Am. J. Hum. Genet. 70:770–775, 2002

Report

A Polymorphism in the Agouti Signaling Protein Gene Is Associated with Human Pigmentation

Peter A. Kanetsky,[1,3,4] Jennifer Swoyer,[1,3] Saarene Panossian,[1,3] Robin Holmes,[4] DuPont Guerry,[2,4] and Timothy R. Rebbeck[1,3,4]

[1]Department of Biostatistics and Epidemiology, [2]Department of Medicine, Division of Hematology-Oncology, [3]Center for Clinical Epidemiology and Biostatistics, and [4]Cancer Center, University of Pennsylvania, Philadelphia

In mice and humans, binding of α-melanocyte–stimulating hormone to the melanocyte-stimulating–hormone receptor (MSHR), the protein product of melanocortin-1 receptor (*MC1R*) gene, leads to the synthesis of eumelanin. In the mouse, ligation of MSHR by agouti signaling protein (ASP) results in the production of pheomelanin. The role of ASP in humans is unclear. We sought to characterize the agouti signaling protein gene (*ASIP*) in a group of white subjects, to assess whether *ASIP* was a determinant of human pigmentation and whether this gene may be associated with increased melanoma risk. We found no evidence of coding-region sequence variation in *ASIP,* but detected a g.8818A→G polymorphism in the 3′ untranslated region. We genotyped 746 participants in a study of melanoma susceptibility for g.8818A→G, by means of polymerase chain reaction and restriction fragment–length polymorphism analysis. Among the 147 healthy controls, the frequency of the G allele was .12. Carriage of the G allele was significantly associated with dark hair (odds ratio 1.8; 95% confidence interval [CI] 1.2–2.8) and brown eyes (odds ratio 1.9; 95% CI 1.3–2.8) after adjusting for age, gender, and disease status. *ASIP* g.8818A→G was not associated independently with disease status. This is the first report of an association of *ASIP* with specific human pigmentation characteristics. It remains to be investigated whether the interaction of *MC1R* and *ASIP* can enhance prediction of human pigmentation and melanoma risk.

The genetic control of human pigmentation is complex and not well understood. Melanogenesis is regulated, in part, by the binding of α-melanocyte–stimulating hormone (α-MSH) to the melanocyte-stimulating–hormone receptor (MSHR [MIM 155555]; Suzuki et al. 1996). This action initiates a signal cascade acting through adenylate cyclase that leads to increased intracellular levels of cyclic adenosine monophosphate (cAMP), increased expression of tyrosinase, and, ultimately, the production of eumelanin (Hunt et al. 1994). Recent investigations published in this journal have demonstrated that the melanocortin-1 receptor (*MC1R*) gene, which codes for MSHR, is highly polymorphic (Harding et al. 2000) and is associated with pigmentation characteristics (Palmer et al. 2000; Bastiaens et al. 2001), risk of melanoma (Palmer et al. 2000), and risk of nonmelanoma skin cancers (Bastiaens et al. 2001). *MC1R* can also act as a modifier of melanoma risk within melanoma-prone families (Box et al. 2001; van Der Velden et al. 2001).

In the mouse, melanogenesis is also regulated, in part, by agouti signaling protein (ASP [MIM 600201]. The binding of ASP to MSHR precludes α-MSH–initiated signaling and thus blocks production of cAMP, leading to a downregulation of eumelanogenesis (Lu et al. 1994; Suzuki et al. 1997; Yang et al. 1997). The net result is increased synthesis of pheomelanin. The human agouti signaling protein gene (*ASIP*; GenBank accession number AL035458) encodes a 132–amino acid protein that is highly similar to mouse *ASIP,* both at the transcriptional and translational levels (Kwon et al. 1994; Wilson et al. 1995). Initial experiments showed that human *ASIP* expression in transgenic mice gives rise to yellow coat colors and decreased cAMP levels in vitro (Wilson et al. 1995).

We sought to characterize genetic variants in *ASIP* in a sample of healthy white subjects from the mid-Atlantic

Received October 2, 2001; accepted for publication December 6, 2001; electronically published February 6, 2002.

