Abstract

Background: Female pattern hair loss (FPHL) is an important cause of hair loss in adult women and has a major impact on patient's quality of life. It evolves from the progressive miniaturization of follicles that leads to a subsequent decrease of hair density, leading to non-scarring diffuse alopecia, with characteristic clinical, dermoscopic, and histological patterns. Vitamin D receptor (VDR) is expressed in follicular keratinocytes and dermal papilla cells and is shown to have important role in hair growth and regulation of hair cycle. VDR polymorphism was not extensively investigated in hair disorders including FPHL.

Aim: To investigate the association between VDR gene polymorphism (Cdx-1 and Taq-1) and FPHL to explore if these polymorphisms affect the disease occurrence or influence its clinical presentation.

Methods: A case-control study was conducted on 30 female patients with FPHL and 30 age-matched female healthy subjects, as a control group. Degree of hair loss was assessed by Ludwig grading. VDR gene polymorphisms, Taq-1 and Cdx-1 were investigated by real time polymerase chain reaction.

Results: CC genotype, TC genotype, and T allele of Taq-1 were more prevalent in FPHL patients than in control group. They increased disease risk by 12.6, 2.1, and 2.9 folds, respectively. AA genotype, GA genotype, and G allele of Cdx-1 were significantly more prevalent among FPHL patients than in control group. They increased disease risk by 7.5, 5.2, and 5.5 folds, respectively. Conclusion: Taq-1 and Cdx-1 can be considered as risk factors for FPHL. They may play role in disease persistence rather than disease initiation. This association may be explained by failure of new anagen growth and decreased proliferation of hair follicle stem cells. Further studies are recommended to confirm current findings.

Key Words: Female pattern hair loss, gene, polymorphism, vitamin D receptor

Introduction

Androgenic alopecia or pattern alopecia is one of the common chronic problems seen by dermatologists worldwide. It has distinctive pattern of hair loss in women in comparison to that in men.\(^1\)

Although the follicular changes that lead to alopecia are similar between men and women, clinical presentation and response to anti-androgen therapy are different and the participation of androgens in the development of female androgenic alopecia has not yet been fully elucidated. Therefore, the term female pattern hair loss (FPHL) has been preferred over female androgenic alopecia.\(^2\)

The disease is characterized clinically by increased hair thinning over the frontal/parietal scalp with greater hair density over the occipital scalp, retention of the frontal hairline and presence of miniaturized hair.\(^1\)

Vitamin D is a steroid hormone that is synthesized in epidermal keratinocytes under influence of UVB light (290–315 nm) or acquired from diet and dietary supplements. Vitamin D needs both 25- and 1-α hydroxylation to become an active hormone – 1, 25-dihydroxyvitamin D. It is estimated that approximately 3% of human genome is regulated directly or indirectly by vitamin D endocrine system.\(^4\)

Vitamin D exerts its effects through high affinity binding to a corresponding nuclear receptor, vitamin D receptor (VDR), in target tissues. This binding induces conformational changes of VDR that lead to heterodimerization with retinoid X receptor (RXR) and to zinc finger mediated binding to vitamin D response elements (VDREs) that are located in regulatory regions of target genes.\(^5\)

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Vitamin D receptor can upregulate or downregulate gene transcription in both a 1,25-dihydroxyvitamin D dependent and a 1,25-dihydroxyvitamin D independent fashion.\[6\]

Vitamin D receptor is expressed in two major cell populations that make up the hair follicle - the mesodermal dermal papilla cells and the epidermal keratinocytes. VDR expression in hair follicle is increased during late anagen and catagen, correlating with decreased proliferation and increased differentiation of keratinocytes.\[7\]

Previous studies showed that VDR is essential for initiation of the first post-natal hair cycle,\[8\] for the start of new anagen\[9\] and for regulating the growth cycle of mature hair follicles. Certain mutations in the VDR lead to mis-regulated gene expression resulting in aberrant hair follicle cycling and alopecia.\[10\]

In spite of these important functions, the role of VDR polymorphism or mutations was not extensively studied in different hair disorders. Akar et al.,\[11\] reported lack of association between VDR gene polymorphisms (Bsm1, Apa1, and Taq-1) and alopecia areata in Turkish Caucasians. No previous studies were done to investigate such association in FPHL.

