Dermatology Practical & Conceptual

Association between Vitamin D Receptor Polymorphisms, Tight Junction Proteins and Clinical Features of Adult Patients with Atopic Dermatitis

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ABSTRACT

Introduction: Few studies have explored the intricate connections between vitamin D receptor (VDR) gene polymorphisms, VDR, tight junction (TJ) protein expression and clinical features of atopic dermatitis (AD).

Methods: From 43 adult AD patients, VDR polymorphisms were genotyped from peripheral blood samples using polymerase chain reaction-restriction fragment length polymorphism. VDR, occludin, claudin-1 and ZO-1 protein expression from skin lesion biopsies were assessed by immunohistochemistry.

Results: The A1012G heterozygous VDR polymorphism exhibited a lower odds ratio (OR) for juvenile AD onset (OR: 0.046, 95% CI 0.004-0.51, p=0.012). In contrast, the presence of ≥2 homozygous

VDR polymorphisms were significantly associated with positive skin prick test (SPT) (10/20, 50%) vs. negative SPT (1/23, 4.3%; p=0.0003). The most highly expressed TJ proteins in lesions of AD patients were claudin-1 and zonulin-1 (ZO-1), while VDR and occludin were less prevalent. A significant correlation was observed between ZO-1 expression and a body mass index ≥30 kg/m^2 (OR: 12.1, 95% CI 1.06-137.9, p=0.045). Claudin-1 expression was associated with a positive SPT (OR: 8.23, 95% CI 1.04-65.5, p=0.046) and serum 25(OH)D levels were negatively correlated with ZO-1 expression (rho= -0.43, p=0.0058).

Conclusion: This study provides novel insights into the relationship between VDR gene polymorphisms, VDR, TJ protein expression, and clinical features in adult AD patients, highlighting a significant role of vitamin D in the pathophysiology of this disease.

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory cutaneous disease with multifactorial etiology [1–3].

Vitamin D and its receptor (VDR) are known to perform antiproliferative, pro-differentiative, anti-inflammatory and immunomodulatory activities [4–7]. The effect of vitamin D on the prevalence and severity of AD has been evaluated in numerous studies with varying results [8–11]. AD patients show lower serum concentrations of 25(OH)D (the major circulating form of vitamin D), compared to healthy controls [12–14] and serum vitamin D levels have been negatively associated with medium and severe AD [15].

Polymorphisms of VDR genes are recognized to drive the biological activity of vitamin D through receptor regulation and activation[16]. Several studies[17–22] and a recent meta-analysis[23] have described associations between AD and single-nucleotide polymorphisms (SNPs) in VDR. Recently, the A1012G (rs4516035) VDR polymorphism has been associated with psoriasis risk by affecting VDR expression [24].

The pathogenesis of AD is primarily characterized by alteration of the skin barrier[1]. The barrier function of the skin is mainly provided by the stratum corneum, the outermost layer of the skin, and tight junctions (TJ), specific junctions that reduce the intercellular space between epithelial cells, until its disappearance[25]. Alteration in TJs is driven by cytokines during the acute phase of inflammation and in psoriasis TJ expression (as well as VDR) is substantially reduced compared to healthy skin, suggesting a potential role of TJ as well as vitamin D in maintaining epithelial barrier integrity[26].

To date, no study has specifically examined the association between the clinical characteristics of patients with AD and VDR polymorphisms, VDR expression, and TJ protein levels. To address this gap, the aim of this study was to explore the potential implications of vitamin D in an adult Italian population with AD, through a comprehensive analysis of VDR polymorphisms, VDR expression, and TJ protein levels.

Materials and Methods

Patients and Study Design

This was a monocentric, cross-sectional, exploratory study on adult AD patients. Forty-three patients with acute phase AD were included. All patients were enrolled in the Dermatological Clinic of the Policlinico Umberto I, University of Rome La Sapienza, Rome, Italy.

