

Association between polymorphisms of estrogen receptor 2 and benign prostatic hyperplasia

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Abstract. Estrogens and estrogen receptors (ESRs) have been implicated in the stimulation of aberrant prostate growth and the development of prostate diseases. The aim of the present study was to investigate four single nucleotide polymorphisms (SNPs) of the *ESR2* gene in order to examine whether *ESR2* is a susceptibility gene for benign prostatic hyperplasia (BPH). In order to evaluate whether an association exists between *ESR2* and BPH risk, four polymorphisms [rs4986938 (intron), rs17766755 (intron), rs12435857 (intron) and rs1256049 (Val328Val)] of the *ESR2* gene were genotyped by direct sequencing. A total of 94 patients with BPH and 79 control subjects were examined. SNPStats and Haploview version 4.2 we used for the genetic analysis. Multiple logistic regression models (codominant1, codominant2, dominant, recessive and log-additive) were produced in order to obtain the odds ratio, 95% confidence interval and P-value. Three SNPs (rs4986938, rs17766755 and rs12435857) showed significant associations with BPH (rs4986938, P=0.015 in log-additive model; rs17766755, P=0.033 in codominant1 model, P=0.019 in dominant model and P=0.020 in log-additive model; rs12435857, P=0.023 in dominant model and P=0.011 in log-additive model). The minor alleles of these SNPs increased the risk of BPH, and the AAC haplotype showed significant association with BPH ($\chi^2=6.34$, P=0.0118). These data suggest that the *ESR2* gene may be associated with susceptibility to BPH.

Introduction

Benign prostatic hyperplasia (BPH), also known as benign prostatic hypertrophy, is the most common prostate disease affecting elderly men. Approximately 50% of men aged 51-60 years and ~90% of men aged 81-90 years have BPH (1,2). Clinically, BPH is associated with lower urinary tract symptoms (LUTS) (3). BPH is commonly viewed as a benign enlargement of the prostate, which contributes to an array of urinary voiding difficulties. The molecular etiology of BPH involves numerous complicated processes, and thus the exact cause remains unknown. Various theories have been suggested, such as embryonic reawakening, aging, androgens, estrogens and inflammation (4).

Estrogen, a female hormone, plays an essential role in the development of female secondary sexual characteristics; however, it is also produced in men. Estrogen is involved in the stimulation of prostate growth and the development of prostatic diseases in men (5,6). Estrogen action is mediated by two estrogen receptors (ESRs), which can be classified into two subtypes: ESR1 (ER α) and ESR2 (ER β) (5). ESR1 and ESR2, however, are not isoforms but are encoded by separate genes on different chromosomes (5). *ESR1* and *ESR2* are located on chromosome 6q25.1 and chromosome 14q23.2, respectively (7). The expression of *ESR2* in the ventral prostate shows a different pattern from that of *ESR1*. While *ESR1* is usually located in the stromal cells of the prostate, *ESR2* is primarily expressed in the epithelial cells (8). The two ESRs have distinct physical characteristics. Different expression locations and affinities may be associated with diverse biological functions of estrogens within the prostate gland (5).

In the prostate tissue of adults, an *ESR2* expression gradient exists, with low proximal levels and increased expression levels distally. This gradient can result in heterogeneity in differentiation and function along the ductal length (9). Although *ESR2* is the predominant ESR expressed in the adult prostate gland, its role has not yet been clearly defined. *ESR2* may play a role in epithelial differentiation (8). It has also been suggested that *ESR2* carries out an anti-proliferative role in the prostate and regulates the androgenic stimulation of prostate growth (8). Previously, several studies investigated the role

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of *ESR2* in the prostate using knockout animal models. The studies suggested that *ESR2* inhibits the proliferation of the prostate and the development of prostatic hyperplasia during the aging process (5,10). The aim of the present study was to investigate whether four single nucleotide polymorphisms (SNPs) (rs4986938, rs17766755, rs12435857 and rs1256049) of the *ESR2* gene were associated with the development of BPH.

