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Placental invasion, preeclampsia risk and adaptive molecular evolution at the origin of the great apes: Evidence from genome-wide analyses

E.J. Crosley [a,*], M.G. Elliot [b], J.K. Christians [a], B.J. Crespi [a]

[a] Simon Fraser University, Biological Sciences, 8888 University Drive Burnaby, BC V5A 1S6, Canada
[b] Simon Fraser University, Human Evolutionary Studies Program, 8888 University Drive Burnaby, BC V5A 1S6, Canada

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ABSTRACT

Introduction: Recent evidence from chimpanzees and gorillas has raised doubts that preeclampsia is a uniquely human disease. The deep extravillous trophoblast (EVT) invasion and spiral artery remodeling that characterizes our placenta (and is abnormal in preeclampsia) is shared within great apes, setting Homininae apart from Hylobatidae and Old World Monkeys, which show much shallower trophoblast invasion and limited spiral artery remodeling. We hypothesize that the evolution of a more invasive placenta in the lineage ancestral to the great apes involved positive selection on genes crucial to EVT invasion and spiral artery remodeling. Furthermore, identification of placentally-expressed genes under selection in this lineage may identify novel genes involved in placental development.
Methods: We tested for positive selection in approximately 18,000 genes using the ratio of non-synonymous to synonymous amino acid substitution for protein-coding DNA. DAVID Bioinformatics Resources identified biological processes enriched in positively selected genes, including processes related to EVT invasion and spiral artery remodeling.
Results: Analyses revealed 295 and 264 genes under significant positive selection on the branches ancestral to Hominidae (Human, Chimp, Gorilla, Orangutan) and Homininae (Human, Chimp, Gorilla), respectively. Gene ontology analysis of these gene sets demonstrated significant enrichments for several functional gene clusters relevant to preeclampsia risk, and sets of placentally-expressed genes that have been linked with preeclampsia and/or trophoblast invasion in other studies.
Conclusion: Our study represents a novel approach to the identification of candidate genes and amino acid residues involved in placental pathologies by implicating them in the evolution of highly-invasive placenta.

1. Introduction

In humans, abnormal placental development is associated with a variety of adverse outcomes, including miscarriage, fetal growth restriction, preterm birth, preeclampsia (PE) and eclampsia [1–3]. PE, with a typical onset of maternal symptoms in the third trimester, is characterized by acute hypertension and proteinuria in the mother. It occurs in 5–7% of all pregnancies, and is a leading cause of maternal mortality [4–6] for which there is presently no cure other than delivery.

PE is associated with deficiency in spiral artery remodeling, a key step in placental development in which extravillous trophoblast (EVT) cells from placental villi invade the maternal decidua and inner myometrium during the first trimester and replace cells of the uterine artery walls, resulting in vessels with increased diameter, decreased resistance and increased blood flow [7–9]. Deficiency in EVT invasion and spiral artery remodeling may trigger distinct maternal inflammatory responses which result in the maternal symptoms of PE. PE therefore results from two processes: insufficient placentation, and the maternal response [3].

Various experimental approaches have been used to improve our understanding of PE, spanning from the genetic to the anthropological [6,9–14]. Previously, it has been suggested that risk for gestational diseases associated with abnormal placentation, such as PE and postpartum hemorrhage, may be a consequence of the evolution of our particularly invasive placental phenotype [15]. The goal of this study is to develop and apply a new approach to

Abbreviations: PE, preeclampsia; EVT, extravillous trophoblast; PAML, phylogenetic analysis by maximum likelihood; DAVID, the database for annotation, visualization and integrated discovery v6.7.

* Corresponding author. Tel.: +1 778 782 3977.
E-mail addresses: ecrosley@sfu.ca (E.J. Crosley), micke@sfu.ca (M.G. Elliot), jkchrist@sfu.ca (J.K. Christians), crespi@sfu.ca (B.J. Crespi).

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uncovering genes crucial to EVT invasion and spiral artery remodeling, by inferring when these steps in placental development first evolved, and assessing selection on placentally-expressed genes along this branch of the primate phylogeny.