Inclusion criteria were: adult male/female patients with diagnosis of mild and moderate-severe AD. Patients were excluded with the following: inflammatory and/or autoimmune pathology of different skin interest from AD, a diagnosis of calcium metabolism and/or bone metabolism, patients in replacement therapy based on vitamin D (or calcium) derivatives, use of corticosteroid treatment in the past 3 months, presence of severe concurrent disease of any interest, HIV 1/2, hepatitis B/C infection, chronic kidney disease, medium-severe liver failure, concomitant anticoagulation therapy, undergoing organ transplantation within 6 months prior to participation in the study and/or with congenital or acquired immunodeficiency. We also excluded patients with dementia, psychosis, history of alcoholism or chronic drug use, and psychoactive substances and currently pregnant, or direct and prolonged exposure to sunlight in the 3 weeks prior to enrollment.

During the screening phase, in addition to blood sample collection, the following data were collected: gender, age, body mass index (BMI), pathological and pharmacological history, age of onset of AD, AD phenotype, disease severity by the Eczema Area and Severity Index (EASI) [27,28], and possible presence of asthma and/or allergic rhino-conjunctivitis.

Within 10 days following the screening visit, patients were recalled for the following procedures: blood sampling for assessment of VDR polymorphisms, as well as a biopsy of the skin lesion for immunohistochemical evaluation and

assessment of gene or protein expression. Skin prick tests (SPT) were also performed for each patient.

This study was conducted in accordance with the the Helsinki Declaration in compliance with GCP, ICH guidelines and applicable national regulatory provisions. The Study Protocol received Ethics Committee approval (Identification Code: DERM/AT/01 - Sponsor: Abiogen Pharma SPA - CRO: Ricerche Nuove Srl).

Analysis of VDR Gene Polymorphisms

Analysis of VDR gene polymorphisms was performed by BMR Genomics (Padua, Italy), subcontractor of Galileo Research (Pisa, Italy), on 43 fasted peripheral blood samples taken from patients affected by AD. The target polymorphisms are summarized in Table 1.

Amplifications were performed by Eppendorf Thermal Cycler 5333, according to a manufacturer protocol (that can be provided upon request to the corresponding author). Each amplicon was sequenced as single filament with specific primers (Table 2). Samples were sequenced by ABI PRISM 3730xl Genetic Analyzer Quality of sequences and single polymorphism status were verified by SeqScape 2.6 software.

Biopsy Sampling

All patients with AD underwent a single biopsy sample at the site of the skin lesion (intralesional) at the first visit. Biopsies were performed after antisepsis with povidone iodine and local anesthesia performed using 2% mepivacaine, taking a flap of the dimensions of 10×5 mm maximum. Following biopsy, incisions were sutured with 1-3 stitches of synthetic thread (nylon 3.0) and a medicated plaster. All patients were instructed to take antibiotic prophylaxis (1g amoxicillin + clavulanic acid tablets) every 12 hours for 5 days. Stitches were removed after 7-15 days.

Immunohistochemistry of Tissue Biopsies

Skin samples were fixed in buffered formalin for 24 hours and embedded in paraffin. Sections of 3-4 µm were cut and stained with hematoxylin–eosin and Masson's trichrome stains. Sections underwent the following processing steps: rabbit polyclonal anti–zonulin-1 (ZO-1) antibody (Invitrogen: Carlsbad, CA, USA, 40-2300) diluted 1:250, goat polyclonal anti-ZO-1 antibody (Santa Cruz Biotechnology: Santa Cruz, CA, USA, sc-8146) diluted 1:50, mouse monoclonal anti-VDR antibody (Santa Cruz, sc-13133) diluted 1:100, rabbit polyclonal anti-VDR antibody (Abcam: Cambridge, United Kingdom, ab3508) diluted 1:200, mouse monoclonal anti-claudin-1 antibody (Santa Cruz, sc 81796) diluted 1:100, rabbit polyclonal anti-occludin antibody (Abcam, ab64482) diluted 1:100, rabbit polyclonal anti-occludin antibody (Santa Cruz, sc-5562) diluted 1:50. Sections were incubated for 1 hour at room temperature with anti-VDR and overnight at 4°C with anti-ZO-1, anti-claudin-1, and anti-occludin. After three washings in phosphate-buffered saline, sections were incubated for 20 minutes with the appropriate secondary biotinylated antibody labeled with the avidin–biotin complex (labeled streptavidin–biotin; Dako: Glostrup, Denmark). Negative controls were performed with normal mouse antiserum instead of the primary antibody, demonstrating no reaction uniformly. Sections were developed with 3,3-diaminobenzidine and finally counterstained with hematoxylin. Estimation of the number of anti-VDR, anti-ZO-1, anti-claudin-1, and anti-occludin immunoreactive keratinocytes was independently performed by two researchers with intra-observer agreement of ≥90%. The number of positive and negative keratinocytes was separately counted for each antibody under a light microscope at 40× magnification.

