTATGTAAATAGTCTATAACCT GTGTGCTAAACTTAAACAGGTTGACTgcctgctgaaataaaacagttatttatga
OCA2	rs7175568	gtgtatgtatgtgtatgtgtgtatgcatatatacatgtataAAtgtgtgtggatgcatataatcatgtgtgcatgcatgtacacatgtataactg tgtgggtatgtatgcatgcatgtat[G/A]aagtgtgtgtttatgtgtgtgcatagctatgtacatgtgcgtgCTGGTCCTCTGGAGATGGCCAA ATGGCTCCTGCAGGCACAGCAGCATGTTTGTGGCCAAGAGGAGCCCCACCTCAAC
OCA2	rs11638265	CCCCATCCCCAGTGCCACTCCTTCCGAGCTGCACAACCCTGGGCACGCCGCTCAAATTCTCTGAGCCTCTGGTTTTGTGTTGTTTCTTTGGTCCT TAAACTCGGCTGTGTACCCCCTGCA[G/A]AGCTCAGTGAGGGTTAGATAAAATGTACTATAAGAGGCTTAGCACAGTGTGCGTCACCTAAATAT CACGTATTAGTATACAGCTAATGTCGCTATTTTGTAGGCCCATGGAATGTTCTGC
OCA2	rs12592159	TGATCTAAAGGGCTGGCTCTGAAAGATGGAATTTCAGCCTTTGCAGAAGTAAAATGTGGGTCAACAGGAATTGGGCCTCAGATGCTGGACATCAG AGTCCTCAAGAATCCCCTGCAATGC[C/T]CCATCTTCTTGCTAGTTACCTGTGTGTATGGCAATCCCTGGAGGCAAAGGGGGCTGATTTTATAG AGCACAAACCGTACCCTCCAGACACAGAGAAGAAATTTAGAATTGATGTTGGCTG
OCA2	rs11074317	TAgatatatatatacatacatacatttcagggtgcatatgataatgtaataattcacataatcaaaccaggtaactaggataaccatcccctta aatatcaatctgttctttaccctgg[G/A]agcattccagttattctcttctagctatcttggagtgtacaatcaattcatgctaactgtattca ccctacAGATCTGTCTAAGCCACATTCTACAGCTGTGGAGCTCCACCATGGCCTC
OCA2	rs7176632	aggatcacttgaggccagcagttggggaagatcagcttTGAAGTCTTTTCTGTCCATGCTGCTCCTGCCGGGGTGGTCTCCTCCCGCCTGTTTCA GCCCCTTCAGTGGCTCCCACTTGCC[T/C]CTTCTTTTTCATGTTTCTGACTCAGCCCACCCCTCCTGGCTGACTTCCTCCCAAACCAATTAGGG AAAAGGGGCTGCACAATGAAGAATGCAGTTTTAGTATCAAAAGAGGCTGACCACA

OCA2	rs4778232	TCCACCATTGTCAAGAACCCTTGCTACCATGATTATTAATAAGACAGGTTCATGTCAGACTGTGAGATGGAAACAGTTTCTTGCCCATGCCTCTG CCTCTTCTGCCCTCTTCTTCAACAG[C/T]TTCCTCATCCCTAGATCCCTTGGTTCTTTCTAGATCAAAACACTTAACAATCCTATGATGCTTAT ATCATCATATTGACAAAAAATGAGCTAAAAAATGTTGAAAATATTGAGTACCTAA
OCA2	rs1498509	gatttgtggtttgatatcattaattttgacaaattcttggctatttttttctgcccatttctctctctctctctctctctctgtctctctctttct gcttcttagattctgattacacatg[T/C]tggatgttttgatgttattccacaattcatgaaagctctgctggttgtttttttctgttcatgtt tcagtttgggcaatttttattgttctattttcaagttcactaattactttctgag
OCA2	rs1562592	GTTCCAATTAACTGTGTCATTCACTAGCAATGGAATACCTAATTTCCACAAATGCACATCATAAACATCACCGAAAAACTGCTTTTTATTATTAA AAGTTTGAGAAGTGAATGGCCACAG[C/T]ACTATTCTCTCATAATTTATTTAAATTCTTCTTCAGATTATTAATGTTGAGGAATTTAAATGTGA CCACAGTGTAGCTTCAGACAAGCAAGTGAAAATGTTGACACAACCTAACTCTTCT
OCA2	rs3794604	GACCAGTGGGCTTCCATTCTGGATCCGACCTGAAGTCGCTGAGCGCCCATCGCAGGGCCTTGCCTGGCCACGCGACGGTACCCTGTCACGCGATG CCCTCCTGGCTTTGTGGCCTCTCAC[A/G]GGCCTACACTAAGATAATATGTGTGAAAGCCCTAGAAAAGTGAAAAAACACTACTTAGTCATCAA ATATTAGCGTCACCGTGTCTCTGTGTGTGGGGACCAGTCTTTTCTGAAGATGCTC
OCA2	rs2594937	TCTTAAGGGTAATGACCAATGAAAAAATTGTAAGTAGTAATCATTCGATGTGAGAATGTTAACAATCTCTTGATGAATGCATATCTAACACCTTA CTCTCCCACCCATAACTCACAGATA[T/C]TTTTATTCAAATAGAGACCACTAACATTTCTGAAAATCCACAGTTTAAAAGCTAATGTGGTACTC CGTCAAAATTATCCAACCTTTTAAAAATCTCGCAGTCTTACTGTTATATGCAGAC
OCA2	rs11858340	ctgacagcgcacgttcccatcaagaagCTCCTTCCCCTGTTTCTAGGGCCTGTGATGCAGGGAGAAGTCTGAGGCCCCCACCGCCATCCCAGCAG TGCTCTTTGGGCCCCCACAACCTGG[A/G]CAGGAGACATAAGTGAGCAGCCTCCAGATGATTCTGGCCTTGGCCTCTGCCAGCTCTGGTGATCC CCAGTGGAATAGTACCAGCTGCCCTTTCAAATTCCATGAAGATGGTAGAGTTAGG
OCA2	rs11631195	CTCTGAGGCCTGCGGAGGCTCTCTCTCCTGTGCTTCTCTGAGGATGTCGCTCCGCCCTACTCAAGGAGTACTAGACCCCTCTGTCTGCTGAGGTC AGCATCCCAGAACACGGCTCCAGCT[G/A]ACGATTGCACTTGGCACCCATATCCTCCGTGCGACAACCTGGTGTCAGCAGTCTCGGAGCAGAGG TCTCCTCTGCACCCTCTTTAATCCAGCAGCGTTCCTCAGAGCTCAGGAGCCACTC
OCA2	rs728404	GTGTGAGTGGATGGGGAAAAATGTTTTCTGGATACAGCAGGCTGATTTTGTGAGGATGTGGAAAGCAAACATCTTTATAGAGCCTTTCCCTGTCC CTGCACGTTGCAGGGCCCGCCCTCT[A/G]AGCGGGTGTCCCTGACCACCAGCCCGCTCCTGGCCCTGAAGGGCAGGCCCAAGGTTCACACTTTG CGGAGGGGAGACCGGCAAGGCATGCTGCATGAAGTGGAGCAGCTTTAGGGGCAGA
OCA2	rs1470608	ATTAATATCCATTTATATTCAATATTTCTTGTGTTAACTGTCCTTACAAATTGTCTGGAAGCATTTTAAAAACAGTTTGTAGACATTCTCTTAAA AATATTAATTTGCACC[A/C]TCTGTAATGAAACTAGTTCTTCGAACAAAGGTTACCATCAACCCTAACATATTTTCCAATTAGGGGGCACTGCT AGGATTGGTGGAGTATAAACGAATAAAATTTTATTATGTTGGCAGA
OCA2	rs2055291	CCACATCCTGGGCGAGCGGCTCCATGCGCTCCCAGGGCCTCGTGCTGGGGTTAGTGCCATGCTGTCGCCGCCTGGAGATTCGGGGTTGGTTTCCG TTTTACACCGGGTCCCCGCGGTGTG[C/T]AGCCATCTGCCTGCTCAGGCCCAGCGAAGCATCTGGAACACCCAGCCGCTTTTCCTGTCGCTGCC TCGCAGCCCAGCCCAGGCCTCCTTCGCGGTTCCCTGCCTGCGGGAGCAGCCCCTA
OCA2	rs7173419	GAGTCTAAGGGGCCAGAGATGCATCTCTCTCTCCCTCCCTCCTGCCCAGGAAACCAAAAGGAAAGGCGCCCTTCTTCCCAGGGCAGGCTGCCCAC CCTCCATCTCAGCCCTCTCTGGCCT[T/C]CCACCAGCCCGGCTGCCTGTGGGCAAAGTCAGTGTCTGGGAACAGGCTCTGAAACCTTCCCATCC AGAATGTGACAAAGCCTATGAACCAAAGCTAAATTAGACTCACCAAGATCAAGAT
OCA2	rs4778129	ccatgttgacctgtaagtccattaaacctctttcctttgtaaattgcccagtctggggtatgtctttattagcagcatgaaaatggactaataca GGAGCCCAGGAAAATGGGAACAGCA[T/C]AGCTGCAGGGAGTATTTCATCCCTCAAAATCAGCATTTCCAGACCAACTCCCAATATAGCAAGAG

		AAGACAGCTAGGTGGGCTTGTTCCTCCCTTGGATGAAAATATATCAGGGGAACAT
OCA2	rs2594906	CTTTCTTTTTGTTTTGtttttgttgttgttttttttgagacggagtcttgctctgttgccccggAGGCACTTTATATTACTGAGAAGAAAACCCTG GAAAGTCCCGGGCTTCCCCCAGGAG[G/C]TTGGAACTCCCCTCGCCCCCTGCTTCCTGCCCTCCATCCCCCAGGAGCGGGTCCCTGCGGCCTAG CGGGAGTGGAGAGCGAGGCTTAGGTCCTTAGTGATGGCTTCAAGGCCTGGGAACC
OCA2	rs1448490	TTTCTAACCTCTACTTTTAAAAAATTAATTGAAATGTTGGCCATGTTTATGATGAGTATTATTCACCACTCCAAACAAGACTCTGCTAACATTTG TACTCAATTTCTGGTACCCTCGGGG[A/C]CTAATAAATGCAGTGATATGTAAAATATGAATATTATGCTCCCTAGATATCATGAAGGAGTAGAA TTAAAATTgtgagtgcttaaaatgatgtcatctcattgtggttaaattctgcatt
OCA2	rs12324648	caggtggggagaaaaacaaatgaaaggcatccaaactggaaaggaagaagtaaaattatctctatttgtagataacacaactttatgtgtagaaa actctaaagactccatttttaaaat[C/A]attaggattaataaattcagcataactgaaggatacaaaatcaacacacaatggtatattcacac aatggaatttcacacaatagtggaaataaatgaactacagctaaacaaatcaata
OCA2	rs11637518	ATTTTATATCCTGGCTTCATCATCTGCTTGCAGGGTTACCATGAGCCAGCAAAGAATAACACTCACACTGAAAGGCATTTTGCACATGCCACAAG ATCCCTGAAAGAGCCCTTATCTCAT[A/G]AAGATGCACACAGCAGGGCTTCCATGAATGAAAATCTCCCCTTCCATTCCAAAGACAACTCAAGG TAAACTGCTCCCTTTTTACAGACCAGGAGCCAACAAACTACAGCCTCAGGCCAAA
OCA2	rs2703958	gctcttgtcttaattgattgaccaaccttacaacattccgctatgacttgttcctgcgctgtcccaactgatggatccatcgacctcatgacatt cttcttctggacaatgagtcttatg[A/T]tctctccagcatgcacgttgtgactcccgccctggcctgcaagagaaaaacccccttaactgta actttccactgcttaccccagtcctataaaactgccccatccctaactcccttcg
OCA2	rs13329466	TCCAGGACAGCTCTCCTCAGGCAATTCCAGAACCACCCTGATTTGTGCCCAAAAAGGACCTGCAAGTTTGCAGGAAATTGAGTTTTCTTGTGAAA CACATATAcagggtttctcaacttc[G/A]tcactactgacatttgtgctggataattctttgtggtgggggctgtcctgtgcattgcagaatgt ttggagcatcccttgcctctacccttagatgccagaagcatctcagccctcagtg
OCA2	rs989869	ggattctcaccactcttattcaacatagtactgaaagcccaagtcagtataataaggcaaaagacacatagactaaaatagatgaaataaaattc tctctatctgcagatgacatgatag[C/T]ttacattaaaaaatcctaagaaattgaaaaaaaaagcaaaacaaaacatttctaaaactaatcag tgagcagtaagttcagcaaggactgcaacacaagatcaacattcaaaccttattt
OCA2	rs16950413	GTTTACAGGCGAGCATTGGCCCCACCCTGGCCAACCCTGAGCAGGGTGGCTCTAGCAACCCCCTTCAAAAAGTGGGGTCCTTGTCACTACTGCTG GACTA[C/T]GCTCTGGGCTCAAGTGAGACTAAGGGAGAGCAGGAAGGGTCCTACTTAACATTCTTAAAACAGGTTATACTTAATAAACGCACAG AAAAAAACGCTAGAG
OCA2	rs2311470	TCCCTTTGAATCACACTGAACAAAGCCAGGTCATTTTCAAATATTGCTTCGAATACAGTATTTTCTAGATGGTTTATTCTCTAGATATTTTTTTC ACTTTTTCATTTAATTTATTAACTT[C/G]ATGTTTTTAAAGCCTCACAATAATTAGCTATGTTCTAAGAAACCACAAAAGAAAACAAAATGAGG TGACAAAAGCTTTACTGGAAGCTAAATTCCACAATAAAATTCAACTTAATGTGTC
OCA2	rs2594938	GCATGATGCTCAGGAAGGGAGATTCAAATGGGCAAAAATGCTCCAGAGTTAGGAACAAATCTTCATAGCAAAAGAAGGCTGTTGTTAATGTAAAT CACAGCTGAAATTTCCAAACAGATG[G/C]AAAAAACAAGTTCACATGTTATTTAGTAaaagagcatttcataaatggccaataagcacatgaaa agatgctcaatgtcattcatcactaggaaatgcaaatcaaagctgtaatgaagta
OCA2	rs1603784	acagatgtcgaaattatctgaaaaggatttcaaagcagctaccataaaaattcttcaacaagcaatgacaaacacacttgaaataagtgaaaaaa aatggaaagtctcagacaagaaatt[A/G]aaactataaaggaggaccacatggaaatttttcagttcaactgaagtaaaactctaacggaatgg gcacaataatataatggagagtataaaggaaagaatcggtgaacttgaagataca
OCA2	rs17651026	TTATTGCCACTGTTTATTTCCTTTTTAATTCAGTCATCTTGTATCAAACTGAAGTCCACCCAGAACTTGTTTCCCAATAAACAGGCTTGTAGACT

		GTCAC[C/G]AAGTCCCTCAAATCTGTTCACTGAATGCTGGTGTCACCTCACCCCACTGTGGGGGGCCAGTGAAGGCCCCATCGAGTGCATTCCA GATGATGTCTCTGCT
OCA2	rs7495174	GCGACTTTTTCCTGGGTCGCCTGGCAGAGAGCCGGAGGGGGTGCTCAGGGGGCAGCTGCTGCAGGCCCAGGCGGACTCAGCCGCGCTAGGTCGGC TCCGTCGCACCCGTCTGTGCACACT[A/G]ACCTTTAGGGGAACTTGCCTTGCTTCCTAAGCCGCGACTCAGCCGGGGCTGTGTAAACCCTCCCT GCCTGTTCCCAGGCTAGTCTGGCTCCTCGCCTCCTTCCTCCCGACCGCGGAGCAC
OCA2	rs977589	TCTCCTAACCAGAATGTTACTTTTCTTTGAATTCTCCAGGGCACGTACCACACAGTTCAGAAGACAAACAGACACTTGCTTATCTGATCATCACA TCTGATGGTGATTCCTTCACCGCTC[C/T]TCTAACACAGATCACCACATCTTTTCTTTAAATTCTCAGTAAGAAATAATTGCTCCAATTTCACT TCTCCTGGGTAAAGCTTTTTGACCCCAGAAATGGCATCCCTTGTTTTCTCTGCTA
OCA2	rs7496968	attaggggaaactgtcatcttaattatgttgttttccaatccaggaacattgaaggctctctacttatctaaatcttctttaagttctctcagc aatattttgtgttgttcaattataa[A/G]tcttgtacttttattaaatttaatcctaaaaatgtgattcttcttaatgattttgtgaaacagt tttcttaattttattttcagattattcattactaacatataaagacaattagttt
OCA2	rs17651375	GGTCTTTGCTAGCAGGGACTGTGCAATCCAGCACCGTCTTCGTGGACAGTCAGGGCACAAGCTCCCACTGGAATATTCAAGAGGACAGCCACACG AAATA[T/C]CTGGCAAGCCCACAGTCATTTCCTGGCCCCCTGACTCAACCTGAGTCATCCAGGATATCTGAACCATCCTCTGTGTTATGGAGCT AATCCCAGCAATTTT
OCA2	rs7174027	ttttagacagtggctccactacctggtaagaacaaagtacaaatgtacataccagctataaaaaagttgtctgatttcacaaattccacgcatga gctaaatgtttcaatatttgcattt[G/A]aaactgatgtggcatgatataaagatgactagtaaaagtaatgctaacaatttaaaatttaaatt tttttatttagaataatgacattaaataccaatttaaaagcaccatgacaagcca
OCA2	rs6497268	gagacaacaccgccagctgcctaggactcgagagggtggttcactcctgcatcaccaatgggaatgtaaaattgtacagccactctggaaagcag tttgacagttttaaaaaaaaacta[A/C]aattgcaactaccagccaacaattgcactcctggacattaatcccagagaaatgaaactttcatt cacacaaaaccctacacgcaaatggtcatagaagctatatgaaaaattggaagcc
OCA2	rs17746978	GCAGGTTGAGAGATCTGCATCACAATTGGATCTTAAGAAAGCCAAACAATGTTTTTCATTAAACAAGAGAGCTCTTAATAAGTAGCTTTTAAAAA CATGA[T/C]TATTCTCATTATTCAACAGAATGGAAGCCACTTTCTTTGCAATCGTCAGGCAAACTCCAGAGGAACAGGGGAGACGATCGCTTCT GCATTTGCAAAGATG
OCA2	rs977588	atcacatactttatatgcatacacaATTTTATGTGATATACATATATATCACACAAGCCTTGCCATTTTAACCATATATTTTTTTAAGCATATGA TATATTATTTTTCTTACTACAACAG[C/A]AACATTTTAAAAAGGAAGATGGGGTTGGGAAGGACATTGTCATTTAGCATGTGAAGGGCAGACCA TGTTTTATATCATGCAAAAATGTTGGGACCCCAGTCTTAGACAGGGAGAAGTCAA
OCA2	rs2305252	GAGCTCTCCGAGTGAGGAAGGGGGACAGCAGGGCACATTCCAGCCAGGACCTGGCTTTCCCTAGCTCCCCGATACTCCTTTCCTCCCCCGTCTCT TTTGC[A/G]TTCAGCGTGACCCATCTTCCAGTCCCTGCTGTCCTGGCTGTGTCACCTTGCTCTGATGACTGCTGTGGCACCTCCTACTGTGCTT GCTCTGGCCTTCTTG
OCA2	rs3947367	TGGATCTTCTGGGAAATGGAGGCTCCCGGTGAACCCGCAGCATCCGTGTCCTGTGTATCTGGAGTCGTCCTGGCAGCGACAGGAAGTCATAACAT CCTCTCTTCAAATAAATCTCCCTCT[A/G]GCTGCCTCCCGGCTGGGCAGCGCATGAAGCATTGAAGCTCCAATCAAGCTGCTTGCATCCCACGG GAGGAAGCAACTGGCTCGGCCCCAGAAGAAACAGGCCAGCCTTTGAAGTGCCAGG
OCA2	rs746861	AAGGACTACAAAAACCAAGACTATGCAACAGATTACAGCAACTGCAAGGAAAGCAAGCCAGTTTCCTAAAATTTCAGTTCACTGAGGCTATCAAG TAAGTAGACTAAAGAAAAAAACACT[C/T]GCCATTTAGCTAACTGAATTAATTCCTTTTTAAATAATAATCATCAAAAATATTTGCCTGATCAG AGCACTGCATCGGAGGGTCCCACCTCTCCAGGACCCTCCACGGATGCCCGCCGCA