Recent evaluations have revealed that trophoblast invasion depth and spiral artery remodeling are essentially the same in humans, chimpanzees and gorillas [16,17]. Similarly, isolated accounts of eclamptic pregnancies in gorillas and chimpanzees [18–20] contest the notion that PE is a uniquely human syndrome [12]. The family Hylobatidae (the gibbons) shares many placental characteristics with Homininae (to which gorillas, chimpanzees and humans belong) including overall discoid shape, hemochorial interhemal interface, and villous fetomaternal interdigitation (Table 1). However, two important characteristics set them apart: shallower trophoblast invasion and the absence of spiral artery remodeling at deeper levels of the myometrium in the gibbons [21,22]. In Homininae, the deep trophoblast invasion that is responsible for spiral artery remodeling occurs via two routes: interstitial, in which EVT invades from the anchoring villi into the underlying decidua and the inner third of the myometrium, and endovascular, in which EVT migrate through the lumen of the spiral arteries [22,23]. In gibbons and old world monkeys, EVT invasion occurs primarily via the endovascular route, but penetrates only the decidual region, while the interstitial route appears to be restricted to the basal plate area with no deeper invasion into the decidua, thus leading to the less invasive hemochorial placental phenotype [21,22,24].

There is currently no description of the route and depth of trophoblast invasion in orangutans [22], the sister species to the Homininae (Fig. 1) and therefore it is not clear whether increased invasiveness evolved before and/or after the divergence of orangutans from the lineage that gave rise to humans, chimpanzees and gorillas. However, several findings suggest that orangutans may also share our more invasive phenotype: the major histocompatibility complex (MHC) class I antigen *HLA-C*, which is expressed on EVT in humans and is implicated in spiral artery remodeling via its interactions with uterine natural killer cells, is present in the orangutan but not gibbons or old world monkeys [21,25–27]. Furthermore, in one case, massive placental infarcts accompanied by maternal proteinuria and post-partum death led to a diagnosis of "toxemia of pregnancy" in an orangutan [28], hinting that PE may also occur within this species, which might reflect a requirement for increased EVT invasion to achieve a normal, healthy pregnancy.

Given that an increased degree of invasion emerged between the time that gibbons diverged from the great apes and the time that gorillas diverged from chimpanzees and humans (Fig. 1, Table 1), we hypothesize that identification of placentally-expressed genes under positive selection during this period of our evolution could identify novel genes involved in EVT invasion and spiral artery remodeling. While others have examined selection on placentally-expressed genes [29], ours is the first to examine selection specifically during the period when increased invasion evolved. We do not consider selection within the Homininae [27,30], since trophoblast invasion and spiral artery remodeling show little variation within this group. We focus especially on the Hominidae-origin branch, but also analyze the data for the branch at the origin of Homininae (immediately after the divergence of orangutans), and we note that increases in placental invasiveness may also have occurred along both of these branches.

2. Methods

Analyses of positive selection on the branch ancestral to Hominidae (humans, chimpanzees, gorillas, and orangutans) and on the branch ancestral to Homininae (humans, chimpanzees, and gorillas) were carried out using the CODEML program in the PAML package using the ratio of non-synonymous to synonymous amino acid substitution for protein-coding DNA (dN/dS ratio). The dN/dS ratio is a commonly-used indicator of selective pressure acting on protein-coding genes. The rationale is that natural selection will have no effect on mutations that do not alter the amino acid sequence (synonymous substitutions), whereas mutations that result in amino acid change (non-synonymous substitutions) may result in a selective advantage or disadvantage. Therefore a dN/dS ratio greater than one implies positive selection, favoring amino acid changes, whereas a ratio less than one implies purifying (stabilizing) selection, reducing or eliminating change at the amino acid level. A ratio of one indicates neutral or no selection. Data (in the form of dN/dS ratios) were generated from ensembl.org for approximately 18,000 aligned protein-coding genes of humans, chimps, gorillas, orangutans, northern white-cheeked gibbon, macaque, common marmoset, and Phillipine tarsier, species for which whole-genome information is available. Maximum likelihood methods were implemented to isolate genes under positive selection solely on the branch ancestral to Hominidae or on the branch ancestral to Homininae (Fig. 1), and not under positive selection across the tree as a whole, where selection was significant at alpha = 0.05.

