Interleukin-1β gene polymorphism is associated with skin photosensitivity

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Abstract

Introduction: Ultraviolet radiation (UVR) exerts both beneficial and harmful effects on humans. Scarce data indicate that cytokine gene polymorphism might determine personal response to UVR. The aim of the study was to analyze the association between polymorphisms in the *IL-1β* gene and individual photosensitivity in healthy volunteers to ultraviolet B radiation.
Material and methods: The study group consisted of 234 healthy Caucasian volunteers (119 F, 115 M) with either II or III skin phototype. In each volunteer phototesting was undertaken and then 24 h later minimal erythema dose (MED) was determined. Three known dimorphic sites within the *IL-1β* gene were analyzed using RFLP-PCR technique: T→C (promoter region) at position-511 (rs16944), G→A (intron 4) known in literature as 5810 (rs1143633) and C→T (exon 5) known in literature as +3953 or 5887 (rs1143634).
Results: The mean value of MED in 234 volunteers was 0.156 J/cm^2. A statistically significant difference was found between MED in subjects with skin phototype II and skin type III: 0.13 vs. 0.17 J/cm^2, P<10^{-5}. A significant association between phototypes and the 5887 (rs1143634) polymorphism was found. Phototype III occurred significantly more often among carriers of the CC genotype; OR (95% CI) = 2.1 (1.2-3.9). Moreover, carriers of the T allele had lower MED as compared to the CC genotype; 0.15 vs. 0.17 J/cm^2, P=0.016.
Conclusions: The obtained results demonstrate the role of genetic factors in skin phototype and individual photosensitivity. It indicates that individual susceptibility to UVB radiation needs to be considered when studying the effects of UVB in humans.

Key words: *IL-1β* gene polymorphism, photosensitivity, skin phototype.

Introduction

Ultraviolet radiation (UVR), a common environmental factor, exerts both beneficial and harmful effects on human beings. Ultraviolet radiation exposure initiates a complex cascade of responses, which finally results in downregulation of the immune system. Deleterious effects of UV exposure include erythema, burns, photo-aging, DNA damage, carcinogenesis and impaired resistance to bacterial, viral, parasitic and fungal infections [1-4]. Ultraviolet radiation is also a well-known aetiopathogenic agent of many skin diseases. The most common

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photosensitivity disorders include polymorphic light eruption (PLE) and cutaneous forms of lupus erythematosus (LE) – subacute cutaneous LE (SCLE) and discoid LE (DLE) [5-8]. The role of UVR in melanoma and non-melanoma skin cancer development has been widely reported. Phenotypic susceptibility (i.e. fair skin and tendency to burn easily) is a well-known predisposing factor to both types of skin cancers.

To establish individual response to UV, assessment of skin phototype and measurements of minimal erythema dose (MED) are performed and their values are considered as an index of cutaneous photosensitivity [9]. Fitzpatrick [10] in 1988 described six skin phototypes (I-VI). Their estimation is based on detailed history taken from an individual's response to the first 30 min of exposure to the sun at noon on a sunny day in a given year. The MED value is assessed by the phototesting procedure and is defined as a dose of selected UV spectrum which induces a just perceptible erythema 24 h after skin irradiation [11].

Under normal or pathological conditions immunoregulatory molecules such as cytokines, circulating and released under various stimuli, can modulate cell activities. Synthesis and release of cytokines have been implicated as one of the mechanisms of UV-mediated immuno-modulation [12]. Evidence, mainly from animal but also human studies, suggests that cytokines are the most important mediators leading to inflammation, immunosuppression and skin cancer development initiated by UVR [13, 14]. For instance, some authors have reported increased IL-1β level after irradiation with a solar simulator [13, 15].

For IL-1β, as well as several other proinflammatory cytokines, differences in secretion levels run in families, which indicates a genetic background [16, 17]. A variant C→T at position 5887 (rs1143634) in the gene encoding for gene *IL-1β* has been reported as affecting the level of IL-1β expression in response to various stimuli. Allelic CC homozygotes produce significantly more IL-1β under exogenous stimulus when compared to hetero-zygotes [17-23]. This minor allelic variant of IL-1β also significantly influenced the immune response to hepatitis B vaccination [24].

