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ORIGINAL ARTICLE

Inheritance of a novel mutated allele of the *OCA2* gene associated with high incidence of oculocutaneous albinism in a Polynesian community

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Oculocutaneous albinism type 2 (OCA2) is a human autosomal-recessive hypopigmentation disorder associated with pathological mutations of the *OCA2* gene. In this study, we investigated a form of OCA in a Polynesian population with an observed phenotype characterized by fair skin, some brown nevi present in the sun-exposed areas and green or blue eyes. Hair presented with a unique red coloration since birth, with tones ranging across individuals from Yellow-Red to Brown-Red, or Auburn. We genetically screened for mutations in the *OCA2* and *MC1R* genes as their products have previously been shown to be associated with red hair/fair skin and OCA2. The *SLC45A2* gene was also screened to identify any possible relation to skin color variation. We have identified a novel missense substitution in the *OCA2* gene (Gly775Asp) responsible for OCA2 in individuals of Polynesian heritage from Tuvalu. The estimated incidence of this form of OCA2 in the primary study community is believed to occur at one of the highest recorded rates of albinism at approximately 1 per 669 individuals. In addition, we have analyzed four unrelated individuals with albinism who have Polynesian heritage from three other separate communities and found they carry the same *OCA2* mutation. We also analyzed an out-group comprising three unrelated individuals with albinism of Melanesian ancestries from two separate communities, one Australian Aboriginal and three Australian Caucasians, and did not detect this mutation. We hypothesize that this mutation may be Polynesian specific and that it originated from a common founder.
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INTRODUCTION

The pigmentary system is dependent on the production of the light-absorbing biopolymer, melanin, within epidermal, ocular and follicular melanocytes.[1] Oculocutaneous albinism (OCA) is a recessive genetic disorder associated with mutations of the genes controlling the biosynthesis of the melanin pigment within the melanocyte. Albinism can be associated with a range of disorders through functional disruption of several classes of pigmentation genes, with this genetic heterogeneity producing a spectrum of pigmentation loss in affected individuals.[2] Mutation in a number of different genes have been reported to be responsible for OCA.[3,4] Four types of albinism specific to melanocytes have been defined on molecular grounds as OCA1–4 (MIM 203100, 203200, 203290, 606574), and two other metabolic diseases have been described as Hermansky–Pudlak syndrome (HSP1-8 (MIM 203300)) and Chediak–Higashi syndrome (CHS (MIM 214500)) with additional health problems and associated OCA. All types of OCA are clinically characterized by visual aberrations and impairment, combined with generalized hyperpigmentation of the skin, hair and eyes when compared with others of the same familial or ethnic background.[5]

Clinically, OCA2 is defined for individuals who have some pigment at birth,[2] with some affected patients accumulating additional pigment over their lifetime. OCA2 is the most common form of albinism worldwide; the incidence in South African Black populations has been reported to be 1:3600,[6,7] 1:10 000 in Black and 1:36 000 in Caucasians in the United States,[8] and 1:10 000 in the Irish.[9] However, the highest incidence of OCA2 so far molecularly characterized at slightly $<$1:2000 is in the Native American Navajos with a carrier frequency of 4.5%, estimated to originate from a single founder.[10] The *OCA2* locus maps to chromosome 15q11.2-q12[11] and is the human homolog of the mouse pink-eyed dilution gene (*p*)[12,13] encoding the P-protein. Structurally, the 838 amino-acid P-protein is predicted to contain 12 transmembrane-spanning domains. The cDNA is encoded in 24 exons,[14,15] and the locus covers over 345 kb.[16] Though initially reported as an integral melanosomal protein potentially associated with transport of some molecule into the melanosome, its subcellular localization has been a subject of debate. It has been proposed to regulate melanosomal pH,[15] with an additional possible role in the processing and trafficking of melanosomal proteins.[17,18] It has recently been confirmed as localizing with mature melanosomes.[19]

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There have been numerous mutations and polymorphisms found in the *OCA2* locus, which are listed on the International Albinism Center Database (http://albinismdb.med.umn.edu). Most of the mutations tend to occur in the C-terminal half of the polypeptide, which contains the majority of the membrane-spanning domains.[20] Polymorphism within the *OCA2* gene is significantly associated with eye color[21] and also underlies the previous assignment of the brown eye (BEY2/EYCL3, MIM227220) and brown hair (HCL3, MIM601800) phenotypes. A single single-nucleotide polymorphism in the neighboring gene, *HERC2*, was recently found to be a common founder polymorphism affecting an *OCA2* regulatory element, which in turn results in blue eye color[22,23] and reduced melanin content in cultured human melanocytes.[24] *MC1R* is associated with red hair in the European population and has been found to modify the OCA2 phenotype to also express red hair.[25]

Type I OCA has been extensively studied and divided into two clinical subtypes; OCA1A is caused by the complete loss of catalytic activity of the tyrosinase enzyme (TYR) and results in the life-long absence of melanin pigment, whereas OCA1B retains residual tyrosinase function allowing for the development of some melanin pigment.[20,26] The two other forms of albinism, OCA3 and OCA4, have been found to result from mutations in the *TYRP1* and *SLC45A2* gene loci, respectively. The *TYRP1* locus has been mapped to chromosome 9p23, and 95% of OCA3-related mutations result in the generation of premature stop codons or frameshifts producing truncated proteins.[27,28] The phenotype is characterized by red-toned hair and reddish-brown pigmented skin, and optical features are not as severe as in other forms of OCA.[29] OCA4 was first defined on presentation in one individual homozygous for a splice-site mutation within the *SLC45A2* gene on chromosome 5p, with the phenotype consisting of generalized and ocular hypopigmentation similar to that seen in OCA2 patients.[30] OCA4 is one of the most common forms of albinism in the Japanese population.[31,32] Polymorphism within the *SLC45A2* gene is a major determinant of normal variation in skin pigmentation,[33] with the rs16891982 b.1122G/C, p.374Leu/Phe change of the encoded protein, MATP, associated with the lighter pigmentation phenotypes of Europeans.[24] A heavier OCA pigmentation phenotype has been noted as being linked with darker familial background pigmentation, indicating that other genes may modify the OCA condition.[5] In addition, compound heterozygosity for *OCA2* combined with a known *TYRP1* (OCA3) mutation has been reported to result in a form of albinism presenting with red hair in the absence of *MC1R* red hair-associated alleles.[34] Hypopigmentation was also suggested as being linked to carriers of OCA2 mutations through *OCA2* gene haploinsufficiency,[35] similar to inheritance of hypopigmentation in Prader-Willi syndrome and Angelman syndrome, which are conditions with deletion or isodisomy of a parental *OCA2* allele.

