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Article type : Correspondence

Argyrophilic Nucleolar Organizing Regions in Patients with Xeroderma Pigmentosum Group E

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Key Words: Xeroderma Pigmentosum Group E, DDB2, Nucleolar Organizing Regions, rDNA, AgNOR

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Running title: AgNOR in Xeroderma Pigmentosum Group E

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/exd.14238](#)

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Xeroderma pigmentosum E (XP-E) is a rare autosomal recessive inherited genodermatosis associated with less severe sun burning with minimal sun exposure. The affected individuals acquire abnormal pigmentation and are at high risk for skin cancer ^[1]. Notably, the DNA damage-binding protein 2 (*DDB2*) gene is responsible for XP- E disease ^[2].

Nucleolar organizer regions (NORs) are sites of the ribosomal genes composed of ribosomal DNA (rDNA) and proteins. Various studies have been conducted regarding the significance of the interphase argyrophilic nucleolar organizer region-associated protein (AgNOR) quantity in different cells ^[3-9]. AgNOR proteins help distinguish between malignant and benign cells and provide information regarding the proliferative and metabolic activities of the cells. This study was conducted to understand whether the AgNOR method could be used to differentiate individuals with XPE from normal individuals and whether the AgNOR method could be helpful in the differential diagnosis of individuals with (homozygous and heterozygous) and without *DDB2* gene mutation that causes XPE. To our knowledge, no study in the literature has evaluated the mean AgNOR number and the total AgNOR area to total nuclear area (TAA/TNA) ratio in individuals with XP-E. Therefore, we performed the current study that was approved by the local ethics committee (2020/96).

Our proband had an ulcerated nodular lesion on the nose at 18 years of age, diagnosed as basosquamous cell carcinoma. Over the last 2 years, basal cell carcinoma in two different areas, squamous cell carcinoma (SCC) in one area, and basosquamous cell carcinoma in one area were

observed on the proband's face. Notably, SCCs were first diagnosed in the right cornea at 13 years of age and then in the left cornea at 15 years of age in our proband. The genetic analysis detected a novel homozygous c.1063C>T(p.Arg355Ter) variation in the *DDB2* gene. The study included a family of eight members (three with homozygous pathogenic *DDB2* gene mutation, three [parents and one sibling] with heterozygous pathogenic *DDB2* gene mutation, and one without the mutation) and age-matched healthy individuals with similar demographic characteristics.

The buccal desquamated epithelial cells and peripheral blood samples of each individual were taken on clean glass slides, and the AgNOR staining technique was performed at 37°C for 15 min in the dark by using a solution containing one volume of 2% gelatin in 1% aqueous formic acid and two volumes of 50% silver nitrate (Figure 1). The mean AgNOR number and TAA/TNA ratio were evaluated using ImageJ image processing software, version 1.47t^[10]. Fifty nuclei on each slide were evaluated. The mean AgNOR number and TAA/TNA ratio of the buccal epithelial cells were significantly higher in the patient group than the control group ($p = 0.003$ and $p = 0.039$). Moreover, the TAA/TNA ratio of lymphocytes was significantly higher in the patient group than the control group ($p = 0.010$). Comparison of the patient subgroups (cases with homozygous *DDB2* mutation, cases with heterozygous *DDB2* mutation, and cases without *DDB2* mutation) revealed statistically significant differences were noted among the groups regarding the mean AgNOR number ($p = 0.009$), TAA/TNA ratio of buccal epithelial cells ($p = 0.030$), and TAA/TNA ratio of lymphocytes ($p = 0.036$). A comparison between two patient subgroups revealed statistically significant differences related to the mean AgNOR number and TAA/TNA of lymphocytes ($p = 0.024$ and $p = 0.014$, respectively) between the cases with heterozygous *DDB2* mutation and cases without *DDB2* mutation. Furthermore, significant differences were noted regarding the mean AgNOR number and TAA/TNA ratio of buccal epithelial cells ($p = 0.014$ and $p = 0.014$, respectively) between the cases with homozygous *DDB2* mutation and cases without *DDB2* mutation. In addition, significant differences were noted regarding the TAA/TNA ratio of buccal epithelial cells ($p = 0.050$) between cases with homozygous and heterozygous *DDB2* mutations (Figure 1).

