RESEARCH NOTE

Analysis of *MC1R* variants in Indian oculocutaneous albinism patients: highlighting the risk of skin cancer among albinos

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Introduction

Variants in melanocortin 1 receptor (MC1R, MIM *155555) are known to be associated with melanoma and non-melanoma skin cancers, either through pigmentary or non-pigmentary processes. Oculocutaneous albinism (OCA), characterized by loss of melanin, is known to predispose the patients towards various forms of skin cancers. We argue that presence of skin cancer associated variants of MC1R would further aggravate the cancer predisposition in OCA patients. In this context, we assessed the *MC1R* variants in our OCA patient pool already screened for OCA causing mutations. Three skin cancer associated *MC1R* nonsynonymous variants were identified in 13 OCA patients; the oldest patient among them was diagnosed with precancerous skin lesions. Interestingly, two other OCA affected family members of this patient did not manifest any suspected skin lesions, nor did they bear any *MC1R* variant. This study underlines the importance of screening for *MC1R* variants in all OCA patients to assess their increased susceptibility to skin cancer.

Exposure to sunlight is regarded as one of the major environmental risk factors for the development of melanoma as well as nonmelanoma skin cancers (NMSCs) like squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). Melanin, the pigment responsible for skin colour in humans, is considered photo-protective against the carcinogenic solar UV radiation. Multiple genes are responsible for the constitutive pigmentation through synthesis and distribution of a basal level of epidermal melanin. Among them *MC1R* plays a central role in the tanning response to UV radiation. MC1R favours the generation of increased levels of photo-protective black–brown polymer eumelanin as opposed to less protective yellow–orange pheomelanin. Loss-of-function variants of MC1R induce increased synthesis of pheomelanin and promote production of ROS, a known DNA damaging agent (de Vijlder *et al.* 2013). Some MC1R variant proteins are reported to be present at reduced level in cell surface and/or have lower affinity for the substrate alpha-melanocortin (α-MSH) (Ringholm *et al.* 2004; Beaumont *et al.* 2005; Scherer *et al.* 2008; Scherer and Kumar 2010). The aforementioned information could very well explain that why different MC1R variants are found to be significantly associated with melanoma (Scherer and Kumar 2010) due to less eumelanin. Interestingly, MC1R variants have also been implicated in NMSCs, independent of the amount or quality of melanin (Scherer *et al.* 2008) through disruption of cytokine regulation and inflammatory responses via modulation of NF-κB, apoptosis and DNA repair etc. (Scherer and Kumar 2010). In accordance, carriers of MC1R p.Arg163Gln variation were found to be at a higher risk of BCC irrespective of their skin pigmentation level suggesting nonpigmentary receptor function of MC1R related to the disease (Scherer *et al.* 2008). In addition to acting as an independent risk factor for skin cancers, MC1R variants have been found to increase the penetrance of *CDKN2A* variants in familial melanoma cases (Scherer and Kumar 2010).

OCA is a heterogeneous group of autosomal recessive disorders characterized by complete or partial loss of pigmentation in the skin and eyes. Four classical OCA subtypes (OCA1 to OCA4) have been described with underlying

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genetic defects in four candidate genes viz. *TYR* (OCA1A and OCA1B), *OCA2* (OCA2), *TYRP1* (OCA3) and *SLC45A2* (OCA4). Except for OCA1A, all other subtypes show some amount of residual pigmentation (i.e. melanin). MC1R, besides being an important player in the melanogenesis pathway, has been reported to modulate the phenotype of OCA patients (King *et al.* 2003).

OCA patients, with less or no melanin, are known to be prone to various types of skin cancers and interestingly, NMSCs are reported to be more prevalent among the albinos than melanoma (Berger *et al.* 2011). Various propositions have been put forward regarding the susceptibility towards skin cancer in OCA patients, but the exact risk status could not be established yet. It is worth mentioning that in albinos skin cancer manifests earlier (Opara and Jiburum 2010) and are often present with additional lesions (Asuquo *et al.* 2010), compared to the nonalbinistic individuals. Thus, one could argue that susceptibility of albinos to skin cancer might increase due to concomitant presence of MC1R variants that are by themselves associated with melanoma and NMSCs, either resulting in further reduction of the residual eumelanin and simultaneous increase in pheomelanin or through perturbation of nonpigmentary pathways.

There are multiple reports of BCCs and SCCs in albinos of African origin, where albinism is quite prevalent (Asuquo *et al.* 2010; Opara and Jiburum 2010; Berger *et al.* 2011), however with unknown MC1R status. To our knowledge, a single case of multiple SCCs with metastasis in a 25-year-old OCA individual (Ramalingam *et al.* 2009) has been reported from Indian population but without MC1R status. In light of the above mentioned information it seems imperative to assess the status of MC1R variations in OCA patients to judge the additional effect of loss of melanin due to OCA and pigmentary or nonpigmentary role of *MC1R* variants towards the increased skin cancer susceptibility.