Most albinism research has centered on individuals from South African, African-American, Asian, Caucasian-American or Northern European populations. There are detailed phenotype and genotype profiles available for most of these populations (http://albinismdb.med.umn.edu). The last published investigation of albinism in the Pacific region were phenotype descriptive studies of OCA(s) in Papua New Guinea.[36–39] The clinical phenotype of one form of albinism termed 'Red-skinned' in the Micronesians of Papua New Guinea has been defined in two published studies.[36,38] The most extensive albinism research in the Pacific in the past few years has involved a photographic project undertaken in collaboration with the National Organization for Albinism and Hypopigmentation of the albinism world alliance (www.positiveexposure.org). There has been little published about albinism in the Polynesians of the South Pacific; moreover, there are no published data of genetic analysis combined with phenotype classification, for OCA in the South Pacific Region. This study was initiated to investigate an original human OCA phenotype in a South Pacific island community of Polynesian descent, establish the nature of its inheritance through extensive pedigree analysis and experimentally determine the genetic cause.

MATERIALS AND METHODS

The study population and pigmentation characteristics

Before the initiation of this study, professional contacts were established with key health administrative groups in the study community, and the project outline assessed and approved by the Human Medical Research Ethics Committee of the University of Queensland. Written informed consent was obtained from all participants. Normal coloration for this South Pacific Island community according to the average phenotype observed in the general population is defined as black/dark brown hair, dark brown eyes and moderately brown skin. Assessment of OCA in the field was based on marked hypopigmentation of skin, hair and irides when compared with other immediate family members. The presence of eye aberrations, such as nystagmus, strabismus, poor vision and photophobia, were considered a definitive feature. Albinism was confirmed clinically for several individuals through independent examination by a dermatologist, optometrist, ophthalmologist or resident medical general practitioner.

A total of 124 Pacific Islander participants were included in the study: 12 individuals with OCA and a further 112 individuals who were part of their immediate or extended families. These participants originated from across the eight Islands that make up the Tuvalu archipelago. In addition, we analyzed an out-group of seven unrelated individuals with OCA, from different South Pacific Island ancestries—Melanesian (one Fijian individual and two Vanuatu/Torres Strait Islander mix siblings) and Polynesian (one Samoan individual, one Tongan individual and one Niuean), plus one individual of mixed Samoan Islander/Caucasian origin. Also included in the out-group were one Australian Aboriginal and three Australian Caucasian individuals with OCA. In addition to OCA individuals, we also included normally pigmented individuals: three immediate family members of the mixed Samoan, four immediate family members of one of the Caucasians, plus one unrelated Niuean Islander. Three observers independently assessed available photographs of 111 Tuvaluan participants to grade the hair, skin and irides by coloration and shade; these results were used to create a consensus for each individual before the genetic profile was known.

Biological sampling and DNA extraction

Whole blood was collected by venipuncture into 9 ml tubes containing liquid EDTA (VACUETTE, Greiner Bio-One, Kremsmunster, Austria), then mixed with equal volumes of storage buffer AS1 (QIAGEN, Doncaster, VIC, Australia) for each individual with albinism and their parents from the study community. The majority of other participants sampled had whole blood collected through fingertip pinprick with a 2-mm 'Safety Flow' semiautomatic lancet (MICROTAINER, Becton Dickinson, Franklin Lakes, NJ, USA) and stored as three blood spots on 1-mm Whatman BFC 180 filter paper 'Sample Collection Cards' provided by the Neonatal Screening Unit of the Royal Brisbane Hospital, Australia.[40] Buccal cells were collected for nine participants sampled either using a sterile long-handled cotton swab (COPAN Diagnostics, Murrieta, CA, USA), FTA Classic Card (Whatman International Ltd, Piscataway, NJ, USA) or Oragene (DNAgenotek, Ottawa, ON, Canada). Genomic DNA was extracted from the peripheral blood leukocytes or buccal cells as previously described (QIAamp Blood Midi/Maxi kit (QIAGEN).[40–42] For longer-term storage, buccal DNA was subjected to phenol–chloroform extraction and recovered by sodium acetate–ethanol precipitation, resuspended in H_2O. PCR reactions were performed using 2 µl extracted product.

Microsatellite analysis

Polymorphic microsatellite markers contained within the *TYR*,[43] *TYRP1*[44,45] and *DCT*[28,45] loci were amplified as described and analyzed on 6% polyacrylamide sequencing gels before visualization by autoradiography. An AC-dinucleotide polymorphic marker derived from an *OCA2* locus BAC clone PR11-322N14 of 149 bp and within intron 17 of the gene was assayed

using amplimer sequences forward 5′-CTTGGCAACATCCCTGTATCA-3′ and reverse 5′-TGAATGCCATTATTTCATTCCTT-3′. PCR amplifications were carried out in a total reaction volume of 25 µl containing 25 ng genomic DNA template; 25 pmol of both primers; 200 µM of each dATP, dTTP, dGTP, 13 µM dCTP (Promega, Sydney, NSW, Australia) and 0.2 µl α-^{32}P-dCTP; 1× PCR buffer (Promega); 1.5 mM $MgCl_2$ (Promega) and 1.25 U Taq polymerase (Promega). For this microsatellite, the PCR protocol run on DNA Thermal Cycler 480 (Perkin Elmer, Waltham, MA, USA) involved initial denaturation for 3 min at 94 °C, followed by 35 cycles of amplification at 94 °C for 1 min, 55 °C for 1 min and at 72 °C for 1 min. A final extension of 72 °C for 7 min was used to terminate the cycle.

