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Review

From PPROM to caul: The evolution of membrane rupture in mammals☆

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ABSTRACT

Rupture of the extraembryonic membranes that form the gestational sac in humans is a typical feature of human parturition. However, preterm premature rupture of membranes (PPROM) occurs in approximately 1% of pregnancies, and is a leading cause of preterm birth. Conversely, retention of an intact gestational sac during parturition in the form of a caul is a rare occurrence. Understanding the molecular and evolutionary underpinnings of these disparate phenotypes can provide insight into both normal pregnancy and PPROM. Using phylogenetic techniques we reconstructed the evolution of the gestational sac phenotype at parturition in 55 mammal species representing all major viviparous mammal groups. We infer the ancestral state in therians, eutherians, and primates, as in humans, is a ruptured gestational sac at parturition. We present evidence that intact membranes at parturition have evolved convergently in diverse mammals including horses, elephants, and bats. In order to gain insight into the molecular underpinnings of the evolution of enhanced membrane integrity we also used comparative genomics techniques to reconstruct the evolution of a subset of genes implicated in PPROM, and find that four genes (*ADAMTS2*, *COL1A1*, *COL5A1*, *LEPRE1*) show significant evidence of increased nonsynonymous rates of substitution on lineages with intact membranes as compared to those with ruptured membranes. Among these genes, we also discovered that 17 human SNPs are associated with or near amino acid replacement sites in those mammals with intact membranes. These SNPs are candidate functional variants within humans, which may play roles in both PPROM and/or the retention of the gestational sac at birth.

Contents

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1. Introduction

Preterm premature rupture of membranes (PPROM) is a leading cause of preterm birth, occurring in approximately 1% of all pregnancies (Parry and Strauss, 1998). A less common occurrence in human delivery is birth in a caul (i.e. intact membranes), and cases have been reported with no ill effects, including an infant born in complete caul that survived 25 min of extrauterine life in intact membranes (Heggarty et al., 1975). Thus, some humans are born with premature membrane rupture, others are born with ruptured membranes at term, while still others fail to have their membranes rupture at the time of delivery. Genetic variation is one possibility to explain differences in the timing of membrane rupture within a species. Indeed, previous work has implicated several genes in PPROM (Anum et al., 2009).

PPROM is a relatively common obstetrical syndrome in humans; however it is unclear whether the syndrome is common in nonhuman therian species (marsupials and placental mammals). It is apparent that a great range of variation in the timing and extent of membrane rupture exists in this group. As in humans, closely related nonhuman primates such as chimpanzees (Lindburg and Hazell, 1972) and Old World monkeys have ruptured membranes at the time of birth. Conversely, common domesticated animals such as cows and horses typically deliver offspring that emerge in intact or partial gestational sacs (Frazer et al., 1999; Schuenemann, 2012). Additionally, the gestation length in therian mammals ranges from two weeks to nearly two years (Asdell and Hubbs, 1964). Therefore, the variability in the timing of membrane rupture in humans is actually less extreme than is observed among mammals as a whole. Fig. 1 shows the typical pattern of gestational sac rupture in humans and three other nonhuman mammalian species. The primates in this figure are born with ruptured membranes; the seal (i.e. Carnivora) and horse (i.e. Perissodactyla) are born with membranes intact. Recent research suggests that Euarchontoglires (a superordinal clade that includes Primates and Rodentia) and Laurasiatheria (a superordinal clade that includes Carnivora and Perissodactyla) last shared a common ancestor approximately ~92 million years ago (mya) (Meredith et al., 2011). It is not possible to accurately reconstruct whether this ancestor had the intact or ruptured gestational sac based solely on these taxa. Instead, an accurate reconstruction of the evolution of fetal membrane integrity at birth would require knowledge of the observed pattern of a diverse range of mammals in addition to each of the two groups depicted in Fig. 1.

Observing mammals giving birth is generally difficult because females tend to retreat from view. This is probably why so little has been published in this area. However, the advent of video sharing websites on the internet has brought an amazing amount of new observations that for the most part are ignored by the scientific community. Recently, social media sites have served as the basis for a wide range of scientific studies (Hua et al., 2013; Lewis et al., 2011; Patel et al., 2009); however video evidence has not yet seen extensive use. From video evidence, we reconstructed the evolution of membrane rupture in 55 species representing all major groups of mammals. This reconstruction served as the framework for a comparative genomics analysis in which we examined a subset of human genes that have been implicated in PPROM.