Only cells that displayed nuclei on the section were considered for VDR. Only keratinocytes that showed stained plasma membrane were considered positive for claudin-1, occludin, and ZO-1. For each slide, at least six microscopic

Table 1. Target polymorphisms

Gene	Polymorphism	SNP ID	Position chromosome 12 (assembly hg38)	Genetic localization (NM 000376)	Position ATG in VDR (NM_000376)
VDR	A-1012G	rs4516045	47906043	Promoter VDR	c.-1172A>G
VDR	FokI	rs2228570 rs107365810	47879112	Exon 3 (coding)	c.2T>C
VDR	BsmI	rs1544410	47846052	Intron 9	c.1024+283G>T
VDR	ApaI	Rs7975232	47845054	Intron 9	c.1025-49G>T
VDR	TaqI	Rs731236	47844974	Exon 10 (coding)	c.1056T>C

SNP = Single-nucleotide polymorphism, VDR = vitamin D receptor

Table 2. List of designed primers

BMR* primer code	Primer name	Primer sequence
7394	FokI rs2228570 Fw	CCAGCTATGTAGGGCGAATCA
7395	FokI rs2228570 Rev	TCTCCCTCCCTTTCCACTGG
7396	BsmI rs1544410 Fw	GAGCGGGGAGTATGAAGGAC
7397	BsmI rs1544410 Rev	GGACCTCATCACCGACATCA
7398	ApaI rs7975232 Fw	GGATGGACAGAGCATGGACA
7399	ApaI rs7975232 Rev	AGTCAGGAGATCTCATTGCCA
7400	A-1012G rs4516035 Fw	GAGTTGTGAGGGGCTGGTTA
7401	A-1012G rs4516035 Rev	CTGGAATTGTGGATGGCTGC

*BMR Genomics SrL (https://www.bmr-genomics.it/), Fw = forward, Rev = reverse

fields were randomly chosen, and the average percentage of keratinocytes with positive staining was then calculated for each group. Level of expression was classified according to the rate of positivity of the specimen in five groups: 0 Arbitrary Unit (AU) (0-19% positive cells); 1 AU (20-39% positive cells), 2 AU (40-59% positive cells), 3 AU (60-79% positive cells) and 4 AU (80-100% positive cells).

Statistical Analysis

Data are presented as mean and standard deviation for continuous variables or number and percent for categorical variables/frequencies. Multivariate logistic regression was used to explore the association between various VDR polymorphisms (dependent variables) or TJ proteins (dependent variables), and clinical and pathological characteristics of AD patients (independent variables). Localization of AD lesions on the hand was not included in logistic regression, as only 2 cases were available. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using MedCalc Software (Broekstraat, Mariakerke, Belgium).

Results

Patient Characteristics

A total of 43 adult patients (60.5% male) aged 42±19 years with AD were included (Table 3). The majority of patients (N= 38; 88.4%) had a moderate-severe disease, with an EASI ≥16 or <16 with involvement of face or hands. Patients presented most frequently with generalized AD, 18 (41.9%) and age of onset was mainly reported in childhood (28 patients; 65.1%).

Asthma and rhinoconjunctivitis were present in 8 and 14 cases (18.6% and 32.6%), respectively and 5 patients (11.6%) had both asthma and rhinoconjunctivitis. Twenty patients (46.5%) were positive for SPT and total IgE were ≥100 IU/ml in 25 (58.1%) patients. Insufficient levels of vitamin D (25-OH-D; <30 ng/ml) were seen in 28 patients (65.1%).

