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GWAS on Prolonged Gestation (Post-term Birth): Analysis of Successive Finnish Birth Cohorts

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Abstract:

Background

Gestation is a crucial timepoint in human development. Deviation from a term gestational age correlates with both acute and long-term adverse health effects for the child. Both being born pre and post-term, *i.e.* having short and long gestational ages, are heritable and influenced by the pre- and perinatal environment. Despite the obvious heritable component, specific genetic influences underlying differences in gestational age are poorly understood.

Methods

We investigated the genetic architecture of gestational age in 9,141 individuals, including 1,167 born post-term, across two Northern Finland cohorts (NNFBC) born in 1966 or 1986.

Results

Here we identify one globally significant intronic genetic variant within the *ADAMTS13* gene that is associated with prolonged gestation ($p=4.85x10^{-8}$). Additional variants that reached suggestive levels of significance were identified within introns at the *ARGHAP42* and *TKT* genes, and in the upstream (5') intergenic regions of the *B3GALT5* and *SSBP2* genes. The variants near the *ADAMTS13*, *B3GALT5*, *SSBP2* and *TKT* loci are linked to alterations in gene expression levels (cis-eQTLs). Luciferase assays confirmed the allele specific enhancer activity for the *BGALT5* and *TKT* loci.

Conclusions

Our findings provide the first evidence of a specific genetic influence associated with prolonged gestation. This study forms a foundation for a better understanding of the genetic and long term health risks faced by induced and post-term individuals. The long-term risks for induced individuals who have a previously overlooked post-term potential may be a major issue for current health providers.

Introduction:

Gestation is a crucial period of human development. Being born too early (preterm, < 37 weeks gestation) or too late (post-term, ≥42 weeks gestation) can have significant acute and long-term health consequences[1]. While preterm birth has received substantial attention[2–4], post-term birth has been scantily explored despite approximately 3-5% of all births each year being post-term[5]. Prolonged gestation poses a unique set of acute and long-term adverse health outcomes, including an increased need for intervention during labour and risk factors for truncal obesity, insulin resistance, altered lipids and elevated blood pressure [6, 7]. Thus, there is a vital need to understand the role of genetic variants on post-term birth.

The acute health risks, for both the mother and the child, of being born post-term are well documented (*for review see* [8]). Consequently, induction of birth at or before 41 weeks gestation is recommended in order to reduce the acute risks associated with post-term birth[5, 8]. As a result, approximately 25% of the routine inductions in Australia in 2010 were primarily performed to prevent prolonged pregnancy. However, the rules regarding the decision to induce labour are not consistently applied across different hospitals, reflecting the influence of opinions of individual practitioners and differing staff routines[9]. Despite this, induction remains an excellent intervention, and its application has reduced post-term births from approximately 20% of all births in the 1960's[10] to the modern-day rate of under 5%[5, 11]. However, there is a possibility that the long-term risks associated with post-term birth are a part of the genetically-informed trajectory for induced individuals. In this case, induction would not change this aspect of the biology of post-term individuals. Family and twin studies attribute 25-40% of the variation in gestational age to genetic factors [12–19] with fetal (26%) and maternal (21%) factors each explaining nearly half of this variation [18]. Thus, there is a large population of individuals who have "post-term potential"[20] and possibly face the long-term health risks of post-term birth without actually having been born post-term (due to pregnancy ending from obstetric management).

Evidence supports the hypothesis that it is the fetus that determines the timing of labor rather than the mother. Therefore, we have investigated the genetic architecture of gestational age in 9,141 Northern Finnish (white European) individuals (1,167 post-term) across two birth cohorts (Northern Finland Birth Cohort [NFBC] 1966 and NFBC1986). Here we identify intronic genetic variants within the *TKT*, *ARGHAP42*, and *ADAMTS13* genes and intergenic upstream (5') of the *B3GALT5* and *SSBP2* genes that are associated with prolonged gestation.

Materials and Methods:

Subjects

We undertook a discovery-replication study of two successive birth cohorts from Northern Finland (for cohort information including loss-to-follow-up, please see the cohort papers: NFBC 1966[10] and NFBC1986[11]). Both cohorts were recruited from the two northernmost provinces of Finland (*i.e.* Oulu and Lapland). Each cohort followed participants prospectively from approximately 12-16 weeks of gestation, providing one of the earliest-known cohorts with accurate gestational age determination[10, 11].

The NFBC1966 dataset consists of 12,231 children born to 12,068 mothers. This cohort represents 96% of all children born in Oulu and Lapland in 1966 with expected delivery dates between Jan 1st and Dec 31st 1966. Blood samples of the children in this cohort were collected for genotyping at age 31 (*i.e*. in 1997) and genetic data was available for 5,402 individuals. Genotyping was completed using Illumina HumanCNV370DUO Analysis BeadChip and the Beadstudio 3.1 algorithm.

The NFBC1986 dataset consists of a prospectively recruited cohort containing 9,432 children born to 9,362 mothers. This cohort represents 99% of all available births in Northern Finland between the 1st of July 1985 and the 30th of June 1986. Blood samples from the children of the NFBC1986 cohort were collected for genotyping at 16 years of age (*i.e.* in 2002-2003). Genetic data

is available for 3,739 individuals in total (~500 were selected as representing individuals with GDM, GHT, and preterm birth; the remaining represented a random sample of the cohort). Genotyping was completed using the OmniExpresse Exome Chip and the Beadstudio 3.1 algorithm.

Defining the post-term dataset:

The gestational ages of the individuals in the 1966 and 1986 NFBC cohorts were calculated at their first antenatal visit. For the NFBC1966 cohort gestational age was calculated through last menstrual period. In the NFBC1986 cohort gestational age was based on ultrasound at <20 weeks of gestation or on the last menstral period, with discrepant cases reviewed in detail from medical records as previously described.[21] The control cohort was restricted to those born at full-term which was defined as between 38 0/7 to 40 0/7 weeks of gestation. Children born between 37 0/7 to 37 6/7 weeks (i.e. Early term) or 41 0/7 to 41 6/7 weeks (*i.e.* Late term) were excluded to reduce mischaracterization due to errors in the calculated gestational age. The post-term case cohorts included those individuals born at ≥42 0/7 weeks of gestation. To further reduce the chances of obscuring the genetic potential of gestational age, we excluded individuals from our control cohort who were born early due to induction of labour or other factors: 1) those born from multiple births; 2) those whose mother had gestational diabetes (prediabetes in 1966 cohort); and 3) those whose birth was by planned caesarean section. Gestational and pre-diabetes were included as an exclusion criteria because it is a strong indicator for induced delivery, which could have biased the selection in the 1966 post-term cohort[22].

Quality control of genetic data:

Genetic data was vetted for quality control. Genetic data for a subject was excluded if: 1) the call rate was < 95% (99 % if the minor allele frequency < 5 %); 2) the mean heterozygosity was < 0.29; 3) there were multidimensional scaling (MDS) outliers; 4) the concordance with other DNA samples in

the cohort ≥ 0.99 (risk of being duplicated sample); 5) identity by state (IBS) pairwise comparisons were > 0.99 with most other samples (suspicion of samples being contaminated); 6) IBS pairwise sharing was > 0.20; 7) consent was not given; 8) comparison to medical records identified a gender-genotype mismatch; 9) there was an elevated heterozygosity rate (4 or more standard deviations from the mean); or 10) there was significant deviation from the Hardy-Weinberg Equilibrium ($p < 0.0001$).

After all QC measures and exclusions were applied, 5,402 and 3,739 individuals remained in the 1966 and 1986 studies, respectively. These included 1034 post-term individuals and 2375 term-born controls from the NFBC1966 cohort and 133 post-term individuals and 1250 term-born controls from the NFBC1986 cohort.

Imputation of genetic data:

Impute version 2 was used to estimate the single nucleotide polymorphisms (SNPs) that were not sampled directly by the genotyping platform for the NFBC 1966 samples. The imputation used HapMap 2 (Build 36) as the reference panel and proper_info > 0.4 as the quality metric[23, 24]. Before imputation, there were 309,948 directly genotyped SNPs. After imputation, 3,855,963 SNPs, including those directly genotyped, were available from the 1966 genotypes for analysis.