Genetic analysis

MC1R was analyzed through nested PCR using amplification conditions and the automated sequence analysis strategy previously described.[46,47] Oligonucleotide primers flanking each coding exon of the *OCA2*[16] and *SLC45A2* genes were designed from genomic DNA sequences, which were entered into Repeat Masker (http://repeatmasker.genome.washington.edu/) to detect repeats. No repeats were detected, with the exception of *OCA2* exon 15, but this repeat was subsequently found to have no impact on amplification, and the exon sequences plus 200 bp of flanking region either side was analyzed with primer analysis software Oligo6.82 (Molecular Biology Insights, West Cascade, CO, USA). Previous publications were used as a guideline for design of *OCA2* gene primers.[14,48] The amplimer sequences OCA4Cex5F 5′-AGAGGTGGAGAA GCAGAGTG-3′ and OCA4Cex5R 5′-AGTTTTTCCTGACGTCCATAGAT-3′ were designed for amplification of exon 5 of the *SLC45A2* gene. PCR amplifications for both *OCA2* and *SLC45A2* exons were carried out in a total reaction volume of 25 µl containing 50–100 ng genomic DNA template, 25 pmol of both 5′ and 3′ primers, 200 µM of each dNTP (Roche Diagnostics, Sydney, NSW, Australia), 1× PCR buffer (Promega), 1.5 mM $MgCl_2$ (Promega) and 1.25 U Taq polymerase (Promega). PCR protocols for *OCA2* exons were as described[16] and run using a MyCycler thermal cycler (BIORAD, Gladesville, NSW, Australia). The PCR protocol for the *SLC45A2* primers involved initial denaturation for 3 min at 94 °C, followed by 30 cycles of amplification at 94 °C for 1 min, 62 °C for 1 min and at 72 °C for 1 min. A final extension of 72 °C for 7 min was used to terminate the cycle. PCR products were separated by agarose gel electrophoresis and purified using the QIAEXII Gel Extraction Kit (QIAGEN). Sequencing reactions performed using each fragment were generated using AB BigDye version 3.1 chemistry (Applied Biosystems, Melbourne, VIC, Australia), fractionated (AGRF, Brisbane, QLD, Australia), and the chromatogram edited using Sequencher 4.2 software (Gene Codes Corporation, Ann Arbor, MI, USA).

Exon 5 of *SLC45A2* was screened for rs16891982 using allele-specific oligonucleotide probes, Leu 5′-GGGGCTT**G**TGCATCA-3′ and Phe 5′-GGGGCTT**C** TGCATCA-3′, which were designed with the mismatch centrally positioned. Probes were radiolabeled and hybridized to fixed *SLC45A2* PCR product using one 2 µl dot blot/sample evenly spaced on two nylon transfer membranes (OSMONICS, Minnetonka, MN, USA, Magna Nylon Transfer Membrane) for each probe, according to the method outlined.[49] Signals were visualized by autoradiography and analyzed on phosphorus screen image scanner Storm 820 (Molecular Dynamics, Sunnyvale, CA, USA).

RESULTS

Observed pigmentation phenotype

The pigmentation phenotype for the majority of the project participants involved normal coloration, especially noted in the extended pedigree family members. Thirteen Tuvaluan Islander individuals presented with an observed OCA phenotype characterized by green through to blue eye color, fair skin that does not tan, with some brown nevi and freckling present in the sun-exposed areas. Hair presented with a unique red coloration since birth with tones ranging across individuals from Yellow-Red to Brown-Red, or Auburn (Figure 1). Of note was the presence of marked sun-damaged skin with grossly enlarged veins in the cheeks and lips. Phenotype characteristics of all individuals with albinism are summarized in Table 1. Nystagmus was present in all but one affected individual (HJ701FUN), and another individual (HJ201FUN) had the darkest auburn hair coloration and additional complications of ataxia, muscular shaking and speech impairment. The Samoan, Tongan, Niuean and mixed Polynesian (Samoan/Caucasian) individuals with OCA all presented with a similar phenotype to that observed in the study community. In contrast, the other Melanesian Islander and Australian individuals showed obvious phenotypic differences. Of note is that the Tongan male grew a very dark brown/almost black beard and his eyes were light brown (Figure 1).

Incidence of albinism in the Tuvaluan community

There were a total of 25 individuals (ages ranging from 1–50 years) with albinism identified within five generations of Tuvaluan Islanders. Although 24 individuals should be alive today, unfortunately, some of them have passed away due to both accidental and relatively early

Figure 1 Comparative phenotype of normal pigmentation and OCA found in Polynesian Islanders. Tuvaluan female (left panel) at 37 years presenting with red hair, fair skin and green/blue eyes. Normally pigmented Tuvaluan male (center panel) has black/dark brown hair, dark brown eyes and moderately brown skin. Tongan male (right panel) at 30 years; he has red/yellow hair, darker beard, fair skin and light brown eyes. Both individuals with OCA are homozygous for the same *OCA2* mutation (b.2324G>A/ p.G775D); of note is the distinct differences in beard/mustache and eye color phenotype indicative of a variable trait, but these are not sex linked.