There is a paucity of literature on the genetic background of individual photosensitivity and its role in susceptibility to skin cancer development. Based on the conducted experiments, mainly in animal models, one may assume that cytokine gene polymorphism might determine personal response to exposure to UVR [25].

The aim of our current study was to evaluate the putative association between functional polymorphisms in the *IL-1β* gene and individual photosensitivity in healthy volunteers using skin phototype and minimal erythema dose (MED) to ultraviolet B radiation.

Material and methods

The study group consisted of 234 healthy Caucasian volunteers (119 F, 115 M) aged from 19 to 54 years (median 24.4, lower and upper quartiles 21.0-30.0 years), with either II (n=123) or III (n=111) skin phototype, as assessed by Fitzpatrick score [10]. They were without any skin or other diseases and were not receiving any medications. People exposed to sunlight or sunlamps two months prior to the study were also excluded. To decrease the influence of natural solar radiation, all the procedures were performed in winter months. Each volunteer gave written informed consent before entry into the study, which was approved by the local ethics committee.

Phototesting of each volunteer was undertaken using a Waldmann Medizintechnik UV 109 device (Waldmann Medizintechnik, Villingen-Schwenningen, Germany) containing TL12 tubes (280-315 nm, biologically weighted with the CIE (Commission Internationale d'Eclairage) erythema action spectrum; Philips, Eindhoven, the Netherlands) emitting 58.6% UVB and 41.4% UVA. The test was performed with an incremental dose series (0.09, 0.11, 0.13, 0.15, 0.17, 0.19 J/cm^2) on 6 squares (1 × 1 cm) on the back. The MED was defined as a just perceptible erythema 24 h later.

Measurement of the intensity of the lamps was performed using a Type 1 UV meter calibrated against a spectrophotometer (Waldmann Medizin-technik, Villingen-Schwenningen, Germany).

Blood samples for genetic analyses were taken from all the volunteers.

Gene analysis

Three known dimorphic sites within the *IL-1β* gene were analyzed using the RFLP-PCR (restriction fragment length polymorphism) technique: T→C within the promoter region at position −511 (rs16944), G→A within intron 4 known in literature as 5810 (rs1143633) and C→T within exon 5 known in literature as +3953 or 5887 (rs1143634). All these single nucleotide polymorphisms (SNPs) are reported as functional by their impact on IL-1β expression [26]. Freshly withdrawn EDTA blood (0.5 ml) was subjected to DNA isolation. Genomic DNA was amplified with primers IL-1β (Table I) on a GeneAmp PCR 9600 (Perkin Elmer). The PCR reaction mixture (20 µl) contained 200 ng genomic DNA, 200 µmol/l of each dNTP, 5 pmol of each primer, 1.5 mmol/l MgCl$_2$, 0.1 U *Taq* DNA polymerase (TIB Molbiol, Germany). Amplification conditions for subsequent restriction endonuclease analysis

were as follows: initial denaturation at 94°C, 5 min, 35 cycles each including: denaturation at 94°C – 30 s, annealing at 57°C – 30 s, and extension at 72°C – 30 s. Final extension was carried out at 72°C for 10 min.

The PCR product (6 μl) in appropriate buffer was subjected to digestion with respective restriction endonuclease: rs16944 – AluI, rs1143633 – ItaI and rs1143634 – TaqI. Both ItaI and TaqI polymorphisms were contained in the fragment amplified with primers IL-1β51: GTA TAT GCT CAG GTG TCC TC and IL-1β52: CAT GGA GAA TTA GCA AGC TG. The rs16944 (AluI) polymorphism was located in the fragment amplified with primers IL-1β11: CAT AGT TTG CTA CTC CTT GC and IL-1β12: CAA AAA GCT GAG AGA GGA GG.

Polymorphic alleles within the *IL-1β* gene were identified: TaqI digestion of primary amplicons produces fragments 211 bp + 116 bp for allele C, and one fragment 327 bp for allele T. ItaI digestion gives 327 bp fragment for allele G, and 288 bp + 39 bp fragments for allele A. Transition T→C at position –511 disrupts the restriction site for AluI position, so the respective amplicon is digested once for allele C, and twice for allele T.

