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

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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.

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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.