OCA2	rs1597196	ccgaatatgatgaaccttgaaaacatcatgctgaatgaataagccaggaacaaaaggacaaatgctgcatgagttcacgtgtatgaaatatctac aaaaggtaaatacagagacagaaag[G/T]aaattgcaggttccagagcctgtggggaggaaggcacggagagttattgcttaatgagcacagag ttcatagttggagtgatgaaaaaattttagaatttgtgttgatggttgcccatta
OCA2	rs749846	CACTCACCCAAAATAACTCCGCAAAGCACAGGCACAACCCATCATGGTCACTGACCACAACCTACACAGCTcacacaggtgcacacacagagaca tacagcaggcacaggggaccacaca[A/C]aacctacacagttcacactggtgcacacacacagacatagagcaggcacaggggaccatacaccc acgtgcagacacgaagacacacagccacagacaggagaccatatactcacctgca
OCA2	rs2122005	TGCCTTGACTTCTGCAGGCATTAGTTGGAGGGTTCATGTAAATCTAGTGAGAATCTGGCTGTGTTGAGGTTTGCGGTTGCTGTGGCAACTGCGGT GCACTAGACACTCAGAATTACCGGG[T/C]GCCCTGCCAGCTGTCTGCCAGACCTTGCTCTATGCCCACCCCTCCCACTGGGGTAAGAGCTACTT TTTCCCCTCCTACCTTCTCATGGCTGCAGGAATTCCCACTGCGCCCTCTGGCTGC
OCA2	rs11074306	AGCAGTGAACACGTAGAGGTCTCTGTGCTCAGGAGCCTTAACCCTAGTTGAGACAGACTGACAATGAACAAATGCATATACACTATATGAGGTAG AAAGCAGTATAGAGAAAAATAGGGA[G/A]GAAAGGAGTGCAAGAGTCCTACTACAATTCGAGTGGTCAAAATTGCCTGTCTGAAAAAGGCAATG TCCCTTACAAACATAGACATAAAATATCTTTTAAGCACTTTGGGAGGCCAAGGCA
OCA2	rs12442147	CCTGCTCAAGATTTCCCTCTTGTCATCCTCTTTGAAAATTTTGGGATCCTAGAATTATTGGGAACAACTCTAGATCTGGAACAATCCAAAGATCA CTGGACTCCCCTCTCCGAAAAAAAT[T/C]TGCCAGCACACACTCCCATGCCTGCCTGTGCATCCCTCTCTTTGGTCAGGCCCTGACACATGCCC TGGCCTATATCCCAGGCCATCTCACAAACTTCAGACTCCCCAAAACACCATTCTC
OCA2	rs17674017	CAAGTTGGCACTACAACCAAACGGGCCCCCCACTCTCACCCTCTCTCTCAGCTGATGCTCCGCCACAGACCAGCATCAGCTCTTCAACAACCATC TCTCT[A/G]GTTCTCCATATCATTAAGGATCTTTTTGAAACAATTATCAGTTCAAGTTTCCAAGACCTGAGAGCAGGGTAGGCTGTCAGAAGCA GAGCTCCAAGGGGTG
OCA2	rs1448489	GAACACAAGTTCTTTTCCATGATTCTGAGAGAAATTTCAGTCATGACAAAAAGCTGGAAGGTCCTTTAGGGCTCTTTTTATAGTTCTGCTTTCTC TTATCAATTGTGGAGGCCTGAGCTT[C/T]CCTCCAGCCAAAACTCCTGCCATAGCACTCATCCATCCAACTTCAGAAAGTTTCCATAGTCACGG AGATTTGTCTTGTTAGAAAAATTCCCTGCAATAGCCTACAATCGAATGAAGACCT
OCA2	rs2871875	GAGTGGTGCTTTCTCCAGAAGCTCCTCCCCTCACAGCAGACACCCTGGGTGTCCTTCACCCAGCCTCCTCCACAGGGCAGGCCAGAGGGCCGGGA GGAGGCCTCGAGCTCCTTGCTCAGA[A/G]GACAGCCCTGAGGACATTGCTGTGGGCCTTTCCAACCAGGCCGAGGCCCCTCCTACCTCACGCCT ATACTGCACGCCCTTTCTCAGGGAATCACCTTGTGGGGGGCAGGGTCCAGCTGGC
OCA2	rs11074304	TAGACTGTTGGTCTTTCAATGCCATGTGCTGGGCCACCATTTAAATCTCTGGATGTTTCCTCTACTTGTGTCATCTTCCAACTTTTCTTCTCTGG AGATTTCCCAGAGATCCCAGCTTCC[A/C]GCAGGCTCCTGAAGTCCCTCACACTTAAGGCCAAGGAGAGCTTGATAAGTAGTGTTGGAAACTAA GGCCAACTGTTGTCTAAGGAAAGGCAAAGGGAAGAAGAAGTTTTTCTTCTGTAGC
OCA2	rs4238497	TTTTTCTCCTTTTGACTTCAATAAGGTAGAAAATATTGATGAGATCTATATTATTCCTGACTTTTCCTCTTCTGAAAAAAGAAATCCCATTGACA TTTTTCTGACTTCAGTCTAAGACTG[G/A]GCACTGTCTAGTTTTACTAATTTCCACATTGTCTGATTTCCCCCATGTCTCAATAAACTCTGTAT ATACATTTACAAAATCCCTATGTGCCTATACATTTATAAGCATTTATAAGCTTAT
OCA2	rs1874841	GGGAACACATTAGCTGTGAAGCATCAGAACCAGTCATCCCCAGGCAATGGGCCC[A/G]GGAAGTCGGGGGCAGCTCTGCAGAGAATGGGAAGGA ACGGAGTTGCTCTGGAATTGCTCTGTCAAAGCTGAATATGTGTGTCCACTCAGGAAATCATCCAGACTCTCCTTCATTTGCTTT
OCA2	rs17566952	AGGCTGCCCACCCTCCATCTCAGCCCTCTCTGGCCTTCCACCAGCCCGGCTGCCTGTGGGCAAAGTCAGTGTCTGGGAACAGGCTCTGAAACCTT CCCAT[C/G]CAGAATGTGACAAAGCCTATGAACCAAAGCTAAATTAGACTCACCAAGATCAAGATGAATGCCAGGGACAAACGAATTGAGGAAA AACATGAAGATAACA

OCA2	rs12900079	gaccctctgaggatctctgtggttcagctgtgtcctctctggactctcgctgccctggtctccccagactttcagcttcatcctctcaactcagg gggtgtcatgggcttggccagggtc[G/A]tccctccctgcactgcactctggaagctctcttgaggtagtgagctggagcagtcatagagctca ccgcatttgtttcccgtctcacagggatcactgtccctttgtgcctgttatccag
OCA2	rs4778137	TTAAAATGATCTAAGAGATGGTTCTTGTTATTGGGAAATGCTATGGATGTGTAAAGAATAAGGACAAGTGGTACACTGTGATGTTTTTATTGAAA TAAATATGTATAAATTACATACATA[C/G]AACAGAGGCCCAAAAGACACACCCTCCAAAACATGGCTACCTCTGGATGGCGAAAGTGCCCACGG GGGTCCTTTTTCTTTGGTTTTGTTTGATCTATGATTTTCACATTTTTTGCATTTA
OCA2	rs7179994	gcccctggcaatgagctgagatggcctccagtcaacagctggcaaggaactgaggccatccatccaacagcccaggaagaaccacacccggccaa caaccacacagatgagctcagaagc[A/G]gaccttgctccagctgaacactgagatgctatggttagagccatacatgacaacttgtcccagcc tgtgaagaccctgagcagaggacctaggtcagctgtgcccagatttctgacccat
MYO5A	rs2290332	GATGTAGAACATCTGCTTGACCACCTGCTTGATCAGTTCAGGGTCCATGCCATGCTGACACATGACCGAGTGGAAGGAGTTGAGCTGCCGGAGGA TGGAGTCCAGTGTGTAGGTGCCCTC[A/G]TCGGCGATACTGGAGGTTCGCTTTCTCAACCCTGTGGGCTTCACCCCAGACACGCCCTGAATCGT TTCATGTTCCAGCATGCCTGAGACTGCAGGAGTATTTCAATTGTTAGAGGAGATG
MYO5A	rs11853114	ACTCTTGGGGATTTTTTGTTTTAATATTGTTGTCACTAAGGTAAAAGTCTTTAAAAAGAGGAGAAATAAAGGAAAGGAAATAACACACAGGCAGA GAAAAAGAAAGACTATGTTACAAAG[G/A]AGAGCAGTATTACACTTTCTCCTTTGTTGCTTCACTAAAGGCAAAGAGGAGTGCGGCCAATTCTA AAGGCCAAGGGTGTACACCTGCCTGACCACATCATGCCCACTGGAATCATCAAAG
MYO5A	rs752865	GTTGGCAAAACTAAGCCGGGGTTTTTGGTTGATGTTGGATGGAGCTCCCAGGTCAGGGAGGTGTGTGTTAGGATTCAGGTTTACATATTTTCTGT TCTTTTAAACAGAACTTGTATAACA[A/G]GTGAGGAAATTGTCTTTTTCCTTCTCAGATTCTAAACCCTGGCATGGAGTCTGACTGttttcttt tctttttttgccttgttccttttttttttctttttGCCAAAAAAATTTTCTAAA
MYO5A	rs1632403	GTGGGCAAATTGATTACTAGCTCCCCACTTAGGGTCTCAGCTTTAGGCAGGAGTCTTTGTGTTCCCCTGTTTATATGGGTCCTGTGTCTTTGCCT TCTGTAGCTCACTTGGCTATGAAAC[C/T]CAAAGCTCAAGGTCACTGGGTTCAGCAAATGCCTTCAAGGTGGGGCAGAACTATCAAATAGAGAT GTCTTACCAGTAACTTTTAAAGCTCATTTAAggccaggcgcagtggctcatgcct
MYO5A	rs7176830	CTTCACACTCAAGCACCCCTTCCTTCTTTCATCCCCCTTTCTCCCAAGTAATCACTTTAGATACAAATCAGCACCACTGCCTGCTCAACTCCCTT AAATAAGCAGCCATATATATGTATG[T/G]ATTTAGAATTCGGCATCATTTAAAAGTGGGTCAggctgggcgtggtggctcacgcctgtaatccc agcacttcgggaggctaaggcaggaggatcacctgaggttaggagtttgagacca
MYO5A	rs1724630	TAATCCTGAATTTTTGACCTACCAGCAAAATTGTTGCCCCTAAATACCAGGCAGCAATTAAGTTTCCTTCTCCTCATTAACTGACTCATGTCTAT TGTTCCTTTCCTTGTACCAGAAGTA[G/C]AAGTTACTCATTTCCCCCAAGTCCTGAGTTCTTATTTATGCTCCTTCTAAAACATAAGATTTGTT ATGCTCAAGTATGAATGAGTTTCATTATTCCTTTGCCCATATTTCATTTTCTGTA
MYO5A	rs1615028	GACACCTTTAATCTGTCTAGCCTCTTCACTGCCCTGATCATTTTACTTCTCCTATTCTGAAATTTCTCTAGTTTCATGTACATTTGTCAAGAAGT AGAAACCCCAACCACACATGCAGGA[T/C]TCCTGGCCAGGACTATATGTTTTTGTATATGTGACAAAAGATGGACAAAACCTATCTTTTTTGTG CATCCAGTCTCCCTCTAGCCCAGCATCTTGCCCACATTTTTGGCCACAGTAGTAC
MYO5A	rs12915892	GCTagatcatcagtgacactgttccgttttatagatgataaaacggagactcagggagaccaggagatttgagcttatatgattagttattggta gatacgtgactagtacagatgtctt[T/C]tTCACTAGACCAAAGTTAATTGCATATAACTTATTTCAAGCAAATTTAAATAAATGAAACTTAGT CAATAAAACTGATTATCACTCACAGTGGAGAGAAAATATAGCAAATTAAAGCCAT
MYO5A	rs7176061	aagtgctgggattacaggtgtgagccaccacgtccagctGAAATTTACTTTTTAAATTAGTAAACAAAAAATATTTTAGCTCTCATAGCACCACC ATTCCCAAATTATATAAAAAGTGTG[C/T]TCTGAAAAAACAGTTATGATCAAAGCTTGACTTTGCTTTACACAGTAAATGCCACCTGAAAGTAT

		AATTTTAGGTTAAAATGTACACATAAGATCTATTTTTGAAGCTATGGTGTTGTGG
MYO5A	rs17614119	AAGTCAATGAAAAGAGAAATGTAGACATAAGGCCACATAATGATGATGACTATGATGATAAGAACAGCTTATATTGGAATAGAACTTAGTATTTT TCGAA[G/A]TGTTTTTGTGTGTATCACCTAATACAATTTTCACCTCCAAAGTAAGCAGGAGAGACATTATATTCTACTCTTTGGAGGAAATAGT AACACCAAGAGGTTA
MYO5A	rs17614255	TTGCGGCAGCTCAAGGCAAGGAGCCTGAGGCCCAGATTCCTAATTTCTTGGCCCTAAATAGTGACTCTATCCCCTGATGCCCCTGGACTACCAAC CTGCC[C/T]GTGGCTCTTCCAGGTTTCTAAGGTCTCCTGTGATTAGGCTTCTATCATTGCAATGGACTGATACTCAAGCTCACTCAGGTGAAGG ATGAGCAGGGCACAC
MYO5A	rs1724625	gccacttcctggctgtgtgaccttgagcaaaactacttaatgactttgaagtttggctttcttatttggaaaatagtgattatatcacctgccaa cccatagggttggtgtgaaatttaa[C/T]gtgagaaaattcactccaaagtgccgaacattttgcctaacaaagcagatgaccaataaatgttA AATAAGTTAATTCCCTAGTTTGGTATTAAAAGCCCTCCTAATTGGCCTAATTCTT
MYO5A	rs10518684	TCTCTTACCACCACAGTTTTAATGGGTATCTTTAGGCTCCCTTCTTATTTTTTCTCTACTCTTTAATCTGGATTTGCTTTCTGAATACCTCCATG TTGAG[G/A]TCCTAGCTGACATATCAAACTTAACACTCCAACAACTGGCCTCATTAATTTGCCTCATAAATGTATACTTTTTTCTCAATATCCG TATTACTAATAACAT
MYO5A	rs10518683	CGTTCTCCTTCAAGATCTGCACCTCCCTCCTCTTCTGCTCCTGATTTCTCCCATGATTCCTTCTTTTCCAGAACATGTTTTATCCCTTTGTCAAG ACTAA[C/T]GACCCCATGATTGACATGCTGGTTTCCCTCCCCTCTCTGGAATTAATTTATCTTTAAACACTTCATAAACCTGGATGCCCATGCC TCATGATCTTTTCAT
MYO5A	rs1615235	CATACACTAAACCTAAATGCCCATTTTCATGAACAAATCACATATGAAAACAGGAATCCTAGATTCAGCCAGCAAGGGAAAGACATGACACAATC AATCATGGCTGGCATGGGCCAGACT[A/G]AACCCGGTCTCTGGCCTGTATCTGTATCCTCATTTCATGTAAATTCACCTCTAGATTTCATTTTT AACAATAATCAGGGTACTAATTTACTTGGAGCTTTGAGAAGGAGCATTTGGATTT
MYO5A	rs1724593	TTGCTTAGTTGAGCAACTTTGGTCCCAATCAGGAGAAAGTGATTCAATGAGATTGTGTTTTTCTTATTCTTCCTTATACAACCTAATCattttga aatcagagatctttctcggtttgaa[G/T]tctgcttctgccatttattagctgagtgactttggggcaagttatctaacttctctCAAGTGTTT TGTTGAATAAGGAACTTCTTTAATAATAATTATAAAGCATATTATGAGGTTAAGT
MYO5A	rs17613475	GCTGATCTATAGGAAAATAAATTAAGCACCAGAGAACTAGAAAGTGGGCATGTTGTTATCAGGTCTGTGGGTGACAAACTGAATTAGGGATCAGT CTCTC[G/A]GAGGCAAGTATTTGAATTTTAAAAAGTGGGACCACTTTGTTAACTAATATTTGCTAAATAAATAAACTGCAGGTCTGCCTCACTC TGTGAAATATGCTTA
MYO5A	rs11637579	GCAGAGGAAAGAGTATGGAATTAGACTATGGGTTCTACTCCCAGGGCCACCAGATGGGCCTTGGCCTTTATTTCAGGCAACAATAAAATGAGGAT GACAACAATAGTCTTCTAGTTTATT[G/T]CAAAGGCTTACGCACATAGAAACTTCATGAAAAGTATAAACCAATATATAAGTACAAGGTGGGGT TGTTACTGTTTTTCCCATAGGAAGTGATTTCTAATGTCATATATCAAACATGTTA
MYO5A	rs2693467	ccaccacatccagccCTGTAACTTATTTATTAGTCCAAGGGCCAAGAGCTAGATGGGCAGACAGTTTGGTTTACAGAAAGCTTGATCCTTACGAT TTTCTTTATGAATATTTGAGGTGTA[G/A]TTTTCCTAAGCCATGGGTCCTAATTTTAGATGCATGATTCCTCCTCCCCTCCCCCAGCCTCTTTT CCTAATGGTGGTTTTGTAAAAGGCAAAATGATGATTCTAATTTAAACCCTCATTT
MYO5A	rs752864	ATCCTCTGCCATCCTAGGTTTATTATGTGATTGTCACAACTGAGACTGGGGACAGGCTTCCTTTACCTTTGGTCCCCAAGCAGCCACTGAGGCCA TC[C/T]CAGGCTCCACCTCTAGCAGACAAGTAGGGTTAGAGTTGAGGTTGAGAAAGGCGGTTGGCAAAACTAAGCCGGGGTTTTTGGTTGATGT TGGATGGAGCTCCCAGGTCAGGGAGGTGTGTG
MATP	rs35415	AATACACGGGATCTTGCTTTGCTGCATCCAGGAAGATTAGATAGAAAAAAATACATATTGATTTGCCAAATGAACGAGCAAGATTAGACACGTTG

		AAAATCCATGCAAGTCCCCTTACAA[C/A]GTATGGTGAAATTAATTATGGAGTAATTGTGAAGTTTGCAGATGACTTTAATGGAGGAGACCTGA GAAATGCTTGTACTGAAGGAGGTATGTTTGCAATTCATGCTGATCATGGTTTTGC
MATP	rs35414	CTGAGATAATATATTTAAAATATTGCTCACTGTTTCCATCCACCTACCTCTAATCCTGGCATCCCTTTCTCTCATTGGGCCCTTCTTTCACATTT AAGCTGGGCCAGGATGCCTCGTTGC[C/T]TTGAGGACAGTATGTAACAGTAGAGAGCAGAGAAATAAGAATGGTTTGTCTTGCTTCAGAGTTCC AAAAGCCCTTAGGCTGCGAAGGCATCGAGAGTAGATTCATTAGAAAATGTGCTTT
MATP	rs35405	TGGGCCTGGGTTTTTTTTTTACCTGTTACTGTATTTCAACCTCACCACCTCCCTGGATGCACATGTTATTATCATTACCCCCATGGTAACAATTA TAATACTAGCTATCACATTTTGAGT[C/T]TTTGTCGGGTGCTAGGGGGCACTATGCTAAGCACATCCACTAATTTGTTTGCTTTTctataaggt aaataacattctttccatttgcctcatgaggaaactgagggcctgcgagcttaaa
MATP	rs7718382	TGGGAAATTGGTAAACTGTCAGTACCAAGGAATCTAAAGGATGAAAGGAAACTCAAGAGAGTCCATCTAAATCAAACTCTGCACCTACCATCTGC CTGTAATTGCCTGCAGGCATCAGGT[G/A]TGTGGCTTGGCAAGCTCAGCACCAGGGCTGACACAGCTCAAGTCATGAGAGCTAGCTATGTGCTG TTTGCTACTTTACCTCTGAAGGAGGACAGACTAAAGAACTCCCATCTTCCCATGG
MATP	rs3756464	AAGGGATTTACCAATATGTGTGCACCCATCATGAATGCTCTGTCTAGCCTGGGAAGCCTGGTTCTTAAAGCACATTTTCTAATGAATCTACTCTC GATGCCTTCGCAGCCTAAGGGCTTT[T/C]GGAACTCTGAAGCAAGACAAACCATTCTTATTTCTCTGCTCTCTACTGTTACATACTGTCCTCAA AGCAACGAGGCATCCTGGCCCAGCTTAAATGTGAAAGAAGGGCCCAATGAGAGAA
MATP	rs6414862	ccttggccgagaggaagggtccttcagttagctgggggttagaattttaattctggtttacaATACTATCATGTATTGGCCTTGGAAAAGTTTCC CTAACACAGAGTTTTGTCTTTTAAT[G/A]TCAAGGCAGCAAAGCATGGGTCTTTTAGCCACATCTAATACAATTGTAAGCTGGGAAGTGGTGGC CACATTGTCCAATGTCCTGTAGATCAATAAACTCTTAATATCGAGTTCAAAAGCC
MATP	rs40132	AGCAGGGATATGGCCCAGGTGAAGGAAGTGGGAAGAGATGGGGAGAATCCCTGTTACCATTCTCAGGAGGACTCACTCTGTCACTGTCAGCCTGC TTCTTGTGAAGGGAAAGTGTGGATG[C/T]ATGTGAGGGCCCCTTCCCCTTCCCTTTACCTAGCCATAGCACAGCAGGAAACCTGCCTCAAGACC TCTTAAAAATTTAGCCCAATTGGGAAAATGGGACCTCATATAAACATGCAAGTTG
MATP	rs16891982	ATCAAATCCAAGTTGTGCTAGACCAGAAACTTTTAGAAGACATCCTTAGGAGAGAGAAAGACTTACAAGAATAAAGTGAGGAAAACACGGAGTTG ATGCA[C/G]AAGCCCCAACATCCAACCTCGACTCCTCTTTCGTAGATGAGAAACTCTGTGGAGTTGTGTGCACTATAGGGATCCCCGCGGTACA CAATCTGAAAGAGAG
MATP	rs26722	GAGCAAGAATATTTTCCCTTGTAAAGAAAAAATGTTGCATCTTTACCTGTTCAGCATGATTTTTGTTTTTTGCTCCCTGCATTGCCAGCTCTGGA TTTACGTAACCATTTTTAACTTTCT[C/T]GATAGAACCATACTCGTACATTCCATCTGATGACAATGGAGGGTCCTGAGGGGTTTGCTGTGGGG GAATGCCCTTTGCAACCTCTGTAAGTGGGGCTTCAGAGATACTGCACAGATGAAC
MATP	rs2287949	CCAGAAGAACCTCAACAGGTGTTAATGGAGGAAATGATGTGTAACAGTGATTGTGTGCACAGACACGTTCATTACCTGGCCCATGAAATCTGTGA AGAACAGCATGTTGGACAGGAAGGC[T/C]GTCCATCCAATGAGGTGGCTGATGCAAAGGTAGCGGTAGTGAGGAGGCATGTTCACCAGTGCTCT CAGCAGTGACTTTAATGTCATTGCCCTGCGAGTCTGAAATAAAACATGAAACAGA
MITF	rs704246	TCCCCTTCTCCTTAAGAGACAATTTCTGCAGGTGGCAGGTGAGCAAGCCCAGGAGAATGCTGCAATCTTGGGGGTGGTTTTATTTATTTCTTTTT TGCCAAATAGAGTGTGGATTCATTT[C/T]AGGGGCTAGCTAAGCCAAGAGGCAGTGGTTTGGGCTTGTTGTTTGTAACAAGAAAATGATCCACA CCACTCCCCCGATTCCCGGGTGCAGAATTGTAACTCGGGGTTGGGCCTCTATATG
MITF	rs7623610	taatatcctcaattttgctacttatcccacaaagccagaagtactaacttcctggctctttgcagaaagtttgctgacttctgCTCTAAGGGGTT AGGTTAGAATTTGGGCTTTCACCAG[A/G]TATTGTTGGATGTCCTCGCCAAGTCCCTAATAACAGTATCTCCAAGGCAATTTCATTATTTGTAA CTAAGGATAGTAACTGATTTCAGGGTTGTTATAAGAACAAATGAGAAATCGTCTG

