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# Molecular and Functional Studies of Tyrosinase Variants Among Indian Oculocutaneous Albinism Type 1 Patients

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## TO THE EDITOR

Oculocutaneous albinism (OCA) refers to a heterogeneous group of autosomal recessive disorders that are characterized by hypopigmentation of the skin, hair, and eyes and associated with developmental eye defects. Because OCA is a major cause of childhood blindness in India, containing it is crucial. Methods of doing so include identification of molecular lesions, detection of carriers, and premarriage genetic counseling; however, the available genetic information is limited. We previously reported that OCA1, which is caused by defects in tyrosinase (*TYR*, 11q14-q21, MIM606933), is the major OCA subtype in India (Chaki *et al.*, 2006). Here, we report the characterization of 11 unrelated OCA1 patients bearing nine different *TYR* mutations (c.655G>A, p.Glu219Lys; c.832C>T, p.Arg278Stop; c.976C>T, p.Gln326Stop; c.1037G>T, p.Gly346Val; c.1265G>A, p.Arg422Gln; c.1379delTT; Deletion 3′*TYR*; c.530T>A, p.Val177Asp; c.1299C>G, p.Tyr433Stop) (Supplementary Table S1 online). To our knowledge, the last two of these were previously unreported. Both mutations were identified in 10 patients, but in one patient a single *TYR* mutation was detected despite analyzing the entire coding region, untranslated regions, the putative locus control region core, and the promoter containing a complex GA-repeat having the potential to induce DNA secondary structure (Ray *et al.*, 2007). In addition, no causal variant was identified in other known OCA loci in this patient

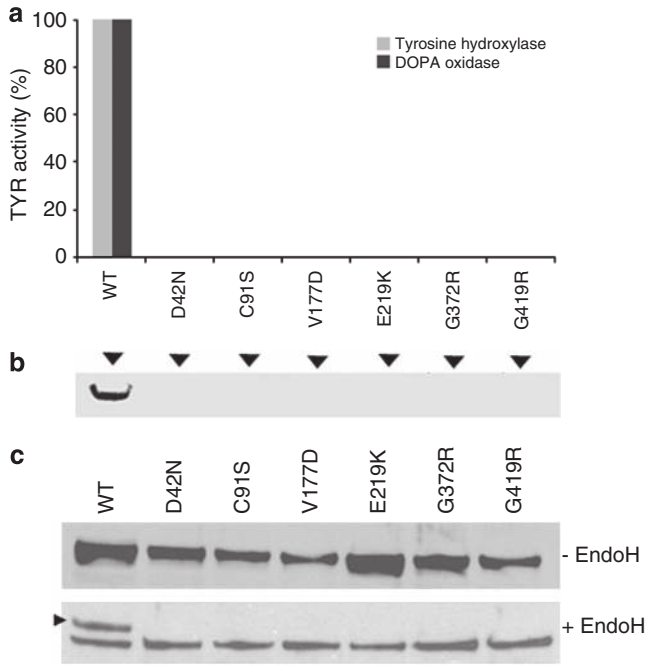


**Figure 1. Functional characterization of mutant TYRs revealed ER retention as the cause of the loss of enzyme function.** (**a**) The enzymatic activity (tyrosinase hydroxylase and DOPA oxidase) of wild-type (WT) TYR is represented as 100%, and all the mutants showed complete lack of enzyme activity. (**b**) On zymogram assay, the DOPA-melanin band is visible only for WT protein. (**c**) The immunoblots of EndoH-untreated (−EndoH) and -treated (+EndoH) cell lysates for WT and mutant TYRs. Only EndoH-treated WT TYR shows a distinct EndoH-resistant form, as represented by the lower-mobility band (marked by an arrow) along with an EndoH-sensitive higher-mobility band, whereas all the mutants show only the EndoH-sensitive higher-mobility band. DOPA, L-3,4-dihydroxyphenylalanine; EndoH, endoglycosidase H; ER, endoplasmic reticulum; TYR, tyrosinase.

*Abbreviations: EndoH, endoglycosidase H; ER, endoplasmic reticulum; L-DOPA, L-3,4-dihydroxyphenylalanine; OCA, oculocutaneous albinism; TYR, tyrosinase*

(Sengupta *et al.*, 2010). Therefore, including the previously identified cases, the frequency of OCA1 with uncharacterized mutations is 8.3% (3/36) in our OCA1 patient cohort, almost twice that of a recent report from China (4.5%, Wei *et al.*, 2010). It is worth noting that we detected two OCA1 patients homozygous for the most prevalent mutation in India (p.Arg278-Stop) but harboring different haplotypes than one reported previously (Chaki *et al.*, 2005), suggesting *de novo* occurrence of the mutation influenced by CpG sequence. Our overall data suggested that, among the OCA-affected Indians, the frequency of OCA1 subtype is ~61% (36/59 unrelated families). All the *TYR* defects reported from Indian OCA1 cases are listed in Supplementary Table S1 online.