Imputation of the missing 1986 genotypes was carried out in two steps: 1) a pre-phasing step that estimated haplotypes for all available samples using the SHAPEIT program with the 1000 genomes reference panel as the guide; and 2) an imputation step (Impute version 2) that imputed the missing alleles directly onto the phased haplotypes[23, 24]. After imputation, 59,683,063 SNPs, including those directly genotyped, were available from the 1986 genotypes for analysis.

Statistical analysis:

SNPTEST version 2[24] was used to perform all genetic analyses on the imputed genetic data for both cohorts. In the regression analysis, the main effects model tested the association between SNP markers and gestational age. SNP genotypes were coded as 0, 1, or 2 (according to the number of copies of the minor allele) and an additive model of genetic variance was assumed where the effect on the trait of the heterozygote was estimated to be midway between the levels of the two homozygotes. This model fits the best assumption for a post-term phenotype that is thought to have a small amount of genetic variance produced by multiple genetic variants in combination.

All genetic analyses also accounted for child's sex, as this was the only trait that has consistently shown a large effect on post-term birth status in any previous post-term studies[15, 25–27].

The p-value for results that were suggestive of statistical significance in the discovery phase in each cohort was set at any p-value less than 1×10^{-5}. In the validation phase, any finding ($p < 0.05$) within the significant LD block was considered validation of significance of the locus. These p-values were selected because we planned functional analyses to confirm the significant variants,

Quantile-quantile plots were generated (qqman package in R), by plotting the expected distribution of p-values versus the observed p-values (assuming a uniform distribution), to test for possible sources of p-value inflation.

Spatial analysis of the validated SNPs for putative regulatory roles within the genome:

HiC spatial genomic connectivity (HiC) data was used to identify genes that SNPs connected to[28, 29]. GWAS3D[30] was used, with default parameters, to identify physical connections (as captured by proximity ligation) that occurred with the most significant GWAS SNPs.

Identification of gene expression alterations associated with the GWAS loci:

eQTL analysis identifies SNPs that associate with altered expression level(s) of one or more genes[31]. The Genotype-Tissue Expression (GTEx) project database (version 6) eQTL data is powered for the global examination of larger eQTL effects[32]. Therefore, we limited false positives in our trans-eQTL results by only testing eQTLs supported by SNP-gene spatial interactions. Significance levels for this analysis were based on evidence from prior literature[29, 32, 33]: cis-eQTL (genes < 1 Mb distance from SNP, $p<1x10^{-4}$), trans-eQTL (longer distance or inter-chromosomal, $p<1x10^{-3}$). Thus, the identification of a SNP-gene spatial interaction that is re-inforced by SNP-gene eQTL has two independent sources of evidence verifying the long-distance transcription regulatory functions.

Luciferase assays

Enhancer activity of the post-term SNPS in proximity to loci (*B3GALT5*, *ARHGAP42*, *ADAMTS13*, *SSBP2*, and *TKT*) was measured by luciferase assay. Briefly, the regions spanning each SNP were PCR amplified from genomic DNA obtained from 1000 genome samples cloned into the Gateway adapted pGL4.23-GW (Addgene Plasmid #60323) [34] and sequenced to confirm the genotype. For rs11170213, no sample genotype information could be obtained from the 1000 genomes, so the region spanning the allele 'A' of the SNP was amplified from MCF-7 genomic DNA. The Allele 'C' version of the SNP was generated by site directed mutageneis (SDM) of the cloned pGL4.23 plasmid using the QuickChange mutageneis protocol (Agilent Technologies). Primers for PCR amplification and SDM are listed in Supplemental Table 1. The *ADAMTS13* locus could not be amplified by PCR. HeLa cells were seeded at $5x10^3$ cells per well in a 96 well plate, grown in DMEM media supplemented with 10% fetal bovine serum (Thermo Scientific, #11995-065) one day prior to transfection. Cells were co-transfected with the cloned pGL4.23 and renilla plasmids using Lipofectamine 3000 (Thermo Scientific, #L3000008) and the the Promega Dual Glo Luciferase Assay System (#PME2920) was used to measure luciferase activity after 48 hours. Luminescence was

normalized to Renilla and expressed relative to the normalized luminescence of empty pGL4.23. Results are from four independent biological replicates.

Results:

Discovery Phase:

NFBC1966 Variants Associated with post-term birth

Analysis of the NFBC1966 post-term cohort identified six GWAS peaks that were suggestive of global significance ($p < 1x10^{-5}$, Figure 1a and 2a). Two clusters of variants were located within introns in the *B3GALT5* (lead SNP rs1534080) and *DNHD1* (rs12285957) genes. An additional four clusters of variants were located within intergenic regions on chromosomes 10 (2 regions), 12, and 15 (Supplemental Table 2).

NFBC1986 Variants Associated with post-term birth

Analysis of the NFBC1986 post-term cohort identified twenty-five significant GWAS peaks ($p < 1x10^{-5}$, Supplemental Table 2, Figure 1b and 2b). The lead SNPs for fourteen of these GWAS peaks were intronic: AC079779.5 (rs72774524), ADAMTS13 (rs655911), ANO4 (rs11609845), ARHGAP42 (rs78598508), ASAH2 (rs75320537), C14orf37/PSMA-AS1 (rs78874632), CTD-2277K2.1 (rs191706929), DCDC2C (rs12612077), DTWD2 (rs17440178), ESR1 (rs117533178), FAT3 (rs7950344), KCNB2 (rs79648768), and RIN3 (rs6575274), and TKT (rs4687715). Only the SNP at the ADAMTS13 locus was globally significant ($p < 5x10^{-8}$).

Eleven intergenic loci were also associated with gestational age in the NFBC1986 cohort. These intergenic loci were located ≤116kbp from a coding exon (gene): AL671972.1 (rs10995050, 7.2 kb downstream), GRIK2 (rs183770336, 724 kb downstream), HMX1 (rs145023824, 75 kb upstream), KCNA5 (rs2239507, 2 kb upstream), LRPPRC (rs62135521, 73 kb upstream), RP11-289F5.1 (rs10780480, 116 kb upstream), RP11-465K16.1 (rs7013779, 40 kb upstream), RP11-644L4.1

(rs72965926, 5.3 kb downstream), SSBP2 (rs2135, 31 kb upstream), ZFR (rs66858738, 7.7 kb upstream), and 7SK (rs11610162, 11 kb upstream)

Of the list of genes located close to the loci that were significantly associated with post-term birth in the 1986 cohort, only ARHGAP42 has previously been associated with a developmental phenotype (age at menarche in a Japanese population, rs12800752)[35].

Validation of Results of the Discovery Phase:

The six NFBC1966 loci and twenty-five NFBC1986 loci were tested for cross-validation (*i.e*. significance) in the opposite cohort. Of these loci, none were associated with gestational age at a p value of $\leq 1x10^{-5}$ in both cohorts. However, the *B3GALT5*, *SSBP2*, and *TKT* GWAS loci (hereafter referred to as post-term loci) were validated as significant in both the 1966 and 1986 cohorts (*i.e.* discovery $p < 1x10^{-5}$ and validation $p<0.05$, Supplemental Tables 2A and 2B). *ADAMTS13* reached global significance ($p < 5x10^{-8}$ [36]) in the NFBC1986 cohort, and was included in the post-term loci for further analyses (Supplemental Table 2B).

The rs78598508 variant, which is located within *ARHGAP42* and associated with gestational age in the 1986 cohort, was not measured in the 1966 cohort. Furthermore, rs78598508 was not in strong LD (> 0.9 r^2) with any other variants. Therefore, rs78598508 could not be tested for cross-validation in this study (Supplemental Table 2). This is a limitation due to the historical use of different platforms for the SNP detection. However, given that ARHGAP42 has previously been associated with a developmental phenotype (age at menarche in a Japanese population, rs12800752)[35] it was included in further analyses in this study.