MITF	rs2131025	CTCAGAACTGTTTTTGGACACATTTAAGGTGTAGTATTAATAGGTTAAAACCAGGCTTTCTAGAAAGAATAAACTTACATATTTATTTTTAGGAC ATGAAAATAGCAATATTCTTGGAGA[C/T]TGATAACCATAGCATTAATACGCCCATTATGGTCATTTAAATTGGGGTTTATTTCAGCAAACTTG TTGAATTTATTTTTAAGAAAGAAATACTGTATTGGGAAGTTACTGTTACTTGATA
MITF	rs13072665	catgatcgtgccactgtcctttagcctgggcaacagagcaagaccctgtttttagaaaacacatttaaaaataaaaaaGTAGAATGAATCCTCAT ACCTCTTCATTCCATCTACATTAGC[G/A]TTGAGGCCTTGTTCCTTCATTATCTCGTTGTTCCGAAAGTATATTAAAGATTAATTTAGCATTTA TGGATATGCCTAATAGGTGGCCAGCACCAGGCAAGGTGCTGTGGATGCGAAGGTG
DCT	rs2031526	CTCTTGAAAAACTAATTGTATCTTTGTTAAATACAAGTTAAAAATGTTAAATACAAGTTAATATCTCTGTAAAGGGTCAAATGTCATTTGAGGGT AGGAA[A/G]CAAAAGCAAAAGTTAAATGAAAGTGTATCATTGGGCTGGGCATGGTGGCCCACACCTGTAATCCCAGCACTTTGTGAGACTGAGG TGGGAGGATCACCCGA
DCT	rs16949829	AAACATGACTTTAAAAACAGTAACTAATGCAACATACACATAGAGTCCCACGTAGCCCAATCTGTTGGTAGACCAATCAATGTCAGCCTTCATGA ACCTA[T/C]CCATCGCAGGTGGTATAGGCTAAATGGGAAATGACTGATTCAGTTATAAGACGATGTGGGTATGAAAAACAAACAAAAGTCTGCC TTTATTTAAAATTTT
DCT	rs9584233	gacataatcactctatcaagatacccactcatagaatcaggaagcatacccctgcttcctgacaacatctgattcagagcaaagcttccaaaagt cctccccaaatcacctagcacaagc[C/T]caaacccttttctgtcttttccaacacccactggctgagactttccacagtcctccattctctgcc tgcaaggagtaacagaccaatgtattcaactacattgtgttcctagaggtcacag
DCT	rs7325046	AXGCTGTGGCTTTTTGTTTATTTATATGTATATTACACATACTACTAAAGAAAAATAAAATTTTAACATTAAGCACATTAGAAACAACGAACACA AAGAT[C/G]ATTAAATGTCTTATATCTCTGAACCACGGGATTTCTCACCCATCAAGTCACAATGTGAGCTTCCACCCTAAAGCAGAAGGAGAAA TATGACTAAAGGGAA
DCT	rs7990565	GCTACTCAGGAGGCTGAGGTAGGAGGATCACTTGAGCCCAGGAGTTTGAGGTTACAATTAGCCATGATTGTGCCACTGCACTCTACCCTAGGTGA CACAG[C/T]GAGATCCTGGCTCTAAAAAAAAGAAAAAAGAAAAAAATTTTCTACCACCATAGAAAGACTTTAAGAATTATGAGAAATATGTTTA TAAATAAGACTTTAA
DCT	rs2892681	GAATCAGTGCTGTAAGAGGCAAATAACTTATAGAAAGTATCAGAATTCACCAAGGGAAAAATGTAAATAATAAAGGAGAAGGCAAGATCCTAAGG CAATTAGTAATCTGGAGAGATAAAA[C/G]AATAGGGGAAAATAATAAGGAGGAATTGAAATTGTAGCTCTCAGGAGGGCTATAAAACATACAAT AAATGCTCTAGTAAggctgcacacagtggctcatgcctgtaatcccagcactttg
DCT	rs9516418	AGTCATGAGCCACCATGCCTGGCAGTTTTCTTCTTTTGTTTTAAAGTGAGACAGAAATCAAACTGATATACCAAAGGAGTTCATCACACTGAAAT TTAAA[C/T]AACTGTGAGGCTCCTGCACAGTCAACAGCAATCCTATTTACTGTCTGTATTATTACATGTCAGAGGTATGAATCAGATTAACAAA TTAAATTGCCTCAAAT
DCT	rs12877284	cataatttgtctgtgcttagacacagttatggagtagtcttgtttttgtcctgatccaccatgatcagagtggccttgtctgctgttgatgttct aggagattgtttatgtccaacaggg[G/A]acaccaaggcctggttgatttcaccaggccagctcttggatgtcaggtgctgcttttcttctttc tcaAGTAAAGCTCACTCAAGTTGGAGTAGGAGGTTTAAGTGTCCAGCACACCCTC
DCT	rs9524494	AGAGGACGAATTACTTTCCCTAATTCTGACTTTTTTGAATATTTAGGGTTTTTTTTTTCTTTTTCCAGAAGTCATGATCACAAAcaaaagttatg aagacagatcacagaaaaggaggtg[C/A]aaatggatttttttcttttgaatgtctgaaaagatgctggacttcacttttttttttgagacaga gtttcgctcttgtcacccaggctggagtgcgatggcacgatcttggctcactgca
DCT	rs4318084	aggcatttgtcttacggttggagacagtgtctcgcttctaggcagctggaagtccaacaccaagatgtcagtggggctggttccttctaagcact gtgagggaaaatctgttccagacct[C/T]actcctaccttctcgggccttgctgacaatctttggcattccttgccttgtagatctctatcttc

		atcctcatgcagtgttctccctgtgtgcaggtgcctatgtccaaattttcccttt
DCT	rs727299	TTCAATGATTCTAAGCTTGCCTGATGGGTGTGAAGTAGCTTTCTGGGGCTCCTAGATAGATTAAAGCATAGTCAGGTGAGCTTCAAGAAATCCTG AGACGGAATTAGTTGGTAGA[G/A]TTGTTCTTTCCTTCTAAAAAATGTTTCTTTCCTCATATTTGCATAGTAGCAATAAATGAAGGGGTTGTCA GAAGTCTGAATTAATGGTCCCAGCCTCTAACAAGGTGGGGGCTTATCTGT
DCT	rs9524493	CATCATTAAAGACCAGCACAGTGTAGCTTTCACCACTTGAGTTCATGTGTTACTGCCCAGAAGGCATCCTTTGTCATCAGACTGGATCCTATTGT GGTGACATGCTATTGCTCTGGTCCT[C/G]TGAGAGTCAGAACTAAGTGACAATTCAAATGTGATCACTTAAAAGCCACATGACAGATAAAAGCA CACACAGGTCCATATGGGAACTCTAATTTAAAACTTAAAGTAGAAGGTATACATT
DCT	rs1325611	GCAGTCATCCCAATGGGTATGAGGTGGTATCTTATTGGGGTTTCTGCCTGGCATCTAAGGCCCTCTGTACCTAGGCTCTTTCATAAATTTGAACT TAATT[C/T]GAGGTAATTCTCTGCCCAAGCGTCCCACTACAGCCAGGCTTGAAAGACTCAGGTCAAAGAGAGAGAGACTGAGCTCTGAAATCAT CTTGATTGCTTTCTA
DCT	rs1407995	TGCAAAACAGTTGGACACAGCATTTAACATAAATCAGTCTGTTCCGATCACACCAACCTGACTGTTGCTTTCTCTAAAGTGGAATAACTTCTTCT TGACATAGGAACTTCTGTATACCCA[C/T]GTGTGGAATACCCCCTTCTGTGTACCACCATGAGGAAGCCCAAATCAACTCATATAGAGTGACTA TGATGGCGAGGATCAAGATTTCGGGAAGAAAAACAGTT
DTNBP1	rs9464795	GAAGATCAAACCTGGGCTAGGGCCTTACAAAACATCTAATTATATCCTAACGGCATGGAGAGGCCTGTGGGGCATGTGGTGGGGCCCAGGCCCCC TCTGCTGCTCAGCCCTCCCGGCACC[G/A]CCTTCAGTCCTCAAGCCTGAGAGCCTCTGTGCTTGGGGGGCCACCTGGCCTCAGCACCATCCAAG TGGGCACATATGTGCCTTCACCCCTTCCTCTGATGATTCTCTGGATAAAACTTTC
DTNBP1	rs875462	ACTCCTTAAAGGGGAACATCTGGGTGAGCCTGGCCTAGCTAGGCTTGTGAAGGGCA[C/T]TGGGCCTTCTTGGAGAAAGATGTGAAGCGTGACA GGGATTTGACACAGGGCCATTATCTGTTGCTGGGTTGGAGGATGGAGGGGGCCACGTGGAAAGGAATGTGGGTAGCATCCT
DTNBP1	rs2619545	GAAGCCAAAATGTGAACAAGGTCTTCAactatttttattcaacattatactagagtttctacccagtataagaaacatgaaaaattaaaagttgt atggcttagaaggaaagaaacaaaC[C/T]TACCATCTACCCAAAACAACTCGCACCCCCAAATACAAAGTAATAGAATAAGAGAATTTAGCAAG ATGGCCAAATATGGGATTAAAATACTGCAAGTCAAACACTGCAGGTTTTTGGTAG
DTNBP1	rs4236167	TTGCATGTGCTGTTCCCTTTGCTGGAACGACCTTCCCACCCGCATCGCCCATTCATCAGTCACAAACAGCTGTTCACCTTCCAAGACCAGCTTCT CAGGT[C/T]GTCCTTCTACTAACTCCAGTGGAGTCTGCTGCTCCATCCAGATGTTTCCACATCCCTGTGTTTAGACTTTTGCAGCTGACTTACA AAAATTGCAGTGCAA
DTNBP1	rs12525702	CTTGTCTTTTTCTTCTCCCAGTGCGCAGAAAGCATGACCACCTCGCTATCCACCAGCTGCAGCACACAAGAAGGGGGGTAAAGTGAAACCAGGTT TTAGGCACAAAGAAAGTACAGTTAA[C/T]GTAATGAGATTTAATGTTATCTTTAACAACCCCTCTACTCAGTAGAGATTACGGTACATTCTAAA AGAGACTGAAGTTGTTTTCATTTTTAGAAAAGAAACAACTTAATATTCAGTGCAG
DTNBP1	rs1011313	AATTGAAGGAACTTCTATCTATGGCAGCTATAACCTTATAAAATGTGTTTCTTAAACAATAACACTTAAAAGTTGATATGACTCCTTAATTCACA GGCTACAGAATGGATGTTGC[G/A]TTAGTGGGCATGAAAACAATGTTAATTAACAGTTGCTTGTGTGCAGTAACTTGGCGTCTCCATGCTGGAC ACATATAAGCCATGCATATTAGCTGTCGATACTCTGTTCATACAGCCTCA
DTNBP1	rs12199640	TGGCTGATGGACAGAACCTATTACTGTGACTACTTGTTCCTGGGAAAGATCTCACAGGCCCACTCTCAAGAAAGAAGCATCTACCATTCATCACC AACAAGTCAGAGGTCTGTTGTTTTC[C/T]GGCAGTCCACAGTCCTGTGGTGCCACCCTACAGGCTTGGTGGGGTTGAGGGGGATTGGGGGTGGG TGGTGGGAGGGACCTCCTTCCAGCTTGAGATGAATTTTCTCAGGACTCTCTATAC
DTNBP1	rs2619540	caggaatagtctgaggctgcagtgagctatgatcacatcaccatgctacagcctgaacaacagagtgagactctgtctcttaaaaaaattaaaat tttaaaacaaaaacaaaaCCCAGAA[T/A]CTTCTGTTCTAGATGAAGGGAACAGTCTTTTAAAAATAGGTCATATAAAATAAACTAAGAGAAAA

		TTCAAAGACAAGGCTTTAACATTTTGAACTTCAATAAAGGAATAGGATTATACTA
DTNBP1	rs12527121	AGACAGAATGCTCAACAATACCTTAGCTTTTATGGGAAGGGCAAGGCAGTTTAGAAGACCGGGGTCTGACTGCAGGGCCAGCTCAGGCAGGCGGG TCTCAGCACACCACTCACCTCCCAC[C/T]ATGCACCCTGGAGGCCTCAGATTTCCCATCGCCAGGAGGGTGGAGCTGGCTGAATATGGATCGGA AGGTCACCTCCAGttcttttttttttttttttttttgagacagagtcttgctctg
DTNBP1	rs3213207	CCTCAAAATTCCTTTATATACTTTATGCAATAAGTATTCCTGTTTAAATGTAAATTGAAACATGGTATATTCTAAATGTATTAGGGAACTTTTCT TTGAAGACTTCCTTTCGTAAAGCCA[G/A]TAATTACCCGTTAATTATGttttatgtttgttttctttttggttgttagtggtagttttttgttt ttgttttttttttTCTAGATTTAGCTTTTCAAATACATGGTCTGCTTAAAACAG
DTNBP1	rs9476886	GTGCAAATAGTATTAACATTTTTGTCCATTAAGATAAAATTTTGGCTAACATCTTTCTCATTAGAAATTTAAAACGAGGCTGGGCGCAGTGACTA ATACC[C/T]ATAATCCCACCACTTCAGGAAGCCTAGGAGGGAGGATCTCTTGTACCCAGGAGTTGGAGGCTGCAGXGAGCTATAATAGCGCCAC TGCGCTCCAGCCTGG
DTNBP1	rs1997679	TTCACATAAAATATCCATTTTTACAGAGATTGGCTTGAGCTCTGGGXAAGACTTTTGGACATACCAAAGTACATACTTCGAAGCCACTCTGGTCT TTGACTTTTACCTTTTGAGCTGTTA[T/C]GACCAAAGGCCTCAGGATTGAGGTATTTAGGCATGCAAACCCTTCAGGGAACTGAAAATCAGATT TACTGACGGGTTTACAGCTGCCTGTCACAGGCTGGTGTTTCCTATTTGTG
DTNBP1	rs7752070	CAGTCTCTTGGCAGGTGAGCTTGAACACTCATTTCTTGAAGCTCAGGAATTTGGCAAGTTAGTCTCTAGAACATACACTGGTCAAACGCCACTAC AGATACGCCATAATCTCTTAAATAT[A/G]TCTGTTATACTTTGTTCTAAAGATTTTCTAAATATAGTCCATAGTCCAAATGTTACCAAAAAACA AAACAAAAAAACACAAACAAAAAGAAGGGCAGAGTCCCTTGGCATTAACTATAGT
DTNBP1	rs742106	TGCACAGTGGTGTGGTTCCGTCATGAATTCTTCTCAAAGATTTGACATGCTCCACTCCGGTAACTTTGGTGAGTTGAGAGCTTTCTTGTTTGTTT TCCCTCCTTTACCA[C/T]CCAGAAATCCATTTGAGTCTGCTCCTTGTGGTTAAGGACTGGCGTTTGCAGGGAGGTGCGGACTCTCCTGCGGGGC TCACGGGAAACTCTTCCCTCTTCGTGCGACAGGCATTTA
DTNBP1	rs2056943	GAACCATCTGCCAGTCACTTACGCATAAACCCCCAAGCTCACAGCCAGCTCCTGGCTCCCTAACCCCACGGTTCCACACGGCTGTGTGGCAGCTG CAACAGTGGTGTGGTTCCGTCATGA[A/G]TTCTTCTCAAAGATTTGACATGCTCCACTCCGGTAACTTTGGTGAGTTGAGAGCTTTCTTGTTTG TTTTCCCTCCTTTACCACCCAGAAATCCATTTGAGTCTGCTCCTTGTGGTTAAGG
DTNBP1	rs9370823	TTACGAGATAGTATGTGACAACAAAAATCTATTATGAATTCAGTAGCACTTTACATAGCTTTGAATTTTGTTTCCCACAACATTTACATATATTT CAAAT[A/G]AAATACTGCATATACTTGCAATACAAAATATTTATCCAAAGCTCAACATGCTTTTGAGTTCAAAGGCCTCTTGTTATAAATATCC TCATGCCACCAGTTG
DTNBP1	rs4712253	AATAAAAGCCAGAAGACTGCGGAGTAAGTTCAAAGAGGAGAGAGAAATGTCATCTCTAAAATGGTGTAACATTAGGTATCGTTTAAGATCCAGGA GAAAAGCAGAGACCAAGAAGAAACA[C/T]GTCATTATGAGCCTGCTCTCGCTAGAGGCATAGTGAAGGAAGGGCTGAGTGCAAGAAAGCATGTT AAGCAAAGAGACGTGTGAGTGTGTGAGTTAATGTAAAACCTGGAATGGAACAGTC
DTNBP1	rs9296981	TAGATAAGCAGACTCGACAGAGACTAGAAAGAACACACGAAAAGCAGTAACAGGGACTATTCTGTACTGGAGAATTAAGGGCTATTTTTCTTTTT TTCCATTTTGGTAATTTTGCAGGTT[C/T]TCTAGAAGCATAATTTGTGTTTGTAATCttttttttttttttttttttttgagacagtctcactc tgccacctaggccggagtgcagtggtgcgatctcggctcatggcaacctccgcct
DTNBP1	rs9396593	GACAATGCTGGTTTATACGTGAAGTATAATAAGCATAAAACAACAAAATCAGAAGTGATTTAATAAGTAATTCAAAAAGTTTTCTCAGTTATTGA AATGA[A/G]TCTCTCGAGAATAGGATTTCTACTACTTAATTGCTCATGAGTAATTAATTGAAAAATGGGAAACTGGAAGAACCTTGAGCTGCTG GAAAGTTTGTGTTTT
DTNBP1	rs1474605	TACAAAGGTGTCTAAAACAGTCTCTGTCCACCAGATGCCCTAGACTGGTGCTGATATAAAGCAAAGCAGAATGAACAGAATGTTAAAGATACAAA

		TAGTGTGGTATGTGAGTCCGAAGAA[A/G]GAGTCATTAGTTAGGATGCATTTACAGAAGGCTTCAAAGAGGAAGGGGTTTCTAAAACTAGGCCT TGAATAATGAGGAGAAATTGCGATAGATTTGGATAAGCACATTTCAGAGATGGAA
DTNBP1	rs9296980	CCTGCCCACCCCTGCTGCACCTCTGAGTCACTCAGTTCTTCCAAAGGAAAAGCTGGGAAGAAGGAGTTCGGTGTCTGGACTTCTAATCAGCTTGT TCCTTACAGCACTATTTACTGAGGG[A/C]AAGATTTTAAATGTTCAGCTAGGAAAACTGGTGGAATATATTAGGATACATCATGACAAAAAGAT GCAGCCTTTGACACACAACCTCTTATAGTATTTTTAACAACAGGCGGAAAGAGCC
DTNBP1	rs1047631	GGACATGGGCGTTGTCTGGCCACACTGGAATCCAGTTTTGGCTGTATGCGGAATTCCACCTGGAAAGCCAGGTTGTTTTATAGAGGTTCTTGATT TTTAC[A/G]TAATTGCCAATAATGTGTGAGAAACTTAAAGAACAGCTAACAATAAAGTGTGAGGACGGTAAACTGAGAGCGCACAGAGCTAGTT TGTACACAAAGGCTT
DTNBP1	rs17470454	GAGTCGGTGCAGGTGGAGGAAGAAGAAGGTGGCTTGGCTCTTAATTCTGAGGGATTTGGAACCTGGAGGGTAATCTCATTCTGACAGGTACTGGA TTCAG[G/A]CCCTGCAAAATAACGTAGGGAAGAAAAAGCCCTGTCATAAAAATATTGTGAATCAGAATTGCCGCCAACAGCTGTGGAATGTGCC CGTCATGAAAGGGGG
HPS1	rs1804689	ACGGGCAGCAGTCAAGACCAGGGCATCTGCCTGCCCTCATATTCTCTCTGTTTTAAGCATTTGCAGGACCAGGGATGCTGTGCTGCTCTCCCATG AGCCAGTCACCGAGTCGGTCTGCTG[A/C]AGCCCTTTCTGAACCTCTGGCCGTCTGGATGCTCCACTGTGCTTGCCAAGGTAGATGATCTTGCC CCACCTGGGGAGGACAGGGAGGCAGGCAGCATCCACCCTGGGGCTTTGGGAGGCA
HPS1	rs11595763	ACTGCTTTCCCCTCCAGTTCCAGCTCAATATAATTTTGTGGGAAAAGGAGGGGAGGGTTGAAGGAACTGTCTTCAAAGATTCCTGTAATTTACAG TGAACCCAAAAATTGCATTAAAAGT[A/C]TGTGTTATCCAGGATCGAGCTGTTTTACCATGGGAAAGCCCATCAGGGTACCCAACCCTCCATAT GGCCAGAAAGAACAACTCTCTCTGAATACACGAGTTTCAGGGGCTGCTTGTGCCT
HPS1	rs17109834	ACACACACACACACACACACACACACGTATAGTTTTTTTGGAGAGTGGCAGGGGTCCTCCCCCTATCAAAAATACAACTTTGTCATGAAAAACAG TCAGT[T/C]AAATAAGGAGAATAAAAGAATTTCCAGGGAATGCTTCCTTAACAGAAATCACCTTGTTTTAAATTCTGATGCCCAACTGATGGAC CTTGCCGTTCTCTCC
HPS1	rs7921146	AATACACATGTGTACATATGTAGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTAAAGTCATAGGAAAAAAAGACTAGAAGGAAACACCCCAA GATGTTAATCTCATTTTGAGGACGG[G/A]ATTAAGAGTAATTTTTATTCCCTTTTTTATAGTTTACTTTTGTATTTCCCGATTTTTCTACAATA AATATACATGCTCACTTAAAAAAAAAATTCTATTGGACTTTCACTCCCAAGAACA
HPS1	rs10786422	CTGGGACTCCCAGGTACATGTGACAAAGCTGACCAGAGTGTATGGGTTAGGGAGCAGTGGGCTATGGAATGGAGGCTGTGGTTGTGAGCGGAGAA GTGAC[C/T]GGCTGGCTGGAGATGGGCAGGTCAAGGTCATGAAGGCGGTCATCTAGGTGCGGGTGTCAGGTAATGAGTGCTCACCCCTTGGTGG GGGCTGGGAGGAGCT
HPS1	rs1061134	GAGTACTTCACACCAGCTCCTTCCCCTGGCGATCAGAGCTCAGGTGAGGACCGGAGGAAAGCAGGAGGAAACAATAGCTAGCTGCGAGCGACAGG CAGCCCAGGACATCTGTCTTAGGCT[T/C]CAGTGGACCCCCGTGCCTCCTAAGGCTTGAGTGCAGGTACCCGTGTTCCTAGAGCACAGGACGCT GTCTGCGGCTCCCCATCTTCCCTGCCAGCTCCAGCCTGAACTCAAGGATTGTTAA
HPS1	rs11189600	GAGAGGAGGGAGAGACAGGAGGAGAGGAGTCCCAGCCACTGCACCCCTCACAGCCCTGTGCAGTGGCTGGGGCAGGGATCCCTGACCCCGTTGTA TGGATGGAGACACAATGATAGTAAG[C/T]TCCCCCCACTGGACCCTACTCTGCAGTAACAGTGAGGCCCAGAAGAAGCAGTGCAGGGCCTGGAG GCCAGGGCCCCGAGCCACACCACCAGTGTCTCCTCAGGCAGCCTGGCTCACTGCT
HPS1	rs12249733	ACCACGGGGCCTACTGGAACCAGACTGGCACAGGAAGGGACCCAGGTCTTGGCCTAGACATGGAGCCGAATGCCCAGGGAGGCCATGCTCACGGC AGCCAGTTAGTGCCTGGTGTGGATC[G/A]GAGGGCACTGGTGCACCTCCATCTCTGGGCAGTCCAGAGATATCTCTCCCTCAGATAAAAACAGT TCCAGggctgggcgcggtggctcacgcctgtaatcccagcactttgggaggccaa

