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Authors

Crawford, Nicholas
Prendergast, D'Arcy
Oehlert, John W
et al.

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Divergent Patterns of Mitochondrial and Nuclear Ancestry Are Associated with the Risk for Preterm Birth

Nicholas Crawford, PhD[1,2,*], **D'Arcy Prendergast, BSc**[3,*], **John W. Oehlert, MSc**[4], **Gary M. Shaw, PhD**[4], **David K. Stevenson, MD**[4], **Nadav Rappaport, PhD**[5], **Marina Sirota, PhD**[5], **Sarah A. Tishkoff, PhD**[1,2], and **Neal Sondheimer, MD, PhD**[1,6]

[1]Department of Genetics, The University of Pennsylvania, Philadelphia, PA

[2]Department of Biology, The University of Pennsylvania, Philadelphia, PA

[3]Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada

[4]Department of Pediatrics, Stanford University, Palo Alto

[5]Department of Pediatrics, University of California San Francisco, San Francisco, CA

[6]Department of Pediatrics, The University of Toronto, Toronto, Ontario, Canada

Abstract

Objective—To examine linkages between mitochondrial genetics and preterm birth by assessing the risk for preterm birth associated with the inheritance of nuclear haplotypes that are ancestrally distinct from mitochondrial haplogroup.

Study design—Genome-wide genotyping studies of cohorts of preterm and term individuals were evaluated. We determined the mitochondrial haplogroup and nuclear ancestry for individuals and developed a scoring for the degree to which mitochondrial ancestry is divergent from nuclear ancestry.

Results—Infants with higher degrees of divergent mitochondrial ancestry were at increased risk for preterm birth (0.124 for preterm vs 0.105 for term infants; $P < .05$). This finding was validated in 1 of 2 replication cohorts. We also observed that greater degrees of divergent ancestry correlated with earlier delivery within the primary study population, but this finding was not replicated in secondary cohorts born preterm.

Conclusions—Individuals with divergent patterns of mitochondrial and nuclear ancestry are at increased risk for preterm birth. These findings may in part explain the higher rates of preterm birth in African Americans and in individuals with a matrilineal family history of preterm birth.

Preterm birth, delivery before 37 completed weeks of gestation, is a critical public health problem that has resisted sustained efforts to develop a deeper understanding of its causes.[1]

Reprint requests: Neal Sondheimer, MD, PhD, Department of Pediatrics, The University of Toronto, 555 University Ave, Toronto, Ontario, M5G1X8, Canada. neal.sondheimer@sickkids.ca.

*These authors contributed equally.

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Epidemiologic studies have strongly indicated that the risk of having a preterm infant is heritable. One distinctive feature of this heritability is that it appears to be maternally biased; pregnancy history studies of a woman's relatives through her mother, but not through her father, are correlated with greater risk.[2,3]

The risk of preterm birth is also unequally distributed between different ethnic groups. African American women are at increased risk for delivering preterm compared with the population as a whole.[4] Determining the etiologies responsible for this heightened risk is complex, because it can be difficult to distinguish environmental and genetic influences. Attempts to elucidate such influences have evaluated carefully controlled populations, such as members of the military, to conclude that African ancestry is an independent risk factor.[5,6] Specifying genetic risk factors in a related group is greatly complicated by false attribution, but 1 possible link between Americans of African descent is that African haplogroup mitochondrial DNA (mtDNA) is highly prevalent in this group.[7]

These 2 risk categories—matrilineal pedigree and mitochondrial ancestry—are consistent with a mitochondria-inherited risk factor. There is also evidence that mitochondrial function is important for the maintenance of pregnancy. Chemically induced damage to mitochondrial activity is sufficient to disrupt pregnancy in a mouse model[8] and mitochondrial parameters such as membrane potential are regulated in embryonic tissues.[9] Furthermore, women who carry the pathogenic mtDNA mutation mt.3243A>G have a higher risk for preterm birth, although they are clinically unaffected.[10] However, attempts to link preterm birth to specific polymorphisms in mtDNA in case-control studies failed to identify polymorphisms associated with preterm birth.[11] Thus, it remains unclear how a mtDNA-specific inheritance pattern could be associated with a common outcome like preterm birth.

In this study, we considered that risk might derive from the interplay of mitochondrial and nuclear inheritance. The mtDNA and nuclear genome must coordinate to create the multisubunit complexes required for the electron transport chain and the generation of cellular energy. Nuclear DNA is biparentally inherited and modern individuals may have highly admixed nuclear ancestry as mobility has increased. By contrast, mtDNA is inherited as a unified haplogroup in a matrilineal fashion, allowing modern individuals to trace their ancestry back to a single geographically isolated region. Differences in ancestral variants in mtDNA affect mitochondrial function.[12] Furthermore, studies in which the mtDNA from 1 mouse strain was transferred into a distinct nuclear background have identified an array of behavioral and metabolic changes that result from such a misalignment of mitochondrial haplogroup and nuclear haplotype.[13–15] In this study, we have developed tools to determine the alignment between nuclear and mitochondrial inheritance, and have used this factor to evaluate previously collected data on genetic risk factors for preterm birth, hypothesizing that differences in the ancestral inheritance of an infant's mitochondrial and nuclear genome would be associated with an increased risk of preterm birth.

Methods

We identified and analyzed all datasets within the database of Genotypes and Phenotypes (dbGAP) that were case-control studies of preterm birth, containing sufficient markers for

nuclear and mitochondrial genotyping that were focused on mothers or infants with diverse ancestries. The primary analysis was performed in the *Eunice Kennedy Shriver* Institute of Child Health and Human Development Genomic and Proteomic Network for Preterm Birth Research (GPN) cohort (phs000714.v1.p1).[16] Validation of the effects was attempted in the Boston Birth Cohort (BBC; phs000332.v3.p2),[17] the Danish National Birth Cohort Study (DNBC; phs000103.v3.p1)[18] and the Genome-Wide Association Studies of Prematurity and Its Complications (GENEVA-AA; phs000353.v1.p1).[19] We also performed validation using the Genome-Wide Association Study for Bronchopulmonary Dysplasia (BPD).[20] Additional controls as needed were obtained from the Genetics Resource with the Health and Retirement Study (HRS; phs000428.v1.p1). All subjects with evaluable genotypes and phenotypes in each dataset were analyzed. The underlying characteristics of each dataset are provided in Table I (available at www.jpeds.com).

The GPN, BBC, DNBC, and HRS datasets were downloaded from dbGAP after approval and Research Ethics Board or Institutional Review Board review. The BPD Study data were evaluated under the approvals of the Institutional Review Board of Stanford University and the Health and Welfare Agency Committee for the Protection of Human Subjects of the State of California.

To determine haplogroup, mitochondrial polymorphisms were extracted from the datasets using the open-source whole genome association analysis toolkit, PLINK.[21] Haplogroups were calculated using Haplogrep.[22] L-branch haplogroups, U6 and U5b1, were scored (H) as 1 and non-L haplogroups were scored as 0. Subjects with haplogrouping confidence scores of ≤0.5 (low confidence) were excluded from further analysis.

Nuclear ancestry was determined using ADMIXTURE.[23] Briefly, autosomal markers were integrated with data from the 1000Genomes project from individuals of African, European, South Asian, East Asian, and Native American descent.[24] Minor allele frequencies (>0.05) were pruned for linkage disequilibrium using PLINK to obtain approximately 25 000 informative markers. ADMIXTURE was run using unsupervised analyses and the k-factor providing best resolution of African ancestry individuals was used for analysis.

Divergent ancestry was calculated with a focus on African ancestry. The degree of African nuclear ancestry (A_A) was defined as the ADMIXTURE-derived fraction of ancestry that aligned to the reference African individuals in the 1000Genomes project. Divergent ancestry was calculated as:

$$\text{Divergent ancestry} = |H - A_A|$$

Divergent ancestry ranged from 0 to 1, with matched ancestries yielding a score of 0 and completely unmatched ancestries yielding a score of 1.