Therefore, AgNOR proteins could be applied in the early diagnosis of patients with XP-E. Notably, the changes in the shape, number, distribution, and amount of these proteins could provide information regarding the prognosis of patients with XPE, thereby aiding the physician in devising effective treatment strategies. This diagnostic modality would, therefore, help eliminate the anxiety and psychological negativity of patients and their families because of the illness, as

well as prevent the loss of time, labor, and money. Hence, we believe that AgNOR protein amounts could be used in detecting skin cancer in patients with chronic sun exposure and photoaging.

CONFLICT OF INTEREST

The authors have no financial or competing interests in relation to this work.

AUTHOR CONTRIBUTION

EK and RE conceived and supervised the project and provided context for biological samples. EK arranged the blood samples; EK and RE analyzed the data; RE designed the research study and laboratory studies, EK and RE wrote the manuscript together. The final version of the manuscript is read and approved by all authors.

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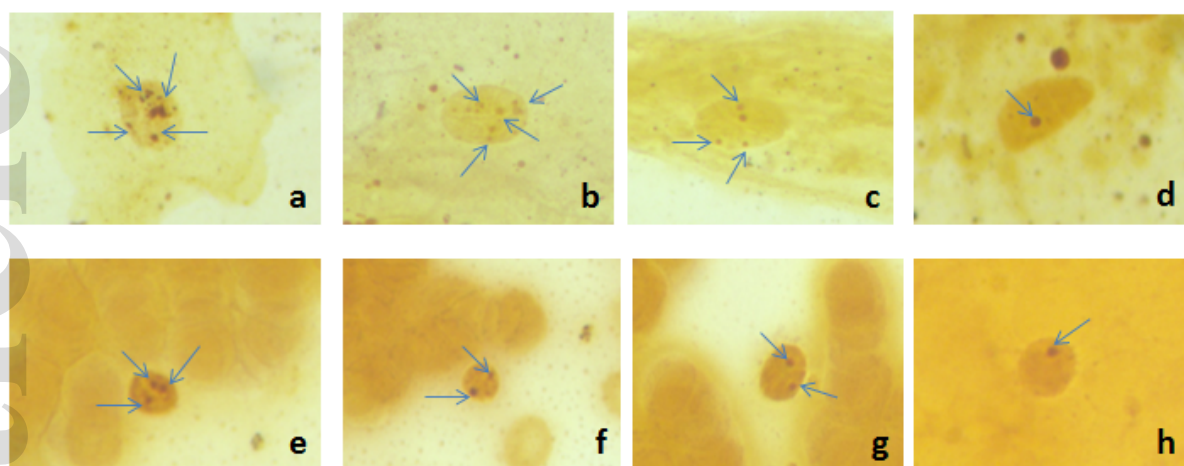


Figure 1. Demonstrative examples of silver stained NOR. The buccal epithelial cells were chosen from cases with homozygous *DDB2* mutation and both SSC and BCS (**a**), cases with homozygous *DDB2* mutation but without lesion (**b**), cases with heterozygous *DDB2* mutation (**c**), and cases without *DDB2* mutation (**d**). The lymphocytes were chosen from cases with homozygous *DDB2* mutation and both SSC and BCS (**e**), cases with homozygous *DDB2* mutation but without lesion (**f**), cases with heterozygous *DDB2* mutation (**g**), and cases without *DDB2* mutation (**h**). The nuclei of both buccal epithelial cells and lymphocytes were photographed at X1,000 magnification ($\times 1000$, oil immersion). Silver stained NORs was shown with arrow for both buccal epithelial cells and lymphocytes. Higher AgNOR proteins amounts were detected from cases with homozygous *DDB2* mutation, cases with heterozygous *DDB2* mutation and cases without *DDB2* mutation, respectively.