



Comprehensive transcriptome profiling between balding and non-balding scalp of female pattern hair loss in Asian

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Abstract

While many gene expression studies have focused on male pattern baldness (MPB), few studies have investigated the genetic differences between bald and non-bald hair follicles in female pattern hair loss (FPHL). This study aimed to identify molecular biomarkers associated with FPHL through genetic analysis of paired bald and non-bald hair follicles from 18 FPHL patients, using next-generation sequencing (NGS) techniques. RNA transcriptome analysis was performed to identify differentially expressed genes (DEGs) between bald and non-bald hair follicles in FPHL. The DEGs were validated using real-time PCR, and protein expression was confirmed through immunohistochemistry and western blot analysis. Our findings suggest that HOXB13, SFRP2, PTGDS, CXCR3, SFRP4, SOD3, and DCN are significantly upregulated in bald hair follicles compared to non-bald hair follicles in FPHL. SFRP2 and PTGDS were found to be consistently highly expressed in bald hair follicles in all 18 samples. Additionally, elevated protein levels of SFRP2 and PTGDS were confirmed through western blot and immunohistochemical analysis. Our study identified SFRP2 and PTGDS as potential biomarkers for FPHL and suggests that they may play a role in inducing hair loss in this condition. These findings provide a foundation for further research on the pathogenesis of FPHL and potential therapeutic targets.

Keywords Female pattern hair loss · Balding and non-balding · DEGs · SFRP2 · PTGDS

Introduction

Female pattern hair loss (FPHL) is a prevalent hair loss disorder in women characterized by hair thinning from the mid-scalp to the vertex without the regression of the frontal

hairline [6]. While FPHL differs from male pattern baldness (MPB) in its clinical features, hair follicle miniaturization is a common histological characteristic observed in both conditions. Androgens have been identified as the primary cause of hair loss in MPB [20], with dihydrotestosterone (DHT) resulting from testosterone conversion causing hair follicles to miniaturize [8, 13, 21]. However, the role of androgens in inducing hair loss in women has not been fully elucidated [3, 12, 14].

Although gene expression profiling studies to identify the genetic etiology of hair loss have largely focused on MPB, few genetic studies have been conducted to compare bald and non-bald hair follicles in FPHL. Previous research on FPHL has typically focused on androgenic factors and aromatase due to the established role of the androgen-dependent pathway in the etiology of MPB, with the assumption that FPHL shares the same underlying mechanism [17, 18, 22]. However, FPHL is influenced by multiple factors, including hormones, environmental factors, and genetics. Recent genome-wide association studies (GWAS) of FPHL have not found significant candidate genetic variants shared

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with MPB, highlighting the need to explore specific genetic biomarkers for FPHL [15, 16, 22].

This study aims to investigate critical genetic biomarkers for FPHL by conducting a whole-transcriptomic analysis of bald and non-bald hair follicles using high-throughput sequencing. Significant differentially expressed genes (DEGs) between balding and non-balding hair follicles in FPHL will be confirmed using real-time PCR and Western blotting.

Prostaglandins are highly expressed in male bald scalps and are associated with alopecia and miniaturization, although this has not been studied in FPHL. SFRP2 is functional role in regulating hair growth is controversial, as previous reports indicate high expression in the catagen phase until telogen in mouse hair follicles and highly expressed in vertex scalp in AGA1, while in *in vitro* study shows that it induces trichogenicity.

Our findings suggest that SFRP2 and PTGDS are significantly upregulated in bald hair follicles compared to non-bald hair follicles in FPHL. SFRP2 and PTGDS were found to be consistently highly expressed in bald hair follicles in all 18 samples.

Materials and methods

Acquisition of scalp tissue

This study involved 18 women diagnosed with FPHL according to the Ludwig classification. Human scalp tissues were collected from the mid-scalp or vertex scalp area with miniaturized hairs, as well as from the occiput area with normal terminal hair follicles in the same participant. The Institutional Review Board of Dankook University Hospital approved this study (IRB No. DKUH 2015-02-014).