Statistical Analyses

For datasets where preterm birth was studied as a quantitative trait using weeks of estimated gestational age (GPN, BBC) the results were evaluated by Pearson correlation. For datasets where preterm birth was studied as a qualitative trait (BPD, HRS), comparisons were made

by 2-tailed Student *t* test. Confounders were evaluated using phenotypes reported in the parent datasets. Associations were tested using 1-way ANOVA or the Student *t* test, depending on the number of classes tested. Statistical testing was performed using Prism 6 (GraphPad, La Jolla, CA), except for logistic regression analyses, which were performed in R (R Core Team, Vienna, Austria). Bonferroni correction was applied to the statistical analysis of the BPD/HRS data to account for multiple testing.

Results

The *Eunice Kennedy Shriver* Institute of Child Health and Human Development GPN study was a multisite, multiethnic study of preterm birth risk of American women experiencing preterm birth at <34 weeks of gestation with comparison to term controls at >39 weeks of gestation.[16] No pregnancies with births between 34 and 39 weeks of gestation were present in the dataset. DNA samples from women and their infants were obtained. Gestational ages determined by a pediatrician were recorded for most infants. Cases and controls were spontaneously in labor, and women were excluded for polyhydramnios, uterine anomalies, cerclage, fetal aneuploidy, or lethal fetal anomaly. Controls were excluded if they had a prior history of preterm birth. Preterm birth cases and controls were balanced for maternal age, self-reported race, and history of prior pregnancy.

Unsupervised ADMIXTURE analysis of the mothers and infants was performed together with the 1000Genomes project individuals of African, European, South Asian, East Asian, and Native American descent to infer autosomal ancestry (Figure 1; available at www.jpeds.com). There was good correlation between self-defined ethnicity and the estimation of ancestry, but there was clear evidence of ethnic admixture; the mean estimate of African ancestry for self-reported African-American individuals was 81% (95% CI, 80%–83%). Haplogrep analysis of the GPN individuals used the 117 mitochondrial single nucleotide polymorphisms available on Affymetrix 6.0 genotyping and demonstrated that 91% of self-described African American mothers had L haplogroup mtDNA (Figure 2; available at www.jpeds.com). Both of these figures are consistent with previous studies.[7]

Using ADMIXTURE-calculated nuclear ancestries and haplogroup, we quantified the difference between the ancestry of one's mitochondrial and nuclear DNA. This measurement, which we term divergent ancestry, ranges from 0 to 1, with values closer to 0 representing inheritance of nuclear and mitochondrial polymorphisms from a similar ancestral background and values closer to 1 representing a maximal divergence. For example, individuals with L, U6, or U5b1 haplogroup mtDNA and primarily African nuclear ancestry were defined as having low levels of divergent ancestry, whereas individuals with non-African haplogroups and high degrees of African nuclear ancestry had high levels of divergent ancestry.

Preterm birth data were initially evaluated categorically, using 34 weeks of gestation as a cutoff, as in the GPN study. Divergent ancestry was higher in preterm birth infants as compared with infants born at term (mean = 0.124 for preterm [n = 705]; 0.105 for term [n = 721] infants; $P = .046$ by a 2-tailed Student *t* test; Figure 3, A). There was a trend toward greater divergent ancestry in mothers with preterm birth (mean = 0.0953 for preterm [n =

697]; 0.0794 for term [n = 713]; $P = .067$). The relationship between divergent ancestry and preterm birth in the infant population was modeled using univariate logistic regression. Divergent ancestry was predictive for preterm birth outcome within the dataset (coefficient estimate = 0.59; standard error = 0.3015; $P = .0473$). When modeled over a population risk of preterm birth of 12%, this finding suggests that hypothetical infants with no divergent ancestry have an 11.1% risk of preterm birth as compared with a risk of 18.5% in infants with completely divergent ancestry.

Next, we explored the relationship between divergent ancestry and estimated gestational age at delivery for the premature infants where weekly estimated gestational age at delivery data were available (Figure 3, B). There was a negative correlation observed, suggesting that greater degrees of divergent ancestry were associated with earlier delivery (infant: r = −0.114, $P = .009$, n = 517; maternal: r = −0.138, $P = .002$, n = 503). No correlation was evident within the infants born at term because their estimated gestational age range (39–41 weeks of gestation) was likely too narrow to detect an association.

We next sought to identify confounding traits that might have driven the observed relationship between divergent ancestry and preterm birth, causing an incorrect correlation. We examined variables within the dataset that were plausibly related to maternal health and psychosocial stressors to determine whether these factors had an association with divergent ancestry. To be consistent with the manner in which the dataset was created, comparisons were made between individuals of the same self-reported ethnicity. Tested confounders included graduation from high school, history of abortions, marital and cohabitation status, employment, income, age, and prepregnancy weight. There was no detected statistical association between divergent ancestry and any of these factors (Table II). We also evaluated whether maternal African nuclear ancestry alone could explain the difference between the preterm and term groups, and found that there was no difference in African nuclear ancestry between the 2 groups (mothers of preterm infants = 0.233; mothers of term infants = 0.244; $P = .63$).

To replicate the findings of the GPN study for maternal samples, we evaluated the BBC. This study of preterm birth excluded pregnancies affected by multiparity, trauma, chromosomal defects, congenital or acquired uterine infections, or cervical incompetence. The study was conducted at a single urban center and term controls (>37 weeks of gestation) were recruited from the same site. By design, the study exclusively recruited individuals of African ancestry in the Boston population.

Divergent ancestry scores were calculated as described, except that a k = 2 unsupervised analysis was used with African and European references (Figure 4 for Haplogroup and ADMIXTURE characteristics; available at www.jpeds.com). The association with preterm birth was tested. There was a trend toward higher levels of divergent ancestry in mothers delivering prematurely (0.16 for term vs 0.17 for preterm; $P = .54$) and toward association between higher levels of divergent ancestry and the weeks of estimated gestational age for mothers delivering prematurely, but both failed to reach statistical significance (Figure 5).

Replication in BPD/HRS Data

As a validation study of the infant studies, we used data collected in the Genome-Wide Association Study for Bronchopulmonary Dysplasia (BPD) in preterm infants from a California population base.[20] The infants in this study were born between 25 and 29 weeks of estimated gestational age. Data from term infants were not collected as part of the study. For our analysis, case infants were compared with individuals from the HRS. Although gestational age data were not available for HRS individuals, the date of birth of the individuals within the HRS dataset (1931–1941) would have been inconsistent with their survival as early premature infants.[25] The HRS data were selected because it was a large dataset with a similar ratio of self-reported African and non-African participants to the premature infants in the BPD data.

The evaluation of mitochondrial haplogroups and ADMIXTURE-derived nuclear ancestry from a k = 5 unsupervised model showed that there was an equivalent degree of African nuclear and mitochondrial ancestry in the 2 datasets with approximately 14% of individuals in both datasets having African origin mitochondrial haplogroups and an equivalent number of individuals with primarily African-aligned nuclear ancestry (Figure 6; available at www.jpeds.com). The principal differences between the 2 groups were in the degree of European ancestry, which was higher in the HRS study, and American/Hispanic ancestry individuals, which was higher in the BPD study.