DAVID Bioinformatics Resources 6.7 [31,32] identified biological processes represented in the two lists of selected genes, from which we selected processes related to EVT invasion and spiral artery remodeling. DAVID functional clustering analysis sorts gene lists into non-mutually exclusive clusters of genes based on commonality in molecular pathway, biological function, protein structure, disease contexts and various other parameters as decided by the user. These clusters are ranked according to statistical significance as denoted by an enrichment score (ES).

3. Results

DAVID functional clustering analysis of genes under positive selection with $p < 0.05$ ($n = 295$ from the branch ancestral to Hominidae shown in Supplementary Table 1, and $n = 264$ from the branch ancestral to Homininae shown in Supplementary Table 2) identified several molecular pathways, gene function categories and disease contexts that were relevant to processes important in placentation.

Table 1
Defining characteristics of placental morphology, EVT invasion and spiral artery remodification across primates as detailed by Carter and Pijnenborg [22,23].

	Strepsirrhini	Haplorhini				
	Lemurs and Lorises	Tarsiers	New world monkeys	Old world monkeys	Lesser Apes (Hylobatidae)	Homininae[a]
Interhemal interface	Epitheliochorial	Hemochorial	Hemochorial	Hemochorial	Hemochorial	Hemochorial
Placental shape	Diffuse	Discoid	Discoid	Discoid	Discoid	Discoid
Fetomaternal interdigitation	Villous (no intervillous space)	Labyrinthine (no intervillous space)	Trabecular (connections persist between villi)	Villous	Villous	Villous
Level of trophoblast invasion	Does not occur	Extent unknown (likely does not occur)	Minimal trophoblast invasion	Shallow trophoblast invasion (endovascular route only)	Shallow trophoblast invasion (endovascular route only)	Deep trophoblast invasion (via interstitial and endovascular routes)
Spiral artery remodification	Does not occur	Extent unknown (likely does not occur)	Endothelial walls of blood vessels remain intact [22]	Extends through decidua but not the inner myometrium	Extends through decidua but not the inner myometrium	Extends through decidua and into the inner myometrium

[a] Susceptible to PE.