Table 1 Observed phenotype and clinical assessment of participants with oculocutaneous albinism

Participant's ID	*Hair color (pigment accumulation)*	*Skin color (pigment accumulation)*	*Freckling/ephelides (brown)*	*Iris color*[a]	*Nystagmus*	*Strabismus*	*Special mention*
HJ1101[b,p]	Red/yellow	White[c] (+)[d]	Moderate	Blue-gray	Present	Present	—
HJ1201[b,e,p]	Red/yellow	White[c]	Mild	Blue	Present	Present	VA 6/18 both eyes
HJ1301[b,e,p]	Red/yellow	White[c] (+)[d]	Moderate	Blue-gray	Present	Present	Several SCCs noted on face
HJ1801[e,p]	Red/yellow	White[c] (+)	Moderate	Blue	Present	Absent	—
HJ901[b,p]	Red/yellow	White[c] (+)[d,f]	Moderate	Blue	Present	Absent	—
HJ201[e,p]	Auburn (++) (Red/brown)	White[c] (+)	Moderate	Blue-green	Present	Present	Marked ataxia, adetoid (shakes), speech impairment
HJ501[e,p]	Red/yellow (+)	White[c] (+)	Moderate	Greenish	Present	Absent	—
HJ401[p]	Red/yellow	White[c] (+)[d]	Moderate	Greenish	Present	Absent	—
HJ101[p]	Red/yellow	White[c] (+)[d]	Moderate	Dark gray	Present	Absent	—
HJ701[b,e,p]	Red/yellow	White[c] (+)	Moderate	Light green	Absent	Absent	—
NF016[p]	Red/yellow (+)	White[c] (+)	Moderate	Blue-green	Present	Absent	—
NF050[b,p]	Red/yellow	White[c] (+)	Moderate	Greenish	Present	Absent	—
SB035[b,p]	Red/yellow (2−)	White	Nil	Blue	Absent	Absent	—
B034[b,p]	Red/yellow (2−)	White (+)[f]	Moderate	Light brown	Present	Absent	Black beard
B060[b,p]	Red/yellow (2−)	White	Moderate	Blue	Present	?	—
B104[b,p,w]	Red/yellow (3−)	White	Nil	Light blue	Present	Absent	White hair at birth
FJ092[m]	Dark yellow/red	White	Extensive freckling	Blue	Present	Absent	—
B020[b,m,t,w]	Dark blonde	Pale, tans	Mild	Green/blue	Present	Absent	—
B021[b,m,t,w]	Golden blonde	Pale, tans	Nil	Green/blue	Present	Absent	—
B015[b,w]	White blonde (+)	White (+)	Nil	Blue	Present	Absent	—
B024[b,w]	Honey blonde	Fair	Nil	Blue	Present	Absent	—
B029[b,w]	White	White (+)	Mild	Blue	Present	Absent	—

Abbreviations: m, Melanesian; p, Polynesian; SCC, squamous cell carcinoma; t, Torres Strait Islander; VA, visual acuity; w, European Caucasian.
[a]All individuals show photophobia, squinting in normal sunlight.
[b]Clinical examination by ophthalmologist confirming oculocutaneous albinism.
[c]Skin for all adults roughened and thickened in the sun-exposed areas and most individuals displayed generalized sunburn redness and their lips also appeared sun damaged.
[d]Show blistering on exposed skin and very visible enlarged blood vessels on the face, especially on cheeks and around lips.
[e]Clinical assessment by dermatologist confirming oculocutaneous albinism.
[f]Tendency for skin on hands to easily blister, associated with proximity to heat from open cooking fire.
(+) Denotes pigment accumulation of a single shade darker than the average reported coloration.
(−) Denotes pigment accumulation of a single shade lighter than the average reported coloration.

death (six individuals), and several others are also living in countries outside Tuvalu. Extensive pedigree analysis found that all identified individuals with albinism were interrelated within a large, extended clan; there was no reported parental consanguinity (Supplementary Figure S1). The 'Tuvalu 2002 Population and Housing Census' information collected by the Tuvalu Government, Central Statistics Division, recorded the resident population of Tuvalu as 9359 individuals. The resident population per island: Nanumea 855 (9.1%), Nanumaga 710 (7.6%), Niutao 817 (8.7%), Nui 610 (6.5%), Vaitupu 1310 (14%), Nukufetau 701 (7.5%), Funafuti 3962 (42.3%), Nukulaelae 392 (4.2%) and Niulakita 2 (0.0%). The resident population structure: 0–59 years 8549 individuals (91.4%); and 60+ years 810 individuals (8.7%). At the time of the census in 2002, there were 14 individuals with OCA residing in Tuvalu (12 females and 2 males); their ages ranged from 6 to 50 years. The prevalence of albinism (14 individuals) calculated against the total population of Tuvalu (9359 individuals) gives an OCA incidence statistic of ~1 in 669 individuals. Assuming the population is in the Hardy–Weinberg equilibrium, for a frequency of 1/669 (q^2) individuals affected with autosomal-recessive OCA, the gene frequency of the recessive allele (q=0.038) is the square root of q^2, which is approximately 1/26. The heterozygote carrier frequency ($2pq$, with p=0.962) is close to 1/14 (0.073). In addition to this, the Tongan, Samoan and Niue participants anecdotally recounted large numbers of individuals in their own family trees and community at large. It was noted that in general OCA did seem to follow a similar trend across the South Pacific region to that observed in the Tuvaluan community.

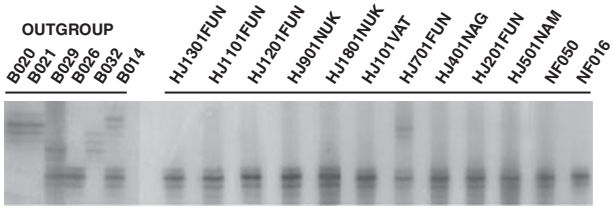

Figure 2 Microsatellite analysis of multiple Tuvaluan participants with OCA. Amplified *OCA2* microsatellite marker located at chr15:25872858-25872901 (UCSC browser) was resolved by 6% polyacrylamide gel electrophoresis (PAGE) and visualized using autoradiography. Lanes 1–6 grouped into control out-group samples: two of outside Islander origin and four of Caucasian origin. Lanes 7–18 represent 12 Polynesian Islanders from Tuvalu. The PAGE displays clear bands with near identical separation between the bands. The analysis shows homozygosity with one allele repeat length for all Tuvaluans (HJ701FUN excepted, which shows heterozygosity with a second longer repeat allele detected), compared with allele variation among out-group individuals.