With the increasing numbers of available mammalian and human genome sequences we find ourselves in a "golden age of human evolutionary genomics" (O'Bleness et al., 2012). This explosion of

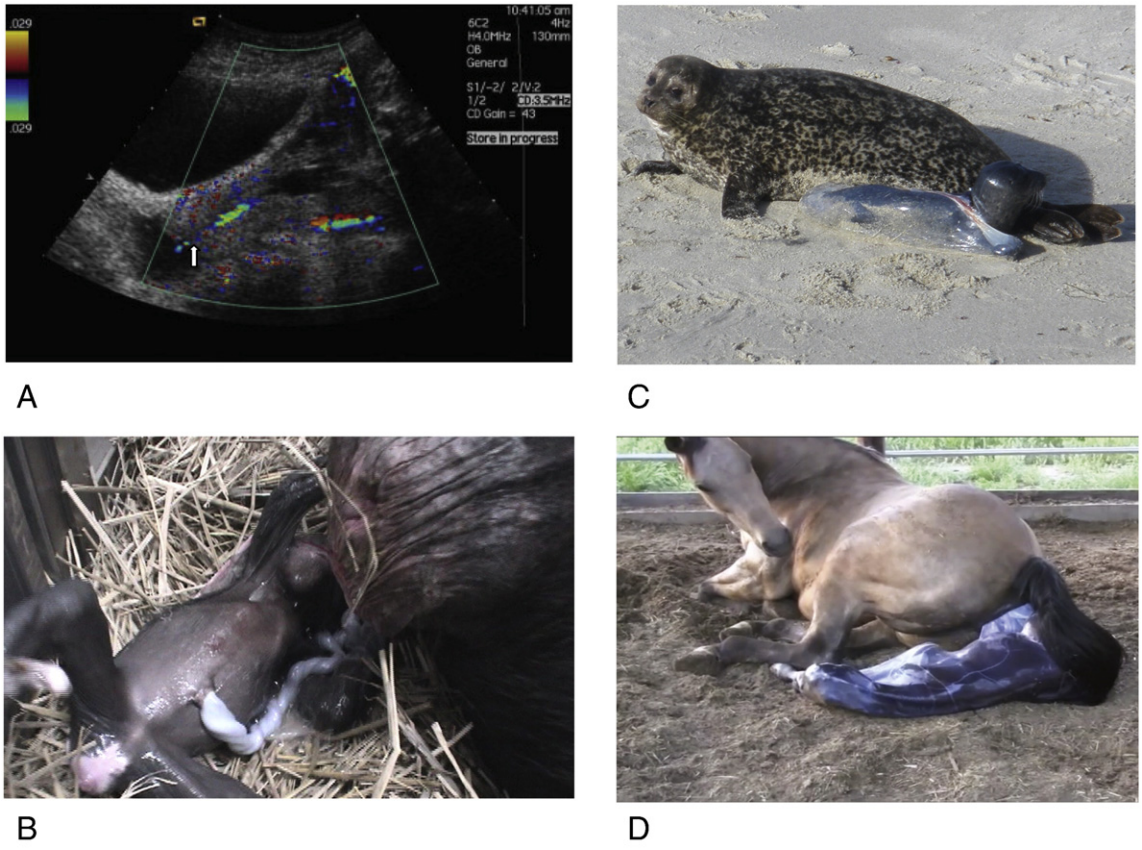

Fig. 1. Examples of intact and ruptured gestational sac at parturition. A. Sonogram of a human fetus in the uterus with premature preterm rupture of fetal membranes (PPROM) as indicated by the white arrow (Devlieger et al., 2003). Image by permission of Wiley InterScience. B. Chimpanzee and neonate with ruptured gestational sac (Hirata et al., 2011). Image by permission of The Royal Society (http://www.youtube.com/watch?v=dfd0fzX9M5g). C. Harbor seal and neonate with intact gestational sac. Photo courtesy of Mauricio Mena. D. Horse and neonate with intact gestational sac. Photo courtesy of Orlando Alamillo. (http://www.youtube.com/watch?v=BHzWgrr7MHI).

comparative data not only allows the determination of unique features of the human genome (O'Bleness et al., 2012) but also enables the determination of convergent features that our species shares with other lineages (Goodman et al., 2009; McGowen et al., 2012). Comparative genomics studies have pointed to mutations that have been implicated in human health and disease, including obstetrical syndromes. By comparing human gene sequences to sequences from other mammals it has become clear that many important human genes associated with health and disease show evidence of positive selection, including the MHC Class I and II genes (Hughes and Nei, 1988, 1989), immunoglobulin genes (Tanaka and Nei, 1989), and the breast cancer associated gene *BRCA1* (Huttley et al., 2000). The comparative analysis of genes across mammals has been used to investigate human diseases to provide clues regarding their dysfunction (i.e. Crespi et al., 2007). This approach has been applied successfully to human parturition, including the adaptive evolution of placenta-specific genes (Hou et al., 2009), a comparison of genes involved in preeclampsia (Crosley et al., 2013), and the identification of genes involved in human birth timing (Plunkett et al., 2011).

