Published in final edited form as:

Mol Phylogenet Evol. 2008 May ; 47(2): 637–649. doi:10.1016/j.ympev.2007.12.026.

The human progesterone receptor shows evidence of adaptive evolution associated with its ability to act as a transcription factor

Caoyi Chen^{1,2}, Juan C. Opazo^{1,3}, Offer Erez⁴, Monica Uddin¹, Joaquin Santolaya-Forgas^{4,5}, Morris Goodman^{1,6}, Lawrence I. Grossman¹, Roberto Romero^{1,4}, and Derek E. Wildman^{1,4,7}

¹ Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI, United States, 48201

⁴ Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, United States, 20892

⁵ Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States, 02115

⁶ Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI, United States, 48201

⁷ Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, United States, 48201

Abstract

The gene encoding the progesterone receptor (*PGR*) acts as a transcription factor, and participates in the regulation of reproductive processes including menstruation, implantation, pregnancy maintenance, parturition, mammary development, and lactation. Unlike other mammals, primates do not exhibit progesterone withdrawal at the time of parturition. Because progesterone-mediated reproductive features vary among mammals, *PGR* is an attractive candidate gene for studies of adaptive evolution. Thus, we sequenced the progesterone receptor coding regions in a diverse range of species including apes, Old World monkeys, New World monkeys, prosimian primates and other mammals. Adaptive evolution occurred on the human and chimpanzee lineages as evidenced by statistically significant increases in nonsynonymous substitution rates compared to synonymous substitution rates. Positive selection was rarely observed in other lineages. In humans, amino acid replacements occurred mostly in a region of the gene that has been shown to have an inhibitory function (IF) on the ability of the progesterone receptor to act as a transcription factor. Moreover, many of the nonsynonymous substitutions in primates occurred in the N-terminus. This suggests that cofactor interaction surfaces might have been altered, resulting in altered progesterone-regulated gene transcriptional effects. Further evidence that the changes conferred an adaptive advantage comes from SNP analysis indicating only one of the IF changes is polymorphic in humans. In chimpanzees, amino acid changes occurred in both the inhibitory and transactivation domains. Positive selection provides the basis for the hypothesis that changes in structure and function of the progesterone

Address correspondence to: Derek E. Wildman, Ph.D. (E-mail: dwildman@med.wayne.edu) or Roberto Romero, M.D. (E-mail: prbchiefstaff@med.wayne.edu), Perinatology Research Branch, NICHD, NIH, DHHS, Wayne State University/Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA, Telephone (313) 993-2700, Fax: (313) 993-2694.

²Current Address: Nantong University, 9 Seyuan Road, Nantong, JS, PR China 226019;

³Current address: Instituto de Ecología y Evolución, Universidad Austral de Chile, Casilla 567, Valdivia, Chile;

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

receptor during evolution contributes to the diversity of primate reproductive biology, especially in parturition.

Introduction

The steroid hormone progesterone is a vital regulator of reproduction in mammals (Csapo, 1961; Etgen et al., 2001; Graham and Clarke, 1997; Hunter, 1996; Pinter et al., 1996; Rider, 2002). The role of progesterone in mammalian pregnancy maintenance is well established (DeMayo et al., 2002; Elovitz and Mrinalini, 2006; Henson, 1998; Liggins et al., 1972; Mesiano, 2001; Romero et al., 2006; Zakar and Hertelendy, 2007). In primates, progesterone participates in the regulation of normal menstrual cycles, ovulation (An et al., 2005; Catt, 1970), and embryo implantation (Bagchi et al., 2005; Goldman and Shalev, 2006). Many of the biological actions of progesterone are mediated through the progesterone receptor (PR; HUGO gene symbol=*PGR*), a member of the nuclear receptor superfamily of ligand-activated transcription factors (Evans, 1988; O'Malley and Tsai, 1992; Tsai and O'Malley, 1994). The gene encoding this receptor arose early in vertebrate evolution via a series of duplications of an ancestral estrogen receptor (Thornton, 2001). The progesterone receptor has been implicated in the initiation of human parturition as the relative abundance of PR isoforms changes near the end of gestation (Condon et al., 2006; Merlino et al., 2007; Mesiano et al., 2002; Mesiano, 2004; Oh et al., 2005; Smith, 2007).

Humans and their closest relatives, the chimpanzees, differ from each other in numerous ways that could be related to progesterone and its receptor. The duration of parturition in humans is, on average, longer than the 80-minute duration observed in chimpanzees (Keeling and Roberts, 1972). Chimpanzee mammary glands, like those of other mammals, are enlarged during lactation whereas human mammarys are consistently evident beyond puberty. Conversely, unlike in humans, cyclic swelling of the skin surrounding the anogenital region of chimpanzees is obvious.

In spite of these differences between humans and chimpanzees, the hormonal profiles and length of the menstrual cycles of humans and chimpanzees are mostly similar. The mean length of gestation in chimpanzees is 227 days compared to 280 in humans (Graham, 1970; Keeling and Roberts, 1972). This difference is actually less than it seems. Human gestational length is measured from the first day of the last menses about two weeks before conception. In contrast, chimpanzee gestations are measured to begin from the last day of maximal sex skin tumescence during a cycle in which copulation was observed. Therefore, the actual difference in gestation length between human and chimpanzees is between 20 to 30 days. In addition, the placental morphology and serum progesterone concentrations of humans and chimpanzees are similar, while rhesus and baboon have lower progesterone levels (Faiman et al., 1981).

Among all of these features, the unique role of *PGR* in human parturition is of considerable interest because of the special challenges humans face during the birth process. Natural selection during human evolution has resulted in anatomical changes including the remodeling of the pelvis during the emergence of bipedalism and the expansion of the cranium associated with encephalization that may have affected parturition (Rosenberg and Trevathan, 2002; Smith, 2007). These anatomical innovations that resulted in a relatively smaller birth canal and larger head are likely to have required adjustments underlying the labor and birth process (i.e., parturition has a longer duration in humans than in chimpanzees). The current study examines the evolution of mammalian *PGR* within this broad context of human evolution.

Structure of Progesterone Receptor

The gene encoding human PR has eight exons and is located on chromosome 11q22.1. PR is characterized by alternatively spliced isoforms (Kastner et al., 1990), as shown in Fig. 1. The two well-studied major isoforms of PR are PR-B and PR-A. Another isoform, PR-C, has also been described (Condon et al., 2006; Wei et al., 1996). Exons one and two can be considered alternatively spliced exons because all of exon one and most of exon two is absent from PR-C and part of exon one is absent from PR-A. The remaining exons are considered constitutively spliced since they are included in all major isoforms.