Microsatellite analysis of candidate albinism-related loci

Microsatellite PCR analysis for the *OCA2* gene seemed to give a homozygous product for the 12 albinism genomic DNA samples analyzed (Figure 2), with only one allele repeat length difference detected (HJ701FUN excepted—the one individual who also did not have nystagmus). In contrast, the other pigmentation pathway-associated genes examined, which included *TYR*, *TYRP1* and *DCT*,

all showed extensive heterozygosity and generalized allelic variation between individuals for each of the diagnostic microsatellite markers used (data not shown).

OCA2 gene mutation detection

To discover the molecular basis for the form of OCA prevalent in the study community, the 23 coding exons (the noncoding exon 1 was excluded) and up to 200 bp of the flanking introns of the *OCA2* gene were analyzed by PCR amplification and direct sequencing for two participants with albinism. A novel pathological missense mutation b.2324G>A changing OCA2 p.Gly775Asp within exon 22 was identified and then confirmed to be homozygous (OCA2 p.775Asp/Asp) in each of the 12 individuals with OCA from the study community (Figure 3a). All 112 immediate and extended family members were then sequenced for this allele; 65 individuals were wild type (OCA2 p.775Gly/Gly) and 46 were found to be heterozygous (OCA2 p.775Gly/Asp) for the *OCA2* mutation, including all 10 participating parents of those individuals with albinism.

Three other Polynesian individuals (Tonga, Samoa and Niue) with OCA were also confirmed to be homozygous for the novel OCA2 p.Gly775Asp missense mutation. One individual of mixed Samoan Islander/Caucasian parentage was found to be heterozygous for the OCA2 p.Gly775Asp missense mutation, as were their Samoan parent and one sibling. A second *OCA2* mutation on the other allele was not