HPS1	rs1061135	CTGTCTGCGGCTCCCCATCTTCCCTGCCAGCTCCAGCCTGAACTCAAGGA[C/T]TGTTAAGACCACTCCACTGATCCCTAAAGCTGTTGATGAC AAGTTGATTC
HPS1	rs10883094	AGTTAAAAGCATCTAACCcagtggttgtcaaccttggctacacacgaaaatcatttggggacttttacaaaatcctgacgcccagggcatagctc agaccaactgagtcagcatctccgg[G/A]tacaggctcagcttcaataattgttaaagatccccaggtgagttcaatatgcagccagggGGCAA AGCATGCTGTAACCTAAATCTGTGCACAGcagtggtttgcacccgaggcacactg
HPS1	rs2296430	TCTCTTTTTGCTCCCCTCCCTACCAGGACAGTGATACCCTCCCAGGAGGGTCTAACACTATGGAACCCTTGATATCAAGGCCTGATCTTGTCCCT TCCTT[A/T]GTTCTTGGTGTCTGGCCCACTCTAAGCTGTGAAATTTTCCCCCATTTTTGCAGCTCCCTGCCCTGGAGGACCAGCTCAGCACCCT CCTAGCCCCGGTCAT
HPS1	rs11592273	AACCCTGTCCCTGCTCTCCCAGGAGGGATGGGAAAGGCCTGGTCGCCTGCAGAGGCCGATCCCCTCCTGCCCCTGACTCCACGAAGTGCACAGCA CACACCGTCTCTGCAGAGCTCCCCC[C/A]AGTTGGGCCCGTGGAGTGAGGGCTCCAGGCCTGCTGCACGGGGATGTTCTGGCTGCTCCGGGCCC TCCGCGGGGAAGGCTGTGCAGGGCAGGGGAGAGGCTGGTTAGCTCCTATCTGACC
HPS1	rs11539873	AGGGCAGGTGAAGGGAGTGCCCTTGGGGCTGGCTGACACTGGCCTGCCTGTGCCTGCAGTTTGGAGAATGCCTGTTCATTGCCATCAATGGTGAC CACAC[T/C]GAGAGCGAGGGGGACCTGCGGCGGAAGCTGTATGTGCTCAAGTACCTGTTTGAAGTGCACTTTGGGCTGGTGACTGTGGACGGTC ATCTTATCCGAAAGG
HPS1	rs3830019	CCTCTCTGCACCTCCTGGCAAGTTTCTTCTATTCCCCACGTTTAAAGCGATGGCACCTCC[G/A]TCCCAGGGTGGTGTGAGGATTACCCAGTGT GGTAGGTGCTCAATAAATGTTGGTCATTGT
HPS1	rs7082570	caataagtctactttggaaaatttgtcctaaggaaaatgatcagagattgggacaaagatacatgcacaaagatgttcactactgcctaatttat aatagcaaacaactggaaaaaaaat[G/A]ttaacatctagtatcagggaaatagttaaataaatagcagcacatcaaaattatggaagggtcta tagccacttaaaatattagagtgtttcaatgacatggaaaattcctctgatacaa
HPS1	rs7071947	TAAAAAATAAAAAAGCCTAACAGTTAGCTTAAATAAAACCACTTGAATGTCTATGATCTCTGATATCTTGTGTTTGCCTAAAGACTGTGATGAGA ACACG[A/G]GTGATGTTGATGGTAAATGGACTCCCTGAGGTGGAGTCAGCTCACTCATTGGCTGGATGATGAGACCCCTTAGAGCAGAAAGGGA CAGAGAGGCAATCAGC
HPS1	rs1801287	GCGACTGATTCACCCCCAGCTCTGTGAGCTGTGCATAGAGGCGCTGGAGCGGCACGTCATCCAGGCTGTCAACACCAGCCCCGAGCGGGGAGGCG AGGAGGCCCTGCATGCCTTCCTGCT[T/C]GTGCACTCCAAGCTGCTGGCATTCTACTCTAGGTGAGCTCAAGGTCTGGCATGTGGTGGCTAAAG GCTCCCTGGCCTTCTCACTCCCCTATGGCCTCTGCCTGAAAAGCACCACCTGCCC
HPS3	rs16861552	AAGTATAAGCTATTTGAATTGGATATTTGTTAGGAATATACATGCTGCAAGAAGCTCCCAAATTATCATGCTCTGTGTTTCAAAAATTGGTTTAT ATCTG[T/C]CTGGAAACTGGAATACCTTTTTCCAGAAGGATAAGCCTTCAGTAATGGTGGTTATCTTAACAAGCCAGCCCCGACTTAGTGCCTA ATGTGTCTGAGATAC
HPS3	rs2689230	ATTTGTCTGTAAAATGAAGATACTGACCCAGATCCATGGTTCCTAACTAGGGATGCACATCAGAGTCACATATGGAGTTTTGTAGAAATGTACAC GTCCAGATTCAGCCCCTAGAGATTG[C/G]GATTCACTAGGGGTGTTATGGGGACCAGTTGTATATTTTTAAAAACACACCACAGGGATTCTGAG GCATTCCTCTTATTGAGAAGAAGTTTAACTTGGCAATATTTGACAAATACTTGAC
HPS3	rs6785780	TGACCCAATGGCTTGATAGCATTTCCCCTTCTCCAGTTCAAGACCCAGTCTAGGATCAGGTAGTGTGTTTAACTATGATGTCTCTTTAGCCTCCT TTGAA[G/T]CTGGAATATTTCTATAGCCTTTCATTATGTTTTATGACATGAGCATTTTTGAAAAATATAATTTTCCCCTCTACTTTTTTTTTGA GAGTATTCCTTATTT
HPS3	rs4681169	GTTTTTAAAAATCTCTATGCCTGGGCCAAACCCTCAGAGATACAGTAGTAGTTTCCCTGGAGTGAGGACAGCATTGGGGTGAGTCAGATATTTAA

		TCAGG[A/G]TTGAGAACCGCTGATCTAGAGCAGTACTGGCTGCAGGAGGCCATGAGTAGCATTGTAACCTCATTAACGAGGCCTTGAACAATAA AGATGTTTTTATAAT
HPS3	rs2254913	AATGTACACGTCCAGATTCAGCCCCTAGAGATTGCGATTCACTAGGGGTGTTATGGGGACCAGTTGTATATTTTTAAAAACACACCACAGGGATT CTGAGGCATTCCTCTTATTGAGAAG[A/C]AGTTTAACTTGGCAATATTTGACAAATACTTGACTGTCACCCCTGCCCATTGCCCTGTTTGCCTG TGAAACCCTCCCTGCTGTGGGTATGTTGGTTCTTGGCCTCACTTTGCTCAGCAGC
HPS3	rs4681487	TTTCTTTATTACCCAGTCTCAGGTATTTGCTTGTAGCAGTAATGTGAAAATGGACTAATACAGTGTGTAAAGCAAAGAAGAGGGGAGACCTGAGA ACTCC[C/T]CACTCCTGAAAGAGAAAGACCTGGTGGCCAGGGTTGAGTGCTGTATGGGGACCTTTGGTGGTAGACACTTGTTTATGGGGTGTCT AGGGGTGGTGGGGCA
HPS3	rs2689229	TTGGATTTGAGTTTGCATAGTCTGTGTTGTCCAGCATAATCCAGGATGGATTTCATCATGAGGWGGTTGAGGAAGAACAACAGTTAAGAAATGGA TTTTA[A/C]TCTTGGTTTTGATAGACACCCATCTGTACCTTGGGCAGGTACTGGGGGCATCATTTGTCTGTAAAATGAAGATACTGACCCAGAT CCATGGTTCCTAACT
HPS3	rs2689234	TCAATATACTTAAGAGCACAAAATTGAATACGATGCATTAAAATGATTTATTTATACTATTATCAGCTATGCCTGTAATTCTGTATTTTATAAGC CTTTGATAACCTTCTATACTTAATC[T/G]AAGAGTTAATGGTTATGGTAGATCAATAATCTCATTTTTAGTGAGAAGTAAAGTGCTTGGTTTCT aaaaaaaaaaaaaaaaaaaaaaaaaaaaaGCCTCTTGACCACAGAATAAATGTAG
HPS3	rs7643410	GGTTTTCTGCAGAAAGGCCTCTGGCTGGATTCCACTCATCTCGGATACTTAAGCAAACAACAACAAAAAACTCTTTAGTGCATCAGATTTCAAAG ACTTA[C/G]CAAAAAAACAAGTAGGAATAAGGAGACTAGTACTGTATCCCGGAACCTCCAAGTACTCCCAGCTTTAACAATTATTCCAACTCTT GGCAGTTCTTTCACA
HPS3	rs7636389	GGTGGGAGAGGAACTGCAGAAGGCTGTGGGAGGGAGACTGCCTTAGTGGGCCCCCATTTCCTGTCTGTTGCACATACAGGAGCTGTTCTGTGGCA CTTGA[A/G]GCAGCCAGTAACAACTCTTTCTACCCTTCTTGCTACCGTTCTTGCTCCTAGCCCATTTCTGTCCTAGCTCATTAGCCATGGCTAA TGGAGGGTTAAGTGG
HPS3	rs3732557	ATGCAATTTCATAAGAAAAGGAGGTATTTTTACTTACCTGTAATTTTGAAAGATCTTCTGTATAATTTTTATCAGCTAAAGAATCAGATGATATT ACGAC[A/G]TGAACCCATGGATAAATTAAGCCATAATGACTACAGGCATTTATCTAAAACATAAAAGAATTTCTCATAAACAAAATATAACAAC AAAAGCTTGCTTATC
HPS3	rs12487928	TTAGAGGGTCAGCTAAAGATCCAGTGAGCTTCCCATAGCACTTGGAATAAAAACTCCAAATCCATTCCATGGCGTATATGACCTTAACTCTAAGC CCATC[G/T]TGTCCCACTCTCACCCTGCTTACTATTCTTCATCCATAGTGGCCTTTCCCTCTTCCTTAGAGACGCCACAGGCACGCTTATCTAA GACCTATTATTTGCT
HPS3	rs6440588	agctaaccagatctaggtttgagtcccagtttcctaatttgttcgttgtgttgccttggacaagttactttgcttgtctgtgcttcagattcctg atgtataaaatgaggagggagattt[G/A]tatcttaaagggctgttgtgagagttaattgaAGGAACTCTAAAGCTCTGATCATAAGGATTGTC AGGCAGGCACATTTAGCAAGTGCTGGATCATCATTTAAAGACCAGGTCATTAATC
HPS3	rs16861514	AAGGCACATACCAGGCTGTGTGTTAACATTGGGTCCTCAAGGGGGAGATCATTATGCTTTTCATTTTGCATCTCTTTACCACGTTACCATTTTGA CTTCC[C/T]ACAACCAATATATGTTTATAAGTTAAAACTTATACAATCAACAATTTAGAGAAAATTAACTTGGATCTGTATTTTCTTTGCTATT TCCAACTTTATCCTG
HPS3	rs2681092	ACATACTGTTAGAGAGATGCCCGGAGGCAGTCATTCCATATGCTAATCATGAACTGAAAGAAGAGAACCGGGTATGCTTTTTCAGATTATGTTTT TAGGCTTGATCAGTGATAATCAGAT[C/T]TGATAACCTCATTTCCTTTCCTGCAAAATGGGGACAATTTATGCTAATCCAACATCATAAGGCTG TGTGGGTCCATTGAGCATATATGTTTAATATGTTACGTGTTTTGAGTACTATTAA

HPS4	rs739289	GGTAACTCRATTCTTGAAGCTCCATAACATCCATTTTCCTATTATGAGCAGAGGAAATAAACATGCAGATGGCTTGGTTTCCTTCGCATAACTTG TACAGGGGTAGGTAGCATAAAAGAC[A/C]GCCGTTCTCAAGAGGCAACCATGCGCCTCASTACTTACCATGTTCCTGYGGGGCATCCYCTCCCG TAGGGAGTCTCTGAAAACAAACACACACAGAAGTTGGCGCTGGGCACCACATTCT
HPS4	rs1894704	CCGTGGAGAGTAGGTTGGGGAGCGACTCAGGGAGGCTCACCTGACAGTCATTTCATAAAGCGCGGGCAGCTGGGCAAATTCGCTATGCATCAGGC TGACGGCCTGGAGGAAGCGGCGATC[A/C]TGCGGGGTGGCCACCTGCGGCAGGTTTGCTTCCAGAAGAGGACACAGAGTTGTGAAGAGCAGACA GTTACCTTCYGCCCTCCCACCCCATGAAGCCCAGTACTGAACACGGGGACTGAGC
HPS4	rs3752589	TTTTCTGAGTTAATCCAGGTGCATGAGACTTTTCCATATACACGGAXAGGCTTTGTACGGTTCCATTTTGGGTCAGTGGAAGAATTTTCACAACA CAAATGACATTATAAAGTTGTCTTC[G/T]CTGGCCTGGTACTGGGGACCACTGGTCAAAGACAACGTGAGACATTGCGGGGACTTCCTGGAGCT GCAGCAACCACAGCAGAGAACTTGTAATCAATTTTTTCTGGGAGAACATAGCAGA
HPS4	rs3752590	GGAGAACATAGCAGAGGAGTAAGACAACATTTATCCTAGCTTCGGATAGGATCCTCTTCCATCTAGGCAGATGGGATCCAAGAGACAGCAGAGAT CCTGGAGAGAGGTGATAGAAGAGGG[A/T]GACACAGGCTTGGTGTATTGGATCAGCTGCAGCTGGTGCTCCCTGGAGCAGCAGGCTGCAGCCTG GCAGCCCTACAAACAGACGGTCCTTACCCAGCCACACTGAACCCAGGGAAGGAAA
HPS4	rs1894706	TGTCATCATGATGCTGGGGCTCAAGTTCTCATCTCCACCCATCTAGAATCTGGCATCCCCGTCGCCCCAGGCCCCTTGGCTGGTTCTTACCCATC AGCAAGCTCTGAATGCGGTCGTAAT[A/G]TGTGAAGTTGTAGGTGCTGCTCGTGGAGGCTGCCTCATCCCTGGGCAGCGTCTCTTTCAGGTGGA CTTCCAGCCCATTCAGTGAAGCCAGGCTGCTGTGGTACTGCAAAGGGGGAGAGGG
HPS4	rs5761554	GGGTGCATGGAAGGAAGGAAGAGCAGAATAAGTAATATCCAAGACAATAAGGCAaaatttgaatacagtctgggtatcaggtaatattaaggaat tactgttactaattactgtaacttt[C/T]gttaggtgtggtaatgttcaaaataatgccctttcagttagagatgttgcttgatactggtgag atgacgcaacatctagaatttgctccaggaaaaaaaaaagagagagaagaagagg
HPS4	rs1894707	AGCCCTGCATGATAGTAAGATCAGCTTAGAATAATACACATTTAAGAATAAAAACAAGGAAAGCCCCAAGACACCATTTGCCCTGCAGCAATTTC AACCTGCTGTTCTTACTGCTACCAC[C/T]TTCCCTGCCTACTAAAGCAAGGCTGTTACTATAGCAACGAATCTCCAGCGTGGGCCCCAGGGAAG GTCAGTTTACCACTCTAAGCCTCTAAGCGGGGAAGGGGCTGCAATAGTTTAAGAG
HPS4	rs5761552	cattcacaaatattaacccaaagtggattacagatctaaatttaaaagctaaaactatcaaattcttacaaaaaataaaaaagggagactatctc ctccaatggagtaggcagtggtttt[T/C]ttcaggcaagacacagaaagcaataaccataaaaagataaaatttataagttagacttcattaaa atgaaaaacttctgctcatcaaaagacaacaagaaaattaacaggcatgccacaa
HPS4	rs9613187	ATCGCCTTCTTCCTTTACCTTGGAACCATCATAAAGAAAAAAATAATTCCACCTGGCAAGAGAACAGAGTGGAGCCATGTTTCCTTTAATCCAGT GATCA[C/T]GGCATTTAAAACACAGCTTCCTGTTTCCCAGCCCGAGGAGGGCTTATTACTGTGTTTGAAAGCCTTCAGGTGGCCCCAGAGAATA AAGAACCCCAGTAGC
HPS4	rs17401652	ACAGAAAGAGCCTCAGGTCAATATCTGAGATTTTGAGGGACAGATTCTTTGGCCAGAGTCCCAACTACCTCAAAAATTGTTCCTCACACAGGAAA CTGAC[C/T]GGAAGGGAGGACCTGTTTTAAACCTATCCCATCACAGATCCACGGCGCCTGCCGCGTGGGGGTGGCAAGCAGGGCCTGCGCTTTC TAAACATCCCAGGGA
HPS4	rs16982145	GAAAGGGGTGCTGTGAGTGCATTCCTCAGCCAGGACACAGGTTGCCAAACTGCACCTCGAACTCCTGGGCCCAGTAAGGAAAGAGTCCCTGCAGG CAACA[T/C]GACTCCCAGCTTTGGAGAAGGAAGATGTTCGGGAGAGAAAGGTTGAAAAGCACTTTTACAAGAAGAAGATATAAGCTAAGAAAGG GATCTGCTCCAACTC
HPS4	rs3747129	CAATCTCCTGGCACTTGACCGAGCAGTCTCACAATAKGAAGTTAAGGAGGGTCTGAAAGCAGGGATTTGACAGCATGCCTAAAATCAKAGCTTTA CCATTAGGGTTCTGGTGGGTAACTC[A/G]ATTCTTGAAGCTCCATAACATCCATTTTCCTATTATGAGCAGAGGAAATAAACATGCAGATGGCT