The major isoforms vary in length but not in amino acid sequence (i.e., there is no frame-shift). PR-B is 933 amino acids in length, while PR-A lacks 164 amino acids at the amino terminus. In vitro, PR-B is a stronger transactivator than PR-A, whereas PR-A acts as a transrepressor of PR-B and some other steroid receptors (McDonnell et al., 1994; Vegeto et al., 1993). The domain architecture of PR is shown in Fig 1C. Structurally, both isoforms consist of an amino-terminal region, a centrally located DNA binding domain (DBD), and a carboxy-terminal hinge region containing nuclear localization signals, as well as a ligand-binding domain (LBD, sometimes called HBD). There are three transcription activation function (AF) domains. AF-1 is located upstream of the DBD domain whereas AF-2 is located in the LBD (Hirata et al., 2003). The other activation function domain (AF-3) is unique to the PR-B isoform, and is located within the N-terminal region (Meyer et al., 1992; Sartorius et al., 1994). There is also an inhibitory function (IF) region located between AF-3 and AF-1, which has been proposed as the region responsible for autoinhibition and transrepression of PR (Abdel-Hafiz et al., 2002; Hovland et al., 1998; Huse et al., 1998). The function of PR-C is still unclear (Hirata et al., 2000, 2002; Yamanaka et al., 2002). It is translated from an in-frame translation start site, Met⁵⁹⁵, at the end of second exon of *PGR*. Evidence of the presence of the PR-C isoform has been found in human (Hirata et al., 2000, 2002, 2003; Saner et al., 2003), mouse (Condon et al., 2006), rat (Fang et al., 2002), guinea pig (Rodriguez et al., 2003), and cow (Schams et al., 2003). Since PR-C lacks the DBD, if bound to PR-B the resulting heterodimer would be unable to function as a transcription factor (Wei et al., 1997). Moreover, the absence of the DBD in PR-C would also prevent PR-C homodimers from acting as a transcription factor.

Adaptive Evolution in Progesterone Receptor

A recent genome-wide scan (Nielsen et al., 2005) of human and chimpanzee genes placed *PGR* among the 50 genes showing the strongest statistical evidence of positive selection as measured by calculating the ratio of nonsynonymous to synonymous nucleotide substitution rates on a per site basis. This ratio is called dN/dS or ω . Generally, $\omega = 1$, >1 , and <1 , indicates neutral evolution, positive selection, and purifying selection, respectively (Yang and Bielawski, 2000; Yang, 2002). Nielsen et al. identified 11 nonsynonymous and 0 synonymous *PGR* substitutions between human and chimpanzee. Therefore, changes in structure and function of the progesterone receptor during evolution may participate in reproductive variation between humans and chimpanzees. As the Nielsen et al. study included only humans and chimpanzees; the authors could not determine whether the positive selection occurred on the human, the chimpanzee, or both lineages. Moreover, the study did not explore whether positive selection was limited to humans and chimpanzees or whether this phenomenon occurred on other mammalian lineages as well. Finally, the study did not describe the locations of the nucleotide substitutions in terms of domains and/or isoforms.

Therefore, in order to determine if positive selection on *PGR* occurred only in humans, in chimpanzees, or among other mammals as well, we collected DNA sequence data from all major primate and non-primate placental mammal lineages. With these nucleotide sequence data in hand, using both probabilistic maximum likelihood and parsimony based approaches, we performed the following analyses: 1) detection of the effects of natural selection on the

PGR during primate, and especially human and chimpanzee, evolution; 2) reconstructions of the evolutionary history of the *PGR* and determination of the specific lineages on which amino acids were replaced; and 3) localization of putatively functionally important amino acid replacements in the gene.

Materials and Methods

DNA Samples and Sequencing

Genomic DNA was used as a template for the polymerase chain reaction to obtain the entire coding region of progesterone receptor in *Homo (Homo) sapiens* (Human), *Homo (Pan) troglodytes* (Common chimpanzee), *Homo (Pan) paniscus* (Bonobo), *Gorilla gorilla* (Gorilla), *Pongo pygmaeus* (Orangutan), *Hylobates lar* (White handed gibbon), *Macaca sylvanus* (Barbary macaque), *Papio anubis* (Olive baboon), *Chlorocebus aethiops* (African green monkey), *Colobus guereza* (Black-and-white colobus monkey), *Trachypithecus obscurus* (Dusky leaf-eating monkey), *Cebus apella* (Tufted capuchin monkey), *Pithecia irrorata* (Gray saki monkey), and *Ateles paniscus* (Black spider monkey). For the following species, exon one was obtained: *Tarsius bancanus* (Western tarsier), *Otolemur crassicaudatus* (Thick-tailed bushbaby), *Mirza coquereli* (Giant mouse-lemur), *Dasypus novemcinctus* (Nine-banded armadillo), *Bradypus sp.* (Three-toed sloth), *Loxodonta africana* (African elephant), *Dugong dugon* (dugong). All sequences have been deposited in GenBank under accession numbers DQ234979-DQ234989 and DQ485133-DQ485143. Published sequences from GenBank for *PGR* used in analyses are *Mus musculus* (Mouse) (NM_008829), *Rattus norvegicus* (Rat) (NM_022847), *Oryctolagus cuniculus* (Rabbit) (M14547) and *Canis familiaris* (Dog) (NM_001003074).

PCR reactions were performed in an Eppendorf (Hamburg, Germany) thermal cycler under the following conditions: 5 min at 95 °C followed by 30–34 cycles of 94 °C for 30 s, a variable annealing step of between 45 and 68 °C for 30–40 s, and 68/72 °C for 30 s–3 min, followed by a final extension at 72 °C for 5 min. *PGR* exons 2–8 were amplified using Taq DNA polymerase (Qiagen Inc., Valencia, CA). The first exon was amplified with the Takara LA PCR Kit, Version 2.1 (Takara, Shiga, Japan). Amplification products were isolated by 1% agarose gel electrophoresis and purified with QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA). Purified PCR products were either sequenced directly with both sense and antisense *PGR*-specific primers or cloned into the pGEM-T easy vector (Promega, Madison, WI) and sequenced with SP6 and T7 primers. Primer sequences are available on request. For some large amplification products, several internal sequencing primers were used. Sequencing was performed using the ABI BigDye terminator cycle sequencing kit v3.1, and sequence electrophoresis was performed on an automated capillary ABI3700 sequencer (Applied Biosystems, Foster City, CA).

Sequence Assembly and Alignment

The DNA sequence data were edited and contigs were assembled using Sequencher 4.5 (Gene Codes, Ann Arbor, MI). Both the full-length coding region and exon 1 sequences of all taxa were aligned using ClustalX 1.83.1 (Higgins, 1994), and visually inspected to ensure that protein coding sequence indels preserved the correct reading frame. The alignment files are available online as supplemental material.

Tests for Selection

Aligned sequences were tested for evidence of positive selection using codon-based models (Goldman and Yang, 1994; Yang and Bielawski, 2000) as implemented in PAML v.3.15 (Yang, 1997). These tests are conducted from estimates of the dN/dS ratio (i.e. ω) that compares the rates of nonsynonymous (amino acid changing) substitution to synonymous (amino acid

maintaining) substitution on a per site basis. We conducted three conventionally applied tests of selection: neutrality, nested lineage, and branch-sites tests (Yang and Bielawski, 2000; Yang, 2002; Zhang et al., 2005).

