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(54) METHOD FOR TESTING THE HORMONAL EFFECT OF SUBSTANCES

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(57) **ABSTRACT**

A method for testing the hormonal effect, especially the androgenic or antiandrogenic effect, of substances is described, in which (a) cells which are transfected with two vectors, one of these vectors containing DNA which codes for a nuclear receptor protein or a fragment thereof, whereas the other vector contains DNA which codes for a co-modulator or a fragment thereof, are exposed to the substance; and (b) the transcription activity which the nuclear receptor or its fragment induces in the presence of the co-modulator or its fragment and/or the effect of the substance on the interaction between the receptor or its fragment and the co-modulator or its fragment is measured by the protein-protein interaction or the protein-protein-DNA interaction. In addition, a method for determining defects in the co-modulation mechanism and means suitable for performing this method are provided.

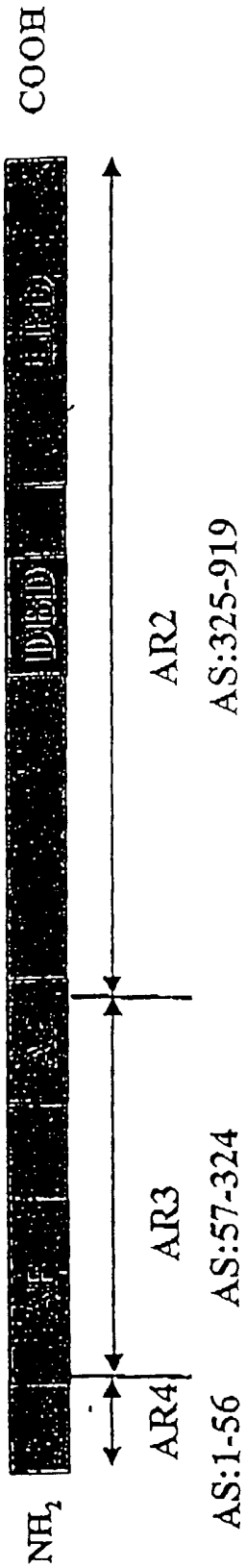

Figure 1. AF = activation function; DBD = DNA binding domain; LBD = ligand binding domain; and AS = amino acid

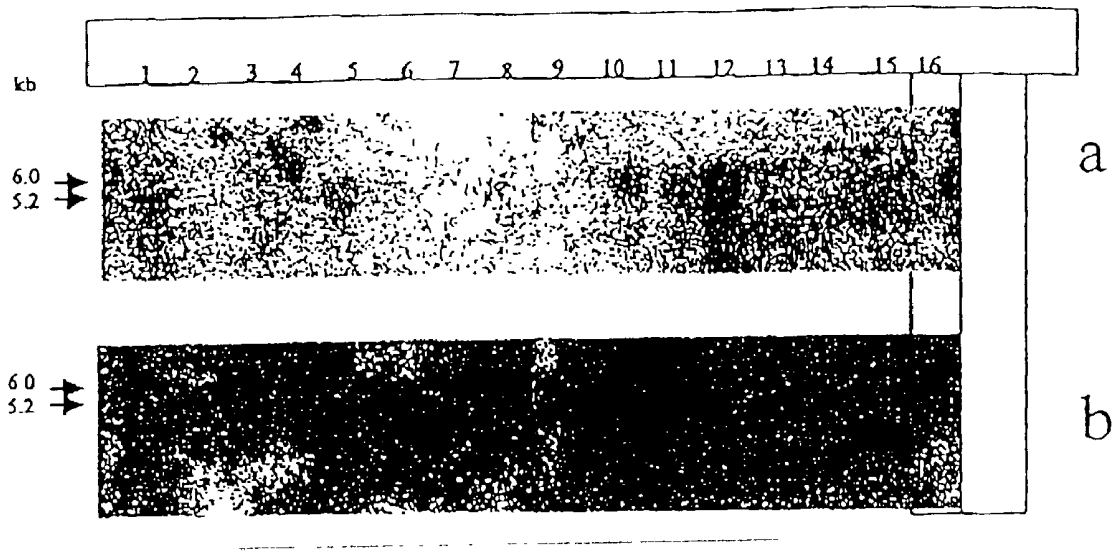

Figure 2. (1) heart; (2) brain; (3) placenta; (4) lung; (5) liver; (6) skeletal muscle; (7) kidney; (8) pancreas; (9) spleen; (10) thymus; (11) prostate; (12) testicles; (13) ovary; (14) small intestine; (15) large intestine; and (16) peripheral leukocytes.

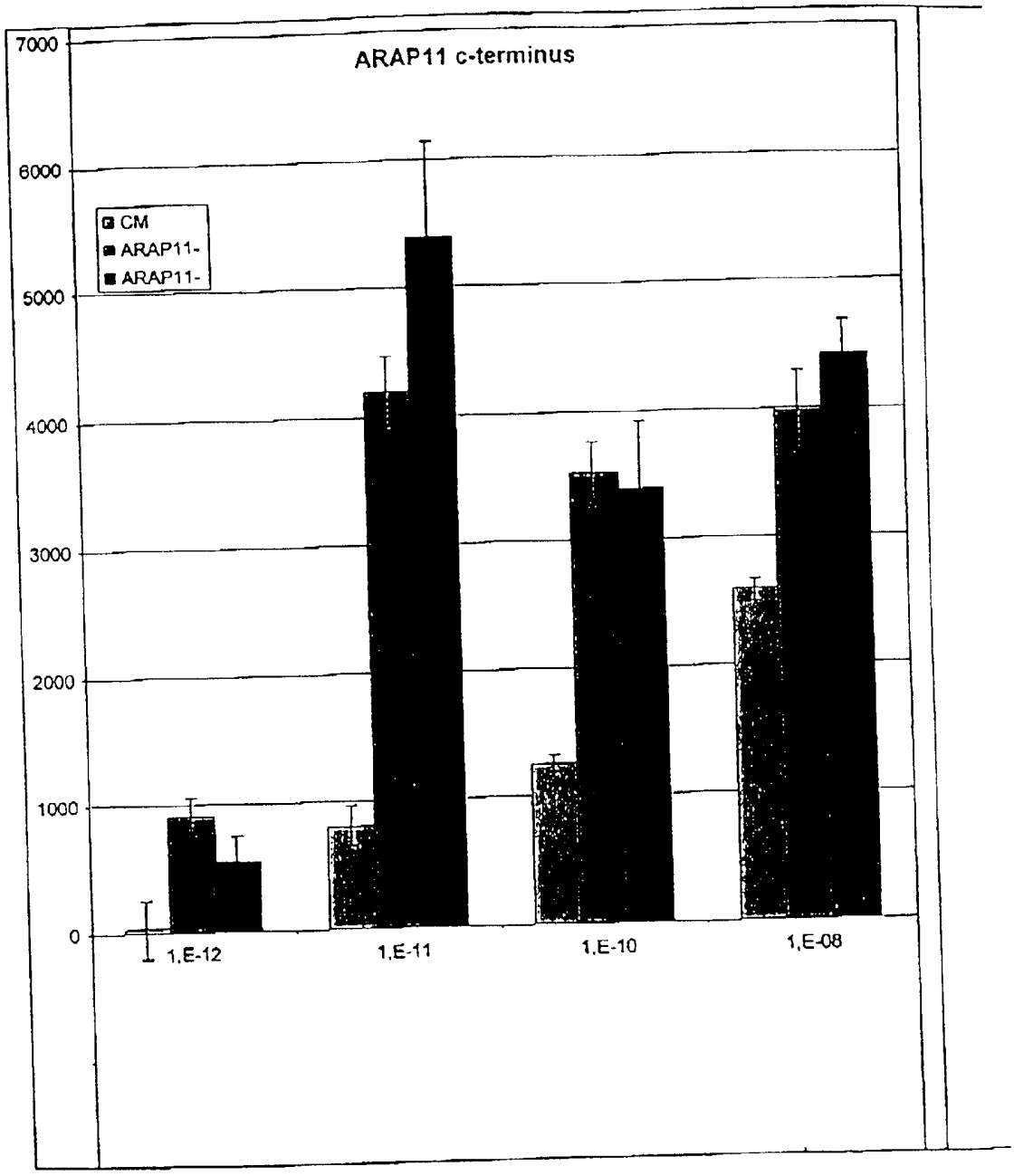
ARAP11 c-terminus
7000
6000
5000
4000
3000
2000
1000
0
CM
ARAP11-
ARAP11-
1,E-12
1,E-11
1,E-10
1,E-08

Figure 3

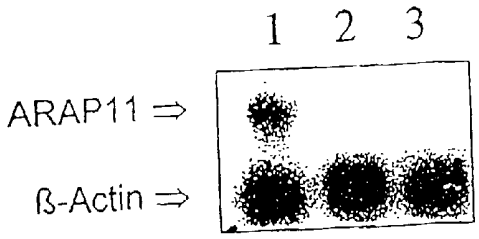

Figure 4.

METHOD FOR TESTING THE HORMONAL EFFECT OF SUBSTANCES

BACKGROUND OF THE INVENTION

[0001] It is well known that a class of compounds known as androgens are the hormonal signals responsible for maleness in mammals in general and human beings in particular. As with most hormonal signals, androgens interact with their targets by binding to a receptor, known as the androgen receptor. Recognition of androgens by the androgen receptor starts a series of transcriptional events giving rise to male-associated processes in certain organs and tissues. The binding of androgens to the androgen receptor is also an important process in many androgen related diseases and conditions, such as baldness and acne, as well as important clinical diseases such as prostate cancer. The androgen receptor belongs to the steroid receptor super family that plays a significant role in male sexual differentiation and prostate cell proliferation. Abnormal expressions or mutations of the androgen receptor in prostate cells may play an important role in the progression of prostate cancer.

[0002] When bound to androgens and androgen responsive elements, the androgen receptor can up-regulate or down-regulate the expression of androgen target genes through a complicated process that may involve multiple adaptors or co-activators. An important problem in the field of steroid hormone regulation is the question or how specific androgen-activated transcription can be achieved in vivo when several different receptors recognize the same DNA sequence. For example, the androgen receptor (AR), the glucocorticoid receptor (GR) and the progesterone receptor (PR) all recognize the same sequence but activate different transcription activities. It has been speculated by some that accessory factors may selectively interact with the androgen receptor to determine the specificity of the androgen receptor target gene activation.

[0003] One of the important uses for the androgen receptor is for testing the androgenic or anti-androgenic effects of specific candidate human pharmaceutical molecules. The androgenic effect of pharmaceuticals is usually an attribute of potential candidate therapeutic medicines that must be evaluated during the process of evaluating a molecule for human therapeutic value. Accordingly, the androgen receptor is used in screens to determine the frequency and specificity by which specific molecules bind to such receptors.

DESCRIPTION OF THE INVENTION

[0004] The present invention relates generally to a method for testing the hormonal effect of substances. More specifically, the invention is directed to a method for determining defects in the co-modulation mechanism of nuclear receptors, and to means suitable for implementing these methods, especially the co-activator ARAP11 for the human androgen receptor and for other nuclear receptors as well as the DNA coding for these means.

[0005] When substances are evaluated for their biological activity with respect to their possible pharmaceutical applications, it is general practice to test these substances for any possible hormonal effects they may have, especially for any androgenic or anti-androgenic activity. When pharmacologically active substances are administered, knowledge concerning the hormonal effects, especially the androgenic and anti-androgenic effects, of these substances are often important, because they can cause adverse side effects in the patient. To test the hormonal action of substances, it is possible in particular to use methods which measure the ability of the substances to bind to hormone receptors and to activate their transcriptional activity.

[0006] Knowledge of the hormonal effects of substances is of interest not only in the case of potential drugs but also in the case of non-pharmaceutical substances, because it is assumed that many substances present in the environment can show androgenic or anti-androgenic effects or estrogenic or anti-estrogenic effects in certain portions of the population. It is therefore possible for undesirable, harmful effects to be produced.

[0007] There is therefore a very considerable need for a method and a means suitable for implementing the method by means of which information concerning the hormonal effect of substances can be obtained in a reliable, sensitive, simple, low-cost, and rapid manner. The methods known so far do not meet these requirements.

[0008] The present invention is therefore based on the task of providing a method and means suitable for implementing the method by means of which information concerning the hormonal effect of substances to be tested can be obtained in a reliable, sensitive, simple, low-cost, and rapid manner.