		TGGTTTCCTTCGCATAACTTGTACAGGGGTAGGTAGCATAAAAGACMGCCGTTCT
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HPS4	rs8142957	atatataCATTTAAGAAATGCAAAATGAGAAAAGGGGAAAAAAAATACTGTCTTTGGTGGGTATGTCACTGACGAACTTGGAAATCCCACAGCTG ATTCAGAACCATCATGATAAGAACC[G/A]GGGCAAGGAAGAAGAGTGCAAAGCCCACCAGGGTCTATGCTCGGTTCTTCCGCATCTGTCATCTC ATTTCATTCTTCACAAGGGatgtctatttcccagaagagaaaattgaggctaagc
HPS4	rs5997095	CCGCATCTGTCATCTCATTTCATTCTTCACAAGGGatgtctatttcccagaagagaaaattgaggctaagcaacttgcccaaggccgcatggctg gcgaaagaagggaggatctggggcc[G/A]agcctgtctggcccaagggcccctgcGAGAGCCTCAGTGCTTCTCCATTTACACAGTAGTTGCCT GCTCCTCAGGAAGCTCCGGGCACAGCGCAATGGCCCTGCCTGCCCTGGGCTGTGA
HPS4	rs16982178	AACCATACCTCATCTCTGCAAATTGAAGACTACAATATTCACATTATCATCAAAGCTCGCTGCATGATCAGGCTGTGGCTATCTTTTCTACAATC TCCCA[G/A]CAATGCCAGACTCATTCAAACATTGGGAGAGGCTTGCAAGGTTAAAACATGTTAGCTTTGGAGAGCGATGGCTCACGTGCTTGAT TCAGCTGCTCTGAGG
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HPS4	rs3747135	GCCAGGAAGCTGCTGGGCGCAGCCCCCGGGGCGCAGGCAGCGACTGGTAACAGCACTAGGGCTGTGCGGTGTCTGGCCCCAGCTCTACGGGACTG GGAAATGGTGGGCGAACGCCTAAGG[A/G]YTAGGCGGGGTCGGGTCACGCTCTAAGCTGGGGGCCCGCCCCTCGGTATCCCCGCGCTCCGAGCG CCGCCCACCCACCGGAGTAGCCAGGCATCCCAGTGCTCGGGGTTCGGGACTCTGG
HPS4	rs722997	ACCATCTTTCCTTCCTTTCAGCTCACTAGATGCTGGAAGAGTCCGTGGCGGGACCATCCACAGTGGCAGCAGCCACAGATCCTGGAGTTATAAGT ATTGCATAAAGGAACTGTGGAGTGC[C/T]GGCTTTTTTGACATACTGGCAGCTATGCAATGTGCTCTGCATACAGTCTCTGCTTTGCCCCTCAC ATAAGCTCTGCAGTGGTGGTATGGTCCCATTTCACAGACAAGGACCCTGAGGCTC
HPS4	rs877593	CTGTGCCATCCCAGCCAGCCATTCTATAAAGCTCTGGGGCCAGCTACAGGAAGTTTTCCAGACTGGAGCAGGGAAAACCCTTCCCCATGTCTGGT GGAGTCATGCTCTGTGAAGGCTGAG[C/T]CACAGTGCTACACCCCTGCTACAGGGACATTATTTACTATCGCAGTGAGACACAGGAGAGGGCTG TGAGTCAGGGCTCAGGTAGCTGGCTCTTAATGGGGCAGCACAGGTGGTCAGGCCA
HPS4	rs4822721	TGGCTGGAGACGCTGAGAGGCAGCAGAACACCTGGCGCTCAAGTGTGGCGGGTGACAGGGAATCAGAACCATCCTAATCACCCACCCAGGCTCCC TGTCAGTCCACCACCCATGCCCCTC[A/G]CACTGGACCACACTGCCCCGACACTGAAATGTGTGTGGAAAGGTCATACATGTGCTTAACAGTTG GAGAAAATGCTTCAAATCCTGGCCCTGCCACTTAAGAAGAATATAACCCTGGGCA
HPS4	rs2014410	GCTTGTTTCCTCTCTGTCCTGGATCTAAGCGAGGCAATAACAAGGGCCTGCGGGTCCTTCTGGGGAGAGGGTCTGCTCTGGGAATGGGGGCTTGG CTGCTATGGCCAGGATGGTCTTCGA[C/G]CTGCTCTTGGGCTCCATGCTGGGTCAGCATCTCAGGAGCAGAGGGAGGGCGCAAGCTGCTGATGG CTGTGTCCTCAGGAGGCGTGGGTTCCAGGCTGCTGGAGGCGCTGAGAGATGCCTT
HPS5	rs4757638	ACTATTAAAAGTTAAAATGAATGGAAAACAGAATAAAGGGTTGACCAGAACAGATGTGATTTTCTACTTAAATCTTTTTTTTAAACCCCAAAATT CAAAACTGCTAATGTTTTTTAATAC[G/A]AATTTCTATCTTTGATAAGGCAATCTGAGTATTACCTTTCAATCCTTCAATAAAAGTATCCCAAA CAGAAGGGCTATTACTGTAACTAAGCTTGATACTCTCCTTCGCTCTTTTCAAGTT
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		TGGAAAACAAGAGACTTCCTTTTTC[T/A]CATTTTCCATTCTCAGCACAAATTACCTAATGTGAGAACTGAAATAAGATCAGTAATGGAATTAA ATGACAAGACTAATTGGTTTACCTTTCTTTGACAGCCTGAAGAGACACAAGAGGA
HPS5	rs2049129	TAAAGTTTCTGGCCTGGATATCTAATTCTCTGTTTGGAGCTGGAGAGAAGAAGAGAGGCCTTCACCAATATTGTGTATCTGAATGATATGAGCCT GATGGAAGGGGACAATGGTATGTCA[G/A]TCCTAACTGGTTACTCTTGGTTGTCAATTTTTAGAAGTTACTCCAGACCTCTCAACAAAAAAATT TGGTGAACCTTTCAATGATCATATATTTAATATGTGATTCCACTTAGTTGTCACA
HPS5	rs10766469	TATTTTGATGTTGTTAGTTGTGTGTGCGCAACAAAAAGCTGACAACTCCTGCTCAacgatgtgccaggagctttattaaaggctctgtctatatt acctaactttgtagaagccagtcaa[G/C]gtagatatactattcttattaaaaagagaaaaagctaaggctcagagaaataaaataatatacca aaggccattcagcatgaaatgtatacgagtcagaatttatactggatatgcagga
HPS5	rs4757637	ATTTTTAGGAAGAAAACACATACATACCTATCTCCCTAACCCCCACACCCAGTTTTATCCTCTCACAGGCTTAATCACCAAGGTCTCTTAATGTG GGCCCATGAAAAACCTGCAGGCGGT[C/A]AGCTGTGCAAAGTTTTGCATGACTGCATTTTTCTAAAAGGAATCATCAGATTCTTATAGGGTGAC CCCAAAAAATTTCAGAACCACTAAGAAATATACTTAATCTCATTACTACCATGAC
HPS5	rs3781945	AACTGGCATTGCTTATTATCCATGTCACACTTCAGTCAGTTATTTATTGCCTGGTGATACTGGCTTATGGCTCCAACTGGATTATAATCTTTTGG AGATAGCAACTTGTGTCTTTCTTTT[T/C]CCTCATAATGTCTATATCTAATTTGTATAGTGGTAGGCACTTGATACATATTTGTTCATTGTAAT GAGGGCTGAGTCTGGCCAGGTGGTGGGTGACAAGATCATGCAGGACCTTGAAAGC
HPS6	rs3737243	TGTGGTGCGAGGAGCGGCAGGCCCGGGCCGAGGGCCCGTCAGGGTCGCCAGCAGCCGCTTTCAGCCACTGTGTGTGCGTCCGGACTCTGGAGCCC AGCGG[A/G]GAAGCTAGCACCAGCCTGGGCCGCACACACGTCCTGCTGCACCACTGCCCTGCCTTCGGGCTGCTGGCCTCCTGCAGACAACTCT TCCTGGTGCCCACTG
HPS6	rs3816	CCTGGGACTTGGAGGGTCCCATGTATGGACCTGTGTATGCAATACTGTTC[G/T]GTCATCTGGAGCTATTTTTAAGATGTGTGTGTTAAATATA TACATAGTTT
HPS6	rs11191206	GTTCTTCCTAACCTGTCCTTAGGAAGTGTTCACATTGTGTAGATGTAGCAGTTTGGTTCACACTGACCCGGGTAATCAGGAAAAGAGGAGAGCCA GAAAG[C/G]GGGGAGGAAGGAGCTCTAGATTCCCAGGTCTATAAGGAGCCACCCTGGGCTGGAATGCTTGGCAGCCTTACTGCCCCAGAATGAA GGGGAAAAAGTCCTG
BLOC1S3	rs7254936	CCGTGTGAAGAAACTCAAACAACACAGTATCCGCCATGCCCCCTCCTCGCACGCCTAAAGATGCGCCCAGCGCTTCCAGCTCTCATTGGCCAGGC TTGGTAAGCCACGCCTCTTGCGTCA[T/C]GGCGCGAGCCCTCCTACTCCGGCTTCCATTGGTGTTTTAGACTAGCCCCGCCTCCCCGCCCCGGA CTCAGGTGGTGTGCGCGTGAGCCTGGGACACTAGGGGGCGGGCCTACGCCTTCTC
SLC24A5	rs17426596	CAGGTAGATGTTCAACAAGCAACATGACCAAAGGCAAACAGAATAGAGGGCCTGGCCTAAATAATTCTAACCACCCCAAGAGAACTTTTCACAAT GGTTT[T/C]TCAGGTGTCAAAATAGCTTGGATTAGGGCTGGTTGGATCTCTCAGACCAAATTACCCCAAGGGATAAAATTAATGACTGGAGCTG GTATCATCAGCAGAC
SLC24A5	rs2675347	CCTTCCTGTAATCCTATCTTCTTTTTCTGTCCATTTGGTTCTACAGGAAAAGAAGCTAATAATTCTCTGAACACAGAGGGCTGAAATGAAATTAA TTGGTGTTGTAGAGGGGACACAAAT[G/A]TATCACCCTCTAGTTGTCCAGGGCATTTCACGTTTGGAGGAAAGCCTTGGCTTCTGGGCTGGGAA ACAGAATGGAGATTCTGGGAAAAGAGTTAAAGATGTGATCAGAAGAGGAGCATTA
SLC24A5	rs4775737	TAGGTTTGGAGGTAAAGTGTCTTGTATAAAAGAGGTACACTTCATAAGATAGGCTAAGAGCAGGTAGAAATCCACTGTAATATTACAGCTGTTAG ATTGGGGATAGGGAGAGTCTTTTTC[G/A]GTGGAATCAGCCTTTAAAAGCATTACAGGTTTCAGAATTCATTCTTTCATTGCTCAGACAAAATA TGAGTTGGTAAATGTAAGGTAATCAAGTTATCTTACTTATTGTTAAAAACATCCC
TP53	rs2909430	tgctccagcctgagtgacagagcaagaccctatctcaaaaaaaaaaaaaaaaaagaaaaGCTCCTGAGGTGTAGACGCCAACTCTCTCTAGCTCG

		CTAGTGGGTTGCAGGAGGTGCTTAC[A/G]CATGTTTGTTTCTTTGCTGCCGTCTTCCAGTTGCTTTATCTGTTCACTTGTGCCCTGACTTTCAA CTCTGTCTCCTTCCTCTTCCTACAGTACTCCCCTGCCCTCAACAAGATGTTTTGC
TP53	rs2078486	GAGTTGAGGAAAGTGCTGGGCACACAGTAAGAGCTCAACAAAGGTTAGCTCTTTCTGCAATTGTTCTATTTCACTTGTTCTATATTATTATTCTA GAGAGAACTGTGTGATTGTTAGTGC[G/A]GATCTGTGGTACTGCTCCCACCCCCACTCCATTAATGCAAGTACACCTCCTTCAGGGATCTATTC AGTCAACAGGCCAGGAGGTGCTGTCCTGAAATGGGGGGCCCAAAGTCTCAATCCC
TP53	rs8064946	gcatcacggagcggttaggggccaaaactcatcttcctgtgcacttgctgtgtgcactggcgctgtgtgtaaatgccacctcgatttaggaaaaA GATGACGTAAGTACGGCACAAAGTG[G/C]CCGGTACGCGGCAGGTGCATGGGAAGAAACTGCGGAATGAAACAACCGCGAGCTAAGAGATGGGG CAGCGGGAGAAATGAATTCGAGTTCCGCCTCCTACCAGGAAGAACCGGCTCGGGC
TP53	rs1042522	AGTCCCCCTTGCCGTCCCAAGCAATGGATGATTTGATGCTGTCCCCGGACGATATTGAACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCT CCCAGAATGCCAGAGGCTGCTCCCC[C/G]CGTGGCCCCTGCACCAGCAGCTCCTACACCGGCGGCCCCTGCACCAGCCCCCTCCTGGCCCCTGT CATCTTCTGTCCCTTCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGG
TP53	rs12951053	TAGGTGGAGGAGAAGCCACAGGTTAAGAGGTCCCAAAGCCAGAGAAAAGAAAACTGAGTGGGAGCAGTAAGGAGATTCCCCGCCGGGGATGTGAT GAGAGGTGGATGGGTAGTAGTATGG[A/C]AGAAATCGGTAAGAGGTGGGCCCAGGGGTCAGAGGCAAGCAGAGGCTGGGGCACAGCAGGCCAGT GTGCAGGGTGGCAAGTGGCTCCTGACCTGGAGTCTTCCAGTGTGATGATGGTGAG
PRKAR1A	rs3785905	CTAATTCATTTTTATTTTACAGGTAATGAATGATATGACATTCTTGTGATTTTTATTTTC[C/T]GCCAATATTATGCCCAATTCCTAGCATTTG CAACCAGTATGTTGACATATTTCACAAAGC
PRKAR1A	rs8905	CTTTTTTTCCTCCTTTTTATTCAGCTAGAATTTCTGGTGGGTTGATGGTAGGGTATAATGTGTCTGTGTTGCTTCAAATTGGTCTGAAAGGCTAT CCTGC[T/G]GAAAGTCCTGCTTTCCTATCTAGCATTTATTCCTCTGGCAAACTTTTCTTTCTTTTCTTTTTTAAAGTAAACTTGTGTATTGAGT CTTAACTGTATTTCA
PRKAR1A	rs6958	ATTATTTCATCTCTTTTTATTACTTGACCTACCATTAAGACAATCTATAACAAAAAAAGTATATTAGCACACATGGTATAATCAGACTGGTAATC AGGAAGCAATACAAAACGGTCCTTT[G/C]GCCATCTATACTTTCTAGAGCAGTAAATCTCATAAATTCACTTACCAAGCCCAGGAATAATGACT TTTAAAGCCTTGAATATCAACTAAGACAAATTAT
PRKAR1A	rs2952275	TGTAAGTTAAGAGGAAAACACCAATTCCAAAGGGGAAAAAAAGAGAGTAACACTTGAAACACTCTCAACAGAATGTACCTCAGATGATAACAGAT GCTATCCTTTATTAATGGGCAAAAG[T/C]CATTAGTACAAGCCATTAGTACTCACATAACATGTGAGTAACAAATCAGATATTCATTAATGTCT TGACAACAATTATCTGTGATGCACACAACAGTAACACACTGCAAGACAAAATCCT
PRKAR1A	rs4281788	CCACTTTTAAAACTAGTGTAATGAAACATGAATCTAGCTTGTGACTGATATATCAGAAATTTTCAATTAGATTCGGTCTATTTTAGTATAGAGTA TATCTGATAAATTACTGTAGAGATA[C/T]TGATATTGATGTTCCTCAAAAATTTTACTCCATTTTATTGGTCAGGACTTGTTGCGCCTTGAAAA AAATTAATCCAATTTTTATTCTTTTTTGCTCTTCCTCCTTGCTTGTATGTCATTG
PRKAR1A	rs11651687	AACCTTGATTACAAAGAGTGATCCTAGCTCTGCTGGGAGAAGTATTATAGATACGATCTCGTTTAAATTCGTGGTTACCATCACATCATTTCATT ATTTGTTTTTATCTGGGATCAAACT[G/A]GAATGTGAATTCCACAGGCTCAGGACCCTCTCCTATTTGTTTTTAATTTCCTGTTGCCTAGCATA GGTCTTGGCCTGGCAGATCCAGAAAATGGCTTATTGAGTTAATGAAATAACTCAG
PRKAR1A	rs16973011	AATGAATGAGAAAGTAGAAGATAACACATTTTTATTTTTTCCCTTTTACTTCAGGCACAACAACTAATTCTTTAGATCTGAGAGGAATTTGTTTT TAAGG[C/G]TCTAGATGTGAAAAAAGGAATGTTTAATAGTTTATCTTAGGTTTTATTTGAATTGGTCCAATAAGTAGTTTTTAAAAGCAAGTAA ACTCTTTTTGTCTTT
PRKAR1A	rs8080306	aaaggagggagaaaaggcagaggcgtcaagggaggccggagggagagtggggtggacagaggagcggagggacgagagggaagCGCACGATAGCT

		GCGCGGAGAGAGAGCGAAGAGCAGG[C/A]GGAGGAACAAAGGCGACCCAAGACACCCAGAGAGGGACAGGTAAGGAGGGGCAGGTGAGCAGGAA GGAGGAGAGAACCGGGCTAGGGAGACTGCGGAAAGGAGAAGTGGCGGGCGTGAGG
ERCC8	rs4235483	CTGTCCTAGCTGACTGGGTGAGCGGGAAGATCAGCTTCTATAGACCACCACCACCCACTCACACTCTGCCTGCCCATCTCAGTGCTGAGAATTAA GGAAATGACCAAGTTCTTTGACATC[A/G]AGGATACCGAGCAAGTCAATGAAGACACCATGGAATGCTTGGCCACTGGAGAATCTGATGAGCTA TTGGGTGACACAGACCCACTCAAAATGGAGATCAAGTTGCTCCATTCTCCGATGA
ERCC8	rs2306351	CAGGAGACAGTGAATTTCAATCCTTTTTTACTGTTATTACAAACTTTTCCATAGTTCACCTGTAGGATTAAAATAATAAGGTTACTCATCTCTGA AATCTGAATTTAAAACACAACAAAG[A/C]CTAGGAGAAATACTTAAAATATTATGCTATAAAAAGGGCTCTTTTAAATTTAAGGAAAATATTCA ACGTTGACAGAACAAGGGCCAATGACATGAATGTACATTTAACAAAAGAGGAAAT
ERCC8	rs158937	gttttggccatctagccagaaactttagggttcagttaccctgttctgccatacgcttccaagtttaggcccaagtctgggctcagaagtgagaa gagaggcaataaacaacagggattg[C/T]ccctaccctcttgggatcgggactctgatgaacagagaaaacaatttcccttcctcagagttctg gcttctgcaggccACTATGCGTAGCCATAGCCATTTCTGTCATGGGATtgtctgt
ERCC8	rs976631	GAGTCTGCGTTGGTGACATATAGCAAAACCCAAACTAATGTTCCTGTGGTGTGACCCTGACTGTGTTCTCTATCCTGACTTCTTTTGTTTTCTGC TCATTTTCTAAGTCTGATTCTCCCA[C/T]CTTCCTGACAGTTATGTGAGATACTAAATATTCTTCCAATAAATTTCGCTTAAGATAAGCAGATT GATT
ERCC8	rs4647128	CAAACTATTTTTTTCTTTCTCTGACATTAGAAATATATGGATACTATAATTGATAAAACTGCTAGGATTTGGGGTGTGTTTGTAAAATTTTCACA CCATTAGTAAGCTCTGACAAATCCT[A/G]ATACTATTTTTAAATACAAAACAAAGCTTTACTAACAAAGAAACAAAGGAAAAACAGCATTGGCC TAGTTTCTTTAGGGAGTAGAAATAGCATTAATCTAATTATTATGACCTAATTATT
ERCC8	rs158938	AGAGCGAGACTCTGTCTCAAAAAGCAACTAAAAGTTATCTAAAAATTAAAAAAAAGAAGTATTGCAACCGGGGAATCCAAACAAACCAGTCTATC AGAAGAAATATCTTTGTGCTAAGGT[T/C]CATAGAAATATTTTACCCAATTTCCCTTCAGGAAAAAAATTAGGAAAGAACGTTAATCAAAAACC AAATGTATTTGAATGAAATCCGAAGGAACATTAAAGATGAAGAGGAGAGGAAACC
ERCC8	rs966497	tctcacataggtctcaatggattataatcaaggtgttgacaggctgcattccttttggaggctctaggagagaatactttctccatcttccaga gaccacccacatttcttggcttgtg[A/G]tcccctaatccatcttcaaagctagcaattttacatttctctggccattctcccagtcaaattt accccgtctccttctgcctcccacttccatttttaagattcttatgattacatt
ERCC8	rs17332991	CTGTGCTTGAACATATTCATCTATATCTCACCAGGTTGTCAATTCTACTGACAGATAGTGTCAATGTTATAGAAATAATGAAGCATGAGTTTAGA GGGGA[C/A]CTAGTTATGAGTTTAGAGAAGGAATAGAACCAAACTTAACATCTACAAGGATATTTCTTGATAACTTTCAAATTTCTGACTGGTA ATTAGTGGCAACTCT
ERCC8	rs976080	TCACATAACACATTTGCATGGCTGTTCCTTGACACTTTCTTGAAAATAGTAACTGACATTCAGGTATAGTTTTTTAACATGTGTCTTCCCCAAAG CAACTTCAGTATCAATTTTCATTAG[T/G]ATTTTATTTTTAAAAAACTTGTGATTATGTCCTTCTTTTTCTTTACCTACATAGCACATCTCAGT AACTAATTAGAGTTTTTAGTACCACTTAGTGTAAGATTCATTTATAGTCCTTCAG
ERCC8	rs7726671	ccccaatcctaccgaaaataatcttcattttaacaagatccctaggtaatccctaggtaaacactaatgcaaatttgagaagcTTTGGGCTAAAT AAGTTGATGGGTTTACTTCCTGATA[A/G]TAATTTTGATAAGCTTGGGGTGAGTGTAAAACCAGTAACTAATCATTTGGGAATCATGATCATGC ATATCAACAAAATCCACTGTCGTCAGGGCTAATATTGAAGTTTAAATAGTGATTT
ERCC8	rs158932	tcatcaaattgtatattctcatttaatgtgcagggtattgcatgtcaattatacctcaataaaGTTGTAAAATGTTTTTTTAGAGGGTGTAAACT GTGAACACTGCATATCAGTGTTCAT[C/G]CTTTTGCTCTTACATGATGACAAGGAAGAACAGTAGACATCTCCTATGAATAAAATGGAACTATA TAATTGTTTAAGCAAAAACATCACTTAATGATGGaaatatttttaaaaaaatttt

