원 저

# The Y153H Variant of the *STOX1* Gene in Korean Patients with Preeclampsia

Shin-Young Kim[1] · So-Yeon Park[1] · Ji-Hyae Lim[1] · Jae-Hyug Yang[2] · Moon-Young Kim[2]
Hyun-Young Park[3] · Kwang-Soo Lee[3] · Young Ju Kim[4] · Hyun-Mee Ryu[1,2]*

[1]Laboratory of Medical Genetics, Cheil General Hospital and Women's Healthcare Center, Seoul, Korea,
[2]Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea,
[3]Division of Cardiovascular Disease, Center for Biomedical Sciences, National Institute of Health Seoul, Korea,
[4]Department of Obstetrics and Gynecology, Ewha Medical Research Institue, College of Medicine, Ewha Womans' University, Seoul, Korea

**Purpose:** Preeclampsia is a multifactorial disorder with genetic and environmental components. Recently, the *STOX1* gene, identified as a candidate gene for preeclampsia in Dutch women, has been shown to be placentally expressed and subject to imprinting with preferential transmission of the maternal allele. The purpose of this study is to investigate whether there is an association between the *STOX1* Y153H variation and preeclampsia in Korean pregnant women.
**Materials and Methods:** This study involved 202 preeclamptic and 204 healthy pregnant women who were genotyped for the Y153H variant of the *STOX1* gene using a commercially available SNapShot assay kit and an ABI Prism 3730 DNA Analyzer.
**Results:** There were no significant differences in genotype frequencies of the Y153H variant of the *STOX1* gene between preeclamptic patients and normal controls ($P$>0.05). The H allele frequency of the *STOX1* Y153H variation was similar in patients with preeclampsia (87.1%) and in normal controls (86.5%). In addition, multiple logistic regression analysis showed that the YH, HH, and YH/HH genotypes were not associated with an increased risk of preeclampsia when compared to the YY genotype.
**Conclusion:** This is the first study to characterize the Y153H variant of the *STOX1* gene in Korean patients with preeclampsia. We found no differences in the genotype and allele frequencies between preeclamptic and normal pregnancies. Although limited by a relatively small sample size, our study suggests that the *STOX1* Y153H variation is not associated with the development of preeclampsia in Korean pregnant women.

**Key Words:** Preeclampsia, *STOX1*, Y153H variation

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**Corresponding auther:** Hyun-Mee Ryu, MD, PhD,
Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Kwandong University College of Medicine, 1-19 Mukjeong-dong, Jung-gu, Seoul 100-380, Korea
Tel: +82-2-2000-7683, Fax: +82-2-2278-4574
E-mail: hmryu@yahoo.com

## Introduction

Preeclampsia is a multifactorial, pregnancy-specific disorder characterized by hypertension and proteinuria and is a major cause of maternal and perinatal morbidity and mortality. Although the etiology of preeclampsia remains elusive, there is a general consensus that the crucial step in the pathogenesis of preeclampsia is a

defect in trophoblast cell differentiation and invasion in early pregnancy[1, 2].

The *STOX1* (also called C10orf24) gene on chromosome 10q22.1 is expressed in the early placenta and is subject to imprinting with preferential expression of the maternal allele. *STOX1* encodes a putative DNA binding protein which is involved in the differentiation of trophoblast cells; specifically, polyploidization of the extravillous trophoblasts and formation of the syncytiotrophoblast[2]. Zhou et al.[2] have demonstrated cell-specific expression of the *STOX1* alleles, with biallelic expression in noninvasive villous and extravillous trophoblasts and monoallelic maternal expression in invasive extravillous trophoblast cells. This expression pattern limits the time and place where the maternal mutations can have their effect. A recent report suggested an association of the *STOX1* gene with preeclampsia in the Dutch population, identifying seven coding variations co-segregating with the preeclamptic phenotype following matrilineal inheritance[3]. A study by van Dijk et al.[4] combined linkage results with expression data of candidate genes in first-trimester placentas and identified *STOX1* as a new imprinted gene in preeclampsia. The authors hypothesized that maternal transmission of the variant allele to the fetus induces premature trophoblast differentiation and results in shallow trophoblast invasion, resulting in preeclampsia[4]. As the paternal imprinted allele is silenced, the maternal allele acts dominantly[4]. Their results showed maternal transmission of the Y153H variant leading to a change of the amino acid Tyrosine to Histidine at position 153 in the *STOX1* protein (p.Tyr153His) in all women with preeclampsia[4].