The human VDR gene is located on chromosome 12q13.11 and is composed of 14 exons spanning ~64 kbp of DNA. The human VDR protein contains either 427 or 424 amino acids depending upon the presence of a T to C polymorphism (ATG to ACG) in a translational start site.\[12\]

Single-nucleotide polymorphisms (SNPs) in the VDR gene include FokI C>T (rs228570), BsmI A>G (rs1544410), ApaI G>T (rs7975232), Taq-1 C>T (rs731236), Cdx-1 (rs731236), and Cdx-2 (rs11568820).\[13\] The Taq-1 polymorphism is a T/C nucleotide substitution (ATT to ATC) leading to a synonymous change at codon 352 (isoleucine) in exon IX.\[14\] Cdx-1 polymorphism is A/G, transition substitution in Cdx-1 binding site on VDR gene.\[15\]

The current study aim was to investigate the association between Cdx-1 and Taq-1 VDR gene polymorphism and FPHL to explore the association between the polymorphisms and the risk or clinical presentation of the disease.

Materials and Methods

Study population

This case-control study was conducted on 30 patients with FPHL and 30 age-matched healthy female volunteers who have no present, past, or family history of FPHL as a control group.

Cases were selected from the Dermatology outpatient clinic at Menoufiya University Hospital during the period from April 2016 to October 2016. Control subjects were selected from the healthy hospital staff.

Ethics

A written informed consent form approved by the Ethical Committee of Menoufiya Faculty of Medicine was signed by every participant before the study initiation. This was in accordance with Helsinki declaration of 1975 (revised in 2000).

Exclusion criteria

Any case or control subject with any systemic and/or scalp disease that might be related to hair loss, on medication that could be related to hair loss (e.g., anticoagulants, retinoids, anticonvulsants, and antidepressants) or receiving drugs containing vitamin D or dietary supplements was excluded. Subjects suffering from recent stressful events (i.e., within the last 6 months) like psychological stress, surgical trauma, high fever, chronic systemic illness, hemorrhage, emotional stress, or pregnancy were excluded. Menopausal females and patients complaining of other skin diseases were also excluded.

Diagnosis was made based on clinical findings and dermoscopic examination.

All studied patients were subjected to complete history taking, general and dermatological examinations. Clinical data describing patients’ age, disease duration, and family history of FPHL were collected. Cases were clinically assessed according to Ludwig.\[14\]

Genetic study

Every case or control subject underwent detection of VDR gene polymorphism, SNP genotyping, for Taq-1 rs731236 and Cdx-1 rs3776082 by real time PCR.

Genomic DNA was extracted from frozen EDTA-treated blood sample using Gene JET™ Whole Blood Genomic DNA Purification Mini Kit (THERMO SCIENTIFIC, EU/Lithuania).

The DNA extract was used for SNP assay in a total reaction volume of 20 µl with 10 µl of TaqMan Genotyping Master Mix, 1.25 µl of 20× TaqMan genotyping assay kits containing both primers and probes and nuclease-free water.

By using the 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA), the reaction steps were as follows: 50°C for 2 min Pre-PCR read, then 95°C for 10 min and 40 cycles of 95°C for 15 s, 60°C for 1 min (cycling), and 60°C for 1 min (post-PCR).

The probe sequence for Taq-1 SNP was rs731236: TGGACACGGCGGTCCTGGATGGCCCTC[T/C]ATCACCGCGGCG TCTGACCCAG.

The probe sequence for Cdx-1 SNP rs3776082 was GGCCCCTTACAGACACTTTCTAC[A/G]TATGGTGGGCTGAAA TCCACATCT.
**Statistical analysis**

Data were collected, tabulated, and statistically analyzed using a personal computer with “SPSS version 11” program. Fisher’s exact test was used for comparison of qualitative variables in $2 \times 2$ tables when expected cell count of more than 25% of cases was less than 5. Chi-square test ($\chi^2$) was used to study the association between two qualitative normally distributed variables. Mann–Whitney $U$-test was used for comparison between two groups not normally distributed having quantitative variables. Odds ratio was used to describe the probability that people who are exposed to a certain factor will have a disease compared to people who are not exposed to the factor. Differences were considered statistically significant with $P < 0.05$.

**Results**

Clinical data of studied cases are summarized in Table 1.

Patient ages ranged from 21 to 44 years with a mean ± SD of 31.53 ± 7.88 years. Control group ages ranged from 19 to 44 years with a mean ± SD of 29.93 ± 6.60 years.

**Taq-1 genotypes and alleles in cases and controls**

TT genotype was more prevalent among control group than FPHL patients (60% vs. 33.3%). TC genotype was significantly more prevalent in FPHL patients than control group (23.3% vs. 3.3%) ($P = 0.03$). It increased disease risk by 2.1 folds. CC genotype was present in 43.3% of cases and 36.7% of controls. It increased the risk of FPHL by 12.6 folds [Table 2].