Sub-Analysis of Patient Clinical Characteristics

A higher proportion of patients with asthma or rhinoconjunctivitis (N=17) were positive for SPT (11/20, 55%) and IgE ≥100 IU/ml (11/25, 44%) compared to those who were negative for SPT (6/23, 26.1%; p=0.05) and IgE <100 IU/ml (4/18, 22%; p=0.049) (Figure 1a-b). In males, flexural lesions were more frequent compared to females (9/26, 34.6% vs. 0/17, 0%; p=0.02) (Figure 1c). Also, lower BMI was associated with IgE ≥100 IU/ml (24±4.1) vs. IgE <100 IU/ml (27.1±4.7; p=0.03) (Figure 1d). Individuals with adult disease onset for AD showed a higher EASI score (25.7±5.8) vs. child/adolescent disease onset (21.3±5.8, p=0.05) and lower vitamin D levels (21±9.8 ng/ml) in patients with generalized and/or hand lesions compared to individuals without generalized and/or hand symptoms (27.6±7.1; p=0.02) (Figure 1e).

Frequency of VDR Gene Polymorphisms in AD Patients

The frequency of different VDR gene polymorphisms according to genotype are summarized in Table 4. The polymorphism A1012G rs4516035 of the promoter region of the VDR, with replacement c.-1172A>G, was found in the wild type in 15 cases (34.9%), while the variants were heterozygous in 23 (53.5%) and homozygous in 5 patients (11.6%). The polymorphism FokI rs2228570 of coding exon 3, with c.2T>C substitution, was wild type in 5 (11.6%), homozygous variated in 21 (48.8%) and heterozygous in 17 cases (39.5%). According to the polymorphism BsmI rs1544410, c.1024+283G>T in intron 9, 18 were homozygous for the reference allele (41.9%), 20 heterozygous (46.5%) and 5 variated homozygous (11.6%). The polymorphism ApaI

Table 3. Clinical characteristics of AD patients (N=43)

Characteristic	N (%)
Gender, n (%)	
Male	17 (39.5)
Female	26 (60.5)
Age	
<60 years	36 (83.7)
≥60 years	7 (16.3)
Age of disease onset, n (%)	
Childhood/adolescent	28 (65.1)
Adulthood*	15 (34.9)
Body Mass Index (BMI)	
<30 kg/m2	35 (81.4)
≥30 kg/m2	8 (18.6)
Phenotype (localisation), n (%)	
Flexural sites	9 (20.9)
Generalised	18 (41.9)
Head/neck	14 (32.6)
Hands	2 (4.7)
EASI score	
Mild (EASI <16)	5 (11.6)
Moderate-to-severe (EASI ≥16 or <16 with face/hand involvement)	38 (88.4)
Asthma, n (%)	
Present	8 (18.6%)
Absent	35 (81.4)
Rhinoconjunctivitis, n (%)	
Present	14 (32.6)
Absent	29 (67.4)
Skin prick test, n (%)	
Positive	20 (46.5)
Negative	23 (53.5)
Total IgE (IU/ml), n (%)	
<100 IU/ml	18 (41.9)
≥100 IU/ml	25 (58.1)
25(OH)D vitamin D	
≥30 ng/ml	15 (34.9)
<30 ng/ml	28 (65.1)

EASI = Eczema Area and Severity Index. *Adult onset of AD was defined as diagnosis of AD at age of 18 years.

rs7975232 c.1025-49G>T, intron 9, has been found in wild type homozygous in 10 cases (23.3%), in heterozygous in 19 (44.2%) and in variated homozygous in 14 (32.6%). TaqI rs731236 in position c.1056T>C on encoding exon 10, was changed to heterozygous in 19 patients (44.2%) and homozygous in 5 (11.6%), while 19 were wild type (44.2%). Polymorphisms detected for each patient are presented in Supplementary Table S1.

Association between VDR Polymorphisms and Clinical Features of AD

Multivariate logistic regression analysis was used to evaluate the association of a range of clinical variables with the five different VDR polymorphisms.

For individuals with A1012G heterozygous status, a significantly lower odds ratio (OR: 0.046, 95% CI: 0.004-0.51, p=0.012) was observed for adolescent disease onset and sex (OR: 0.23, 95% CI: 0.047-1.14, p=0.07); a trend for females to have a higher likelihood of possessing the polymorphism being observed, although this association just failed to reach statistical significance. Individuals with IgE levels >100 IU/ml showed an increased odd (OR: 8.43, 95% CI: 0.98-72.6, p=0.05) of carrying this polymorphism.