In this study, we investigate the genotype and allele frequency of the *STOX1* Y153H variation in preeclamptic and normotensive pregnancies; we also evaluate whether the Y153H variant in the coding region of the *STOX1* gene is associated with susceptibility to preeclampsia in Korean pregnant women.

## Materials and methods

### 1. Subjects

All subjects were recruited from the Obstetrics and Gynecology Department at Kwandong University Cheil General Hospital and Ewha Womans' University Mokdong Hospital in Seoul, Korean between 2003 and 2004. The study population included 202 preeclamptic patients and 204 normotensive pregnant women of Korean ethnicity. Normal pregnancy controls were selected randomly from contemporaneous women who were normotensive and who had no proteinuria throughout pregnancy and who delivered healthy neonates at term (>37 weeks of gestation) without medical or obstetric complications such as chronic hypertension, diabetes, renal insufficiency, congenital anomalies, intrauterine growth restriction (IUGR), or fetal demise. Preeclampsia was defined as hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) and proteinuria (≥300 mg in a 24 h urine collection and/or ≥1+ on dipstick testing) after 20 weeks of gestation, in accordance with the Committee Terminology of the American College of Obstetricians and Gynecologists. Severe preeclampsia was defined as one or more of the following: diastolic blood pressure ≥110 mmHg, severe proteinuria (urinary protein excretion ≥5 g per 24 h and/or ≥3+ on dipstick testing), evidence of pulmonary edema, seizures, oliguria (<500 mL/d), thrombocytopenia (platelet count <100,000/mL), or severe central nervous system symptoms such as altered mental status, headaches, blurred vision, or blindness. Exclusion criteria included major congenital anomalies, fetal chromosomal abnormalities, recurrent miscarriage, prior preeclampsia, illicit drug use, alcohol consumption, smoking, and preexisting medical conditions such as diabetes, chronic hypertension, autoimmune disease, or renal disease. The Ethics Committee at Cheil General Hospital approved both the use of clinical information and the collection of samples for

research purposes (# SCH-IRB-2005-12). Written informed consent was obtained from all enrolled subjects before blood sampling, which was approved by the Ethics Committee of Kwandong University Cheil General Hospital and Ewha Womans' University Mokdong Hospital.

### 2. DNA Extraction and Genotyping

Peripheral blood was collected in EDTA vacutainer tubes (Becton Dickinson, USA) and genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations.

Genotypes corresponding to the single nucleotide polymorphism (SNP) rs1341667 in exon2 of the *STOX1* gene were determined in all available individuals. The SNP is located at position 19193015 from the human genomic contig NT_008583.16 (NCBI build 36.1 and van Dijk et al.[4]) and consists of a T/C change (in the reverse strand) leading to a change of amino acid Tyrosine to Histidine at position 153 of the *STOX1* protein (Y153H).

The *STOX1* Y153H variation was genotyped by a single base primer extension assay using the SNapShot kit according to manufacturer recommendations (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction (PCR) was used to amplify the region of genomic DNA containing the *STOX1* Y153H variation. The forward primer was 5'-GGGTGAAGTTCTTTGCTGTGC-3' and the reverse primer was 5'-ATCAGCGTTCCCAGAGTGGTA-3'. The PCR reaction was performed in a final volume of 10 μL containing 30 ng of genomic DNA, 1X PCR Buffer, 3 mM of $MgCl_2$, 250 mM of dNTPs, 1.25 pmol of each primer, and 0.15 U of Taq DNA polymerase (Applied Biosystems). Amplification was carried out in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) under touch-down conditions.