Our purpose was to test whether genes involved in PPROM showed evidence for adaptive evolution (i.e. positive Darwinian selection) on mammalian lineages at times coincident with evolutionary modification of the pattern of gestational sac integrity. We reasoned that evidence for adaptive evolution at these times would strengthen the assertion that these genes are indeed involved in promoting membrane integrity. Moreover, we further considered that our evolutionary approach would have implications in translational medicine. If our hypothesis was correct we could identify specific functionally important amino acid replacements and identify variants in human genes that correspond to these functionally important sites that may play a role in gestational sac rupture timing defects.

2. Methods

2.1. Observation of live births

We searched video sharing websites such as YouTube for video footage of parturition in a wide range of mammals using search terms of specific species included in every order of mammals as defined by Meredith et al. (2011). Although the recorded documentation of the presence of the gestational sac is rare, we were able to document the live birth of 55 mammal species from 12 out of approximately 22 orders of extant therian mammals (Primates, Rodentia, Lagomorpha, Eulipotyphla, Carnivora, Chiroptera, Perissodactyla, Cetartiodactyla, Proboscidea, Xenarthra, Dasyuromorphia, Diprotodontia) (Supplemental Table 1). These orders provide a broad sampling across the mammalian tree, including at least one representative of all four major clades of eutherian mammals (Laurasiatheria, Euarchontoglires, Afrotheria, Xenarthra), as well as two orders of marsupials. Viewers recorded whether upon birth, the gestational sac was present and intact (0), present and ruptured (1), or absent (2). For each species anywhere from 1 to 6 births were recorded; species with more than one character state were recorded as polymorphic.

2.2. Reconstruction of evolutionary history

Character state evolution was reconstructed using a maximum parsimony algorithm in the software Mesquite v.2.75 (Maddison and Maddison, 2011). Character states were considered unordered, meaning that a transition from any character state to any other was considered as a single step, and the minimum number of steps was considered the most parsimonious reconstruction of evolutionary history. We traced the evolution of character states using a recently inferred mammalian phylogenetic tree (Meredith et al., 2011). We pruned the tree of Meredith et al. to include only taxa from which we were able to obtain video evidence.

2.3. Molecular evolution of genes implicated in PPROM

The protein coding DNA sequences for 11 genes known to potentially predispose fetuses to preterm birth due to PPROM (Anum et al., 2009) were downloaded from available mammalian genomes in Ensembl (Flicek et al., 2012) or from GenBank. The genes analyzed included: *COL5A1*, *COL5A2*, *COL3A1*, *COL1A1*, *COL1A2*, *PLOD1*, *ADAMTS2*, *CRTAP*, *LEPRE1*, *TNXB*, and *ZMPSTE24*. A complete list of genes, species and accession numbers is provided in Supplemental Table 2. We downloaded sequences for species from which we had recorded an observation of gestational sac rupture and related taxa, which resulted in a minimum of 13 species (*COL5A1*) and a maximum of 20 species (*COL1A2*) per gene. The genome assemblies in some species do not contain all 11 PPROM-related genes. For a gene to be analyzed we required that orthologous sequences for at least one marsupial and one afrotherian were present. We then included all remaining placental mammals that did not have an excess of missing data. The species selected for subsequent analysis include human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), gorilla (*Gorilla gorilla*), orangutan (*Pongo abelii*), rhesus macaque (*Macaca mulatta*), rabbit (*Oryctolagus cuniculus*), guinea pig (*Cavia porcellus*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), panda (*Ailuropoda melanoleuca*), dog (*Canis lupus familiaris*), cat (*Felis catus*), alpaca (*Vicugna pacos*), pig (*Sus scrofa*), cow (*Bos taurus*), dolphin (*Tursiops truncatus*), horse (*Equus caballus*), megabat (*Pteropus vampyrus*), hedgehog (*Erinaceus europaeus*), elephant (*Loxodonta africana*), hyrax (*Procavia capensis*), and Tasmanian devil (*Sarcophilus harrisii*).

Sequences were aligned using MUSCLE (Edgar, 2004) via the European Bioinformatics Institute. Alignment files for each gene were imported into Mesquite and manually edited to match the predicted protein sequence. With this data set, we generated alignment files using 13 to 20 species for all the genes listed except the highly variable *TNXB*, which was removed from analysis due to the difficulty in aligning across multiple species.