Materials and methods

Study subjects. The present study involved 173 Korean men who had visited Kyung Hee University Medical Center (Seoul, Korea) for LUTS between January 2002 and December 2006. The clinical symptoms of the patients were assessed using the International Prostate Symptom Score (IPSS) and quality of life (QoL) questionnaires (<http://www.urospec.com/uro/Forms/ipss.pdf>). The prostate volumes of the patients were measured using transrectal ultrasonography, and the serum prostate-specific antigen (PSA) level of each subject was tested. Peak urinary flow rate (Qmax) and average urinary flow rate (Qavg) were measured using an uroflowmetry system. Subjects were excluded from the study if they had suffered from prostate cancer, neurogenic bladder, urethral stricture, acute/chronic prostatitis, urinary tract infection, uncontrolled diabetes mellitus, previous pelvic surgery or hypertension. Based on their symptoms, the subjects were allocated to either the control (prostate volume, <30 ml) or the BPH [prostate volume, ≥30 ml; IPSS, >8; Qmax, <15 ml/sec] group (11,12). The demographic characteristics of the BPH and control groups are shown in Table I. All subjects provided written, informed consent. This study was approved by the institutional review board of Kyung Hee University Medical Center in 2009.

SNP selection and genotyping. The SNP database was searched in order to select SNPs of the *ESR2* gene for study (<http://www.ncbi.nlm.nih.gov/SNP>, BUILD 141). SNPs with heterozygosity >0.1, minor allele frequency >10% and associations with other diseases were selected (13-16). Four promoter polymorphisms [rs4986938 (intron), rs17766755 (intron), rs12435857 (intron) and rs1256049 (Val328Val)] of the *ESR2* gene were eventually selected. Genotypes were determined by direct sequencing. A polymerase chain reaction (PCR) was conducted prior to sequencing. Genomic DNA was amplified using the following primers (Macrogen, Inc., Seoul, Republic of Korea): rs4986938 sense, 5'-TGTATGACC TGCTGCTGGAG-3' and anti-sense, 5'-AGGCCATTGAGT GTGGAAAC-3'; rs17766755 sense, 5'-GTAGCTCAAGCC ACTTGCTG-3' and anti-sense, 5'-ACATGGAAGCCAGAG TGACC-3'; rs12435857 sense, 5'-CAAGGATTCAGGGTC CTGTG-3' and anti-sense, 5'-GGCAGCAGGATCACTTGA ATC-3'; and rs1256049 sense, 5'-TCAACTCCCTAATGGTTT GTGT-3' and anti-sense, 5'-AGTGAAGGAGCTGATGAT GCTA-3'. During the PCR, 39 cycles were performed at 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min. Finally, the PCR was conducted at 72°C for 7 min to terminate the reaction. Each PCR product was identified with 1.8% agarose gel electrophoresis, and the products were sequenced using an ABI Prism® 377 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using SeqManII software, v2.3 (DNASTAR Inc., Madison, WI, USA).

Table I. Clinical data of participants included in the study.

| Parameter | Control | BPH |
|----------------------------|-----------|----------|
| Subjects (n) | 79 | 94 |
| Age (years) ^a | 63.2±10.2 | 67.2±8.1 |
| Total prostate volume (ml) | 22.54 | 53.07 |
| PSA (ng/ml) | | |
| Total | 2.16 | 6.52 |
| Free | 0.45 | 1.46 |
| IPSS | 15.41 | 18.29 |
| QoL | 3.32 | 3.92 |
| Uroflowmetry (ml/sec) | | |
| Qmax | 13.69 | 9.33 |
| Qavg | 8.01 | 5.60 |

^aPresented as the mean ± standard error of the mean. BPH, benign prostatic hyperplasia; PSA, prostate-specific antigen; IPSS, international prostate symptom score; QoL, quality of life; Qmax, peak urinary flow rate; Qavg, average urinary flow rate.

Statistical analysis. For the analysis of genetic data, SNPStats (<http://bioinfo.iconcologia.net/index.php>) was used. A Hardy-Weinberg equilibrium test was conducted for each SNP in the control group, and logistic regression models (codominant1, codominant2, dominant, recessive and log-additive) were then applied to obtain odds ratios (ORs), 95% confidence intervals (CIs) and P-values. A linkage disequilibrium (LD) block of *ESR2* gene SNPs was analyzed using Haploview version 4.2 and the algorithm of Gabriel *et al* (17). The level of significance was set at P<0.05.