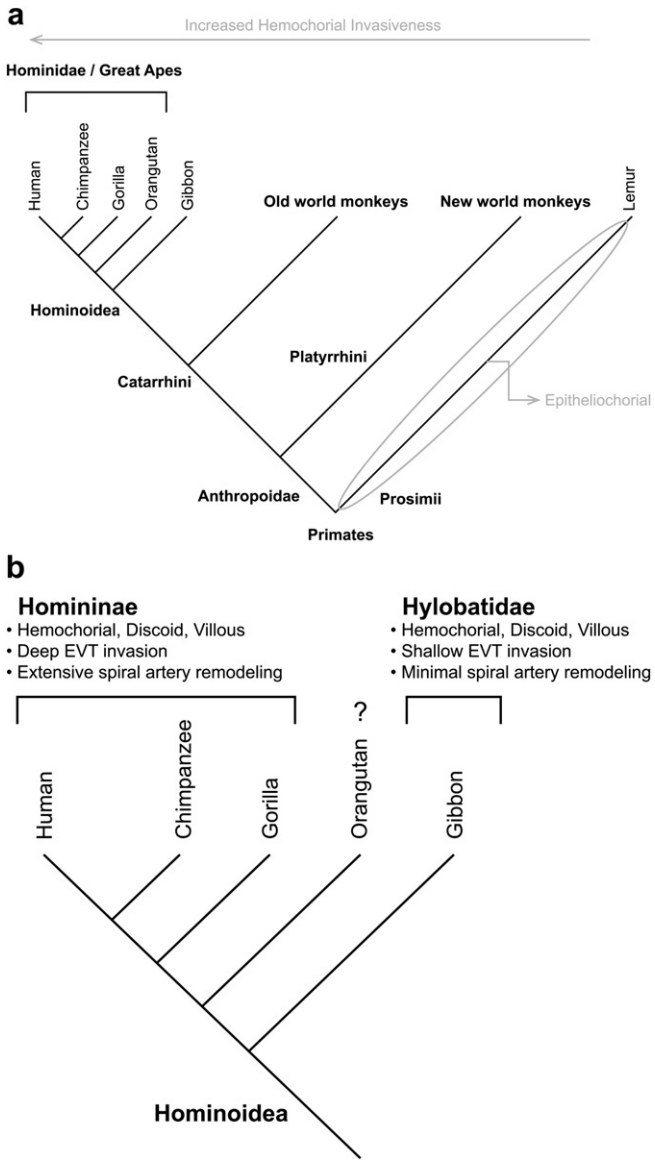

Fig. 1. a) Phylogenetic tree of select primates (adapted from Ref. [62]), highlighting trend toward increased hemochorial invasiveness throughout evolution of great apes [22,23]. b) Phylogenetic tree of Hominoidea, highlighting divergence of Hominidae from Hylobatidae (gibbons), and the defining placental characteristics of Homininae compared to Hylobatidae [22].

Clusters functionally related, or potentially related, to placentation are summarized in Table 2. Specifically, from highest to lowest enrichment levels from Analysis 1 (DAVID analysis of genes found to be under positive selection on the branch ancestral to Hominidae), these included immunoglobulin-binding, MHC class I mediated immunity, reproductive cellular process, immunoglobulin-related immune response, sexual reproduction, pregnancy-induced hypertension, lipid transport, regulation of blood vessel size, zinc-finger related proteins, hypertension, apoptosis and regulation of cell migration (Table 2). Analysis 2 (selection on the branch ancestral to Homininae) identified almost all of these clusters, as well as metallopeptidase activity (Table 2). We excluded clusters considered to be unrelated to placentation from further analysis (Supplementary Tables 3 and 4). Excluding clusters was not expected to exclude important candidates, since clusters are not mutually exclusive, and so genes of interest would be expected to be present in multiple clusters.

Within each relevant cluster, genes that were found to be expressed in the placenta, implicated in pregnancy/pregnancy-associated disease and/or directly implicated in EVT invasion were identified using the information included in individual DAVID gene reports, and are summarized in Supplementary Table 5 and Supplementary Table 6. These genes are discussed below, with relevance to their roles in placental invasion and PE risk. Within Supplementary Tables 5 and 6 the genes are ranked from top to bottom according to the significance with which they were inferred to be under positive selection. CODEML also allowed generation of individual amino acid sites inferred to be under positive selection according to both Bayes empirical Bayes estimation of selected sites as well as naive empirical Bayes estimation of selected sites for each gene of interest from the branch ancestral to the great apes (see Supplementary Material 7).

4. Discussion

Genes under positive selection on the branch ancestral to the great apes included genes with verified or hypothesized roles in EVT invasion and spiral artery remodeling. This set of genes is thus implicated by evolutionary and physiological-developmental evidence in the emergence of a more invasive hemochorial phenotype. The most highly enriched clusters of genes included those involved in immune function, particularly in relation to the immunoglobulin-like proteins and MHC class I antigen functioning, as well as gene clusters implicated in hypertension and pregnancy-induced hypertension in the analysis focused on the Hominidae origin branch.

Several genes under positive selection from our results are already viewed as important mediators in EVT invasion and/or spiral artery remodeling, illustrating the validity of our approach. Genes that code for MHC class I antigens play important roles in spiral artery remodeling by facilitating the concerted actions of EVT and uterine natural killer cells [21], and deficits in communication between these two cell types increases the risk of PE [3,21,27,33,34]. Important among the MHC class I antigens is *HLA-G*, as it has been found to play a central role in the protection of the trophoblast from cytotoxic effects of natural killer cells, facilitating EVT invasion [35–37]. Unlike villous trophoblast, which does not express surface HLA class I or class II antigens, EVT expresses a unique repertoire of HLA class I, including high levels of HLA-G, further reinforcing *HLA-G's* importance in invasive processes [9]. We found that *HLA-G* shows a significant level of positive selection on the branch ancestral to Hominidae as well as on the branch ancestral to Homininae, suggesting it may also have played an important evolutionary role in the invasive placental phenotype. *HLA-G* also appeared in several enriched clusters via DAVID analysis, including immunoglobulin-related, MHC class I histocompatibility antigen, and immune response (Table 2). Further implicating *HLA-G* in placental invasiveness are findings that both reduced plasma HLA-G concentrations and genetic polymorphisms have been clinically linked to PE in humans [38,39].

