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Detection of Heterozygotes for Tyrosinase-Negative Oculocutaneous Albinism by Hairbulb Tyrosinase Assay

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INTRODUCTION

Human oculocutaneous albinism (OCA), an easily recognized autosomal recessive disorder of melanin metabolism, is described in at least four distinct forms [1]. All forms share to varying degrees the triad of hypopigmentation of hair, skin, and eyes. Tyrosinase-negative (ty-neg) OCA is the most hypopigmented form and is characterized by marked skin sensitivity to sunlight, photophobia, nystagmus, and a severe reduction in visual acuity. These changes result from the absence of active tyrosinase, the enzyme responsible for the oxidation of tyrosine, and dopa (3,4-dihydroxyphenylalanine) in the melanocyte [2]. The frequency of ty-neg OCA is 1:39,000 in the United States white population and 1:28,000 in the United States black population, with a heterozygote frequency of approximately 1% in the total population [1].

Heterozygotes for all forms of OCA are fully pigmented and are indistinguishable within the general population. Froggatt [3] and Waardenberg [4] have suggested that heterozygotes for ty-neg OCA have an abnormal iris translucency, but this has not been a consistent finding. Biochemical detection of heterozygotes has not been possible because of the lack of suitable methods for studying human pigment metabolism. We report the application of a recently described method for quantitating tyrosinase activity in single human hairbulbs [2] to detect heterozygotes for ty-neg OCA.

MATERIALS AND METHODS

Subjects

The test subjects were five obligate heterozygotes from three families containing a ty-neg albino. In the first two families, normally pigmented mothers of an albino child were tested (subjects 1 and 2). In the third family, three normally pigmented offspring of an albino mother were tested (subjects 3, 4, and 5). Controls were normally pigmented subjects with no family history of OCA.

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Source of Enzyme

Scalp hairbulbs were used as a source of melanocytes. Eight to 10 hairs were grasped at the scalp with a small pair of orthodontic pliers and plucked with a single pull. The hairs were separated and anagen or growing hairbulbs identified [5, 6]. Catogen (transitional) and telogen (resting) hairbulbs were not used for the assay. Single hairbulbs were cut from the hair shaft and immediately placed in buffer for assay. After the assay, the hairbulbs were observed at 80 × magnification for positive identification of growth phase. All nonanagen hairbulbs were excluded from analysis. Usually 6–8 anagen hairbulbs were assayed per individual.

Tyrosinase Assay

The tyrosine hydroxylase activity of tyrosinase was assayed by determining the rate of ^{3}HOH formed with the oxidation of L-tyrosine-3,5-^{3}H to L-dopa-5-^{3}H as described by Pomerantz [7]. Single anagen hairbulbs were placed in an incubation mixture containing 0.2 nmol L-tyrosine-3,5-^{3}H (1 Ci/mmol); 0.1 nmol L-dopa; 6 μmol phosphate buffer, pH 6.8; and 0.5% Triton X-100 in a total volume of 0.06 ml. Nonspecifically formed ^{3}HOH in the stored L-tyrosine-3,5-^{3}H was eliminated by evaporating the sample to dryness immediately before use. Incubation was at 37°C. At 0, 60, and 120 min a sample of the incubation mixture was obtained and placed on a small Dowex-50W column equilibrated with 0.1 M citrate. The ^{3}HOH was recovered from the column with two washes of 0.4 ml of 0.1 M citrate, and the total wash was counted. Blank reactions containing no hairbulb were run with each assay, and values for the blank were subtracted from the hairbulb values before determining the amount of tyrosine oxidized. A mean value was determined from results of all hairbulbs for each subject, and enzyme activity was expressed in picomoles (pmol) as the mean tyrosine oxidized per 120 min. The 60 min value was used only to follow the linearity of the reaction. Statistical analysis was by Mann-Witney rank order test.

RESULTS

Tyrosinase activity in hairbulbs from normally pigmented control subjects is given in table 1. For brown hair, the overall mean activity was 1.41 pmol of tyrosine oxidized per 120 min with a range of 0.46–2.52 for 17 subjects. Hair color was light to medium brown with no correlation between hairbulb activity and intensity of pigmentation. Reproducibility for brown hair was shown by repeated testing of three subjects, giving a mean ± SD of 1.51 ± 0.34 (n = 23 tests), 1.70 ± 0.36 (n = 13), and 2.52 ± 0.47 (n = 7) pmol of tyrosine oxidized per 120 min. Blond hairbulbs had an overall mean of 1.11 pmol of tyrosine oxidized per 120 min with a range of 0.18–3.94. Hair color varied from light white-blond to dark blond, with no correlation between activity and intensity of pigmentation. One subject tested on five occasions had a mean ± SD of 0.18 ± 0.06 pmol of tyrosine oxidized per 120 min. All control subjects were readily classified as blond or brown with no overlap. Enzyme activity in black and red