Results

Clinical characteristics. Prostate volumes were used to categorize the 173 subjects into the BPH group (≥30 ml, n=94) and the control group (<30 ml, n=79); the clinical characteristics of the two groups are shown in Table I. The mean prostate volume in the BPH group was significantly higher than that in the control group (P<0.001). The total PSA, free PSA, IPSS, QoL, Qmax and Qavg in the BPH group were higher than those in the control group (P<0.05).

Genotype and allele distributions. Genotype and allele distributions of the four SNPs of the *ESR2* gene (rs4986938, rs17766755, rs12435857 and rs1256049) in the BPH patients and control subjects are shown in Table II. Table II also shows the association between each genotype/allele and the risk of BPH by logistic regression analysis. Among the four SNPs, three polymorphisms (rs4986938, rs17766755 and rs12435857) were found to be associated with BPH (Table II), with the minor alleles of these polymorphisms increasing the risk of BPH: i) rs4986938: OR, 1.98; 95% CI, 1.14-3.44; P=0.023 in the log-additive model (C/C genotype vs. C/T genotype vs. T/T genotype); ii) rs17766755: OR, 2.10; 95% CI, 1.06-4.17; P=0.033 in the codominant1 model (G/G genotype vs. G/A genotype); OR, 2.18; 95% CI, 1.13-4.20; P=0.019 in the dominant model

Table II. Genotype and allele frequencies of promoter polymorphisms of the *ESR2* gene in BPH patients and controls.

| SNPs | Genotype or allele | Control, n (%) | BPH, n (%) | Models | OR (95% CI) | P-value |
|------------|--------------------|----------------|------------|--------------|-------------------|--------------|
| rs4986938 | C/C | 56 (71.8) | 53 (56.4) | Codominant1 | 1.65 (0.84-3.25) | 0.150 |
| Intron | C/T | 21 (26.9) | 34 (36.2) | Codominant2 | 6.85 (0.80-58.82) | 0.080 |
| | T/T | 1 (1.3) | 7 (7.5) | Dominant | 1.89 (0.98-3.64) | 0.060 |
| | | | | Recessive | 5.82 (0.68-49.39) | 0.050 |
| | | | | Log-additive | 1.91 (1.08-3.37) | 0.023 |
| | C | 133 (85.3) | 140 (74.5) | | 1 | |
| | T | 23 (14.7) | 48 (25.5) | | 1.98 (1.14-3.44) | 0.015 |
| rs17766755 | G/G | 58 (73.4) | 52 (55.3) | Codominant1 | 2.10 (1.06-4.17) | 0.033 |
| Intron | G/A | 19 (24.1) | 36 (38.3) | Codominant2 | 2.81 (0.52-15.06) | 0.230 |
| | A/A | 2 (2.5) | 6 (6.4) | Dominant | 2.18 (1.13-4.20) | 0.019 |
| | | | | Recessive | 2.21 (0.42-11.66) | 0.330 |
| | | | | Log-additive | 1.94 (1.09-3.43) | 0.020 |
| | G | 135 (85.4) | 140 (74.5) | | 1 | |
| | A | 23 (14.6) | 48 (25.5) | | 2.01 (1.16-3.49) | 0.013 |
| rs12435857 | G/G | 43 (54.4) | 35 (37.2) | Codominant1 | 1.86 (0.98-3.54) | 0.060 |
| Intron | G/A | 33 (41.8) | 49 (52.1) | Codominant2 | 4.15 (1.03-16.66) | 0.050 |
| | A/A | 3 (3.8) | 10 (10.6) | Dominant | 2.05 (1.10-3.83) | 0.023 |
| | | | | Recessive | 3.02 (0.78-11.62) | 0.090 |
| | | | | Log-additive | 1.94 (1.15-3.27) | 0.011 |
| | G | 119 (75.3) | 119 (63.3) | | 1 | |
| | A | 39 (24.7) | 69 (36.7) | | 1.77 (1.11-2.82) | 0.017 |
| rs1256049 | C/C | 38 (48.1) | 53 (56.4) | Codominant1 | 0.64 (0.34-1.22) | 0.180 |
| Val328Val | C/T | 38 (48.1) | 35 (37.2) | Codominant2 | 1.30 (0.30-5.64) | 0.730 |
| | T/T | 3 (3.8) | 6 (6.4) | Dominant | 0.70 (0.38-1.29) | 0.250 |
| | | | | Recessive | 1.58 (0.38-6.67) | 0.520 |
| | | | | Log-additive | 0.82 (0.49-1.38) | 0.460 |
| | C | 114 (72.2) | 141 (75.0) | | 1 | |
| | T | 44 (27.8) | 47 (25.0) | | 0.86 (0.54-1.40) | 0.550 |