T allele was more prevalent among control than patient group (80% vs. 55%). C allele was significantly associated with patient group (45% vs. 20%) ($P = 0.007$). It increased the risk of occurrence by 2.9 folds [Table 2].

**Cdx-1 genotypes and alleles in cases and controls**

GG genotype was more prevalent among control group than FPHL patients (90% vs. 60%). AA genotype was significantly more prevalent among FPHL patients than control group (16.7% vs. 3.1%) ($P = 0.03$). It increased disease risk by 7.5 folds. GA genotype was present in about 23.3% of cases and about 6.7% of controls. It increased the risk of FPHL by 5.2 folds [Table 2].

G allele was more prevalent among control than patient group (93.3% vs. 75%). A allele was significantly associated with patient group (25% vs. 6.7%) ($P = 0.002$). It increased the risk of occurrence by 5.5 folds [Table 2].

| Variable                                    | No. | Percentage |
|----------------------------------------------|-----|------------|
| Family history                              |     |            |
| Absent                                       | 15  | 50.0       |
| Present                                      | 15  | 50.0       |
| Age of onset (years)                         |     |            |
| Min.-Max.                                    | 18.0-42.0 |
| Means±SD.                                    | 29.30±7.42 |
| Median                                       | 26.50  |
| Duration of hair loss (months)               |     |            |
| Min.-Max.                                    | 8.0-48.0 |
| Means±SD.                                    | 27.07±13.24 |
| Median                                       | 24.0  |
| Bitemporal recession                         |     |            |
| Absent                                       | 29  | 96.7       |
| Present                                      | 1   | 3.3        |
| Miniaturized hair in affected area           | 30  | 100.0      |
| Hair pull test                               |     |            |
| Negative                                     | 30  | 100.0      |
| Positive                                     | 0   | 0.0        |
| Ludwig grading                               |     |            |
| I                                            | 13  | 43.3       |
| II                                           | 12  | 40.0       |
| III                                          | 5   | 16.7       |

**Relationship between the Taq-1 genotypes and alleles and different parameters in cases**

There was statistically significant association between CC genotype and C allele of Taq-1 and grade I hair loss ($P = 0.003$, 0.004, respectively) [Figure 1].

**Relationship between the Cdx-1 genotypes and alleles and different parameters in cases**

There was statistically significant association between GA genotype of Cdx-1 and older age and older age of onset ($P = 0.03$, 0.01, respectively) [Figure 2].

There was statistically significant association between A allele and younger age of onset and grade I hair loss ($P = 0.03$, 0.02, respectively) [Figure 2].

**Discussion**

In the current work, CC genotype, TC genotype and T allele of Taq-1 were more prevalent in FPHL patients than in control group. They increased disease risk by 12.6, 2.1, and 2.9 folds respectively. AA genotype, GA genotype, and G allele of Cdx-1 were significantly more prevalent among FPHL patients than control group. They increased disease risk by 7.5, 5.2, and 5.5 folds, respectively.

Therefore, VDR gene polymorphisms Taq-1 and Cdx-1 may be considered as risk factors for FPHL in Egyptian patients.
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Taq‑1 polymorphism, located near the 3' UTR of the VDR gene, does not alter the protein’s amino acid sequence, and it remains difficult to explain how this variant might influence VDR function. However, even if it does not have a direct action, it can be in linkage with other gene polymorphisms and act as marker of other sequences in the VDR gene that regulate transcription, translation, or RNA processing.

It was reported that the 3' UTR is known to be involved in regulation of gene expression, especially through regulation of mRNA stability, and protein translation efficiency.[18] It was also postulated that VDR polymorphisms could be functional themselves and affect the transcriptional activity of the VDR.[19]

As VDR is important in anagen initiation, we can postulate that VDR polymorphism Taq‑1 may be involved in FPHL through prevention of new anagen growth due to its effect on RNA stability and/or protein translation efficiency - a conclusion that needs further investigation to be proved or denied.