After amplification, the PCR products were purified through incubation with 1 U each of shrimp alkaline phosphatase (SAP) (Rhoche/Roche Diagnostic Systems, Norwalk, CT, USA) and exonuclease I (USB Corporation) at 37℃ for 1 hour and then 72℃ for 15 minutes. Primer extension reactions were performed with the SNapShot ddNTP Primer Extension Kit (Applied Biosystems) as recommended by the manufacturer. One microliter of the purified amplification products was added to a SNapShot Multiplex Ready reaction mixture containing 0.15 pmol of genotyping internal primer. The extension primer was 5'-GATGATTCTTCCGATGGAATTGCAATGCCTGGGT-3'. The extension primer reaction was carried out for 25 cycles at 96℃ for 10 seconds, 50℃ for 5 seconds, and 60℃ for 30 seconds. One microliter of the final reaction samples, plus extension products and GeneScan 120 Liz size-standard solution, were added to 9 microliters of Hi-Di formamide (Applied Biosystems). This mixture was incubated at 95℃ for 5 minutes followed by 5 minutes on ice, and then electrophoresed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). The results were analyzed using the ABI Prism GeneScan and Genotyper program software version 3.7 (Applied Biosystems).

### 3. Statistical analysis

Data are presented as the mean±SD or number (%). The clinical characteristics of preeclamptic patients and normal controls were compared with the Student's *t*-test and chi-square test. The comparison of allele and genotype frequencies of the *STOX1* Y153H variation between the two groups was performed with the chi-square test or Fisher's exact test. Hardy-Weinberg equilibrium (HWE) was separately tested in patients and controls by means of chi-square analysis (www.fourmilab.ch/rpkp/experiments/analysis/chiCalc.html). Odds ratio (OR) and 95% confidence intervals (CI) were calculated to assess the disease risk conferred by the *STOX1* Y153H variation. Multiple logistic regression analysis was carried out with adjustment for covariates (maternal age, nulliparity, delivery week, and body mass index). $P<0.05$ was considered stati-

stically significant. The statistical analysis was performed with the Statistical Package for Social Sciences version 12.0 (SPSS Inc., Chicago, USA).

## Results

Polymorphism analysis of the *STOX1* gene was performed for 202 preeclamptic patients and 204 normotensive pregnancies. The clinical characteristics of the study population are shown in Table 1. The delivery week and birth weight were significantly lower in the preeclamptic group than in the control group, whereas nulliparity and blood pressure were significantly higher. There were no significant differences in the maternal age and body mass index (BMI) between the two groups ($P$>0.05).

The genotype and allele frequencies of the Y153H variant of the *STOX1* gene of preeclamptic patients and normal controls are displayed in Table 2. The genotype frequencies of the *STOX1* Y153H variation in the preeclamptic and control groups did not indicate a departure from HWE ($P$=0.300 and $P$=0.104, respectively). The frequencies of the YY, YH, HH, and YH/HH genotypes were 2.5%, 20.8%, 76.7%, and 23.3 % in preeclamptic patients and 0.5%, 26.0%, 73.5%, and 13.2% in normal controls, respectively. The frequencies of the Y and H alleles were 12.9% and 87.1% in preeclampsia and 13.5% and 86.5% in controls, respectively.

**Table 1.** Clinical Characteristics of the Study Population

| Characteristics | CON (n=204) | PE (n=202) | P value |
|---|---|---|---|
| Age (y) | 31.4±4.3 | 31.1±4.4 | 0.431 |
| Nulliparity (n) | 121 (59.3%) | 140 (69.3%) | 0.036 |
| Systolic BP (mmHg) | 116.9±13.5 | 158.6±18.0 | <0.001 |
| Diastolic BP (mmHg) | 76.0±10.0 | 99.8±12.2 | <0.001 |
| Delivery week (wk) | 39.1±1.7 | 35.8±3.9 | <0.001 |
| BMI (kg/m2) | 27.4±4.9 | 28.1±4.5 | 0.533 |
| Birth weight (g) | 3171.8±509.7 | 2398.6±896.6 | <0.001 |
| IUGR (cases) | 0 (0%) | 57 (34.8%) | – |
| Proteinuria (dipstick) | – | 2.7±0.9 | – |

Abbreviations : CON, controls; PE, preeclampsia; BP, blood pressure; BMI, Body Mass Index; IUGR, intrauterine growth restriction; y, year; n, Number
Data are presented as mean±SD for continuous variables and as number (percentage) for categorical variables.
P values were calculated using the Student's t-test or chi-square test. P<0.05 was considered significant.