The relationship between divergent ancestry and preterm birth was evaluated using preterm birth as a binary measure. Divergent ancestry was increased in the preterm BPD individuals (mean = 0.1126) compared with the HRS controls (mean = 0.052; $P < .0001$; Figure 7). To confirm that differences in the input populations did not impact this association, we also performed a subanalysis comparing individuals of primarily (>50%) African ancestry between the 2 datasets. The magnitude of the difference between term and preterm groups was unchanged (0.257 vs 0.205; $P < .0001$). We also used logistic regression models to confirm that divergent ancestry was predictive of preterm birth outcome, even after considering any difference between African ancestries in the 2 groups (data not shown). Finally, the BPD dataset of premature infants alone was analyzed for correlation between week of gestational age and divergent ancestry. There was a modest negative correlation, not attaining statistical significance, similar in magnitude to that observed in the BBC and GPN datasets (data not shown).

Replication in the GENEVA Datasets

Data from the DNBC Study of preterm birth was also evaluated. There was no significant difference in divergent ancestry in preterm and term infants (cases = 0.06545; controls = 0.06497; $P = .927$). The population under study was likely not sufficiently diverse to generate a signal of divergent mitochondrial ancestry. Of the 1844 infants in the dataset, only 73 had a non-European haplogroup. The GENEVA-AA did not contain sufficient mitochondrial markers for haplogroup identification and was not evaluated.

Discussion

In this study, we correlated divergence between nuclear and mitochondrial ancestry and the occurrence and timing of preterm birth. In an ethnically diverse cohort of spontaneous preterm birth (GPN), we observed that higher degrees of divergent ancestry were observed in infants experiencing spontaneous preterm birth and greater degrees of divergent ancestry were correlated with earlier delivery times. There was a trend toward this finding in mothers within this dataset that did not reach statistical significance. We repeated the maternal analysis in another dataset composed of exclusively African-ancestry women (BBC). Here we observed a trend toward earlier preterm birth with increasing divergent ancestry that failed to reach statistical significance. The failure to replicate the observation may have been due to the smaller number of women enrolled or may have been due to the inclusion of women where preterm birth was medically induced.

The principal finding of greater divergent ancestry in preterm birth infants was replicated in the BPD dataset, which had the advantage of considerably greater size than the other studies. The notable weakness of this analysis in terms of interpretation is that the (term) controls were not obtained in the same study, making exact calculation of confounding effects impossible. One important confounder that may exist is the increasing frequency of interracial partnerships at the time of the BPD study recruitment vs the period when the HRS subjects were born. This factor would tend to increase divergent ancestry in the preterm infants of the BPD study as compared with HRS controls.

The observation of an association between divergent ancestry and preterm birth is not unprecedented; other studies have compared groups with distinct degrees of divergent ancestry and identified differences in preterm birth risk. It has been observed previously that parents of different self-described ethnicities may have increased rates of preterm birth.[26–28] In addition, studies of immigrant and nonimmigrant mothers of African ancestry have observed that immigrant mothers, who may have less admixture than women whose families have been in the US for many generations, have lower rates of preterm birth.[29,30] One caveat is that environmental exposures and stressors could also be responsible for the difference in preterm birth risk between immigrant and nonimmigrant populations.[29]

Because African Americans tend to have higher degrees of divergent ancestry, the observation that increased divergent ancestry is associated with preterm birth may explain a portion of the increased risk of preterm birth in African Americans. However, it is important to note that it cannot explain all of the observed increased risk. The estimated difference in preterm birth risk between infants where there is no divergent ancestry and those with completely divergent ancestry is 7.5%. This number approximates the entire difference in risk between people of European and African American ancestry,[29] but the actual difference in divergent ancestry that we observed between African American and European ancestry infants was much smaller.

Previous studies that evaluated the impact of mtDNA variation on preterm birth did not find an association between individual mitochondrial single nucleotide polymorphisms and preterm birth.[11] Our findings are not in conflict with these studies, because our methodology

considered the divergence between mitochondrial and nuclear ancestries as a whole, rather than examining mitochondrial variants themselves.

The mechanism through which divergent ancestry might impact preterm birth may arise from the interaction of haplogroup-defining mitochondrial polymorphisms with geographically local nuclear variation. Haplogroup-defining polymorphisms are not merely markers of ancestry, and have consequences for mitochondrial function, including the regulation of mitochondrial gene expression.[12,31] Furthermore, the disruption of co-evolved nuclear and mitochondrial polymorphisms in subunits of the electron transport complexes can produce detectable cellular phenotypes.[32] Studies of conplastic animals, where the mitochondrial haplogroups have been bred into a distinct nuclear background, also show the potential for widespread changes in health, with the incidence of obesity, insulin signaling, and lifespan all affected.[15] Thus, divergent ancestry may alter common phenotypes such as preterm birth by directly impairing mitochondrial potential.

Previous studies have evaluated the ranges of both nuclear and mitochondrial divergence between individuals as a way of evaluating the potential for “mismatch” in offspring created using mitochondrial replacement therapy (commonly known as “3-parent embryos”).[33] The study illustrated the potential for creating mismatch when parents come from distinct geographical regions. However, to our knowledge, our study marks the first attempt to evaluate how differences in mitochondrial and nuclear inheritance contribute to the presence of a distinct phenotype such as preterm birth. Matching of haplogroups between the nuclear and mitochondrial donor has been considered a safeguard to prevent problems arising from genomic divergent ancestry.[34]

The co-evolution of mitochondrial and nuclear polymorphisms and its impact on mitochondrial function may have particular importance in pregnancy and development. It has been confirmed recently that women with pathogenic mtDNA mutations were more likely to have an obstetrical history of preterm birth.[10] Animal studies have shown that common haplogroup polymorphisms have consequences for reproductive fitness.[35] In this study, we have found that the inheritance of nuclear and mtDNA from distinct ancestral origins is associated with an increased risk for preterm birth. This finding adds to a growing understanding of genetic influences on gestational duration,[36] a common and serious issue whose complete causes remain unknown. A deeper understanding of the mechanism linking divergent ancestry and preterm birth may drive future therapies for the maintenance of pregnancy, focusing on the stabilization of cellular energy supply. This process could include antioxidant strategies to reduce free radical generation or rigorous maintenance of the caloric needs of the maternal–fetal pair.

One key question that is unresolved by this study is whether the risk for preterm birth caused by distinct mitochondrial ancestry is specific to mothers, to their infants, or is shared between both. Our data indicate that the effect may be more related to infant genotype, but the necessarily close genetic linkage between mothers and their infants makes it impossible to answer this question explicitly. Future research in this area will be directed toward the use of animal models where specific crosses can allow the direct comparison of the risks of divergent ancestry in mother and infant. These studies will also allow us to explore the

specific linkage between mitochondrial physiology and pregnancy maintenance that is impacted by divergent ancestry.

We have identified an association between genomic divergent ancestry and increased risk of preterm birth. We propose that this finding explains a fraction of the increased risk of preterm birth in African American infants. The ultimate goal is to apply this knowledge to patient care to reduce the incidence and severity of preterm birth. Divergent ancestry could be used to counsel for increased risk for preterm birth. In addition, studies in animal models may define the physiological impairment associated with preterm birth, allowing us to explore ways to mitigate divergent ancestry-associated risk and improve pregnancy outcomes.