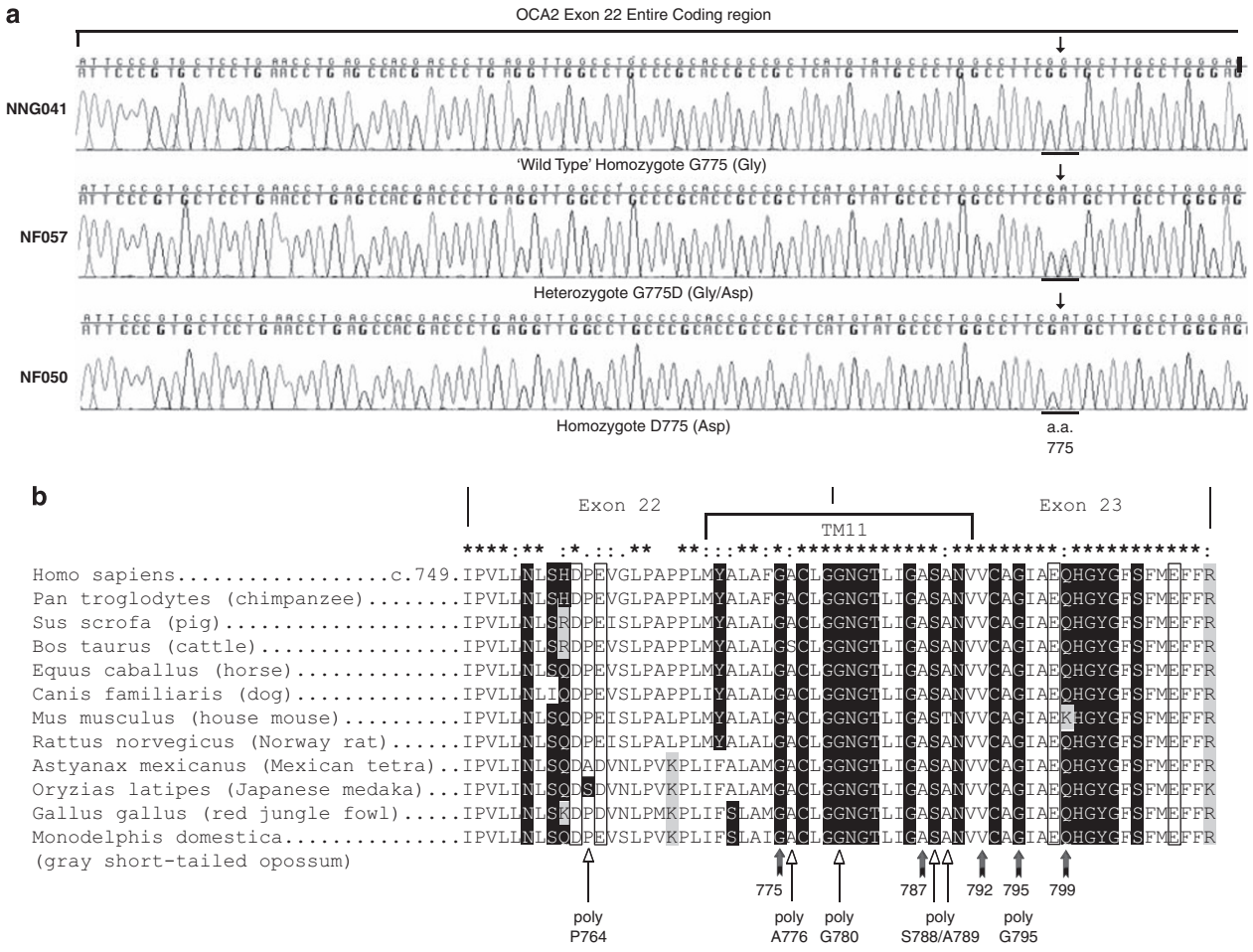

Figure 3 Sequencing of the missense mutation b.2324G>A identified within exon 22 of the *OCA2* gene. (**a**) Alignment of automated sequence traces of three separate subjects for the OCA2 exon 22 coding region. Amino acid c.775 is underlined and the mutation site b.2324G>A is indicated by an arrow. NNG041 shows the wild-type homozygous sequence (b.2324G) in an individual of normal coloration. NF057 is heterozygous (b.2324G/A) and NF050 shows the mutated variant (b.2324A) in homozygous form in an individual with OCA. (**b**) OCA2 protein sequence p.G775 occurs in a highly conserved region across multiple species. The region displayed is labeled as for the human OCA2 protein, incorporating transmembrane domain 11 (TM11), plus its flanking region. Sequences derived using BLAST search of the NCBI genomic database using *OCA2* reference sequence (www.ncbi.nlm.nih.gov). Transmembrane domains predicted by *SOSUI* engine ver. 1.11 (http://bp.nuap.nagoya-u.ac.jp/sosui) for reference sequence NM_000275. Multiple sequence alignments were compiled using ClustalW alignment (www.ebi.ac.uk/Tools/clustalw) and are arranged to show maximum homology. Proteins represented: Human, Mexican tetra, Pig, Horse—OCA2 protein; Mouse, Norway rat—pink-eyed dilution protein; Chimpanzee, Japanese tetra, cattle, dog, opossum, fowl—P-protein (NCBI accession Nos: AAH12097; ABB29299; NP_068679; XP_510251; AAT58049; NP_999259; XP_001494337; BAE03195; XP_001789723; XP_545800; XP_001366525; XP_425579, respectively). Wide arrows indicate the positions of human OCA2 mutations: exon 22, p.G775D in South Pacific Islander individuals with OCA2 and p.G775R in Chinese individuals with OCA2. Exon 23, p.A787V/T; p.V792del; p.G795R; p.Q799H. Thin arrows list polymorphisms (poly) (p.P764; p.A776 (rs1800419); p.G780 (rs1800420); S788 (rs12592307); p.A789; G795). The alignment is displayed according to the physiochemical characteristics of the amino acids. Dark shading are hydroxyl, amine or basic; nil shading represents small hydrophobic and aromatic amino acids except Y; open boxes are acidic; light shading are basic. Consensus symbols: '*' indicates residues or nucleotides identical in all sequences; '**:**' conserved substitutions observed; and '.' indicates the semiconserved substitutions observed. Consensus figure based on Lee *et al*. (Figure 4).[15]

identified in these individuals, although they were not sequenced across all exons for this study. One Australian Caucasian with OCA was found to be a compound heterozygote for one novel apparent pathological missense mutation b.1185G>T, OCA2 p.Met395Ile and one common hypopigmentation-related allele b.1327G>A, p.Val443Ile.[14,50–53] Each of their parents were heterozygotes for one of these changes and their maternal uncle and sibling heterozygous for OCA2 p.Val443Ile.

OCA2 mutations were not identified for the remaining five outgroup participants as they were not sequenced across all exons for this study, but none were found to have the novel OCA2 p.Gly775Asp mutation. A novel synonymous change variant (b.2271C/T, OCA2 p.His757) was found only in the Australian Aboriginal. The Fijian Islander was the only participant found to harbor the previously described polymorphism IVS22+37, b.2338+37C>T.[53] Two mixed Vanuatu/Torres Strait Islander individuals shared two common polymorphisms both previously described;[14,51] substitution IVS21+25G, c.2240+25G/C plus, synonymous variant b.2364G/A, OCA2 p.Ser788, which was also in the Tuvaluan population.

Cross-species comparison of the OCA2 protein shows that Gly at c.775 is a highly evolutionary conserved residue. The 12 species used originate from separate phylogenic families (Figure 3b). It is noteworthy that a different pathological OCA2 mutation b.2323G>C, p.Gly775Arg has been previously described at the same codon in the Chinese population.[54,55] This variant was also confirmed as being possibly damaging when tested by PolyPhen analysis (http://genetics.bwh.harvard.edu/pph/), which predicted potential improper substitution in the transmembrane region. The nonpolar hydrophobic glycine residue is located within the transmembrane 11 region[14] and is surrounded by small, hydrophobic residues. There are several conserved glycine residues in the transmembrane 11 domain in close proximity to each other, with Gly at c.775 also conserved in equivalent protein positions in an additional four species of simple bacteria. Glycine (NH2-CH2-COOH) is the smallest side chain, it is an uncharged polar amino acid and adds flexibility to the protein chain. The substitution with aspartic acid (HOOC-CH2-CH(NH2)-COOH), which is structurally larger and less 'flexible', may possibly inhibit OCA2 P-protein folding. In addition, it carries a hydrophilic acidic group with strong negative charge, which is usually located on the outer surface of a protein, thus making it more water soluble so as to decrease the hydrophobicity of this transmembrane domain.

Heterozygous effect of the OCA2 p.Gly775Asp allele on pigmentation characteristics

The χ^2 contingency tests were used to investigate if heterozygosity for the identified OCA2 mutation p.Gly775Asp influences overall coloration of the eyes, skin or hair in the 111 unaffected participants sampled from the general community. It is noteworthy that a statistically significant correlation was identified associating with hypopigmentation in each of these pigmentary traits and heterozygous status (Table 2) of the Tuvaluan participants. A significant proportion of heterozygous participants have light brown skin, with medium brown skin being the next prevalent. The heterozygous individuals are not likely to have dark brown skin pigmentation, compared with participants homozygous for the wild-type allele, who tended to have medium brown skin followed by light brown skin and then dark brown skin pigmentation. Eye color in heterozygotes segregated evenly across light brown/hazel green and blue, medium brown and dark brown, whereas in wild-type genotypes it predominately presented as dark brown eye color, with some medium brown and light brown. Only heterozygotes showed significantly lighter eye color with several being light brown with a greenish tinge; four participants had green eye color and one had blue eyes. All those heterozygous for the Polynesian OCA2 mutation p.Gly775Asp with green or blue eyes had known Caucasian ancestors, suggesting possible interaction with other pigmentation-influencing variants.[16,22,23] There was a predominance for gray hair in heterozygous carriers; however, the majority of participants expressed black hair irrespective of carrier status and age-related hair graying must be taken into account.