Catechol-O-methyltransferase (COMT) is another enzyme found to show robust expression in the placenta, and was also found to be under positive selection on the branch ancestral to Hominidae. *COMT* functions in the generation of 2-methoxyoestradiol (2-ME), a natural metabolite of estradiol which in the placenta functions to destabilize microtubules and inhibit hypoxia inducible factor-1α (*HIF-1α*), a transcription factor that senses tissue oxygen tension and regulates the expression of hypoxia induced genes [40]. HIF-1α protein accumulation has been previously associated with vascular defects characteristic of PE, including poor spiral artery remodeling due to shallow trophoblast invasion [40]. *COMT* knock-out mice

Table 2
DAVID functional clustering of genes found to be under positive selection ($p < 0.05$) on the branch ancestral to Hominidae (analysis 1) and on the branch ancestral to Hominidae (analysis 2); clusters presented are functionally related to placentation.

DAVID functional clustering annotation category	Enrichment score[a] analysis 1	Genes identified analysis 1	Enrichment score[a] analysis 2	Genes identified analysis 2
Immunoglobulin; immunoglobulin-like	2.50	*OBSCN, FAM55A, ADAMTSL3, TARM1, HLA-B, MCAM, HLA-G, GP6, LILRB5, LILRA2, HEPN1, LILRB4, TREML4, FCGR2A, MR1, STBD1*	0.84	*VSIG10, WFIKKN1, LAIR2, TARM1, HLA-B, LRRC24, HLA-G, CD1E, LILRA2, FSTL5, LILRB4, VSIG8, STBD1, IZUMO1*
MHC class I histocompatibility antigen; MHC class I receptor activity; immune response	1.22	*HLA-B, MR1, HLA-G*	0.72	*BPI, APOL1, LILRA2, LILRB4, TFEB, HLA-B, CD1E, HLA-G*
Reproductive cellular process; cell–cell recognition	1.14	*FCGBP, PCSK4, TNP2*	N/A	No cluster generated
Peripheral blood leukocyte; immunoglobulin-like; immune response	1.13	*ADAMTSL3, FCGR2A, HEPN1, HLA-B, HLA-G, LILRA2, LILRB4, LILRB5, MR1, APOL1, CXCL2, IL32, POLM, MCAM, OBSCN, TREML4*	0.73	*LILRA2, LILRB4, HLA-B, HLA-G, VSIG10, WFIKKN1, FSTL5, VSIG8, LRRC24, CD1E, BPI, APOL1, TFEB*
Reproductive process in a multicellular organism; multicellular organism reproduction; sexual reproduction;	1.12	*FAM50A, APOL2, PRKACG, PARN, AGT, PDILT, COMT, SOD1, GGNBP2, PCSK4, TNP2*	0.28	*APOL2, CCND2, DRD5, GGN, GGNBP2, INHA, IZUMO1, MUC2, NPPB, PDILT, QKI*
Hypertension, pregnancy induced; circulatory system process	1.09	*ADRB2, F5, AGT, CACNA1G, NPPB, SOD1*	N/A	No cluster generated
Lipid transport; lipid binding	0.90	*APOL2, KCNN4, APOL1, APOL4, FFAR1, ADH7*	1.19	*APOL2, PNLIP, ABCA7, APOL1, OSBPL3, SLC27A4, AKR1B15*
Regulation of blood vessel size; vascular process in circulatory system; blood circulation; positive regulation of apoptosis;	0.53	*ADRB2, AGT, CACNA1G, NPPB, SOD1, F5, ARHGEF3, OBSCN, PCBP4*	N/A	No cluster generated
Zinc finger, C_2H_2-type/integrase, DNA-binding, regulation of transcription	0.48	*ADAMTSL3, ADH7, ADRB2, AGT, COG8, CYP2S1, EAF1, EDF1, ETV7, F5, HMGA1, INSR, IRX2, LOXL2, MARCH1, MEIS1, METAP2, MGMT, NCOR2, NTHL1, OLIG2, PARK2, PCBP4, PDXK, POLM, PPM1A, PPM1N, SNRPC, SOD1, SPRYD5, TCOF1, TFB2M, TNP2, ZNF20, ZNF300, ZNF358, ZNF561, ZNF563, ZNF594, ZNF611, ZNF645, ZNF80, ZNF814, ZNF837, ZNF846, ZNF99*	0.66	*ADAM19, AGRN, ARID2, C17ORF48, C4ORF21, CEP290, CHAF1B, COG8, FRYL, GDA, GTF3C6, INSM1, KLF14, LOXL2, MAML1, MEF2D, MMP28, NUP153, NUP62, ONECUT3, P4HA3, PCBP4, PDLIM3, PPFIBP2, PPM1N, PPP1R13L, REPIN1, RFX2, RIPK1, SPRYD5, STAMBPL1, TFEB, TOE1, TRIM47, TRIM66, TWIST1, ZDHHC4, ZKSCAN4, ZNF141, ZNF197, ZNF25, ZNF467, ZNF480, ZNF496, ZNF563, ZNF594, ZNF611, ZNF785, ZNF80,INSM1, PHYHD1, ZNF563, ZNF594, ZNF611, ZNF80*
Hypertension	0.39	*ADRB2, CYP2S1, AGT, NPPB, ADRA1A, COMT, INSR*	N/A	No Cluster Generated
Apoptosis; programmed cell death	0.27	*FASTKD3, ARHGEF3, OBSCN, APOL1, RHOT2, ADRA1A, SOD1, WDR92, BLCAP*	0.15	*ALS2, MUC2, MEF2D, APOL1, TTBK2, NUP62, RIPK1, PPP1R13L, TWIST1*
Regulation of cell migration	0.21	*AGT, INSR, VCL*	0.48	*MUC2, PLD1, DRD5, FURIN*
Serine-type endopeptidase activity; Metallopeptidase activity	N/A	No cluster generated	0.36	*TOR1A, TMPRSS9, TPSAB1, FURIN, MMP28, ADAM19, STAMBPL1*