In order to determine whether *PGR* dN/dS ratios were variable among the different branches of the tree, we tested the neutral prediction that ω is the same on all lineages by comparing a fixed ratio model (model 0) to the free ratio model (model 1). In addition, to test lineage-based hypotheses four nested models as well as model 0 were examined using the species with full coding region sequences (Fig. 2). A two ω model (Fig. 2a) estimated one ratio for primates (as a total group) and another ratio for the outgroup species. A three ω model (Fig. 2b) estimated one ratio for non-primates, and two ratios for primates, namely the human-chimpanzee total group (all descendant branches in the human-chimpanzee clade including their stem lineage), and all the other primates. A four ω model (Fig. 2c) estimated one ratio for non-primates and three ratios for primates- the human-chimpanzee crown group (the descendent lineages from the last common ancestor of humans and chimpanzees), the human-chimpanzee stem lineage, and all the other primates. A five ω model (Fig. 2d) estimated five different ratios as follows: 1) non-primates, 2) the human terminal branch, 3) the chimpanzee clade, 4) the human-chimpanzee stem, and 5) the other primate lineages. In every model tested, the likelihood values were calculated three times, with different starting seed values for the ω parameter (0.5, 1, and 2) to increase the probability of reaching the optimal likelihood score.

Branch sites tests of Zhang et al. (2005) were conducted to detect individual *PGR* codons that had experienced positive selection. These tests compare *a priori* determined foreground and background branches. In our test, branches in the human and chimpanzee crown group were the foreground lineages. The remaining branches were the background. Model A is the alternative hypothesis and assumes that on the foreground branch(es) some codons have undergone purifying selection while other codons are neutral or positively selected. Selection is not allowed on background branches. Model A was compared to the null model which fixed $\omega=1$ on the foreground in the two site classes that allow selection. Bayes Empirical Bayes (BEB) posterior probabilities for site classes were examined to infer sites that likely were under positive selection..

Likelihood scores for alternative models were compared by likelihood ratio tests, and a χ^2 test was used to determine significance. A result was considered significant if $p<0.05$. All tests were conducted using presumed phylogenetic trees (Goodman et al., 2005; Murphy et al., 2001)

Evolutionary rates

In addition to maximum likelihood tests for selection, maximum parsimony ancestral sequences (ACCTRAN and DELTRAN) were inferred using the software PAUP* (70) to identify the specific lineages and sites where nucleotide substitutions and amino acid replacements occurred. With these data, we compared the alternatively spliced exons (i.e. exons 1 and 2) to the exons found in all *PGR* isoforms (i.e., the constitutively spliced exons 3–8). In particular, we assessed the number of nucleotide substitutions per site in human descent from the last common ancestor of anthropoid primates for different exon classes: each alternatively spliced exon (one and two), exons 3–8, and the full gene. The exon classes were compared to one another by either a χ^2 test or Fisher's exact test. Previously published phylogenetic trees of primate and mammalian relationships were employed to describe the pattern of nucleotide substitution during evolution (Goodman et al., 2005; Kriegs et al., 2006; Murphy et al., 2001).

Results

Characterization of sequences and evolutionary changes in progesterone receptor

The multiple sequence alignment of coding nucleotide sequences including indels spanned 2853bp. Aligned exon 1 sequences are 1688 bp. Exons 2–8 have an alignment length of 1165 bp. We obtained two slightly different clones from *Tarsius*, suggesting that there is a second copy of *PGR* in that taxon.

All the taxa included in our analysis have alternative translation initiation sites (TIS) for the three major isoforms, PR-B/PR-A/PR-C. PR-B/A TIS, Met¹ and Met¹⁶⁵ are inside the first exon region. PR-C uses an in-frame translation start site Met⁵⁹⁵ at the end of the second exon. The PR-C in-frame TIS not only exists in the taxa included in the full *PGR* gene analysis, but also in the publicly available whole genome assemblies and Ensembl gene builds for armadillo (*Dasypus novemcinctus*), tenrec (*Echinops telfairi*), elephant (*Loxodonta africana*), opossum (*Monodelphis domestica*), chicken (*Gallus gallus*), frog (*Xenopus tropicalis*), spotted green pufferfish (*Tetraodon nigrovirdis*), Japanese pufferfish (*Fugu rubripes*), and zebrafish (*Danio rerio*) (data not shown).

In primates the first exon is more variable than the other exons. Human-specific amino acid changes are mostly in the first exon, specifically in the IF region (Table 1). According to parsimony ancestral reconstructions, there were 17 unambiguous amino acid replacements in the human and chimpanzee clade (Fig. 3). Eight of these replacements occurred on the human lineage and eight in the chimpanzee lineages. There was one replacement on the human-chimpanzee stem lineage. Six of the eight inferred amino acid replacements on the human terminal lineage were in the inhibitory function region. The other two amino acid replacements were in AF3 and hinge region, respectively. All human replacements occurred in exon one with the exception of one replacement in the hinge region. Searches of the NCBI single nucleotide polymorphism database (dbSNP) revealed only one nonsynonymous SNP among the codons changed on the human lineage (Table 1). There were eight amino acid replacements observed in chimpanzees (two on the chimpanzee stem, three on the bonobo terminal lineage, and three in the common chimpanzee terminal lineage). Of these eight replacements, four are in the IF region (one of the amino acid changes was on the chimpanzee stem and three were on the terminal lineage of the common chimpanzee). The remaining replacements are scattered among the transactivation AF1 and AF3 domains, and the hinge region (Fig. 3).

Parsimony analysis inferred a range of three to five amino acids replacements on the stem lineage leading to macaque and baboon: 1) a minimum of three changes, all in the IF region; or 2) a maximum of five amino acids changes, of which four are in the IF region and one is in the AF1 domain. Additionally, there were four unambiguous amino acid replacements on the haplorhine (anthropoid and tarsier) stem (two in the IF region and two in the AF3 domain).

Functionally important conserved amino acid sites, including ⁵K, ⁷K, ⁵⁵LxxLL(L1), ¹¹⁵LxxLL(L2), ¹⁴⁰W, ³⁸⁷IKEE, and ⁵³¹K (amino acid numbers are from the human protein sequence) have been identified in PR (Man et al., 2006; Takimoto et al., 2003; Tung et al., 2006). According to our data, three of these sites (⁷K, ³⁸⁷IKEE, and ⁵³¹K) are uniformly conserved. The others have experienced amino acid replacements during mammalian evolution. ⁵K is replaced by Q in mouse and rat and T in dog. ¹⁴⁰W is replaced by ¹⁴⁰R in dog. L1 is replaced by LxxLV in *Otolemur*, SxxLL in dog and LxxLF in *Bradypus*. L2 is replaced by LxxLW in NWMs and PxxAL in dog.

