

# Endothelial Protein C Receptor gene Expression in a Female with Homozygous EPCR gene 23-bp Insertion

## *Homozigot EPCR 23-baz çifti İnsersiyona Sahip Kadın Bireyde Endotelyal Protein C Reseptörü Gen Ekspresyon Seviyesi*

Afife Karabıyık, Nejat Akar

Ankara University, School of Medicine, Department of Pediatric Molecular Genetics, Ankara, Turkey

### To the Editor,

Endothelial protein C receptor (EPCR) is an essential component of the protein C (PC) anticoagulant pathway and is important for regulation of coagulation [1-3]. EPCR has a transmembrane domain, extracellular domains, and a very short cytoplasmic tail, and is primarily localized on the endothelial cells of large blood vessels. The human *EPCR* gene is located on chromosome 20q11.2, and has 4 exons and 3 introns [4,5]. EPCR has both an endothelial cell-specific transmembrane form and a soluble form that arises via metalloprotease cleavage [6].

To date, several polymorphisms and mutations—including a 23-bp insertion—have been reported on the human *EPCR* gene. The *EPCR* gene 23-bp insertion TATC-CACAGTTCCTCTGACCATC is located between intron 2 and exon 3 (nt4031), and is related to thrombotic risk and myocardial infarction [7-10]. Exons 2 and 3 encode most of the extracellular region of the *EPCR* gene [5]. This insertion of 23 nucleotides preceding the insertion point (nt4031) introduces a frameshift and premature stop that deletes the entire alpha 2 domain of the gene [10]. The truncated protein results in absence of the cytoplasmic tail, transmembrane domain, and part of the extracellular domain. As such, this mutation is probably a good model for an EPCR null-allele.

Homozygous null mice embryos died prior to embryonic d 10.5, and it was reported that EPCR is essential for normal embryonic development and plays a key role in preventing thrombosis at the maternal-embryonic interface [11,12]. The homozygous state of *EPCR* gene 23-bp insertion is very rare, and we only reported it once before in a 8-month-old boy with sepsis [13].

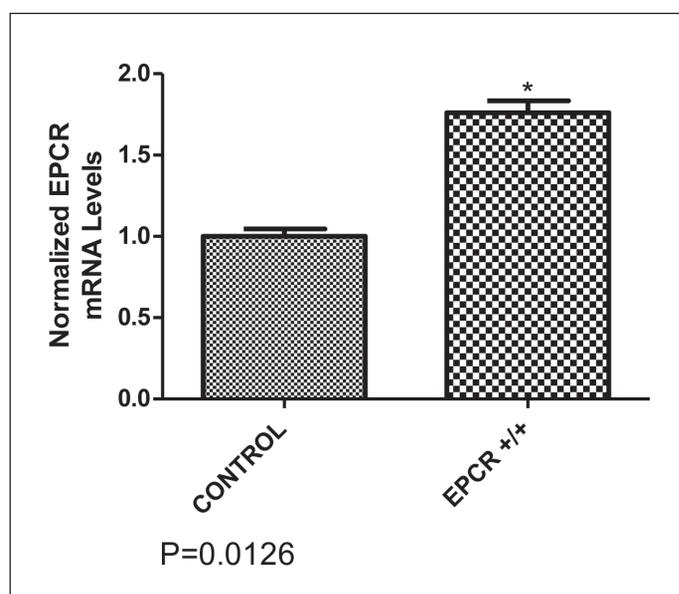
Herein we report a 25-year-old female with homozygous 23-bp insertion of the *EPCR* gene. The patient had experienced abortus twice, and then gave birth following antiplatelet (aspirin) therapy. She was referred for evaluation of thrombotic risk factors to us. The family history was negative for thrombotic disease. Informed consent was provided by the patient. Her DNA was examined for factor V 1691 and prothrombin 20210 mutations, and she carried normal alleles. Factor VIII, factor IX, protein C, antithrombin, protein S, homocysteine, and lipoprotein (a) levels were normal. Plasma sEPCR was 227 ng mL<sup>-1</sup> (38-132 ng mL<sup>-1</sup>), which was measured via enzyme-linked immunosorbent assay (ELISA) (Diagnostica Stago Asserachrom sEPCR, Asnieres-France). *EPCR* gene exon 3 amplification was performed using primers 5'-ACACCTG-GCACCCTCTCT-3' and 5'-CATCCTTCAGGTCCATCC-3' at an annealing temperature of 58 °C. To detect the 23-bp insertion the PCR product was electrophoresed in 3% agarose gel and stained with ethidium bromide. The patient

**Address for Correspondence: Afife KARABIYIK, M.D.,**

Suadiye Cad. 38/3 06190 Yenimahalle, Ankara, Turkey  
Phone: +90 312 595 63 48/115 E-mail: afifekrbyk@gmail.com

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**Figure 1:** The EPCR mRNA level in the patient (1.7-fold higher) and control.

was homozygous for the *EPCR* gene 23-bp insertion mutation, and her father, mother, and child were heterozygous for the insertion.

RNA was isolated from blood samples obtained from the index case and a control, and then the level of expression of EPCR mRNA was determined (Roche Light Cycler 1.5, Basel, Switzerland), following RNA isolation and cDNA synthesis (Roche, Switzerland). Quantitative real-time (RT)-PCR was used to measure gene expression using EPCR-specific fluorescent marked UPL probes (Probe 50) and primers (EPCR<sub>F</sub> 5'-GTAGCCAAGACGCCT-3', EPCR<sub>R</sub> 5'-GATAGGGGTCGCGGA-3') (Roche, Switzerland). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene was used for normalization of *EPCR* gene expression data. All experiments were performed twice. Statistical analysis of the results was performed using two-way ANOVA (GraphPad Prism v.5.00, GraphPad Software, San Diego, California, USA, <http://www.graphpad.com>).

The patient's EPCR mRNA level was 1.7-fold higher than that of the control, as shown in the Figure 1. Because of the premature stop of the EPCR protein, which was due to the 23-bp homozygous insertion, her EPCR expression level could be higher than the control that has normal EPCR gene 23-bp mutation allele. This can be explained by the EPCR protein requirement.

Disruption of the *EPCR* gene in mice leads to early embryonic death [12]. The presented case is not only

alive, but also gave birth to a healthy child. As the patient had abortus twice, we think that homozygous 23-bp insertion might affect the fetus negatively by causing hypercoagulability. There is a strong association between anti-EPCR autoantibodies and the risk of fetal death. High levels of IgM and IgG anti-EPCR in humans are associated with a high risk of a first episode of fetal death [14]. *EPCR* gene 23-bp insertion in women with fetal loss is more prevalent than in women that have given birth to  $\geq 1$  healthy baby and have no history of late fetal death [11]. The data obtained in the presented case show that 23-bp homozygous mutation of the *EPCR* gene in humans is compatible with life. Additional research—including cases with homozygous 23-bp insertion mutations—is needed to clarify the possible effects of the insertion.

#### Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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