Address for correspondence and reprints: Dr. Peter A. Kanetsky, Department of Biostatistics and Epidemiology, 903 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104-6021. E-mail: pkanetsk@cceb.med.upenn.edu

0002-9297/2002/7003-0021$15.00

Table 1

Cycling and Reagent Conditions for *ASIP* Amplification and Sequencing

Exon	Primers and PCR Products	Reagents	Cycling Parameters
2	For PCR: forward 5′-GTCCCACTCAGGCCTCCT-3′, reverse 5′-GAGCCCAGAGGCTAGGTGA-3′, 205-bp product; for sequencing: 5′-GTCCCACTCAGGCCTCCT-3′ and 5′-GAGCCCAGAGGCTAGGTGA-3′	10 μl DNA template; .5 μM forward and reverse primers; 200 μM each dATP, dCTP, dGTP, dTTP; 1.5 mM $MgCl_2$, 1.5 μl 1 M tetramethylammonium chloride (TMAC); 5 μl 10× Buffer (PE Applied Biosystems); .5 μl Ampli*Taq* DNA polymerase (PE Applied Biosystems); ddH_20 to 50 μl	Robocycler 96 (Stratagene); 1 cycle of 5 min at 95°C; 35 cycles of 1 min at 94°C, 1 min at 57°C, and 1.5 min at 68°C; and 1 cycle of 10 min at 72°C
3	For PCR: forward 5′-CGCTTCTTCTCCTCTC-3′, reverse 5′-CTTCCCTGCCCTTAC-3′, 195-bp product; for sequencing: 5′-CGCTTCTTCTCCTCTC-3′	10 μl DNA template; .5 μM forward and reverse primer; 200 μM each dATP, dCTP, dGTP, dTTP; 1.5 mM $MgCl_2$, 1.5 μl 1M TMAC; 5 μl 10× buffer; .5 μl Ampli*Taq* DNA polymerase, ddH_20 to 50 μl	Robocycler 96; 1 cycle of 5 min at 95°C; 35 cycles of 1 min at 94°C, 1 min at 57°C, and 1.5 min at 68°C; and 1 cycle of 10 min at 72°C
4	For PCR: forward 5′-TGGAGGTGAGGGAATCTGAC-3′, reverse 5′-ACTGGGAGTGCAGCTGTGA-3′, 648-bp product; for sequencing: 5′-TCTGGTCCGGGATCTCCCTAG-3′	6 μl DNA template; .4 μM forward and reverse primer; .4 μl 50× dNTP mix[a]; .88 μl 25mM $Mg(OAc)_2$[a], 4 μl GC-Melt[a]; 4 μl 5× GC PCR buffer[a]; .4 μl Advantage-GC polymerase mix[a]; ddH_20 to 25 μl	Robocycler 96; 1 cycle of 5 min at 95°C; 40 cycles of 1 min at 94°C, 1 min at 57°C, and 1 min at 72°C; 1 cycle of 10 min at 72°C
3′ UTR	For PCR: forward 5′-GTGCTCAGCCTCAACTGCT-3′, reverse 5′-CACACCTTCGAGCAGCCTAT-3′, 208-bp product	10 μl DNA template; .5 μM forward and reverse primer; 200 μM each dATP, dCTP, dGTP, dTTP; 2 mM $MgCl_2$; 5 μl dimethyl sulfoxide; 1.5 μl bovine serum albumin; 5 μl 10× buffer; 1.2 μl Ampli*Taq* DNA polymerase; ddH_20 to 50 μl	Robocycler 96; 1 cycle of 5 min at 95°C; 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and 1 cycle of 7 min at 72°C

[a] Advantage-GC Genomic PCR kit (Clontech).

region of the United States. We also determined whether sequence variants in *ASIP* were associated with pigmentation characteristics and an elevated risk of melanoma or dysplastic nevi (DN), which are atypical moles that are epidemiological risk markers of melanoma and nonobligate precursors (Tucker et al. 1997).

Our study population consisted of participants enrolled in a previously described molecular epidemiological investigation of melanoma susceptibility and included 147 healthy control subjects, 176 persons with DN, and 423 patients with melanoma with or without a diagnosis of DN (Kanetsky et al. 2001). The University of Pennsylvania's institutional review board that oversees research involving human beings approved the study protocol. In brief, patients with a histologically confirmed diagnosis of incident melanoma or a clinical diagnosis of DN were eligible to participate. These patients were each asked to refer a healthy non–blood relative or friend to be control subjects. Each participant signed informed consent, completed a questionnaire eliciting information on pigmentation characteristics, gave buccal-swab samples containing genomic DNA, and underwent a skin examination to enumerate and characterize nevi, eye color, and freckling.

