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Rapid Communication: Genetic Linkage and Physical Mapping of the Porcine Androgen Receptor (AR) Gene

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Abstract

Source and Description of Primers. Primers were designed from published bovine and human androgen receptor (AR) sequences (GenBank accession number Z75315 and M27430, respectively). These primers were used to obtain a porcine sequence (submitted to GenBank: accession number AF079783). A total of 247 bp out of the entire 793-bp PCR product shared 90.3% identity with sections of exons 7 and 8 of the human AR gene. Porcine specific primers (AR-3 and AR-4) were then designed to amplify a 160-bp fragment in intron 7 of the AR gene region from porcine genomic DNA.

Keywords

Pigs, Polymorphism, Microsatellites, Receptors

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Rapid Communication: Genetic Linkage and Physical Mapping of the Porcine *Androgen Receptor* (*AR*) Gene[1]

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Primer Sequences. Forward primer: AR-3: 5′-TGT TTT CCC CCT CTT CCT T-3′ and reverse primer: AR-4: 5′-TCC TTT TTT CCA GCA TAG ACC-3′. Position ranges in GenBank AF079783 (F: 332–350, R: 475–495).

Method of Detection. Polymerase chain reaction was conducted utilizing primers AR-3 and AR-4. Each reaction (10 μL total volume) contained 1 μL of genomic DNA (12.5 ng/μL), 5 pmol of each primer, .25 m*M* of each dNTP, .75 U of *Taq* polymerase (Promega), 1.5 m*M* $MgCl_2$, and 1 × PCR buffer (Promega). An MJ Research model PTC-100 (Watertown, MA) was used for thermocycling as follows: 4 min at 94°C, followed by 33 cycles of 45 s at 92°C, 1 min at 58°C, and 1 min at 72°C, with a final 5-min extension at 72°C. After amplification, the products were electrophoresed on an 8% acrylamide gel and then visualized by ethidium bromide staining for 15 to 20 min. Physical mapping of the marker was completed by genotyping the Somatic Cell Hybrid Panel containing 27 hybrid lines (Yerle et al., 1996) and comparing the results with the established regional assignment (http://www.toulouse.inra.fr/lgc/pig/hybrid/chromo19/chromo19.htm). Linkage mapping of *AR* was accomplished using CRIMAP (version 2.4, Green et al., 1990) and the existing USDA-MARC porcine linkage map (Rohrer et al., 1996).

Description of Polymorphism. A microsatellite polymorphism was recognized in the intron between exon 7 and exon 8. The sequenced products from a Meishan and a Yorkshire pig displayed a compound dinucleotide repetitive sequence: $(GT)_8(GC)(GT)_2(GC)(GT)_4(GC)_{2–3}(GT)_{16}(GA)_8$. Primers were designed flanking a 160-bp region containing a group of these repeats within the intron. Genotyping of the USDA-MARC mapping reference families revealed four alleles. Alleles 1 to 4 were 160 bp, 158 bp, 156 bp, and 154 bp, respectively.

Pattern of Inheritance. Sex-linked segregation was observed in eight two-generation USDA-MARC reference families (Rohrer et al., 1996).

Allelic Frequency. Allele frequencies were determined in 55 unrelated animals representing five breeds from Iowa State University. Allele 1 occurred with a frequency of .11 in Duroc (n = 8) and .15 in Hampshire (n = 11). Allele 2 occurred with a frequency of .22 in Duroc, .44 in Chester White (n = 10), .33 in Yorkshire (n = 9), .75 in Landrace (n = 10), and .69 in Hampshire. Allele 3 occurred with a frequency of .67 in Duroc, .56 in Chester White, .5 in Yorkshire, .16 in Landrace, and .15 in Hampshire. Allele 4 occurred with a frequency of .16 in Yorkshire and .08 in Landrace.

Chromosomal Location. The *AR* gene was physically mapped to the porcine X chromosome (SSCX) q13 (Figure 1) with a correlation coefficient of .86 and an error risk of less than .1%. Based on the linkage analysis with the USDA-MARC pig linkage map (Rohrer et al., 1996), the *AR* gene was confirmed to be on SSCX and closely linked to several previously mapped markers. Linked markers and their 2-point LOD scores (in parenthesis) were *SW2470* (27.98), *SWR1861* (28.08), and *SW2476* (28.00), *SW1943* (23.66), *HPRT1* (12.11), and *SW2453* (21.61).

Comments. The AR is a member of the steroid receptor family and serves as a receptor for dihydrotestosterone, which controls differentiation of

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the male sex organs (Lubahn et al., 1989). The *AR* gene contains eight exons and has a total genomic size of approximately 90 kb in humans (Kuiper et al., 1989). The *AR* mutation effects can include infertility and receptor insensitivity in humans (Lubahn et al., 1989). The particular microsatellite location found in this study was also discovered to be similar in the bovine *AR* gene (Altschul et al., 1990), and this may reflect a common origin and a conserved genomic structure.

Literature Cited

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Green, P., K. Falls, and S. Crooks. 1990. Documentation for CRI-MAP, version 2.4, Washington University School of Medicine, St. Louis, MO.

Kuiper, G.G.J.M., P. W. Faber, H.C.J. van Rooij, J.A.G.M. van der Korput, C. Ris-Stalpers, P. Klaassen, J. Trapman, and A. O. Brinkmann. 1989. Structural organization of the human androgen receptor gene. J. Mol. Endocrinol. 2:R1–R4.

Lubahn, D. B., T. R. Brown, J. A. Simental, H. N. Higgs, C. J. Migeon, E. M. Wilson, and F. S. French. 1989. Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. Proc. Natl. Acad. Sci. USA 86:9534–9538.

Rohrer, G. A., L. J. Alexander, Z. Hu, T. P. Smith, J. W. Keele, and C. W. Beattie. 1996. A comprehensive map of the porcine genome. Genome Res. 6:371–391.

Yerle, M., G. Echard, A. Robic, A. Mairal, C. Dubut-Fontana, J. Riquet, P. Pinton, D. Milan, Y. Lahbib-Mansais, and J. Gellin. 1996. A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. Cytogenet. Cell Genet. 73(3):194–202.

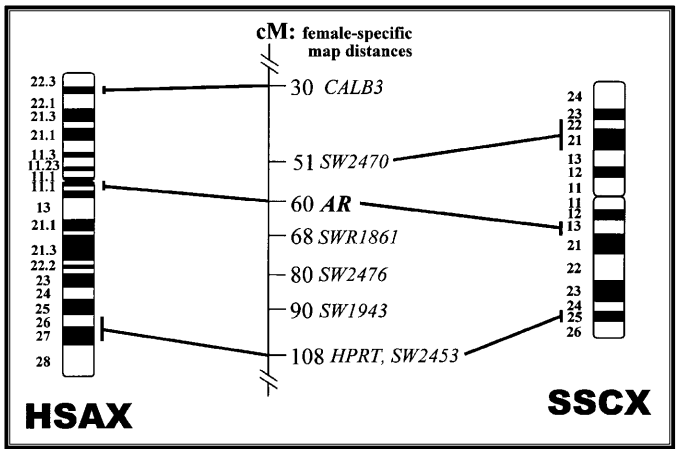

Figure 1. Schematic diagram showing the physical and linkage map assignment of the *AR* gene to porcine chromosome X. The linkage map position of the *AR* gene is presented with a vertical bar showing the closest estimates of linkage distances in centimorgans (cM) relative to the selected markers on the published USDA-MARC porcine gene map. The genes or markers physically assigned to human and porcine X chromosomes are indicated with solid lines connecting their physical map positions.

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