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Glossary

BBC	Genome Wide Association Study of Preterm Birth/Boston Birth Cohort
BPD	Genome-Wide Association Study for Bronchopulmonary Dysplasia
DNBC	Danish National Birth Cohort Study
GENEVA-AA	Genome-Wide Association Studies of Prematurity and Its Complications
GPN	Genomic and Proteomic Network for Preterm Birth Research
HRS	Genetics Resource with the Health and Retirement Study
mtDNA	Mitochondrial DNA

References

1. Jain J, Gyamfi-Bannerman C. Future directions in preterm birth research. Semin Fetal Neonatal Med. 2015; 21:2–5. DOI: 10.1016/j.siny.2015.11.002 [PubMed: 26732078]
2. Boyd HA, Poulsen G, Wohlfahrt J, Murray JC, Feenstra B, Melbye M. Maternal contributions to preterm delivery. Am J Epidemiol. 2009; 170:1358–64. DOI: 10.1093/aje/kwp324 [PubMed: 19854807]
3. Svensson AC, Sandin S, Cnattingius S, Reilly M, Pawitan Y, Hultman CM, et al. Maternal effects for preterm birth: a genetic epidemiologic study of 630,000 families. Am J Epidemiol. 2009; 170:1365–72. DOI: 10.1093/aje/kwp328 [PubMed: 19854802]
4. Culhane JF, Goldenberg RL. Racial disparities in preterm birth. Semin Perinatol. 2011; 35:234–9. [PubMed: 21798403]
5. Alexander GR, Baruffi G, Mor JM, Kieffer EC, Hulsey TC. Multiethnic variations in the pregnancy outcomes of military dependents. Am J Public Health. 1993; 83:1721–5. DOI: 10.2105/AJPH.83.12.1721 [PubMed: 8259802]

6. Kistka ZAF, Palomar L, Lee KA, Boslaugh SE, Wangler MF, Cole FS, et al. Racial disparity in the frequency of recurrence of preterm birth. Am J Obstet Gynecol. 2007; 196:1–6. DOI: 10.1016/j.ajog.2006.06.093 [PubMed: 17240216]
7. Stefflova K, Dulik MC, Pai AA, Walker AH, Zeigler-Johnson CM, Gueye SM, et al. Evaluation of group genetic ancestry of populations from Philadelphia and Dakar in the context of sex-biased admixture in the Americas. PLoS ONE. 2009; 4:e7842.doi: 10.1371/journal.pone.0007842 [PubMed: 19946364]
8. Thouas GA. Mitochondrial dysfunction in mouse oocytes results in preimplantation embryo arrest in vitro. Biol Reprod. 2004; 71:1936–42. DOI: 10.1095/biolreprod.104.033589 [PubMed: 15286028]
9. Acton BM, Jurisicova A, Jurisica I, Casper RF. Alterations in mitochondrial membrane potential during preimplantation stages of mouse and human embryo development. Mol Hum Reprod. 2004; 10:23–32. DOI: 10.1093/molehr/gah004 [PubMed: 14665703]
10. de Laat P, Fleuren LHJ, Bekker MN, Smeitink JAM, Janssen MCH. Obstetric complications in carriers of the m.3243A>G mutation, a retrospective cohort study on maternal and fetal outcome. Mitochondrion. 2015; 25:98–103. DOI: 10.1016/j.mito.2015.10.005 [PubMed: 26455484]
11. Alleman BW, Myking S, Ryckman KK, Myhre R, Feingold E, Feenstra B, et al. No observed association for mitochondrial SNPs with preterm delivery and related outcomes. Pediatr Res. 2012; 72:539–44. DOI: 10.1038/pr.2012.112 [PubMed: 22902432]
12. Suissa S, Wang Z, Poole J, Wittkopp S, Feder J, Shutt TE, et al. Ancient mtDNA genetic variants modulate mtDNA transcription and replication. PLoS Genet. 2009; 5:e1000474.doi: 10.1371/journal.pgen.1000474 [PubMed: 19424428]
13. Roubertoux PL, Sluyter F, Carlier M, Marcet B, Maarouf-Veray F, Chérif C, et al. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. Nat Genet. 2003; 35:65–9. DOI: 10.1038/ng1230 [PubMed: 12923532]
14. Yu X, Gimsa U, Wester-Rosenlöf L, Kanitz E, Otten W, Kunz M, et al. Dissecting the effects of mtDNA variations on complex traits using mouse conplastic strains: dissecting the effects of mtDNA variations on complex traits using mouse conplastic strains. Genome Res. 2009; 159:65.doi: 10.1101/gr.078865.108
15. Latorre-Pellicer A, Moreno-Loshuertos R, Lechuga-Vieco AV, Sánchez-Cabo F, Torroja C, Acín-Pérez R, et al. Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. Nature. 2016; 535:561–5. DOI: 10.1038/nature18618 [PubMed: 27383793]
16. Zhang H, Baldwin DA, Bukowski RK, Parry S, Xu Y, Song C, et al. A genome-wide association study of early spontaneous preterm delivery. Genet Epidemiol. 2015; 39:217–26. DOI: 10.1002/gepi.21887 [PubMed: 25599974]
17. Tsai H-J, Yu Y, Zhang S, Pearson C, Ortiz K, Xu X, et al. Association of genetic ancestry with preterm delivery and related traits among African American mothers. Am J Obstet Gynecol. 2009; 201:94, e1–e10. DOI: 10.1016/j.ajog.2009.02.027 [PubMed: 19446788]
18. Olsen J, Sørensen HT. The Danish National Birth Cohort - a valuable tool for pharmacoepidemiology in pregnancy. Int J Risk Saf Med. 1997; 10:197–8. DOI: 10.3233/JRS-1997-10307 [PubMed: 23511373]
19. Ehn NL, Cooper ME, Orr K, Shi M, Johnson MK, Caprau D, et al. Evaluation of fetal and maternal genetic variation in the progesterone receptor gene for contributions to preterm birth. Pediatr Res. 2007; 62:630–5. DOI: 10.1203/PDR.0b013e3181567bfc [PubMed: 17805208]
20. Wang H, St Julien KR, Stevenson DK, Hoffmann TJ, Witte JS, Lazzeroni LC, et al. A genome-wide association study (GWAS) for bronchopulmonary dysplasia. Pediatrics. 2013; 132:290–7. DOI: 10.1542/peds.2013-0533 [PubMed: 23897914]
21. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–75. DOI: 10.1086/519795 [PubMed: 17701901]
22. Weissensteiner H, Pacher D, Kloss-Brandstätter A, Forer L, Specht G, Bandelt H-J, et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. Nucleic Acids Res. 2016; 44:W58–63. DOI: 10.1093/nar/gkw233 [PubMed: 27084951]
23. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 2009; 19:1655–64. DOI: 10.1101/gr.094052.109 [PubMed: 19648217]

24. Roslin NM, Li W, Paterson AD, Strug LJ. Quality control analysis of the 1000 Genomes Project Omni2.5 genotypes. Am Soc Hum Genet. 2016 Abstract 576.
25. Shapiro AB, Austin CA. A high-throughput fluorescence anisotropy-based assay for human topoisomerase II β-catalyzed ATP-dependent supercoiled DNA relaxation. Anal Biochem. 2014; 448:23–9. [PubMed: 24309019]
26. Fulda KG, Kurian AK, Balyakina E, Moerbe MM. Paternal race/ethnicity and very low birth weight. BMC Pregnancy Childbirth. 2014; 14:385–93. DOI: 10.1186/s12884-014-0385-z [PubMed: 25406725]
27. Palomar L, DeFranco EA, Lee KA, Allsworth JE, Muglia LJ. Paternal race is a risk factor for preterm birth. Am J Obstet Gynecol. 2007; 197:152, e1–7. DOI: 10.1016/j.ajog.2007.03.035 [PubMed: 17689630]
28. Shachar BZ, Mayo JA, Lyell DJ, Stevenson DK, Shaw GM, Blumenfeld YJ. Risk for spontaneous preterm birth among inter-racial/ethnic couples. J Matern Fetal Neonatal Med. 2017; :1–7. DOI: 10.1080/14767058.2017.1293029
29. Elo IT, Vang Z, Culhane JF. Variation in birth outcomes by mother's country of birth among non-Hispanic black women in the United States. Matern Child Health J. 2014; 18:2371–81. DOI: 10.1007/s10995-014-1477-0 [PubMed: 24756226]
30. Urquia ML, Frank JW, Moineddin R, Glazier RH. Immigrants' duration of residence and adverse birth outcomes: a population-based study. BJOG. 2010; 117:591–601. DOI: 10.1111/j.1471-0528.2010.02523.x [PubMed: 20374596]
31. Cohen T, Levin L, Mishmar D. Ancient out-of-Africa mitochondrial DNA variants associate with distinct mitochondrial gene expression patterns. PLoS Genet. 2016; 12:e1006407.doi: 10.1371/journal.pgen.1006407 [PubMed: 27812116]
32. Gershoni M, Levin L, Ovadia O, Toiw Y, Shani N, Dadon S, et al. Disrupting mitochondrial-nuclear coevolution affects OXPHOS complex I integrity and impacts human health. Genome Biol Evol. 2014; 6:2665–80. DOI: 10.1093/gbe/evu208 [PubMed: 25245408]
33. Rishishwar L, Jordan IK. Implications of human evolution and admixture for mitochondrial replacement therapy. BMC Genomics. 2017; 18:140.doi: 10.1186/s12864-017-3539-3 [PubMed: 28178941]
34. Morrow EH, Reinhardt K, Wolff JN, Dowling DK. Risks inherent to mitochondrial replacement. EMBO Rep. 2015; 16:541–5. DOI: 10.15252/embr.201439110 [PubMed: 25807984]
35. Tsai T-S, Rajasekar S, St John JC. The relationship between mitochondrial DNA haplotype and the reproductive capacity of domestic pigs (Sus scrofa domesticus). BMC Genet. 2016; 17:67.doi: 10.1186/s12863-016-0375-4 [PubMed: 27188709]
36. Zhang G, Feenstra B, Bacelis J, Liu X, Muglia LMLJ, Juodakis J, et al. Genetic associations with gestational duration and spontaneous preterm birth. N Engl J Med. 2017; 377:1156–67. DOI: 10.1056/NEJMoa1612665 [PubMed: 28877031]