To describe human *ASIP,* we use nomenclature adopted from mouse *ASIP,* which consists of a noncoded first exon, followed by three coding exons, designated 2, 3, and 4 (reviewed by Siracusa [1994]). We completely characterized the coding regions of *ASIP* exons 2 (g.1-160), 3 (g.2458-2519), and 4 (g.8617-8793), including a portion of the 3′ UTR of exon 4 (g.8794–8968). *ASIP* gene regions were PCR-amplified and were sequenced directly on an ABI Prism 377 (Applied Biosystems) using Big Dye Terminators (Applied Biosystems) according to the manufacturer's specifications. Table 1 lists the primers, reagents, and reaction conditions used for PCR amplification and sequencing of *ASIP.*

We developed a PCR-RFLP assay to genotype the *ASIP* g.8818A→G polymorphism. The PCR conditions are given in table 1. Presence of the G allele gave rise to a unique *BsrB*I cleavage site within the expected 208-bp amplicon, resulting in products 161 bp and 47 bp in size. As an internal control that indicated incomplete or failed digestion, we added a known *MC1R* PCR product that contained a single, nonpolymorphic *BsrB*I recognition site that produced fragments 766 and 359 bp in size, after appropriate digestion. We digested 10 μl of *ASIP* PCR template and 2 μl of control *MC1R* PCR template with 3 μl of *BsrB*I (New England Biolabs), 2.5 μl of *BsrB*I buffer, and 7.5 μl of ddH_20 for at least 4 h at 55°C. Genotyping was completed by visualization of banding patterns on a 3% agarose gel after ethidium bromide staining.

Statistical analyses were undertaken using SAS for Windows v8.01 (SAS Institute). χ^2 analysis was used to test for differences in disease status and pigmentation characteristics, as well as disease status and *ASIP* genotype. We used logistic regression to estimate the odds ratio (OR) and 95% CI for associations of *ASIP* genotype (G/− vs. A/A) and pigmentation characteristics after adjustment for age, sex, and disease status. Logistic regression models containing indicator variables were

Table 2

***ASIP* g.8818A→G Genotype and Allele Frequency by Disease Status**

	No. (%) of Subjects[a]		
Genotype	Control (n = 147)[b]	with DN (n = 176)[c]	with Melanoma (n = 423)[d]
A/A	114 (78)	132 (75)	330 (78)
A/G	30 (20)	44 (25)	87 (21)
G/G	3 (2)	0 (0)	6 (1)

[a] P = .34.
[b] Frequency of G allele = .12.
[c] Frequency of G allele = .13.
[d] Frequency of G allele = .12.

used to assess associations of heterozygous (A/G vs. A/A) and homozygous (G/G vs. A/A) carriage and pigmentation characteristics.

From a convenient sample of 105 healthy control subjects, we successfully completed PCR amplification and directly sequenced 92 samples for *ASIP* exon 2, 97 samples for *ASIP* exon 3, and 55 samples for *ASIP* exon 4, including the 3′ UTR. Overall, samples from 52 individuals (104 chromosomes) were completely sequenced for all three *ASIP* regions. We did not detect a sequence variant in the *ASIP* coding regions. However, we detected a polymorphism in the 3′ UTR of *ASIP,* defined by the presence of an A or G at a position 25 bp downstream of the TGA termination codon in the noncoding exon 4. On the basis of the genomic DNA sequence available for *ASIP,* we designated this polymorphism as g.8818A→G (Beaudet and Tsui 1993; Antonarakis 1998). Among the 147 healthy control subjects genotyped for the polymorphism, the G allele occurred at a frequency of .12 and was in Hardy-Weinberg proportions (df 1; χ^2 = .18; P = .67). Table 2 contains results from *ASIP* g.8818A→G genotyping among the healthy control, DN, and melanoma groups. *ASIP* genotypes were similar across the groups.