For ApaI in homozygosity an association was observed with disease onset, indicating higher odds of developing the disease earlier (OR: 5.99, 95% CI: 0.93-38.59; p=0.059), however, this association just failed to reach statistical significance.

Among samples analyzed, patients with 2 or more homozygotic SNPs were evaluated. We observed that 11 out of 43 (25.6%) patients had a cumulative homozygous (independent variable) and, therefore, a multivariate logistic analysis was performed with each clinical-pathological dependent variable. A statistically significant association was observed among the presence of cumulative homozygous >2 and a SPT positivity (10/20, 50%) vs. negative SPT (1/23, 4.3%; p=0.0003).

Immunohistochemical Analysis of VDR and TJ Proteins from AD Biopsy Samples

From AD biopsy lesions, immunohistochemistry was performed for occludin, claudin-1, and ZO-1, as well as for VDR expression analysis. Our findings revealed that the majority of samples for VDR, occludin, and ZO-1 fell into class 0 (72.1%, 81.4%, 44.2%, respectively) (Figure 2). In contrast, for claudin, class 0 was entirely absent, with class 1 and class 2 being the most frequent, each accounting for 39.5% of the samples. Additionally, for ZO-1, both class 1 and class 2 were well represented, constituting 39.5% and 14.0%, respectively. Regarding VDR, class 1 represented a significant portion at 23.3%. The least common classes were 3 and 4, although class 3 was also well represented for claudin (16.3%). Based on these distributions, it appears that