Identification of Spatial and Functional Connections to Post-Term Birth

Spatial Associations:

We screened Haploreg v4.1 (1000 genomes haplotype data), to show that none of the lead SNPs in the *ADAMTS13*, *ARHGAP42*, *B3GALT5*, *SSBP2*, and *TKT* loci are in linkage disequilibrium (LD, r^2 > 0.95 and D' > 0.95) with any variants located within exons or critical transcriptional processing sequences (*e.g.* intronic branch sites, polyA signals, or transcription termination signals). Thus, there is no evidence that these SNPs directly impact on protein function through aberrant transcript processing. As such, we hypothesized that the post-term SNPs were affecting enhancer regions (*i.e*. short genomic regions that are bound by transcription factors) and altering the transcriptional regulation of distant genes.

Interactions between the lead SNPs at each post-term locus and distant genes were screened for using GWAS3D (Figure 3, Supplemental Table 3). The *ADAMTS13* locus spatially connects to the *SLC2A6* (9q34), *COL5A1* (9q34.2-q34.3), and *RABGAP1L* (1q24) loci. This is notable as *ADAMTS13*, *SLC2A6*, *COL5A1*, and additional genes within 9q34 have been implicated in the coagulation process and associated with ovarian function[37]. The *ARHGAP42* locus spatially connects to an intergenic region downstream of FAM133A. The *B3GALT5* locus spatially connects to the Down Syndrome Cell Adhesion Molecule (*DSCAM*) locus. *DSCAM* is a member of the immunoglobulin superfamily of cell adhesion molecules (Ig-CAMs) that are involved in human central and peripheral nervous system development. The *SSBP2* locus shows no significant spatial connections in the Hi-C data in GWAS3D. The *TKT* locus spatially connects to an intergenic region in 21p11.2 which contains predicted open reading frames that encode undefined proteins.

Locus-Specific Transcriptional (eQTL) Associations with Gene Expression:

Functional-regulatory roles for the SNPs we identified in this study were refined by testing the spatial SNP-gene pairs for significant eQTLs using the GTEx database (version 6). Variants in the *ADAMTS13* (Tibial Nerve Tissue, $p=1.50\times10^{-8}$), *B3GALT5* (Thyroid Tissue, $p=9.0\times10^{-5}$), and *TKT* (Left Ventricular Heart Tissue, $p=2.0\times10^{-5}$) loci associate with altered expression changes within these genes, confirming that these SNPs fall within loci that regulate their local gene landscape

(Supplemental Table 4). In addition, rs655911 (intronic, *ADAMTS13*) also showed eQTL associations with the expression of *SLC2A6*, reinforcing the significance of the spatial connection. The variant associated with the *ARHGAP42* locus (rs78598508) showed no significant eQTLs.

The SNP rs2135, which was 44kb upstream of *SSBP2,* did not show any evidence of spatial connections to other regions. Moreover, there was no evidence of a cis-eQTL between this SNPs and the *SSBP2* gene itself. A global survey of eQTL associations with the *SSBP2* SNPs, within GTEx, did not identify any globally significant eQTLs (Supplemental Figure 1). Analyses indicated putative eQTLs between the SSBP2 lead SNP (rs2135; 5q14.1) and: HBG1 (11p15.5, Lung, $1.10x10^{-5}$); HLA-DRB5 (6p21.3, Whole Blood, $2.50x10^{-5}$); and FYB (5p13.1, Mucosa of the Esophagus, $4.00x10^{-5}$) (Supplemental Figure 1).

Locus-Specific Enhancer (Luciferase) Associations with Gene Expression:

The lead SNPs that were proximal to *ARHGAP42* (rs78598508), *B3GALT5* (rs111702173, rs560928), *SSBP2* (rs2135) and *TKT* (rs4687715) were screened for enhancer activity (Figure 4). *ADAMTS13* (rs655911) was unable to be cloned and could not be tested. Cloning loci with alternate alleles allowed the measurement of the effect of genetic variation on the observed enhancer activity. Luciferase assays in Hela cells revealed a pronounced enhancer effect for the loci containing *ARHGAP42* (rs78598508), *B3GALT5* (rs111702173, rs560928) and *TKT* (rs4687715), and a repressive effect for *SSBP2* (rs2135) (Figure 4). For rs4687715 and rs560928, the region shows a differential enhancer allelic effect. Therefore, for two of the five loci tested, the enhancer activity associated with these gestational age associated regions is sensitive to the identity of the haplotype at the SNP position. For the remaining three regions, the SNP tested did not have a measurable allelic effect in HeLa cells, but still showed significant enhancer/insulator capabilities.

Discussion:

Previously, there has been indirect evidence of a genetic component to post-term birth, as children born post-term are more likely to have a sibling or mother born post-term[6]. This study is the first to identify specific genetic variants in proximity to the *ADAMTS13*, *B3GALT5*, *SSBP2*, and *TKT* genes as being associated with prolonged gestation. Data on the spatial connections with these loci, eQTLs, and enhancer activity is consistent with these post-term variants acting as functional determinants of gestational length. Thus, the SNPs associated with the *ADAMTS13*, *B3GALT5*, *SSBP2*, and *TKT* loci may alter the expression of these genes, contributing to the post-term phenotype by affecting regulation of processes involved in human development such as growth and metabolism, and, more specifically, hematopoiesis.

Post-term Associated SSBP2 and TKT are linked to Alterations in Cellular Growth, Proliferation, and Metabolism

Alterations in cellular growth, proliferation, and metabolism pre-program biological development (*e.g.* pentose phosphate pathway) resulting in an amplified risk of chronic non-communicable disease (*i.e.* diseases of long duration and slow progression including cardiovascular disease, diabetes and obesity) [38]. Proteins encoded by the *SSBP2* and *TKT* genes are involved in cellular growth, proliferation, and metabolism and are thus capable of altering developmental trajectories. For example, TKT is involved in carbohydrate metabolism[39] and could contribute to the later-in-life increased adiposity and risk of metabolic syndrome in children and adults born post-term[6, 7].

Post-term Associated TKT is linked to Alterations in Cell Cycle and Growth

TKT dysregulation has an important role in cellular growth rates, oocyte cell cycle progression and maturation[39]. The *TKT* gene encodes a protein that contributes to the main carbohydrate metabolic pathways by connecting the pentose phosphate pathway (PPP) to glycolysis. This process results in NADPH synthesis. NADPH is part of the control for reactive oxygen species, which were

found to be imbalanced in post-term births[40]. Under-expression of *TKT* in maternal mice contributes to pregnancy resulting in fewer progeny, retarded postnatal growth, and reduced levels of adipose tissue in offspring[41]. This phenotype has similarities to post-term infants, who are typically born lean[14]. Collectively, the effects of aberrant *TKT* expression are consistent with TKT variation in humans contributing to aberrant gestational timing. The links between variation in TKT function and metabolism may help to partially explain the observed links between post-term birth and the later development of symptoms of the metabolic syndrome [6].

Post-term associated ADAMTS13 and *SSBP2 are linked with hematopoiesis and blood disorders*

Alterations in *ADAMTS13* and *SSBP2* levels could be affecting gestation through alterations in hematopoietic pathways. There is a large developmental aspect to hematopoiesis during different phases of gestation. Maturity of the hematopoietic system occurs late in gestation and tracks with gestational age, with the proportions of fetal hemoglobin decreasing during the progression from preterm - term - post-term (83.93% to 68.59% to 60.03%, respectively) [42]. In post-term births, cord blood collected at birth shows differences in levels of polycythemia (increased concentration of hemoglobin in the blood), erythropoietin levels (increased erythropoiesis), mean corpuscular hemoglobin, red blood cell count, neutrophil count, and monocyte count [43].

The *ADAMTS13* gene encodes a protease that has previously been shown to disrupt the regulation of platelet thrombosis by cleaving Von Willebrand factor [44]. Critically, ADAMTS13 variants are also known to cause neonatal platelet disorders. These disorders include Upshaw-Schulman syndrome (hemolytic anemia) and blood hyper-coagulation (thrombophilia), the second of which is associated with fetal loss[37]. Additionally, the identification of a significant eQTL between a post-term associated *B3GALT5* SNP and *B3GALTL* expression reinforces the significance of the pathways altered by the ADAMTS13 variants (Supplemental Table 4) [35]. Specifically, *B3GALTL*

encodes a beta-1,3 glucosyltransferase that is required to glycosylate the *ADAMTS13* gene product as part of its pre-processing for Von Willebrand factor cleavage [45].