We then conducted branch-specific tests of adaptive evolution on the remaining 10 genes using the codeml package of PAML (Yang, 2007) to determine whether on specific lineages a gene showed evidence for an increase in rates of nonsynonymous nucleotide substitution. Specifically, the ratio of the rate of nonsynonymous substitution per site to the rate of synonymous substitution per site ($\omega = dN/dS$) was used as an indicator for potential adaptive evolution in each gene. In the free-ratio model, a branch which has a $\omega < 1$ is considered to be under purifying selection, a branch with $\omega = 1$ is said to be neutrally evolving, and a $\omega > 1.0$ implies that positive selection may have occurred (Yang and Nielsen, 1998). Moreover, statistical evidence for accelerated nonsynonymous rates was obtained by conducting likelihood ratio tests among nested branch models (Yang, 2007). The amino acid tree topology of Meredith et al. (2011) was used for all analyses, with excluded species pruned from each tree.

We tested three branch models, a one-ratio model (m0) with a fixed ω for all branches, a free-ratio model (m1) where ω is allowed to vary between all branches, and a two-ratio model (m2), where the two ω parameters are used to for two sets of branches corresponding to the presence of an intact or partially intact sac (ω_0) or a ruptured sac (ω_1). We used the reconstruction of character states in the phylogenetic analysis of the presence of the gestational sac to classify internal branches. In cases where branches were reconstructed as polymorphic or equivocal in character state, branches were classified as having an intact/partially intact sac. A likelihood ratio test was used to compare the models m0 versus m2 and m0 versus m1 (Yang, 2007). The resulting statistic was compared using a chi-square distribution to determine the model that best fits the data.

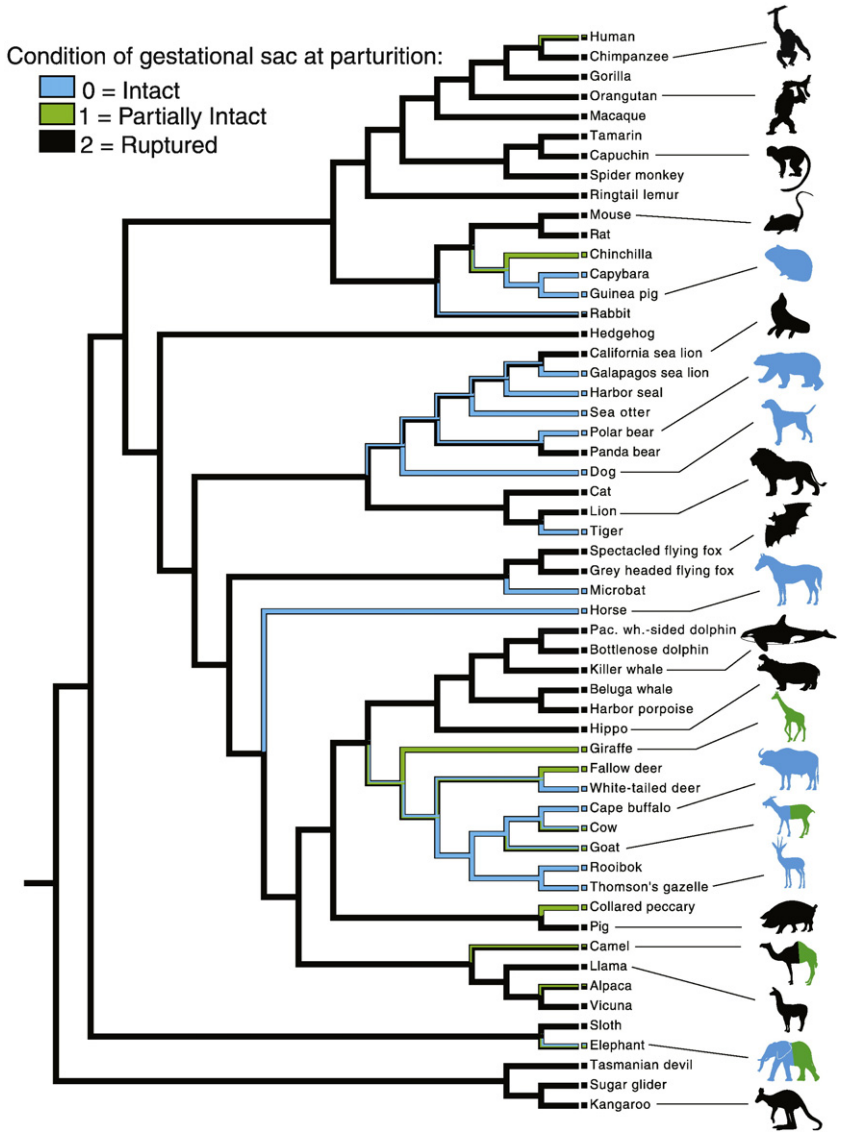

Fig. 2. Evolution of intact and ruptured gestational sacs at parturition. Reconstruction of the evolution of the gestational sac at parturition on a phylogenetic tree of 55 mammal species from 12 orders using maximum parsimony. Blue = species with intact membranes; green = partially intact; black = ruptured membrane at birth; Pac. wh.-sided dolphin = Pacific white-sided dolphin.