Statistical analysis

Univariate analyses were examined for skewness and kurtosis and Shapiro-Wilk approach was used to test variables' distribution for normality. Since quantitative variables were not normally distributed, non-parametric analysis was applied using the Mann-Whitney U and Wilcoxon's test and the data are presented as medians and upper and lower quartiles. The categorized variables were analyzed using non-parametric χ^2 test in Yates' modification. Odds ratio (OR) with 95% confidence interval (95% CI) was also calculated.

All statistical analyses were conducted using the statistical package Statistica, version 6.1 (StatSoft, Inc., USA). P values <0.05 were considered as statistically significant.

Hardy-Weinberg equilibrium and linkage disequilibrium (LD) between studied polymorphisms were defined using Haploview and EH software [27, 28]. Since studied polymorphisms were not in tight LD (D'<0.6) SNPs were treated separately and haplotype analysis was not performed.

Results

The mean value of MED in 234 volunteers was 0.156 J/cm^2 (median 0.15, lower and upper quartiles 0.13-0.17). A statistically significant difference was found between MED in subjects with skin phototype II and skin type III: 0.13 (0.13-0.15) vs. 0.17 (0.15-0.19) J/cm^2, P<10^{-5}.

The distribution of genotypes for each polymorphism in the study group did not deviate significantly from the Hardy-Weinberg equation.

Analysis of genotypes and alleles distribution of −511 (rs16944) and 5810 (rs1143633) polymorphisms in the *IL-1β* gene showed no statistically significant differences between subjects with phototype II and III (Table I). Similarly, no association with MED value was found (data not shown).

Interestingly, a statistically significant association between phototypes and and the 5887 (rs1143634) polymorphism was found (Table I). Phototype III occurred significantly more often among carriers of the CC genotype; OR (95% CI) =2.1 (1.2-3.9). Moreover, carriers of the T allele had lower MED as compared to the CC genotype; 0.15 (0.13-0.17) vs. 0.17 (0.15-0.17) J/cm^2, P=0.016 (Figure 1).

Table I. Distribution of genotypes and alleles of three polymorphisms studied in *IL-1β* gene among individuals with skin phototype II and III

	Phototype II	Phototype III	P
−511 polymorphism (rs16944)			
TT	51 (40.48%)	48 (45.7%)	
TC	54 (42.86%)	41 (39.81%)	NS
CC	21 (16.67%)	16 (15.53%)	
Allele			
T	156 (61.9%)	137 (65.2%)	NS
C	96 (38.1%)	73 (34.8%)	
5810 polymorphism (rs1143633)			
GG	43 (34.13%)	32 (30.48%)	
GA	62 (49.21%)	49 (46.67%)	NS
AA	21 (16.67%)	24 (22.86%)	
Allele			
G	148 (58.7%)	113 (53.8%)	NS
A	104 (41.3%)	97 (46.2%)	
5887 polymorphism (rs1143634)			
CC	24 (19.05%)	35 (33.33%)	
CT	73 (57.94%)	56 (53.33%)	0.015 (df=2)
TT	29 (23.02%)	14 (13.33%)	
Carriers			
CC	24 (19.05%)	35 (33.33%)	0.013 (df=1) OR (95% CI) =2.1 (1.2-3.9)
T+	102 (80.95%)	70 (66.67%)	0.013 (df=1)
Allele			
T	131 (52.0%)	84 (40%)	0.01 (df=1)
C	121 (48.0%)	126 (60%)	