The frequencies of the Y153H genotypes and alleles did not differ between the two groups ($P$>0.05). The association between this polymorphism and preeclampsia was estimated by multiple logistic regression analysis adjusting for covariates such as maternal age, nulliparity, delivery week, and BMI. The Y153H genotypes were not associated with risk of preeclampsia (YH: adjusted OR=0.85, 95% CI 0.42-1.35, $P$=0.340; HH: adjusted OR=1.15, 95% CI 0.65-2.02, $P$=0.629; YH/HH: adjusted OR=0.20, 95% CI 0.02-1.95, $P$=0.164; Table 2).

## Discussion

Van Dijk et al. originally proposed that mutations in *STOX1* could be causal for predisposition to preeclampsia[4], a hypertensive disorder originating from placental defects which affects up to 10% of human pregnancies[5]. However, three recent studies have shown that the polymorphism described as a causal mutation by van Dijk and colleagues is widely shared by non-preeclamptic women from various populations [6-8]. As presented in the original *STOX1*-preeclampsia study, a specific feature of *STOX1* is its allele-specific expression from maternal alleles, demonstrated by the absence of *STOX1* mRNA in hydatidiform moles (which are exclusively of paternal origin)[4]. This discovery is in concordance with the hypothesis that imprinted genes may be particularly involved in preeclampsia[9].

In this study, we investigated the relationship between *STOX1* Y153H variation and the occurrence of preeclampsia. Our study is the first nested case-control study that investigates the association of the *STOX1* Y153H variation with preeclampsia. There were no significant differences in genotype or allele frequencies of the Y153H variant in the *STOX1* gene between preeclamptic patients and controls. Intere-

**Table 2.** Genotype and Allele Frequencies of the STOX1 Y153H Variant

| | CON n (%) | PE n (%) | Unadjusted OR (95% CI) | *P* value | Adjusted* OR (95% CI) | *P* value |
|---|---|---|---|---|---|---|
| Genotype | | | | | | |
| YY | 1 (0.5) | 5 (2.5) | 1.00 (reference) | – | 1.00 (reference) | – |
| YH | 53 (26.0) | 42 (20.8) | 0.16 (0.02–1.41) | 0.094 | 0.85 (0.42–1.35) | 0.340 |
| HH | 150 (73.5) | 155 (76.7) | 0.21 (0.02–1.79) | 0.215 | 1.15 (0.65–2.02) | 0.629 |
| YH/HH | 54 (13.2) | 47 (23.3) | 0.17 (0.02–1.54) | 0.106 | 0.20 (0.02–1.95) | 0.164 |
| Allele | | | | | | |
| Y | 55 (13.5) | 52 (12.9) | 1.00 (reference) | – | – | – |
| H | 353 (86.5) | 352 (87.1) | 1.06 (0.70–1.58) | 0.798 | – | – |

Abbreviations : CON, controls; PE, preeclampsia; OR, odds ratio; CI, confidence interval
Data are expressed as number (%).
*P* values were calculated using the chi-square test or Fisher's exact test. $P<0.05$ was considered significant.
*Adjusted for maternal age, nulliparity, delivery week, and BMI.

stingly, the disease-associated allele (H allele) of the *STOX1* gene appeared to be the predominant allele in our study population. Berends et al.[6] showed that the H allele frequencies of the *STOX1* Y153H variation were similar in 157 women with preeclampsia (65%) and in 157 controls (64%). The H allele frequencies we observed were higher than the 50% described by van Dijk et al.[4]. Furthermore, the authors reported a preferential transmission of the *STOX1* H allele from mothers with preeclampsia to their offspring[4]. This indicates a distortion in transmission in favour of the H allele, supporting the concept of maternal preferential transmission in women with shared aetiological placental pathology leading to abnormal pregnancy outcomes.