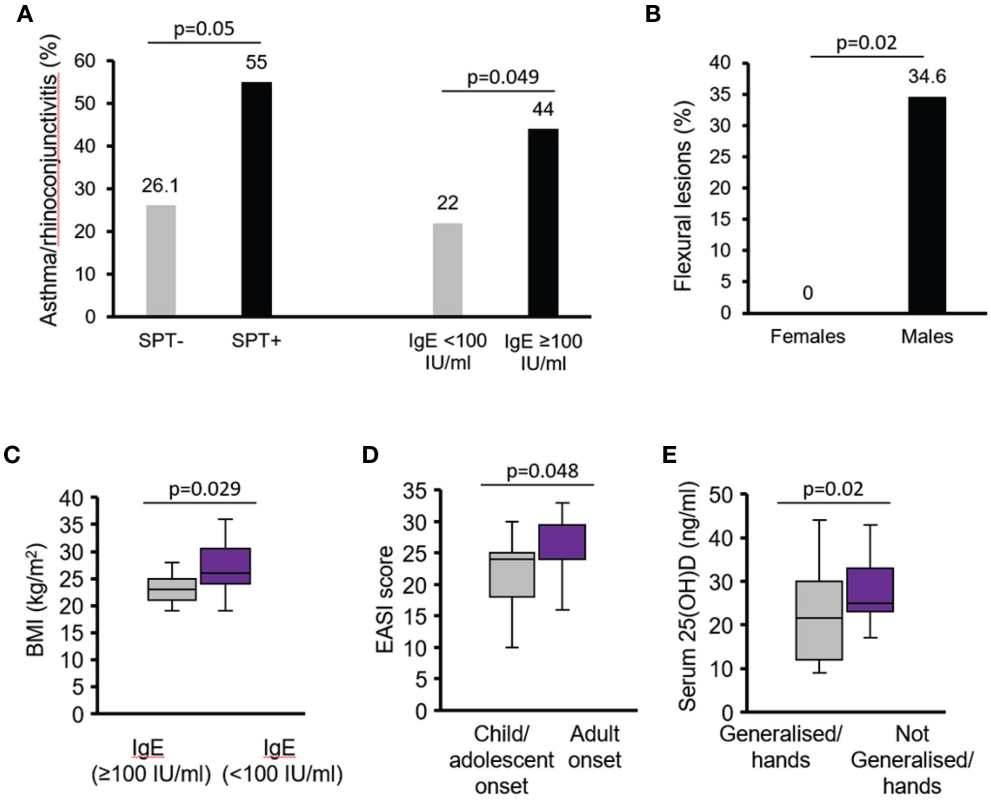

Figure 1. Sub-analysis of AD patient clinical characteristics. A) The proportion of patients with asthma or rhinoconjuntivitis in SPT positive vs. negative groups, B) for asthma or rhinoconjuntivitis in IgE <100 IU/ml vs. IgE ≥100 IU/ml; C) the proportion of male and female patients with flexural AD lesions, D), mean BMI mean in IgE ≥100 IU/ml vs. IgE <100 IU/ml patients, E) EASI score in child/adolescent disease onset vs. patients having adult disease onset, F) serum 25(OH) D levels in patients with generalized and/or hand lesions vs. those without generalized and/or hand lesions. SPT = skin prick test; IgE = immunoglobulin E; BMI = body mass index. P-values represent statistically significant differences between groups. Data are presented as box-whisker plots whereby error bars denote range, boxes denote upper and lower interquartile range and horizontal lines within boxes denote the median value.

occludin exhibits the lowest expression, given its substantial presence in class 0 (81.4%), followed by VDR (72.1%), while claudin and ZO-1, particularly in class 2-3, demonstrate the highest level of expression compared to the other TJ proteins.

Association between VDR and TJs Protein Expression, VDR Polymorphisms, and Clinical Features of AD

Multivariate logistic regression analysis was used to explore the association between VDR/TJ protein expression and VDR polymorphisms. We observed an association between ApaI in the heterozygous state with VDR (OR of 4.9; 95% CI: 0.92-26.25; p=0.063), indicating potential influences, although it just failed to reach statistical significance.

Regarding the association between VDR/TJ protein and clinical parameters, we observed a significant association between ZO-1 expression and BMI ≥30 (OR: 12.1, 95% CI: 1.06-137.9, p=0.045), as well as 25(OH)D levels <30 ng/ml (OR: 10.8, 95% CI: 1.08-108.3, p=0.043). Furthermore, 25(OH)D levels were negatively correlated with ZO-1 expression by univariate regression analysis (rho = -0.43, p=0.0058; Figure 3). Claudin was observed to be associated with a positive SPT (OR: 8.23, 95% CI: 1.04-65.5, p=0.046). Finally, VDR was observed to be associated with generalized AD localization (OR: 0.031, 95% CI: 0.001-1.42, p=0.070), that just failed to reach statistical significance.

Discussion

Findings from this cross-sectional study identify a link between VDR polymorphisms, VDR and TJ protein expression and clinical features in a cohort of patients with AD in Italy. Specifically, we observed a lower OR for AD onset in individuals with the A1012G homozygous VDR polymorphism, while the cumulative homozygous ≥2 VDR polymorphisms was linked to a higher likelihood of developing allergic reactions.

Table 4. Frequency of specific genotypes for different VDR single nucleotide polymorphisms in AD patients.

Polymorphism	Number of cases (%)
rs4516035 A1012G	
Genotype	
AA (wild type)	15 (34.9)
AG (heterozygous)	23 (53.5)
GG (homozygous)	5 (11.6)
rs2228570 FokI	
Genotype	N (%)
TT (wild type)	5 (11.6)
TC (heterozygous)	17 (39.5)
CC (homozygous)	21 (48.8)
rs1544410 BsmI	
Genotype	N (%)
GG (wild type)	18 (41.9)
GT (heterozygous)	20 (46.5)
TT (homozygous)	5 (11.6)
rs7975232 ApaI	
Genotype	N (%)
GG (wild type)	10 (23.3)
GT (heterozygous)	19 (44.2)
TT (homozygous)	14 (32.6)
rs731236 TaqI	
Genotype	N (%)
TT (wild type)	19 (44.2)
TC (heterozygous)	19 (44.2)
CC (homozygous)	5 (11.6)

EASI = Eczema Area and Severity Index. *Adult onset of AD was defined as diagnosis of AD at age of 18 years.