RNA isolation and sequencing with NGS technique

Total RNA was extracted from balding and non-balding hair follicles of 18 female individuals using Trizol reagent, and RNA quality was assessed by analyzing rRNA band integrity using an Agilent RNA 6000 Nano kit (Agilent Technologies, CA). mRNA sequencing libraries were constructed by enriching mRNA with oligo-dT attached to magnetic beads from total RNA and fragmenting it into short sizes. The fragmented mRNA was reverse-transcribed to cDNA, modified by end-repair, poly-A addition, and linked with 5' and 3' adaptors on both ends using the TruSeq RNA Sample Prep Kit (Illumina, CA) according to the manufacturer's instructions. The modified mRNA fragments were separated on a BluePippin 2% agarose gel cassette (Sage Science, MA), and the suitable fragments were amplified

as PCR templates for enrichment. The final products were quantified by an Agilent High Sensitivity DNA Kit (Agilent Technologies, CA) on an Agilent Bioanalyzer 2100 system. High-throughput RNA sequencing was then performed on an Illumina HiSeq2500 platform (Illumina, CA). All processes were carried out by the Theragen Bio Institute (Suwon, Korea).

Mapping and assembly of transcriptome data

To ensure exact and high-quality analysis, the total output reads underwent quality control and filtering for sequence quality score, GC content, and duplicated sequences before mapping and assembly. Raw quality-reads were those with more than 10% skipped bases, those including more than 40% of bases with less than Q20, and those with average quality scores less than Q20. The entire filtering process was performed using in-house scripts, and this step resulted in clean reads that were mapped to the human reference genome [7] using the STAR aligner v.2.3.0e [4]. The expression level of each gene was measured based on fragments per kilobase of exon per million mapped reads (FPKM), calculated by Cufflinks v2.1.1 [19] from the gene annotation database of Ensembl release 72.

Quantitative real-time PCR

One microgram of total RNA was used to synthesize cDNA following the M-MLV reverse transcription protocol (Promega, Madison, WI, USA). The mRNA expression levels of HOXB13, SFRP2, PTGDS, CXCR3, SFRP4, SOD3, and DCN were quantified by real-time RT-PCR using the Eco Real-time PCR System with Illumina. All reactions were performed in triplicate and analyzed by the delta-delta Ct method. The primer sequences used in this study are listed in Table 1, which were designed using the Primer3 software (V. 0.4.0).

Western blots for hair follicle

Two additional pairs of bald and non-bald HF samples were collected for Western blot analysis. The HF samples were homogenized using a lysis buffer containing 1mM PMSF and protease inhibitor, and the resulting lysates were centrifuged. The supernatants were collected, and the protein concentration was measured. The primary antibodies used were against SFRP2 (Abcam), PTGDS (Thermo Fisher scientific), and β -actin (Santacruz).

Immunohistochemistry in HF

Two additional pairs of bald and non-bald HF samples were collected for immunohistochemistry. The HF samples were embedded in paraffin, and 10- μ m-thick histological sections were produced. The HF slides were incubated overnight at 4 °C with primary antibodies against SFRP2 or PTGDS. The following day, the slides were incubated with the secondary antibody for 1 h at room temperature. After removing the secondary antibody, hematoxylin staining was performed for nucleus staining. The stained HF were observed under a microscope (Olympus CX35, Tokyo, Japan).

Statistical analysis

Data are presented as mean \pm standard deviation (SD) and were analyzed using the Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

Result

Demographics

A total of 18 female patients with FPHL were included in this study, of whom 10 were under 50 years old (pre-menopause) and 8 were over 50 years old (menopause). The Ludwig classification revealed that 11 FPHL cases were L1, 5 were L2, and 1 was L3 type.

Differential gene expression analysis between bald and non-bald HF

To identify the differentially expressed genes (DEGs) between bald and non-bald HF, we considered a fold change (FC) of ≥ 2 and a corrected *p*-value ≤ 0.05 as a cut-off value. We identified a total of 360 DEGs, among which 345 were up-regulated and 15 were down-regulated in bald HF. Figure 1 shows the hierarchical clustering of the differentially expressed genes. To further investigate FPHL-specific genes' regulation, we selected hair growth-related genes from the 345 up-regulated genes based on previous reports. As a result, HOXB13, SFRP2, PTGDS, CXCR3, SFPR4, SOD3, and DCN were significantly upregulated in bald HF compared to non-bald HF. Individual DEG analysis showed that HOXB13, SFRP2, PTGDS, CXCR3, SFPR4, SOD3, and DCN expression levels were elevated in the balding area compared to the non-balding area in all 18 samples.