Tests for selection- variable dN/dS rates among lineages and sites

The ω values estimated among sites are shown in Fig. 4. For the full gene (Fig. 4a), the free ratio model (model 1, log likelihood = -1440.52) fits the data significantly better ($p < 0.001$)

than the one ratio model (model 0, log likelihood = -11500.53; Table 2). Similar results are observed in the analysis of the first exon (Fig. 4b). These results indicate that the dN/dS ratios are indeed different among lineages. The complete gene shows the signature of purifying selection; the background ω value (i.e., one ratio or Model 0) is 0.25. In contrast to the low background ω value, a few branches do show evidence of positive selection. The branch with the highest ratio for the full gene is the human terminal branch ($dN/dS = 1.63$; $N*dN=8.3$, $S*dS=2.1$). Most of this change occurred in exon 1 (Fig. 4b). In addition to the human terminal branch, accelerations occur in the chimpanzee clade (for both the full gene and for exon 1), the stem papionan (macaque and baboon) lineage for the full gene, and the stem papionan and stem haplorhine lineages for exon 1.

The dN/dS ratios estimated from the various nested models and likelihood ratio tests are shown in Table 2. The best-fit model ($p=0.021$; Table 3, Fig. 2c) for *PGR* evolution is the 4 ω model that estimated one ratio for non-primates and three rates for primates: 1) the human-chimpanzee crown group (the descendent lineages from the last common ancestor of humans and chimpanzees); 2) the human-chimpanzee stem lineage; and 3) the other primates. In this statistical model *PGR* has a > 7 fold higher dN/dS in the human and chimpanzee crown group (2.1682) compared with all other lineages thus providing strong evidence for positive selection in the gene. The branch sites tests identified a proportion of sites as potentially being positively selected (17%, $\omega=5.5$) although the null hypothesis could not be rejected ($p=0.22$).

Evolutionary rates

Our data demonstrate that the 5' region of *PGR* containing the sites associated with the transactivation and transrepression function is highly variable, whereas other coding regions are well conserved. The comparison of number of substitutions per site on the anthropoid lineages leading to human between alternatively and constitutively spliced exons is presented in Table 4. Exon 1 evolves much more rapidly than exon 2, even though both are alternatively spliced. During human descent from the last common anthropoid ancestor, exon 1 had significantly more substitutions per site in comparison to both the other alternatively spliced exon 2 and to the constitutively spliced exons 3–8. Most of this acceleration is due to nonsynonymous substitution. The background dN/dS value as calculated by PAML in alternatively spliced exons (exons 1 and 2) is more than four times higher than in other constitutive exons ($dN/dS=0.27$ vs. 0.06). The absolute value of dN is also increased when the alternatively spliced exons are compared to the constitutively spliced exons ($dN = 0.53$ vs. 0.07).

Discussion

This study investigated three aspects of *PGR* evolution: 1) the strength of positive selection (i.e., adaptive evolution) in human and chimpanzees lineages; 2) the prevalence of positive selection within primates; and 3) the specific isoforms and domains at which amino acids were replaced. Positive selection occurred on both the human and chimpanzee lineages. Exon 1 (particularly the IF region) was the region of the gene that showed the most amino acid replacements during human evolution after the split from the chimpanzee lineage. The chimpanzees also showed changes in the IF region but fewer than in the human lineage. In primates, positive selection occurred rarely and was mostly limited to the human and chimpanzee clade although the papionan (macaque and baboon) and haplorhine stem lineages also showed evidence of selection.

Comparatively, humans and chimpanzees showed a significantly higher nonsynonymous than synonymous substitution rate. This result is in general accord with the finding of Nielsen et al. (2005), who showed evidence for positive selection in human and chimpanzee *PGR*. However, with the addition of data from other species we demonstrate that positive selection occurred

on both the human and chimpanzee lineages. Furthermore, among chimpanzees there is evidence for positive selection in both common and bonobo chimpanzees as well as on their stem lineage.

An alternative interpretation to the finding of adaptive evolution is that the human and chimpanzee changes have no functional consequences and have evolved neutrally under relaxed selection. This is unlikely because in humans there is only one reported nonsynonymous SNP in the codons encoding the amino acids that changed on the human lineage, which suggests that substitutions were fixed before the last common ancestor of modern humans (Table 1). We propose that natural selection first spread these amino acid replacements through the human lineage then preserved these sites at which point no further changes took place.

Among all sampled taxa, the first exon includes a CpG island. CpG dinucleotides have high mutation rates and increased frequency of transitions relative to transversions because they are prone to be mutated by cytosine deamination (Antequera, 2003) (Hellmann et al., 2003). As such, one possible interpretation of the results is that the observed amino acid replacements are due solely to CpG status rather than adaptive evolution. However, CpG islands differ from methylated CpG dinucleotides. They usually escape methylation in the germline and remain consistently nonmethylated in normal tissues, with the exception of some imprinted genes and inactivated X chromosomes (Antequera, 2003; Filippova et al., 2005; Mohandas et al., 1981; Li et al., 1993; Weber et al., 2007). In our dataset, the ratio of transitions over transversions (ts/tv) in the CpG island region (2.88) is lower than in the non- CpG island region (exon 2–8) (3.09). This suggests that the island escapes the effects of rapid mutation due to CpG status. More importantly, none of the nonsynonymous substitutions in the human-chimpanzee clade took place at CpG dinucleotides. Finally, progesterone receptors are unmethylated in normal human tissues such as uterine endometrium, breast tissues, and bone marrow (Lapidus RG et al., 1996; Liu et al., 2004; Sasaki et al., 2001). Therefore, we do not attribute the increase in nonsynonymous substitution rate observed in human and chimpanzees to CpG status.

Conservation in PGR

The pattern of selection observed among humans and chimpanzees is fairly rare in the context of primate and even mammalian *PGR* evolution. Of 33 mammalian lineages examined for exon 1 (Fig. 4b), only six had dN/dS ratios greater than 1, and four of these fall within the human chimpanzee clade. Throughout the majority of primate evolution, purifying selection has acted strongly on *PGR*, even in the most variable region of the gene, exon 1. As mentioned before, some key sites for PR autoinhibitory and transrepression function in the exon one IF region are ⁷K, ³⁸⁷IKEE, and ⁵³¹K. These residues are uniformly conserved among all the species in our study as well as in the chicken (Takimoto et al., 2003). This implies that the inhibitory function of the IF region is highly conserved.

PGR is characterized by several alternatively spliced isoforms, and alternative splicing is a powerful and economical way to increase protein diversity from a single gene locus in the course of evolution. The three major alternative N-terminal isoforms PR-A/B/C have some overlapping and some unique functions (Conneely et al., 2002; Condon et al., 2006; Hovland et al., 1998; Meyer et al., 1992; Mulac-Jericevic et al., 2000; Mulac-Jericevic and Conneely, 2004; Vegeto E, 1993). Our study provides evidence for the existence of these three *PGR* isoforms in all species studied (as measured by conservation of the three alternative TIS). Until the transcripts of the different *PGR* isoforms of the study species are available, a definitive description of the effects of alternative splicing in *PGR* evolution will be lacking. Finally, the DBD and LBD are highly conserved among all the mammals we sequenced as part of this study, and we found no evidence of rapid evolution in either the DBD or LBD.