Two recent studies performed in Dutch and Finnish populations could not confirm the involvement of the *STOX1* gene in preeclampsia[7, 8]. No differences were detected in the expression of *STOX1* mRNA between placentas from preeclamptic and uncomplicated pregnancies. Additionally, Iglesias-Platas et al.[7] were able to demonstrate biallelic expression in fetal tissues and in both preeclamptic and control placentas - both in human and mice - which challenges the imprinting status of *STOX1*. Despite the fact that the imprinting status of the *STOX1* gene is questioned, there is still substantial support for epigenetic phenomena, such as genomic imprinting, in placental pathology. Altered expression of imprinted genes has been described in association with IUGR[10]. Furthermore, Graves et al.[9] had postulated years ago that imprinted genes were involved in preeclampsia as a solution for the genetic conflict between maternal genes that limit growth and paternal genes that promote growth.

It should be noted that there are some limitations to this study. First, the study was confined to Korean patients. Preeclampsia is a multifactorial disease with varied clinical manifestations wherein individual exposure to various environmental factors and genetic susceptibility can have a significant influence on disease outcomes. This should be taken into account when evaluating the potential applicability of our findings to other ethnic populations. In addition, the sample size was relatively small because there was a limited selection of women who subsequently developed preeclampsia among the pregnant women in the two hospitals studied. Finally, demonstration for a preferential transmission of the C allele of *STOX1* gene from mothers with preeclampsia to their offspring is impossible because there were no data for such a distortion in transmission in our population.

In conclusion, we found that neither the genotype nor the allele frequencies of the Y153H variant in the *STOX1* gene differed significantly between preeclamptic patients and controls. Our result suggests that the

*STOX1* Y153H variation is not associated with susceptibility to development of preeclampsia in Korean pregnant women. The relevance of *STOX1* Y153H variation in preeclamptic patients in larger and more diverse ethnic populations remains to be studied.

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## 국문초록

**목 적**: 자간전증은 유전적·환경적 요인에 의해 발생하는 다요인성 질환이다. 최근 네덜란드 산모에서의 자간전증 후보 유전자로 확인된 *STOX1* 유전자는 태반에서 발현되며 모계 대립유전자의 선택적 유전에 의해 각인된다. 본 연구에서는 *STOX1* 유전자의 단일염기변이형 부위인 Y153H와 한국인 자간전증의 연관성에 대하여 알아보고자 하였다.

**대상 및 방법**: 자간전증 산모 202과 임신기간 동안 자간전증이 발생하지 않은 정상 산모 204명의 혈액으로부터 *STOX1* 유전자의 Y153H 변이 양상을 SNapShot assay kit와 ABI Prism 3730 DNA analyzer를 사용하여 분석하였다.

**결과**: *STOX1* Y153H 변이의 유전자형 빈도는 자간전증 환자군과 정상 대조군 간에 유의한 차이가 없었다($P$>0.05). 또한 자간전증 환자군(87.1%)에서 대립유전자형인 H의 빈도도 정상 대조군(86.5%)과 유사하였다. 다중회귀분석결과 YH, HH, YH/HH 유전자형들은 자간전증 발생 위험에 있어서 연관성이 없었다.

**결 론**: 본 연구진은 처음으로 한국인 자간전증 환자에서 *STOX1* 유전자의 Y153H 변이형의 특징을 파악 하였다. 연구결과 자간전증 산모와 정상 산모 간의 유전자형과 대립유전자형의 빈도는 차이가 없었다. 비록 실험 대상군의 수적인 제한이 있지만, 이상의 결과를 통해서 한국인에서 *STOX1* 유전자의 Y153H 변이형은 자간전증과 연관성이 없는 것으로 사료된다.

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