[a] Enrichment scores are calculated as the geometric mean of the *p*-values of annotation terms within each cluster expressed on a negative log scale to rank the significance of gene function clusters.

show a preeclamptic placental phenotype that can be rescued by 2-ME administration, which has led to the hypothesis that variation in *COMT* genotype may affect levels of HIF-1α, leading to angiogenic dysfunction and placental insufficiency [40]. Although mouse placentation is different morphologically from that in humans and great apes [41], the data from COMT knock-out mice paired with our findings suggest that *COMT* may have contributed to increased placental invasiveness at the emergence of the great apes.

The insulin receptor (*INSR*), which is activated by insulin, and insulin-like growth factors -I and -II (IGF-I and IGF-II, respectively), is highly expressed in placental trophoblast, and was found to be under positive selection on the branch ancestral to Hominidae [42–44]. Choriocarcinoma models of EVT invasion have shown that INSR inhibition blocks the cell adhesion and chemotaxis effects of IGF-II, and thus inhibits choriocarcinoma cell invasion [44].

Other genes under positive selection on the branch ancestral to Hominidae have received less attention in the context of placental development, including proprotein convertase subtilisin/kexin type 4 (*PCSK4*) and melanoma cell adhesion molecule (*MCAM*). PCSK4 is implicated in the processing of IGF-II, an insulin-like growth factor that is paternally imprinted, and highly expressed in the fetus and placenta [45]. IGF-II functions to stimulate the EVT migration and invasion that is key to spiral artery remodeling [45]. Previous studies have hypothesized that abnormal processing of IGF-II from its precursor, pro-IGF-II, can lead to abnormal placentation resulting in intrauterine growth restriction, a leading cause of perinatal mortality [45]. *PCSK4* has been shown to be highly expressed in placental trophoblast cells, and inhibition of PCSK4 *in vitro* resulted in blocked processing of pro-IGF-II to IGF-II and reduced trophoblast cell migration [45]. While no IGFs were found to be under positive selection, their modulation via PCSK4 may have been a contributor to the increased invasiveness of the hemochorial placenta at the origin of the great apes.