TABLE 1

HAIRBULB TYROSINASE ACTIVITY IN NORMALLY PIGMENTED CONTROL SUBJECTS

HAIRCOLOR	No.	TYROSINE OXIDIZED (PMOL/120 MIN)	
		Mean	Range
Blond	12	1.11	0.18–3.94
Brown	17	1.41	0.46–2.52
Black	11	1.86	0.54–3.16
Red	9	3.63	1.35–5.44

hairbulbs is given in table 1. The complete control group contained 21 females and 28 males, and there was no correlation between sex and hairbulb activity.

The five test subjects were either a parent or an offspring of a classical ty-neg albino. The albino daughter in the second family and the albino mother in the third family had no detectable tyrosinase activity in their hairbulbs [2]. The albino son in the first family had not been studied by the quantitative hairbulb tyrosinase assay. He was classified as ty-neg because his qualitative hairbulb incubation test was negative [1]; his hairbulbs formed no visible pigment after prolonged incubation in tyrosine. Clinically, the three albinos had marked hypopigmentation, with white skin, an absence of pigmented nevi, white-light yellow hair, photophobia, nystagmus, and reduced visual acuity.

Results of the hairbulb tyrosinase assay for the ty-neg heterozygotes are given in table 2. Values ranged from 0 to 0.12 pmol of tyrosine oxidized per 120 min. Each subject was tested once except for subject 3 who had identical results on two tests. All had brown hair, with two having light brown hair; the hair of these two subjects was lighter than that of any of the control subjects with brown hair, but pigmented enough to be definitely classified as brown. Eye color was brown or blue, and none had translucent irides. Clinically, none were distinguishable from control subjects.

TABLE 2

HAIRBULB TYROSINASE ACTIVITY IN TY-NEG HETEROZYGOTES

Subject	Sex	Age	Hair Color	Eye Color	Mean Tyrosine Oxidized (pmol/120 Min)
1	F	40	Brown	Blue	0.00
2	F	45	Brown	Blue	0.08
3	F	19	Light brown	Brown	0.05
4	F	18	Light brown	Brown	0.00
5	M	16	Brown	Brown	0.12

With all five classified as having brown hair, the level of hairbulb tyrosinase activity in the ty-neg heterozygotes was significantly different than that of the controls with brown hair ($P < .001$). The lowest level in a brown control was 3.7 times greater than the highest level in a heterozygote. If the heterozygote with the lightest hair (subject 3) was classified as blond rather than brown, then the difference between her level of activity and that of the blond controls was not statistically significant ($.1 < P < .05$), although the lowest level in a blond control was 3.8 times greater than her level of activity.

DISCUSSION

Tyrosinase-negative OCA results from an absence of active tyrosinase in melanocytes [2]. The data presented here show that normally pigmented individuals heterozygous for ty-neg OCA have markedly reduced or absent tyrosinase activity in their hairbulb melanocytes when assayed by the present method and that their levels of activity are significantly different from those of the control subjects. There was no overlap between control and heterozygous values, and no control had absent activity.

These levels of activity in hairbulbs from ty-neg heterozygotes are unexpectedly low

and are much less than a level that is 50% of normal, which might be expected in a biochemical defect of this type. Adequate enzyme activity is present in heterozygous subjects since their pigmentation is indistinguishable from that of control subjects. Why enzyme activity is undetectable by assay appears to be related to the method used for assay and to the biology of the melanocyte.

Melanin forms within the melanosome, a specialized organelle of the melanocyte [8]. Tyrosinase is thought to be the single enzyme controlling the formation of melanin by catalyzing the hydroxylation of tyrosine to dopa and the oxidation of dopa to dopaquinone [9, 10]. Dopaquinone undergoes nonenzymatic oxidation and polymerization to form melanin. Tyrosinase, synthesized on small ribosomal granules, is transferred through the endoplasmic reticulum to the golgi where it is incorporated into the developing melanosome [11]. It is in a soluble phase before incorporation into the melanosome, but once packaged into the melanosome, the enzyme becomes protein-bound and insoluble [11, 12]. Bound tyrosinase is active in melanin formation and appears to be inactivated within the melanosome as melanin fills the organelle [11, 13]. Soluble tyrosinase does not form melanin in the melanocyte.