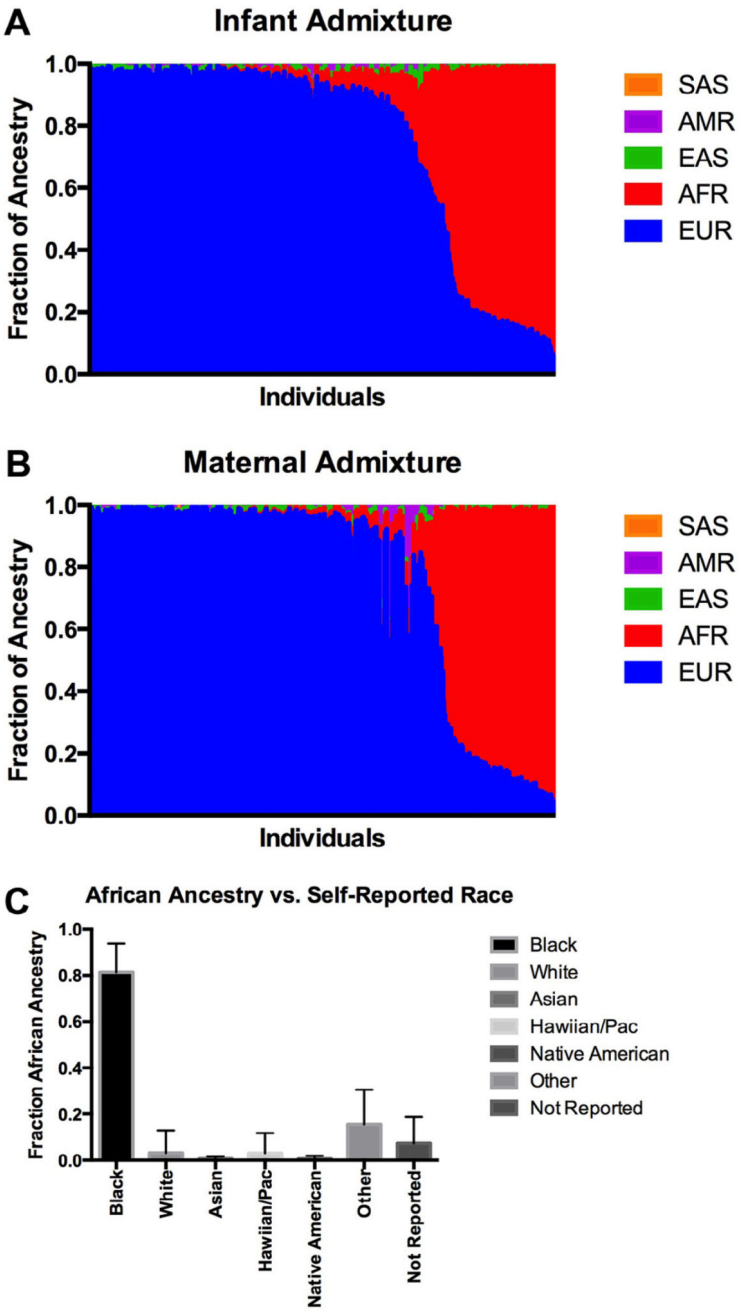

Figure 1.

The ADMIXTURE-determined fractions of nuclear ancestry (South Asian [SAS], American [AMR], East Asian [EAS], African [AFR] and European [EUR]) are plotted for **A,** infants and **B,** mothers in the GPN dataset with individuals along the *x*-axis ordered by degree of African ancestry from least to greatest. **C,** The degree of African ancestry for mothers is also shown, with the group divided by the self-reported race.