[0009] The objective of the invention is achieved surprinsingly by a method for testing the hormonal effect, especially of the androgenic or anti-androgenic effect, of substances in which:

[0010] (a) cells which are transfected with two vectors, one of which contains DNA coding for a nuclear receptor protein or a fragment thereof, especially a human nuclear receptor or a fragment thereof, whereas the other vector contains DNA which codes for a co-modulator or a fragment thereof, are exposed to the substance; and

[0011] (b) the transcription activity which the nuclear receptor or its fragment induces in the presence of the co-modulator or its fragment and/or the effect of the substance on the interaction between the receptor or its fragment and the co-modulator or its fragment is measured by the protein-protein interaction or the protein-protein-DNA interaction.

[0012] The surprising discovery was made that the method according to the invention makes it possible to determine whether or not substances which can be of interest from, for example, an environmental or a pharmacological standpoint exert a hormonal effect, especially an androgenic or anti-androgenic effect, in a reliable, sensitive, simple, rapid, and low-cost manner.

[0013] In the process according to the invention, cells which have been transformed with a vector are used. The vectors contain DNA which codes for a nuclear receptor protein or a fragment thereof.

[0014] The superfamily of nuclear receptors (NRs), to which more than 50 different proteins belong, is a group of related transcription factors, which control the transcription of the individual target gene in reaction to specific ligands, e.g., hormones. The family can be divided into several

subfamilies on the basis of certain characteristics such as dimerization status, type of ligand, and structure of the DNA reaction element (Beato et al., *Human Reproduct. Update*, Vol.6, pp. 225-236, 2000). A characteristic feature of the NRs is the similarity of the structure of their functional domains (with the designations A-F), consisting of a highly variable, only weakly preserved N-terminal region with an autonomous constitutive activation function (AF-1); a strongly preserved DNA binding domain (DBD), which is responsible for the detection of specific DNA reaction elements and consists of two Zinkfinger motifs; a variable hinge domain; and a preserved, multi-functional C-terminal ligand binding domain (LBD) with a dimerization-dependent and ligand-dependent transactivation function (AF-2). Following after this is the region the farthest away from the C-terminal, the function of which is not known and which is absent in receptors such as PR (progesterone receptor), PPAR (peroxisome proliferator-activated receptor), and RXR (retinoid-X receptor) (Mangelsdorf & Evans, *Cell*, Vol. 83, pp. 841-850, 1995; Robyr et al., *Mol. Endocrinol.*, Vol. 14, pp. 329-347, 2000). For some of the NRs (e.g., the androgen receptor (AR)), it has been found that the N-terminal region is able to interact with the C-terminal region (Brinkmann et al., *J. Steroid Biochem. and Mol. Biol.*, Vol 69, pp. 307-313, 1999). Steroid hormonal receptors such as estrogen receptor (ER), progesterone receptor (PR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and androgen receptor (AR) bind steroidal ligands derived from pregnenolone such as the progestins, the estrogens, the glucocorticoids, the mineralocorticoids, and the androgens. The binding of the ligand activates the receptor and controls the expression of the corresponding target genes.

[**0015**] As explained above, the cells used in step (a) of the method according to the invention contain a vector with a DNA which codes for a co-modulator or a fragment thereof.

[**0016**] The co-modulators are a class of proteins which serve as bridge molecules between the transcription initiation complex and the NRs during the activation (co-activators) or repression (co-repressors) of gene transcription (McKenna et al., *Endocr. Rev.*, Vol. 20, pp. 321-347, 1999). A co-activator must be able to intensify the receptor function and interact directly with the activation domain of NRs in the presence of an agonist. It must also interact with the basic transcription apparatus, but, finally, it may not itself intensify the basic transcription apparatus. Most co-modulators interact with the help of one or more LXXLL motifs (NR boxes) with the AF-2 domain of NRs, but several co-modulators have also been described which interact with other NR regions (Ding et al., *Mol. Endocrinol.*, Vol 12, pp. 302-313, 1998). In addition, many co-modulators have been identified which interact in a similar manner with several different NRs, which suggests that it would be useful to determine the degree of specificity of each co-modulator.

[**0017**] In a preferred embodiment of the method according to the invention, the co-modulator designated ARAP11 or the fragment of ARAP11 containing the amino acids 813-1390 is used. SEQ ID No. 1 and SEQ ID No. 2 show, respectively, the cDNA sequence of the co-modulator ARAP11 and the amino acid sequence of this co-modulator containing the 1390 amino acids. When these proteins are used, it is possible to implement the method according to the invention in an especially reliable, sensitive, simple, low-cost, and rapid manner. In addition, the ARAP11 fragments, especially the fragment of ARAP11 containing amino acids 813-1390, offer the advantage that they are easier to manage and are clonable while still having the functional properties of ARAP11.

[**0018**] ARAP11 is a co-activator for the human androgen receptor and other nuclear receptors; it increases the interaction between an androgen and the receptor. A portion of the sequence of ARAP11 has already been described as Pro2000 in the gene bank XM 005253, but no function is indicated there for it. In comparison with the sequence already known from the gene bank, it has now been established that the amino acid sequence of ARAP11 is larger than the known sequence: it has additional amino acids in the N-terminal region. In addition, it has also been possible to establish that there is an interaction between nuclear receptors, especially AR, and ARAP11, as well as an intensification of AR-mediated transactivation. ARAP11 is a protein which functions as a co-mediator, in that it intensifies or represses the transcription effect after steroids have become bound to the nuclear receptor, and it also promotes the binding and activation of the nuclear receptor to molecules to which no hormonal action was ascribed in the past.

[**0019**] The protein ARAP11 represents a co-activator for the androgen receptor and other nuclear receptors such as estrogen receptor α, estrogen receptor β, progesterone receptor A, progesterone receptor B, glucocorticoid receptor, mineralocorticoid receptor, thyroid hormone receptor, vitamin D receptor, peroxisome proliferator-activated receptor, retinoic acid receptor, retinoid-X receptor, and orphan receptors; in the method according to the invention, these are the preferred receptors, because with them the above-indicated advantages of the method according to the invention can be achieved in an especially favorable manner.

[**0020**] In the process according to the invention, it is also possible to use vectors which code for fragments of the above proteins. "Fragments" in conjunction with the above proteins are understood to be those which have one amino acid or several amino acids less than the full-length proteins but which still have the functional properties of a nuclear receptor or of a co-modulator.

[**0021**] As already explained above, cells which are transfected with two vectors which contain DNA coding for special proteins are used in step (a) of the method according to the invention. These cells are therefore able to express these two different proteins.

[**0022**] The cells are preferably established cell lines and/or eukaryotic cells, especially prostate cells, nerve cells, glial cells, fibroblasts, blood cells, osteoblasts, osteoclasts, hepatocytes, epithelial cells, or muscle cells. By the use of established cell lines, the process according to the invention can be implemented in an especially low-cased and rapid manner. When eukaryotic cells are used, especially the eukaryotic cells listed above, the method according to the invention makes it possible to obtain especially informative results in an advantageous manner.

[**0023**] In a preferred embodiment of the process according to the invention, eukaryotic expression vectors are used such as pCMX or pSG5. When these vectors are used, especially when they are used in conjunction with the above established cell lines and/or eukaryotic cells, the process according to the invention can be carried out especially favorably and quickly, and especially informative results are obtained.

[0024] The expert is familiar with methods and the materials required for inserting the DNA coding for the above proteins into a vector, for introducing this vector into the cells, and for cultivating the cells thus obtained under suitable culture conditions so that they can express these proteins.

[0025] According to step (b) of the invention, the transcription activity which the nuclear receptor or its fragment induces in the presence of the co-modulator or its fragment is measured. This can be done, for example, by the detection of a reporter gene.

[0026] Reporter genes are genes or gene fragments which are coupled with other genes or regulatory sequences in such a way as to make the activity of these sequences detectable. Reporter genes generate gene products which are extremely easy to detect with a photometer as a result of color reactions, for example. Frequently used reporter genes are the gene for β-galactosidase, the gene for alkaline phosphatase, the gene for chloramphenicol acetyl transferase, the gene for catechol dioxygenase, the gene for "green fluorescent protein", and various luciferase genes, which can cause the cells to produce light.

[0027] Such reporter genes can also be introduced into the cells by vectors, especially eukaryotic expression vectors. An example of a vector which contains a reporter gene-coding DNA is the vector MMTV-luciferase, which is used to measure the androgenic effect of substances.

[0028] Substances with a hormonal effect, especially with a androgenic/antiandrogenic effect, can then be recognized by the elevated or reduced activity of the reporter gene.

[0029] The influence of the test substance on the interaction between the receptor or its fragment and the co-modulator or its fragment can also be measured by determining the protein-protein interaction, e.g., by the use of yeast two hybrid systems, by immunoprecipitation, by GST pull-down assays, by FRET analysis, and by ABCD assays. It can also be measured by determining the protein-protein-DNA interaction by means of gel retardation assays.

[0030] It has also been found that ARAP11 can be used very effectively as an indicator of androgen-caused disorders, some of which do not occur until mature years. Relevant androgen-caused disorders such as prostate cancer, erectile dysfunction, infertility, baldness, acne, and hypogonadism and androgen resistance syndromes such as testicular feminization are based on defects in the co-modulation mechanism between AR and ARAP11. A possibility in patients with these types of disorders thus consists in measuring the relative concentrations of AR and ARAP11. This measurement can be made favorably outside the body in body fluids, body cells, or body tissue. This is possible through the use of quantitative methods for measuring the relative quantity of the two molecules in the patient in question, in which methods, for example, antibodies against both AR and against ARAP 11 or nucleic acid probes against their mRNA can be used. There are several methods for measuring these comaprative values, which are known to the expert. The expert also knows suitable materials and devices such as radioimmunoassay, the ELISA test, immunostaining, RT-PCR, Western Blot, Northern Blot, DNA microarrays, and protein microarrays. With the help of ARAP11-cDNA, it is also possible to construct probes in the conventional manner for a PCR assay, by means of which, in certain patients, mutations of the normal DNA sequence can be detected or transcripts for the Northern Blot Assay or a DNA for in-situ hybridization assays can be produced.

[0031] The measured ratio of AR to ARAP11 can be greater or less than that present in healthy persons. The normal value of a healthy person can be easily determined by measuring the ratio of AR to ARAP11 in a large number of healthy test subjects. By comparison of the normal value with the ratio of AR to ARAP11 found in the patients to be studied, it can be established whether the value for the determined ratio is greater or less than the normal value.

[0032] The concentration of ARAP11 and/or of AR in tissues can vary. For example, it is possible for the concentration of ARAP11 to be very high in the testicles but lower in the liver, heart, thymus, and prostate. It is therefore necessary to take the differing tissue concentrations into consideration when making an evaluation; that is, the test value and the normal value should originate from the same tissue.

[0033] Another way in which defects in the co-modulation mechanism between AR and ARAP11 can be determined is to measure only the concentration of ARAP11, it being assumed here that the AR concentration is at least approximately constant. If a lower than normal ARAP11 concentration has been measured, this means that the ratio of AR to ARAP11 has shifted, which serves in turn as an indication of a defect in the co-modulation mechanism.

[0034] It is also possible to use an ARAP11-specific probe to determine changes in the expression of ARAP11 and thus changes in the ratio to AR. Such changes can be causally involved in various diseases or occur as a consequence of such diseases.

[0035] These types of measurements of the AR/ARAP11 ratio or of ARAP11 are based on the surprising insight, which is based on the discovery and characterization of ARAP11, that an androgen resistance syndrome, for example, can be traced back to a disturbance in the equilibrium between AR and ARAP11 prevalence in the target cells. Too much ARAP11 could lead to a hypersensitivity of the AR system, so that it reacts to molecules which normally do not have any androgenic effect. Conversely, the absence or a malfunction of ARAP11 leads to androgen resistance on all levels. The detection of too much ARAP11 in a patient would suggest the need for down-regulation agents such as antisense or similar medications to reduce the ARAP11 titer in the patient in question under clinical conditions. The same goal can be achieved by molecules which are able to inhibit the interaction between AR and ARAP11. If a patient has too little ARAP11, he can be supplied with ARAP11-cDNA, ARAP11-protein, or ARAP11-DNA via various mechanisms known in and of themselves to increase the titer of active ARAP11. It is also possible to elevate the concentration or the activity of ARAP11 by low-molecular drugs or by stimulation of natural synthesis by means of specific ARAP11-promoter proteins.