Pigmentation characteristics for the total study population are given in table 3. As expected, there were significant differences among the healthy control, DN, and melanoma groups for most characteristics, including hair color, eye color, freckling, and total number of common acquired nevi. Differences between the groups in skin reaction to first intense and repeated sun exposure approached but did not reach significance. Table 4 presents results from crude and adjusted analyses estimating the association between specific pigmentation characteristics and carriage of the *ASIP* variant. These data indicate that carriage of the G allele was significantly associated with having dark hair (OR 1.8; 95% CI 1.2–2.8) and brown eyes (OR 1.9; 95% CI 1.3–2.7) after adjustment for age, sex, and disease status. The associations with dark hair and brown eyes remained significant even after additional adjustment for the remaining pigmentation characteristics in the table (dark hair: OR 1.7 and 95% CI 1.1–2.8; brown eyes: OR 1.6 and 95% CI 1.1–2.4). Although only nine samples were genotyped as G/G, results from logistics models provide some indication that there may be an *ASIP* allele-dosage effect such that homozygous carriers may have an increased likelihood of having dark hair and brown eyes compared with heterozygous carriers (see table 4). We also reran logistic models among the subset of 679 subjects who had complete information on all pigmentation variables. Results were nearly identical for *ASIP* associations with hair and eye color.

Although we statistically adjusted for DN and melanoma status in the above analyses, we further explored the possibility that the observed association between *ASIP* genotype and pigmentation could have been mediated, in part, by disease status. Our data do not suggest that the *ASIP* g.8818A→G polymorphism is associated with an alteration in susceptibility to melanoma. We did not note any difference in the proportion of carriers when subjects with melanoma were compared with control subjects (OR .97; 95% CI .62–1.5) or with subjects with DN (OR .85; 95% CI .56–1.3). As well, no difference was noted when subjects with DN were compared to control subjects (OR 1.2; 95% CI .69–1.9). Hardy-Weinberg proportions were evident among patients with melanoma (df 1; χ^2 = .025; P = .88) and the total study sample (df 1; χ^2 = .53; P = .47). However, among subjects with DN, we noted a slight deviation from Hardy-Weinberg proportions (df 1; χ^2 = 4.6; P = .033), caused by a deficiency of homozygous G/G carriers. In post hoc analyses, the addition of one homozygous variant carrier or reassignment of one heterozygous carrier to homozygous status would have resulted in Hardy-Weinberg proportions in the DN group (df 1; χ^2 = 2.4; P = .12 and df 1; χ^2 = 2.3; P = .13, respectively).

This is the first study to show that an *ASIP* polymorphism may play a role in human pigmentation characteristics, specifically hair and eye color. Two previous reports have investigated *ASIP* sequence variation. In 1999, Norman et al. genotyped 24 samples collected from Pima Indians (Norman et al. 1999); coding-region variants were not detected, nor did they characterize noncoding regions of *ASIP.* A recent publication (Voisey et al. 2001) reported screening for *ASIP* polymorphisms in coding and noncoding regions, including noncoding exon 1, in several small sample sets derived from different populations, including white, African American, Spanish Basque, Hispanic, Apache, and Australian Aboriginal ones. Although coding-region variation was not found, Voisey et al. (2001) detected the g.8818A→G polymorphism in 3 of 10 African American, 1 of 4 Asian, and 1 of 34 white samples. We calculated the polymorphism frequency among whites in the report by Voisey

Table 3

Frequency of Pigmentation Characteristics by Disease Status

Pigmentation Characteristic[a]	No. (%) of Subjects		
	Control (n = 147)	with DN (n = 176)	with Melanoma (n = 423)
Hair color:[b]			
Red or reddish brown	9 (6)	5 (3)	53 (13)
Blond	26 (18)	34 (20)	85 (20)
Dark	111 (76)	133 (77)	277 (67)
Eye color:[c]			
Blue or gray	49 (37)	55 (34)	184 (45)
Green or hazel	26 (20)	46 (29)	114 (28)
Light or dark brown	58 (44)	60 (37)	111 (27)
Skin reaction to first intense sun exposure:[d]			
Burn with blistering	13 (9)	13 (8)	57 (14)
Burn without blistering	48 (33)	74 (43)	156 (38)
Mild burn then tan or no burn	84 (58)	84 (49)	201 (49)
Skin reaction to repeated sun exposure:[d]			
No tan	6 (4)	7 (4)	30 (7)
Light tan	32 (22)	43 (25)	126 (31)
Medium or dark tan	106 (74)	120 (71)	254 (62)
Freckling:[e]			
Extensive	56 (42)	70 (43)	258 (62)
Moderate	29 (22)	40 (25)	95 (23)
Mild	32 (24)	44 (27)	49 (12)
Absent	16 (12)	8 (5)	11 (3)
No. of common acquired nevi:[e]			
⩾75	1 (1)	90 (56)	82 (20)
50–74	6 (5)	19 (12)	40 (10)
25–49	12 (9)	28 (17)	75 (18)
⩽24	114 (86)	25 (15)	216 (52)