2.4. Mining human SNP data for functional variants implicated in membrane integrity

Variation within nonhuman genomes can potentially inform investigations into the functional evolution of human genes (Schaner et al., 2001). We therefore further investigated single nucleotide polymorphisms (SNPs) in genes that were shown to differ significantly between species with intact vs. ruptured gestational sacs. We downloaded a list of human missense SNPs within these genes from the 1000 Genomes Project (Altshuler et al., 2012). The location of these SNPs in alignments of each gene was cross-referenced against a list of nucleotide changes inferred on the branches leading to each of the species with intact gestational sacs at birth. Nucleotide substitutions were inferred using marginal reconstruction of ancestral sequences in codeml. To examine patterns of convergent evolution between human variants and nonhuman amino acid replacements, we determined whether these nonhuman substitutions/replacements occurred in gene regions near (within five codons) each human SNP in the human sequence.

3. Results

3.1. Evolution of membrane integrity at parturition

We mapped the presence of an intact gestational sac on a tree of 55 species spanning the phylogenetic breadth of therian mammals (Fig. 2). We infer that a ruptured gestational sac upon birth is ancestral for therian and eutherian mammals. Based on the data here, we infer that an intact or partially intact sac evolved a minimum of 13 and a maximum of 16 times in mammals. In addition, parturition with purely intact membranes has evolved independently at least eight times. A ruptured gestational sac is ancestral for primates, and all primates with the exception of humans in caul are born with a ruptured sac. Humans are coded here for a sac that is both ruptured and partially intact (although partially intact membranes are rare in humans). A partially intact or intact gestational sac is a consistent feature of the Ruminantia within the order Cetartiodactyla, and is inferred as the ancestral state of this clade.

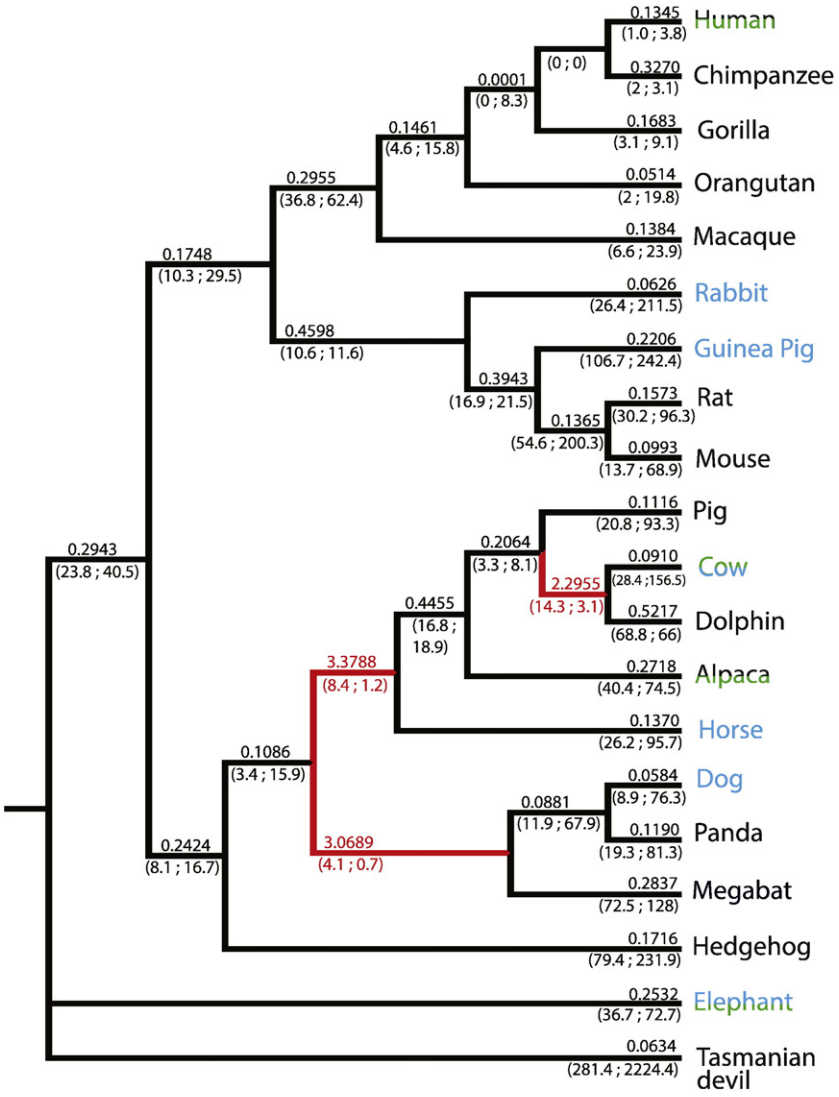

Fig. 3. Adaptive evolution in COL1A2. Phylogenetic tree of mammals used in this study with COL1A2 branch-specific dN/dS above each branch, N*dN and S*dS values below each branch. COL1A2 shows evidence of adaptive evolution (dN/dS > 1.0) on three branches highlighted in red. Names of each species are colored with blue for intact gestational sac, green for partial gestational sac, and black for ruptured gestational sac. Abbreviations: dN/dS = ratio of non synonymous substitution rate/synonymous substitution rate; N*dN = nonsynonymous changes; S*dS = synonymous changes.