Also potentially modulating EVT invasion and found to be under positive selection on the branch ancestral to Hominidae is melanoma cell adhesion molecule (*MCAM*). In the human placenta, *MCAM* has been shown to be specifically expressed on invasive EVT, but not on noninvasive cytotrophoblast or syncytiotrophoblast, and functions to regulate migration of EVT on smooth muscle cells [46,47]. In preeclamptic pregnancies however, MCAM was no

longer detectable on EVT, supporting the hypothesis that *MCAM* may play a role in facilitating spiral artery remodeling, and is an appropriate marker for the successful differentiation of cytotrophoblast into invasive EVT [46].

A novel gene implicated in increased EVT invasion and spiral artery remodeling by our analysis of selection on the branch ancestral to Hominidae is interleukin 32 (*IL32*). Interleukins are known to stimulate cells into pathways involved in cell death, inflammation and autoimmunity [48]. A microarray study examining differential gene expression between EVT and villous trophoblast demonstrated an upregulation of *IL32* mRNA in the EVT compared to villous trophoblast [9], implicating *IL32* as a contributor to EVT invasion. *IL32* is otherwise uncharacterized in terms of its role in EVT invasion and spiral artery remodeling, and is thus a new candidate.

Analysis of genes under positive selection on the branch ancestral to Homininae, after the divergence of orangutan from the lineage leading to human, chimpanzee and gorilla, identified a suite of other genes implicated in EVT invasion not found to be under selection on the branch ancestral to Hominidae. This gene set includes *TWIST1* and *PPP1R13L*, two genes implicated in modulating the differentiation of cytotrophoblast [49,50]. Other genes we found to be under positive selection include *ADAM19*, *FURIN*, and *MMP28* which are all highly expressed in placental villi and modulate EVT invasiveness according to various *in vitro* models for EVT invasion [51–55]. Notably, *ADAM19* is upregulated on EVT versus villous trophoblast as is *LAIR2* [9], and the latter gene is down-regulated in the placental tissue of women with PE [9,11,56]. Lastly, *INHA* is more highly expressed in term placental tissue from women with PE, and has been suggested to play a role in modulating placental vascularization via antagonistic effects on *ACTA* [57–59].

Other genes under positive selection on the branches ancestral to either Hominidae or Homininae that were found via DAVID clustering analysis and identified as expressed in the placenta and/or associated with pregnancy-related diseases include *F5*, *AGT*, *ADRB2*, *HLA-B*, *APOL1*, *APOL2*, *LILRB4*, *LILRB5*, *LOXL2*, *SOD1*, *KCNN4*, *FSTL5*, *PHYHD1*, *CEP290*, *STBD1*, *QKI*, *RIPK1* and *P4HA3* (summarized in Supplementary Tables 8 and 9). These are currently not directly implicated in EVT invasion and spiral artery remodeling (either by absence of evidence or evidence of absence of role). For example, those found in the "Hypertension, pregnancy-induced; Circulatory System Process" cluster (Table 2, Analysis 1), are likely involved in the development of preeclamptic symptoms on the maternal side, downstream of the onset of deficient spiral artery remodeling. In other cases, these genes are implicated in the risk of PE in few studies with weak replication of results. For example, *SOD1*, an antioxidant enzyme, has been shown to regulate differentiation of cytotrophoblast to syncytiotrophoblast, but polymorphisms in this gene have not been associated with risk of PE [35,60,61].

5. Conclusions

We describe a novel approach that identifies genes, pathways and specific amino acid sites under positive selection during the evolution of the particularly invasive placenta of the great apes. This approach is valuable in that it provides a novel set of candidate genes, and candidate functional amino acid sites, for further investigation with respect to roles in the evolution of placental invasiveness, spiral artery remodeling and PE risk.

Interpretation of these results is subject to several caveats. First, many of the genes are expressed in tissues other than the placenta, and could have been under selective pressures unrelated to placental development. Second, the use of dN/dS ratios focuses on changes in amino acid sequence but not regulatory sequences. Further work should include functional studies of our candidates in placental explants and cell lines, as well as candidate-gene SNP-based association studies from normal and preeclamptic pregnancies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.placenta.2012.12.001.

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