Our eQTL analysis showed that the variants in SSBP2 were likely eQTLs with *HBG1* and *FYB* (Supplemental Figure 1). *HBG1* is a key component of the fetal hemoglobin locus that is normally expressed in the fetal liver, spleen and bone marrow, as a part of the constitution of fetal hemoglobin (HbF)[46]. In adults, the beta-globin locus is only accessible (chromatin open, DNase I hypersensitive) in adult erythroid cells, however HBG1 shows chromatin accessibility in both fetal and adult erythroid cells[46]. The FYB gene encodes a hematopoietic-specific protein involved in platelet activation[47]. In mice, FYB knockout affects platelet function and causes mild thrombocytopenia[48]. Therefore, our results are consistent with rs2135 being involved in dysregulation of genes that contribute to the production and development of fetal blood.

Therefore, the role of SNPs in both *the ADAMTS13* and *SSBP2* loci could result in a post-term birth phenotype arising through alterations in hematopoiesis. In future work, identifying the regulatory role of these regions could help elucidate the cause-and-effect relationships between alterations of hematopoiesis and gestational length.

Post-term Birth Versus the Rise of More Intensive Obstetric Management of Birth

Analysing two cohorts from the same geographical region (Northern Finland) enabled us to control for regional and culture-specific differences in routine management of pregnancies. However, two major confounders remain between the 1966 and 1986 cohorts: 1) technological changes led to better estimation and certainty of gestational age in the 1986 cohort; and 2) management practices led to changes in the incidence of induced labor and post-term birth. Firstly, the uncertainty surrounding gestational age prediction in 1966 raises issues around phenotype definition in this cohort. The impact of this ambiguity on our study was minimized through the use of a narrow term gestational age range of 38 0/7 to 40 0/7.

There have been significant changes to obstetric management over the last 50 years, with a shift from conservative monitoring of prolonged pregnancies through to the current recommendations to induce women who are beyond 41 weeks gestation[5, 8, 49]. The induction of labor became a therapeutic option between 1966 and 1986. Therefore, the NFBC1986 cohort contains births that would have been post-term if not for induction and/or Caesarean-section. Consistent with this, we observed a reduction in numbers of post-term births from approximately 20% in the 1966 NFBC cohort to less than 5% in the 1986 NFBC cohort. However, while the induction of labour is an improvement in obstetric management, it is possible that these individuals have "post-term potential" and carry genetic risks that were not mitigated by the act of induction. Therefore, we excluded all term-born induced births from the 1986 cohort from the analyses.

Conclusion

We have identified genetic variants in proximity to the *ADAMTS13*, *B3GALT5*, *SSBP2*, and *TKT* loci as being associated with post-term birth in two birth cohorts (NFBC1966 and NFBC1986). This finding is consistent with previous observations that suggested there was a genetic component to post-term birth[12, 13, 15–20, 26]. Spatial and mRNA expression analyses further provided novel clues about how these loci contribute to the regulation and consequences of post-term birth. This study forms a foundation for a better understanding of the genetic and long term metabolic health risks faced by induced and post-term individuals. Since nearly 20% of births in the NFBC cohort were post-term, the long-term risks for induced individuals who have a previously overlooked post-term potential may be a major issue for current health providers.

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The authors declare no conflicts of interest.

Contributors:

WS, JO, and WC contributed to the first draft of the manuscript. All authors critically reviewed the manuscript. JA and JH performed the molecular analyses. WS contributed to bioinformatic analyses. VK, MV, SF, PE, EK, SS, AB, and MJ generated and reviewed clinical data. WS, JO, and WC contributed to interpretation of molecular and bioinformatic data. WC, MJ and JO conceptualised the study.

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Signed, informed consent and written permission to use their data for scientific research was obtained from the NFBC 1966 study participants at age 31. For the 1986 cohort, adolescents and parents received written and oral information and gave their written informed consent. The studies were approved by the ethics committees of each of the participating medical university study sites in

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Legends to figures

Figure 1. Manhattan plots of the discovery phase of the post-term GWAS for the (A) NFBC1966 and (B) NFBC1986 cohorts. The –log10 observed p-values (2-tailed) for the GWA (y-axis) are plotted versus the chromosomal position of each SNP (x-axis). The blue line indicates significance for follow-up ($p<1x10^{-5}$) through cross-validation, while the red line indicates global significance (5x10-8). Only SNPs in the ADAMTS13 locus in the NFBC1986 cohort reach genome-wide significance in the discovery phase.

Figure 2. Q-Q plots of the of the quantiles of expected versus observed $-\log_{10}$(p-value) of the association with gestational age in the (A) 1966 and (B) 1986 cohort. The negative logarithm of the expected (x-axis) and the observed (y-axis) p-values for the GWA analysis is plotted for each SNP (black dots). Deviation from the red line indicates points whose observed values are deviating from the null hypothesis of no true association. Inflation factors (λ) near 1 suggest that population stratification was adequately controlled.

Figure 3. Spatial Results from GWAS3D identify 4 significant spatial connections between loci in the validated GWAS data and distant genomic regions. The SNP associated with the *ADAMTS13* locus had multiple spatial connections, *SSBP2* had none, while the others only exhibited a single spatial association. Only the spatial connections with high confidence scores are plotted here (thickness of the red line).

Figure 4. Post-term associated SNPs show allele specific enhancer and repressor effects. All amplified regions, except that containing rs21355, acted as enhancers. There were significant differences ($p<0.0001$) between the enhancer activity of the 'A' and 'G' versions of rs4687715. Similarly, there were significant ($p<0.05$) differences between the enhancer activity of the 'C' and 'T' alleles of rs560928 in HeLa cells. Notably, DNA amplicons containing the A and G alleles of rs2135 acted as a repressor of basal activity. PCR-amplified genomic DNA with the indicated SNP variants were assayed for their ability to drive luciferase expression in HeLa cells. The increase in luminescence indicates that competence for transcription depends on the alellic version of these SNPs. Error bars represent ±SEM from four biological replicates and significance was determined by One-Way ANOVA. Asterisks above the bars denote significant differences compared to the empty

pGL4.23 vector control. Allele specific differences that are significant are indicated. (****p<0.0001, ***p<0.001, **p0.01, *p<0.05).

Supplamental Figure S1. Global eQTL analysis reveals little evidence of trans-eQTLs in the ADAMTS13 (A), B3GALT5 (B), and SSBP2 (C) post-term loci. The –log10 of the eQTL p-values of the association between the ADAMTS13 (A), B3GALT5 (B), and SSBP2 (C) loci and expression in various tissue types in the GTEx database. For rs655911 (ADAMTS13 locus), the only significant peak is at the ADAMTS13 gene ($p=1.5x10^{-8}$). For rs1534080 (B3GALT5 locus), no globally significant ($p < 5 x 10^{-8}$) peaks were identified for, but the highest peak is at the B3GALT5 gene ($p=9x10^{-5}$). For rs2135 (SSBP2 locus), the highest peaks are not at the SSBP2 locus, but are spread out amongst other areas of the genome. Tissue types tested: subcutaneous adipose, aortic artery, tibial artery, heart (left ventricle), lung, tibial nerve, sun-exposed skin (lower leg), skeletal muscle, mucosa and muscularis of the esophagus, thyroid, mammary (breast) tissue, and whole blood.