OR (95% CI) – odds ratio with 95% confidence interval

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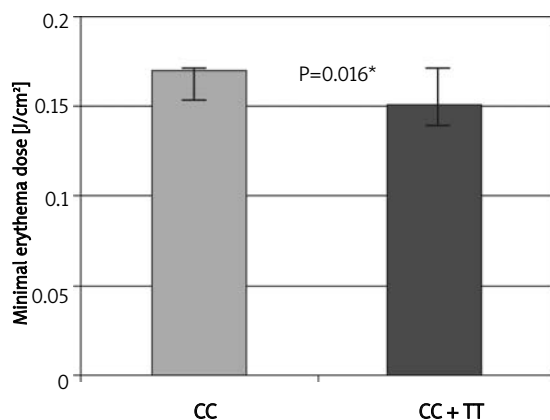
Figure 1. Minimal erythema dose among carriers of T allele and CC genotype of the 5887 polymorphism (rs1143634) in IL-1β. Values presented as median and upper and lower quartile
P value for Mann-Whitney U-test

Discussion

Solar UVR has been shown to be the main environmental causative factor of skin cancer. It seems that skin cancer susceptibility is definitely connected with genetic background including polymorphisms in the *MC1R* gene. Four *MC1R* variant alleles have been shown to be associated with fair skin and red hair phenotype and higher sun-sensitivity and consequently loss-of-function variants of *MC1R* could influence the risk of developing skin cancer [21, 29-31]. On the other hand fair skin and red hair are typical findings in humans representing lower Fitzpatrick phototypes [31].

A variety of cytokines such as IL-1, IL-6, IL-10, IL-12 and TNF-α were found to be secreted by UV-irradiated keratinocytes. Some were also detected in the serum of UV-irradiated mice [5, 6]. Previous studies have revealed that 10 days of sub-erythemal UVB whole body irradiation of human subjects with phototype II or III caused an increase in serum concentration of TNF-α [32]. In another study we also reported that the same doses of UVB resulted in enhanced expression of IL-1β and TNF-α mRNAs in human skin [33]. Granstein and Sauder [34] demonstrated elevated serum levels of IL-1 four hours after whole body irradiation with an erythemal dose of UV. Also Barr et al. [13] observed higher IL-1β concentrations after exposure to solar simulator radiation.

In the light of these findings it would be useful to disclose further genetic and molecular factors to identify a population with higher sun-sensitivity and risk of developing skin cancer in order to introduce appropriate preventive strategies. Therefore, IL-1β seemed to be one of the most important cytokines involved in immunomodulation after the set of events following UV exposure. Hence, in our study we attempted to find a relationship between polymorphism within the gene encoding for IL-1β and individual UV-sensitivity.

Different polymorphisms have been described in the *IL-1β* gene and some of them could influence protein production [26, 35-37]. There are only scarce data on the role of IL-1β gene polymorphism in photoimmunosuppression. Slejiffers et al. [25] showed that pre-irradiation with UVB of subjects with +3953 *IL-1β* gene polymorphism, followed by hepatitis B vaccination, resulted in lower titre of anti-HBV antibodies. Moreover in the same individuals lower UVB sensitivity as assessed by MED was observed. We decided to study the functional polymorphisms in the *IL-1β* gene as a putative genetic factor involved in individual photosensitivity. We documented an association between C→T polymorphism at 5887 (rs1143634) and skin phototype. Phototype III occurred significantly more often among carriers of the CC genotype. Coherently, carriers of the T allele had lower values of minimal erythema dose than carriers of the genotype CC. This documented a recessive effect of genetic variation in IL-1β on photosensitivity in the general Caucasian population. This observation is also consistent with the reports which have revealed that individuals homozygous for the C allele at 5887 produce approximately fourfold more IL-1β, and the heterozygous cells produce approximately twofold more IL-1β, than do individuals homozygous for the T allele [36]. Moreover, the C→T polymorphism at 5887 has been associated with development or severity in a number of immune and inflammatory diseases, such as type 1 diabetes, psoriasis, rheumatoid arthritis, ulcerative colitis, systemic lupus erythematosus, Graves' disease and periodontal disease [16-18, 23, 38]. Thus, it is likely that we documented not a spurious but a real genetic effect on photosensitivity.

In conclusion, the obtained results demonstrate the role of genetic factors in skin phototype and individual photosensitivity. It indicates that individual susceptibility to UVB radiation needs to be considered when studying the effects of UVB in humans. The obtained results are not enough to draw definite conclusions, and replication in different populations is also needed. Thus, further research on the genetic background of photosensitivity is required.

Acknowledgments

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