Because our study was based mostly on small families (sometimes only the proband sample was available), an unequivocal causal relationship of the putative mutations with the disorder cannot be argued very strongly. Although in/del and nonsense changes are predicted to translate to aberrant and/or truncated proteins, nonsynonymous changes remain open to speculation without functional clarification. Hence, we characterized six missense mutations (p.Asp42Asn, p.Cys91Ser, p.Val177Asp, p.Glu219Lys, p.Gly372Arg, p.Gly419Arg) identified in our OCA1 cohort. Because p.Gly346Val was a recent finding and p.Arg422Gln had already been validated as a temperature-sensitive mutation (King *et al.*, 1991), those were excluded from our study. Notably, TYR, the most important protein in melanogenesis, catalyzes the first two rate-limiting steps: hydroxylation of L-tyrosine and oxidation of L-DOPA (L-3,4-dihydroxyphenylalanine). Relevant enzyme assays revealed that none of the mutants had any tyrosine hydroxylase and DOPA oxidase activities in comparison with the wild-type TYR (Figure 1a and b). Thus, it was evident that OCA1 individuals with these mutations lacked functional TYRs, leading to melanin deficiency and consequently to OCA1 pathogenesis. It is likely that these mutations lead to either immature, mislocalized proteins or fully processed, but catalytically inactive, proteins. To resolve this, subcellular localization of the mutant proteins was assessed through immunofluorescence study with calnexin (an endoplasmic reticulum (ER) marker) and α-1,2-mannosidase II (Golgi marker), which demonstrated that all the mutants colocalized with calnexin but not with α-1,2-mannosidase II, indicating ER retention of the mutant TYRs as the cause of pathogenesis (data not shown). To confirm this observation, an endoglycosidase H (EndoH) assay was performed with the wild-type and mutant TYRs, followed by western blot analysis (Figure 1c). EndoH can cleave TYRs in high-mannose forms, characteristic of ER processing, but not in those with complex carbohydrate modifications (attained in Golgi). Our study revealed that, unlike the wild-type TYR, all the mutant proteins were immature ER-retained species, as suggested by