Functional consequences of rapid evolution in PGR

PGR, and particularly the inhibitory function region, have evolved rapidly during recent human evolution. Indeed, 75% (6/8) of the amino acid changes on the human lineage since the human-chimpanzee most recent common ancestor (MRCA) were in the first exon in the IF region (Table 1), and in chimpanzees the amino acid changes were in both the IF region and transactivation domains (Fig. 3).

Although the exact functional consequences of the observed amino acid replacements are unclear, we can speculate about their effects based on their location in the protein. The AF3, IF, and AF1 regions provide surfaces for transcriptional activation and repression of progesterone-modulated genes, and also are involved in *PGR* autoinhibitory function. Additional functional consequences that could be implicated from changes in these regions include cofactor recruitment, cofactor-binding affinity, the conformation of the protein complexes of PR and its coregulators, and the transcriptional activity of PR-B. In this last example it is important to note that AF3 is unique to PRB. Nonsynonymous substitutions took place in AF3 domain in humans and chimpanzees, and this region might directly influence the intramolecular communication between the PR-B N terminus and downstream domains (Takimoto et al., 2003). The two highly conserved L1 boxes and ¹⁴⁰W that define the AF3 related-activity (Man et al., 2006; Takimoto et al., 2003) had amino acid replacements in New World monkeys, *Otolemur*, dog, and *Bradypus*. These observed amino acid replacements might also affect coactivator recruitment and direct N-/C- terminal interaction. Coactivators known to act on progesterone receptor include c-AMP responsive element binding protein 1 (*CREB1*) and members of the steroid receptor coactivator (SRC) family (Condon et al., 2003). An additional interpretation is that the observed amino acid replacements are compensatory substitutions for replacement in protein cofactors, which are recruited by the activated *PGR* to the promoters of target genes in each species.

Implications of this study

PGR is an important factor in the normal development and function of the reproductive tract (Bagchi et al., 2005; Briton-Jones et al., 2005; Schams et al., 2003), mammary glands (Schams et al., 2003), and even reproductive behavior (Condon et al., 2003; Auger, 2004). Changes in the expression of PR-B and PR-A have been reported in reproductive tract malignancies such as endometrial (Sasaki et al., 2001) and ovarian tumors (Akahira et al., 2000) as well as in breast cancer (Lapidus RG et al., 1996). Possible clinical implications of the rapid evolutionary divergence in the progesterone receptors of humans and chimpanzees should therefore be considered.

The observed amino acid replacements may represent species-specific changes in the biologic inhibitory/repressor activity of the PR in human and chimpanzees. The six human amino acid replacements found in the IF region combined with the lack of nonsynonymous human SNPs at five of the six codons raises the possibility that the changes on the human lineage were fixed before the mrca of modern humans. This finding strengthens the evidence for adaptive evolution in human *PGR* and the conclusion that observed amino acid replacements have functional importance. The IF is essential for PR-A function, and researchers have suggested that one of the functions of the AF3 domain in the PR-B isoform is to block the inhibitory effect of the IF (Challis et al., 2000; Tung et al., 2006).

A possible implication of the evolutionary changes in the IF region can be related to the mechanism of human labor, many aspects of which are unique among mammals (Smith, 2007). For example, it has been proposed that primates have a functional progesterone withdrawal leading to labor (Brown et al., 2004; Challis et al., 1999; Challis et al., 2000; Condon et al., 2003,2006; Goldman et al., 2005; Goldman and Shalev, 2007; Mesiano et al.,

2002; Mesiano, 2004). One of the proposed mechanisms that effectively cause functional progesterone withdrawal during parturition is the change in the relative expression of PR-A, PR-B, and PR-C in myometrium, fetal membranes, and placenta during the onset of labor (Condon et al., 2006; Merlino et al., 2007; Mesiano et al., 2002; Mesiano, 2004). It is possible that the expression and interaction among the different PR isoforms in functional progesterone withdrawal during human and chimpanzee parturition are uniquely modulated via changes in the IF region. Human parturition is different from that of chimpanzees and other mammals because in the past six million years human anatomy has been modified by the emergence of bipedalism (resulting in the remodeling of the pelvis) as well as encephalization (resulting in an increased cranial size relative to body mass) (Rosenberg and Trevathan, 2002). These anatomical changes were certainly adaptive, and the mechanism of human parturition may have undergone modification to account for the modified morphology. Chimpanzees have also been subject to evolutionary pressures on their reproductive biology as evidenced by the presence of sex skin swelling and extreme differences in sexual behavior observed between common and bonobo chimpanzees (Graham 1981; de Waal and Lanting, 1997). Positively selected changes in *PGR*, particularly in its IF region, might well have participated in the emergence of these adaptations. However, elucidating the exact functional consequences of progesterone receptor evolution must await further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was funded by the Intramural Research Program of the National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services and by the National Science Foundation, BCS-0550209. The authors acknowledge the following sources of biological materials and DNA samples: The Biochain Institute (Hayward, CA), the Brazilian National Primate Center (BCNP), CRES at the San Diego Zoological Society, the Duke University Primate Center, the Detroit Zoological Society, the Southwest Foundation for Biomedical Research (San Antonio, TX), Kathy Neiswanger (University of Pittsburgh), Caro-Beth Stewart (SUNY, Albany), and Jerry Slightom.

Reference List

- Abdel-Hafiz H, Takimoto GS, Tung L, Horwitz KB. The inhibitory function in human progesterone receptor N termini binds SUMO-1 protein to regulate autoinhibition and transrepression. *J Biol Chem* 2002;277:33950–33956. [PubMed: 12114521]
- Akahira J, Inoue T, Suzuki T, Ito K, Konno R, Sato S, Moriya T, Okamura K, Yajima A, Sasano H. Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies. *Br J Cancer* 2000;83:1488–1494. [PubMed: 11076658]
- An BS, Choi JH, Choi KC, Leung PC. Differential role of progesterone receptor isoforms in the transcriptional regulation of human gonadotropin-releasing hormone I (GnRH I) receptor, GnRH I, and GnRH II. *J Clin Endocrinol Metab* 2005;90:1106–1113. [PubMed: 15562029]
- Antequera F. Structure, function and evolution of CpG island promoters. *Cell Mol Life Sci* 2003;60:1647–1658. [PubMed: 14504655]
- Auger AP. Steroid receptor control of reproductive behavior. *Horm Behav* 2004;45:168–172. [PubMed: 15047011]
- Bagchi IC, Li Q, Cheon YP, Mantena SR, Kannan A, Bagchi MK. Use of the progesterone receptor antagonist RU 486 to identify novel progesterone receptor-regulated pathways in implantation. *Semin Reprod Med* 2005;23:38–45. [PubMed: 15714388]
- Briton-Jones C, Lok IH, Cheung CK, Po AL, Chiu TT, Haines C. Ratio of mRNA expression of progesterone receptor isoforms AB is to B in human oviduct mucosal cells during the ovulatory cycle. *J Assist Reprod Genet* 2005;22:429–435. [PubMed: 16331541]