In the assay used in the present study, tyrosinase is readily released from the hairbulb melanocyte with the detergent Triton X-100, the majority solubilized within 30 min after exposure of the hairbulb to the detergent [2]. That rapidity suggests that the detergent, by disrupting the cell wall, has released soluble cytoplasmic tyrosinase. Bound tyrosinase might also be available since detergents have been shown to release or activate melanosomal-bound tyrosinase [14, 15]; however, it seems unlikely that this contributes greatly to the quantity of active enzyme available for assay. The inactivation of bound tyrosinase within the melanosome is thought to result from the binding of melanin or melanin intermediates to the active site of the enzyme [11], and Triton should not rapidly reverse this inactivation.

The most likely explanation for the finding of absent or markedly reduced tyrosinase activity in the hairbulb of ty-neg heterozygotes is an absence of soluble tyrosinase in these hairbulb melanocytes. Adequate amounts of bound melanosomal tyrosinase are present for the subjects are normally pigmented. The presence of only one normal tyrosinase gene in the heterozygote leads to the production of approximately half the normal amount of enzyme. After production, the enzyme must be packaged directly into the melanosome for melanin formation with little remaining in the soluble cytoplasmic phase. This explanation suggests that there are mechanisms that control the incorporation of tyrosinase into the melanosome, but at present these mechanisms are unknown. Recent work by Varga et al. [16] suggests that melanocyte stimulating hormone (MSH) may be involved.

SUMMARY

Five heterozygotes for ty-neg OCA had absent or markedly reduced hairbulb tyrosinase activity, when compared to normally pigmented control subjects. Clinically, all five were fully pigmented and could not be distinguished from the normal control subjects. It is proposed that the quantitative hairbulb tyrosinase assay can be used to detect heterozygotes for ty-neg OCA. A mechanism to explain the low levels of activity in the hairbulbs from heterozygotes is discussed.

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REFERENCES

1. WITKOP CJ JR, WHITE JG, KING RA: Oculocutaneous albinism, in *Heritable Disorders of Amino Acid Metabolism*, edited by NYHAN W, New York, John Wiley 1974, pp 177–261
2. KING RA, WITKOP CJ JR: Hairbulb tyrosinase activity in oculocutaneous albinism. *Nature* 263:69–71, 1976
3. FROGGATT P: Albinism in Northern Ireland. *Ann Hum Genet* 24:213–238, 1960
4. WAARDENBURG PJ: *Remarkable Facts in Human Albinism and Leukism*. The Netherlands, Van Gorcuin, 1970, pp 14–18
5. CHASE HB, RAUCH H, SMITH VW: Critical stages of hair development and pigmentation in the mouse. *Physiol Zool* 21:1–8, 1951
6. MONTAGNA W, PARAKKAL PF: *The Structure and Function of the Skin*. New York, Academic Press, 1974, pp 186–222
7. POMERANTZ SH: The tyrosine hydroxylase activity of mammalian tyrosinase. *J Biol Chem* 241:161–168, 1966
8. SEIJI M, SHIMAO K, BIRBECK MSC, FITZPATRICK TB: Subcellular localization of melanin biosynthesis. *Ann NY Acad Sci* 100:497–533, 1963
9. LERNER AB, FITZPATRICK TB, CALKINS E, SUMMERSON WH: Mammalian tyrosinase: preparation and properties. *J Biol Chem* 178:185–195, 1949
10. LERNER AB, FITZPATRICK TB: Biochemistry of melanin formation. *Physiol Rev* 30:91–126, 1950
11. SEIJI M: Subcellular particles and melanin formation in melanocytes. *Adv Biol Skin* 8:189–222, 1967
12. BURNETT JB: The tyrosinase of mouse melanoma. Isolation and molecular properties. *J Biol Chem* 246:3079–3091, 1971
13. SEIJI M, FITZPATRICK TB: The reciprocal relationship between melanization and tyrosinase activity in melanosomes (melanin granules). *J Biochem (Tokyo)* 49:700–706, 1961
14. BURNETT JB, SEILER H, BROWN IV: Separation and characterization of multiple forms of tyrosinase from mouse melanoma. *Cancer Res* 27:880–889, 1967
15. MENON IA, HABERMAN HF: Activation of tyrosinase in microsomes and melanosomes from B16 and Harding-Passey melanomas. *Arch Biochem Biophys* 137:231–242, 1970
16. VARGA JM, MOELLMAN G, FRITSCH P, GODAWSKA E, LERNER AB: Association of cell surface receptors for melanotropin with the golgi region in mouse melanoma cells. *Proc Natl Acad Sci USA* 73:559–562, 1976