ERCC8	rs1038144	TTTTCTAAAATATTTTTACTGAGAAAAAATGTACCTAAATATTAAGAACAATGTTTACTCATTAGCCAGAATATTAAATAATACATATGAGACAA GTACACTGATGTGAGTTGCATTAGT[C/T]TTTTTAATGTAGAAATAACACAAGGTTTTGAGTTAGAACTATTTTTCATATTGTTTGAGCTTCCC CAATGATCATTGTTCATATAAATGTGTTTTCAATGGTTCAGTTTTATAAGAATAA
ERCC8	rs4647068	TCTAAAAGATGAGTAATAATTCTTTTCTTTTTCCTGACCTTTTCCAGGCTCAGTGTAACACCTTCTATTCAAGGACAGCTGGGGGGGCACTCACT TATGAAACATGCAGACATGAGCAGC[A/G]GGAGATAGCTGACTTTTGGGTTTGGATGCTGGTCAGGAAAGTAATCAATACTGTTACTCCAGCCA GCCACTTGGTGTAGTAATCAGTTGTGTGGCCTGTGATGTATGAGAAGCCAGTAGA
ERCC8	rs4647078	GAGGGAAAACTTTGTAAACATTAACATCAACTATCTATTACAGCTCTTCCCAAAGCATTAGAATATATGCTATGGAATATTTCGGAGGAGATTTG CTATTGTTTGTTGGCTCTGGTGTTT[C/T]GCATTAGAAATACAAATTTGGGAAAATCAGTTAATCTCAGCTTCACATTATTTTATTTTAGAAAT CAGTATACATGCCCTGTGGTTCTTATACGTTTCTATGAGAATTAAATGAAATACT
ERCC6	rs1018603	AACATTTTTTAAAAGTCCTGCCCGGTGACCCCACGCTACACTTCCTAAGTCAGCGTCTCCAGTGTTAGGGGCTCCCAGTGACACTGCATAATCAG TCTGTGGGTGGGGAAGCTTTGCTCT[A/C]GTCTGTGCTCGTTGCCCTCACTGTAGTCTGCGAGGGCAGCAACTTAAATAGTAACTGCCGATACA TGAAAATAGAGTAAGAACTTGGGGGAATGAGTTTAAGTCAAATGATAAACACTAA
ERCC6	rs2228526	AAATGATGCCACATCATCTGAAGAGAAATCTGAGGCTAAAGGAGCTGAAGTAAATGCAGTAACTTCTAATCGAAGTGATCCTTTGAAAGATGACC STCAC[A/G]TGAGTAGTAATGTAACTAGCAATGATAGGCTTGGAGAAGAGACAAATGCAGTATCYGGACCAGAAGAGTTGTCAGTGATTAGTGG AAATGGGGAATGTTC
ERCC6	rs3810945	AGAGCATACTGAATAGCAAAGTAATTAATGTGTGCTGTAAACTGGCTAATTATCTCATTAAGGTTTAATCATTATCCTGTATCTCTTACTATTTT TAACAACACCATCTCTCAXATAAAA[T/C]CAATCACTTCTCCTGAAGCCACTTTGGAAAACAGGGGATGAAAGCAAGGAGGAAGGCAAAGTTAT AACAGTATTTCTCCAAGTCACCCAGATTTTGCTGCTATAATCCCTCCCTG
ERCC6	rs4838519	AGAAAACACTGAAAAGGCAAAGTAACACCCTATTGGACAATAAAACACGCTCCAATTACAACAGTTTGGTGCTGAGGAAAGAAGAGACACAACAG GAGATTGACCAGAAAATAGAAATAG[A/C]TTGAACTGCATTTGGAAATGTACTACATGATAAAGGCATCTCTAATCACTGAGAAAAACTTATTT GAAATAAATGATGCTACAAACGTTGGGGCACAAACGTTGGGGCATTTTATCTTTG
ERCC6	rs2228528	GACTCTGAGGGTGAAGAGTCTGAGTATTTCCCCACAGAGGAGGAGGAAGARGAGGAAGATGACGAGGTGGAGGGGGCAGAGGCGGACCTGTCTGG AGATG[A/G]TACTGACTATGAGCTGAAGCCTCTGCCCAAGGGCGGGAAACGGCAGAAGAAAGTGCCAGTGCAGGAGATTGATGATGMCTTTTTC CCAAGTTCTGGGGAA
ERCC6	rs4253211	GAGAAACATCTGAGACCAAAGCAAAAGCCTAAGAACTCTAAGCATTGCXGAGACGCCAAGTTTGAAGGAACTCGAATTCCACACCTGGTGAAGAA AAGGC[C/G]TTACCAGAAGCAAGACAGTGAAAACAAGAGTGAGGCCAAGGAACAGAGCAATGACGATTATGTTTTGGAAAAGCTTTTCAAAAAA TCAGGTAATCCATTT
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ERCC6	rs2229760	TACAATATGTGGAGGCAGAGGCTGAGAACTGTAACTGGTCTTAAGTGTGTGTGCTCAGTGTTGTGTGTCTTACCTCTAGGACACATCCATATTTG TGTTTCTTCTGACCACGCGGGTGGG[C/T]GGCTTAGGTGTCAACCTGACGGGGGCAAACAGAGTTGTCATCTATGACCCAGACTGGAACCCAAG CACGGACACGCAGGTTTGTTTTTATTTTTTTTTTAAAAGAATGTATTAGTAGAAA
ERCC6	rs4253231	ATCTGTGCACTTTCCATAGAACTTCTGGTGGTGAAGGAATTTGGAAACTCAAGCCAGAATACTGCTAAACAACATTGCTTCCTAAACTTTCAAGT CCCTTTTTCTAACGGGCATTTCTGa[C/T]tattaatttattattaataatCATGTTTGTCAATGGAAGTTGGCTGCACTTGATGTTTGTTTGCA

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ERCC5	rs1998876	ATGTAAAATTAGACGACCTGAAAATATTCACATAAATTGTTAGACAAGAAATATGAAAAATGAGACAATCTGAAAATATTTGTATAATTTGACTT GTCTAATTTCAGTAAGTTTATATAC[A/G]CAAAATTAAATTTCTGCGGACAGAAAAGTTAAAATCTGCAGCAAAGCCAAAAATCACTTTCGGTA TAAGACCCAAAGACCAATAACATTTCAATACAAAAAATAAGTTTCTTTAGTTCTT
ERCC5	rs4771436	TGGACCTACATTTTTTTTTATGTTAGTAAAAGTTAGGCAGTGTACATTCATTTAAATACTAAACTGTTCAACTTATTTAATAGCAGATATTTATT GTGTA[G/T]AAGAGTCCCTGTGAAAGGGAGTGCAGTGCACAAATTGCCCGGGCAAGCCATGGAGCACACAGGCTGGGTTTTTTCCCTGCTAGAT TATAAACTCCATAGGG
ERCC5	rs7325708	GTAAATCTTAAAGTTATTATCCTGTCCGGGAGGTCAGTAAGGAGAGCAGAGTAGACTTCGACGATTAGTTTTGCTTGAGTCTTGCCCCATTTATG TTTCTTAGAGGAAGGATAGTGTGGA[C/G]AGGTGTTTTACCCATTTTTTAAATTGACTTTTTAAGGACTATTGTTTCTGTACATGTTTGGCTGG TTTTGTTTGTCATTGGAATTAAATTCTTTTTTCATTAGCAAAACGTGATACTGCT
ERCC5	rs4150386	CTCATTTGAGATGTGGACTAAAGACTTAGTTGACAGAGATGGTACAAGTACTGCTTTAGGTATGATTTAGAAAGTGAAAATTACTGTCAGTAATT CACTGGGAGAGAACTGGGTTTTGGG[A/C]GATAATGAATTAATATTCTCTGACATAGTAATCCAATGTGAGTGATCAAGGTTGAGCTTGTTGAT TTGGTTTAGAAACTTGACTTACTTGTCTGATTTATTATTATTATTCTTTTGTTAT
ERCC5	rs4150351	GCCAGGCCCTCCTGCTTGAAGGAGTAACCATAGCTTTGGCTTGATGAGCTCACAAGTGCACTGATTCTAACTGCCAGTCATGATTACAGTTTCAA AAACGAAAATCTGCAAGTAAAGTAG[A/C]GGAGAATTATCGGTATTCTCTCAGTGCACTCCTGGAAAGAATGTTGTAAAAGTAATGAAGAAAAA TTATTTTTTCCTTTTTGCATTTTGTTGTTATAATACTTTGAAGACCGCAATTAGT
ERCC5	rs873601	ACTAAGACGTGCGAGGGGAAGAAAAAGGAAAACCTAATTAAAAAATATGTATCCTCTATAATTAGTTATGACAGCCATTTGTAATGAATTTGTCG CAAAGACGTAATAAAATTAACTGGT[A/G]GCACGGTCTTTGTATTTAGTGTGTGGTTCCTAAAAACAAATGCTAAATCTGACATTTGTTTTTTA ATGTTTTACTTTTCTAGTATTTTTTAGCTGAATATTTCAAGTATCATTGGATATT
ERCC5	rs2227869	GTGATGAGTCTATGATTAAGGACAGAAAAGATCGGCTGCCTCTGGAGAGTGCAGTGGTTAGACATAGTGACGCACCTGGGCTCCCGAATGGAAGG GAXCTGACACCGGCATCTCCAACTT[G/C]TACAAATTCTGTGTCAAAGAATGAAACACATGCTGAAGTGCTTGAGCAGCAGAACGAACTTTGCC CATATGAGAGTAAATTCGATTCTTCTCTTCTTTCAAGTGATGATGAAACA
ERCC5	rs2296147	GTTTCCGTCGCCTGCGTGGCCCTTGCACCCCGGTCTTCCATTAGCGGCGCAGACGTTTGGGCCTAAGCGCTGGGCGAGGCGAGGCCCTGCCCCTC CCCGCCAACGGCCATTCTCTGGACC[C/T]GTCTTTCTTCCGGGAGGCGGTGACAGCTGCTGAGACGTGTTGCAGCCAGAGTCTCTCCGCTTTAA TGCGCTCCCATTAGTGCCGTCCCCCACTGGAAAACCGTGGCTTCTGTATTATTTG
ERCC5	rs4150383	TCCACCCACCTTGGCCTCCCAGAGTGCTGGGGTTACAGGTGTGAGCCACCATGCCCGGCCAAAGAATATCTTCTTAAAAGTGAATTTACTGAATA

		AAAGGCATGAATATTTCTTACAGTT[A/G]CTAATATATACTGTGAACTTGCCTCTCAAAGGTATTGTATGATGATAATGTTTTTAAAAGAAAGA TATAGTAGGACTTAGAAACAGGCCCCATGAAGTCGTGTTGCTCCTGAGGAAGATG
ERCC4	rs3136130	aggcggaggttgcagtgagctgagatcacactactgcactccagcctgggcaacagagtgagactcggtctcaaaaaaaaaaaaaaaaaaaaac acaaaaacttttaaagtagcctggc[G/T]aggtgatgtgcacttatcgtctcagctacttgagaggctaaggtgagcccaggagttgaaggtga caccaagatatgatcacaccactatactccagcctgggtgacagagtacgactgt
ERCC4	rs1800067	TCTTCCCTTCGGGTGAAGGAATAAGGGGGCACAGGGAAACTAGGAGGACAAGTGAGGTAATAGTAACATAATGTTGTTTTCTATTTTCAGGTCAA GTACTGATTTGTGCAAGTGATGACC[A/G]AACATGTTCCCAGCTGAGAGACTATATCACTCTTGGAGCGGAGGCCTTCTTATTGAGGCTCTACA GGAAAACCTTTGAGAAGGATAGCAAAGCTGAAGAAGTCTGGATGAAATTTAGGAA
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ERCC3	rs4150471	TTTGATGGAGCACATGTTCCAGTAGGTTCCTGAGAGAAGGGATGTAGGAGGAGAAGCTTTGGAGACTTCACATGTTTACAGATGTCTTTACTCGA CTTTA[C/T]ACTTGATCAGTAGTTTGGCCAGGTCCTAGAACTAACATTGGAGCTTCCAGTGCAGCAGTTTGGCAGTCTGATGCCCTTTGCTTCC TCTCAGTGTGTTGTC
ERCC3	rs4150506	GCTACAGGGCTTACAGTAACCAAAACAGCATGGTACTGGTACAGAACAGGCACATAGACCAATGGAGCAGAATAGAGAGCTCAGAAATAATGCCA TGTAC[C/T]TGCAGCCAACTGATGTTCAACAAAATTGGCAAAAATATGCAGTGGGGAAAGTACTCCCTATTCAATAAATGGTGCTGGGGAAACT GGCTAACTGTATGCA
ERCC3	rs4150459	ACTCAGTAGCAGTGCTGACTCGTGTGTGGAAGTGTGGCCTAAATGGAAGAATTGCTGACACTACCAATTGAGAATCTGGTTTCGCACACTTGCAG CTGGTGTGAAAATAACTCCCTGGAT[A/G]TGATTGCATTTAAAAATCTTTTAAGTGGTTAACATGTGAAAACGTCCAGTTCAATAGGAGGTATT AGGTTGGGTTTAGTTTTGTGCATGCATTTATTTTCAGTTGTACTGATTTCTAATT
ERCC3	rs4150403	CCAGCAATTCCACCTGCCCTGCTAATGTAACAATRGGCATAGGTGCTCCATGTTGTCAGCCTGTGGCAGCCATGAGAATGTGCCATTCAGATTTC CTGTG[A/G]GGAGCATAACGACTGATTACCCCAGCCGCTACACTCTGGATCCAGCATTGTGTTCTGGGTGAGGCCAYGCTTCTCATGAGCCGCT ACACTCTGGATCCAG
ERCC2	rs1618536	CAGGCAAGCACACTTCCTCCAGGGAGTCTTCAAATTCTACCCAGGCCTAGGAGAGGTTAAAAAGGCAGCTCTTAGAGTCTCTGGCAAgcctggtt tcctggctctgaaacttactagccc[G/A]gtatttatggagaggcattttctgccacctgggcctcagtcttccttttctgtaaaatggggataa ttatgtacctgcttcataaggttgctgatgataaaattgggtgaaatgttgcagc
ERCC2	rs238415	TCCAGGTATTGGAAAGGCACGCATGGGAGGCGGGAACGTCATCCTCACAGCCAGGACATCGGCCTTGTGCTTCAATAGGCGGCTTCCACGCGGCG GGAAAGGTGACCCAGTCCCCACAGC[C/G]CAGgtgccttttgtgtcctacttaagacacctttgcctaccccaaggtcatagagagaggctcct ttgtttccttctaggagcttgtggttttagctttcacatccaggtctatgaggta
ERCC2	rs1799788	CCTCCCCTCCCCTCTGTCCCTAGGCACCCGGCGGGAACACACCCTCTCCCCCCAACTCAGACACAGCATCCTGGGTYGCGTGAGGCTGTCCCTGT CCCACCAGCCACACTTGTAACCCCC[C/T]TCGCCCCCTCTCCTCTGYCYACCAGTGCACCACTACGGGCGGGCCGTCATCATGTTTGGYGTCCC CTACGTCTACACACAGAGCCGCATTCTCAAGGTGAGTAGCTCTGTCTCCCAGGGA
ERCC2	rs13181	AGACCTTCTAGCACCACCGCCGCTGGGAACCAGGGCCAGGCAAGACTCAGGAGTCACCAGGAACCGTTTATGGCCCCACCCGCCCCACTCAGAGC TGCTGAGCAATCTGCTCTATCCTCT[T/G]CAGCGTCTCCTCTGATTCTAGCTGCTCCAGGCTGAGCAGGGACAGGCCCAGCTGATCCTCCTGCA GAGAACAGAGGAAAGGGAGAGGGGGGCACTGTTGGGCAGGGGCCCAGGCAGCTGC

ERCC2	rs3916874	atccacttgctccagtaaaagctctccccactgaactgcACAGGTGGCTGTGTGTTTCTGGACTCTCCATTCTGTCCCAGTGGTCTGTCCTTGCA TTGGCTCCACACTGTCTCTATTGTA[C/G]TGTCATATGGGaagtctcagggcagcctctgattggctctgcctgcctcacagtcagcccctcca ccaatcgctggcaggggttggggtgccctgattggccacccgcttgggccacatg
ERCC2	rs50872	ATCCCTACCTTTGTCATTCTCTACCCACTATTTCCCATCTGCCAACACCTCAtggtctttcacctggatcatccttcctcatcttcctcatcccc caaccacctccccctcatccttagg[C/T]tctcagcttagtagagatgtcttgtcctccaggaagccctccctgacccccaggctgagtcaggc ccctcctctgggcacctctgtcccagcactgcccactctcagtcatcactatctg
ERCC2	rs50871	GGGCTGGGTAGCGAGGCCGGCTGGCTGCTGCTTCTGCCTCTGTCTCTCTGTCCTGCCCCATCTTCCATCCCTACCTTTGTCATTCTCTACCCACT ATTTCCCATCTGCCAACACCTCAtg[T/G]tctttcacctggatcatccttcctcatcttcctcatcccccaaccacctccccctcatccttagg ttctcagcttagtagagatgtcttgtcctccaggaagccctccctgacccccagg
ERCC1	rs3212961	CAACCTGCACCCAGACTACATCCATGGGCGGCTGCAGAGCCTGGGGAAGAACTTCGCCTTGCGGGTCCTGCTTGTCCAGGTGGATGTGGTAAGCA GGGGCTGCTCCCTAGCCAGCCTCAC[A/C]GGGCTTTGAGGTTGTGCAGTGGGCTGTTGAATCCCTTCCAAGAGGAAATGCAGCTAAAACCTCAG CGAGGAACGTTTTTCACATCTGAGATGACATGGGTCACCAAGACATTATGTTGGC
ERCC1	rs3212948	TGAAACCCGGGGCAAAATCCAACAGCATCATTGTGAGCCCTCGGCAGGTGAGGAGGGAGACGGAGAAGTGAGGCCTTGAGGTTTTCAGTGGGAAA CGCTGTTCTAGGGATGACTCCAGTG[G/C]AAGGAGGTCTGAGTTCCAGCCACTTTGTTCTCCCcattcattcattcattcattcattcattcGA CAGCAGTGTGTGGGTTGAGAGTGCCCCAGCTCTACTTGCTGGGGCTGTGGCTGTA
ERCC1	rs2298881	CCCCTTTGACCTCCACCTTCGGCTCGCCCCGCCCCTTACAGGTCCACAAGTCCCATCGCTCCGCCCCTCGCCCCCACCGGATTCTATTGGCTCCG TCCCCACCATCCCCCGCCTTCCGTT[C/A]GTCCGGCCCCCGAGGCTAGCATCTGGACGCCCTCCCCACGCCTGGCCTTGTCCATCTCTCAGACT CGGCAATGATTGGCTTCCGCGGCGCCAATCTCCACCCGCAGTCTCCGCCTCCCGC
ERCC1	rs3212955	GTGCTGGGCCAGAGCACCTGTGCCCTGTTCCTCAGGTGAGCTCTGCGGCGCCACCCCAGACTTCAGGAAGGGCACCCCACTGGCCTGGGAGGGTC ATGTCCCAGTGTTCTGGACTGTTCT[G/A]TGAGAAGCCTCTGGGCATGGTCCTGCAGGTTCTGGGCCTTGGTGGGACCCTGTGTTGGAGGCAGG GGGTCTTGGCCTGGAGGTGCCCAGTGCAACaagagctggagcccgaactcctggg
Gnas	rs919197	TGACCCTAAGCATTTAGGGAAATAGGGCTGTGCTCACATTTTGAATAATGTAAAAATACTATATTTTATTGCTGTAATTTTAAAACATAACAAAG AGGAA[C/T]GGTGAAGACATAGCTACGCCTCCTCTCTCCTTGGTAGTTGTTGATTGATCTATTTCTGCTCTCAGGTAAGCGACTCTAGTAGCTG CCTAGTGTTACTAGA
Gnas	rs8121252	GAAGAGCAGTGGTGCAGTGACCTGTGAAAGGGGCTTCACGTAGGAAGGGGGCGAGATGGGATCCTTCAGCAGGTGAAGGCCCAGAAGTACACAGA CATTTATTCAGAAAGAATAAATTTG[T/C]AAGTATAATTCTTTGAGAAAGGAAAGACCAAATGATTTGTGGAAGGCCTGTCGACAGGCCCGCAC TTGACCCCACCGTCCGTCCCCGCCCCCCACCCCTGCTGCTCCACTGACTGCCCTG
Gnas	rs6026593	TGTAACCTTAATTTAATAATAATAATCATTAGGGCTTTGTTCAAGATGGATGAGCAAAATTCTGTCACCCCTTCTACATCTTAGCTCACCTGTCC TCACAAATAAACATCACTCTTGAAT[A/G]CTACAATCTCACTTTATTAGATTGTAAATTTTTATGAGGAAAAAGGTCCTGAGCTATGGCAGGCT TAATTATTCCCTCATTCACATCTTAGGACAAAACTGTATGTTAAATATGGCACAC
Gnas	rs7121	CCAGTGCTGTTCCCTGACCGCTTTGCTAAATCATTTTCAGACCATTGTGGCCGCCATGAGCAACCTGGTGCCCCCCGTGGAGCTGGCCAACCCCG AGAACCAGTTCAGAGTGGACTACAT[C/T]CTGAGTGTGATGAACGTGCCTGACTTTGACTTCCCTCCCGTAAGCTACACCCCGACTTGTGTGGC CTTAGCCCCGCCCACCTGAGCACAGTGTCCATATAGGAACATGAGTGACAGCCCT
Gnas	rs6092704	TTTTGGGGGAAAAAAATGTAATGAAATGACCAAAAGAATTACACAGCATTAATTAAAAATGGAAGTTTTCCACTTCCTTGATAATTTGGCTATCT GAATA[A/C]ATTTGTGAATTTGCTAGGTTAAGACCTAGTTCGTGGTCACATTTCAACAAAACAGCTTGAGTATAAAGAAAATAATAAAAGGCTG