- Brown AG, Leite RS, Strauss JF 3rd. Mechanisms underlying “functional” progesterone withdrawal at parturition. *Ann NY Acad Sci* 2004;1034:36–49. [PubMed: 15731298]
- Catt KJ. IV. Reproductive endocrinology. *Lancet* 1970;1:1097–1104. [PubMed: 4191971]
- Challis J, Sloboda D, Matthews S, Holloway A, Alfaidy N, Howe D, Fraser M, Newnham J. Fetal hypothalamic-pituitary adrenal (HPA) development and activation as a determinant of the timing of birth, and of postnatal disease. *Endocr Res* 2000;26:489–504. [PubMed: 11196419]
- Challis JR, Patel FA, Pomini F. Prostaglandin dehydrogenase and the initiation of labor. *J Perinat Med* 1999;27:26–34. [PubMed: 10343931]
- Condon JC, Hardy DB, Kovacic K, Mendelson CR. Up-Regulation of the Progesterone Receptor (PR)-C Isoform in Laboring Myometrium by Activation of Nuclear Factor- κ B May Contribute to the Onset of Labor through Inhibition of PR Function. *Mol Endocrinol* 2006;20:764–775. [PubMed: 16339279]
- Condon JC, Jeyasuria P, Faust JM, Wilson JW, Mendelson CR. A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. *Proc Natl Acad Sci U S A* 2003;100:9518–9523. [PubMed: 12886011]
- Conneely OM, Mulac-Jericevic B, DeMayo F, Lydon JP, O’Malley BW. Reproductive Functions of Progesterone Receptors. *Recent Prog Horm Res* 2002;57:339–355. [PubMed: 12017551]
- Csapo AI. The onset of labour. *Lancet* 1961;2:277–280. [PubMed: 13718694]
- de Waal, FBM.; Lanting, F. *The Forgotten Ape*. University of California Press; Berkeley: 1997.
- DeMayo FJ, Zhao B, Takamoto N, Tsai SY. Mechanisms of action of estrogen and progesterone. *Ann N Y Acad Sci* 2002;955:48–59. [PubMed: 11949965]discussion 86–48, 396–406
- Elovitz MA, Mrinalini C. The use of progestational agents for preterm birth: lessons from a mouse model. *Am J Obstet Gynecol* 2006;195:1004–1010. [PubMed: 17000233]
- Etgen AM, Ansonoff MA, Quesada A. Mechanisms of ovarian steroid regulation of norepinephrine receptor-mediated signal transduction in the hypothalamus: implications for female reproductive physiology. *Horm Behav* 2001;40:169–177. [PubMed: 11534978]
- Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* 1988;240:889–895. [PubMed: 3283939]
- Faiman, C.; Reyes, FL.; Winter, JSD.; Hobson, WC. Endocrinology of pregnancy in apes. In: Graham, CE., editor. *Reproductive Biology of the Great apes: Comparative and Biomedical Perspectives*. Academic Press; London: 1981. p. 45–68.
- Fang X, Wong S, Mitchell BF. Messenger RNA for progesterone receptor isoforms in the late-gestation rat uterus. *Am J Physiol Endocrinol Metab* 2002;283:E1167–E1172. [PubMed: 12388156]
- Filippova GN, Cheng MK, Moore JM, Truong JP, Hu YJ, Nguyen DK, Tsuchiya KD, Disteché CM. Boundaries between chromosomal domains of X inactivation and escape bind CTCF and lack CpG methylation during early development. *Dev Cell* 2005;8:31–42. [PubMed: 15669143]
- Goldman N, Yang Z. A codon-based model of nucleotide substitution for protein-coding DNA sequences. *Mol Biol Evol* 1994;11:725–736. [PubMed: 7968486]
- Goldman S, Shalev E. Difference in progesterone-receptor isoforms ratio between early and late first-trimester human trophoblast is associated with differential cell invasion and matrix metalloproteinase 2 expression. *Biol Reprod* 2006;74:13–22. [PubMed: 16135696]
- Goldman S, Shalev E. Progesterone receptor profile in the decidua and fetal membrane. *Front Biosci* 2007;12:634–648. [PubMed: 17127324]
- Goldman S, Weiss A, Almalah I, Shalev E. Progesterone receptor expression in human decidua and fetal membranes before and after contractions: possible mechanism for functional progesterone withdrawal. *Mol Hum Reprod* 2005;11:269–277. [PubMed: 15764807]
- Goodman M, Grossman LI, Wildman DE. Moving primate genomics beyond the chimpanzee genome. *Trends Genet* 2005;21:511–517. [PubMed: 16009448]
- Graham, CE. Reproductive physiology of the chimpanzee. In: Bourne, GH., editor. *Immunology, Infections, Hormones, Anatomy, and Behavior*. S. Karger; New York: 1970. p. 183–220.
- Graham, CE. Menstrual cycle of the great apes. In: Graham, CE., editor. *Reproductive Biology of the Great apes: Comparative and Biomedical Perspectives*. Academic Press; London: 1981. p. 1–44.