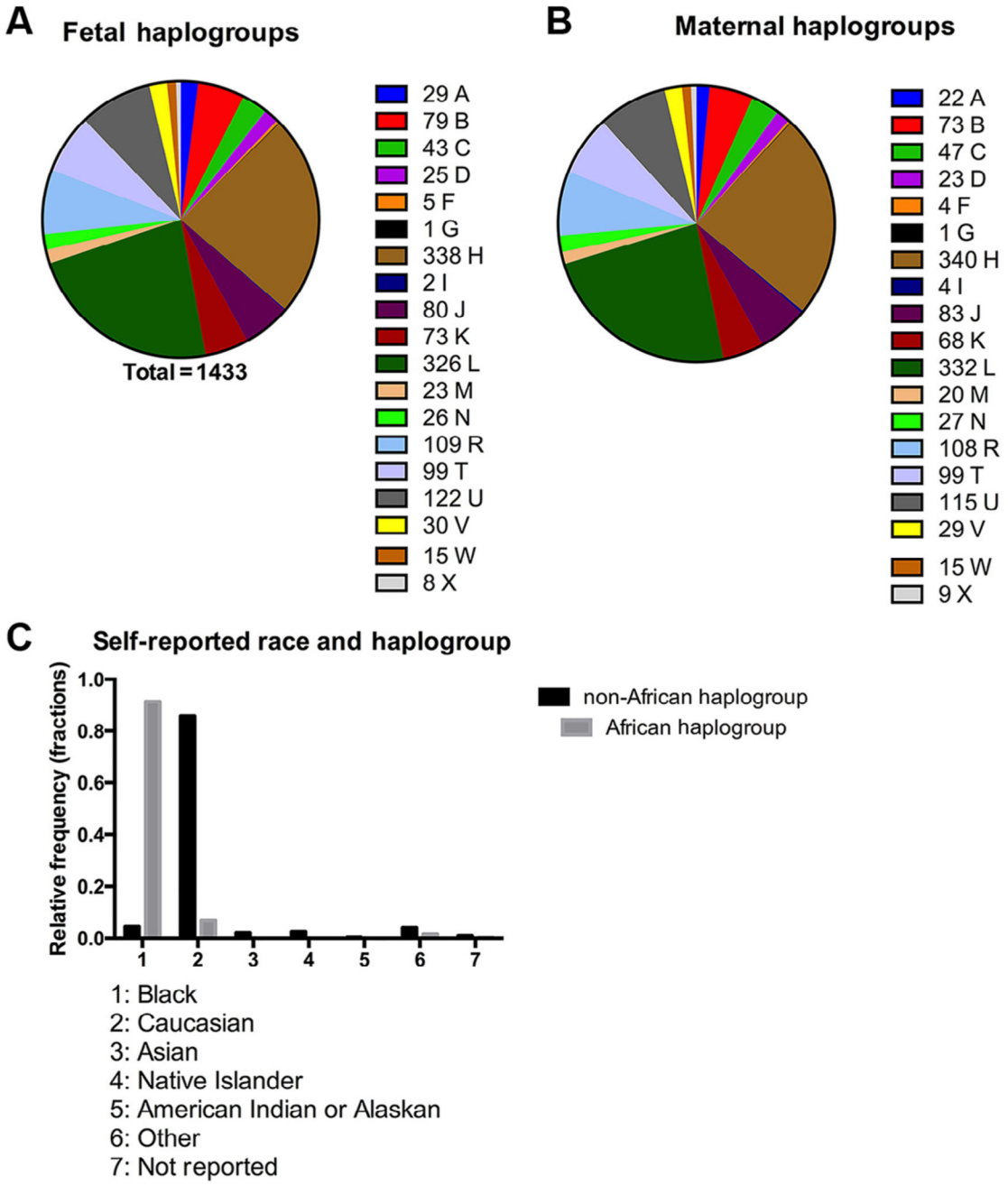

Figure 2.

The haplogroups of **A,** infants and **B,** mothers are shown. The small differences in the number of sampled individuals in the individual haplogroups are due to missing samples in the original dataset. **C,** Frequency of African and non-African haplogroups observed broken down by self-described race.

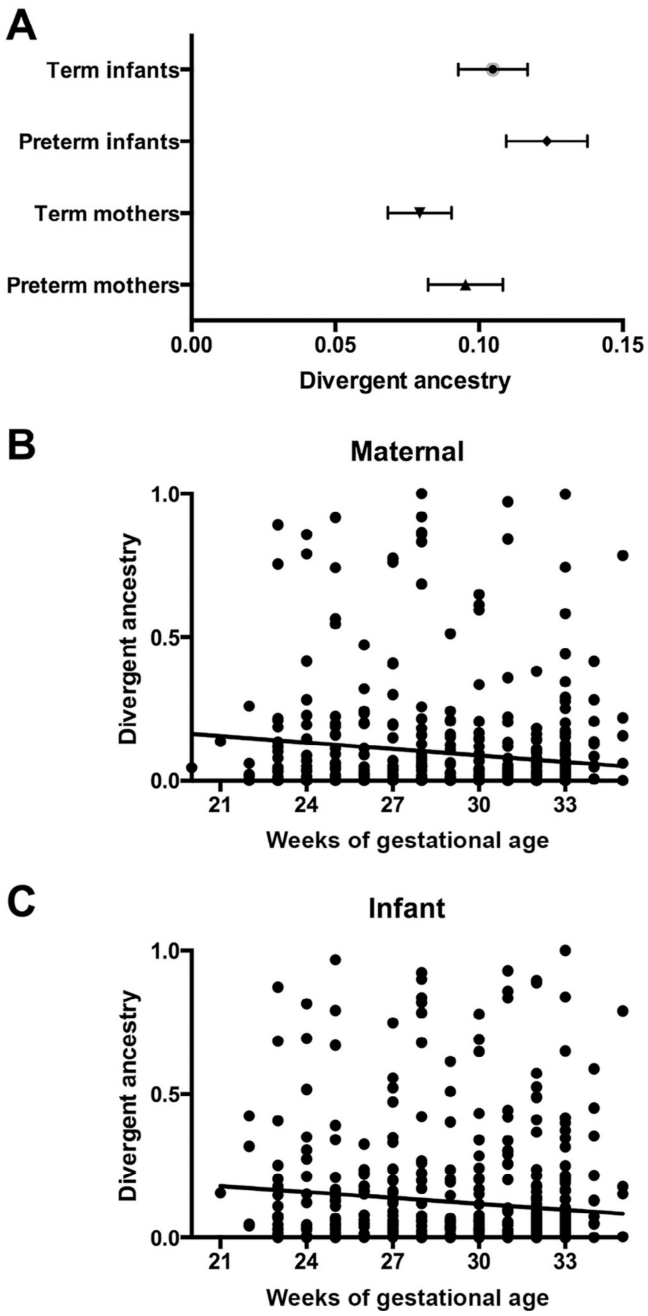

Figure 3.

Divergent ancestry in the GPN dataset. **A,** The values for all individuals in the dataset were plotted by term (n = 717) or preterm (n = 702) status for mothers and infants. Divergent ancestry is higher in the preterm infants ($P = .046$) and there was a trend toward greater values in mothers with preterm birth ($P = .067$). The degree of divergent ancestry for preterm deliveries was then plotted against the estimated weeks of gestational age for all infants and mothers where data was available. For both **B,** mothers and their **C,** infants, there was a significant negative correlation between divergent ancestry and estimated gestational age indicating that more divergent infants were born earlier (infant Pearson r = −0.114, $P = .$

009 n = 517; maternal Pearson r = −0.138, *P* = .002 n = 503). A best-fit interpolation is shown.