Tables

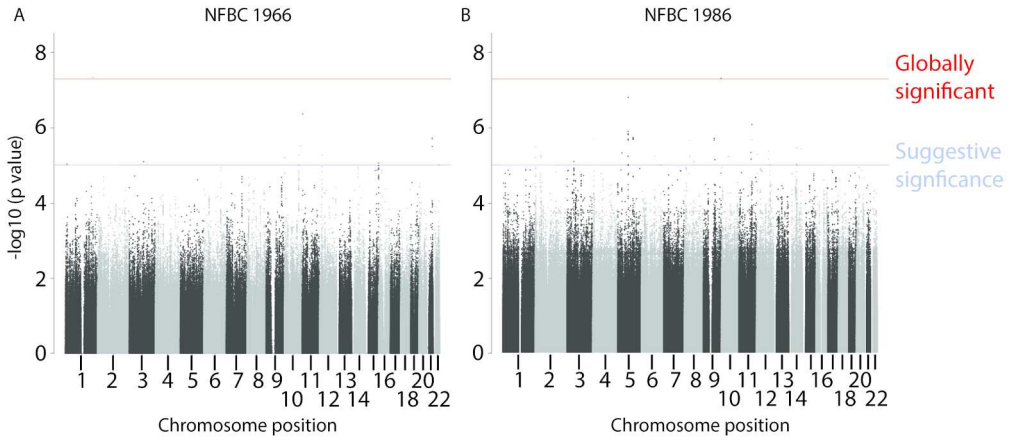
Supplemental Table 1. Primer sequences used to amplify genomic DNA regions to test for enhancer activity in the Post-term loci.

Supplemental Table 2. Cross-Validation of the NFBC1966 and NFBC1986 cohorts resulted in five significant loci: B3GALT5, SSBP2, TKT, ARGHAP42, and ADAMTS13. Using a cross-validation methodology of a discovery phase (A, GWAS $p < 1x10^{-5}$) and a validation phase (B, GWAS p<0.05), it was found that the B3GALT5, SSBP2, TKT, and ARGHAP42 loci are significantly associated with post-term birth. Additionally, the *ADAMTS13* locus reached global significance ($p < 5x10^{-8}$).

Supplemental Table 3. Spatial Results from GWAS3D identify significant spatial connections between loci in the validated GWAS data and distant genomic regions. The *ADAMTS13* locus had multiple spatial connections, *SSBP2* had none, while the others only exhibited a single spatial association.

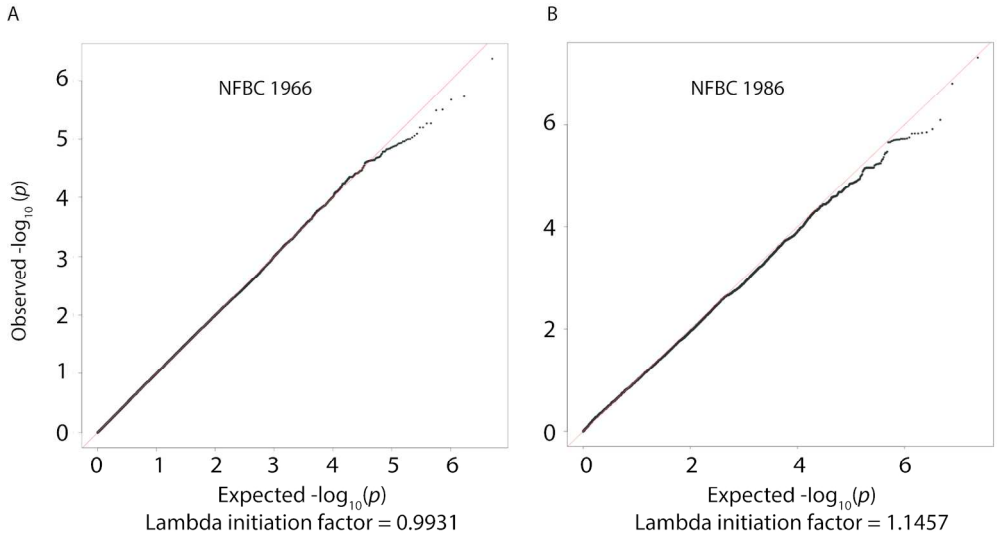
Supplemental Table 4. eQTL analysis supports the ADAMTS13-SLC2A6 spatial connection, but also confirms self-eQTLs for the ADAMTS13, TKT, and B3GALT5 post-term loci. Using GTEx to determine effect size and significance of SNP-gene expression associations, it was determined that a number of

eQTLs exist that support the spatial connections. This table includes all self- and spatial-eQTLs with p < 5 x 10^{-3} in GTEx (version 6).



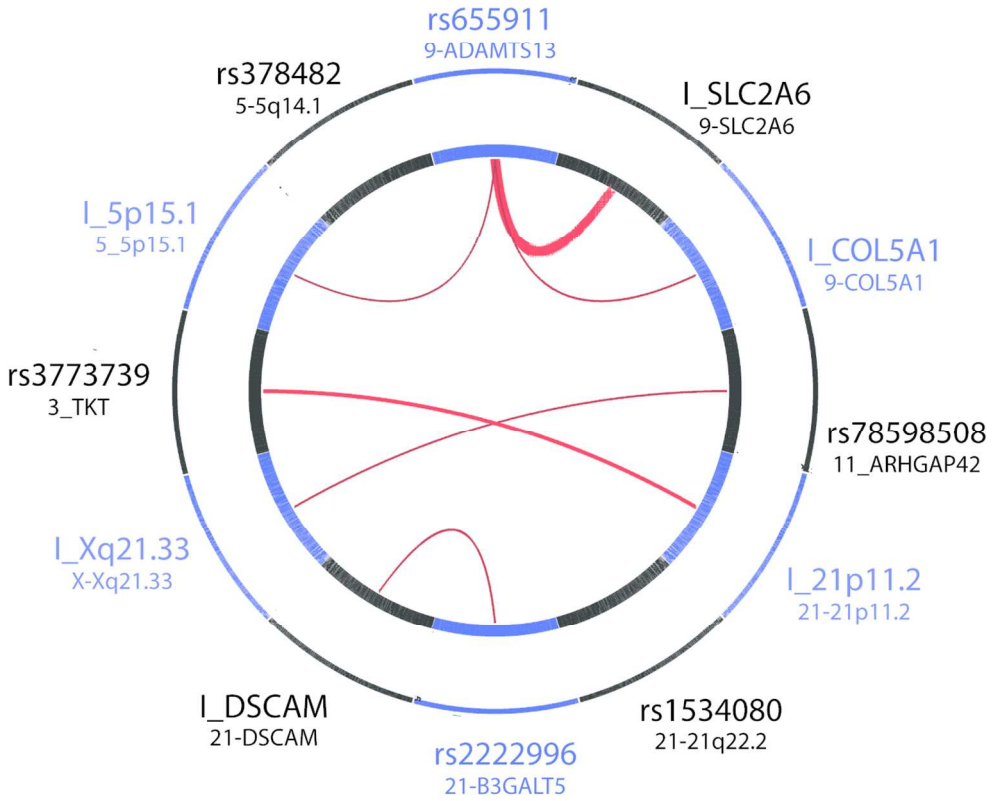
Manhattan plots of the discovery phase of the post-term GWAS for the (A) NFBC1966 and (B) NFBC1986 cohorts. The –log10 observed p-values (2-tailed) for the GWA (y-axis) are plotted versus the chromosomal position of each SNP (x-axis). The blue line indicates significance for follow-up (p<1x10-5) through cross-validation, while the red line indicates global significance (5x10-8). Only SNPs in the ADAMTS13 locus in the NFBC1986 cohort reach genome-wide significance in the discovery phase.