Validation of DEG expression levels by real-time PCR

To validate the expression levels of the selected genes (HOXB13, SFRP2, PTGDS, CXCR3, SFRP4, SOD3, and DCN), we performed real-time PCR on the 18 pairs of HF samples used for RNA sequencing. The results showed that the expression levels of these seven genes were significantly up-regulated in bald HF in real-time PCR analysis, consistent with NGS results (Fig. 2). Moreover, individual mRNA expression of these seven genes was analyzed in all 18 pairs of samples. The relative mRNA expression levels of HOXB13, SFRP2, PTGDS, CXCR3, SFPR4, SOD3, and DCN were higher in the balding area than in the non-balding area in 17, 18, 18, 15, 9, 11, and 17 samples, respectively, out of the total 18 FPHL samples (Table 1). In the RNA sequencing data, the FPKM values of HOXB13, SFRP2, PTGDS, CXCR3, SFPR4, SOD3, and DCN were higher in the balding area of all 18 samples.

Protein expression of SFRP2 and PTGDS in bald and non-bald scalp

Next, we analyzed the protein expression levels of SFRP2 and PTGDS, which were highly expressed in the mRNA of all 18 bald samples. First, immunohistochemistry staining showed that SFRP2 and PTGDS were strongly expressed in bald scalp than in non-bald scalp, particularly around the epidermis, including the hair shaft, rather than the dermis (Fig. 3A). Second, western blot analysis revealed that SFRP2 and PTGDS were up-regulated in the balding area compared to the non-balding area, consistent with the tissue staining results (Fig. 3B).

Discussion

Although MPB and FPHL share many common characteristics, their exact patho-mechanisms are different. The important genetic biomarkers such as androgen, 5 alpha reductase in MPB have not been validated in FPHL, highlighting the need to investigate the different genetic expression between balding and non-balding areas of FPHL. This study presents the first whole transcriptomic analysis of the balding and non-balding area of FPHL in Asians.

Differential gene expression (DEG) was screened using high output RNA sequencing based on NGS technology, which is a useful technique for investigating DEG between normal and pathological states quickly and widely. However, NGS results often need to be validated by real-time PCR. In this study, HOXB13, SFRP2, PTGDS, CXCR3, SFRP4, SOD3, and DCN were selected as important biomarkers in the DEG analysis using RNA sequencing by