Multiple molecular genetic studies from our lab reported the relative contribution of classical OCA genes towards OCA in Indian population and identified the prevalent and rare genetic variants (Chaki *et al.* 2006; Sengupta *et al.* 2007, 2010; Chaki *et al.* 2011; Mondal *et al.* 2012). We hereby take the opportunity to investigate the status of risk enhancing MC1R variants in these previously screened albino patients to assess their increased predisposition towards skin cancer. To the best of our knowledge, no such study has yet been undertaken in OCA patients of any population group.

It should however be noted that skin cancer has a multifactorial pathogenesis, and the sole objective of this study was to assess the prevalence of those *MC1R* variants, which have been previously associated with skin cancer in different association-studies including GWAS, among the albino population to evaluate their gross predisposition. The finer assessment of the imparted risk due to the individual *MC1R* variants is beyond the scope of the current study.

Materials and methods

Sixty-five albinistic individuals were screened for *MC1R* variants through PCR-sequencing based approach. The single 954- bp exon of *MC1R* was amplified by PCR done in a total volume of 20 μL using 20–50 ng of DNA, 20 pmol of primers and 10 μL Taq Premix (Genet Bio, Daejeon, Korea). The amplicons were purified with ExoSap (USB®, Cleveland, USA) and sequencing was performed in the ABI Prism 3130xl DNA sequencer (ABI™, Carlsbad, USA). The primer sequences are provided in supplementary table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/. Heterozygous variants were identified from the double-peaks in chromatograms while homozygous variants were identified with the help of NCBI-BLAST. In this study all the OCA patients screened for *MC1R* variants had been screened previously for defects in classical OCA genes (Chaki *et al.* 2006; Sengupta *et al.* 2007, 2010; Chaki *et al.* 2011; Mondal *et al.* 2012). Clinical diagnosis of OCA and other features was done by our clinical collaborators (SS and AS). Novel MC1R variants were screened in 50 normal chromosomes.

Results and discussion

Eight different changes in *MC1R* were identified in 37 OCA patients including seven coding region changes (four nonsynonymous and three synonymous) and a single 5′ UTR variant, out of which two were novel (see table 2 in electronic supplementary material). The novel changes were not found in 50 control alleles studied. Among the reported changes identified, five were reported SNPs and a single mutation. Three of the reported SNPs identified have been previously associated with different forms of skin cancer viz. rs2228479 (p.Val92Met), rs1805007 (p.Arg151Cys) and rs885479 (p.Arg163Gln). In our patient pool, 13 individuals harboured skin cancer associated MC1R variants; while 10 patients harboured the frequent p.Arg163Gln allele, two patients carried the p.Val92Met change and a single patient had the much severe p.Arg151Cys change.

The underlying causal genes for OCA in those patients who contain skin cancer associated MC1R variants are furnished in table 1 along with other relevant information. It suggests that while four patients who are OCA type 1, likely without any residual melanin, would be more susceptible for NMSCs than melanoma (de Vijlder *et al.* 2013); rest of the patients who are either OCA type two or type four or the cases where the causal gene for OCA could not be identified, would have the risk of developing either melanoma or NMSCs due to the presence of MC1R variations.

It is worth noting that among all the patients in table 2, the oldest individual (OCA75, 50 yrs), a heterozygote for MC1R variant p.Arg163Gln, was identified with precancerous lesions in her hand (figure 1), while two other albinos (aged 35 and 50 years) of the same family lacking any variant in MC1R did not have any suspected lesion. Incidentally,

Table 1. OCA patients containing skin cancer associated *MCIR* variants.

	Patient ID	Age (yrs)	Sex	MC1R variant	OCA type	Causal gene for OCA (mutation and genotype)
1	OC60	NA	M	p.Arg163Gln (het)	Not known	Not found
2	OC70	NA	M	p.Val92Met (het)	OCA4*	*SLC45A2* (p.Gly64Ser, het)
3	OC154	NA	F	p.Arg163Gln (hom)	OCA1*	*TYR* (p.Arg278Stop, het)
4	OC157	NA	M	p.Arg163Gln (het)	OCA1	*TYR* (p.Tyr433Stop, hom)
5	OCA37	18	M	p.Arg151Cys (het)	OCA1	*TYR* (p.Arg278Stop , c.1379_1380delTT)
6	OCA68	16	M	p.Arg163Gln (het)	OCA1*	*TYR* (p.Gly372Arg, het)
7	OCA75	50	F	p.Arg163Gln (het)	OCA1	*TYR* (p.Asp42Asn, hom)
8	OCA79	42	F	p.Arg163Gln (het)	OCA1	*TYR* (p.Cys91Ser, hom)
9	OCA80	24	M	p.Arg163Gln (het)	Not known	Not found
10	OCA91	32	M	p.Val92Met (het)	OCA4	*SLC45A2* (p.Leu325Pro, hom)
11	OCA93	22	M	p.Arg163Gln (het)	OCA1	*TYR* (c.1379_1380del TT, hom)
12	OCA95	22	F	p.Arg163Gln (het)	Not known	Not found
13	OCA96	40	M	p.Arg163Gln (het)	OCA2	*OCA2* (c.775_776insG, p.Leu674Val)