P-values were derived from logistic regression analyses with the codominant, dominant, recessive and log-additive models. Bold numbers indicate a significant association. SNP, single nucleotide polymorphism; BPH, benign prostatic hyperplasia; OR, odds ratio; CI, confidence interval.

Table III. Haplotype analysis for the association between *ESR2* gene cluster polymorphisms and BPH.

| Haplotype | Frequency | Control (n) | | BPH (n) | | χ^2 | P-value |
|-----------|-----------|-------------|-----|---------|-----|----------|---------------|
| | | + | - | + | - | | |
| GGC | 0.425 | 75 | 83 | 72 | 116 | 2.955 | 0.0900 |
| GGT | 0.263 | 44 | 114 | 47 | 141 | 0.359 | 0.5500 |
| AAC | 0.205 | 23 | 135 | 48 | 140 | 6.340 | 0.0118 |
| GAC | 0.107 | 16 | 142 | 21 | 167 | 0.098 | 0.7500 |

Haplotype consists of rs17766755, rs12435857 and rs1256049. Bold numbers indicate a significant association. BPH, benign prostatic hyperplasia; (+), ratio a specific haplotype of case or control; (-), ratio not a specific haplotype of case or control.

(G/G genotype vs. G/A genotype + A/A genotype); OR, 1.94; 95% CI, 1.09-3.43; P=0.02 in the log-additive model (G/G genotype vs. G/A genotype vs. A/A genotype); iii) rs12435857:

OR, 2.05; 95% CI, 1.10-3.83; P=0.023 in the dominant model (G/G genotype vs. G/A genotype + A/A genotype); OR, 1.94; 95% CI, 1.15-3.27; P=0.011 in the log additive model (G/G

genotype vs. G/A genotype vs. A/A genotype). In addition, allele distributions of the three SNPs (rs4986938, rs17766755 and rs12435857) showed significant association with BPH (rs4986938: OR, 1.98; 95% CI, 1.14-3.44; $P=0.015$; rs17766755: OR, 2.01; 95% CI, 1.16-3.49; $P=0.013$; and rs12435857: OR, 1.77; 95% CI, 1.11-2.82; $P=0.017$) (Table II).

The four SNPs (rs4986938, rs17766755, rs12435857 and rs1256049) underwent LD and haplotype analysis. One LD block was constructed using the method of Gabriel *et al* (17) during pair-wise comparisons among these SNPs (data not shown). The LD block was constructed with rs17766755, rs12435857 and rs1256049. Among the eight possible haplotypes in the LD block, four haplotypes (frequency >0.1) were analyzed (Table III). The AAC haplotype in the *ESR2* gene exhibited a significant association with BPH [χ^2 , 6.34; $P=0.0118$].

Discussion

Although the prostate is commonly considered as a target of androgens, it is also an important target of estrogens. Previous studies have found that estrogens directly and indirectly affect the growth of the prostate and the development of prostate diseases (4-6). In the estrogen pathway, ESRs play an important role in modulating estrogen action. It has been reported that, while ESR1 is an oncogenic factor involved in cell proliferation and survival, ESR2 is a protective factor that is anticarcinogenic and proapoptotic. Numerous previous studies have investigated the association between ESRs and the development of different types of cancer and have focused on the contribution of ESR1 and ESR2 to the onset of such cancers as prostate, ovarian and breast cancer (18-21). The present study, however, focused on investigating the association between ESR2 and the risk of BPH.