Among TJ proteins evaluated, claudin and ZO-1 were the most highly expressed. VDR protein expression was associated with the presence of generalized AD lesions, whereas claudin demonstrated a significant association with a positive SPT. While earlier studies have investigated differences in VDR polymorphism frequency and levels of VDR and TJ expression between AD patients and healthy controls, the specific aim of this uncontrolled study was to characterize the interrelationships between SNPs, VDR, and TJ protein expression in skin biopsies from this AD cohort and associate them with clinical features of AD patients.

To date, no studies have correlated VDR polymorphisms with the clinical characteristics of AD. The results of our study suggest that VDR polymorphisms may indeed be associated with the clinical features of AD. We found that individuals with A1012G heterozygous status had significantly lower odds of developing AD earlier (OR: 0.046, 95% CI: 0.004-0.510, p=0.012), suggesting a potential protective effect of this polymorphism on the onset of the disease. Richetta et al. reported a lower risk of developing psoriasis when this polymorphism, whether in heterozygosity or homozygosity, was present compared to the wild-type genotype[24].

We also observed that the presence of ApaI in homozygosity exhibited a trend in higher odds (OR of 5.99) of developing the disease earlier, indicating a potential risk-associated with this polymorphism. It is noteworthy that ApaI (rs7975232) is associated with lower levels of expression and decreased stability of VDR mRNA[16], and low levels of vitamin D are associated with AD. Moreover, Heine et al.[19], demonstrated an association between ApaI polymorphism and severe forms of AD.

Our findings showed a statistically significant association among the presence of cumulative homozygous >2 VDR polymorphisms and a positive SPT (10/20, 50%) compared with negative SPT (1/23, 4.3%; p=0.0003). Supporting this observation, it has been reported that low vitamin D status is associated with higher IgE levels in atopic conditions[29,30].

VDR polymorphisms were also correlated with receptor expression in lesional skin biopsies (LS) from our AD patient cohort. We observed a positive association for the ApaI polymorphism when in the heterozygous state with VDR expression. This result is in contrast with others reporting that polymorphisms in ApaI are associated with decreased stability of messenger RNA and lower levels of expression[31,32]. However, another study on Turkish children described a significant link between ApaI in heterozygosity and asthma risk[21]. Nevertheless, there is a lack of functional studies on the association between ApaI polymorphisms and VDR protein expression.

It is known that alterations to TJs are driven by cytokines during the acute phase of inflammation and that in psoriasis TJs' expression is reduced by 50% (as also VDR), compared to healthy skin, granting reason to assume a role of vitamin D in maintaining the barrier epithelia integrity[26].

Among TJ proteins, we observed that claudin and ZO-1 were the most highly expressed in the skin biopsies of lesions from patients with AD, while VDR and occludin were the lowest. No study has reported the relative expression levels for these proteins; only case-control studies are present in the literature. Furthermore, our study revealed a negative correlation between vitamin D and ZO-1 expression (rho=-0.43; p=0.0058). In a study by Yuki et al.[33], TJ protein levels were quantified in the epidermal tissues of three patients with AD and three normal subjects. Skin biopsies were taken from AD nonlesional sites (NLS) and lesional skin sites (LS).