MC1R genotype in the Tuvaluan community

Given the prevalence of reddish hair in this presentation of OCA2 albinism, 29 Tuvaluan Islander participants were analyzed for *MC1R* variants: 12 with OCA, 5 normally pigmented, plus 12 individuals showing varying degrees of hypopigmentation that were different to other individuals classed as having albinism, or being of average normal pigmentation. Three previously published *MC1R* variants

Table 2 Phenotypic effect on pigmentation characteristics in heterozygous carriers of albinism mutation OCA2 *p*.Gly775Asp within Tuvaluan Islanders

Pigmentation variable	*Phenotype*	*No. (%) of wt/mt diplotype 775G/D*[a]	*No. (%) of wt/wt diplotype 775G/G*[b]	χ^2/ P-*value*[c]
Eye color	Dark brown	15 (32.6)	52 (80.0)	χ^2=65.9
	Medium brown	15 (32.6)	7 (10.8)	$P<0.0001$
	Light brown/hazel (green and blue)	16 (34.8)	6 (9.2)	
Total		*n*=46	*n*=65	
Skin color	Dark brown	1 (2.2)	9 (13.8)	χ^2=33.2
	Medium brown	15 (32.6)	38 (58.5)	$P<0.0001$
	Light brown	30 (65.2)	18 (27.7)	
Total		*n*=46	*n*=65	
Hair color	Black	32 (69.6)	51 (78.5)	χ^2=6.6
	Brown/brown-red	1 (2.2)	4 (6.2)	$P<0.05$
	Predominately gray	13 (28.3)	10 (15.4)	
Total		*n*=46	*n*=65	
Grand total				*N*=111

[a]*wt/mt*=22 male and 24 female; *SLC45A2* genotypes p.374L/L=44, p.374F/L=2.
[b]*wt/wt*=28 male and 37 female; *SLC45A2* genotypes p.374L/L=58, p.374 F/L=6.
[c]χ^2 contingency test, d.f. (2).

were detected in individuals irrespective of OCA status: b.274A>G, p.Val92Met; b.488G>A, p. Arg163Gln; and b.942A>G, p.T314.[33] Two Islanders were found to be heterozygous for a red hair-associated variant b.451, p.Arg151Cys. In addition, the Melanesian Islander with OCA from Fiji FJ092 and a normally pigmented Australian Caucasian B013 were analyzed for *MC1R* and both were found to be of consensus wild-type genotype.

SLC45A2 genotype in the Tuvaluan community

A polymorphism screen of *SLC45A2* exon 5 was performed in participants both with and without OCA to assess in the Tuvalu population the level of the b.1122C/G, p.374Phe/Leu polymorphism that has previously been associated with variation in normal skin pigmentation phenotype.[33] One novel polymorphic change b.1156+108T/C, IVS5+108 and one previously published b.1033-44A/C, IVS4-44, rs35398[56] were detected in sequence analysis of samples. Allele-specific oligonucleotide hybridization analysis was conducted on 120 Tuvaluan participants and 9 out-group participants. The genotype determined at this position was confirmed for seven random Tuvaluan samples (three OCA, four non-OCA) by sequencing of the *SLC45A2* exon 5 coding region. Eight Tuvaluan samples (six OCA, two non-OCA) plus one out-group OCA (Samoan SB035) were analyzed by sequencing alone. The majority of Islander participants plus the Australian Aboriginal were found to be homozygous MATP p.374Leu/Leu; the exceptions were eight non-OCA Tuvaluans (NF026, NF067, NF069a, NF076, NF077, NF091, NF093, NF095) who were heterozygous p.374Phe/leu. All Australian Caucasians genotyped (B013, B014, B024, B029) were found to be homozygous MATP p.374Phe/Phe.

DISCUSSION

Tuvalu is a small country, which consists of eight established communities on separate islands and atolls of the group (six atolls and three islands) spread over 25 km^2. The geographical structure of the group and relative difficulty in internal transport, especially in the past with canoe being the major vessel, has resulted in each of these communities evolving somewhat independently of each other over time. Although these internal communities are both interlinked as a country and people with a common language shared across the group, apart from certain local variations, they are also distinctly separated with differing customs and attitudes. Extensive pedigree analysis found that all identified individuals with albinism were interrelated within a large, extended clan with individuals originating from one specific island of the archipelago (Nukufetau) strongly associated with the pedigree. However, it was also found that there are three other islands (Vaitupu, Niutao and Nukulaelae) that also exert a major influence within the extended linking pedigree. It was found that this form of albinism is expressed as a recessive trait throughout the pedigree.

The aim of this study was to classify the type and prevalence of OCA in Polynesian Islanders from Tuvalu. The phenotype presented with the ability to form some pigmentation featuring unique red hair coloration since birth and development of brown skin nevi and freckling in the sun-exposed areas. Analysis to determine the frequency of microsatellite alleles across several candidate albinism genes, including *TYR*, *TYRP1*, *DCT*, and *OCA2*, found that on average there was generalized variation in alleles for each participant across all genes with the exception of the *OCA2* gene, which showed homozygosity with one allele repeat length for Tuvaluan albinism samples, compared with allele variation among out-group individuals. This homology across one pigmentation gene for individuals derived from different immediate families established *OCA2* as the most likely candidate gene. In addition, the allele variation detected across the other genes gave evidence that the population gene pool was diverse enough to be able to discern the mutation.

A novel pathological missense mutation b.2324G>A, p.Gly775Asp of the *OCA2* gene exon 22, first identified through direct sequencing of each exon, was then confirmed to be homozygous A, p.775Asp in all the 12 representative individuals with OCA from the Polynesian population of Tuvalu. Moreover, homozygosity of this allele was not found in any normally pigmented individual. All 10 participating parents were found to be heterozygous G/A carriers for this change consistent with an autosomal-recessive genetic inheritance of this mutation, as were 36 other immediate and extended family members. Several individuals with albinism from different countries within the same South Pacific region have also been included in this study as potential out-groups to determine the extent of the identified *OCA2* mutation. Three other Polynesian individuals (Tonga, Samoa and Niue) with OCA were confirmed to be homozygous for the novel OCA2 p.Gly775Asp missense mutation. One individual of mixed Samoan Islander/Caucasian parentage was found to be heterozygous for the p.Gly775Asp missense mutation, as were their Samoan parent and one sibling. Extensive pedigree analysis also confirmed a Tuvalu and Tokelau couple having a child with OCA, suggesting that Tokelau potentially has the same OCA2 p.Gly775Asp mutation. These results suggest that the Gly775Asp mutation associated with OCA2 could be considered as a 'Polynesian Islander'-specific mutation. The previously described polymorphism IVS22+37, b.2338+37C>T[53] was found in homozygous form only in the Fijian Islander with OCA and was not present in any of the Polynesian Islanders, which offers probable evidence of a different recessive allele in the *OCA2* gene for that Melanesian population.

