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Liver X Receptor-α polymorphisms (rs11039155 and rs2279238) are associated with susceptibility to vitiligo

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ABSTRACT

Vitiligo is a complex genetic skin depigmentation disorder caused by the destruction of melanocyte from the lesional site. Liver X Receptor-α (LXR-α) expression is upregulated in the melanocytes from perilesional skin as compared to the normal skin of vitiligo patient suggesting its involvement in vitiligo pathogenesis. Polymorphisms in LXR-α have been associated with several diseases including cardiovascular disease, polycystic ovary syndrome, cancer, inflammatory bowel disease and diabetes. In this study, for the first time, we have investigated the association of LXR-α gene polymorphisms and risk of vitiligo. Sixty six vitiligo patients and 75 matched healthy control subjects who did not have any history of vitiligo or any other autoimmune disorder were recruited. The DNA isolated from patients and healthy controls was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for both rs11039155 (−6 G>A) and rs2279238 (+1257 C>T) variants. Our data suggest significant association between the LXR-α gene polymorphisms and vitiligo susceptibility (rs11039155: odds ratio (OR) = 1.99, 95% CI = 1.07–3.71, p = 0.03; rs2279238: OR = 1.70, 95% CI = 1.06–2.73, p = 0.027). Our results provide an evidence that the LXR-α −6A and +1257T alleles contribute to risk of vitiligo in North Indian population and highlight the importance of this gene in the vitiligo pathogenesis.

1. Introduction

Vitiligo, an idiopathic skin depigmentation disorder, is caused by the destruction of melanocytes from the basal layer of the epidermis as a result of which irregularly shaped milky white patches appear on the lesional skin (Shajil et al., 2006). Vitiligo affects approximately 1% of the world population including both men and women unbiasedly from all ethnicities (Tang et al., 2013). Although, it is not a life threatening disorder but this could be psychologically devastating leading to mild embarrassment to a severe loss of self-confidence for the affected person especially the dark skinned people because of the presence of white patches on the skin (Ongenae et al., 2006).

Melanocytes are solely responsible for the melanin pigment production by the process named melanogenesis which imparts color to the skin. Loss of melanocyte results in lack of melanin and thus, milky white patches appear at the lesion site. The exact pathophysiology of vitiligo is hard to pin down but various theories based on autoimmunity (Kemp et al., 2001; Laberge et al., 2005), oxidative stress (Denat et al., 2014), neural factors (Lerner, 1959) and genetic defects (Shajil et al., 2006) have been proposed to explain the reason of melanocyte destruction. Various researchers are focusing their efforts to explicate the mechanism of skin pigmentation and its regulation with the purpose of better management of pigmentary disorder. More than 150 genes are identified that affect pigmentation in skin, hair and eye along with multiple transcription factors, signaling factors and other biological factors involved in its regulatory pathway known to interfere with pigmentation process (Bennett and Lamoreux, 2003). Candidate gene association studies have reported more than 33 genes involved in generalized vitiligo (Spritz, 2011).

Liver X Receptor (LXR) is ligand (such as oxysterol, high concentration of D-glucose) activated nuclear transcription factor which regulate the expression of target genes involved in various physiological processes. LXR exists in two isoforms: LXR-α and LXR-β. LXR-α is highly expressed in several metabolically active tissues including liver, adipose tissue, macrophages, and intestine, whereas LXR-β is ubiquitously expressed in most tissues (Steffensen and Gustafsson, 2004; Jamroz-Wisniewska et al., 2007; Lee et al., 2013). Recently, LXR has also been shown to express in skin tissue such as sebaceous gland, hair follicle, epidermal keratinocyte and fibroblast and was found to be linked with various skin disorder pathogeneses like psoriasis and acne vulgaris

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(Russell et al., 2007). Melanocytes express LXR but the functions of LXR in these cells are still elusive (Kumar et al., 2010).

Kumar et al. observed high expression of LXR-α at both transcriptional and translational levels in the melanocytes obtained from perilesional skin compared to the normal skin of vitiligo patient (Kumar et al., 2010). The involvement of LXR-α in the development of vitiligo. Various researchers have reported the effect of activated LXR on melanogenesis (Kumar et al., 2010; Lee et al., 2013). Kumar et al. further showed that LXR-α agonist 22(R)-hydroxycholesterol treatment significantly downregulates the cell adhesion molecule, suggesting the existence of melanocytorrhagy in which melanocytes with defective adhesion system induces detachment from the basement membrane in perilesional vitiligo skin followed by melanocyte apoptosis (Kumar and Parsad, 2012). Lei et al. demonstrated that matrix metalloproteinase 2 (MMP 2) plays an important role in melanoblast (melanocyte precursor) migration from the outer root sheath of hair follicle into clinically depigmented epidermis and crucial for the repigmentation of vitiliginous skin (Lei et al., 2002). But, the study by Kumar et al. reported that MMP-2 and MMP-9 were downregulated in vitiligo patient and furthermore, it was shown that LXR-α gene knock-down significantly increased the expression of MMPs (Kumar et al., 2011). Recently, Lee et al. reported that the activated LXR inhibits the melanogenesis through the accelerated activation of extracellular signal-regulated kinase (ERK) mediated microphthalmia-associated transcription factor (MITF) degradation (Lee et al., 2013). Thus, upregulated expression of LXR-α in perilesional skin melanocytes significantly decreases the adhesion, proliferation and matrix metalloproteinases and increases apoptosis. Based on all these studies, LXRs appear to play a key role in vitiligo pathogenesis and considered as a potential therapeutic target for vitiligo (Kumar et al., 2012). Variation in gene expression which is considered as an intrinsic factor in a disease may be associated with both genetic factor (mutational changes, single nucleotide polymorphism, epigenetic change) and non-genetic factors (level of agonists e.g. oxysterol, drugs such as statins, fibrates, thazolidinedione derivatives, level of antagonists e.g. oxysterol sulfonates).