Several association studies have investigated the SNPs assessed in the present study (rs4986938, rs17766755 and rs12435857). In the study conducted by Ryan *et al* (13), it was found that an allele of the rs4986938 SNP resulted in a decline in psychomotor speed, and it was thus concluded that this allele was associated with cognitive function. Marini *et al* (14) also showed that rs4986938 SNP gene polymorphism was associated with plasma fibrinogen levels in postmenopausal women, while Yang *et al* (16) reported that the rs17766755 SNP was significantly associated with human height. Kristiansen *et al* (15) showed that rs12435857 was associated with an increased risk of testicular germ cell tumor.

In the present study, an association was found between the rs4986938, rs17766755 and rs12435857 SNPs of the *ESR2* gene and the susceptibility to BPH. The minor alleles of these SNPs were associated with an increased risk of BPH. Previous studies have shown that the serum estrogen level is associated with prostate volume and other characteristics of BPH (22,23). Although the exact role of ESR2 in the mechanism of BPH has not yet been fully determined, it is suggested that ESR2 located in epithelial cells inhibits the proliferation of the prostate and activates apoptosis in BPH (10,24). In the study by Krege *et al* (10), *ESR2*-knockout mice developed prostatic hyperplasia during the aging process. McPherson *et al* (24) demonstrated that ESR2 also activates apoptosis in BPH in an androgen-independent manner. Thus, ESR2 plays an important role in the estrogen-induced proliferation of the prostate (10).

In conclusion, this is the first study, to the best of our knowledge, to investigate whether SNPs of the *ESR2* gene are associated with the susceptibility to BPH. Three polymorphisms (rs4986938, rs17766755 and rs12435857) of the *ESR2* gene were found to be correlated with the development of BPH. Although these three SNPs are intronic and, thus, are unlikely to be directly involved in the development of the disease, they may interfere with the mRNA splicing process and even the gene expression level (25). These results suggest that these polymorphisms of the *ESR2* gene may contribute to the susceptibility to BPH in the Korean population. In the future, additional studies should be conducted with larger study populations to confirm these results.

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References

1. Karatzas A, Tzortzis V, Giannatou E, Gravas S, Zachos I, Oeconomou A, Melekos M and Tsezou A: Lack of association between the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene polymorphism and the risk of benign prostatic hyperplasia in Caucasian men. *Mol Biol Rep* 40: 6665-6669, 2013.
2. Roehrborn CG and Rosen RC: Medical therapy options for aging men with benign prostatic hyperplasia: Focus on alfuzosin 10 mg once daily. *Clin Interv Aging* 3: 511-524, 2008.
3. Roehrborn CG: Male lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH). *Med Clin North Am* 95: 87-100, 2011.
4. Bianco JJ, McPherson SJ, Wang H, Prins GS and Risbridger GP: Transient neonatal estrogen exposure to estrogen-deficient mice (aromatase knockout) reduces prostate weight and induces inflammation in late life. *Am J Pathol* 168: 1869-1878, 2006.
5. Prins GS and Korach KS: The role of estrogens and estrogen receptors in normal prostate growth and disease. *Steroids* 73: 233-244, 2008.
6. McPherson SJ, Ellem SJ and Risbridger GP: Estrogen-regulated development and differentiation of the prostate. *Differentiation* 76: 660-670, 2008.
7. Menasce LP, White GR, Harrison CJ and Boyle JM: Localization of the estrogen receptor locus (ESR) to chromosome 6q25.1 by FISH and a simple post-FISH banding technique. *Genomics* 17: 263-265, 1993.
8. Weihua Z, Makela S, Andersson LC, Salmi S, Saji S, Webster JJ, Jensen EV, Nilsson S, Warner M and Gustafsson JA: A role for estrogen receptor beta in the regulation of growth of the ventral prostate. *Proc Natl Acad Sci USA* 98: 6330-6335, 2001.
9. Prins GS, Marmer M, Woodham C, Chang W, Kuiper G, Gustafsson JA and Birch L: Estrogen receptor-beta messenger ribonucleic acid ontogeny in the prostate of normal and neonatally estrogenized rats. *Endocrinology* 139: 874-883, 1998.
10. Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA and Smithies O: Generation and reproductive phenotypes of mice lacking estrogen receptor beta. *Proc Natl Acad Sci USA* 95: 15677-15682, 1998.
11. Ban JY and Yoo KH: Promoter polymorphism (rs12770170, -184C/T) of microseminoprotein, beta as a risk factor for benign prostatic hyperplasia in Korean population. *Int Neurourol J* 18: 63-67, 2014.
12. Yoo KH, Kim SK, Chung JH and Chang SG: Nitric oxide synthase 2 gene polymorphisms are associated with prostatic volume in Korean men with benign prostatic hyperplasia. *Asian J Androl* 12: 690-696, 2010.
13. Ryan J, Carrière I, Amieva H, Rouaud O, Berr C, Ritchie K, Scarabin PY and Ancelin ML: Prospective analysis of the association between estrogen receptor gene variants and the risk of cognitive decline in elderly women. *Eur Neuropsychopharmacol* 23: 1763-1768, 2013.