[0036] As can be seen from the discussion of the method according to the invention presented above, the protein ARAP11 is highly suitable for implementing the method. Another object of the present invention is therefore the ARAP11 with the following amino acid sequence:

```
Met Val Val Leu Arg Ser Ser Leu Glu Leu His Asn His Ser Ala Ala
  1               5                  10                  15

Ser Ala Thr Gly Ser Leu Asp Leu Ser Ser Asp Phe Leu Ser Leu Glu
             20                  25                  30

His Ile Gly Arg Arg Arg Leu Arg Ser Ala Gly Ala Ala Gln Lys Lys
        35                  40                  45

Pro Ala Ala Thr Thr Ala Lys Ala Gly Asp Gly Ser Ser Val Lys Glu
     50                  55                  60

Val Glu Thr Tyr His Arg Thr Arg Ala Leu Arg Ser Leu Arg Lys Asp
 65                  70                  75                  80

Ala Gln Asn Ser Ser Asp Ser Ser Phe Glu Lys Asn Val Glu Ile Thr
                85                  90                  95

Glu Gln Leu Ala Asn Gly Arg His Phe Thr Arg Gln Leu Ala Arg Gln
            100                 105                 110

Gln Ala Asp Lys Lys Lys Glu Glu His Arg Glu Asp Lys Val Ile Pro
        115                 120                 125

Val Thr Arg Ser Leu Arg Ala Arg Asn Ile Val Gin Ser Thr Glu His
    130                 135                 140

Leu His Glu Asp Asn Gly Asp Val Glu Val Arg Arg Ser Cys Arg Ile
145                 150                 155                 160

Arg Ser Arg Tyr Ser Gly Val Asn Gln Ser Met Leu Phe Asp Lys Leu
                165                 170                 175

Ile Thr Asn Thr Ala Glu Ala Val Leu Gln Lys Met Asp Asp Met Lys
            180                 185                 190

Lys Met Arg Arg Gln Arg Met Arg Glu Leu Glu Asp Leu Gly Val Phe
        195                 200                 205

Asn Glu Thr Glu Glu Ser Asn Leu Asn Met Tyr Thr Arg Gly Lys Gln
    210                 215                 220

Lys Asp Ile Gln Arg Thr Asp Glu Glu Thr Thr Asp Asn Gln Glu Gly
225                 230                 235                 240

Ser Val Glu Ser Ser Glu Glu Gly Glu Asp Gln Glu His Glu Asp Asp
                245                 250                 255

Gly Glu Asp Glu Asp Asp Glu Asp Asp Asp Asp Asp Asp Asp Asp Asp
            260                 265                 270

Asp Asp Asp Asp Asp Glu Asp Asp Glu Asp Glu Glu Asp Gly Glu Glu
        275                 280                 285

Gln Asn Gln Lys Arg Tyr Tyr Leu Arg Gln Arg Lys Ala Thr Val Tyr
    290                 295                 300

Tyr Gln Ala Pro Leu Glu Lys Pro Arg His Gln Arg Lys Pro Asn Ile
305                 310                 315                 320

Phe Tyr Ser Gly Pro Ala Ser Pro Ala Arg Pro Arg Tyr Arg Leu Ser
                325                 330                 335

Ser Ala Gly Pro Arg Ser Pro Tyr Cys Lys Arg Met Asn Arg Arg Arg
            340                 345                 350

His Ala Ile His Ser Ser Asp Ser Thr Ser Ser Ser Ser Ser Glu Asp
        355                 360                 365

Glu Gln His Phe Glu Arg Arg Arg Lys Arg Ser Arg Asn Arg Ala Ile
    370                 375                 380

Asn Arg Cys Leu Pro Leu Asn Phe Arg Lys Asp Gln Leu Lys Gly Ile
385                 390                 395                 400

Tyr Lys Asp Arg Met Lys Ile Gly Ala Ser Leu Ala Asp Val Asp Pro
                405                 410                 415
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-continued

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Met Gln Leu Asp Ser Ser Val Arg Phe Asp Ser Val Gly Gly Leu Ser
            420                 425                 430

Asn His Ile Ala Ala Leu Lys Glu Met Val Val Phe Pro Leu Leu Tyr
        435                 440                 445

Pro Glu Val Phe Glu Lys Phe Lys Ile Gln Pro Pro Arg Gly Cys Leu
    450                 455                 460

Phe Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Val Ala Arg Ala Leu
465                 470                 475                 480

Ala Asn Glu Cys Ser Gln Gly Asp Lys Arg Val Ala Phe Phe Met Arg
                485                 490                 495

Lys Gly Ala Asp Cys Leu Ser Lys Trp Val Gly Glu Ser Glu Arg Gln
            500                 505                 510

Len Arg Leu Leu Phe Asp Gln Ala Tyr Gln Met Arg Pro Ser Ile Ile
        515                 520                 525

Phe Phe Asp Glu Ile Asp Gly Leu Ala Pro Val Arg Ser Ser Arg Gln
    530                 535                 540

Asp Gln Ile His Ser Ser Ile Val Ser Thr Leu Leu Ala Leu Met Asp
545                 550                 555                 560

Gly Leu Asp Ser Arg Gly Glu Ile Val Val Ile Gly Ala Thr Asn Arg
                565                 570                 575

Leu Asp Ser Ile Asp Pro Ala Leu Arg Arg Pro Gly Arg Phe Asp Arg
            580                 585                 590

Glu Phe Leu Phe Ser Leu Pro Asp Lys Glu Ala Arg Lys Glu Ile Leu
        595                 600                 605

Lys Ile His Thr Arg Asp Trp Asn Pro Lys Pro Leu Asp Thr Phe Leu
    610                 615                 620

Glu Glu Leu Ala Glu Asn Cys Val Gly Tyr Cys Gly Ala Asp Ile Lys
625                 630                 635                 640

Ser Ile Cys Ala Glu Ala Ala Leu Cys Ala Leu Arg Arg Arg Tyr Pro
                645                 650                 655

Gln Ile Tyr Thr Thr Ser Glu Lys Leu Gln Leu Asp Leu Ser Ser Ile
            660                 665                 670

Asn Ile Ser Ala Lys Asp Phe Glu Val Ala Met Gln Lys Met Ile Pro
        675                 680                 685

Ala Ser Gln Arg Ala Val Thr Ser Pro Gly Gln Ala Leu Ser Thr Val
    690                 695                 700

Val Lys Pro Leu Leu Gln Asn Thr Val Asp Lys Ile Leu Glu Ala Leu
705                 710                 715                 720

Gln Arg Val Phe Pro His Ala Glu Phe Arg Thr Asn Lys Thr Leu Asp
                725                 730                 735

Ser Asp Ile Ser Cys Pro Leu Leu Glu Ser Asp Leu Ala Tyr Ser Asp
            740                 745                 750

Asp Asp Val Pro Ser Val Tyr Glu Asn Gly Leu Ser Gln Lys Ser Ser
        755                 760                 765

His Lys Ala Lys Asp Asn Phe Asn Phe Leu His Leu Asn Arg Asn Ala
    770                 775                 780

Cys Tyr Gln Pro Met Ser Phe Arg Pro Arg Ile Leu Ile Val Gly Glu
785                 790                 795                 800

Pro Gly Phe Gly Gln Gly Ser His Leu Ala Pro Ala Val Ile His Ala
                805                 810                 815

Leu Glu Lys Phe Thr Val Tyr Thr Leu Asp Ile Pro Val Leu Phe Gly
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-continued

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           820                 825                 830

Val Ser Thr Thr Ser Pro Glu Glu Thr Cys Ala Gln Val Ile Arg Glu
        835                 840                 845

Ala Lys Arg Thr Ala Pro Ser Ile Val Tyr Val Pro His Ile His Val
    850                 855                 860

Trp Trp Glu Ile Val Gly Pro Thr Leu Lys Ala Thr Phe Thr Thr Leu
865                 870                 875                 880

Leu Gln Asn Ile Pro Ser Phe Ala Pro Val Leu Leu Leu Ala Thr Ser
                885                 890                 895

Asp Lys Pro His Ser Ala Leu Pro Glu Glu Val Gln Glu Leu Phe Ile
            900                 905                 910

Arg Asp Tyr Gly Glu Ile Phe Asn Val Gln Leu Pro Asp Lys Glu Glu
        915                 920                 925

Arg Thr Lys Phe Phe Glu Asp Leu Ile Leu Lys Gln Ala Ala Lys Pro
    930                 935                 940

Pro Ile Ser Lys Lys Lys Ala Val Leu Gln Ala Leu Glu Val Leu Pro
945                 950                 955                 960

Val Ala Pro Pro Pro Glu Pro Arg Ser Leu Thr Ala Glu Glu Val Lys
                965                 970                 975

Arg Leu Glu Glu Gln Glu Glu Asp Thr Phe Arg Glu Leu Arg Ile Phe
            980                 985                 990

Leu Arg Asn Val Thr His Arg Leu Ala Ile Asp Lys Arg Phe Arg Val
        995                1000                1005

Phe Thr Lys Pro Val Asp Pro Asp Glu Val Pro Asp Tyr Val Thr Val
    1010                1015                1020

Ile Lys Gln Pro Met Asp Leu Ser Ser Val Ile Ser Lys Ile Asp Leu
1025                1030                1035                1040

His Lys Tyr Leu Thr Val Lys Asp Tyr Leu Arg Asp Ile Asp Leu Ile
                1045                1050                1055

Cys Ser Asn Ala Leu Glu Tyr Asn Pro Asp Arg Asp Pro Gly Asp Arg
            1060                1065                1070

Leu Ile Arg His Arg Ala Cys Ala Leu Arg Asp Thr Ala Tyr Ala Ile
        1075                1080                1085

Ile Lys Glu Glu Leu Asp Glu Asp Phe Glu Gln Leu Cys Glu Glu Ile
    1090                1095                1100

Gln Glu Ser Arg Lys Lys Arg Gly Cys Ser Ser Ser Lys Tyr Ala Pro
1105                1110                1115                1120

Ser Tyr Tyr His Val Met Pro Lys Gln Asn Ser Thr Leu Val Gly Asp
                1125                1130                1135

Lys Arg Ser Asp Pro Glu Gln Asn Glu Lys Leu Lys Thr Pro Ser Thr
            1140                1145                1150

Pro Val Ala Cys Ser Thr Pro Ala Gln Leu Lys Arg Lys Ile Arg Lys
        1155                1160                1165

Lys Ser Asn Trp Tyr Leu Gly Thr Ile Lys Lys Arg Arg Lys Ile Ser
    1170                1175                1180

Gln Ala Lys Asp Asp Ser Gln Asn Ala Ile Asp His Lys Ile Glu Ser
1185                1190                1195                1200

Asp Thr Glu Glu Thr Gln Asp Thr Ser Val Asp His Asn Glu Thr Gly
                1205                1210                1215

Asn Thr Gly Glu Ser Ser Val Glu Glu Asn Glu Lys Gln Gln Asn Ala
            1220                1225                1230
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Ser Glu Ser Lys Leu Glu Leu Arg Asn Asn Ser Asn Thr Cys Asn Ile
       1235                1240                1245

Glu Asn Glu Leu Glu Asp Ser Arg Lys Thr Thr Ala Cys Thr Glu Leu
   1250                1255                1260

Arg Asp Lys Ile Ala Cys Asn Gly Asp Ala Ser Ser Ser Gln Ile Ile
1265                1270                1275                1280

His Ile Ser Asp Glu Asn Glu Gly Lys Glu Met Cys Val Leu Arg Met
               1285                1290                1295

Thr Arg Ala Arg Arg Ser Gln Val Glu Gln Gln Gln Leu Ile Thr Val
           1300                1305                1310

Glu Lys Ala Leu Ala Ile Leu Ser Gln Pro Thr Pro Ser Leu Val Val
       1315                1320                1325

Asp His Glu Arg Leu Lys Asn Leu Leu Lys Thr Val Val Lys Lys Ser
   1330                1335                1340

Gln Asn Tyr Asn Ile Phe Gln Leu Glu Asn Leu Tyr Ala Val Ile Ser
1345                1350                1355                1360

Gln Cys Ile Tyr Arg His Arg Lys Asp His Asp Lys Thr Ser Leu Ile
               1365                1370                1375

Gln Lys Met Glu Gln Glu Val Glu Asn Phe Ser Cys Ser Arg
           1380                1385                1390
```

[0037] or the ARAP11 fragment with amino acids 813-1, 390 of this protein.

[0038] The object of the present invention is also a DNA coding for ARAP11 or its fragment, especially the fragment with amino acids 813-1,390, and a DNA which hybridizes with them. The term "hybridizing DNA" indicates a DNA which hybridizes with the coding DNA under standard conditions, especially at 20° C. below the melting point of the DNA.

[0039] The invention is explained in greater detail below with reference to the following figures, where:

[0040] **FIG. 1** is a schematic diagram of the androgen receptor with identification of the androgen receptor domain (AR2) extending from amino acid 325 to amino acid 919, this being the domain which is able to interact with ARAP11 in the absence of androgen;

[0041] **FIG. 2** shows the tissue distribution of ARAP11;

[0042] **FIG. 3** shows the co-activation of the androgen receptor signal in SH-SY5Y cells; and

[0043] **FIG. 4** shows the expression of ARAP11 and β-actin in the testicles of rats.

