XbaI and PvuII estrogen receptor alpha gene polymorphism and Y chromosome deletions in infertile vs. fertile men

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The most frequently studied single nucleotide polymorphisms are the ***Pvu*II** (known as IVS1-397 T/C, or rs2234693; where the T and C allele are often reported as the p and P allele) and ***Xba*I** (also known as IVS1-351 A/G or rs9340799; where the A and G allele are often reported as the x and X allele) both located in intron 1 of the ESR1. ESR1 XbaI polymorphism was suggested to have an effect on azoospermia or severe oligospermia in males.

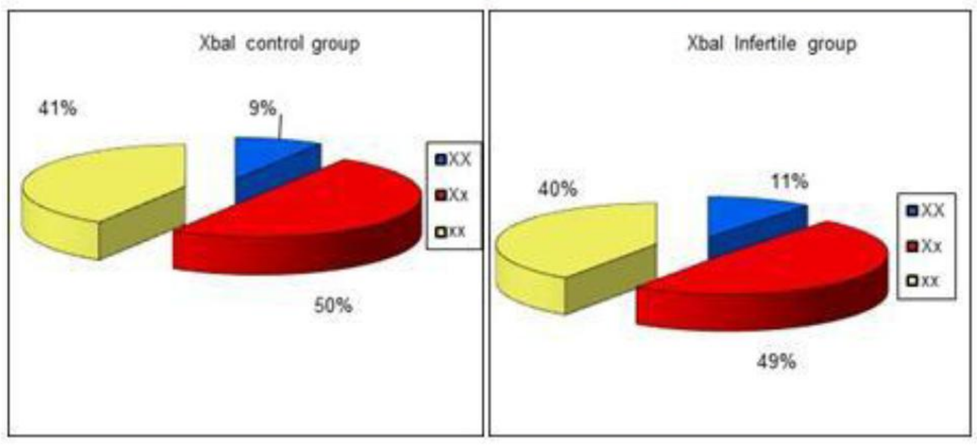
Objective: The aim of this study was to determine the frequencies of estrogen receptor α (ESR1) PvuII and XbaI polymorphisms and Y chromosome deletion in infertile Romanian patients and to investigate their involvement in male infertility.

Subjects and methods: The study was carried out on 49 infertile men, aged between 20 and 50 years, divided into three groups, based on spermatic parameters: group 0-men with azoospermia (17 subjects), group 1-men with severe oligospermia (14 subjects), group 2-men with oligospermia (18 subjects), and 34 controls with the same age without pelvic radiotherapy and/or chemotherapy in the last 6 months, known genetic aberrations, urogenital infections, bilateral orchiectomy, vasectomy, occupational exposure to noxious hydrocarbons organophosphates, ionizing radiation, heavy metals. They were enrolled after signing a written consent form approved by our ethic committee. The hematological, biochemical and hormonal profiles were evaluated. ESR1 (*XbaI* and*PvuII*) polymorphisms were determined by PCR-RFLP method on genomic DNA. Genomic DNA was prepared from whole blood using the Wizard DNA Blood Purification Kit (Promega Inc.). ESR alpha (XbaI and PvuII) polymorphisms were determined by RFLP method. Screening for microdeletions in the azoospermia factor (AZF) region of Y chromosome was performed by multiplex polymerase chain reaction (PCR) with Y chromosome deletion selection system, version 2.0 (Promega Corporation).

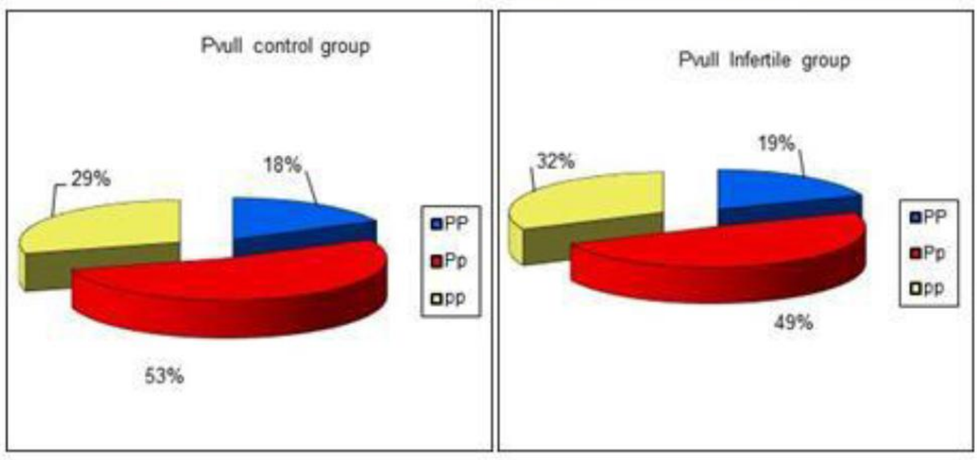
Polymorfism	Primer	PCR-RFLP products, pb
*Pvu*II ESR1 IVS-1 -397 T/C	F: 5'CTGCCACCCTATCTGTATCTTTTCCTATTCTCC3' R: 5'TCTTTCTCTGCCACCCTGGTGTCGATTATCTGA3'	TT (pp) - 438,939 TC (Pp)– 438,939,1374 CC (PP) – 1374
*Xba*I ESR 1 IVS-1 -351 A/G	F: 5'CTGCCACCCTATCTGTATCTTTTCCTATTCTCC3' R: 5'TCTTTCTCTGCCACCCTGGTGTCGATTATCTGA3'	AA (xx) – 393,984 AG (Xx)- 393,984,1374 GG (XX) – 1374