183x90mm (300 x 300 DPI)



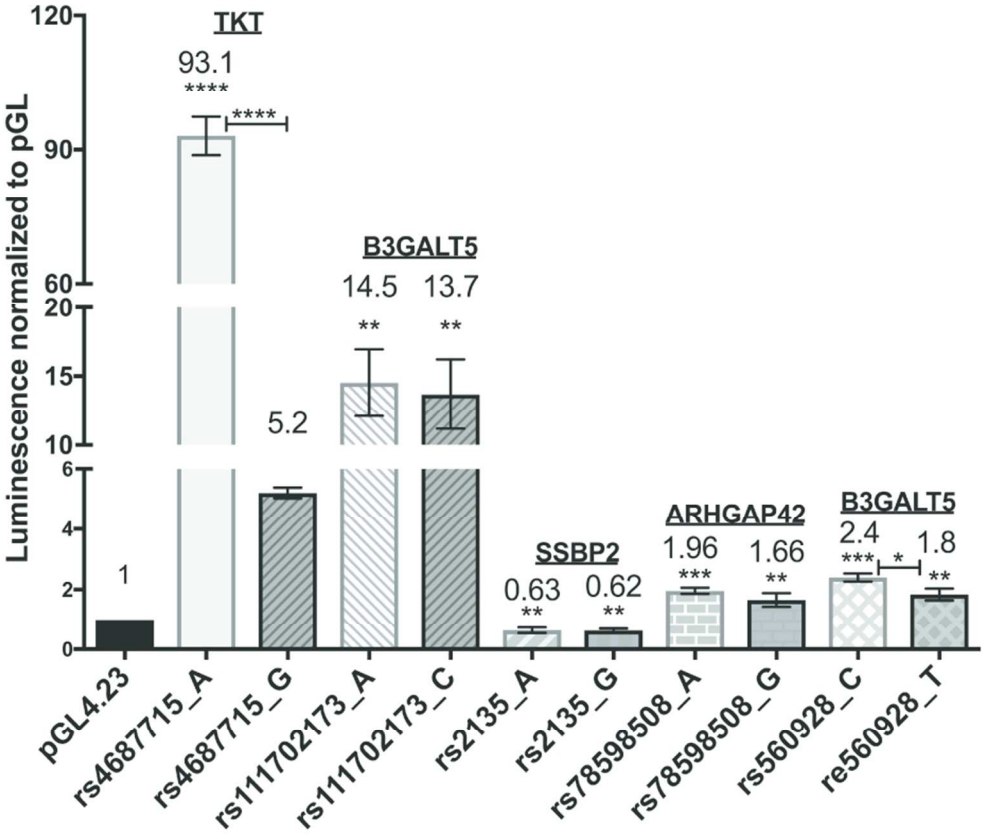
Q-Q plots of the of the quantiles of expected versus observed –log10(p-value) of the association with gestational age in the (A) 1966 and (B) 1986 cohort. The negative logarithm of the expected (x-axis) and the observed (y-axis) p-values for the GWA analysis is plotted for each SNP (black dots). Deviation from the red line indicates points whose observed values are deviating from the null hypothesis of no true association. Inflation factors (λ) near 1 suggest that population stratification was adequately controlled.

170x93mm (300 x 300 DPI)



Spatial Results from GWAS3D identify 4 significant spatial connections between loci in the validated GWAS data and distant genomic regions. The SNP associated with the ADAMTS13 locus had multiple spatial connections, SSBP2 had none, while the others only exhibited a single spatial association. Only the spatial connections with high confidence scores are plotted here (thickness of the red line).

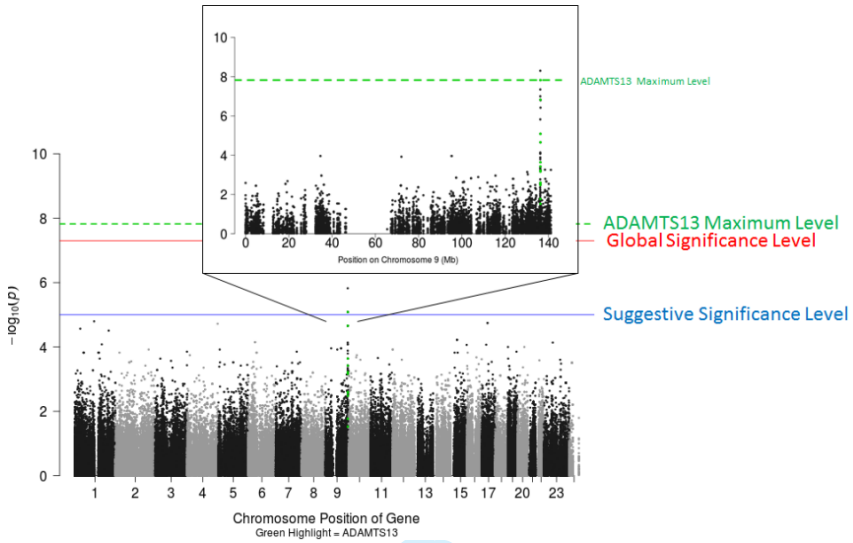
116x101mm (300 x 300 DPI)



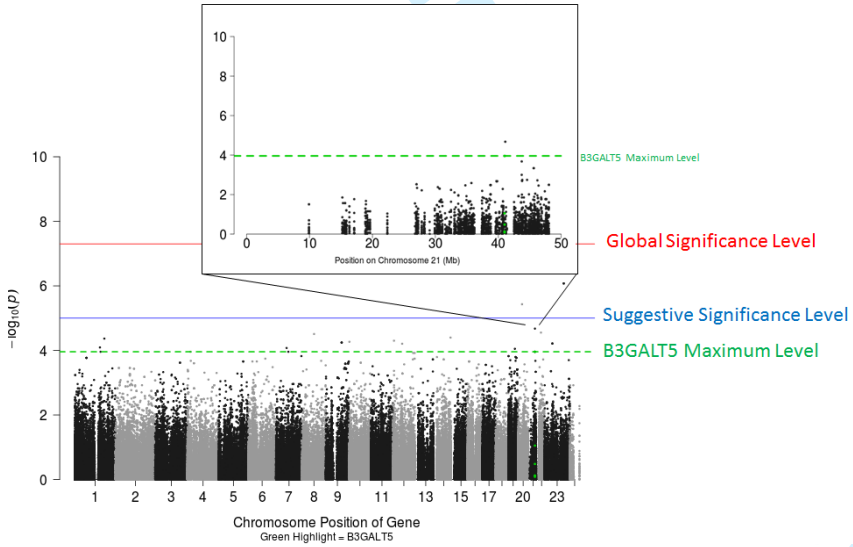
Post-term associated SNPs show allele specific enhancer and repressor effects. All amplified regions, except that containing rs21355, acted as enhancers. There were significant differences ($p<0.0001$) between the enhancer activity of the 'A' and 'G' versions of rs4687715. Similarly, there were significant ($p<0.05$) differences between the enhancer activity of the 'C' and 'T' alleles of rs560928 in HeLa cells. Notably, DNA amplicons containing the A and G alleles of rs2135 acted as a repressor of basal activity. PCR-amplified genomic DNA with the indicated SNP variants were assayed for their ability to drive luciferase expression in HeLa cells. The increase in luminescence indicates that competence for transcription depends on the alellic version of these SNPs. Error bars represent ±SEM from four biological replicates and significance was determined by One-Way ANOVA. Asterisks above the bars denote significant differences compared to the empty pGL4.23 vector control. Allele specific differences that are significant are indicated. (****$p<0.0001$, ***$p<0.001$, **p0.01, *$p<0.05$).

80x68mm (300 x 300 DPI)

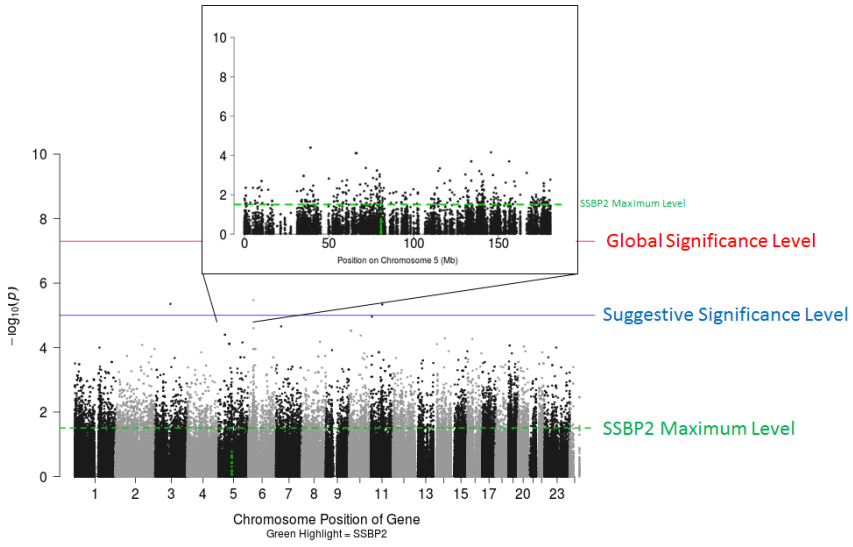
A



B



C



Supplemental Fig. S1. Global eQTL analysis reveals little evidence of trans-eQTLs in the ADAMTS13 (A), B3GALT5 (B), and SSBP2 (C) post-term loci. The –log10 of the eQTL p-values of the association between the ADAMTS13 (A), B3GALT5 (B), and SSBP2 (C) loci and expression in various tissue types in the GTEx database. For rs655911 (ADAMTS13 locus), the only significant peak is at the ADAMTS13 gene ($p=1.5x10^{-8}$). For rs1534080 (B3GALT5 locus), no globally significant ($p < 5 \times 10^{-8}$) peaks were identified for, but the highest peak is at the B3GALT5 gene ($p=9x10^{-5}$). For rs2135 (SSBP2 locus), the highest peaks are not at the SSBP2 locus, but are spread out amongst other areas of the genome. Tissue types tested: subcutaneous adipose, aortic artery, tibial artery, heart (left ventricle), lung, tibial nerve, sun-exposed skin (lower leg), skeletal muscle, mucosa and muscularis of the esophagus, thyroid, mammary (breast) tissue, and whole blood.