[0044] The following examples illustrate the invention in greater detail without limiting it.

EXAMPLE 1

Co-Activation of the Androgen Receptor Signal by ARAP11

[0045] With the use of a cDNA library from fetal brain (Clontech MATCHMAKER) and of a human AR fragment which codes for amino acids 325-919 as a probe (**FIG. 1**), a screening process was carried out by means of a conventional two-hybrid yeast system in the absence of androgen. In agreement with the instructions of the manufacturer (Clontech), the number of screened clones was 6×10^7. The number of independent clones according to the manufacturer was 3.5×10^8. From these, 350 positive clones were selected and tested by a β-galactosidase assay; 240 were confirmed as being lacZ-positive. The inserts of these clones were amplified by PCR. At least 17 different clones were identified by restriction fragment analyses and sequencing. One of these was a clone with an insert comprising 1,169 bp (3,243 bp-4,412 bp), which codes for a part of the ORF (Open Reading Frame). This sequence also contains almost the entire part of the ORF already described in Pro2000 (Gene Bank Access No. XM005253).

[0046] By means of a conventional PCR method, the coding ARAP11-cDNA which codes for a protein (SEQ ID No. 2) consisting of 1390 amino acids and extending considerably beyond the previously know Pro 2000 sequence, which describes a protein with 362 amino acids, was cloned in its full length. Together with the 5' and 3' nontranslated regions, the sequence described here has a length of 4,412 bp (SEQ ID No. 1).

[0047] **FIG. 2** shows the tissue distribution of ARAP11, which was studied by means of Northern Blot analysis in the standard manner. Poly-A+-RNA (2 μg) isolated from various human tissues was separated by a formaldehyde-containing agarose gel, blotted onto a Nylon membrane, and hybridized with a labeled ARAP11-cDNA fragment. For the experiment illustrated in **FIGS. 2***a* and **2***b*, a fragment of 3,111-4,217 bp of the cDNA sequence of ARAP11 was used; for the experiment illustrated in **FIG. 2***c*, a fragment of 2,065-2,476 bp was used. After washing, the membrane was laid on a piece of film and developed after exposure to light for either 24 hours (**FIGS. 2***a* and **2***c*) or 8 days (**FIG. 2***b*). As can be seen from **FIG. 2**, very strong expression of ARAP11 was detected in the testicles, whereas weaker expression was found in the liver, in the heart, in the thymus, and in the prostate. Two transcripts (6.0 kb and 5.2 kb) were discovered.

[0048] When the probe consisting of the fragment with 2,065-2,476 bp of the cDNA sequence of ARAP11, containing 411 bp, was used, two transcripts of equal size were again found in the testicles. It can therefore be assumed that the transcripts which were found are identical to the transcripts which were detected in **2***a* and **2***b* with the probe of 3,111-4,217. This is evidence that the sequence of Pro 2000 filed in the gene bank under XM005253 is incomplete and is 2,480 bp longer in the 5' region.

[0049] The ARAP11-cDNA which codes for the ARAP11 fragment of amino acids 813-1,390, obtained by PCR, was cloned in the standard manner into the vector CMX and transfected with pSG5-AR and MMTV-luciferase in SH-SY5Y cells, also in the standard manner.

[0050] As can be seen from **FIG. 3**, the transient transfection of ARAP11-cDNA in SH-SYT5Y cells led to a strong co-activation of the AR signal activity, especially at low androgen concentrations of 10^{-12}-10^{-10} M. For this purpose, in a cell culture tray with wells, 3×10^5 cells per well were transfected with 1 μg of co-activator (ARAP11-3 or ARAP11-1, coding in each case for amino acids 813-1,390 of ARAP11) in CMX or with 1 μg of CMX as control plasmid, with 1.5 μg of MMTV-luciferase plasmid, and with 0.75 μg of pSG5AR plasmid. After 24 hours, the cells were treated with dihydroxytestosterone (DHT) as the androgen in the indicated concentrations. The transfected cells were harvested after another 24 hours, and the activity of the reporter gene luciferase was measured. In addition, the total quantity of cell protein was determined for the sake of normalization. One experiment and four measurements were conducted per transfection batch and substance concentration. The error range is given as the SD. The values of the corresponding controls without DHT were subtracted from all signals. The activity is expressed in relative units.

EXAMPLE 2

Determination of ARAP11 in the Testicles of Rats

[0051] **FIG. 4** shows the expression of ARAP 11 and β-actin in the testicles of rats. Poly-A+RNAs (4 μg) were isolated from the tissues of rat testicles, separated with a formaldehyde-containing agarose gel, transferred to a Nylon membrane, and hybridized either with a labeled ARAP11-cDNA fragment (2,226-4,228 bp) or with a labeled β-actin CDNA (rat). After washing, the membrane was laid on a piece of film, exposed to light for 5 days, and developed. A RNA transcript (6.0 kb) could be detected in the testicular tissue of the rats. The isolated RNA of 3-week-old animals is plotted in column 1, that of 6-week-old animals in column 2, and that of 2-year-old animals in column 3. It is easy to see that the expression of the ARAP11 gene is clearly age-dependent, whereas the expression of the β-actin gene shows no change. Six weeks after birth, the expression of ARAP11 is considerably reduced (by more than 50%), and in old animals (2 years) only a very low level of expression of the ARAP11 gene can still be detected. Similar behavior in terms of changes in the gene expression of the co-modulator ARAP11 can be expected in certain disease pictures.

[0052] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents.

[0053] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety.

[0054] The foregoing description and Examples detail certain preferred embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1
<211> LENGTH: 4412
<212> TYPE: DNA
<213> ORGANISM: fetal brain
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (45)..(4214)
<223> OTHER INFORMATION: cDNA library from fetal brain

<400> SEQUENCE: 1

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Met Val Val Leu
1

cgc agc agc ttg gag ctg cac aac cac tcc gcg gcc tcg gcc acg ggc 104

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Arg Ser Ser Leu Glu Leu His Asn His Ser Ala Ala Ser Ala Thr Gly
5                   10                  15                  20

tcc ttg gac ctg tcc agt gac ttc ctc agt ctg gag cac atc ggc cgg      152
Ser Leu Asp Leu Ser Ser Asp Phe Leu Ser Leu Glu His Ile Gly Arg
                25                  30                  35

agg cgg ctc cgc tcg gcc ggc gcg gcg cag aag aaa ccc gcg gcg acc      200
Arg Arg Leu Arg Ser Ala Gly Ala Ala Gln Lys Lys Pro Ala Ala Thr
            40                  45                  50

aca gcc aaa gcg ggc gat ggg tca tca gtt aag gaa gtt gaa acc tac      248
Thr Ala Lys Ala Gly Asp Gly Ser Ser Val Lys Glu Val Glu Thr Tyr
        55                  60                  65

cac cgg aca cgt gct tta aga tct ttg aga aaa gat gca cag aat tct      296
His Arg Thr Arg Ala Leu Arg Ser Leu Arg Lys Asp Ala Gln Asn Ser
    70                  75                  80

tca gat tct agt ttt gag aag aat gtg gaa ata acg gag caa ctt gct      344
Ser Asp Ser Ser Phe Glu Lys Asn Val Glu Ile Thr Glu Gln Leu Ala
85                  90                  95                  100

aat ggc agg cat ttt aca agg cag ttg gcc aga cag cag gct gat aaa      392
Asn Gly Arg His Phe Thr Arg Gln Leu Ala Arg Gln Gln Ala Asp Lys
                105                 110                 115

aaa aaa gaa gag cac aga gaa gac aaa gtg att cca gtt act cgg tca      440
Lys Lys Glu Glu His Arg Glu Asp Lys Val Ile Pro Val Thr Arg Ser
            120                 125                 130

ttg agg gct aga aac atc gtt caa agt aca gaa cac tta cat gaa gat      488
Leu Arg Ala Arg Asn Ile Val Gln Ser Thr Glu His Leu His Glu Asp
        135                 140                 145

aat ggt gat gtt gaa gtg cgt cga agt tgt agg att aga agt cgt tat      536
Asn Gly Asp Val Glu Val Arg Arg Ser Cys Arg Ile Arg Ser Arg Tyr
    150                 155                 160

agt ggt gta aac cag tcc atg ctg ttt gac aaa ctt ata act aac act      584
Ser Gly Val Asn Gln Ser Met Leu Phe Asp Lys Leu Ile Thr Asn Thr
165                 170                 175                 180

gct gaa gct gta ctt caa aaa atg gat gac atg aag aag atg cgt aga      632
Ala Glu Ala Val Leu Gln Lys Met Asp Asp Met Lys Lys Met Arg Arg
                185                 190                 195

cag cga atg aga gaa ctt gaa gac ttg gga gtg ttt aat gaa aca gaa      680
Gln Arg Met Arg Glu Leu Glu Asp Leu Gly Val Phe Asn Glu Thr Glu
            200                 205                 210

gaa agc aat ctt aat atg tac aca aga gga aaa cag aaa gat att caa      728
Glu Ser Asn Leu Asn Met Tyr Thr Arg Gly Lys Gln Lys Asp Ile Gln
        215                 220                 225

aga act gat gaa gaa aca act gat aat caa gaa ggc agt gtg gag tca      776
Arg Thr Asp Glu Glu Thr Thr Asp Asn Gln Glu Gly Ser Val Glu Ser
    230                 235                 240

tct gaa gag ggt gaa gac caa gaa cat gaa gat gat ggt gaa gat gaa      824
Ser Glu Glu Gly Glu Asp Gln Glu His Glu Asp Asp Gly Glu Asp Glu
245                 250                 255                 260

gat gat gaa gat gat gat gat gat gac gat gat gat gat gat gat gat      872
Asp Asp Glu Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp
                265                 270                 275

gat gaa gat gat gaa gat gaa gaa gat gga gaa gaa gag aat cag aag      920
Asp Glu Asp Asp Glu Asp Glu Glu Asp Gly Glu Glu Glu Asn Gln Lys
            280                 285                 290

cga tat tat ctt aga cag aga aaa gct act gtt tac tat cag gct cca      968
Arg Tyr Tyr Leu Arg Gln Arg Lys Ala Thr Val Tyr Tyr Gln Ala Pro
        295                 300                 305

ttg gaa aaa cct cgt cac cag aga aag ccc aac ata ttt tat agt ggc     1016
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Leu Glu Lys Pro Arg His Gln Arg Lys Pro Asn Ile Phe Tyr Ser Gly
    310                 315                 320

cca gct tct cct gca aga cca aga tac cga tta tct tcc gca gga cca     1064
Pro Ala Ser Pro Ala Arg Pro Arg Tyr Arg Leu Ser Ser Ala Gly Pro
325                 330                 335                 340

aga agt cct tac tgt aaa cga atg aac agg cga agg cat gca atc cac     1112
Arg Ser Pro Tyr Cys Lys Arg Met Asn Arg Arg Arg His Ala Ile His
                345                 350                 355

agt agt gac tcg act tca tct tcc tcc tct gaa gat gaa cag cac ttt     1160
Ser Ser Asp Ser Thr Ser Ser Ser Ser Ser Glu Asp Glu Gln His Phe
            360                 365                 370

gag agg cgg agg aaa agg agt cgt aat agg gct atc aat agg tgc ctc     1208
Glu Arg Arg Arg Lys Arg Ser Arg Asn Arg Ala Ile Asn Arg Cys Leu
        375                 380                 385

cca cta aat ttt cgg aaa gat gaa tta aaa ggc att tat aaa gat cga     1256
Pro Leu Asn Phe Arg Lys Asp Glu Leu Lys Gly Ile Tyr Lys Asp Arg
    390                 395                 400

atg aaa att gga gca agc ctt gcc gat gtt gat cca atg caa cta gat     1304
Met Lys Ile Gly Ala Ser Leu Ala Asp Val Asp Pro Met Gln Leu Asp
405                 410                 415                 420

tct tca gta cga ttt gat agt gtt ggt ggc ctg tct aat cat ata gca     1352
Ser Ser Val Arg Phe Asp Ser Val Gly Gly Leu Ser Asn His Ile Ala
                425                 430                 435

gct cta aaa gag atg gtg gtg ttt cca tta ctt tat cca gaa gtc ttt     1400
Ala Leu Lys Glu Met Val Val Phe Pro Leu Leu Tyr Pro Glu Val Phe
            440                 445                 450

gaa aaa ttt aaa att caa ccc cca aga ggt tgt ttg ttt tat ggg cca     1448
Glu Lys Phe Lys Ile Gln Pro Pro Arg Gly Cys Leu Phe Tyr Gly Pro
        455                 460                 465