3.2. Molecular evolution of genes implicated in PPROM

We tested for the presence of adaptive evolution in 10 of the 11 genes related to PPROM using both a one-ratio and free-ratio model. The free-ratio model (m1) is a significantly better fit (Chi-square $p < 0.05$) to the data than the one-ratio model (m0) for 9 of the 10 genes analyzed. The free-ratio model is one in which each individual branch of the tree has a distinct ω; therefore, rates of nucleotide substitution vary across the tree in a majority of the genes tested. ω did not significantly vary across the tree in only one gene (*ZMPSTE24*).

Using the free-ratio model, adaptive evolution can be detected within individual branches in the phylogenetic tree used in the analysis. One gene, *COL1A2*, showed clear evidence for adaptive evolution, (i.e. dN/dS > 1.0) on three branches, all within the Laurasiatheria (Fig. 3). These three branches are the stem branch of Carnivora, the stem branch of both the Perissodactyla and Cetartiodactyla, and the stem branch of Cetruminantia (cow and dolphin in the present study). Of the eight analyzed extant species included within these adaptively evolving clades, four are born with intact membranes and four are born with ruptured membranes. Free-ratio values for the remaining nine analyzed genes are provided in Supplemental Table 3. Five of these genes (*COL3A1*, *CRTAP*, *LEPRE1*, *PLOD1*, and *ZMPSTE24*) have limited evidence for positive selection but are not discussed here as dS = 0 on these branches.

We also examined a two-ratio (m2) model, where dN/dS is modeled to vary between two character states (i.e. one rate for intact membranes and another rate for ruptured membranes). This model is a significantly better fit (Chi-square $p < 0.05$) to the data than the one-ratio (m0) model in five genes (*ADAMTS2*, *COL1A1*, *COL5A1*, *CRTAP*, *LEPRE1*). Four of these five genes showed a higher dN/dS ratio in species with intact membranes (*ADAMTS2*, *COL1A1*, *COL5A1*, and *LEPRE1*) than those with ruptured membranes. Complete results are shown in Table 1. *COL1A1* had a highly significant result when comparing model m0 and m2, and it also had the largest difference between the dN/dS ratio of the species with intact and ruptured membranes (Table 1). However, there is a large section of the horse sequence that is significantly divergent from the other species. When the horse is removed from the alignment the m2 and m0 models do not significantly differ for this gene.

3.3. Human SNPs in adaptively evolving PPROM-related genes

In order to gain insight into the evolution of membrane integrity at parturition in mammal species we tested for adaptive evolution in

Table 1
Comparison of substitution rates in species with intact vs. ruptured gestational sac. List of genes used in the branch model tests and their corresponding p-values of the likelihood ratio tests. In the one-ratio (m0) branch model, the dN/dS value (ω) is fixed across all branches. In the two-ratio (m2) branch model, two different ω parameters are used, each corresponding to an intact or a ruptured membrane at birth. In the one-ratio (m1) branch model, ω is allowed to vary at each branch point. Bolded numbers represent significant p values < 0.05.

Gene name	Gene symbol	ln likelihood		p value	dN/dS	
		m2	m0		Ruptured	Intact
Collagen, type I, alpha 1	*COL1A1*	−15104.92	−15150.63	**1 E-21**	0.1656	0.4215
ADAM metallopeptidase with thrombospondin type 1 motif, 2	*ADAMTS2*	−12701.67	−12707.51	**6E-04**	0.0505	0.0741
Cartilage associated protein	*CRTAP*	−3281.68	−3286.72	**0.001**	0.0794	0.0324
Collagen, type V, alpha 1	*COL5A1*	−12961.53	−12966.03	**0.003**	0.0368	0.0611
Leucine proline-enriched proteoglycan (leprecan) 1	*LEPRE1*	−3671.43	−3674.38	**0.015**	0.0590	0.1005
Collagen, type III, alpha 1	*COL3A1*	−20778.47	−20779.32	0.191	0.1652	0.1473
Collagen, type I, alpha 2	*COL1A2*	−19872.86	−19873.32	0.339	0.1669	0.1524
Zinc metallopeptidase STE24 homolog (S. cerevisiae)	*ZMPSTE24*	−3616.79	−3616.99	0.530	0.0751	0.0878
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	*PLOD1*	−8417.60	−8417.69	0.672	0.0474	0.0445
Collagen, type V, alpha 2	*COL5A2*	−14036.80	−14036.85	0.753	0.0778	0.0749