- Graham JD, Clarke CL. Physiological action of progesterone in target tissues. *Endocr Rev* 1997;18:502–519. [PubMed: 9267762]
- Hellmann I, Zollner S, Enard W, Ebersberger I, Nickel B, Paabo S. Selection on human genes as revealed by comparisons to chimpanzee cDNA. *Genome Res* 2003;13:831–837. [PubMed: 12727903]
- Henson MC. Pregnancy maintenance and the regulation of placental progesterone biosynthesis in the baboon. *Hum Reprod Update* 1998;4:389–405. [PubMed: 9825854]
- Higgins DG. CLUSTAL V: multiple alignment of DNA and protein sequences. *Methods Mol Biol* 1994;25:307–318. [PubMed: 8004173]
- Hirata S, Shoda T, Kato J, Hoshi K. The novel isoform of the progesterone receptor cDNA in the human testis and detection of its mRNA in the human uterine endometrium. *Oncology* 2000;59(Suppl 1): 39–44. [PubMed: 11096355]
- Hirata S, Shoda T, Kato J, Hoshi K. The novel exon, exon T, of the human progesterone receptor gene and the genomic organization of the gene. *JSteroid Biochem Mol Biol* 2002;80:365–367. [PubMed: 11948021]
- Hirata S, Shoda T, Kato J, Hoshi K. Isoform/variant mRNAs for sex steroid hormone receptors in humans. *Trends Endocrinol Metab* 2003;14:124–129. [PubMed: 12670738]
- Hovland AR, Powell RL, Takimoto GS, Tung L, Horwitz KB. An N-terminal inhibitory function, IF, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors. *J Biol Chem* 1998;273:5455–5460. [PubMed: 9488667]
- Hunter RH. Ovarian control of very low sperm/egg ratios at the commencement of mammalian fertilisation to avoid polyspermy. *Mol Reprod Dev* 1996;44:417–422. [PubMed: 8858611]
- Huse B, Verca SB, Matthey P, Rusconi S. Definition of a negative modulation domain in the human progesterone receptor. *Mol Endocrinol* 1998;12:1334–1342. [PubMed: 9731702]
- Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J* 1990;9:1603–1614. [PubMed: 2328727]
- Keeling, ME.; Roberts, JR. Breeding and reproduction of chimpanzees. In: Bourne, GH., editor. *Histology, Reproduction, and Restraint*. University Park Press; Baltimore: 1972. p. 127–163.
- Kriegs JO, Churakov G, Kieffmann M, Jordan U, Brosius J, Schmitz J. Retroposed elements as archives for the evolutionary history of placental mammals. *PLoS Biol* 2006;4:e91. [PubMed: 16515367]
- Lapidus RG, Ferguson AT, Ottaviano YL, Parl FF, Smith HS, Weitzman SA, Baylin SB, Issa JP, NED. Methylation of estrogen and progesterone receptor gene 5' CpG islands correlates with lack of estrogen and progesterone receptor gene expression in breast tumors. *Clin Cancer Res* 1996;2:805–810. [PubMed: 9816234]
- Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. *Nature* 1993;366:362–365. [PubMed: 8247133]
- Liggins GC, Grieves SA, Kendall JZ, Knox BS. The physiological roles of progesterone, oestradiol-17 and prostaglandin F₂ in the control of ovine parturition. *J Reprod Fertil Suppl* 1972;16:85–10. [PubMed: 4345772]
- Liu ZJ, Zhang XB, Zhang Y, Yang X. Progesterone receptor gene inactivation and CpG island hypermethylation in human leukemia cancer cells. *FEBS Letters* 2004;567:327–332. [PubMed: 15178346]
- Man JH, Li HY, Zhang PJ, Zhou T, He K, Pan X, Liang B, Li AL, Zhao J, Gong WL, Jin BF, Xia Q, Yu M, Shen BF, Zhang XM. PIAS3 induction of PRB sumoylation represses PRB transactivation by destabilizing its retention in the nucleus. *Nucl Acids Res* 2006;34:5552–5566. [PubMed: 17020914]
- McDonnell DP, Shahbaz MM, Vegeto E, Goldman ME. The human progesterone receptor A-form functions as a transcriptional modulator of mineralocorticoid receptor transcriptional activity. *J Steroid Biochem Mol Biol* 1994;48:425–432. [PubMed: 8180103]
- Merlino AA, Welsh TN, Tan H, Yi LJ, Cannon V, Mercer BM, Mesiano S. Nuclear progesterone receptors in the human pregnancy myometrium: evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. *J Clin Endocrinol Metab* 2007;92:1927–1933. [PubMed: 17341556]
- Mesiano S. Roles of estrogen and progesterone in human parturition. *Front Horm Res* 2001;27:86–104. [PubMed: 11450438]

- Mesiano S. Myometrial progesterone responsiveness and the control of human parturition. *J Soc Gynecol Investig* 2004;11:193–202.
- Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *J Clin Endocrinol Metab* 2002;87:2924–2930. [PubMed: 12050275]
- Meyer ME, Quirin-Stricker C, Lerouge T, Bocquel MT, Gronemeyer H. A limiting factor mediates the differential activation of promoters by the human progesterone receptor isoforms. *J Biol Chem* 1992;267:10882–10887. [PubMed: 1587864]
- Mohandas T, Sparkes RS, Shapiro LJ. Reactivation of an inactive human X chromosome: evidence for X inactivation by DNA methylation. *Science* 1981;211:393–396. [PubMed: 6164095]
- Mulac-Jericevic B, Conneely OM. Reproductive tissue selective actions of progesterone receptors. *Reproduction* 2004;128:139–146. [PubMed: 15280552]
- Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of Reproductive Functions of Progesterone Mediated by Progesterone Receptor-B Isoform. *Science* 2000;289:1751–1754. [PubMed: 10976068]
- Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, Teeling E, Ryder OA, Stanhope MJ, de Jong WW, Springer MS. Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* 2001;294:2348–2351. [PubMed: 11743200]
- Nielsen R, Bustamante C, Clark AG, Glanowski S, Sackton TB, Hubisz MJ, Fledel-Alon A, Tanenbaum DM, Civello D, White TJ, Sninsky J, Adams MD, Cargill M. A scan for positively selected genes in the genomes of humans and chimpanzees. *PLoS Biol* 2005;3:e170. [PubMed: 15869325]
- O'Malley BW, Tsai MJ. Molecular pathways of steroid receptor action. *Biol Reprod* 1992;46:163–167. [PubMed: 1536890]
- Oh SY, Kim CJ, Park I, Romero R, Sohn YK, Moon KC, Yoon BH. Progesterone receptor isoform (A/B) ratio of human fetal membranes increases during term parturition. *Am J Obstet Gynecol* 2005;193:1156–1160. [PubMed: 16157129]
- Pinter JH, Deep C, Park-Sarge OK. Progesterone receptors: expression and regulation in the mammalian ovary. *Clin Obstet Gynecol* 1996;39:424–435. [PubMed: 8734007]
- Rider V. Progesterone and the control of uterine cell proliferation and differentiation. *Front Biosci* 2002;7:d1545–1555. [PubMed: 12045023]
- Rodriguez HA, Kass L, Varayoud J, Ramos JG, Ortega HH, Durando M, Munoz-De-Toro M, Luque EH. Collagen remodelling in the guinea-pig uterine cervix at term is associated with a decrease in progesterone receptor expression. *Mol Hum Reprod* 2003;9:807–813. [PubMed: 14614043]
- Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, Chaiworapongsa T, Mazor M. The preterm parturition syndrome. *BJOG* 2006;113(Suppl 3):17–42. [PubMed: 17206962]
- Rosenberg K, Trevathan W. Birth, obstetrics and human evolution. *BJOG* 2002;109:1199–1206. [PubMed: 12452455]
- Saner KJ, Welter BH, Zhang F, Hansen E, Dupont B, Wei Y, Price TM. Cloning and expression of a novel, truncated, progesterone receptor. *Mol Cell Endocrinol* 2003;200:155–163. [PubMed: 12644308]
- Sartorius CA, Melville MY, Hovland AR, Tung L, Takimoto GS, Horwitz KB. A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. *Mol Endocrinol* 1994;8:1347–1360. [PubMed: 7854352]
- Sasaki M, Dharia A, Oh BR, Tanaka Y, Fujimoto S-i, Dahiya R. Progesterone Receptor B Gene Inactivation and CpG Hypermethylation in Human Uterine Endometrial Cancer. *Cancer Res* 2001;61:97–102. [PubMed: 11196205]
- Schams D, Kohlenberg S, Amselgruber W, Berisha B, Pfaffl MW, Sinowatz F. Expression and localisation of oestrogen and progesterone receptors in the bovine mammary gland during development, function and involution. *J Endocrinol* 2003;177:305–317. [PubMed: 12740019]
- Smith R. Parturition. *N Engl J Med* 2007;356:271–283. [PubMed: 17229954]
- Takimoto GS, Tung L, bdel-Hafiz H, Abel MG, Sartorius CA, Richer JK, Jacobsen BM, Bain DL, Horwitz KB. Functional properties of the N-terminal region of progesterone receptors and their mechanistic relationship to structure. *J Steroid Biochem Mol Biol* 2003;85:209–219. [PubMed: 12943706]