We selected two LXR-α single nucleotide polymorphisms (SNPs; rs11039155 and rs2279238) to investigate their contribution to the risk of vitiligo. These SNPs have been reported to be associated with many metabolic indicators and conditions, including circulating LDL and HDL-cholesterol concentrations (Robitaille et al., 2007; Sabatti et al., 2009), type 2 diabetes mellitus (Dahlman et al., 2009; Ketterer et al., 2011) and obesity (Dahlman et al., 2006). To the best of our knowledge, this is the first study to assess the association of LXR-α polymorphisms with vitiligo disorder.

2. Material and methods

2.1. Study population

The studied group included 66 vitiligo patients (27 females/39 males; mean age: 27.38 ± 14.06) attending in Department of Dermatology, Dr. Rajendra Prasad Government Medical College, Kangra, Himachal Pradesh and Dr. Bansal's Skin Laser Center Clinic, Manimajra, Chandigarh. The diagnosis of vitiligo was established using standard diagnostic criteria. Clinical history of vitiligo and any other autoimmune disease in patients was taken by clinical interview. A total of 75 (31 females/44 males) healthy individuals with no history of vitiligo or apparent autoimmune disease were included as control; they matched to patients with regard to age, sex and geographical distribution. The study was approved by Institutional Ethical Committees of Dr. Rajendra Prasad Government Medical College and Jaypee University of Information Technology, Solan, Himachal Pradesh. The importance of the study was explained to all participants and written consent was obtained from patients and controls.

2.2. Sampling, DNA isolation and genotyping

Peripheral blood (2 ml) was collected from the patients and healthy subjects in Na_2EDTA coated tubes. Genomic DNA was extracted from peripheral blood using a standard salting out procedure described earlier (Miller et al., 1988).

Genotyping of LXR-α −6 G>A and +1257 C>T SNP was done using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described earlier (Legry et al., 2008; Fukae et al., 2011). Briefly, the DNA fragments containing these SNPs were amplified in 25 μl reaction mixture containing 12.5 μl PCR master mix (Promega, Madison, US), 0.2 μM of each primer and 40 ng of DNA template. The primer pairs used were, for −6G>A (Forward 5′-GTG AGA GGA TCA CTT GAG C-3′ and Reverse 5′-CAG ACC GCA GGC TCC ACG C-3′; amplifies 366 bp fragment), and for +1257C>T (Forward 5′-CTT TCT GAG CCT CAC TTT CC-3′ and Reverse 5′-CGC AGC TCA GAA CAT TGT AG-3′; amplifies 377 bp fragment). The PCR amplification conditions were: 3 min initial denaturation at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing for −6G>A for 30 s at 62 °C & for +1257C>T for 45 s at 60 °C and 30 s of extension at 72 °C followed by final extension at 72 °C for 5 min. Amplified PCR products were analyzed on 1.5% agarose gel by ethidium bromide staining. For RFLP analysis, PCR products were digested with 1 U of respective restriction enzyme (Hpy188III for −6G>A and Fnu4HI for +1257C>T) for 4 h at 37 °C. Digested products were analyzed on 3% agarose gel containing ethidium bromide.

2.3. Statistical analysis

Allelic and genotypic frequencies were calculated using Microsoft Excel software (Microsoft Corporation). Hardy–Weinberg equilibrium (HWE) was tested to determine if the population fulfilled the HWE at the variant locus. The observed genotype distribution of control was assessed for deviation from HWE using a chi square test (http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html). Odds ratios and 95% confidence intervals were calculated to assess the risk associated with alleles and genotype. RevMan v5.3 was used to calculate odds ratio. The significance of the odds ratio was determined by Z test ($p < 0.05$ was considered statistically significant).

3. Results

Clinical and demographic characteristics of vitiligo patients are shown in Table 1. The average ages of patients and controls were 27.38 ± 14.06 yr and 23.05 ± 7.08 yr, respectively. The average age at onset of disease was 18.09 ± 10.44 yr. The majority of patients (75%) had less than 25% body coverage of the depigmented patches.

Table 1
Clinical and demographic characteristics of vitiligo cases and control group.

Characteristics	Vitiligo (N = 66)	Controls (N = 75)
Sex (n/N [%])		
Female	27/66 [41]	31/75 [41]
Male	39/66 [59]	44/75 [59]
Age	27.38 ± 14.06 yr	23.05 ± 7.08 yr
Age at the onset	18.09 ± 10.44 yr	
Changing size of depigmented patches (n/N[%])	40/60 [67]	
Body coverage of depigmented patches (%)		
1–25	75	
26–50	5	
51–75	9	
76–100	11	
Reported family history of vitiligo (n/N [%])	5/66 [7]	