cct gga act gga aag act ctg gtt gcc aga gca ctt gcc aat gag tgc     1496
Pro Gly Thr Gly Lys Thr Leu Val Ala Arg Ala Leu Ala Asn Glu Cys
    470                 475                 480

agt caa ggg gat aaa aga gta gca ttt ttc atg agg aaa ggt gct gat     1544
Ser Gln Gly Asp Lys Arg Val Ala Phe Phe Met Arg Lys Gly Ala Asp
485                 490                 495                 500

tgt cta agt aaa tgg gta gga gaa tct gaa aga cag cta cga ttg ctg     1592
Cys Leu Ser Lys Trp Val Gly Glu Ser Glu Arg Gln Leu Arg Leu Leu
                505                 510                 515

ttt gat cag gcc tat cag atg cgc cca tca att att ttt ttt gac gaa     1640
Phe Asp Gln Ala Tyr Gln Met Arg Pro Ser Ile Ile Phe Phe Asp Glu
            520                 525                 530

att gat ggt ctg gct cca gta cgg tca agc agg caa gat cag att cac     1688
Ile Asp Gly Leu Ala Pro Val Arg Ser Ser Arg Gln Asp Gln Ile His
        535                 540                 545

agt tct att gtt tcc acc ctg cta gct ctt atg gat gga ttg gac agc     1736
Ser Ser Ile Val Ser Thr Leu Leu Ala Leu Met Asp Gly Leu Asp Ser
    550                 555                 560

aga ggg gaa att gtg gtc att ggt gct acg aac agg cta gat tct ata     1784
Arg Gly Glu Ile Val Val Ile Gly Ala Thr Asn Arg Leu Asp Ser Ile
565                 570                 575                 580

gat cct gct tta cga agg cct ggt cgc ttt gat aga gaa ttc ctc ttt     1832
Asp Pro Ala Leu Arg Arg Pro Gly Arg Phe Asp Arg Glu Phe Leu Phe
                585                 590                 595

agc ctg cct gat aaa gag gct cga aaa gag att cta aag att cac acc     1880
Ser Leu Pro Asp Lys Glu Ala Arg Lys Glu Ile Leu Lys Ile His Thr
            600                 605                 610

agg gat tgg aat ccc aaa cca ctg gac aca ttt tta gaa gag cta gca     1928
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Arg Asp Trp Asn Pro Lys Pro Leu Asp Thr Phe Leu Glu Glu Leu Ala
        615                 620                 625

gaa aac tgt gtt gga tac tgt gga gca gat att aaa tca ata tgt gct     1976
Glu Asn Cys Val Gly Tyr Cys Gly Ala Asp Ile Lys Ser Ile Cys Ala
    630                 635                 640

gaa gct gct tta tgt gct tta cga cga cgc tac cca cag atc tat acc     2024
Glu Ala Ala Leu Cys Ala Leu Arg Arg Arg Tyr Pro Gln Ile Tyr Thr
645                 650                 655                 660

act agt gag aaa ctg cag ttg gat ctc tct tca att aat atc tca gct     2072
Thr Ser Glu Lys Leu Gln Leu Asp Leu Ser Ser Ile Asn Ile Ser Ala
                665                 670                 675

aag gat ttc gag gta gct atg caa aag atg ata cca gcc tcc caa aga     2120
Lys Asp Phe Glu Val Ala Met Gln Lys Met Ile Pro Ala Ser Gln Arg
            680                 685                 690

gct gtg aca tca cct ggg cag gca ctg tcc acc gtt gtg aaa cca ctc     2168
Ala Val Thr Ser Pro Gly Gln Ala Leu Ser Thr Val Val Lys Pro Leu
        695                 700                 705

ctg caa aac act gtt gac aag att tta gaa gcc ctg cag aga gta ttt     2216
Leu Gln Asn Thr Val Asp Lys Ile Leu Glu Ala Leu Gln Arg Val Phe
    710                 715                 720

cca cat gca gaa ttc aga aca aat aaa aca tta gac tca gat att tct     2264
Pro His Ala Glu Phe Arg Thr Asn Lys Thr Leu Asp Ser Asp Ile Ser
725                 730                 735                 740

tgt cct ctg cta gaa agt gac ttg gct tac agt gat gat gat gtt cca     2312
Cys Pro Leu Leu Glu Ser Asp Leu Ala Tyr Ser Asp Asp Asp Val Pro
                745                 750                 755

tca gtt tat gaa aat gga ctt tct cag aaa tct tct cat aag gca aaa     2360
Ser Val Tyr Glu Asn Gly Leu Ser Gln Lys Ser Ser His Lys Ala Lys
            760                 765                 770

gac aat ttt aat ttt ctt cat ttg aat aga aat gct tgt tac caa cct     2408
Asp Asn Phe Asn Phe Leu His Leu Asn Arg Asn Ala Cys Tyr Gln Pro
        775                 780                 785

atg tct ttt cga cca aga ata ttg ata gta gga gaa cca gga ttt ggg     2456
Met Ser Phe Arg Pro Arg Ile Leu Ile Val Gly Glu Pro Gly Phe Gly
    790                 795                 800

caa ggt tct cac ttg gca cca gct gtc att cat gct ttg gaa aag ttt     2504
Gln Gly Ser His Leu Ala Pro Ala Val Ile His Ala Leu Glu Lys Phe
805                 810                 815                 820

act gta tat aca tta gac att cct gtt ctt ttt gga gtt agt act aca     2552
Thr Val Tyr Thr Leu Asp Ile Pro Val Leu Phe Gly Val Ser Thr Thr
                825                 830                 835

tcc cct gaa gaa aca tgt gcc cag gtg att cgt gaa gct aag aga aca     2600
Ser Pro Glu Glu Thr Cys Ala Gln Val Ile Arg Glu Ala Lys Arg Thr
            840                 845                 850

gca cca agt ata gtg tat gtt cct cat atc cac gtg tgg tgg gaa ata     2648
Ala Pro Ser Ile Val Tyr Val Pro His Ile His Val Trp Trp Glu Ile
        855                 860                 865

gtt gga ccg aca ctt aaa gcc aca ttt acc aca tta tta cag aat att     2696
Val Gly Pro Thr Leu Lys Ala Thr Phe Thr Thr Leu Leu Gln Asn Ile
    870                 875                 880

cct tca ttt gct cca gtt tta cta ctt gca act tct gac aaa ccc cat     2744
Pro Ser Phe Ala Pro Val Leu Leu Leu Ala Thr Ser Asp Lys Pro His
885                 890                 895                 900

tcc gct ttg cca gaa gag gtg caa gaa ttg ttt atc cgt gat tat gga     2792
Ser Ala Leu Pro Glu Glu Val Gln Glu Leu Phe Ile Arg Asp Tyr Gly
                905                 910                 915

gag att ttt aat gtc cag tta ccg gat aaa gaa gaa cgg aca aaa ttt     2840
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Glu Ile Phe Asn Val Gln Leu Pro Asp Lys Glu Glu Arg Thr Lys Phe
            920                 925                 930

ttt gaa gat tta att cta aaa caa gct gct aag cct cct ata tca aaa     2888
Phe Glu Asp Leu Ile Leu Lys Gln Ala Ala Lys Pro Pro Ile Ser Lys
        935                 940                 945

aag aaa gca gtt ttg cag gct ttg gag gta ctc cca gta gca cca cca     2936
Lys Lys Ala Val Leu Gln Ala Leu Glu Val Leu Pro Val Ala Pro Pro
    950                 955                 960

cct gag cca aga tca ctg aca gca gaa gaa gtg aaa cga cta gaa gaa     2984
Pro Glu Pro Arg Ser Leu Thr Ala Glu Glu Val Lys Arg Leu Glu Glu
965                 970                 975                 980

caa gaa gaa gat aca ttt aga gaa ctg agg att ttc tta aga aat gtt     3032
Gln Glu Glu Asp Thr Phe Arg Glu Leu Arg Ile Phe Leu Arg Asn Val
                985                 990                 995

aca cat agg ctt  gct att gac aag cga  ttc cga gtg ttt act  aag      3077
Thr His Arg Leu  Ala Ile Asp Lys Arg  Phe Arg Val Phe Thr  Lys
            1000                1005                1010

cct gtt gac cct  gat gag gtt cct gat  tat gtc act gta ata  aag      3122
Pro Val Asp Pro  Asp Glu Val Pro Asp  Tyr Val Thr Val Ile  Lys
            1015                1020                1025

caa cca atg gac  ctt tca tct gta atc  agt aaa att gat cta  cac      3167
Gln Pro Met Asp  Leu Ser Ser Val Ile  Ser Lys Ile Asp Leu  His
            1030                1035                1040

aag tat ctg act  gtg aaa gac tat ttg  aga gat att gat cta  atc      3212
Lys Tyr Leu Thr  Val Lys Asp Tyr Leu  Arg Asp Ile Asp Leu  Ile
            1045                1050                1055

tgt agt aat gcc  tta gaa tac aat cca  gat aga gat cct gga  gat      3257
Cys Ser Asn Ala  Leu Glu Tyr Asn Pro  Asp Arg Asp Pro Gly  Asp
            1060                1065                1070

cgt ctt att agg  cat aga gcc tgt gct  tta aga gat act gcc  tat      3302
Arg Leu Ile Arg  His Arg Ala Cys Ala  Leu Arg Asp Thr Ala  Tyr
            1075                1080                1085

gcc ata att aaa  gaa gaa ctt gat gaa  gac ttt gag cag ctc  tgt      3347
Ala Ile Ile Lys  Glu Glu Leu Asp Glu  Asp Phe Glu Gln Leu  Cys
            1090                1095                1100

gaa gaa att cag  gaa tct aga aag aaa  aga ggt tgt agc tcc  tcc      3392
Glu Glu Ile Gln  Glu Ser Arg Lys Lys  Arg Gly Cys Ser Ser  Ser
            1105                1110                1115

aaa tat gcc ccg  tct tac tac cat gtg  atg cca aag caa aat  tcc      3437
Lys Tyr Ala Pro  Ser Tyr Tyr His Val  Met Pro Lys Gln Asn  Ser
            1120                1125                1130

act ctt gtt ggt  gat aaa aga tca gac  cca gag cag aat gaa  aag      3482
Thr Leu Val Gly  Asp Lys Arg Ser Asp  Pro Glu Gln Asn Glu  Lys
            1135                1140                1145

cta aag aca ccg  agt act cct gtg gct  tgc agc act cct gct  cag      3527
Leu Lys Thr Pro  Ser Thr Pro Val Ala  Cys Ser Thr Pro Ala  Gln
            1150                1155                1160

ttg aag agg aaa  att cgc aaa aag tca  aac tgg tac tta ggc  acc      3572
Leu Lys Arg Lys  Ile Arg Lys Lys Ser  Asn Trp Tyr Leu Gly  Thr
            1165                1170                1175

ata aaa aag cga  agg aag att tca cag  gca aag gat gat agc  cag      3617
Ile Lys Lys Arg  Arg Lys Ile Ser Gln  Ala Lys Asp Asp Ser  Gln
            1180                1185                1190

aat gcc ata gat  cac aaa att gag agt  gat aca gag gaa act  caa      3662
Asn Ala Ile Asp  His Lys Ile Glu Ser  Asp Thr Glu Glu Thr  Gln
            1195                1200                1205

gac aca agt gta  gat cat aat gag acc  gga aac aca gga gag  tct      3707
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Asp Thr Ser Val  Asp His Asn Glu Thr  Gly Asn Thr Gly Glu  Ser
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Ser Val Glu Glu  Asn Glu Lys Gln Gln  Asn Ala Ser Glu Ser  Lys
            1225                 1230                 1235

ctg gaa ttg aga  aat aat tca aat act  tgt aat ata gag aat  gag      3797
Leu Glu Leu Arg  Asn Asn Ser Asn Thr  Cys Asn Ile Glu Asn  Glu
            1240                 1245                 1250

ctt gaa gac tct  agg aag act aca gca  tgt aca gaa ttg aga  gac      3842
Leu Glu Asp Ser  Arg Lys Thr Thr Ala  Cys Thr Glu Leu Arg  Asp
            1255                 1260                 1265

aag att gct tgt  aat gga gat gct tct  agc tct cag ata ata  cat      3887
Lys Ile Ala Cys  Asn Gly Asp Ala Ser  Ser Ser Gln Ile Ile  His
            1270                 1275                 1280

att tct gat gaa  aat gaa gga aaa gaa  atg tgt gtt ctg cga  atg      3932
Ile Ser Asp Glu  Asn Glu Gly Lys Glu  Met Cys Val Leu Arg  Met
            1285                 1290                 1295

act cga gct aga  cgt tcc cag gta gaa  cag cag cag ctc atc  act      3977
Thr Arg Ala Arg  Arg Ser Gln Val Glu  Gln Gln Gln Leu Ile  Thr
            1300                 1305                 1310