- Thornton JW. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. *Proc Natl Acad Sci U S A* 2001;98:5671–5676. [PubMed: 11331759]
- Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem* 1994;63:451–486. [PubMed: 7979245]
- Tung L, Abdel-Hafiz H, Shen T, Harvell DME, Nitao LK, Richer JK, Sartorius CA, Takimoto GS, Horwitz KB. Progesterone Receptors (PR)-B and -A Regulate Transcription by Different Mechanisms: AF-3 Exerts Regulatory Control over Coactivator Binding to PR-B. *Mol Endocrinol* 2006;20:2656–2670. [PubMed: 16762974]
- Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol Endocrinol* 1993;7:1244–1255. [PubMed: 8264658]
- Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schubeler D. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* 2007;39:457–466. [PubMed: 17334365]
- Wei LL, Hawkins P, Baker C, Norris B, Sheridan PL, Quinn PG. An amino-terminal truncated progesterone receptor isoform, PRC, enhances progestin-induced transcriptional activity. *Mol Endocrinol* 1996;10:1379–1387. [PubMed: 8923464]
- Wei LL, Norris BM, Baker CJ. An N-terminally truncated third progesterone receptor protein, PRC, forms heterodimers with PRB but interferes in PRB-DNA binding. *J Steroid Biochem Mol Biol* 1997;62:287–297. [PubMed: 9408082]
- Yamanaka T, Hirata S, Shoda T, Hoshi K. Progesterone receptor mRNA variant containing novel exon insertions between exon 4 and exon 5 in human uterine endometrium. *Endocr J* 2002;49:473–482. [PubMed: 12402980]
- Yang Z. PAML: a program package for phylogenetic analysis by maximum likelihood. *Bioinformatics* 1997;13:555–556.
- Yang Z. Inference of selection from multiple species alignments. *Curr Opin Genet Dev* 2002;12:688–694. [PubMed: 12433583]
- Yang Z, Bielawski JP. Statistical methods for detecting molecular adaptation. *Trends Ecol Evol* 2000;15:496–503. [PubMed: 11114436]
- Zakar T, Hertelendy F. Progesterone withdrawal: key to parturition. *Am J Obstet Gynecol* 2007;196:289–296. [PubMed: 17403397]
- Zhang J, Nielsen R, Yang Z. Evaluation of an improved branch-site likelihood method for detecting positive selection at the molecular level. *Mol Biol Evol* 2005;22:2472–2479. [PubMed: 16107592]

Fig. 1. Location and structure of the human progesterone receptor

A) Location and orientation of *PGR* in the human genome. The gene usually contains 8 exons and the full-length receptor spans 92 kb. **B)** Gene structure of *PGR*. Translation initiation sites for PR isoforms are indicated with arrows. Exons 1 and 2 are alternatively spliced and shown in gray. Not drawn to scale. **C)** Schematic illustration of previously identified functional domains in the human progesterone receptor. Numbers indicate the amino acid positions that delineate the beginning and end of each domain while bent arrows point to the translation initiation site of the three isoforms (PR-A, PR-B, and PR-C). AF, activation function region; IF, inhibitory function region; DBD, DNA-binding domain; NLS, nuclear localization signal; H, hinge region; HBD, hormone/ligand binding domain.

Fig 2. Nested Models of Progesterone Receptor Evolution

In addition to the one ω ratio (model 0) the maximum likelihood scores from alternative scenarios of nucleotide substitution rate variation were calculated using PAML and tested against one another using likelihood ratio tests. **A)** two ω ratio model estimated separate ratios for non-primates and primates; **B)** three ω model estimated one ratio for non-primates, one ratio for the human-chimpanzee total group, and one for the other primates; **C)** four ω model estimated one ratio for non-primates, a ratio for the human-chimpanzee crown group, a ratio for the human-chimpanzee stem lineage, and a ratio for the other primates; **D)** a five ω model estimated a separate ratio for each of the following five groups: non-primates, the human terminal branch, the chimpanzee clade, the human-chimpanzee stem, and the other primates. Hypotheses tests were conducted using a nested procedure.

Fig 3. Amino acid replacements in PGR during human and chimpanzee evolution

A phylogram depicts the amino acid replacements that occurred in PGR during recent human and chimpanzee evolution. Numbers refer to the amino acid position in the multiple sequence alignment of the complete gene. This alignment included all anthropoid primates sampled (see Methods), as well as mouse, rat, rabbit, and dog. Branch lengths are proportional to the amount of amino acid replacement.

A)

B)

Fig 4. Adaptive evolution in progesterone receptor

The free ratio model (model 1) of nucleotide substitution, which estimates a separate ω value for each branch in the tree, is shown. The numbers shown along each branch are ω , and the maximum-likelihood estimates of the numbers of nonsynonymous (N*dN) and synonymous (S*dS) substitutions along that branch. **A)** Complete coding region **B)** First exon. Lineages showing evidence of adaptive evolution are depicted in red.

Table 1

Amino acid replacements in human since the divergence from chimpanzees

Amino acid position		Change	Domain	Nonsynonymous SNPs
Human protein	Multiple species alignment			
159	171	G to R	AF3	no
172	184	A to V	IF	no
192	204	S to A	IF	no
201	213	G to E	IF	no
216	228	P to A	IF	no
347	359	S to C	IF	yes
430	444	P to T	IF	no
662	679	I to V	Hinge	no

AF-Activation function region

IF- Inhibitory function region

Table 2
Likelihood ratio scores and ω values of the different nested models

Model	Likelihood (-lnL)	ω Values					
		Non primates	Primates	Homo-Pan clade(total)	Homo-Pan clade(crown)	Homo-Pan stem	Pan clade
1 ω	11500.53	0.2442	0.2442	--	--	--	--
2 ω	11495.5	0.2166	0.3123	--	--	--	--
3 ω	11490.95	0.217	0.2933	1.1584	--	--	--
4 ω	11488.3	0.217	0.2934	--	2.1682	0.1351	--
5 ω	11488.16	0.217	0.2934	--	--	0.217	3.2449
							1.6286

Table 3

Significance tests of the nested models

Models	$2\Delta \ln L$	<i>P</i> value
1 ω : 2 ω	10.06	0.0015
2 ω : 3 ω	9.01	0.0025
3 ω : 4 ω	5.29	0.021
4 ω : 5 ω	0.28	>0.05

Parsimony inferred rates of nucleotide substitutions in *PGR* during human descent from the most recent common ancestor of anthropoids

Table 4

Exon	ACCTRAN	DELTRAN	Number of sites	ACCTRAN sub/site	DELTRAN sub/site
Exon1	175	50	1689	0.104	0.03
Exon 2	2**	1	152	0.03	0.013
Exon 3–8	38**	13*	1013	0.038	0.013
Full gene	215	64	2853	0.075	0.022

The significance of the comparisons between exon 1 and the other exons are labeled as follows:

- *
-p<0.05;
- **
-p<0.005. The comparisons between exon 2 and exon 3–8 were not statistically significant. Sub=substitution.