		TTCTTATTTATTTTC
Gnas	rs3730168	TCCCTCCYGTAAGCTACACCCCGACTTGTGTGGCCTTAGCCCCGCCCACCTGAGCACAGTGTCCATATAGGAACATGAGTGACAGCCCTGCACAT GGGCA[A/G]GAGCATCCAAACCACACTTCAGGCAAAACTACATTTCAGTGATGTCCATCCTTAGGAAAAAGTTAATTTCATGTGTAACCTTAAT TTAATAATAATAATC
Gnas	rs2295583	ATGATTGCTAAGGCAATTTGCTAATCTGCCCCGATTGGGCGTGTCCTCAGGGCACATTTGGGAGGTTATAATTTGCAACTATGTTTATTCAGCTA CCTCC[A/T]ATCTTTGCACAGATCCGAACCCACAACTCCCTGAAGAACAGAATACTATGCTTTTTAGTCGGGATGTCTTTATGAAAGCAGTACT CCTAACTGACATGGT
Gnas	rs13831	TTGTCATATCTTGCTCAAATGCCAACTTTTGGTACAGCAGATTGTCAATTAGTCATTTCTTATAGAGATGCCTTTTTGGATAGTTGTCATACTAC ATTTCTGCCAATTAAGGATTTCAGT[C/T]CATTTTTCGTTTTTTCAATCATCGTTTAAGGTCAGGTAGCAGGGGCCCCATCCCCCCTCCCCCCT CAGATAACAGAATAGTATTCTTATTAGAAGTTCCTAAGACCGAGCAATTCCAGCA
Gnas	rs234630	TAAAAAGTAAAAAGGAAGGGATACAGATTCTCAGTAACTAAAACAATCTCGTGTGCCCTTGAGGGGAAAGTCCTTGATGTTTTTAAGAATGTCAC TTTATTGTTTTTTAACTGAATGATA[T/C]AGAGGTATACAATTTTCAAACTGTTTGCCATTTTAATCAAGCAATTTGAAAATTAAAATGTTTTT GTCAGGCATTACCAAATGGCACAGAATGTGATAGGCCAGCCTGGTTTTGGGGTCC
Gnas	rs2057291	CCAATCCTTCTGAATTCAGGGTTAAAGTAGAAAAAGCATCCTCAACTAACAGGACTATTCGTATGGGTGATGTCATCCTTCTCTAAACAATTATT TGATCTAACAAATTCTAGAATTCTC[C/T]ATAAGGAGCTGCCTGCCTTGCTCTGAAAGGTGAAAAAGTTCACCTTTGTTTCCTGGATGCTTAGT GACTAGTAAAGGTTTTTTTGTTTGTTTTGTTTGTTTTTTTGGGGGGTGGT
Gnas	rs6123836	TCTGTCTAGTAGCATCTCTAAGCTTGCAGTAGCTGCTCACGTGTCTGCCACGCCCCTTAGAAGGACGAATCCTTGCCTGGTCGGTGAGTTTGCCT CAATT[C/T]GGGCATTTTCATTTGTTCATTCATTCGATATACATGTATTGAGTGCCAAATATGTGCCCTTCCCCGTGGGGAAGACAAAAGTATG AGACACTGCTACTCC
Gnas	rs6026567	CTCAACCCCTCTCCACTTCTCAGTGCTTCACGGCACACTGGGGCATTCTCACTAGCGAAGGTGTCCTCTCTAAGGATGGGACCCCTACTGTCCAT CTCAGGCTCAGCACTGCCTTGGGGC[A/G]GGCCACTTCTGGCTTCTTTAGGCCTCGTTTCCACGGGAGGGGAAGCTGGGTCCGATGGTGTCTGG GTCAAATTCCTTGGTTCCAAGAACAGGTACCGTTGCCATTCCCTTCCATTTCTGC
Gnas	rs6128461	CTTCGCCTTTCCTCTGCCTTAACTGTCGTGTTCTAGTCTGGAGAGATTATATGTTTTAATTTCTACTCCAGTCTATGAATTGGTGAATCAGCCAA GTGAATGCTTCAAAAACTGGGACTC[T/C]CAAAAGATTAAAAAATATATATATACAAACCGTGAAAAAGATAAACTCTGTCCCCTCCATCTCCC ATTGGTTTCTGCCTCGGTGACTCCCCCCCTCTTGGCCTCAGTTTCCCTGTCAGAG
Gnas	rs6026574	CCATTTCCTTTGTATGGTTGAGCTATTTGGCACCATCTTGGCAGAAGACAGGGATCCAGGGCCTTTGCTACCAGTGGAAGGGATTGAGGAAAGCT TCCTC[A/T]GTGATAAGTCTTGGCTTGGCAAAACATAGCCTAGCCTTCTTCCTTCCCTCTCCAGACCAGGGCACCACGTGGAGATAGCGCCGTT TGCTTGGGCTGGCCT
Gnas	rs8125112	GACACGCGGGGAAGGTGGCGGGGCCTCCCGGGAAATAAGCGGGGCCCTTGGCCTGGGGGAGCGGGGAATCGCTTTTCGCCGGCCTCCGCGTAACC TTGTT[C/T]CTGTATGTCTTTCTCTCTTTTTCCTTTTGCGCTAAGCGTTTCCTCACTTCTCATTCGTTCGCCAAACCCTCCCGCCTTTACAGTT GAAAAACCAGACACA
Gnas	rs3787496	TGACAATGTCGGAAACATTGACTGACTCATAATAAATTTGAGATTGGATAACAATTTATACTACCTTTATACCTCTCTCCTCGCCCCAAATTGAA TCATTAAAAAACATTTAGTAAGATC[G/C]AATGTGCAGTTCTGTGGGCAGAACTCCACAAGTCAAAATACTCCTGAATTTAAATACTCCTGAGG TTTAACAAATTACTCTGCAATGCAATTTGGGATTTTACTCTAAGGAAGTACAACA
Gnas	rs2145288	ATTAAATAAGTTTTAAAGTGAGAAAGACATCCCTCCTGTTTTTTCAATTGAGAAAAACTAGAACCAGGGATTTGTCCAAGATCTCAGGACCAGTT

		CCCAGGAGAACCGGGGCACCAGGGC[T/G]GTGAATCTGGAGGTTACCTGATCGTGTGCTTTCCTGCCTTGGCTAAGATCTTCAGATCCACTCGT TAAAAGTTGCCGGGATTTGTCCTTAGATGTCAATTGCTCAACTGAAACCTTTTAA
Gnas	rs2180696	CCTTTGGCCTCCAGGCTCTGCAAACCTCTACATCTGATTTGTCTCCctccccccccacccgcacccccaccaccaccgctgcctcTGCATTAAA ACTCTTTCTAAAATAAAACCACTAA[C/G]CAGCCACGCGGTCATAAGCCAGGATGCTGCTCCTGCATCTCCCTCCTTCCAGACAGAACTGCCGG ACCCTAATGTTGCTTTCCAGAAGGAATAGGGGGAAAATGCATTTTCACATATTTT
Gnas	rs7271854	TTCTAGTCCAGAACAGAGGAGAGGATCAGGTTGCAAGTGATTATCCTTCTACCAGGCAGGGGGTGGAGCGCTGCCAAGGAGGGTGTGGCTGGCAC AGCTGAGGGGCCAGGCTGGCTAGAC[C/T]GGACAGGAGTACGGGGAGGAGAGGCAGGTTCACAATCCAGGCTGGGGCATCCTCTCCTCCCCATC TCACTGCCCCATGAGGTCCCTTGTTCGTTCAAGTGCCAACCAGAAGAGCTGGGAC
Gnas	rs4812042	AGCCTAGCTGACCACAAACCAGCTTGTGTTTCAGCACTCTGGGTGTCATTTGCTTACCTGTAAACCCATGATTCCACCTGCTGTCATTTATTTAC AAAGT[A/G]AATGCTTTATTTATCCATCAGTAGTTAGTCATAATAAAATATAGGTGAAACCTACACTTAAAAAATTTTTGTTTTGAAGAGTGGC CTCATAGATTGAGAG
Gnas	rs6064716	TTCCCAAGGACACGGCCCCTGCCCCTAAGGCTGAGGAATGTTTTTAAAAGGTTTCAGTTGAGCAATTGACATCTAAGGACAAATCCCGGCAACTT TTAAC[C/G]AGTGGATCTGAAGATCTTAGCCAAGGCAGGAAAGCACACGATCAGGTAACCTCCAGATTCACXGCCCTGGTGCCCCGGTTCXCCT GGGAACTGGTCCTGA
Gnas	rs12625436	GCCACACAGGCCTCCCACACAAGGACCTACAAAGTCCTCCAATGGGAATTTATTTCTTTCTGATCAAATTTACCCTCCATACCCCATTCATATAC ACATTCTTTCTCCCCTCTCTCTTAA[G/A]ATTGTAAAGTTAAAGAAACTGAATACACAGGGTTGATTTAAGGTAAACGTCCCTCCCACCCATCT ATTTAAAAAATTTTCTTTTACTTGATAACTTTCCTACAACATGATTCTCTTATGC
Gnas	rs6100269	TGTAAATTTTTATGAGGAAAAAGGTCCTGAGCTATGGCAGGCTTAATTATTCCCTCATTCACATCTTAGGACAAAACTGTATGTTAAATATGGCA CACAAATACTAATTGTCCATTTACT[C/T]CACTGGAAGTGCCCTAAGGTACCTTGGGATGCCTCAGTGAAGTGCCAAATGGAAGACATTCTCAT TTTAAACAGGTGACCTTTTTATTAAGAGTTTTCTAAAGTCCTTGGGGCAAATGAA
Gnas	rs6123832	TTGCCTTGTTGCAXGATGAATAAACAAGTTTTTTAAAAGCCCTCCGTGTCGAGCCCACACCTCACACCCATCTTGCTGTTTTTTAAACAGAAGCC CAACA[C/T]AGGGATGTTTCCTGAACTGTAAGCAATGTACCATGTTCACATGTAGCGAGGAGGGGCAATCTAGGAACACACACTGTCTTTCAGA CAGTCTTCCACAAAC
Gnas	rs4810147	AGGTGTGGAGTTTTCATRCAAGTACTTCAATTGCTACACTCAAAGAGAAAGATTTAACACCTAGAAATCTAGCTGTCTRGCTCCAAGGCTAAAGC TTTGG[A/G]TTTTGGTTCACAGCAAGGTTGGTCTTCAAGGTCCCCACTCTCAAACTTGAGGGGTCCAAGGGGCACAGGCCAAAGACCGGCAGCC AGCAGAAGACCACGT
Gnas	rs6026561	AAACCACCCTAAACCGGCATTAAACCCCAACCAAAATCCCCTTTAACCCTTCCCTAGAACAGCAGGACCTGCGAAACTCTGAGGCCGCTTTGTGA GGTCC[C/T]CCTCTGCGCAGCACGCCCCCCACCCCTCTCTTGGTGCCGCCGCAGCTACTCCCTAGGGGGCTTTGCTCTTGGTGGTACGCACCCG AGACCTTCTTATTCC
CYP1A2	rs2470890	ACAGCAACTGGAGTTCAGCGTGCCGCCGGGCGTGAAAGTCGACCTGACCCCCATCTACGGGCTGACCATGAAGCACGCCCGCTGTGAACATGTCC AGGCGCGGCTGCGCTTCTCCATCAA[C/T]TGAAGAAGACACCACCATTCTGAGGCCAGGGAGCGAGTGGGGGCCAGCCACGGGGACTCAGCCCT TGTTTCTCTTCCTTTCTTTTTTTAAAAAATAGCAGCTTTAGCCAAGTGCAGGgcc
CYP1A2	rs762551	AGAAGTGGAAACTGAGATGATGTGTGGAGGAGAGAGCCAGCGTTCATGTTGGGAATCTTGAGGCTCCTTTCCAGCTCTCAGATTCTGTGATGCTC AAAGGGTGAGCTCTGTGGGC[A/C]CAGGACGCATGGTAGATGGAGCTTAGTCTTTCTGGTATCCA
CYP2C8	rs11572093	CCATTTATTCAAGGTTGTAGGGAAGACTTGGTTTAAAAATGAGAAAATTGATACTAAAATGCTTTTATACAATAAAAATGATGTATGAGTGAAGA

		AAATAATTACCACCTTTGATTTCCT[A/G]TTCAAAATTTTCAGCCTCCAATCTTTAGGTACAGAAAATTGCTATATGTGCACAATAAAAATTTC CCCATCAGAAGTGCAAGGGGTCAGGGAATTCCCTTTCCTAGCCAAGCAAAGCTGT
CYP2C8	rs11572169	TAAATTTTCACAATTTTTCTAGAATATTTTTATGGCTTTTTTCTCCTGCAGTTTACTATACAGGCAAGGAACAAGCATATAGTTCATTTATGTTG ACCTTTTCACCTCCTGTAATCAAGG[A/G]AAGTTCCTTTCCTGGTTTGTTTCTTTTCAATCAGTTGACCTTTTTGAGACCAGCAGTCATTTAAA ATGTTCCTCAGGCTGGTTTTGTATGACTGTTTTCTAGAGGCAGATTCCAGCTACA
CYP2C8	rs3752988	TTAAAAGATTTGCATTTGTTAAGACATAAAGGAAATTTAGAAATTTTAAACAATATCTTACAAATTCCCCATGTGTCCAAAAAAAATCAGCATGG ATGAAATAAACACATTACTTTTACC[C/T]TAAATATGAGTTGAGCATTACAGGCTAGCTAAACAATGTCATTTCGCATGTGGTTATTCACATCC ACTGCCTTTATTTAAACTCTTTTAACCCCAAGTGTCTTTTATTTTGGAAGGAAAA
CYP2C8	rs1934980	tatatatttaaatcaaggtgcatgctcatctgattttggttcttatgaaggtacattttgtgtagatagctgttaaactgatgtctttgcttgggg gaataatcaatgaagcattcaattc[C/T]gtcatcttgctccactctcccATTTGTatatcttcttttgagaaatttctgttcatgttgtttgc ccactttctaatgggattattcggatttttttcttgctgttttgagtttcttgta
CYP2C8	rs1934984	gttagcattgaacagaattggtgatagagggcatccttatttcatttctgatctgctgggggtagagtataagaatattaatatttcactactg agaatgatgttTTAAGTATTTTTTT[T/A]AAATAATTGACTTTTATTTAACTTCATTTATAAAAGAATTTTCACGAATATGGTTTGAAATGATA CTCTACATTTGTTATGAAACAGATAGTTGGTATTTTCATAAAATCCCAGCCCCCA
CYP2C8	rs1891071	TCAGAGAAGGCTCATTCTTTAAATTTCTGTGTCATCAGCTGTAATCTGTCTAAATTTGATGACACAATTTAAAATGACATCTTTGTACAATGGAG GAGGATGACAGAGATCAGTA[G/A]AAACAGTATGGCAGTAGCAAAATAAGTAAAGCACTGATGAAGTGTCTGGATTTCAGCAAAGGTAATTTGT GGTAAGGAGAGCCAGCATAAATTGCCCTAGTATTGAATGTTGGTTTTATT
CYP2C8	rs11572177	AAAGAGCTGTTTTAATGTGGGAATAAATAAAGAAATGACTGTTATGGAGCTGATAATCAATGAATATTTGTTGAATGAAGGGTGCCTATTGAGAT TAGATGTTAGACAGATAGCAAATAT[A/G]TCTCTTTTTGTACATTTGTTTGTCCCACCATCCATTAATCAATCCATCATGTCATCCATCCATTC ATCCACATGTTCATTCATCTACCCAATCATTAATCAATTATTTACTGCATATTCT
CYP2C8	rs11572126	ATTTCAATTTTATTCCACTGGTCTTTATTTCTTTCCTTTTGCCAGTACCATGCTGTTTTGACTACTATAGTTTTGTTTTGAAGTCTGGAAATTTG GAAATTGAGTCCTCTCCCTGTAGTT[A/G]CATATAAATTCAGGATTGGCTTTTCCATTTTTGCACAAATAAAAATTTTAAAAAGGACATTGGAA TTGTGACAGTGATTACATTCAATCTTTAGATTACTTTTGGAAGCATTGCCATCTT
CYP2C8	rs1934956	AGCACCACAAAGTTAGAATCAGGACATTCAGCATCTCCTGGAAGAACAATTTCTTCCATTCTCCAGCAACCTTGGCCACAGACAGTGGCTTTCTT CAGAATCCAAAAGAATTCTGTTAAC[T/C]ACCTATTGGGCCCTGACTGATTACTTCTTTCTTTCATGCACTTATGTTTGCCCTGAACCCACAGA TATTTATTTCACATCAGCCATCAAATGAATCTTCAGAGAAGTTCAATTTT
CYP2C8	rs11572172	ATCTCCTTCCTTTATTCTTTGATCTTTTATGTCTGCTTTATATTTGGCACTGTAGATACCAACATGACAAGGACAGAGACCTTCCTTCAAGAATT CACATTTTACCACAATAGATAAATA[A/C]ATACAGAATTACGATTCCTGGTAATCTGTGCTTTAATGAAAGCATGAGTATGCTGTTGAGGAAAA CCAGAGAGCATAGGATTTGATTACTTGAGGGGATTGTAAAGAGTGTGAAGATGAA
CYP2C8	rs1341164	ccaaaatatcttatgaatacagaagtcctgaacaataagtaacagaacaaatccagcTGTTTGTGTATACTTATGTATATTTAGTGAGGGATGTG AGGCCAGTATatacaatcatataaa[T/C]agatgcagagaatcaatttgataaaattgcacacacattcatgacacaaatttatataaaaatga gagaagagaaaaaacttccctaatctgataaaggatatctactaaaatcttaaag
CYP4B1	rs4646493	CTTCTTCTGACGGTAGAACCTGATTACCCTTCTGGGTCCCTGGATAGAGTAGGGAGAGCTGGGAGAGAAAGAGGTGGAAATGTGGGGGTGAACAG AGCTG[A/G]GACAGCTGGGAGAGCCAGTTCCTTGCTACTTGCCTTTGGATGGCAATACTCAGCTTCCAGATGCCATATCTCTCCTGCTGGTGAA GGAGAAAAGACTTCC

CYP4B1	rs837401	AGGGAAAGAAAGAAAAGTCTCCGGCATCTCTACCTGGATTCCCCAAGCTGGCTGCAGATCAAAACACTAACACTTGGACAGGTTTATAAATGTAA ACTCCTAGTTCCAATCTGACAAATT[G/A]GAATCTCCAGGAACTGGAGGTCCAGGAATTTGCTCCTCCCAGTTGATTCCAATTCCCTGCCCTGT GTCTAATTAGAAACCTCTGGTCTAGATCCCCAATACAGTAGGAAACTAAGAGATC
CYP4B1	rs4646491	CTGTCAGATGCAGACCTCCGGGCTGAAGTGGACACATTCATGTTTGAAGGCCATGACACCACCACCAGTGGTATCTCCTGGTTTCTCTACTGCAT GGCCCTGTACCCTGAGCACCAGCAT[C/T]GTTGTAGAGAGGAGGTCCGCGAGATCCTAGGGGACCAGGACTTCTTCCAGTGGTGAGTCTGAGGG TGGGCCCGGTTTATCCTGCTCAGCCCTTGGGAAGGGCGATGCCCATCCTGTCCTG
CYP4B1	rs2065996	GTCCAAGCTCTGGAGTTTGTGGGGAGGCATGGtaatgactaatctttattaagcacttgctgtgtgctagaccttgttctaagcaccttacattt tgacctgattgaatcctcacaacaa[C/T]tctaagtggtcccattttagggaagaggagcctaaggaatagagaggttaggttacAGTCCCATA AAATTCCTGTTGAGGCCTGAGCCTGTGCTGGGCCTGCTTTTTCTCTCCGTAAAAT
CYP4B1	rs3766209	TCATGCTCACTCTCATTTCCTGCCTTACAAATGGAAATCTTTACACTACACACAACAACACGGACGCTGATTGAGCTTTCAATGGATTGCCTACT TCTAT[A/G]TACTATCCAGAGGTTTTGGATTTCTGCCATTGCCTTTCTGTCATTGCTGAGCTTTTAGCAGAACCTGGTCCAAACTTTGGGCCTC AGAATGAGGAGAAGG
CYP4B1	rs4646481	GGCAGTGGCTTGGACTCATGCACACATGGAGCTCTGGTAATTTTGGGGTGTGGGGAGAGG[G/A]CCCATAGTAGGGAGACAGCTGAGACTAGGG CACCAAAGGGCACCTAAGGGTGTCCATCCT
CYP4B1	rs837398	TGAACCCCAGGCCTGGGTCTGATTCATCTCTCTGACCCACACTTTCCTTTCCTGGATGTCTTCAGGATGCATGTTACAGGGTTATCTGTCAGGGC AAGAATAAGCCTAGAGGTCAAGCTC[A/T]TTTCTAGTGTCAATCAAGGAAGAGAAGCAGTGCCCCCATCCAGACTGAGAATGAGGAAAGTGAGT TCAGAAGGTAGGAGAAGGAACTTGCCTCACTCTGAGCCTCAATCTCTGGCATGTC
CYP4B1	rs4646485	GGTCAGGGTTAGAGTCTTAGGGCTGGGTAATCCCTAAGTTATCCAGGCTAGTCTTTCCCCTTGTAGGAAACTCCCCTCCAGGATTACTTACATAT ACACC[A/G]TGCGGGGGAGCTCACCACCTCTCAGCCATCCTACTCCCTTATAGGCTGCCCTGACTGGGAGAAAACGTCTGTGTGCTGTGCAAGA TCTGCCTCCTGGTCC
CYP4B1	rs1572603	TTACAGGACTGGAAGTTCTGGGACGGCTTGTTTTTTCTCCAGAAGGAAGGAAACAGGAAGAGAGCAGGGTATGAAAATAATTCACTGATCCCAGC TCCCCAGCCCCACCTCCCACCCTCA[C/T]ATTGGCACTCCTTGTTGACCTCACCAGAAATAAACCACTGGAGGGATTGGGAAGAGCAAGCATAC GTCACCACTTTCCTCTCTGCTTCTCAAGAGCCTAAAGTAAGCTTTGCTCCAAGAA
KIT	rs6843170	TAGTTGTGGCCTTTTTGACATCATTTTAGCTGAGCCAGTTACATATGTAGGTGTACTGTATGGTTTATTTTGTAAATAAGCTGAGCCTCAAGGAC AAAAGGGATTGTGTTCTGCAAAAAT[C/A]AGAATTGATAAAAATCAGAATAATGAATGCATAGTGTTTGTTAGTGCAGAAATTCAGCCTACTTT TACTCTGTGACACTAATGCGTAATTGCTTAATAGATTCATCTGCAAGAAATGCTA
KIT	rs17084733	CAATCCTGTCTTTCTGAGCACACTTTAGTGGCCGATGATTTTTGTCATCAGCCACCATCCTATTGCAAAGGTTCCAACTGTATATATTCCCAATA GCAAC[G/A]TAGCTTCTACCATGAACAGAAAACATTCTGATTTGGAAAAAGAGAGGGAGGTATGGACTGGGGGCCAGAGTCCTTTCCAAGGCTT CTCCAATTCTGCCCA
KIT	rs3822213	CTGCCTGCAGGTTCTCCGCCGCGCTGAGGGCAAAGTTAGAGA[G/T]CCCTTTCTCTGCTCTGCGGCTACTGCCTTCCAACCGCAGGCGCTCTTT GTGCCCAGAGCT
KIT	rs2237023	TTCCTACCTGACTTCCCCCTCTCGGGCAGCACATCCTAGCATTCTTTCCTTCAGAGCCCCCTCCACACACCCTGTATCCCTTCCATTCCTGCTGT TCCAC[C/T]TGAGACCAGCCAACTACTTCACAGAAGTAATATCCTCTCCTAAAAGACTCCCCGCCAGCCGCCACCCACCCCACCCCAACTCATC CTCCCTCAAAACTCT
KIT	rs12643468	ccatgtccatggtccaagatgaccaccaggtggatagccagccatcatatccagaaggcagggggcccttgcactagagaagagagggcagaggg