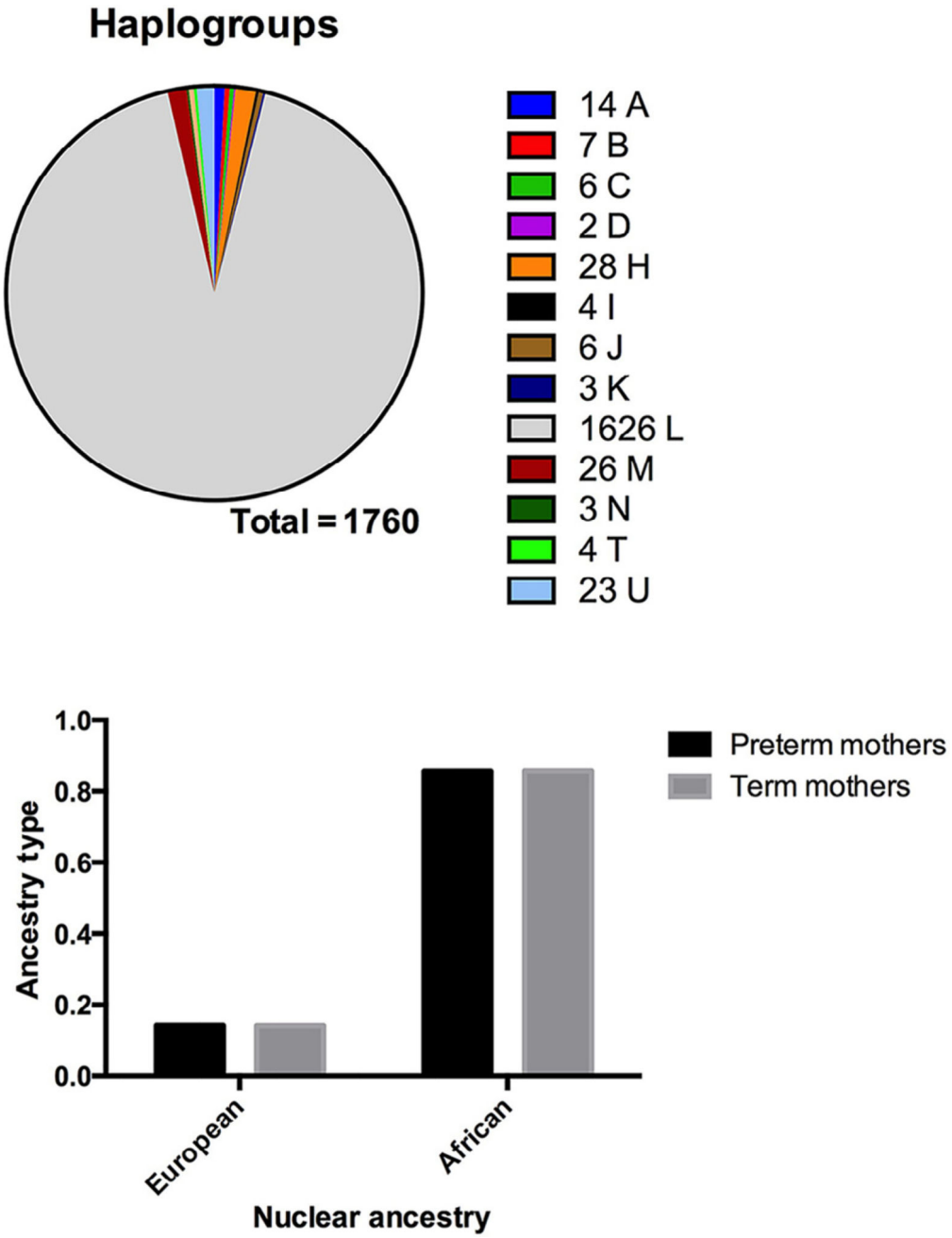

Figure 4.

Characteristics of individuals in the BBC dataset. The haplogroups and nuclear ancestry observed were consistent with the recruitment of an African American population.

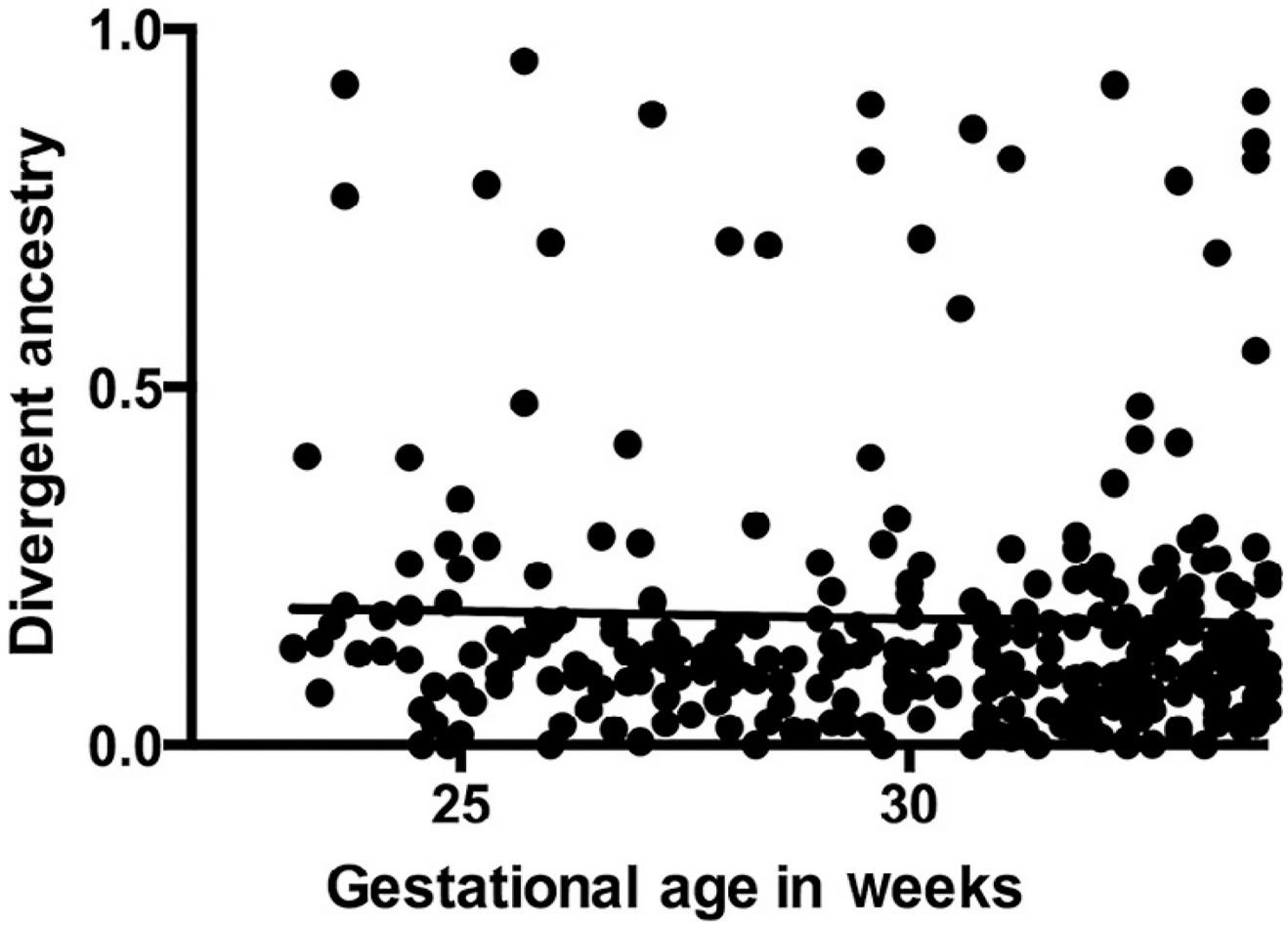

Figure 5.

Divergent ancestry in the BBC dataset. The divergent ancestry of maternal samples (n = 294) was plotted by weeks of gestational age. There is a trend toward correlation that did not reach statistical significance (Pearson r = −0.043 with $P > .05$).