SNP	Chromosome	5' position	sequence	Forward/Reverse	Product size (bp)
rs4687715	3	53,235,493	AGGAAAGTGAGGAAGGGTGG	Forward	1431
rs4687715	3	53,236,923	CCCCACCCCTAACTCTAACA	Reverse	
rs2135	5	81,795,000	CAGGCTGTCATCCAAGCAAG	Forward	1491
rs2135	5	81,796,490	AGAGGGATGCTAGCTCTCCT	Reverse	
rs78598508	11	100,769,075	AGGCAGTTGTAACACAGTGG	Forward	894
rs78598508	11	100,769,968	CAGCCAGGATGTGCAGTTTT	Reverse	
rs111702173	21	39,651,024	TGTCTTCCCCTGAATCGGTG	Forward	728
rs111702173	21	39,651,751	TAGCTTCGCCGGTATTTGGA	Reverse	
rs111702173	SDM primers		CTGGAGTAGATTCTCCCGGACAGCCTCAGAAGAAC	Forward	
rs111702173	SDM primers		GTTCTTCTGAGGCTGTCCGGGAGAATCTACTCCAG	Reverse	
rs560928	21	39,656,207-	GCAGGGACGTTGATGTTGTT	Forward	159
rs560928	21	39,656,703	TGCAGAACGTGTAGACCTCC	Reverse	

Supplemental Table S1. Primer sequences used to amplify genomic DNA regions to test for enhancer activity in the Post-term loci.

A

Cohort	rsID	Chr	Coordinates (NCBI Build 38)	GENECODE Gene	P-Value
NFBC1966	rs12571151	10	2416828	RP11-446F3.2	7.04E-06
	rs12257796	10	2418708	RP11-446F3.2	7.04E-06
	rs11248532	10	123445888	RP11-282I1.1	5.56E-06
	rs12285957	11	6542816	DNHD1	2.14E-07
	rs1463732	12	19803450	RP11-405A12.2	4.10E-06
	rs999227	12	19844354	RP11-405A12.2	9.52E-06
	rs10841383	12	19845405	RP11-405A12.2	9.41E-06
	rs11635432	15	101580136	TM2D3	8.65E-06
	rs2121206	15	101580301	TM2D3	8.87E-06
	rs12902757	15	101580692	TM2D3	9.07E-06
	rs12101912	15	101580774	TM2D3	7.70E-06
	rs10854398	21	39649825	B3GALT5	1.57E-06
	rs1534080	21	39651826	B3GALT5	9.79E-07
	rs8132770	21	39653957	B3GALT5	8.43E-07
	rs560928	21	39656644	B3GALT5	5.00E-04
NFBC1986	rs6734412	2	291276	AC079779.4	7.56E-06
	rs72774523	2	304478	AC079779.5	6.24E-06
	rs72774524	2	305346	AC079779.5	3.37E-06
	rs12612077	2	3843037	DCDC2C	6.92E-06
	rs55804313	2	3843667	DCDC2C	6.92E-06
	rs11679758	2	3846292	DCDC2C	6.92E-06
	rs55742273	2	3847416	DCDC2C	6.92E-06
	rs17018173	2	3848103	DCDC2C	6.92E-06
	rs17018176	2	3848430	DCDC2C	6.92E-06
	rs12477884	2	3849890	DCDC2C	6.92E-06
	rs62107652	2	3851348	DCDC2C	6.92E-06
	rs61512202	2	3851386	DCDC2C	6.92E-06
	rs11693904	2	3856403	DCDC2C	7.68E-06
	rs17018208	2	3856717	DCDC2C	6.92E-06
	rs60124171	2	3856929	DCDC2C	6.92E-06
	rs60852654	2	3856940	DCDC2C	6.92E-06
	rs62107654	2	3857010	DCDC2C	6.92E-06
	rs12477500	2	3857589	DCDC2C	6.92E-06
	rs12464001	2	3858107	DCDC2C	7.10E-06
	rs12475409	2	3858328	DCDC2C	6.92E-06
	rs17018215	2	3861268	DCDC2C	7.09E-06
	chr2:3896520:D	2	3896520	DCDC2C	6.92E-06
	chr2:3909181:I	2	3909181	DCDC2C	7.09E-06
	rs62135521	2	44068863	LRPPRC	5.69E-06
	rs75199129	2	44071636	LRPPRC	5.69E-06
	rs62135525	2	44072740	LRPPRC	5.69E-06
	rs62135536	2	44098889	U6	6.86E-06

	rs62136969	2	44132737	U6	6.19E-06
	rs4687715	3	53235888	TKT	8.04E-06
	rs145023824	4	8946821	HMX1	2.07E-06
	rs66858738	5	32452481	ZFR	4.83E-06
	chr5:81088891:D	5	81088891	RASGRF2	1.22E-06
	rs12521503	5	81782627	SSBP2	5.86E-06
	rs378482	5	81784169	SSBP2	5.86E-06
	rs401996	5	81790535	SSBP2	1.49E-06
	rs384075	5	81791185	SSBP2	1.49E-06
	rs391229	5	81793043	SSBP2	1.41E-06
	rs456778	5	81794669	SSBP2	1.45E-06
	rs463247	5	81795097	SSBP2	1.50E-06
	rs2135	5	81795581	SSBP2	1.55E-07
	rs457700	5	81796274	SSBP2	2.21E-06
	rs386424	5	81796968	SSBP2	2.01E-06
	rs462122	5	81797718	SSBP2	1.96E-06
	rs72784027	5	118225881	DTWD2	1.96E-06
	rs72784032	5	118235946	DTWD2	1.98E-06
	rs11739538	5	118822305	DTWD2	1.89E-06
	rs11750860	5	118885894	DTWD2	1.89E-06
	rs11741257	5	118894945	DTWD2	1.89E-06
	rs17440178	5	118903953	DTWD2	1.78E-06
	rs183770336	6	102794582	GRIK2	5.82E-06
	rs117533178	6	151989489	ESR1	9.98E-06
	rs7013779	8	40942080	RP11-465K16.1	2.19E-06
	rs1553932	8	40949972	RP11-465K16.1	5.96E-06
	rs79648768	8	72706578	KCNB2	8.27E-06
	chr8:73557501:D	8	73557501	STAU2	7.81E-06
	rs10780480	9	80987559	RP11-289F5.1	1.86E-06
	rs1582027	9	80994776	RP11-289F5.1	1.95E-06
	rs10780482	9	80995813	RP11-289F5.1	2.20E-06
	chr9:90067785:I	9	90067785	N/A	7.23E-06
	rs655911	9	133447776	ADAMTS13	4.85E-08
	rs75320537	10	50188977	ASAH2	5.90E-06
	rs10995050	10	62123476	AL671972.1	6.07E-06
	rs7950344	11	92763108	FAT3	4.68E-06
	rs72965926	11	95705915	RP11-644L4.1	6.95E-06
	chr11:100631998:I	11	100631998	ARHGAP42	2.10E-06
	rs78598508	11	100769446	ARHGAP42	8.08E-07
	rs2239507	12	5041968	KCNA5	5.16E-06
	rs79766994	12	93997910	7SK	6.99E-06
	rs11610162	12	94030905	7SK	4.30E-06
	rs11609845	12	100902544	ANO4	9.85E-06
	rs78874632	14	58276011	C14orf37/PSMA-AS1	9.39E-06
	rs191706929	14	61884310	CTD-2277K2.1	3.55E-06

	rs77835182	14	61929868	CTD-2277K2.1	3.55E-06
	chr14:62234490:D	14	62234490	CTD-2277K2.1	9.26E-06
	rs6575274	14	92680353	RIN3	3.81E-06