PPROM related genes. This analysis enabled us to determine the specific amino acid replacements that occurred in lineages and genes that evolved membrane integrity at parturition. We next asked whether these or nearby amino acid positions were variable in human populations. We reasoned that if such human sites were variable then they would potentially play a functional role for membrane integrity. We therefore examined the SNP data generated from the 1000 Genomes Project (Altshuler et al., 2012), and searched for human missense SNPs in such proximity. Among the five genes we analyzed, we identified 17 human missense SNPs proximate to nucleotide substitutions in lineages that evolved the character state of intact membranes during parturition. Table 2 summarizes the number of missense SNPs per gene as well as the number of nearby nonsynonymous substitutions among species with intact membranes at birth.

In our survey of the five genes with significant evidence for positive selection (*ADAMTS2*, *COL1A1*, *COL5A1*, *CRTAP*, *LEPRE1*), there were four to ten human missense SNPs per gene (Table 2). Four out of five genes had replacements in lineages leading to species with an intact gestational sac within five codons of a human SNP (Table 2). Of these, *ADAMTS2* had the most (n = 33) and *COL5A1* had the fewest (n = 1). In *ADAMTS2*, there were eight amino acid replacing substitutions within the same codon of a human SNP. These included changes in the terminal lineages leading to the guinea pig, cow, elephant, dog, and rabbit. For example, the human *ADAMTS2* SNP rs59567206 results in a GAC(D) → GGC(G) substitution. In both the guinea pig and cow, a substitution at this same nucleotide results in a GGC(G) → GAC(D) replacement, a situation opposite that of the human. *CRTAP* has six replacements close to a human SNP including one within the same codon. This SNP (rs114245114) results in a AAG(K) → AGG(R) replacement. This identical substitution occurs in the cow. *LEPRE1* has 16 replacements close to a human SNP including one within the same codon. This SNP (rs146982397) results in a CGT(R) → TGT(C) substitution. In elephants, there is a change at the same codon, but a different nucleotide with a different amino acid change, CGT(R) → CAT(H). A full list of replacements at each SNP is available in Supplemental Table 4.

4. Discussion

4.1. Principal findings

The present study examined the evolution of membrane integrity at term in 55 species. Tracing the evolution of this feature allows us to confidently infer that the last common ancestral population of 1) viviparous mammals, 2) placental mammals, and 3) Primates typically gave birth to individuals with ruptured extraembryonic membranes. The transition from ruptured membranes at parturition to intact membranes at parturition occurred frequently during mammalian evolution; however, the transformation from intact to ruptured membranes occurred less frequently. Parturition with intact membranes has evolved independently at least eight times. We next examined the molecular evolution of genes implicated in PPROM and from these analyses we describe two principal findings. First, 50% of these genes show significant evidence for different rates of evolution in lineages that have intact vs. ruptured membranes at parturition. Moreover, four of the five genes that have significantly different nucleotide substitution rates between the two groups evolved more rapidly in the lineages that evolved intact membranes. When the 17 human missense SNPs in these genes are interrogated we find that 10 codons are variable in humans and have undergone nonsynonymous substitutions at the same position in at least one lineage that has evolved intact membranes.

The evolution of an intact gestational sac at parturition raises the possibility of morphological adaptation. Alternatively, the accelerated evolution of connective tissue genes may reflect selective pressure on tissues and phenotypes other than the gestational sac. However, we may speculate that the utility of intact membranes at delivery could relate to both the protection of the newborn against injury. An intact gestational sac may help to cushion the newborn upon expulsion from the birth canal, especially in animals that give birth standing up, such as the giraffe, alpaca, and the elephant. Mammals that are born with a sac tend to be precocial, such as those in Ruminantia, some carnivores, and the guinea pig. The ability to break out of an intact gestational sac may be

Table 2
Number of replacement substitutions in lineages leading to species with an intact gestational sac at birth close to the site of a human SNP. The number of SNPs as listed includes missense SNPs obtained from the 1000 Genomes Project. SNPs listed correspond to amino acid replacements that occurred in significantly adaptively evolving genes implicated in PPROM. Replacement substitutions are those missense amino acid changes that occurred along the lineages leading to mammals born with intact gestational sacs (guinea pig, rabbit, cow, horse, dog, and elephant).