[a] Data were missing on hair color for 13 persons, on eye color for 43 persons, on skin reaction to first intense sun exposure for 16 persons, on skin reaction to repeated sun exposure for 22 persons, and on freckling and number of common acquired nevi for 38 persons.
[b] $P = .0014$ for these values.
[c] $P = .0022$ for these values.
[d] $P = .059$ for these values.
[e] $P \leqslant .0001$ for these values.

et al. (2001) to be .015, nearly 10-fold lower than that reported here. This discrepancy in allele frequency is likely due to the white population selected for genotyping by Voisey et al. (2001), of whom half had either red or blond hair color. However, this finding is consistent with the predicted direction of effect for the g.8818A→G variant that would be expected to be underrepresented in individuals with low-eumelanin pigmentation types.

Although novel within the field of human pigmentation, our results parallel those of two investigations that molecularly characterized recessive mutations in *ASIP* that are associated with varied patterns of darkened coat colors seen in several mouse strains (Bultman et al. 1994; Hustad et al. 1995). Two pigmentation patterns arose from insertion mutations in *ASIP* intron 1 (Bultman et al. 1994)—one pattern from a point mutation and one from a base-pair deletion in *ASIP* coding regions—and four other patterns arose from mutations in the *ASIP* regulatory region (Hustad et al. 1995). These data indicate that decreased ASIP function may result from genetic alterations in nearly all gene domains of *ASIP*. Importantly, the 3′ UTR domains of primary transcripts have been shown to be critical to mRNA stability (reviewed by Ross [1995]). If the G allele destabilizes *ASIP* mRNA, leading to the premature degradation of transcript, it is possible that decreased levels of ASIP could preclude the blocking of α-MSH–mediated signaling, leading ultimately to a bias toward eumelanin synthesis and away from production of pheomelanin. Investigation of the functional significance of this polymorphism and of whether the G allele effects mRNA stability is necessary to corroborate our findings at a mechanistic level. In addition, it remains possible that sequence variation in other noncoding domains of *ASIP* (e.g., the promoter or intronic regions) may be associated with human pigmentation or disease risk. Although Voisey et al. did not find evidence of variants in *ASIP* noncoding exon 1, the search for polymorphic sites in other noncoding regions should be targeted for future investigation.

In mouse, an effect of ASIP was not observed against the genetic background of a nonfunctional MSHR (Ollmann et al. 1998; Abdel-Malek et al. 2001). Furthermore, against a genetic background of limited tyrosinase production (Tyr^{ch}/Tyr^{cb}), mouse strains that expressed

Table 4

Association of Pigmentation Characteristics for *ASIP* 8818G Allele Carriers

	Carrier				Heterozygote		Homozygote	
	G/− vs. A/A		G/− vs. A/A		A/G vs. A/A		G/G vs. A/A	
Pigmentation Characteristic	OR	95% CI	OR[a]	95% CI	OR[a]	95% CI	OR[a]	95% CI
Hair color:								
Red, reddish brown, or blond	1.0		1.0		1.0		1.0	
Dark	1.9	1.2–2.8	1.8	1.2–2.8	1.8	1.2–2.7	3.3	.40–27
Eye color:								
Blue, gray, green, or hazel	1.0		1.0		1.0		1.0	
Light or dark brown	1.9	1.3–2.7	1.9	1.3–2.8	1.9	1.3–2.7	3.4	.74–16
Skin reaction to first intense sun exposure:								
Burn with or without blistering	1.0		1.0					
Mild burn then tan or no burn	1.2	.86–1.7	1.2	.81–1.6				
Skin reaction to repeated sun exposure:								
No or light tan	1.0		1.0					
Medium or dark tan	1.5	1.0–2.3	1.4	.96–2.1				
Freckling:								
Extensive or moderate	1.0		1.0					
Mild or none	1.1	.70–1.6	.99	.64–1.5				
No. of common acquired nevi:								
⩾25	1.0		1.0					
⩽24	1.2	.87–1.8	1.2	.87–1.8				

[a] Adjusted for age, sex, and disease status.

both *ASIP* (A^y/a) and a functional *MC1R* produced a lighter-colored coat than those strains expressing a nonfunctional *MC1R* ($Mc1r^e/Mc1r^e$), regardless of *ASIP* expression (Ollmann et al. 1998). Taken together, these results indicate that ASIP may affect melanogenesis to a greater degree than lack of MSH signaling alone.