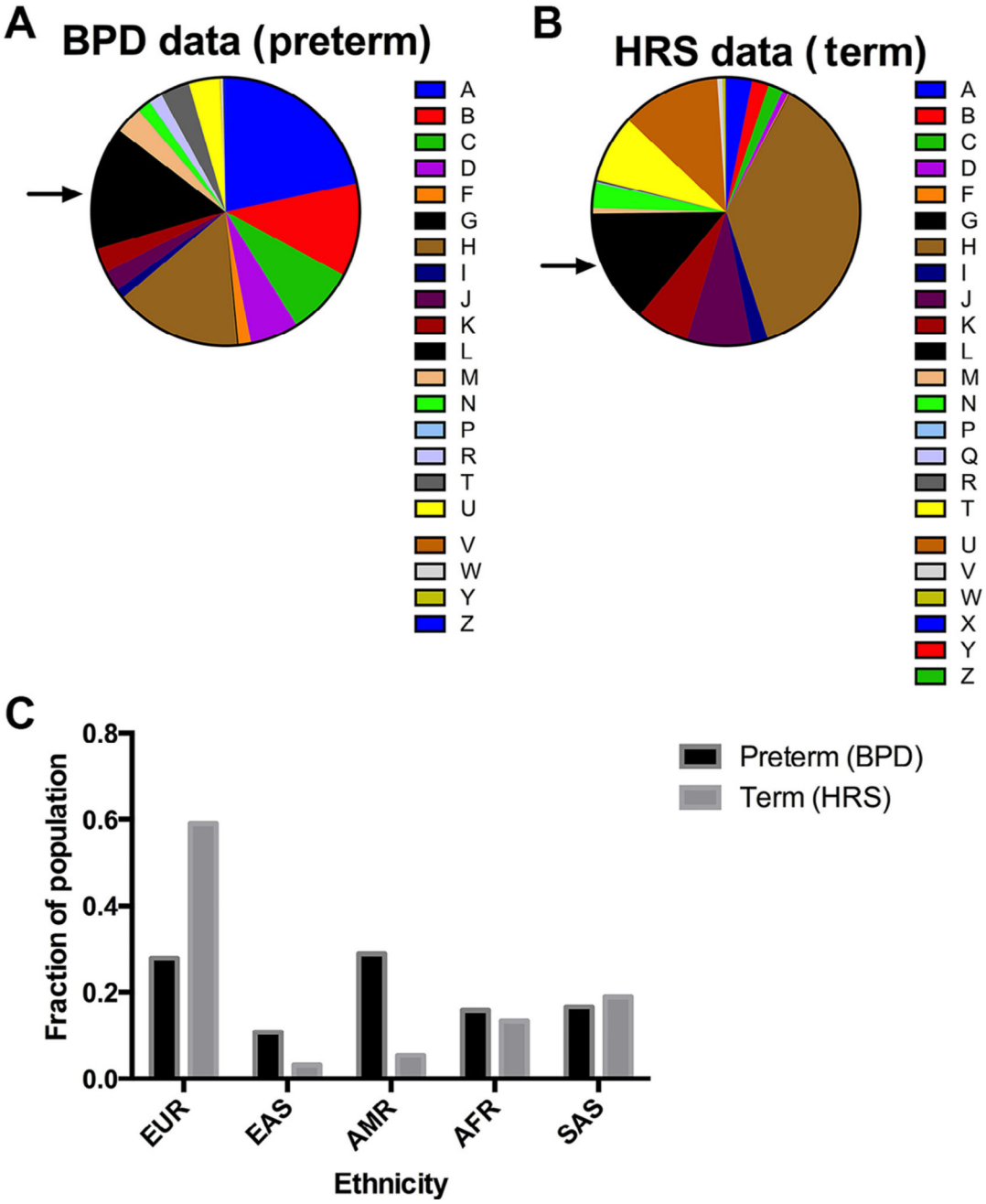

Figure 6.

Characteristics of individuals in the BPD and HRS datasets. The haplogroups identified showed a comparable frequency of African haplogroup between the 2 datasets, 14.9% in the BPD group, and 13.7% in the HRS group (compare **A** and **B**; L haplogroups marked with *arrows*). **C,** The average distribution of nuclear ancestries within each dataset is shown, showing comparable levels of African nuclear ancestry, 15.7% in the BPD group and 13.4% in the HRS group.

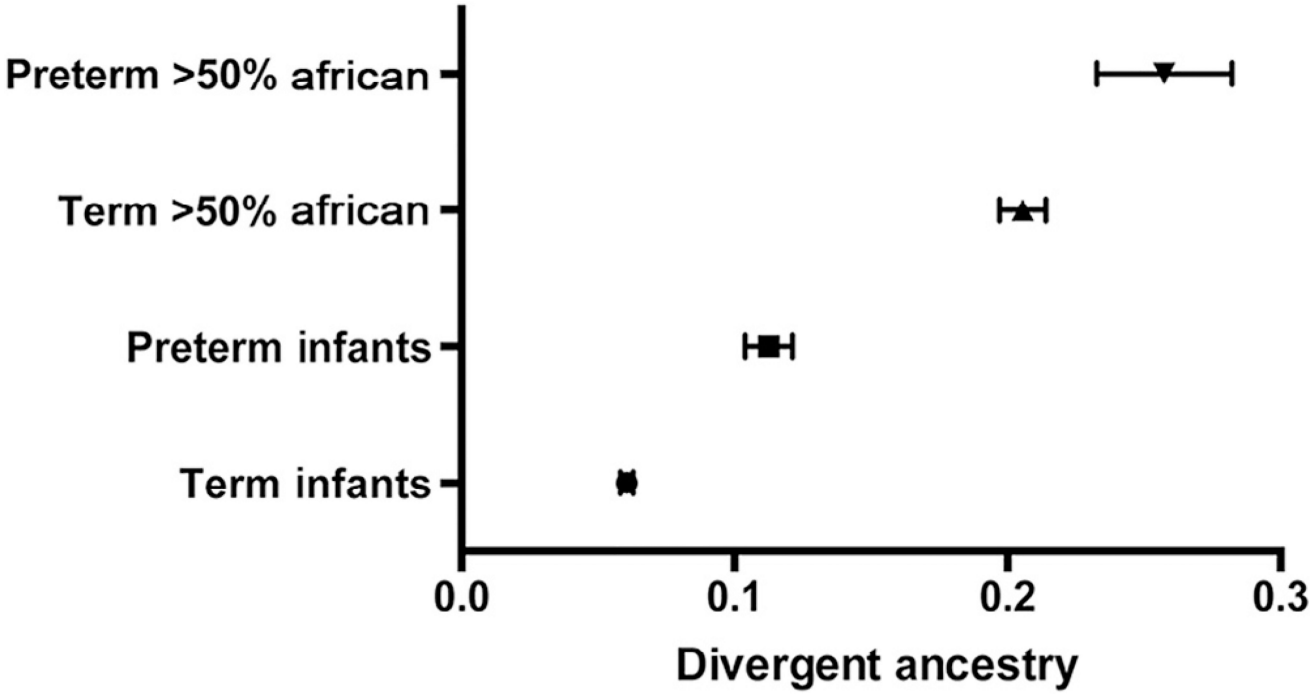

Figure 7.

Divergent ancestry in the BPD dataset. The divergent ancestry of the BPD (preterm n = 1726) and HRS (control n = 12 570) data are plotted as the mean and 95% CIs ($P < .0001$). A subgroup analysis was performed with individuals with >50% African ancestry (preterm n = 225; term n = 1665) and was also significantly different ($P < .0001$).

Table I

Characteristics of studies analyzed

Studies	Accession	Genotyping platform	Platform MtSNPs	Patients			Study features
				Cases	Controls	Total	
GPN	phs000714.v1.p1	Affymetrix Genome-Wide 6.0	117	702	717	1419	Populations matched for race and parity. Cases born before 34 weeks of gestation, controls born after 39 weeks of gestation.
BBC	phs000332.v3.p2	Illumina HumanOmni2.5	93	722	1057	1779	Patients all recruited from the same hospital. Cases born before 37 weeks of gestation, controls after 37 weeks of gestation.
DNBC	phs000103.v3.p1	Illumina Human660W-Quad	135	884	960	1844	Study population insufficiently diverse to measure trends in divergent mitonuclear ancestry.
GENEVA-AA	phs000353.v1.p1	Illumina HumanOmni1-Quad	26	1035	508	1543	Genotyping platform used in the study did not assess enough MtSNPs to accurately haplogroup the study subjects.
BPD	N/A	Illumina HumanOmni2.5	93	1724	0	1724	All patients born prematurely at 25–29 weeks of gestation.
HRS	phs000428.v1.p1	Illumina HumanOmni2.5	93	0	12 507	12 507	Patients assumed to be born at term.

MtSNP, mitochondrial single nucleotide polymorphism; *N/A*, not applicable.

Table II

Potential confounders are not associated with divergent ancestry

Variables	Discrete Ancestry	*P* value
Self-reported African American		
No prior abortion	.224	
Prior abortion	.220	.92
Married	.219	
Unmarried and with partner	.220	
Unmarried and lives alone	.226	.95
Not a high school graduate	.195	
High school graduate	.235	.08
Unemployed	.226	
Employed	.220	.82
Income <$12K	.204	
Income $12–$24K	.244	
Income $24–$50K	.237	
Income $50–$100K	.172	
Income >$100K	.129	
Unknown income	.239	
No answer income	.242	.58
Self-reported White		
No prior abortion	.034	
Prior abortion	.044	.22
Married	.031	
Unmarried and with partner	.039	
Unmarried and lives alone	.053	.09
Not a high school graduate	.047	
High school graduate	.033	.13
Unemployed	.041	
Employed	.030	.16
Income <$12K	.058	
Income $12–$24K	.039	
Income $24–$50K	.038	
Income $50–$100K	.017	
Income >$100K	.027	
Unknown income	.040	
No answer income	.031	.10