gtt gaa aag gct  ttg gca att ctt tct  cag cct aca ccc tca  ctt      4022
Val Glu Lys Ala  Leu Ala Ile Leu Ser  Gln Pro Thr Pro Ser  Leu
            1315                 1320                 1325

gtt gtg gat cat  gag cga tta aaa aat  ctt ttg aag act gtt  gtt      4067
Val Val Asp His  Glu Arg Leu Lys Asn  Leu Leu Lys Thr Val  Val
            1330                 1335                 1340

aaa aaa agt caa  aac tac aac ata ttt  cag ttg gaa aat ttg  tat      4112
Lys Lys Ser Gln  Asn Tyr Asn Ile Phe  Gln Leu Glu Asn Leu  Tyr
            1345                 1350                 1355

gca gta atc agc  caa tgt att tat cgg  cat cgc aag gac cat  gat      4157
Ala Val Ile Ser  Gln Cys Ile Tyr Arg  His Arg Lys Asp His  Asp
            1360                 1365                 1370

aaa aca tca ctt  att cag aaa atg gag  caa gag gta gaa aac  ttc      4202
Lys Thr Ser Leu  Ile Gln Lys Met Glu  Gln Glu Val Glu Asn  Phe
            1375                 1380                 1385

agt tgt tcc aga  tgatgatgtc atggtatcga gtattcttta tattcagttc       4254
Ser Cys Ser Arg
            1390

ctatttaagt catttttgtc atgtccgcct aattgatgta gtatgaaacc ctgcatcttt  4314

aaggaaaaga ttaaaatagt aaaataaaag tatttaaact ttcctgatat ttatgtacat  4374

attaagataa atgtcatgtg taagataact gataaata                          4412


<210> SEQ ID NO 2
<211> LENGTH: 1390
<212> TYPE: PRT
<213> ORGANISM: fetal brain

<400> SEQUENCE: 2

Met Val Val Leu Arg Ser Ser Leu Glu Leu His Asn His Ser Ala Ala
1               5                  10                  15

Ser Ala Thr Gly Ser Leu Asp Leu Ser Ser Asp Phe Leu Ser Leu Glu
            20                  25                  30

His Ile Gly Arg Arg Arg Leu Arg Ser Ala Gly Ala Ala Gln Lys Lys
        35                  40                  45

Pro Ala Ala Thr Thr Ala Lys Ala Gly Asp Gly Ser Ser Val Lys Glu
    50                  55                  60
```

-continued

```
Val Glu Thr Tyr His Arg Thr Arg Ala Leu Arg Ser Leu Arg Lys Asp
65                  70                  75                  80

Ala Gln Asn Ser Ser Asp Ser Ser Phe Glu Lys Asn Val Glu Ile Thr
                85                  90                  95

Glu Gln Leu Ala Asn Gly Arg His Phe Thr Arg Gln Leu Ala Arg Gln
            100                 105                 110

Gln Ala Asp Lys Lys Lys Glu Glu His Arg Glu Asp Lys Val Ile Pro
        115                 120                 125

Val Thr Arg Ser Leu Arg Ala Arg Asn Ile Val Gln Ser Thr Glu His
    130                 135                 140

Leu His Glu Asp Asn Gly Asp Val Glu Val Arg Arg Ser Cys Arg Ile
145                 150                 155                 160

Arg Ser Arg Tyr Ser Gly Val Asn Gln Ser Met Leu Phe Asp Lys Leu
                165                 170                 175

Ile Thr Asn Thr Ala Glu Ala Val Leu Gln Lys Met Asp Asp Met Lys
            180                 185                 190

Lys Met Arg Arg Gln Arg Met Arg Glu Leu Glu Asp Leu Gly Val Phe
        195                 200                 205

Asn Glu Thr Glu Glu Ser Asn Leu Asn Met Tyr Thr Arg Gly Lys Gln
    210                 215                 220

Lys Asp Ile Gln Arg Thr Asp Glu Glu Thr Thr Asp Asn Gln Glu Gly
225                 230                 235                 240

Ser Val Glu Ser Ser Glu Glu Gly Glu Asp Gln Glu His Glu Asp Asp
                245                 250                 255

Gly Glu Asp Glu Asp Asp Glu Asp Asp Asp Asp Asp Asp Asp Asp Asp
            260                 265                 270

Asp Asp Asp Asp Asp Glu Asp Asp Glu Asp Glu Glu Asp Gly Glu Glu
        275                 280                 285

Glu Asn Gln Lys Arg Tyr Tyr Leu Arg Gln Arg Lys Ala Thr Val Tyr
    290                 295                 300

Tyr Gln Ala Pro Leu Glu Lys Pro Arg His Gln Arg Lys Pro Asn Ile
305                 310                 315                 320

Phe Tyr Ser Gly Pro Ala Ser Pro Ala Arg Pro Arg Tyr Arg Leu Ser
                325                 330                 335

Ser Ala Gly Pro Arg Ser Pro Tyr Cys Lys Arg Met Asn Arg Arg Arg
            340                 345                 350

His Ala Ile His Ser Ser Asp Ser Thr Ser Ser Ser Ser Ser Glu Asp
        355                 360                 365

Glu Gln His Phe Glu Arg Arg Arg Lys Arg Ser Arg Asn Arg Ala Ile
    370                 375                 380

Asn Arg Cys Leu Pro Leu Asn Phe Arg Lys Asp Glu Leu Lys Gly Ile
385                 390                 395                 400

Tyr Lys Asp Arg Met Lys Ile Gly Ala Ser Leu Ala Asp Val Asp Pro
                405                 410                 415

Met Gln Leu Asp Ser Ser Val Arg Phe Asp Ser Val Gly Gly Leu Ser
            420                 425                 430

Asn His Ile Ala Ala Leu Lys Glu Met Val Val Phe Pro Leu Leu Tyr
        435                 440                 445

Pro Glu Val Phe Glu Lys Phe Lys Ile Gln Pro Pro Arg Gly Cys Leu
    450                 455                 460
```

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```
Phe Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Val Ala Arg Ala Leu
465                 470                 475                 480

Ala Asn Glu Cys Ser Gln Gly Asp Lys Arg Val Ala Phe Phe Met Arg
                485                 490                 495

Lys Gly Ala Asp Cys Leu Ser Lys Trp Val Gly Glu Ser Glu Arg Gln
            500                 505                 510

Leu Arg Leu Leu Phe Asp Gln Ala Tyr Gln Met Arg Pro Ser Ile Ile
        515                 520                 525

Phe Phe Asp Glu Ile Asp Gly Leu Ala Pro Val Arg Ser Ser Arg Gln
    530                 535                 540

Asp Gln Ile His Ser Ser Ile Val Ser Thr Leu Leu Ala Leu Met Asp
545                 550                 555                 560

Gly Leu Asp Ser Arg Gly Glu Ile Val Val Ile Gly Ala Thr Asn Arg
                565                 570                 575

Leu Asp Ser Ile Asp Pro Ala Leu Arg Arg Pro Gly Arg Phe Asp Arg
            580                 585                 590

Glu Phe Leu Phe Ser Leu Pro Asp Lys Glu Ala Arg Lys Glu Ile Leu
        595                 600                 605

Lys Ile His Thr Arg Asp Trp Asn Pro Lys Pro Leu Asp Thr Phe Leu
    610                 615                 620

Glu Glu Leu Ala Glu Asn Cys Val Gly Tyr Cys Gly Ala Asp Ile Lys
625                 630                 635                 640

Ser Ile Cys Ala Glu Ala Ala Leu Cys Ala Leu Arg Arg Arg Tyr Pro
                645                 650                 655

Gln Ile Tyr Thr Thr Ser Glu Lys Leu Gln Leu Asp Leu Ser Ser Ile
            660                 665                 670

Asn Ile Ser Ala Lys Asp Phe Glu Val Ala Met Gln Lys Met Ile Pro
        675                 680                 685

Ala Ser Gln Arg Ala Val Thr Ser Pro Gly Gln Ala Leu Ser Thr Val
    690                 695                 700

Val Lys Pro Leu Leu Gln Asn Thr Val Asp Lys Ile Leu Glu Ala Leu
705                 710                 715                 720

Gln Arg Val Phe Pro His Ala Glu Phe Arg Thr Asn Lys Thr Leu Asp
                725                 730                 735

Ser Asp Ile Ser Cys Pro Leu Leu Glu Ser Asp Leu Ala Tyr Ser Asp
            740                 745                 750

Asp Asp Val Pro Ser Val Tyr Glu Asn Gly Leu Ser Gln Lys Ser Ser
        755                 760                 765

His Lys Ala Lys Asp Asn Phe Asn Phe Leu His Leu Asn Arg Asn Ala
    770                 775                 780

Cys Tyr Gln Pro Met Ser Phe Arg Pro Arg Ile Leu Ile Val Gly Glu
785                 790                 795                 800

Pro Gly Phe Gly Gln Gly Ser His Leu Ala Pro Ala Val Ile His Ala
                805                 810                 815

Leu Glu Lys Phe Thr Val Tyr Thr Leu Asp Ile Pro Val Leu Phe Gly
            820                 825                 830

Val Ser Thr Thr Ser Pro Glu Glu Thr Cys Ala Gln Val Ile Arg Glu
        835                 840                 845

Ala Lys Arg Thr Ala Pro Ser Ile Val Tyr Val Pro His Ile His Val
    850                 855                 860

Trp Trp Glu Ile Val Gly Pro Thr Leu Lys Ala Thr Phe Thr Thr Leu
```

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```
865                 870                 875                 880

Leu Gln Asn Ile Pro Ser Phe Ala Pro Val Leu Leu Leu Ala Thr Ser
                885                 890                 895

Asp Lys Pro His Ser Ala Leu Pro Glu Glu Val Gln Glu Leu Phe Ile
            900                 905                 910

Arg Asp Tyr Gly Glu Ile Phe Asn Val Gln Leu Pro Asp Lys Glu Glu
        915                 920                 925

Arg Thr Lys Phe Phe Glu Asp Leu Ile Leu Lys Gln Ala Ala Lys Pro
    930                 935                 940

Pro Ile Ser Lys Lys Lys Ala Val Leu Gln Ala Leu Glu Val Leu Pro
945                 950                 955                 960

Val Ala Pro Pro Pro Glu Pro Arg Ser Leu Thr Ala Glu Glu Val Lys
                965                 970                 975

Arg Leu Glu Glu Gln Glu Glu Asp Thr Phe Arg Glu Leu Arg Ile Phe
            980                 985                 990

Leu Arg Asn Val Thr His Arg Leu  Ala Ile Asp Lys Arg  Phe Arg Val
        995                 1000                1005

Phe Thr  Lys Pro Val Asp Pro  Asp Glu Val Pro Asp  Tyr Val Thr
    1010                1015                1020

Val Ile  Lys Gln Pro Met Asp  Leu Ser Ser Val Ile  Ser Lys Ile
    1025                1030                1035

Asp Leu  His Lys Tyr Leu Thr  Val Lys Asp Tyr Leu  Arg Asp Ile
    1040                1045                1050

Asp Leu  Ile Cys Ser Asn Ala  Leu Glu Tyr Asn Pro  Asp Arg Asp
    1055                1060                1065

Pro Gly  Asp Arg Leu Ile Arg  His Arg Ala Cys Ala  Leu Arg Asp
    1070                1075                1080

Thr Ala  Tyr Ala Ile Ile Lys  Glu Glu Leu Asp Glu  Asp Phe Glu
    1085                1090                1095

Gln Leu  Cys Glu Glu Ile Gln  Glu Ser Arg Lys Lys  Arg Gly Cys
    1100                1105                1110

Ser Ser  Ser Lys Tyr Ala Pro  Ser Tyr Tyr His Val  Met Pro Lys
    1115                1120                1125

Gln Asn  Ser Thr Leu Val Gly  Asp Lys Arg Ser Asp  Pro Glu Gln
    1130                1135                1140

Asn Glu  Lys Leu Lys Thr Pro  Ser Thr Pro Val Ala  Cys Ser Thr
    1145                1150                1155

Pro Ala  Gln Leu Lys Arg Lys  Ile Arg Lys Lys Ser  Asn Trp Tyr
    1160                1165                1170

Leu Gly  Thr Ile Lys Lys Arg  Arg Lys Ile Ser Gln  Ala Lys Asp
    1175                1180                1185

Asp Ser  Gln Asn Ala Ile Asp  His Lys Ile Glu Ser  Asp Thr Glu
    1190                1195                1200

Glu Thr  Gln Asp Thr Ser Val  Asp His Asn Glu Thr  Gly Asn Thr
    1205                1210                1215