Human *MC1R* is highly polymorphic, and sequence variants have been associated with several cutaneous pigmentary phenotypes and with elevated risks of skin neoplasms (Valverde et al. 1995; Palmer et al. 2000; Bastiaens et al. 2001). Most *MC1R* variants are point mutations, some of which have been shown to compromise the quantity of α-MSH–induced cAMP production (Schioth et al. 1999). In one investigation, the Arg151Cys variant resulted in MC1R loss of function (Frandberg et al. 1998). Arg151Cys is one of several *MC1R* variants that, alone or in combination, have been strongly associated with red hair color (Valverde et al. 1995; Box et al. 1997; Palmer et al. 2000; Bastiaens et al. 2001). We can speculate that, analogous to mouse models, against a genetic background devoid of MC1R function, there could be limited or no effect of g.8818A→G; however, against a background of decreased MC1R function, it is plausible that *ASIP* could effect melanogenesis. Thus, *ASIP* genotyping may help explain those instances where *MC1R* genotyping predicts red hair although the subject has brown hair.

The addition of *ASIP* g.8818A→G genotyping to studies exploring human pigmentation may prove to be of interest and utility. Because *ASIP* and *MC1R* interact at the level of ligand and receptor, it is possible that the combination of *ASIP* and *MC1R* may associate better with pigmentation, such as hair and eye color, than either gene does alone. Even though, in our data, *ASIP* did not demonstrate a direct association with melanoma, it remains biologically plausible that interactive effects of *ASIP* may exist in combination with *MC1R* variants or one of the many other genes involved in pigmentation pathways. Hence, *ASIP* g.8818A→G genotyping may be meaningful in further investigations of melanoma and nonmelanoma skin neoplasms and may contribute independent information in the construction of future multivariable risk models of melanoma.

Acknowledgments

This work was supported, in part, by American Cancer Society grant IRG-78-002-22 and by National Institutes of Health grant P01 CA75434. We are indebted to past and present physicians of the Pigmented Lesion Clinic—Drs. David Elder, Rosalie Elenitsas, Stuart Lessin, Michael Ming, Lynn Schuchter, and Jonathan Wolfe—without whom this study would not have been possible. We thank Drs. Paska Permana and Jeannette Vaughn for sharing their experience and cycling conditions for the PCR amplification of *ASIP*.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *ASIP* [accession number AL035458])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MC1R [MIM 155555] and ASP [MIM 600201])

References

Abdel-Malek ZA, Scott MC, Furumura M, Lamoreux ML, Ollmann M, Barsh GS, Hearing VJ (2001) The melanocortin 1 receptor is the principal mediator of the effects of agouti signaling protein on mammalian melanocytes. J Cell Sci 114: 1019–1024

Antonarakis SE (1998) Recommendations for a nomenclature system for human gene mutations. Hum Mutat 11:1–3

Bastiaens MT, JAC ter Huurne, Kielich C, Gruis NA, Westendorp RGJ, Vermeer BJ, Bavinck JNB (2001) Melanocortin-1 receptor gene variants determine the risk of nonmelanoma skin cancer independently of fair skin and red hair. Am J Hum Genet 68:884–894

Beaudet AL, Tsui LC (1993) A suggested nomenclature for designating mutations. Hum Mutat 2:245–248

Box NF, Duffy DL, Chen W, Stark M, Martin NG, Sturm RA, Hayward NK (2001) Mc1r genotype modifies risk of melanoma in families segregating cdkn2a mutations. Am J Hum Genet 69:765–773

Box NF, Wyeth JR, O'Gorman LE, Martin NG, Sturm RA (1997) Characterization of melanocyte stimulating hormone receptor variant alleles in twins with red hair. Hum Mol Genet 6:1891–1897

Bultman SJ, Klebig ML, Michaud EJ, Sweet HO, Davisson MT, Woychik RP (1994) Molecular analysis of reverse mutations from nonagouti (a) to black-and- tan (a(t)) and white-bellied agouti (Aw) reveals alternative forms of agouti transcripts. Genes Dev 8:481–490

Frandberg PA, Doufexis M, Kapas S, Chhajlani V (1998) Human pigmentation phenotype: a point mutation generates nonfunctional MSH receptor. Biochem Biophys Res Commun 245:490–492