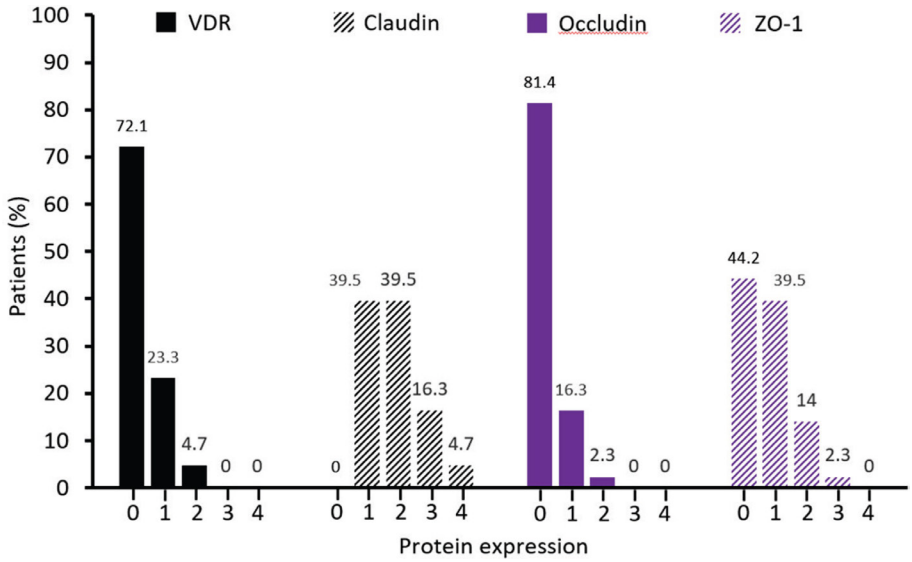

Figure 2. Immunohistochemical protein expression of VDR and tight junctions (TJs) in the 5 classes, 0, 1, 2, 3, 4. Abbreviations. VDR, Vitamin D Receptor; ZO-1, Zonulin-1.

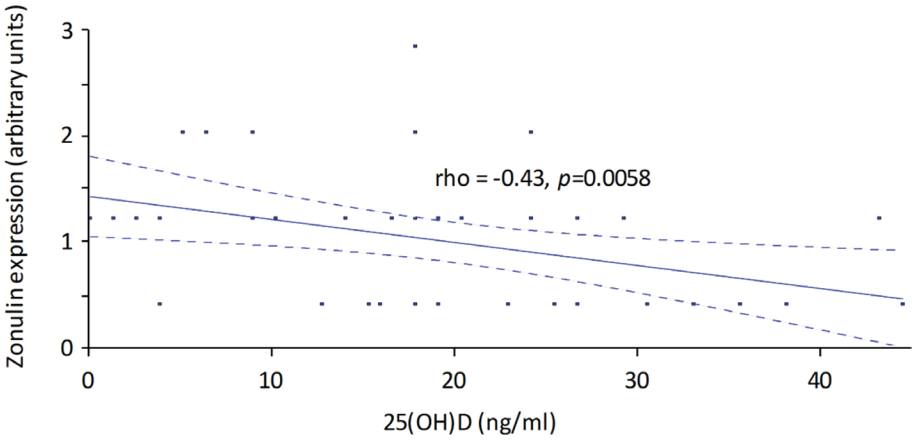

Figure 3. Scatter plot depicting the association between ZO-1 expression and 25(OH)D levels. Solid line represents trend line and dotted line represents 95% confidence interval.

In the NLS of AD, claudin-1, occludin, and ZO-1 proteins occurred similarly to those in normal skin. However, in the LS, the signal intensities of claudin-1 and ZO-1 were markedly reduced. These data appear to be in contrast with our results, even though only three patient samples were examined and our analysis was restricted to LS.

Meckel et al.[34] observed an inverse correlation between serum 25(OH)D concentrations and mucosal inflammation in 230 subjects with ulcerative colitis, along with altered protein expression concentrations of VDR, occludin, and decreased protein expression of ZO-1. These results align with our findings. We also found a positive association between ZO-1 expression and BMI ≥30. Zonulin is considered the sole physiological mediator known to regulate intestinal permeability reversibly by modulating intercellular TJs, and obesity has been associated with increased intestinal permeability and absorption[35].

We also observed a higher level of claudin expression in patients presenting with positive SPT. De Benedetto et al.[36] reported reduced expression of claudin-1 in AD NLS, while Gruber et al.[37] and Yuki et al.[33] found that claudin-1 was upregulated in NLS of AD subjects. Overall, these results suggest a complex and context-dependent role of claudin-1 in AD, influenced by genetic factors and environmental considerations.

Study Limitations

Although the sample size was lower than desired, significant associations were still detected and confirm findings observed elsewhere. While other genotyping studies in

this setting have compared the frequency of VDR polymorphisms to a control population, for ethical reasons (i.e. taking of biopsy samples) was not possible. This was a cross-sectional analysis and therefore temporal effects were not assessed.

Conclusion

In our study in Italian AD patients, we have identified significant associations between VDR polymorphisms, VDR expression, TJ proteins, and clinical characteristics of AD. These findings provide important insights into the complex interplay of genetic factors, vitamin D deficiency, and TJ proteins in AD pathology, emphasizing the multifaceted nature of AD pathophysiology and the identification of potential markers for the early diagnosis of AD.

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