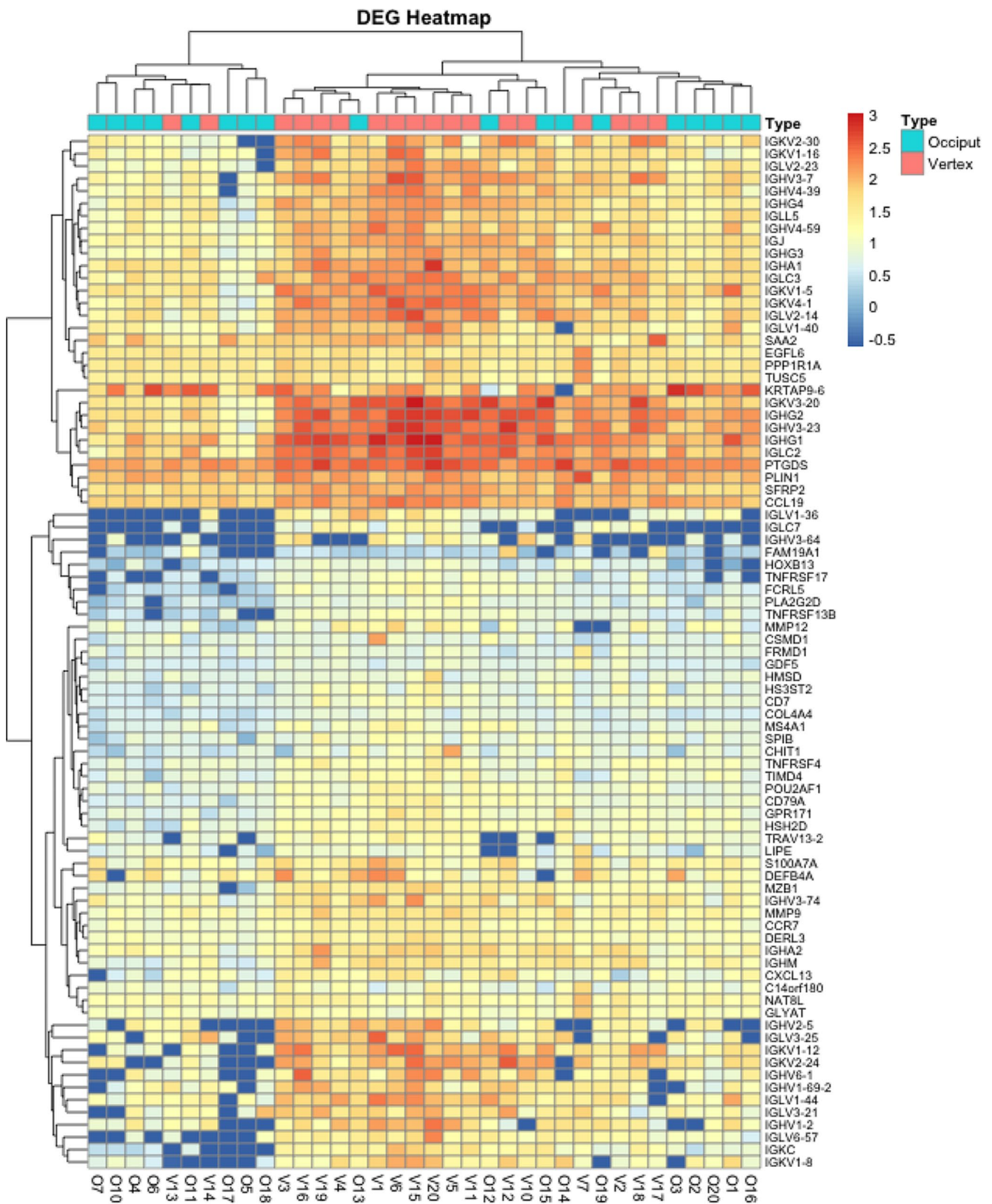


Fig. 1 Transcriptome profiling expression of FPHL hair follicle. Heatmaps showing expression values (TPM) in the non-balding (occiput) and balding (vertex) hair follicle. Fold change count of up- and down-regulated contigs. (Fold Change $\geq |4|$; FDR = 0.05)

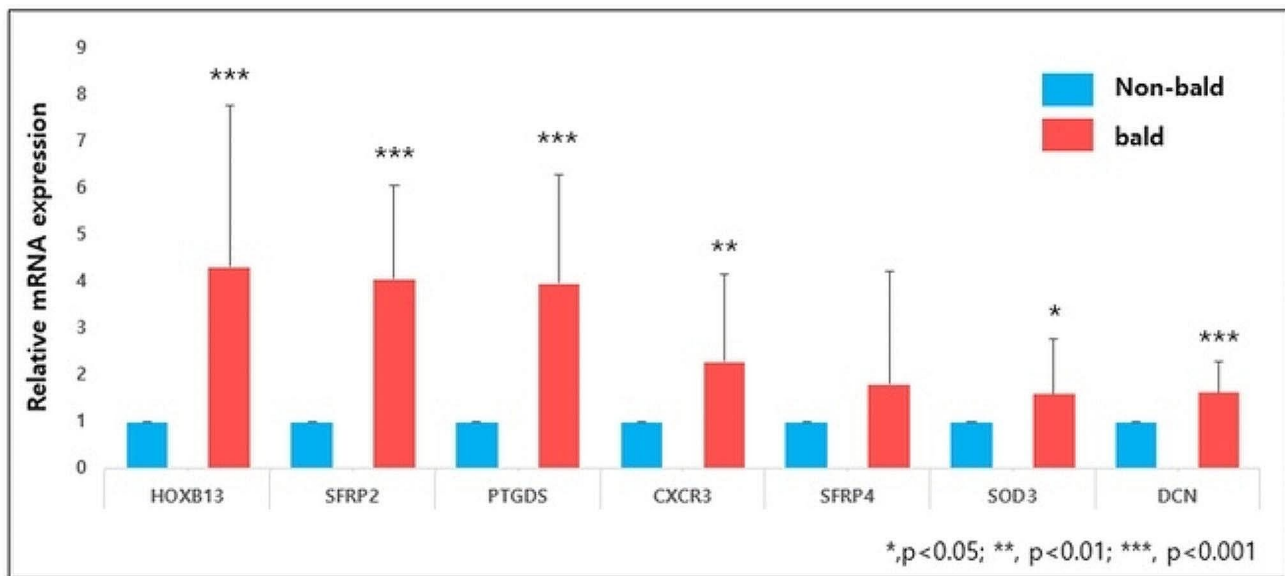
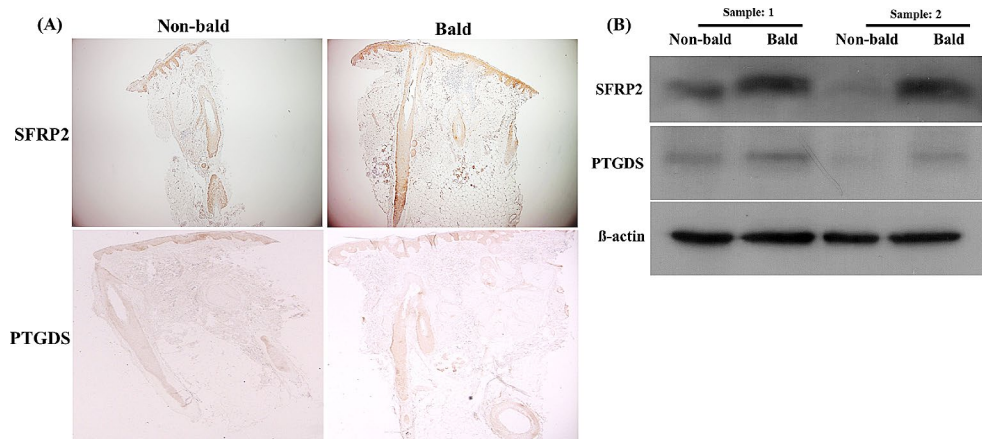