14. Marini H, Curro M, Adamo EB, Polito F, Ferlazzo N, Bitto A, Atteritano M, D'Anna R, Alibrandi A, Altavilla D, *et al*: The ESR2 AluI 1730G>A (rs4986938) gene polymorphism is associated with fibrinogen plasma levels in postmenopausal women. *Gene* 508: 206-210, 2012.
15. Kristiansen W, Andreassen KE, Karlsson R, Aschim EL, Bremnes RM, Dahl O, Fosså SD, Klepp O, Langberg CW, Solberg A, *et al*: Gene variations in sex hormone pathways and the risk of testicular germ cell tumour: A case-parent triad study in a Norwegian-Swedish population. *Hum Reprod* 27: 1525-1535, 2012.
16. Yang TL, Xiong DH, Guo Y, Recker RR and Deng HW: Comprehensive association analyses of IGF1, ESR2 and CYP17 genes with adult height in Caucasians. *Eur J Hum Genet* 16: 1380-1387, 2008.
17. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, *et al*: The structure of haplotype blocks in the human genome. *Science* 296: 2225-2229, 2002.
18. Schuler S, Lattrich C, Skrzypczak M, Fehm T, Ortmann O and Treeck O: Polymorphisms in the promoter region of ESR2 gene and susceptibility to ovarian cancer. *Gene* 546: 283-287, 2014.
19. Gu Z, Wang G and Chen W: Estrogen receptor alpha gene polymorphisms and risk of prostate cancer: A meta-analysis involving 18 studies. *Tumour Biol* 35: 5921-5930, 2014.
20. Chattopadhyay S, Siddiqui S, Akhtar MS, Najm MZ, Deo SV, Shukla NK and Husain SA: Genetic polymorphisms of ESR1, ESR2, CYP17A1 and CYP19A1 and the risk of breast cancer: A case control study from North India. *Tumour Biol* 35: 4517-4527, 2014.
21. Attia DM and Ederveen AG: Opposing roles of ER α and ER β in the genesis and progression of adenocarcinoma in the rat ventral prostate. *Prostate* 72: 1013-1022, 2012.
22. Hammarsten J, Damber JE, Karlsson M, Knutson T, Ljunggren O, Ohlsson C, Pecker R, Smith U and Mellström D: Insulin and free oestradiol are independent risk factors for benign prostatic hyperplasia. *Prostate Cancer Prostatic Dis* 12: 160-165, 2009.
23. García-Flórez M, Oliveira CA and Carvalho HF: Early effects of estrogen on the rat ventral prostate. *Braz J Med Biol Res* 38: 487-497, 2005.
24. McPherson SJ, Hussain S, Balanathan P, Hedwards SL, Niranjana B, Grant M, Chandrasiri UP, Toivanen R, Wang Y, Taylor RA and Risbridger GP: Estrogen receptor-beta activated apoptosis in benign hyperplasia and cancer of the prostate is androgen independent and TNF α mediated. *Proc Natl Acad Sci USA* 107: 3123-3128, 2010.
25. Sadee W, Wang D, Papp AC, Pinsonneault JK, Smith RM, Moyer RA and Johnson AD: Pharmacogenomics of the RNA world: Structural RNA polymorphisms in drug therapy. *Clin Pharmacol Ther* 89: 355-365, 2011.