There was an observed high incidence of generalized hypopigmentation of the eye and skin in immediate family members of those with albinism, and this effect was also noticed in other extended family members. Statistical analysis was conducted to quantify and show a direct link between hypopigmentation of the eye, skin or hair color and heterozygote status for the OCA2 p.Gly775Asp mutation. This represents the first quantified evidence for a pigmentation carrier effect in individuals heterozygous for an OCA2 mutation. This large-scale study confirms the hypothesis of haploinsufficiency of *OCA2* gene function, previously proposed to explain hypopigmentation within a single Hispanic family.[34]

Several other pigmentation genes, including *MC1R* and *SLC45A2*, were analyzed to assess whether they were possibly influencing the spectrum of pigmentation in this form of albinism and the Polynesian population at large in addition to the *OCA2* heterozygous effect. Several individuals from across the extended pedigree showing varying degrees of hypopigmentation were also included in the study. This hypopigmentation ranged from affecting only one category (skin tone, irides or hair) to affecting multiple categories. It was noted that most of those individuals showing a different phenotype tended to have a Caucasian ancestor. It was noted that even though as a collective group all individuals with albinism tended to present with a similar phenotype, there was also some individual variation, with hair and eye color ranging across lighter to darker pigmentation scales. Red hair associated with albinism is a rare trait and it has previously been shown that *MC1R* red hair variants are able to modify the OCA2 phenotype;[25] however, this cannot explain this phenotype in this population. In a previous study,[34] the *TRYP1* gene was shown to modify the OCA2 phenotype, resulting in red hair in the absence of MC1R R alleles and was also proposed as a likely candidate for reddish hair or highlights in

the African, Hispanic and European populations in general. Although *TYRP1* (OCA3) was excluded as being the gene responsible for OCA in this population, it warrants further consideration for variants that could potentially modify the red hair phenotype in Polynesians.

This study reports a high prevalence of genetically defined OCA2 among the Polynesian islanders of Tuvalu of ~1 in 669. This is one of the highest recorded rates of OCA and is the highest molecularly characterized incidence of OCA2 in the world, with a calculated heterozygote carrier frequency of 1 in 14. The nature of the data collected both experimentally and defined in family trees genealogically has led us to hypothesize that the albinism mutation detected has been in the community for many generations and potentially may have arrived with the original inhabitants of the Pacific Islands. There is a significant founding history of interaction between Tuvalu and other South Pacific nations; hence it is significant that only one common mutation was identified in all albinism individuals. This leads to the second hypothesis that this mutation may explain other instances of albinism throughout the South Pacific and may even emerge as one of the most common albinism-related mutations among the Islander communities in this region of the world. The first recorded description of OCA in Tuvalu was during Wilkes' visit to Nukufetau (*Tuvalu: a history*)[57] in 1841 describing a grown man with normally pigmented biological parents, representing at least two generations already carrying the recessive gene. Bottleneck effect is a possible explanation for high frequency of albinism in the Tuvalu population, similar to that described in the Navajo population.[10] In 1866, the total population of Tuvalu was recorded as 2810 individuals with the population of Nukufetau recorded as being 220,[57] thus representing a recent historical bottleneck. The relatively high incidence of albinism in the community suggests that the responsible mutation has been present for many generations. There may be a common mutation causing albinism being repeated throughout the extended region, perhaps first brought to Tuvalu through direct ancestral links with neighboring Polynesian countries. Of the founding population, a significant fraction would be post-breeding age and as many as half of the population may have been children of the same founders with subsequent expansion of the population leading to widespread distribution of the OCA2 allele. Similar considerations to that of the Navajo population also apply[10] with the population history of Tuvalu, including one or more known bottlenecks that could lead to high frequency of the OCA2 missense mutation p.Gly775Asp from a single founder.

A recent genome scan of autosomal microsatellite markers for 952 individuals from 41 Pacific populations showed that Polynesians (represented by Samoans and Maoris) are a distinctive group that also tend to cluster with Micronesians, Taiwan Aborigines, East Asians, but not with Melanesians.[58] This indicates that the ancestors of Polynesians moved through Melanesia relatively rapidly and only intermixed to a very modest degree with the indigenous populations there. It was also noted that the Polynesian genetic variation is relatively homogeneous and comparatively reduced when compared with other human groups. The authors suggest that this has resulted in no discernable signal that has survived that can be identified as specifically ancestral to Polynesians.[58] Recently published genetic segregation of the human pathogenic bacterium, *Helicobacter pylori*, into two distinct populations (hpSahul and hspMaori) provides an alternative support for two distinct waves of migrations into the Pacific.[59] Distribution and sequencing found that all hpSahul strains clustered into a monophyletic clade verifying a single colonization event, followed by independent evolutionary trajectories for each subsequent human population. This confirmed early migrations to New Guinea and Australia accompanied by hpSahul, and second, a much later dispersal of hspMaori from Taiwan through the Pacific by the Malayo-Polynesian-speaking Lapita culture. hsbMaori is a marker for the entire Austronesian expansions rather than only for Polynesians.[59] This newly described p.Gly775Asp Polynesian OCA2 mutation described in this study also mirrors the findings of being distinct from Melanesian populations. It may also help to decipher the migration patterns of humans across the Pacific in a similar manner to hspMaori by providing a marker that is specifically ancestral to Polynesians.

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