Fig. 2 Validation of 7 differentially expressed genes by quantitative real-time PCR. Seven genes were up-regulated in the balding HF statistically significance, except for SFRP4 (* p -value < 0.05, ** p -value < 0.01, and *** p -value < 0.001)

Table 1 Balding and non-balding Individual expression of 7 DEG genes in paired 18 FPHL samples (Count of higher sample in bald /Total sample)

HOXB13	SFRP2	PTGDS	CXCR3	SFRP4	SOD3	DCN
17/18	18/18	18/18	15/18	9/18	11/18	17/18

Fig. 3 Protein expression of SFRP2 and PTGDS in balding and non-balding HF of FPHL scalp. Immunohistochemical staining (A) and western blot analysis (B) identified highly expression both SFRP2 and PTGDS in bald than in non-bald HF



NGS technology. The mRNA expression of these genes was verified by real-time PCR, and the RNA expression patterns of these genes between the balding and non-balding areas were consistent with the real-time PCR analysis. However, individual analysis of mRNA expression by real-time PCR showed that only SFRP2 and PTGDS were significantly higher expressed in balding hair follicles (HFs) of all 18 FPHL samples, which was consistent with the RNA sequencing analysis. Although the individual pattern of mRNA expression in real-time PCR between the lesion and control was generally similar to the FPKM value in RNA sequencing analysis, the data was not identical and requires validation.

Based on the RNA sequencing data and real-time PCR results, the protein expression pattern of SFRP2 and PTGDS in the hair follicles of FPHL was investigated. SFRP2 is a representative Wnt inhibitor that plays an important role in various cellular regeneration and differentiation. Its functional role in regulating hair growth is controversial, as previous reports indicate high expression in the catagen phase until telogen in mouse hair follicles and highly expressed in vertex scalp in AGA [9, 11], while in in vitro study shows that it is induce trichogenicity [10]. Considering our results, it can be known that SFRP2 is significantly upregulated in both M-AGA and FPHL vertex scalp although the

functional role of SFRP2 in regulating hair growth still should be studied.

Prostaglandins are highly expressed in male bald scalps and are associated with alopecia and miniaturization [1], although this has not been studied in FPHL. In our study, PTGDS mRNA and PGD2 protein were elevated in bald compared to haired scalp, which is consistent with previous reports implicating the prostaglandin pathway in the pathogenesis of AGA in both males and females. Although PGE2 and PGF2a have been reported to be more generated in female than male scalps [2], contributing to hair growth induction, they were not identified in the DEGs analysis between balding and non-balding HFs. The recent Phase 2 clinical trial of the PTGDR2 inhibitor did not show efficacy on AGA, suggesting that further study on the PTGDS pathway and alopecia is needed [5].

In conclusion, this study identified the genetic expression pattern between balding and non-balding scalp of FPHL and found that SFRP2 and PTGDS are significantly involved in FPHL. These findings provide a foundation for further research on the pathogenesis of FPHL and potential therapeutic targets.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00403-024-03114-w>.

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Author contributions S.K. and Y.K. wrote the main manuscript text and J.K., S.K., W.P. and S.K. made contributions to the analysis and interpretation of the study. J.C. approved the version to be published and M.C. and M.K. revised the work for important intellectual content. B.P. was in charge of the whole process of the work.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate The patients in this manuscript have given written informed consent to publication of their case details.

Competing interests The authors has no conflict of interest to declare.

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