		cacgtgccagctatattgtaaggag[G/A]ttttcctgaagctgctatacagcacttacaacttagtggccaaaaccgagtcacctaactacagc tagctagctgcaaaggaggctgggaaatgtagtcctaattgtgagaggccgtgtt
KIT	rs2703488	CTGACACACTTTTATATCCCCATAGGAATAAACTGGATGAAAACCATGATGGAAGGATCCCAGTAATCGCCCACAACACCCATGAGGCTTTACAA ACAAAGAGATGATACTGGAAATAGC[G/A]TGCAGATGGGGGAGGGGGAGTATGAGCTCAGTGAAAACCACACAAGTCCTCAAAACACTCTTGCC CAGCAGTGGGGAGACTAAATCAACTCTGAATCACCCACATGAACCAAAGGAAGCC
KIT	rs2237037	CCAGGAGACAGACCTTAAAATTCCCTTTGCCAAAAATCTATTGGGGAGAAGAAAACCATTGATTCTTTTAATTTTTTAAAAGGGAGATATTCGAT GTGGTCTTTCTAGTCTCCTACTAGG[G/T]TCTAGTTCACATAGCTTAACTGATATTAAAAAGGTCAAGGCACAAAAACATAAAGACCAGATGTG TTGTTTTAAGCTAGGTGATCAAATCCTCCAGAGAATCCGAACACTCCCTCGTGAC
KIT	rs2237028	TAGGAGTTCGGTCATATTTTAAGAAAAACATGAATGAATTTTAAAAAGAGGGGCAGAATCTCCAGTCCTTGGAGAGATACCAAAGAGCATCTAGC CTGGACCCTGAAACATCTCTCTCAG[C/A]TGTGAAAGAGCATTCCGGCACATTCCAGAACAAACAGTTCCAAACCTGCAGACTAAAGCACATTA AAGGCTCCAAAGGTCCTGAAAGTGAAGACACCTATCACTAGTCAATCCTCTTGGG
KIT	rs4864920	CCAGGTTAACATCACAATTGAATAGTTAGTATGGTCGCAGAAATTTTATTTTTCATGGAATAAGCCTCTTTATCACAACAAGTTGGAAGGGGTTA CATCGGATTGCTGTTTTCAGCTGGG[G/T]TTTAGGAGAGTGTTTTCTTTTGGGGAATACTAAGTAGGGTTCAAGGCCCCTGGGAGGAATGGCCA CAGCAGAAGCAAGCCCTGTAGTTACAGGcattcattcagtagatacacagggagt
KIT	rs2237032	TCTTACAGGGACCTGACCCTAAGATTTCATTGACGTCTTCCCTCCATGTCTGTGAAATTGAGTTTCAAGAAAATGCAGCTCACAAATAATCAATG AATAAACCCAGCAGAGTCCAACATC[G/A]TATTCTCTCCCTCAAATAGGCCACCCCTCCTCTTGGCTTTATTTCCACTAATTCCATAGTATCAA CAATACTCCCAGTTACCTACACCCATGATAATTCCAGTTCTCCTTCCCCTCAATC
KIT	rs3020827	TTTCACTGGATATACAGATGAGGATCATGTTGGTCCTGACCTCAAGCTCTTCAGAAAGTCTTTTCCTTGAAGTTAGTTTGTTACAAATAACAAAC TGACA[A/G]AGGCATTTATTTCACTTACCTAACACGTTGGATTTATTGATTTGCAAACGTTTGGACAGAGGTAGTAGCATTTATGTTTGAGCTT TAAACTGTCTATGAG
KIT	rs13135792	CCATTTCATCAATAAGGACACGTTACACAAGTGCTTGCTTTGTTACTGATCCAGCCTTGTTTGCCATTAATTACTGACACTCTAGCTTTAGCTTT ATTTCTCTGTTCTGTGATTCCCCTG[T/C]GTCAATTAGTTCATCATCTTTAGACCAACCTTGAAATATACTTAAGGAGCTACTTAAAAAAAAAA ACTAAATATGCTGTAATGAAGTCTGTTATTTTGTGAATTTTAAATGGCTAATCAT
KIT	rs11721352	ACAGAGGCTAATAATAGAGGCCAGTGTGATCAGATAAAAAAGTGTCAAGATAGCTGAGGCAGGTTTTAGGTTTAGCCGGGCCTTAAAGAATTGTT GAGTTTTTAAAACAttaattgcagt[A/G]aaatacatgtaacttaggatgtactatttttaagtgtcccaattctgtgtggcaccaagtatctt cacaatgttgtgtaaccatcactactaaccatttccagaacttcttcatatccca
KIT	rs12646437	AGAGTAGGAAAAAAGAGGCTTTTGTCCCATGCCTGCACTCCTTGGCAGCAAGCAGAGTGGCATTGTGATGTGTGTACAGGTCTTCCTCCCCACGG CTGGGTGATACAGCAATGGTGCTCC[T/A]GTAAGTTGAGCAGTGATTCTGGCTGAAGAAGTCTCTCTCCAAGTAGTTTCATCATTTTTCAACGT AATCTCGTGATTGGGATATCTTTTTGATTGCTGTTTAGAACAGGGGTTGGTAGAA
KIT	rs17084644	TATTGAAATTAGGGAAAATTTGATTATTTAAAACCTGGGATTTCCAGAGGTGAGCAGGGTAGGATTTGCATGAGAAAGACGCCTGTTTGCTTAAA AGAAT[A/G]TAAGTAGTTGTTGGTCACATCCTCCTGACACCATCAAGGTTGTCTGAAATAAAGATTAAAGCAGCTTCTCTATGCTAGGGCTTCT ACTTGGTTTGGAAAA
KIT	rs2237029	AACACATTCAGATCTACTAAAAAAACTCACAAACCCTTCTACATATGCTGGTTTATTTTAAAAAAGCAAGGGGGGGGGGTCCTCTTTTATCAAAG TGATGCTTTTTGGTGCGGCTAAAAA[T/C]GTATATACTATTTCCCAGCTGCACATATACAGACTATACTAAAATAAGTAGAGAAACATTTAAAC ATTCCAATTAAGTATTTTCATTAGCTATCCTAACATATTTTAGGTTAAGAATCCT

KIT	rs11735550	agagatgtagaaacttactcaagttcaaagaactgagaaatgtaagagctggtattcAGATGTGTCCACTATGCATGGGGCTCCCGTAATTCTGT GCTTAGACTTGGCACAATCAATGCT[A/C]TTCTTCAGAGTACAGGGTGAGCATATCAGCATCCCTGCCATCCTGTTGCTTCTGTTGAGCTTATT TATTCTTAGAGGGTTGGAATCCTTCAGATGTATTGCCATCAGAACCACCGAGTAT
KIT	rs11946333	CAAATTATTTAACCATTCTTTCCTTTCTGCCCCTTTGCATCTTCTGTACTGTAGACATTCCTTCCCCCTGCACTTAGCAGGTGGTATATTCCTGG CACTGGACAGAGACCTTACTTTGGA[T/C]TGTGAGAGCTCACAGTTTGGTTGGGTTGGGGTCCCCATAGGACTAGCTTTTCCTGATGCTTTGGA CACCCCTTCATGCTTTGAGAACTTTGTCTTCGCCTTCCTTTTCCTTGAATACACA
KIT	rs1008658	TATTATTGAATGTTAGTTGTAGTAATGTTCAGCATACCATGCAAATTTTGCTGAAGTATACTTAATTTGACTGCTAAAATGTGTGATATCCCTAG ACAGGATTTACATTATGAAA[A/G]TCACAGGAAACAATTTTTATCGAAAGTTGAAACTAAAAATCCTTTGCAGGACTGTCAAGCAGAGAATGGG TACTCACGTTTCCTTTAACCACATAATTAGAATCATTCTTGATGTCTCTG
KIT	rs2237034	ATCTTAAAAGAGTAGGCATTATTCCCATTTTAGGGACAATAATAATTCCTGTTTTACAGA[C/A]GATaattatggctaacacatattgagcctg tactatgaggcaccactctaaaagttttac
KIT	rs2213180	aatatgattttgagatacctataaagacttaataggatatgaagatatttatgattactgttcatgacaaagtcacaggtacagctgatactact gcgttttatcacctttgttagtaat[T/G]taaagaaatgctaaatttcatctagaaattagcgaaaataaggatgtcatttttcctttccaagt tcacaggcccctgaatttgacacccatagatctcaggttaggagcccttATAGGA
KIT	rs3134889	CATATAGATGGCTTCACCTGTTTGCCAGCTCTCTGGGTCACCCACCTGGCTGGAATCTCCAACTACCTTTATCCAGAGTAAAATAATAACGGCAG TCTTGCCTGCAAGGACTGCTCAGAT[T/C]TGGTCCTTAAGGCCACTGTGCCCTTCAGGAAGATCCTCACAAAACAGAGCAAAGTTACTAGAACT TCAACTTTTTTGGAAGATGGCAATGTCTTCCCACCACTTAACCTATTAGCTTTTT
KITLG	rs3782174	GCTTTGTTCATCCCGAAATCATACATGGTTCAAAAAAGCATGTAAGATCTCCCCTAGATCGTGACGGTTGGGAAGGATGCATTCCAGATGTGTTA TGCCC[A/T]CTCTGTGGCTTGATCTTGAGAGAAAGCATCTGGAGAAGCATAGCATGGAGCTGGCAGTGACTTGGGGAAGCAATGCAAATGCTTT CGCTGAGGGAAGAGT
KITLG	rs1907702	ATAGTATCTTCTTCTGGGAATATATAAAACATGATCACTACCTGCTTAAAATCTCCCAATGCCTTCCCATTACATTTACAGTAAGACTCCAACTC CTAATCAAGGCCTACAGAGACCTTC[G/A]GAAGACAGACTTGTCCAACTCCATGTTTACGCCTTAGTAACCTTTATCTTCCCACCATGCTCTAG CCACACTGGCCTCCTTTATGTTCCTGAAATACCTGGAAGGTAATTCCTGCCTCAA
KITLG	rs10777129	tataaacctcattttacaagtgagaaaatcagaatgttatccgaagaatcaagtgactcaaagtcatacagttTGAAGAGATGATTCCAGATTTA TGAGTCTCCAAAGCTATTCATGATC[A/G]TACTATACAAAAACTAATTTGAGGCTCATTTTAGAAGACATTAATAAACTAAAGTTTTCATGTAC TTGGAAAATTTATATTTTTTATTGCTCAAGTCTGAGAACAATACAACAACACTAG
KITLG	rs1492354	AAATCACTTCTCATATTCATAGAGAAAAAAAGGCTTTTTTTCTGTATTTTATTTTTTTGTTTTTCCAAGTTTAGAGGATTGGTATCTTCTGGTTT CTTGAACAGCATATGTCCCCCTGAT[A/G]TTCTTAGGCTGCATGCATTTTAATTTCTACTGAAGGAAAGGAATCTGCAGTGTGACTTATGTGTT GCAACACACGTTCAGATTAAGTCCATGGTTTGTTTTGGGTGTTTGTAGCTCCTTT
KITLG	rs11610915	gtcactgcactccagcctgggcaacagagcaagactctgtctttaaaaaaaaaaaaGGAGCAGAATGATTCACGAATTAAATAAACAAAACACAT CTCCCACAAGGGTCTCTTCCAACAT[G/A]AAACTTATTTTGACAGATTTTTCTATATACCTATACCTATTTAACATACAAAGTACCTAGAGTAC ATAATATAAAATAATAGATCTACTATGTGTGTATCAACATCTTACAGGGAAAAAT
KITLG	rs3782171	AGAACTACTTAAAATAGCATTTAATGTCATATTTTATTCATTCAACTAACATTTTATGCTTATTCCTTTTAAATAAATACTTTCTTATTACATGG GAGAT[C/T]GAGGCAGATAAATAGTTGACTACAAGTGTCTATTTGGCTGACGTCTATTTTTATAGAGAGAATGCCGCATCATAGGAGTGGAGCC TCAGAATCCATGCTT

KITLG	rs10858753	TCAAAGATTCAAGTATTTAGACTCTTACAACTAGCACTTTTAGATGGCTGTTTTAACGTATGTAATTCAGATTGGTAATGCAGGGTAGTGATCCC AGTGGGATCACAAGTTGAATCTGGG[G/T]CAGTGAACACAATGTCATGGCaaaaaataaaataaaataaataaCCTTAAAAACAAGCTGGGGAT ATTTTTCTTTAGAGATGCTCATTAAAAACATGAGGGTTTATTTTTAAAAGACCCC
KITLG	rs1388789	CATGATTTCTGAATGAGTTTTTCTCTTTATTTATAACTTTAGTTGAATTAGAGTAGGAAAACGTGCCCCAAAGTTTCTCCAAAGTACTTTAAAGC TCAATGAAATGTATTTAATTCTTAC[C/T]AAAACCAATACTTCATTAGGTATGCATATTTTTTCATAATTTTCTTTTCACATACTTTTTAAGAG AATAGGCTACTTGTTCTATTATTGTATTGAAGAAAAATTAATTTTTCTCTGACTC
KITLG	rs4842477	GAAggtgtcctggaaccaatttcctaaggatagtgaagaacaactATACAGGTCTAGGATATATGTGCTGAAATGATCACTGATAAAGTTTAACA GTTGCTTAAGAGGGGGAAAAAAAAG[T/C]CTGCAAGTCAAATTCCAAAGATAAATTCTTCAAATCCTGACAATAAGCAAGCTCCTAAATAGCAG CTAGTGTACTATCTCAATATGAATGATCCCATTTCTTCTATGAGCTACTAAAATG
DTNBP1	rs9396592	GTTGACTGTAAAAATGAAAAAGGACAACCTGAGTGCCTTACCTTACGTAAATATTTCCTAACTCCTGCCCTGTGTCTGTGGCAGACACCCTGCTC CGCTGAGTTTGGGTGCTATTACTCC[G/A]GAAAATCTAGTCATCTGCAGAAACCATTTCAGCTACCTTCTGTCCTGGGATACACTGTAACAAAC CATTTCACCCAAGGCATTGTTTCCTCTGGTTCAGCTCAGGGGAGTATCAACATCT
DTNBP1	rs6909929	agggctcacctcacttgtttcctatctatcgggtaccatcacatctcttctttgtccaaaatccagtgtcttaaaaacattctctcatttatttc acctggtttttcattgtttcggggg[A/G]gagaataaatctggtccctgttactccatcctggtggatgcagaagtctgctCCCTTTTTGTAAA GTCATTGCCTGCGGAAAAGTTCTCCTGCTCCCCTTTTCATTCAGCATCCAAACAG
OCA2 exon 9 R305W C-T	ij-opa1-305	GGAGGAAGAGCCAGTTCAATGGCTGGCGTCTTCTGTGCAGGCCCCTACTCACTGTTCATTGTCGGGTGGTGTCACAGGAAGGCTGAGGGGCCTGC TTGGACCAGTCGGGCTTGGCTGCAGGGAGGCTGCATCCTATGTCTCACGCCGCTGGCCTGTGCTCACTGCTCTTCCAGCTGTGAGATTGGGCGTT GGGCTGAATTGTTCCATTTGCACTCTGGTTAATGCCATGGCTGATACAGAGGGAGGTCCCCTAACTGTTGACCTTGTGAACAGTAAGGTCGTTGT TTCGTTCTGCAGAGAGACGGTGTCCATCAGCATC[C/T]GGGCCTCCCTGCAGCAGACCCAGGCTGTCCCTCTTTTGATGGCTCATCAGTACCTC CGCGGAAGTGTAGAAACCCAGGTGACCATCGCGACGGCCATCCTCGCGGGCGTCTACGCGCTGATCATATTTGAGGTAACTTTCACACCTGCTCC CCCGATCTGTCTGGGCCCACAGTCAGGGAGGCTTGAGATCCGTGAGACACTCTGGATGGGCTCAGTCCTGACTCCTTAATCAAACTGGACTAGTG TCATCATTCCTAAAGATTAGCGTGTCCCTCTCTCTAGGTAGAAAGGGAACCAT
OCA2 exon 13 R419Q G-A	ij-opa1-419	CCACCAGCTTCTGAGGCCCCACTGCTTGTAGCATGTACTGCAGTCACACAAAGCAGACACGAGCTGGACTGGAATGCAGTGAGCTGTGGGCTCAA CCGCCCCCACCTTTTCATGCACCTGAGAATGGAACCTGGAGCCAGGCAGTGCAGGCAGAGCCCCTGCCTGCCAGAACCTGGCCGCAACTCCCACG GCAGAGGTGCTTTGCGTACCTTATGGTCACAGGCGTGAAGAGGAGCATGGTGGTGACGTTGTCCAAGAAGGCAGAGAGGACGGCCGCGATGAGAC AGAGCATGATGATCATGGCCCACACCCGTCCCC[G/A]GGAGAGCCGGTATGCCTGGCCACACACACACAGAGAGAGTACAAGCCAGAGTGAGCA GGCTCGTAGAACAGAGGCAGCCTTTCATTAGTGACTTTAAGAACAGGGAGCCAAACTAACATTACCCCATGGGTTAAGACATAGACCCACGGAGT CCTAGGGGGGCCGAGATGAGACAGTGCTGGCCATCGAAGTTCCTAGACATTCTAAATCCTCCCTAGCCAGCACTGTCACCCCTCTGATCCTCTTC CTTCACAGAGCCCCTGTGATGGTTAATTTGTCACAAGGTGATGACCCTCAG
OCA2 exon 21 I722T T-C	ij-opa1-722	TGGTCTCGTGATGGGTAAGAGGAAGGACTAACACCCTCGGGCCCCTCTGAGTCTCGCGGCTGGTGGGTCTGACCCTAAGTGCATGCGATGGAACA CTGCAGCTGCTATTGTCCTCCTTCCAGATGGTCCCAGAGGAGCAGCGCCTCA[T/C]AGCCGCCATTGTCCTGGTGGTGTGGGTCTCAGCCCTGG CGTCGTCCCTGATTGACAACATCCCGTTCACTGCTACCATGGTGAGTTGCACATGTCCATGTCGACGGCTCAACTTTAGCCTGGACATAGCCTGG GGCTCACCCTCCCTTCCTAAGGCAGCAGAGGATGAAGCCTGCCCCTCTGCTGCACTCACAGGTGTAGAGGACGAAAGTG

OCA2 exon 9 R305W C-T, OCA2 exon 13 R419Q G-A, OCA2 exon 21 I722T T-C (courtesy of and personal communication, Professor M. H. Brilliant)

Appendix 7

MC1R consensus sequence

MC1R consensus sequence from Africa according to (Harding *et al.*, 2000):

GGAGGCCTCC AACGACTCCT TCCTGCTTCC TGGACAGGAC
TATGGCTGTG CAGGGATCCC AGAGAAGACT TCTGGGCTCC
CTCAACTCCA CCCCCACAGC CATCCCCCAG CTGGGGCTGG
CTGCCAACCA GACAGGAGCC CGGTGCCTGG AGGTGTCCAT
CTCTGACGGG CTCTTCCTCA GCCTGGGGCT GGTGAGCTTG
GTGGAGAACG CGCTGGTGGT GGCCACCATC GCCAAGAACC
GGAACCTGCA CTCACCCATG TACTGCTTCA TCTGCTGCCT
GGCCTTGTCG GACCTGCTGG TGAGCGGGAG CAACGTGCTG
GAGACGGCCG TCATCCTCCT GCTGGAGGCC GGTGCACTGG
TGGCCCGGGC TGCGGTGCTG CAGCAGCTGG ACAATGTCAT
TGACGTGATC ACCTGCAGCT CCATGCTGTC CAGCCTCTGC
TTCCTGGGCG CCATCGCCGT GGACCGCTAC ATCTCCATCT
TCTACGCACT GCGCTACCAC AGCATCGTGA CCCTGCCGCG
GGCGCGGCGA GCCGTTGCGG CCATCTGGGT GGCCAGTGTC
GTCTTCAGCA CGCTCTTCAT CGCCTACTAC GACCACGTGG
CCGTCCTGCT GTGCCTCGTG GTCTTCTTCC TGGCTATGCT
GGTGCTCATG GCCGTGCTGT ACGTCCACAT GCTGGCCCGG
GCCTGCCAGC ACGCCCAGGG CATCGCCCGG CTCCACAAGA
GGCAGCGCCC GGTCCACCAG GGCTTTGGCC TTAAAGGCGC
TGTCACCCTC ACCATCCTGC TGGGCATTTT CTTCCTCTGC
TGGGGCCCCT TCTTCCTGCA TCTCACACTC ATCGTCCTCT
GCCCCGAGCA CCCCACGTGC GGCTGCATCT TCAAGAACTT
CAACCTCTTT CTCGCCCTCA TCATCTGCAA TGCCATCATC
GACCCCCTCA TCTACGCCTT CCACAGCCAG GAGCTCCGCA
GGACGCTCAA GGAGGTGCTG ACATGCTCCT GGTGA

Appendix 8

Publication