Table 2: Distribution of Taq‑1 and Cdx‑1 genotypes and alleles in cases and controls

| Variable | Case (n=30) | Control (n=30) | χ² (P) | OR | 95% C.I |
|----------|-------------|---------------|--------|----|---------|
| Taq‑1 genotypes | No. | Percentage | No. | Percentage |     |        |        |
| TT       | 10   | 33.3     | 18   | 60.0     | 6.8 | -      | -      |
| TC       | 13   | 43.3     | 11   | 36.7     | 2.1 | 0.698-6.485 |
| CC       | 7    | 23.3     | 1    | 3.3      | 12.6 | 1.35-117.57 |
| Taq‑1 alleles |     |          |     |          |     |        |        |
| T        | 33   | 55.0     | 47   | 80.0     | 7.3 | -      | -      |
| C        | 27   | 45.0     | 13   | 20.0     | 2.1 | 0.698-6.485 |
| Cdx‑1 genotypes | No. | Percentage | No. | Percentage | χ² (P) | OR | 95% C.I |
| GG       | 18   | 60.0     | 27   | 90.0     | 6.9 | -      | -      |
| GA       | 7    | 23.3     | 2    | 6.7      | 2.1 | 0.698-6.485 |
| AA       | 5    | 16.7     | 1    | 3.1      | 12.6 | 1.35-117.57 |
| Cdx‑1 alleles |     |          |     |          |     |        |        |
| G        | 43   | 75.0     | 56   | 93.3     | 9.7 | -      | -      |
| A        | 17   | 25.0     | 4    | 6.7      | 2.1 | 0.698-6.485 |

OR: Odds ratio; C.I: confidence interval, *: Statistically significant

Figure 1: (a) Significant association between CC genotype of Taq‑1 and grade I hair loss (P = 0.003). (b) Significant association between C allele of Taq‑1 and grade I hair loss (P = 0.004)

It has been hypothesized that the role of the VDR in the hair cycle is to repress the expression of gene(s) in a ligand-independent manner.[12]

Mutations in the VDR that disrupt the ability of the unliganded VDR to suppress gene transcription are hypothesized to lead to the derepression of gene(s) whose product, when expressed inappropriately, disrupts the hair cycle that ultimately leads to alopecia. Potential candidates include inhibitors of the Wnt signaling pathway.[20] Cdx‑1 is a downstream target of the Wnt signaling pathway[21] and Wnt signaling in gut requires the binding of the Tcf4/β‑catenin complex to the Cdx‑1 gene promoter, which induces the expression of Cdx‑1.[22] Scientists have found strong evidence that Wnt signaling is a crucial component in the maintenance of hair follicle stem cells (HFSCs).[23]

The finding overturns the previous assumption that Wnt signaling has a negligible impact on the maintenance of HFSCs, and is only critical in activating their proliferation for hair growth.[24]
Unlike most other tissue stem cells that are dependent on sustaining signals from adjacent niche stem cells, HFSCs can maintain their stemness and growth potential by producing their own Wnt signals. Disrupting Wnt signaling caused the HFSCs to lose their stemness, with the hair follicle arrested in the telogen phase and no growth observed. This mechanism of self-maintenance is relatively novel in the field of stem cells.\(^{[25]}\)

In AGA, the follicle miniaturization is accompanied by a decrease of anagen, with an increase in the percentage of resting (telogen) hair follicles containing microscopic hair in bald scalp.\(^{[26]}\) In balding scalp, the number of HFSCs remains intact, whereas the number of more actively proliferating progenitor cells markedly decreases. This suggests that balding scalp either lacks an activator or has an inhibitor of hair follicle growth.\(^{[27]}\)

Therefore, based on above mentioned facts, we can assume that Cdx-1 polymorphism may lead to loss of VDR inhibitory action on inhibitors of Wnt signaling which adversely affect HFSCs proliferative capacity and may share in FPHL pathogenesis.

From the current results we can assume that, Taq-1 and Cdx-1 are not involved in FPHL initiation but rather in its maintenance and persistence.

In the present work, CC genotype and C allele of Taq-1 and AA genotype and A allele of Cdx-1 were significantly associated with Ludwig grade I hair loss. This finding was not reported before and needs further research to be clarified. However, we can postulate that these alleles do not play a role in FPHL severity, but rather in disease maintenance.

The significant association between GA genotype and G allele of Cdx-1 with older age and older age of onset, detected in the present study, and the lack of association between Taq-1 genotypes or alleles and patient age may give evidence that old aged FPHL cases may have Cdx-1 polymorphism rather than Taq-1 polymorphism; a suggestion that needs further investigation.

**Conclusion**

Taq-1 and Cdx-1 may be considered as risk factors for FPHL. They may play role in disease persistence rather than disease initiation. This association may be explained by failure of new anagen growth and decrease proliferative effect of HFSCs. These findings might open a new era for gene therapy in FPHL. Further studies are recommended to prove or deny current findings. The effect of other types of VDR polymorphisms on FPHL should be investigated as well as the interplay between VDR polymorphisms and polymorphisms of other genes involved in FPHL pathogenesis for better understanding of this fairly common hair disorder.

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**Conflicts of interest**

There are no conflicts of interest.

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