All the OCA causing mutations have been cited in Indian Genetic Disease Database (http://www.igdd.iicb.res.in).
NA, not available; het, heterozygous; hom, homozygous.
* In these cases the status of OCA has been described on the basis of the single mutation identified.

the remaining 12 OCA patients bearing skin cancer associated MC1R variants (table 1), but not showing any sign of skin cancer, are mostly much younger (16–42 yrs).

It is worth noting that the p.Arg151Cys variant allele has been found to be strongly associated with the red hair colour (RHC) phenotype caused due to increased pheomelanin, while the p.Val92Met and p.Arg163Gln have a relatively weak association with RHC. p.Arg163Gln and p.Arg151Cys showed moderate and marked reduction in cell surface receptors respectively, thus impairing MC1R activity to variable extent (Beaumont *et al.* 2005). The p.Val92Met allele with normal receptor level (Beaumont *et al.* 2005) was found to have 100-fold lower affinity with α-MSH as compared to the wild type (Ringholm *et al.* 2004). The p.Arg163Gln variant, most frequent in East and Southeast Asians including Indians (Rana *et al.* 1999), also showed lower potency for α-MSH binding (Ringholm *et al.* 2004). Thereby, all of these variants with impaired MC1R activity could presumably lead to increased phaeomelanin production and lesser eumelanin synthesis. Again, the risk of nonmelanotic BCC in the p.Arg163Gln variant carriers with fair complexion was found to be almost twice as much as in the corresponding noncarriers, whereas, in case of medium skin complexion the carriers were at a 3-fold higher risk than the noncarriers. This indicated a nonpigmentary receptor function of p.Arg163Gln variant related to BCC (Scherer *et al.* 2008). p.Arg151Cys has been found to be associated with cutaneous malignant melanoma in an Australian population irrespective of skin colour (Palmer *et al.* 2000). p. Val92Met and p.Arg151Cys have also been independently found to be associated with nonmelanoma skin cancer (Bastiaens *et al.* 2001). A meta-analysis by Raimondi *et al.* (2008) found p.Arg151Cys and p.Arg163Gln to be significantly associated with melanoma risk. We argue that presence of these MC1R variants associated with both melanoma and NMSCs when present in OCA patients would further increase the risk of skin cancer. In general, the risk alleles of MC1R have been found to affect in a dose-dependent manner; risk increases with the number of associated variants present in an individual (Helsing *et al.* 2012). However, we did not find any patient with more than one skin cancer associated MC1R variant.

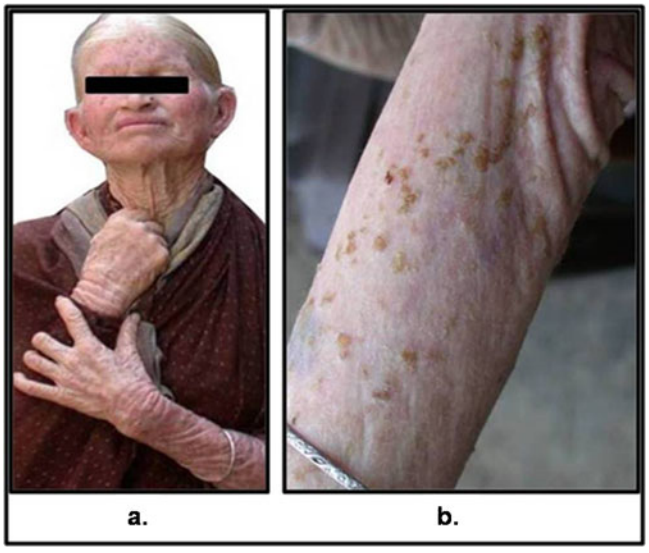

Figure 1. (a) Photograph of OCA75 (age 50 yrs) harbouring precancerous lesions. (b) Enlarged photograph of forehand showing the precancerous lesions, that later could develop into squamous cell carcinoma.

The proposed higher risks of skin cancer among albinos harbouring specific MC1R variants need to be further substantiated in replicate studies in larger cohort of the OCA patients and follow them for longer period of time. Our report nevertheless underlines the importance of screening for MC1R variants in all mutation-screening studies involving OCA patients, to assess their skin cancer risk and for

proper follow up throughout their lifetime (minimizing sun exposure, regular screening for suspected skin lesions).

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