B

Cohort	rsID	Chr	Coordinates (NCBI Build 38)	GENECODE Gene	P-Value
NFBC1966	rs12612077	2	3843037	AC019172.2	2.60E-01
	rs11679758	2	3846292	AC019172.2	2.72E-01
	rs17018173	2	3848103	AC019172.2	2.69E-01
	rs17018176	2	3848430	AC019172.2	2.69E-01
	rs11693904	2	3856403	AC019172.2	2.13E-01
	rs17018208	2	3856717	AC019172.2	2.26E-01
	rs12477500	2	3857589	AC019172.2	2.19E-01
	rs12464001	2	3858107	AC019172.2	2.25E-01
	rs4687715	3	53235888	TKT	1.53E-02
	rs12521503	5	81782627	SSBP2	1.95E-02
	rs378482	5	81784169	SSBP2	1.95E-02
	rs401996	5	81790535	SSBP2	2.06E-02
	rs384075	5	81791185	SSBP2	2.13E-02
	rs391229	5	81793043	SSBP2	2.23E-02
	rs456778	5	81794669	SSBP2	1.99E-02
	rs463247	5	81795097	SSBP2	2.13E-02
	rs2135	5	81795581	SSBP2	2.19E-02
	rs457700	5	81796274	SSBP2	2.48E-02
	rs386424	5	81796968	SSBP2	2.21E-02
	rs462122	5	81797718	SSBP2	3.92E-03
	rs11739538	5	118822305	DTWD2	1.69E-01
	rs11750860	5	118885894	DTWD2	6.23E-01
	rs11741257	5	118894945	DTWD2	5.81E-01
	rs17440178	5	118903953	DTWD2	4.49E-01
	rs10780480	9	80987559	RP11-289F5.1	9.32E-01
	rs652600*	9	133445896	ADAMTS13	2.87E-01
	rs2239507	12	5041968	KCNA5	2.02E-01
	rs11609845	12	100902544	ANO4	NA
NFBC1986	rs12571151	10	2416828	RP11-446F3.2	7.40E-02
	rs12257796	10	2418708	RP11-446F3.2	7.40E-02
	rs11248532	10	123445888	RP11-282I1.1	3.58E-01
	rs12285957	11	6542816	DNHD1	8.81E-01
	rs1463732	12	19803450	RP11-405A12.2	2.70E-01
	rs999227	12	19844354	RP11-405A12.2	2.63E-01
	rs10841383	12	19845405	RP11-405A12.2	2.63E-01
	rs10854398	21	39649825	B3GALT5	6.91E-01
	rs1534080	21	39651826	B3GALT5	6.90E-01

	rs8132770	21	39653957	B3GALT5	5.33E-01
	rs560928	21	39656644	B3GALT5	2.60E-02
	rs111702173	21	39651360	B3GALT5	1.69E-02

*rs652600 is in LD with rs655911

Supplemental Table 2. Cross-Validation of the NFBC1966 and NFBC1986 cohorts resulted in five significant loci: B3GALT5, SSBP2, TKT, ARGHAP42, and ADAMTS13. Using a cross-validation methodology of a discovery phase (A, GWAS $p < 1x10^{-5}$) and a validation phase (B, GWAS $p<0.05$), it was found that the B3GALT5, SSBP2, TKT, and ARGHAP42 loci are significantly associated with post-term birth. Additionally, the *ADAMTS13* locus reached global significance ($p < 5x10^{-8}$).

SNP	Locus	Spatial Connections
rs560928	B3GALT5	chr21:42080001-42090000
rs2135	SSBP2	None
rs4687715	TKT	chr21:9650001-9660000
rs655911	ADAMTS13	chr9:136340001-136350000, chr9:136330001-136340000, chr9:137560001-137570000, chr5:18160001-18170000, chr1:174900001-174910000
rs78598508	ARHGAP42	chrX:93780001-93790000
rs111702173	B3GALT5	chr21:42080001-42090000

Supplemental Table 3. Spatial Results from GWAS3D identify significant spatial connections between loci in the validated GWAS data and distant genomic regions. The *ADAMTS13* locus had multiple spatial connections, *SSBP2* had none, while the others only exhibited a single spatial association.

Self/Spatial	SNP	Chr	Coordinates (NCBI Build 37)	SNP Locus	eQTL Gene	Effect Size	P-Value	Tissue
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.31	1.50E-08	Nerve - Tibial
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.33	1.50E-07	Skin - Sun Exposed (Lower leg)
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.52	3.00E-07	Pituitary
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.18	6.30E-06	Cells - Transformed fibroblasts
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.44	7.10E-06	Brain - Cortex
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.26	8.20E-06	Adipose - Subcutaneous
Self	rs4687715	3	53235888	TKT	TKT	-0.21	2.00E-05	Heart - Left Ventricle
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.27	2.20E-05	Esophagus - Mucosa
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.36	5.80E-05	Brain - Cerebellum
Self	rs8132770	21	39653957	B3GALT5	B3GALT5	0.29	9.00E-05	Thyroid
Self	rs1534080	21	39651826	B3GALT5	B3GALT5	0.28	1.10E-04	Thyroid
Self	rs10854398	21	39649825	B3GALT5	B3GALT5	0.28	1.20E-04	Thyroid
Self	rs4687715	3	53235888	TKT	TKT	-0.18	1.30E-04	Esophagus - Mucosa
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.22	2.30E-04	Lung
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.17	3.80E-04	Thyroid
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.27	4.40E-04	Skin - Not Sun Exposed (Suprapubic)
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.24	6.00E-04	Breast - Mammary Tissue
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.13	6.80E-04	Muscle - Skeletal
Self	rs4687715	3	53235888	TKT	TKT	-0.24	7.80E-04	Brain - Caudate (basal ganglia)
Spatial	rs655911	9	133447776	ADAMTS13	SLC2A6	-0.2	1.20E-03	Brain - Anterior cingulate cortex
Self	rs10854398	21	39649825	B3GALT5	B3GALT5	-0.24	1.70E-03	Stomach
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.29	2.00E-03	Pancreas
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.24	2.40E-03	Adipose - Visceral (Omentum)
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.21	2.50E-03	Artery - Aorta
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.15	2.70E-03	Whole Blood
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.17	3.30E-03	Artery - Tibial
Self	rs1534080	21	39651826	B3GALT5	B3GALT5	-0.23	3.30E-03	Stomach

Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.25	4.30E-03	Uterus
Self	rs8132770	21	39653957	B3GALT5	B3GALT5	-0.22	4.60E-03	Stomach
Self	rs78598508	11	100769446	ARHGAP42	ARHGAP42	-0.53	4.60E-03	Brain - Hypothalamus
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.21	4.90E-03	Brain - Frontal Cortex (BA9)
Spatial	rs8132770	21	39653957	B3GALT5	B3GALTL	0.33	4.90E-03	Small Intestine - Terminal Ileum
Self	rs655911	9	133447776	ADAMTS13	ADAMTS13	0.3	5.00E-03	Brain - Hippocampus

Supplemental Table 4. eQTL analysis supports the ADAMTS13-SLC2A6 spatial connection, but also confirms self-eQTLs for the ADAMTS13, TKT, and B3GALT5 post-term loci. Using GTEx to determine effect size and significance of SNP-gene expression associations, it was determined that a number of eQTLs exist that support the spatial connections. This table includes all self- and spatial-eQTLs with $p < 5 \times 10^{-3}$ in GTEx (version 6).