

SUPPLEMENTAL DATA

This material is an online-only supplement to the following article: Binkley CJ, Beacham A, Neace W, Gregg RG, Liem EB, Sessler DI. Genetic variations associated with red hair color and dental anxiety, fear of dental pain and avoidance of dental care. *JADA* 2009;140(7):896-905. Copyright © 2009 American Dental Association. All rights reserved.

GENETIC ANALYSIS

We obtained venous blood samples (5 milliliters) from the volunteer's arm as in our previous studies.^{1,2} We analyzed these samples for genes related to hair color only.

None of the results were disclosed to the volunteers. We extracted DNA from the blood samples by using the PureGene DNA isolation kits (Gentra Systems, Minneapolis).

We genotyped samples for 29insA, V60L, K65N, D84E, V92M, R142H, R151C, Y152X, I155T, R160W, R163Q, I293M, D294H and A299T variants using a Pyromark Pyrosequencing system (Biotage, Charlottesville, Va.). The polymerase chain reaction (PCR) primers and detection primers are listed in Table 1. To generate the PCR products, we used a PCR mix containing the following in 40 microliters of 1x Accuprime buffer II (Invitrogen, Carlsbad, Calif.): 50 to 100 nanograms of DNA, 2 units of Accuprime Taq polymerase and accompanying buffer (Invitrogen) and 0.3 micromolars of each primer. We amplified the DNA for 36 cycles (0.5 minute at 95°C, 0.5 minute at 55°C, 0.5 minutes at 68°C). We analyzed amplified products by means of agarose gel electrophoresis to ensure PCR success. We purified the biotinylated single-stranded DNA and did pyrosequencing reactions as recommended by the manufacturer (Biotage).

We also sequenced a subset of 53 samples. We generated two overlapping PCR amplicons that cover the entire open reading frame using primers: P5-F: 5'-ACTCCTTCCTGCTTCCTGGA and P12-R: 5'- TGGTCGTAGTAGGCGATGAA; P9-F: 5'- GCAGCAGCTGGACAATGTCA and 5'- ACTGCCAGGGTCACACAGG. We purified the PCR fragments by using a Qiaquick PCR purification system (Qiagen, Valencia,

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Calif.), and we sequenced approximately 20 ng using the ABI Big Dye sequencing system and an ABI3130 XL DNA sequencer (Applied Biosystems, Foster City, Calif.). We analyzed the sequence and detected single nucleotide polymorphisms by using onboard sequencing analyses software for heterozygote detection. Our genotyping results are shown in Table 2.³ ■

1. Liem EB, Joiner TV, Tsueda K, Sessler DI. Increased sensitivity to thermal pain and reduced subcutaneous lidocaine efficacy in redheads. *Anesthesiology* 2005;102(3):509-514.
2. Liem EB, Lin CM, Suleman MI, et al. Anesthetic requirement is increased in redheads. *Anesthesiology* 2004;101(2):279-283.
3. García-Borrón JC, Sánchez-Laorden BL, Jiménez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. *Pigment Cell Res* 2005;18(6):393-410.

Table 1
Primers used for SNP* genotyping.

MELANCORTIN-1 RECEPTOR GENE VARIANT	BIOTINYLATED PRIMER*	PAIRED PRIMER	SNP SEQUENCING PRIMER
V60L, K65N	TGAAGCAGTAC ATGGGTGAG	CTGGTGAGCTT GGTGGAGAA	GTGGAGAACGC GCTG
R142H, R151C, Y152X, I155T, R160W, R163Q	CGATGAAGAGC GTGCTGAAG	CTGCAGCTCCA TGCTGTCC	CGCCATCGCCG TGGA
I293M, D294H, A299T	TGCCCAGGGTC ACACAGG	ACGTGCGGCTG CATCTTC	CATCTGCAATG CCAT

* SNP: Single nucleotide polymorphism.

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Table 2
MC1R Variants* Hair Color Crosstabulation.

MC1R VARIANTS	HAIR COLOR		TOTAL
	Dark	Red	
No Variants	30	1	31
Variants Most Likely to Decrease Melanocyte Function³			
29insA;N279S	0	1	1
D294H	4	0	4
D294H;D294H	0	1	1
D294H;R151C	0	5	5
D294H;R160W	0	1	1
D84E;D294H	0	1	1
D84E;R151C	0	1	1
I155T;V60L	1	0	1
R142H; V60L	1	0	1
R142H;R151C	0	3	3
R142H;R160W	0	1	1
R151C	5	1	6
R151C;29insA	0	4	4
R151C;D294H	0	2	2
R151C;I155T	0	1	1
R151C;R142H	0	1	1
R151C;R151C	0	11	11
R151C;R160W	1	10	11
R151C;R160W;D294H	0	2	2
R151C;R163Q	1	0	1
R151C;S83P	0	1	1
R151C;V60L	1	4	5
R151C;V92M	1	1	2
R160;V60L	0	1	1
R160W	5	0	5
R160W;D84E	0	1	1

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R160W;R160W	0	9	9
R160W;V60L	0	2	2
Variants Least Likely to Decrease Melanocyte Function (Neutral Alleles)			
R163Q	4	0	4
R163Q;V92M	2	0	2
V60L	12	1	13
V60L;R163Q	1	0	1
V60L;V60L	2	0	2
V60L;V92M	1	0	1
V92M	4	0	4
V92M;V92M	1	0	1
TOTAL	77	67	144