Propensity for DNA Damage in Psoriasis Patients Genotyped for Two Candidate Genes

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Abstract

Studies assessing genetic damage and its association with disease-candidate genes in patients belonging to geographically distinct populations are scanty. The present study evaluated DNA damage using the alkaline Single Cell Gel Electrophoresis assay in peripheral blood leukocytes of Psoriasis Punjabi Patients on systemic-topical therapy who had been genotyped for two disease-candidate genes (HLA-C, human leukocyte antigen and the coiled-coil alpha-helical rod protein 1(CCHCR1). Genetic damage was disease gene-influenced as homozygous mutants for CCHCR1 Exon 4 site 386* (C→T) and heterozygous mutants for 404* (C→T) alleles had significantly more damage (p<0.05) compared to respective homozygous wild types. The arginine to tryptophan substitution alters the protein, triggering keratinocyte proliferation and probably inflammation/ oxidative stress. This along with drug treatment probably caused the observed DNA damage. Population sub-groups had no within group differences but larger sizes can explore this possibility. Studies of this type can provide disease-gene-damage prone information for exploring DNA-safe therapeutics.

Keywords: Genetic damage; Gene association; Population sub-groups

Background

Studies for associations between polymorphisms of candidate disease genes and genetic damage in geographically defined populations have not come to attention. Such studies can explore ethnic and racial differences in disease as well as propensity for DNA damage since the latter is an early indicator of carcinogenesis. Assessment of patients for genetic damage can assist in its management. Psoriasis, an immune-mediated inflammatory skin disease requiring long term treatment, affecting 2–3% of the population [1] with varying ethnic-frequency [2], mediated via genetic-environmental factors, causes keratinocyte hyperproliferation, inflammatory response [3] with an impaired oxidant/antioxidant status [4-6] which is damaging to proteins, lipids and DNA. Patients with/without various treatment modalities have been assessed for genetic damage [7-11] but none on both, systemic and topical treatments which is a regular, local prescription. Therefore DNA damage was investigated in peripheral blood leukocytes (PBL) of patients applying coal tar ointment and prescription. Gel Electrophoresis assay in peripheral blood leukocytes of Psoriasis Punjabi Patients on systemic-topical therapy who had been genotyped for two disease-candidate genes (HLA-C, human leukocyte antigen and the coiled-coil alpha-helical rod protein 1(CCHCR1). Genetic damage was disease gene-influenced as homozygous mutants for CCHCR1 Exon 4 site 386* (C→T) and heterozygous mutants for 404* (C→T) alleles had significantly more damage (p<0.05) compared to respective homozygous wild types. The arginine to tryptophan substitution alters the protein, triggering keratinocyte proliferation and probably inflammation/ oxidative stress. This along with drug treatment probably caused the observed DNA damage. Population sub-groups had no within group differences but larger sizes can explore this possibility. Studies of this type can provide disease-gene-damage prone information for exploring DNA-safe therapeutics.

Questions Addressed

As propensity for genetic disease is ethnicity-dependent and as susceptibility for genomic insult has an association with metabolic genotypes, there could also be an association underlying disease gene-specificity and genetic damage. With this hypothesis and in view of cited literature, Psoriasis patients on systemic and topical therapy, already genotyped for some polymorphisms of CCHCR1 gene and HLA-C region, were assessed for DNA damage (pre-cancerous lesions) in order to correlate the level of genetic damage, if any, with their genotypic status, population sub-group besides routine variables. To the best of our knowledge, no such studies have been reported, at least not in Psoriasis patients from this region.

Experimental Design

Patients and healthy, matched controls participated after voluntary, written informed consent and study’s institutional ethical clearance. Disease history, demographic information and pedigree were recorded on a questionnaire. PBL from genotyped patients were processed for DNA damage by alkaline SCGE assay [15] except use of local chemicals and silver staining. Under electrophoresis, breaks in the super coiled DNA migrate towards the anode and appear as a comet indicative of DNA damage. Coded slides were visually scored at 400X. DNA migration was measured using a calibrated ocular micrometer and cells were graded into categories based on tail length. Arbitrary score, damage frequency (DF) and damage index (DI) were calculated [16] as mean ± S.E.M. Mann-Whitney U test was applied for significance of DNA damage. Chi-square (χ2) test compared attributes of patients and controls and their cells in different damage categories. Regression analysis, analysis of variance and odds ratio at 95% confidence interval were performed for confounders of DNA damage. Values were taken significant at p≤0.05, p<0.01 and p<0.001. All analysis was done using SPSS (version 10.0).