Gene name	Gene symbol	Number of SNPs	Replacements within 5 codons	Replacements within same site
ADAM metallopeptidase with thrombospondin type 1 motif, 2	*ADAMTS2*	9	33	8
Collagen, type I, alpha 1	*COL1A1*	6	0	0
Collagen, type V, alpha 1	*COL5A1*	10	1	0
Cartilage associated protein	*CRTAP*	4	6	1
Leucine proline-enriched proteoglycan 1	*LEPRE1*	4	16	1

a result of the limb strength that allows these newborns to walk immediately following birth.

4.2. Translational implications

Alexander Pope famously noted in *Essay on Man* (1734) that, "the proper study of mankind is man." There is still a strong argument that in order to understand human biology it is best to focus on the study of humans. Indeed, this perspective drives the vast majority of clinical research. However, the advent of transgenic and other animal model approaches have also been shown to have value in understanding normal and abnormal phenotypes, and FDA drug approval often requires preliminary animal studies (Conn, 2013). At first glance it seems unlikely that the study of horses, elephants, guinea pigs, dogs, and other species used less frequently as model organisms can inform understanding of PPROM. However, it is well appreciated that an understanding of evolution can inform our understanding of human medicine (Gluckman et al., 2009). From a translational perspective it is possible to look at human variants at codons that have evolved adaptively in nonhuman mammals. Perhaps these sites are functionally relevant to human membrane integrity during gestation. Future work could test this idea by comparing these variants in PPROM cases vs. controls. In the present study we asked which species had intact vs. ruptured membranes at parturition. We found that ruptured membranes represented the ancestral state, and that intact membranes had evolved multiple times on different mammalian lineages. Thus, the evolution of intact membranes is a good example of convergent evolution. Convergent evolution of phenotypic traits is a common occurrence, and probably the most famous example of convergent evolution is the multiple origins of eyes across the animal kingdom (Fernald, 2000). Both the octopus (a mollusk) and the human (a vertebrate) possess eyes, yet their last common ancestor did not possess this feature. Eyes have independently evolved dozens of times, and interestingly it appears that the evolution of the eye is strongly influenced by the actions of a single gene, *PAX6*, a transcription factor involved in morphogenesis (Gehring and Ikeo, 1999). It is thus reasonable to ask whether the evolution of membrane integrity could also be under the control of a limited number of genes because like eyes, membrane integrity appears to evolve convergently.

The extraembryonic membranes that constitute the gestational sac include two fetally derived tissues (amnion, chorion) and one maternally derived tissue (decidua) (Mossman, 1987). This fact makes determining the genetic underpinnings of PPROM difficult because both fetal and maternal alleles can confer risk or resilience to PPROM and associated obstetrical syndromes. Therefore, when considering human genetic variants in the context of PPROM it is important to consider both maternal and fetal effects, and to note that genomic conflict theory predicts that maternal and fetal adaptations may at times be at odds with one another (Haig, 1993). Therefore, any clinical study testing convergently evolving PPROM variants would need to account for the genotypes of both mothers and their offspring.

4.3. Study limitations

While the results yielded in the current study are promising, a number of important caveats temper the interpretations of our results. First, we are basing our analyses of adaptive evolution on sequences derived from reference genome draft assemblies. Second, our video analyses are based on one or a few individuals per species. Because our study is not population based it is entirely possible that variation exists both phenotypically and genotypically within our study taxa. In that case we have likely missed several interesting aspects of this variation. Moreover, the current study examined only protein coding changes. It is well appreciated that much functionally important evolution occurs outside of protein coding regions (e.g. gene regulatory sequences) (Prud'homme et al., 2007), and the present study did not examine this and other important classes of nucleotide sites. Additionally, we used genes that were annotated as orthologs according to Ensembl. The algorithm used to identify orthologous sequences is based upon reciprocal BLAST strategies. If the "true" orthologous gene is located in an unassembled portion of the genome it is possible that a paralogous gene would be falsely identified as orthologous, and this could result in an erroneous inference of positive selection. As genome assemblies are refined and new drafts are released it will be crucial to repeat the analyses presented in the present paper. Finally, there are more than 5000 extant species of mammals, and our relatively small sample size may incorrectly infer the timing of some of the major transitions in membrane integrity at parturition. Despite these limitations, we are confident that a comparative approach can identify key genes and lineages that provide insight into the evolution of mammalian parturition.

4.4. Conclusions

Our survey of the evolution of membrane integrity has identified a large number of potential mammalian models for the study of membrane rupture in humans. We propose that the disparate taxa that have evidence for convergent and adaptive evolution of intact membranes during parturition may share similar underlying molecular mechanisms in strengthening membranes, particularly pathways involving collagens. DNA substitutions in genes associated with membrane strength in these species may provide clues toward identifying fetuses at risk for PPROM.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.atg.2013.08.002.

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