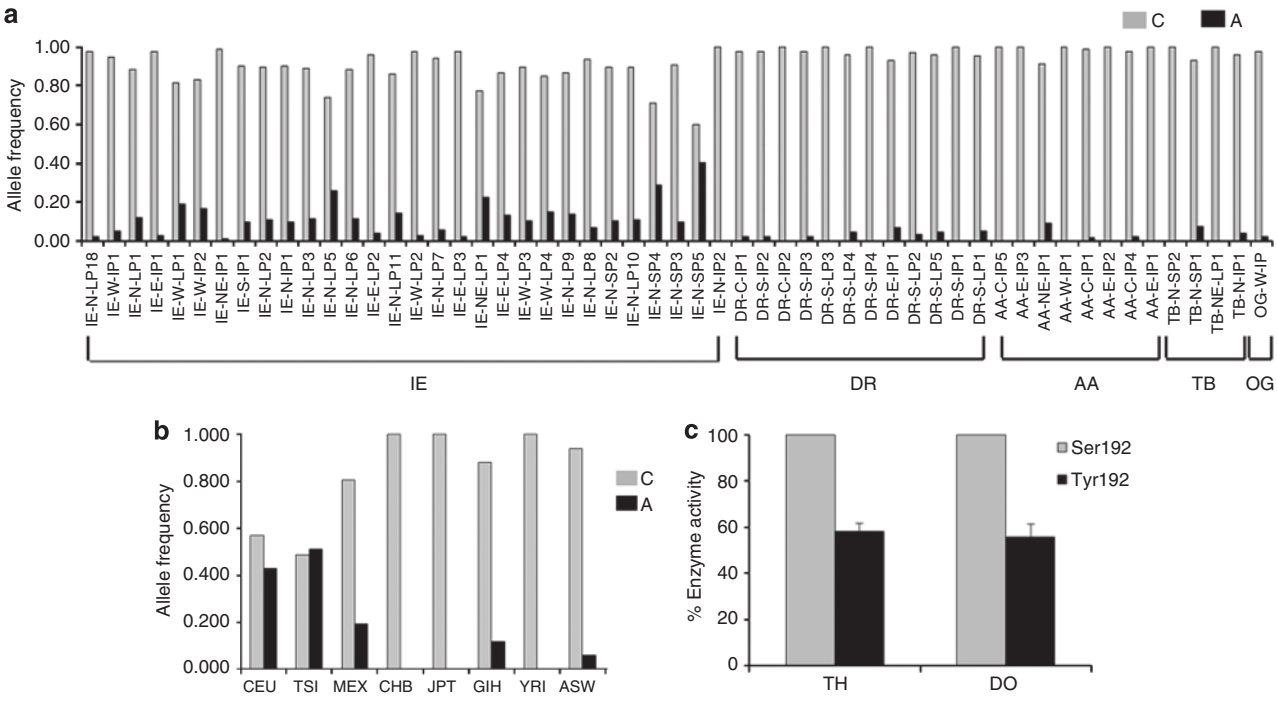


**Figure 2. Distribution of rs1042602 (c.575C>A, p.Ser192Tyr) in different population groups and an estimate of its enzymatic activity.** (**a**) The distribution of the derived allele A and ancestral allele C of tyrosinase among Indians. The Indian population comprises 55 ethnic groups representing 4 major linguistic groups—Indo-European (IE), Dravidian (DR), Austro-Asiatic (AA), and Tibeto-Burman (TB)—and the outgroup (OG). Panel **b** shows the same for the HapMap populations. These populations are designated CEU, TSI, MEX, CHB, JPT, GIH, YRI and ASW. Details are available at the HapMap website (http://snp.cshl.org/cgi-perl/gbrowse/hapmap27_B36/). (**c**) Relative enzyme activities (tyrosine hydroxylase (TH) and DOPA oxidase (DO)) of TYR, encoded by both Ser192 and Tyr192 alleles. DOPA, L-3,4-dihydroxyphenylalanine; TYR, tyrosinase.

the presence of only the higher-mobility band in the gel (Figure 1c). As expected in the dynamic milieu of a living cell, a fraction of wild-type TYR was always being processed in the ER. Thus, the EndoH-treated wild-type TYR showed two distinct bands; the higher one corresponding to the mature protein (hence, EndoH resistant) and the lower one comigrating with the mutant TYRs, signifying its immature status (that is, EndoH sensitive). Our observation corroborates an earlier finding that OCA1 is an ER-retention disease (Halaban *et al.*, 2000). Given that the selected mutations spanned entire TYR polypeptides, it is likely that ''sorting failure'' is the key mechanism of OCA1 pathogenesis in most missense mutations. However, it would be interesting to determine whether all missense mutations cause OCA1 in a similar manner.

Previously, we identified a *TYR* cSNP p.Ser192Tyr (c.575TCT>TAT, rs1042602, Giebel and Spritz, 1990) among some OCA1 individuals. This single-nucleotide polymorphism has biased distribution among the HapMap population—the frequency of the derived allele (Tyr192) is quite high (0.43–0.51) among populations of European descent, but it was very low or completely absent among all others. In addition, it has recently been proposed to be a risk factor for squamous cell carcinoma (Nan *et al.*, 2009). We, as a part of the Indian Genome Variation Consortium, genotyped p.Ser192Tyr in 1,871 normal healthy Indians, comprising 55 ethnicities belonging to four linguistic groups representing the overall population diversity of India (Indian Genome Variation Consortium, 2008). As compared with the Dravidian and Austro-Asiatic groups, the likely natives of the Indian subcontinent, Tyr192 was found to be overrepresented among the Indo-Europeans (Figure 2a). Notably, because the 192nd residue is located within the copper-A catalytic site with H-bonded His residues at 180, 202, and 211 (García-Borrón and Solano, 2002; Schweikardt *et al.*, 2007), Ser>Tyr substitution might perturb the substrate binding or catalytic property as a result of steric hindrance. Our functional study revealed ~40% reduced enzymatic activity for Tyr192, but further insight into the molecular event would require elucidation of the crystal structure of the protein, which is not yet available.

Considering that 5–8% residual enzyme activity is enough to influence partial pigmentation in OCA1B patients (King and Witkop, 1977), it would be valuable to assess the role, if any, that Tyr192 plays in pigmentation variation as reported by genome-wide association studies (Stokowski *et al.*, 2007). It should be noted that none of our OCA1 individuals with uncharacterized mutations harbors Tyr192, thereby preventing us from exploring its role in OCA1 pathogenesis. Nevertheless, this information would be useful in future epidemiological studies pertaining to the wide variation in skin pigmentation profiles across India.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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## SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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