Results

Characteristics of patients and matched controls are given in...
supplementary material (Table S1, Table S2). DNA migration length, $DI$ and $DF$ reflect cellular events

**Table 1: HLA-C and CCHCR1 genotypes and DNA damage in Psoriasis patients and controls.**

| Allele | Genotype | PATIENTS | CONTROLS |
|--------|----------|----------|----------|
|        |          | DI S.E.M. (n) | DF S.E.M. (n) | Mean DNA migration length ± S.E.M. (n) | DI S.E.M. (n) | DF S.E.M. (n) | Mean DNA migration length ± S.E.M. (n) |
|        |          |          |          |          |          |          |
| 22222* (G–A) | GG | 25.38***±1.97 (12) | 55.23***±3.28 (12) | 37.51***±1.56 (12) | 4.88±1.28 (9) | 11.77±5.9 (9) | 9.38±1.94 (9) |
|        | GA | 28.41***±1.94 (10) | 55.29***±3.62 (10) | 38.78***±2.60 (10) | 6.75±2.56 (4) | 13.50±5.12 (4) | 12.29±3.36 (4) |
|        | AA | 34.46±1.32 (7) | 62.85±1.37 (7) | 33.41±1.49 (7) | 6.00±1.30 (2) | 12.00±2.00 (2) | 5.07±0.68 (2) |
| 22333* (A–G) | GG | 26.25***±1.95 (12) | 54.83***±3.54 (12) | 36.92***±1.58 (12) | 10.68±3.10 (9) | 11.33±2.76 (9) | 8.79±2.17 (9) |
|        | AG | 28.31***±2.06 (10) | 55.12***±3.85 (10) | 33.88***±2.77 (10) | 6.75±2.56 (4) | 13.50±5.12 (4) | 12.29±3.36 (4) |
|        | AA | 35.57±1.70 (9) | 62.85±1.37 (9) | 33.41±1.49 (9) | 6.00±1.30 (2) | 12.00±2.00 (2) | 5.07±0.68 (2) |
| 24118* (T–C) | TT | 26.90***±2.01 (11) | 56.18***±3.59 (11) | 36.76***±1.72 (11) | 9.00±3.60 (11) | 11.07±2.28 (11) | 8.69±1.77 (11) |
|        | TC | 27.72±2.17 (18) | 53.86±3.33 (18) | 36.04±2.27 (18) | 9.00±3.00 (11) | 10.00±0.00 (11) | 7.56±0.00 (11) |
|        | CC | 34.83±1.88 (6) | 65.33±2.34 (6) | 33.66±4.26 (6) | 9.00±0.00 (1) | 8.00±0.00 (1) | 7.59±0.00 (1) |
| 386* (C–T) | CC | 26.09***±2.02 (11) | 50.36***±3.33 (11) | 32.83***±2.38 (11) | 9.28±3.32 (11) | 14.28±3.32 (11) | 8.96±1.82 (11) |
|        | CT | 29.40±1.91 (21) | 57.40±3.06 (21) | 36.51±2.11 (21) | 9.00±0.00 (11) | 10.00±0.00 (11) | 8.39±0.00 (11) |
|        | TT | 37.06±1.57 (18) | 66.98±1.55 (18) | 36.37±2.66 (18) | 9.00±0.00 (11) | 10.00±0.00 (11) | 8.39±0.00 (11) |
| 404* (T–C) | CC | 25.69***±1.95 (9) | 48.33***±2.94 (9) | 31.32***±2.50 (9) | 9.28±3.32 (14) | 12.14±3.21 (14) | 8.96±1.82 (14) |
|        | CT | 30.30***±1.70 (20) | 59.20***±3.21 (20) | 35.07±2.05 (20) | 5.00±0.00 (1) | 10.00±0.00 (1) | 8.39±0.00 (1) |
|        | TT | 31.50±3.41 (6) | 66.85±3.55 (6) | 39.24±3.85 (6) | 9.00±0.00 (11) | 10.00±0.00 (11) | 8.39±0.00 (11) |
| 1364* (C–T) | TC | 29.01***±2.47 (10) | 56.20***±2.99 (10) | 33.54***±3.81 (10) | 6.40±1.39 (10) | 10.84±2.76 (10) | 8.63±2.22 (10) |
|        | CC | 30.31***±1.47 (17) | 59.12***±3.57 (17) | 34.64***±1.93 (17) | 5.25±1.93 (14) | 10.50±3.86 (14) | 7.63±2.54 (14) |
|        | TT | 25.60±2.37 (16) | 10.00±0.00 (8) | 30.00±4.23 (8) | 50.00±0.00 (1) | 10.00±0.00 (1) | 7.55±0.00 (1) |

**Very highly significant (p<0.001), ** highly significant (p<0.01), *significant (p<0.05) when compared to parallel control groups (Mann Whitney-U-test)**

**Very highly significant when compared to parallel control group (p<0.005) within patient group (404* (C–T)).**

**Very highly significant (p≤0.001), ** highly significant (p≤0.01), *significant (p≤0.05) when compared to parallel control groups (Mann Whitney-U-test).**
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