Results: ESR XbaI polymorphism study in infertile patients revealed 5 homozygote (XX), 23 heterozygote (Xx) and 19 homozygote cases (xx). The frequency of X allele in infertile patients was 0.35, and 0.65 for x allele, χ^2=1.299. ESR XbaI polymorphism study in normal patients revealed 3 homozygote (XX), 17 heterozygote (Xx) and 14 homozygote (xx) cases. The frequency of X allele in population was 0.34, for x allele the frequency was 0.66, χ^2=0.464. ESR PvuII polymorphism study in infertile patients pointed to homozygote (PP), 23 were heterozygote (Pp) and 15 were mutant homozygote (pp) cases. The frequency of P allele was 0.44, and 0.56 for P allele, χ^2=0.137. ESR PvuII polymorphism study in normal subjects pointed to 6 homozygote cases for PP allele, 18 heterozygote (Pp) and 10 homozygote cases (pp). The frequency of p allele was 0.44, for P allele the frequency was 0.56, χ^2=0.184. 5.4% of all patients presented microdeletions in AZFc region and 2.7% in AZFb region, 8.11% in AZFb and AZFc regions.

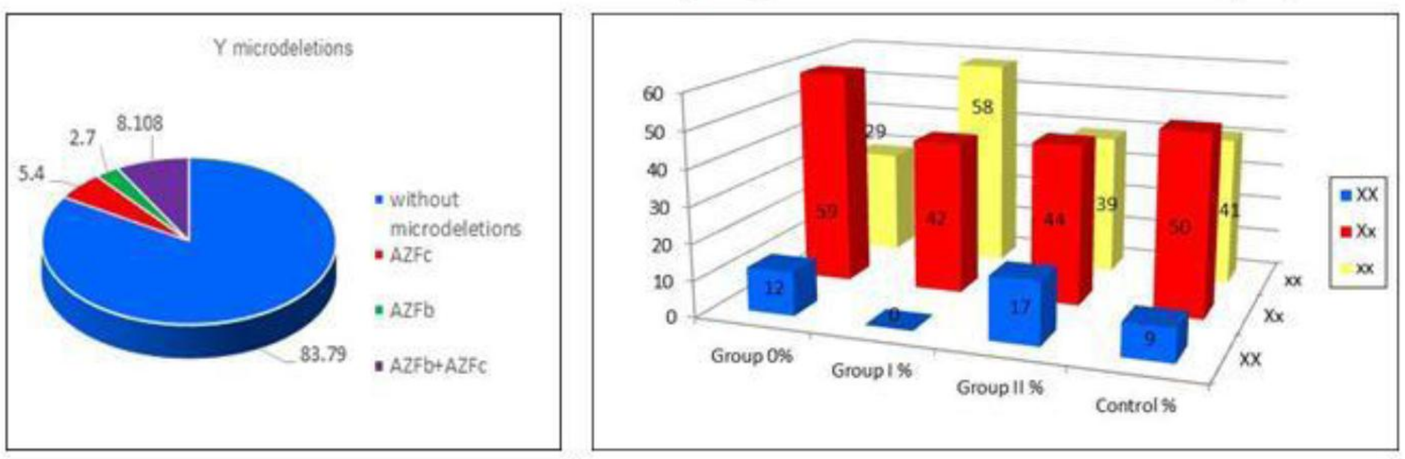
XbaI genotype distribution in control and infertile group



PvuII genotype distribution in control and infertile group

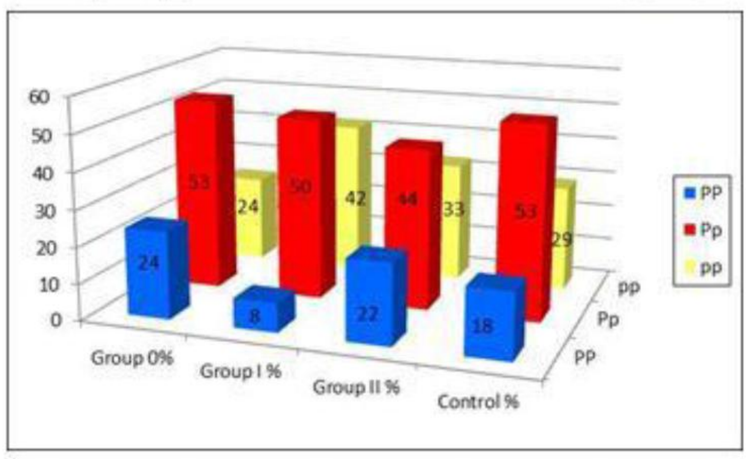


XbaI genotype distribution in control and infertile groups



	Group 0%	Group I %	Group II %	Control %
XX	12	0	17	9
Xx	59	42	44	50
xx	29	58	39	41

PvuII genotype distribution in control and infertile groups



	Group 0%	Group I %	Group II %	Control %
PP	24	8	22	18
Pp	53	50	44	53
pp	24	42	33	29

Conclusion: Even if no statistic significance can be established, a higher percentage of homozygote x and p allele was found in group with severe oligospermia. No significant differences were found for the alleles frequency between the infertile patients and the control group.

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