Gly Glu  Ser Ser Val Glu Glu  Asn Glu Lys Gln Gln  Asn Ala Ser
    1220                1225                1230

Glu Ser  Lys Leu Glu Leu Arg  Asn Asn Ser Asn Thr  Cys Asn Ile
    1235                1240                1245

Glu Asn  Glu Leu Glu Asp Ser  Arg Lys Thr Thr Ala  Cys Thr Glu
    1250                1255                1260
```

-continued

```
Leu Arg  Asp Lys Ile Ala Cys  Asn Gly Asp Ala Ser  Ser Ser Gln
    1265                1270                1275

Ile Ile  His Ile Ser Asp Glu  Asn Glu Gly Lys Glu  Met Cys Val
    1280                1285                1290

Leu Arg  Met Thr Arg Ala Arg  Arg Ser Gln Val Glu  Gln Gln Gln
    1295                1300                1305

Leu Ile  Thr Val Glu Lys Ala  Leu Ala Ile Leu Ser  Gln Pro Thr
    1310                1315                1320

Pro Ser  Leu Val Val Asp His  Glu Arg Leu Lys Asn  Leu Leu Lys
    1325                1330                1335

Thr Val  Val Lys Lys Ser Gln  Asn Tyr Asn Ile Phe  Gln Leu Glu
    1340                1345                1350

Asn Leu  Tyr Ala Val Ile Ser  Gln Cys Ile Tyr Arg  His Arg Lys
    1355                1360                1365

Asp His  Asp Lys Thr Ser Leu  Ile Gln Lys Met Glu  Gln Glu Val
    1370                1375                1380

Glu Asn  Phe Ser Cys Ser Arg
    1385                1390


<210> SEQ ID NO 3
<211> LENGTH: 578
<212> TYPE: PRT
<213> ORGANISM: fetalbrain
<220> FEATURE:
<223> OTHER INFORMATION: ARAP11 fragment from amino acid residues 813-
      1390

<400> SEQUENCE: 3

Val Ile His Ala Leu Glu Lys Phe Thr Val Tyr Thr Leu Asp Ile Pro
1               5                   10                  15

Val Leu Phe Gly Val Ser Thr Thr Ser Pro Glu Glu Thr Cys Ala Gln
            20                  25                  30

Val Ile Arg Glu Ala Lys Arg Thr Ala Pro Ser Ile Val Tyr Val Pro
        35                  40                  45

His Ile His Val Trp Trp Glu Ile Val Gly Pro Thr Leu Lys Ala Thr
    50                  55                  60

Phe Thr Thr Leu Leu Gln Asn Ile Pro Ser Phe Ala Pro Val Leu Leu
65                  70                  75                  80

Leu Ala Thr Ser Asp Lys Pro His Ser Ala Leu Pro Glu Glu Val Gln
                85                  90                  95

Glu Leu Phe Ile Arg Asp Tyr Gly Glu Ile Phe Asn Val Gln Leu Pro
            100                 105                 110

Asp Lys Glu Glu Arg Thr Lys Phe Phe Glu Asp Leu Ile Leu Lys Gln
        115                 120                 125

Ala Ala Lys Pro Pro Ile Ser Lys Lys Lys Ala Val Leu Gln Ala Leu
    130                 135                 140

Glu Val Leu Pro Val Ala Pro Pro Pro Glu Pro Arg Ser Leu Thr Ala
145                 150                 155                 160

Glu Glu Val Lys Arg Leu Glu Glu Gln Glu Glu Asp Thr Phe Arg Glu
                165                 170                 175

Leu Arg Ile Phe Leu Arg Asn Val Thr His Arg Leu  Ala Ile Asp Lys
            180                 185                 190

Arg Phe Arg Val Phe Thr Lys Pro Val Asp Pro Asp Glu Val Pro Asp
```

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```
        195                 200                 205

Tyr Val Thr Val Ile Lys Gln Pro Met Asp Leu Ser Ser Val Ile Ser
    210                 215                 220

Lys Ile Asp Leu His Lys Tyr Leu Thr Val Lys Asp Tyr Leu Arg Asp
225                 230                 235                 240

Ile Asp Leu Ile Cys Ser Asn Ala Leu Glu Tyr Asn Pro Asp Arg Asp
                245                 250                 255

Pro Gly Asp Arg Leu Ile Arg His Arg Ala Cys Ala Leu Arg Asp Thr
           260                 265                 270

Ala Tyr Ala Ile Ile Lys Glu Glu Leu Asp Glu Asp Phe Glu Gln Leu
        275                 280                 285

Cys Glu Glu Ile Gln Glu Ser Arg Lys Lys Arg Gly Cys Ser Ser Ser
    290                 295                 300

Lys Tyr Ala Pro Ser Tyr Tyr His Val Met Pro Lys Gln Asn Ser Thr
305                 310                 315                 320

Leu Val Gly Asp Lys Arg Ser Asp Pro Glu Gln Asn Glu Lys Leu Lys
                325                 330                 335

Thr Pro Ser Thr Pro Val Ala Cys Ser Thr Pro Ala Gln Leu Lys Arg
            340                 345                 350

Lys Ile Arg Lys Lys Ser Asn Trp Tyr Leu Gly Thr Ile Lys Lys Arg
        355                 360                 365

Arg Lys Ile Ser Gln Ala Lys Asp Asp Ser Gln Asn Ala Ile Asp His
    370                 375                 380

Lys Ile Glu Ser Asp Thr Glu Glu Thr Gln Asp Thr Ser Val Asp His
385                 390                 395                 400

Asn Glu Thr Gly Asn Thr Gly Glu Ser Ser Val Glu Glu Asn Glu Lys
                405                 410                 415

Gln Gln Asn Ala Ser Glu Ser Lys Leu Glu Leu Arg Asn Asn Ser Asn
            420                 425                 430

Thr Cys Asn Ile Glu Asn Glu Leu Glu Asp Ser Arg Lys Thr Thr Ala
        435                 440                 445

Cys Thr Glu Leu Arg Asp Lys Ile Ala Cys Asn Gly Asp Ala Ser Ser
    450                 455                 460

Ser Gln Ile Ile His Ile Ser Asp Glu Asn Glu Gly Lys Glu Met Cys
465                 470                 475                 480

Val Leu Arg Met Thr Arg Ala Arg Arg Ser Gln Val Glu Gln Gln Gln
                485                 490                 495

Leu Ile Thr Val Glu Lys Ala Leu Ala Ile Leu Ser Gln Pro Thr Pro
            500                 505                 510

Ser Leu Val Val Asp His Glu Arg Leu Lys Asn Leu Leu Lys Thr Val
        515                 520                 525

Val Lys Lys Ser Gln Asn Tyr Asn Ile Phe Gln Leu Glu Asn Leu Tyr
    530                 535                 540

Ala Val Ile Ser Gln Cys Ile Tyr Arg His Arg Lys Asp His Asp Lys
545                 550                 555                 560

Thr Ser Leu Ile Gln Lys Met Glu Gln Glu Val Glu Asn Phe Ser Cys
                565                 570                 575

Ser Arg
```

What is claimed is:

1. A method for testing the hormonal effect, especially the androgenic or antiandrogenic effect, of substances, in which

(a) cells which have been transfected with two vectors, one of these vectors containing DNA which codes for a nuclear receptor protein or a fragment thereof, the other vector containing DNA which codes for a co-modulator or a fragment thereof, are exposed to the substance; and

(b) the transcription activity which the nuclear receptor or its fragment induces in the presence of the co-modulator or its fragment and/or the effect of the substance on the interaction between the receptor or its fragment and the co-modulator or its fragment is measured by the protein-protein interaction or protein-protein-DNA interaction.

2. The method according to claim 1, where the co-modulator is ARAP11, which comprises the following amino acid sequence:

```
Met Val Val Leu Arg Ser Ser Leu Glu Leu His Asn His Ser Ala Ala
  15                  10                  15

Ser Ala Thr Gly Ser Leu Asp Leu Ser Ser Asp Phe Leu Ser Leu Glu
             20                  25                  30

His Ile Gly Arg Arg Arg Leu Arg Ser Ala Gly Ala Ala Gln Lys Lys
         35                  40                  45

Pro Ala Ala Thr Thr Ala Lys Ala Gly Asp Gly Ser Ser Val Lys Glu
     50                  55                  60

Val Glu Thr Tyr His Arg Thr Arg Ala Leu Arg Ser Leu Arg Lys Asp
 65                  70                  75                  80

Ala Gln Asn Ser Ser Asp Ser Ser Phe Glu Lys Asn Val Glu Ile Thr
                 85                  90                  95

Glu Gln Leu Ala Asn Gly Arg His Phe Thr Arg Gln Leu Ala Arg Gln
            100                 105                 110

Gln Ala Asp Lys Lys Lys Glu Glu His Arg Glu Asp Lys Val Ile Pro
        115                 120                 125

Val Thr Arg Ser Leu Arg Ala Arg Asn Ile Val Gln Ser Thr Glu His
    130                 135                 140

Leu His Glu Asp Asn Gly Asp Val Glu Val Arg Arg Ser Cys Arg Ile
145                 150                 155                 160

Arg Ser Arg Tyr Ser Gly Val Asn Gln Ser Met Leu Phe Asp Lys Leu
                165                 170                 175

Ile Thr Asn Thr Ala Glu Ala Val Leu Gln Lys Met Asp Asp Met Lys
            180                 185                 190

Lys Met Arg Arg Gln Arg Met Arg Glu Leu Gln Asp Leu Gly Val Phe
        195                 200                 205

Asn Glu Thr Glu Glu Ser Asn Leu Asn Met Tyr Thr Arg Gly Lys Gln
    210                 215                 220

Lys Asp Ile Gln Arg Thr Asp Glu Glu Thr Thr Asp Asn Gln Glu Gly
225                 230                 235                 240

Ser Val Glu Ser Ser Glu Glu Gly Glu Asp Gln Glu His Glu Asp Asp
                245                 250                 255

Gly Glu Asp Glu Asp Asp Glu Asp Asp Asp Asp Asp Asp Asp Asp Asp
            260                 265                 270

Asp Asp Asp Asp Asp Glu Asp Asp Glu Asp Glu Glu Asp Gly Glu Glu
        275                 280                 285

Glu Asn Gln Lys Arg Tyr Tyr Leu Arg Gln Arg Lys Ala Thr Val Tyr
    290                 295                 300

Tyr Gln Ala Pro Leu Glu Lys Pro Arg His Gln Arg Lys Pro Asn Ile
305                 310                 315                 320

Phe Tyr Ser Gly Pro Ala Ser Pro Ala Arg Pro Arg Tyr Arg Leu Ser
```

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```
                325                 330                 335

Ser Ala Gly Pro Arg Ser Pro Tyr Cys Lys Arg Met Asn Arg Arg Arg
            340                 345                 350

His Ala Ile His Ser Ser Asp Ser Thr Ser Ser Ser Ser Ser Glu Asp
        355                 360                 365

Glu Gln His Phe Glu Arg Arg Arg Lys Arg Ser Arg Asn Arg Ala Ile
    370                 375                 380

Asn Arg Cys Leu Pro Leu Asn Phe Arg Lys Asp Glu Leu Lys Gly Ile
385                 390                 395                 400

Tyr Lys Asp Arg Met Lys Ile Gly Ala Ser Leu Ala Asp Val Asp Pro
                405                 410                 415

Met Gln Leu Asp Ser Ser Val Arg Phe Asp Ser Val Gly Gly Leu Ser
            420                 425                 430

Asn His Ile Ala Ala Leu Lys Glu Met Val Val Phe Pro Leu Leu Tyr
        435                 440                 445

Pro Glu Val Phe Glu Lys Phe Lys Ile Gln Pro Pro Arg Gly Cys Leu
    450                 455                 460

Phe Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Val Ala Arg Ala Leu
465                 470                 475                 480

Ala Asn Glu Cys Ser Gln Gly Asp Lys Arg Val Ala Phe Phe Met Arg
                485                 490                 495

Lys Gly Ala Asp Cys Leu Ser Lys Trp Val Gly Glu Ser Glu Arg Gln
            500                 505                 510

Leu Arg Leu Leu Phe Asp Gln Ala Tyr Gln Met Arg Pro Ser Ile Ile
        515                 520                 525

Phe Phe Asp Glu Ile Asp Gly Leu Ala Pro Val Arg Ser Ser Arg Gln
    530                 535                 540

Asp Gln Ile His Ser Ser Ile Val Ser Thr Leu Leu Ala Leu Met Asp
545                 550                 555                 560

Gly Leu Asp Ser Arg Gly Glu Ile Val Val Ile Gly Ala Thr Asn Arg
                565                 570                 575