Harding RM, Healy E, Ray AJ, Ellis NS, Flanagan N, Todd C, Dixon C, Sajantila A, Jackson IJ, Birch-Machin MA, Rees JL (2000) Evidence for variable selective pressures at MC1R. Am J Hum Genet 66:1351–1361

Hunt G, Donatien PD, Lunec J, Todd C, Kyne S, Thody AJ (1994) Cultured human melanocytes respond to MSH peptides and ACTH. Pigment Cell Res 7:217–221

Hustad CM, Perry WL, Siracusa LD, Rasberry C, Cobb L, Cattanach BM, Kovatch R, Copeland NG, Jenkins NA (1995) Molecular genetic characterization of six recessive viable alleles of the mouse agouti locus. Genetics 140: 255–265

Kanetsky PA, Holmes R, Walker A, Najarian D, Swoyer J, Guerry D, Halpern A, Rebbeck TR (2001) Interaction of glutathione S-transferase M1 and T1 genotypes and malignant melanoma. Cancer Epidemiol Biomarkers Prev 10: 509–513

Kwon HY, Bultman SJ, Loffler C, Chen WJ, Furdon PJ, Powell JG, Usala AL, Wilkison W, Hansmann I, Woychik RP (1994) Molecular structure and chromosomal mapping of the human homolog of the agouti gene. Proc Natl Acad Sci USA 91:9760–9764

Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO (1994) Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371:799–802

Norman RA, Permana P, Tanizawa Y, Ravussin E (1999) Absence of genetic variation in some obesity candidate genes (GLP1R, ASIP, MC4R, MC5R) among Pima indians. Int J Obes Relat Metab Disord 23:163–165

Ollmann MM, Lamoreux ML, Wilson BD, Barsh GS (1998) Interaction of Agouti protein with the melanocortin 1 receptor in vitro and in vivo. Genes Dev 12:316–330

Palmer JS, Duffy DL, Box NF, Aitken JF, O'Gorman LE, Green AC, Hayward NK, Martin NG, Sturm RA (2000) Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? Am J Hum Genet 66:176–186

Ross J (1995) mRNA stability in mammalian cells. Microbiol Rev 59:423–450

Schioth HB, Phillips SR, Rudzish R, Birch-Machin MA, Wikberg JE, Rees JL (1999) Loss of function mutations of the human melanocortin 1 receptor are common and are associated with red hair. Biochem Biophys Res Commun 260: 488–491

Siracusa LD (1994) The agouti gene: turned on to yellow. Trends Genet 10:423–428

Suzuki I, Cone RD, Im S, Nordlund J, Abdel-Malek ZA (1996) Binding of melanotropic hormones to the melanocortin receptor MC1R on human melanocytes stimulates proliferation and melanogenesis. Endocrinology 137:1627–1633

Suzuki I, Tada A, Ollmann MM, Barsh GS, Im S, Lamoreux ML, Hearing VJ, Nordlund JJ, Abdel-Malek ZA (1997) Agouti signaling protein inhibits melanogenesis and the response of human melanocytes to alpha-melanotropin. J Invest Dermatol 108:838–842

Tucker MA, Halpern A, Holly EA, Hartge P, Elder DE, Sagebiel RW, Guerry DT, Clark WH Jr (1997) Clinically recognized dysplastic nevi. A central risk factor for cutaneous melanoma. JAMA 277:1439–1444

Valverde P, Healy E, Jackson I, Rees JL, Thody AJ (1995) Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nat Genet 11:328–330

van Der Velden PA, Sandkuijl LA, Bergman W, Pavel S, van Mourik L, Frants RR, Gruis NA (2001) Melanocortin-1 receptor variant r151c modifies melanoma risk in dutch families with melanoma. Am J Hum Genet 69:774–779

Voisey J, Box NF, van Daal A (2001) A polymorphism study of the human Agouti gene and its association with MC1R. Pigment Cell Res 14:264–267

Wilson BD, Ollmann MM, Kang L, Stoffel M, Bell GI, Barsh GS (1995) Structure and function of ASP, the human homolog of the mouse agouti gene. Hum Mol Genet 4:223–230

Yang YK, Ollmann MM, Wilson BD, Dickinson C, Yamada T, Barsh GS, Gantz I (1997) Effects of recombinant agouti-signaling protein on melanocortin action. Mol Endocrinol 11:274–280