Leu Asp Ser Ile Asp Pro Ala Leu Arg Arg Pro Gly Arg Phe Asp Arg
            580                 585                 590

Glu Phe Leu Phe Ser Leu Pro Asp Lys Glu Ala Arg Lys Glu Ile Leu
        595                 600                 605

Lys Ile His Thr Arg Asp Trp Asn Pro Lys Pro Leu Asp Thr Phe Leu
    610                 615                 620

Glu Glu Leu Ala Glu Asn Cys Val Gly Tyr Cys Gly Ala Asp Ile Lys
625                 630                 635                 640

Ser Ile Cys Ala Glu Ala Ala Leu Cys Ala Leu Arg Arg Arg Tyr Pro
                645                 650                 655

Gln Ile Tyr Thr Thr Ser Glu Lys Leu Gln Leu Asp Leu Ser Ser Ile
            660                 665                 670

Asn Ile Ser Ala Lys Asp Phe Glu Val Ala Met Gln Lys Met Ile Pro
        675                 680                 685

Ala Ser Gln Arg Ala Val Thr Ser Pro Gly Gln Ala Leu Ser Thr Val
    690                 695                 700

Val Lys Pro Leu Leu Gln Asn Thr Val Asp Lys Ile Leu Glu Ala Leu
705                 710                 715                 720

Gln Arg Val Phe Pro His Ala Glu Phe Arg Thr Asn Lys Thr Leu Asp
                725                 730                 735
```

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```
Ser Asp Ile Ser Cys Pro Leu Leu Glu Ser Asp Leu Ala Tyr Ser Asp
            740                 745                 750

Asp Asp Val Pro Ser Val Tyr Glu Asn Gly Leu Ser Gln Lys Ser Ser
        755                 760                 765

His Lys Ala Lys Asp Asn Phe Asn Phe Leu His Leu Asn Arg Asn Ala
    770                 775                 780

Cys Tyr Gln Pro Met Ser Phe Arg Pro Arg Ile Leu Ile Val Gly Glu
785                 790                 795                 800

Pro Gly Phe Gly Gln Gly Ser His Leu Ala Pro Ala Val Ile His Ala
                805                 810                 815

Leu Glu Lys Phe Thr Val Tyr Thr Leu Asp Ile Pro Val Leu Phe Gly
            820                 825                 830

Val Ser Thr Thr Ser Pro Glu Glu Thr Cys Ala Gln Val Ile Arg Glu
        835                 840                 845

Ala Lys Arg Thr Ala Pro Ser Ile Val Tyr Val Pro His Ile His Val
    850                 855                 860

Trp Trp Glu Ile Val Gly Pro Thr Leu Lys Ala Thr Phe Thr Thr Leu
865                 870                 875                 880

Leu Gln Asn Ile Pro Ser Phe Ala Pro Val Leu Leu Leu Ala Thr Ser
                885                 890                 895

Asp Lys Pro His Ser Ala Leu Pro Glu Glu Val Gln Glu Leu Phe Ile
            900                 905                 910

Arg Asp Tyr Gly Glu Ile Phe Asn Val Gln Leu Pro Asp Lys Glu Glu
        915                 920                 925

Arg Thr Lys Phe Phe Glu Asp Leu Ile Leu Lys Gln Ala Ala Lys Pro
    930                 935                 940

Pro Ile Ser Lys Lys Lys Ala Val Leu Gln Ala Leu Glu Val Leu Pro
945                 950                 955                 960

Val Ala Pro Pro Pro Glu Pro Arg Ser Leu Thr Ala Glu Glu Val Lys
                965                 970                 975

Arg Leu Glu Glu Gln Glu Glu Asp Thr Phe Arg Glu Leu Arg Ile Phe
            980                 985                 990

Leu Arg Asn Val Thr His Arg Leu Ala Ile Asp Lys Arg Phe Arg Val
        995                1000                1005

Phe Thr Lys Pro Val Asp Pro Asp Glu Val Pro Asp Tyr Val Thr Val
    1010                1015                1020

Ile Lys Gln Pro Met Asp Leu Ser Ser Val Ile Ser Lys Ile Asp Leu
1025                1030                1035                1040

His Lys Tyr Leu Thr Val Lys Asp Tyr Leu Arg Asp Ile Asp Leu Ile
                1045                1050                1055

Cys Ser Asn Ala Leu Glu Tyr Asn Pro Asp Arg Asp Pro Gly Asp Arg
            1060                1065                1070

Leu Ile Arg His Arg Ala Cys Ala Leu Arg Asp Thr Ala Tyr Ala Ile
        1075                1080                1085

Ile Lys Glu Glu Leu Asp Glu Asp Phe Glu Gln Leu Cys Glu Glu Ile
   1090                1095                1100

Gln Glu Ser Arg Lys Lys Arg Gly Cys Ser Ser Ser Lys Tyr Ala Pro
1105                1110                1115                1120

Ser Tyr Tyr His Val Met Pro Lys Gln Asn Ser Thr Leu Val Gly Asp
               1125                1130                1135

Lys Arg Ser ASP Pro Glu Gln Asn Glu Lys Leu Lys Thr Pro Ser Thr
           1140                1145                1150
```

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```
Pro Val Ala Cys Ser Thr Pro Ala Gln Leu Lys Arg Lys Ile Arg Lys
       1155                1160                1165

Lys Ser Asn Trp Tyr Leu Gly Thr Ile Lys Lys Arg Arg Lys Ile Ser
   1170                1175                1180

Gln Ala Lys Asp Asp Ser Gln Asn Ala Ile Asp His Lys Ile Glu Ser
1185                1190                1195                1200

Asp Thr Glu Glu Thr Gln Asp Thr Ser Val Asp His Asn Glu Thr Gly
               1205                1210                1215

Asn Thr Gly Glu Ser Ser Val Glu Glu Asn Glu Lys Gln Gln Asn Ala
           1220                1225                1230

Ser Glu Ser Lys Leu Glu Leu Arg Asn Asn Ser Asn Thr Cys Asn Ile
       1235                1240                1245

Glu Asn Glu Leu Glu Asp Ser Arg Lys Thr Thr Ala Cys Thr Glu Leu
   1250                1255                1260

Arg Asp Lys Ile Ala Cys Asn Gly Asp Ala Ser Ser Ser Gln Ile Ile
1265                1270                1275                1280

His Ile Ser Asp Glu Asn Glu Gly Lys Glu Met Cys Val Leu Arg Met
               1285                1290                1295

Thr Arg Ala Arg Arg Ser Gln Val Glu Gln Gln Gln Leu Ile Thr Val
           1300                1305                1310

Glu Lys Ala Leu Ala Ile Leu Ser Gln Pro Thr Pro Ser Leu Val Val
       1315                1320                1325

Asp His Glu Arg Leu Lys Asn Leu Leu Lys Thr Val Val Lys Lys Ser
   1330                1335                1340

Gln Asn Tyr Asn Ile Phe Gln Leu Glu Asn Leu Tyr Ala Val Ile Ser
1345                1350                1355                1360

Gln Cys Ile Tyr Arg His Arg Lys Asp His Asp Lys Thr Ser Leu Ile
               1365                1370                1375

Gln Lys Met Glu Gln Glu Val Glu Asn Phe Ser Cys Ser Arg
           1380                1385                1390
```

3. The method according to claim 1, where the fragment of the co-modulator contains the amino acids 813-1390 of ARAP11.

4. The method according to claims **1**, **2** or **3**, where the nuclear receptor is selected from androgen receptor, estrogen receptor α, estrogen receptor β, progesterone receptor A, progesterone receptor B, glucocorticoid receptor, mineralocorticoid receptor, thyroid hormone receptor, vitamin D receptor, peroxisome proliferator-activated receptor, retinoic acid receptor, retinoid X receptor, and orphan receptors.

5. The method according to claim 4 where the cells are established cell lines and/or eukaryotic cells.

6. The method according to claim 5, where the eukaryotic cells are selected from prostate cells, nerve cells, glial cells, fibroblasts, blood cells, osteoblasts, osteoclasts, hepatocytes, epithelial cells, or muscle cells.

7. The method according to claims **1**, **2**, or **3** where the vector is a eukaryotic expression vector.

8. A method for determining defects in the co-modulation mechanism between androgen receptors and ARAP11, wherein the concentrations of ARAP11 or a fragment thereof and of androgen receptor and/or a fragment thereof are measured.

9. The method according to claim 8, where the concentration measurement is carried out by radioimmunoassay, an ELISA test, immunostaining, RT-PCR, Western Blot, Northern Blot, DNA microarrays, or protein microarrays.

10. A protein or a fragment thereof with co-modulator properties for the androgen receptor, having the amino acid sequence according to claim 2.

11. The protein according to claim 10, where the fragment contains amino acids 813-1390.

12. A DNA sequence coding for the proteins according to claim 10 or **11** or DNA hybridizing with said DNA sequence.

13. A method for testing the hormonal or anti-hormonal effect of a chemical compound in vitro comprising the steps of:

(a) providing cells which are transfected with two vectors, wherein one of said vectors contains DNA coding for a nuclear receptor protein or a fragment thereof, especially a human nuclear receptor or a fragment thereof, and the other vector contains DNA which codes for a co-modulator or a fragment thereof;

(b) exposing the transformed host cells to the chemical compound; and

(c) measuring the level of transcriptional activity caused by the hormone receptor.

14. The method according to claim 13, where the co-modulator is ARAP11, having the amino acid sequence SEQ ID No. 2.

15. The method according to claim 13, where the fragment of the co-modulator contains the amino acids 813-1390 of ARAP11.

16. The method according to claims **13**, **14** or **15**, where the nuclear receptor is selected from androgen receptor, estrogen receptor α, estrogen receptor β, progesterone receptor A, progesterone receptor B, glucocorticoid receptor, mineralocorticoid receptor, thyroid hormone receptor, vitamin D receptor, peroxisome proliferator-activated receptor, retinoic acid receptor, retinoid X receptor, and orphan receptors.

17. The method according to claim 16 wherein the cells are established cell lines and/or eukaryotic cells.

18. The method according to claim 17, wherein the eukaryotic cells are selected from the group consisting of prostate cells, nerve cells, glial cells, fibroblasts, blood cells, osteoblasts, osteoclasts, hepatocytes, epithelial cells, or muscle cells.

19. The method according to claims **13**, **14**, or **15** where the vector is a eukaryotic expression vector.

20. A method for testing the androgenic or antiandrogenic effect of a chemical compound in vitro comprising the steps of:

(a) transforming host cells with a genetic construct effective in that host cell to produce both human androgen receptor protein and ARAP11 protein;

(b) exposing the transformed host cells to the chemical compound; and

(c) measuring the level of transcriptional activity caused by said androgen receptor.

21. The method of claim 20 wherein the host cells are selected from the group consisting of prostate cells, nerve cells, glial cells, fibroblasts, blood cells, osteoblasts, osteoclasts, hepatocytes, epithelial cells, or muscle cells.

22. The method of claim 20 wherein the genetic construct producing the ARAP11 protein has the DNA sequence of SEQ ID NO. 1.

23. The method of claim 20 wherein the genetic construct also includes a reporter gene, the expression of which can be detected and quantified.

24. The method of claim 20 wherein the chemical compound is a pharmaceutical.

25. The method of claim 20 wherein the chemical compound is contained in an environmental sample.

26. The method of claim 23 wherein the reporter gene is selected from the group consisting of: the gene for β-galactosidase, the gene for alkaline phosphatase, the gene for chloramphenicol acetyl transferase, the gene for catechol dioxygenase, the gene for "green fluorescent protein", and the luciferase genes.

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专利名称(译)	测试物质的激素作用的方法		
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摘要(译)

描述了一种测试物质的激素作用，特别是雄激素或抗雄激素作用的方法，其中（a）用两种载体转染的细胞，这些载体之一含有编码核受体蛋白或其片段的DNA。而另一种载体含有编码共调节剂或其片段的DNA，暴露于该物质；（b）核受体或其片段在共调节剂或其片段存在下诱导的转录活性和/或该物质对受体或其片段与共调节剂或其共有物之间相互作用的影响。通过蛋白质 - 蛋白质相互作用或蛋白质 - 蛋白质-DNA相互作用来测量片段。另外，提供了一种用于确定共调制机制中的缺陷的方法和适合于执行该方法的装置。

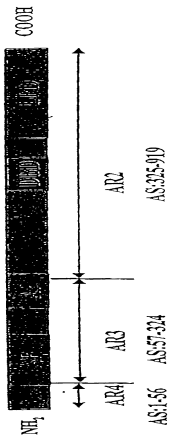

Figure 1. AF = activation function; DBD = DNA binding domain; LBD = ligand binding domain; and AS = amino acid