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Genetic Variation in Pentraxin (PTX) 3 Gene Associates with PTX3 Production and Fertility in Women[1]

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ABSTRACT

Pentraxin 3 (PTX3) plays an important role in innate immune responses and in female fertility, as discovered with studies in mice. However, the role of PTX3 in human fertility is unknown. Here, we report on a population-based study from a rural area of Upper East Ghana (n = 4346). We studied the association between the number of children given birth by women during their lifetime and ex vivo, lipopolysaccharide (LPS)-induced PTX3 production (n = 362). In addition, we studied the association of genetic variation in the *PTX3* gene with PTX3 production (n = 617) and with female fertility (n = 1999). We found that ex vivo LPS-induced PTX3 production was associated with fertility (*P* = 0.040). Furthermore, we identified genetic variants in the *PTX3* gene that influence PTX3 production, and also fertility. The strongest associations were observed for the rs6788044 single-nucleotide polymorphism (SNP). We found that carriers of this SNP had higher PTX3 production capacity (*P* = 0.003) and higher fertility (*P* = 0.043). The results reported here provide the first evidence, based on protein production and analysis of polymorphisms, that the long pentraxin PTX3 plays a role in female fertility in humans.

Africa, female fertility, immunology, polymorphisms, PTX3

INTRODUCTION

Pentraxin 3 (PTX3) is a soluble pattern-recognition receptor, a member of the acute-phase reactants superfamily [1]. Studies with mouse models have shown that PTX3 has a dual role in innate immunity as well as in female fertility [2–4]. In innate immunity, PTX3 plays a nonredundant role in recognition of selected pathogens and immune responses, mainly to *Aspergillus*, *Pseudomona*, *Salmonella*, cytomegalovirus, and influenza [2, 5, 6]. Upon inflammatory signaling, PTX3 is produced to coat pathogens and initiate complement activation and opsonization [7]. Regarding female fertility, mouse studies have shown that PTX3 plays an important role in the formation of the extracellular matrix of the cumulus oophorus in ovarian follicles, as well as implantation and decidualization [4, 8, 9]. Because PTX3 is highly conserved in evolution in terms of function, structure, and regulation [1], it is expected to be a major player also in human reproductive immunology.

Regarding the maternal-fetal interface, studies in mouse models have shown that *Ptx3* knockouts have lower amounts of fertilized oocytes and embryos and were compromised in the formation of the extracellular matrix of the cumulus oophorus [3, 4]. Also, implantation was severely compromised [8]. In addition, at the site of implantation, it was found that stromal cells upregulated *Ptx3* gene expression [9]. The same was observed by in vitro simulation with human stromal cells [10, 11], indicating an important function for PTX3 during the human implantation and decidualization process. In humans, PTX3 is under tight hormonal control [12] and is upregulated during the time of ovulation [10, 11]. Also in humans, the extracellular matrix of the cumulus is made of PTX3 and is now suggested as a marker for oocyte quality [13]. To date, genetic regulation of PTX3 production has not been studied extensively. One study described a haplotype that was associated with resistance to tuberculosis [14]. It has not been evaluated whether genetic variation in the *PTX3* gene influences PTX3 production and whether this variation is also resulting in difference in fertility.

We had the unique opportunity to study the natural fertility of women in a population living under adverse environmental conditions, where the use of contraceptives has been virtually absent. Here, in a gene-wide association study, we tested whether ex vivo lipopolysaccharide (LPS)-induced PTX3 production capacity is influenced by genetic variation in the *PTX3* gene, and whether these variants influence fertility. Because PTX3 is also important in pathogen recognition, and genetic variants in the PTX3 gene have been associated with resistance to tuberculosis [14], we also tested the role of *PTX3* gene in survival.

MATERIALS AND METHODS

Study Population

This study was conducted in the remote Garu-Tempane district in the Upper East region of Ghana. This densely populated, agricultural area is inhabited by several tribes, mainly Bimoba (63%) and Kusasi (27%). The whole Ghana Upper East region, and especially the Garu-Tempane district, is underdeveloped and poor, and mortality rates are high. Main causes of death are malaria, diarrhea, and poor nutrition [15, 16]. The vast majority of the people are farmers, and the total agricultural process is done by hand labor. Families, which live in clay compounds, consist of one landlord with several wives and

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TABLE 1. Characteristics of the study population.

Characteristic	Genotyped (n = 4346)	Ex vivo PTX3 production (n = 656)
Age range (yr)	0–95	8–95
Male:female	1373:2973	114:542
Mean compound wealth (SD)	$1450 (1260)	$1570 (1410)
Female fertility: Mean number of offspring (SD)[a]	6.3 (2.7)	6.7 (2.8)

[a] Female fertility was measured in 2461 women over 20 years of age.

their children. During a field visit in 2003, the fertility history of women older than 20 years was obtained, including the number of children conceived at the time of questionnaire. The use of contraceptives is virtually absent, and most women reported to strive for as many children as possible [17]. Also, mouthswabs were obtained from these women, newborn boys and girls, and elderly men and women, for DNA analyses. In 2006, a series of whole-blood assays were performed for a subset of the population. The Medical Ethical Committee of the Ghana Health Service, as well as the Medical Ethical Committee of the Leiden University Medical Center, approved the study. Witness-observed informed consent was obtained from all participants.

Whole-Blood and PTX3 Assays

Ex vivo whole-blood stimulation was performed as described elsewhere [18, 19]. All venous blood samples were drawn in the morning to exclude circadian variation, were diluted 2-fold with RPMI-1640, and within 2 h after collection were cultured with medium and an optimal dose of 10 μg/ml *Escherichia coli*-derived LPS (Sigma Aldrich, Zwijndrecht, the Netherlands) in 24-well plates at duplicate volumes of 1 ml for 24 h in 37°C incubators. Ambient CO2 levels were induced by a candle jar incubation system [20]: culture plates were placed in an airtight container with a burning candle enclosed and were transferred as a whole to a 37°C incubator once the candle had faded. Supernatants were collected and kept at −20°C in Ghana until transported on dry ice to the Netherlands.

In the Netherlands, all samples were stored at −80°C until PTX3 production was determined by ELISA, according to the manufacturer's guidelines (Quantikine Human Pentraxin 3/TSG-14 Immunoassay, catalog no. DPTX30; R&D Research). Each sample was assessed in duplicate.

Genotyping

We saturated the *PTX3* gene and flanking regions by selecting 17 single-nucleotide polymorphisms (SNPs) from the HapMap database release no. 21 (www.hapmap.org), using the Yoruba in Ibadan, Nigeria (Yoruba) data. The Haploview's program Tagger [21] was used to derive a set of tag SNPs from the whole-gene region such that each common SNP (≥5%) in that set was captured with $r^2 \geq 0.8$. All SNPs were genotyped using mass spectrometry (Sequenom Inc., San Diego, CA), according to the manufacturer's guidelines.

Statistical Analyses

As the LPS-induced PTX3 production was nonnormally distributed, *z* scores were calculated on log-transformed data. Linear mixed models were used to test for differences in LPS-induced PTX3 production over categories of female fertility. Because reduced fertility of the landlord might result in a lower number of offspring, we adjusted for this by clustering on compound. The program Haploview [22] was used to estimate allele frequencies and to estimate pairwise linkage disequilibrium (LD). PLINK [23] was used to test for Hardy-Weinberg equilibrium. Haplotypes and haplotype frequencies were calculated using Phase [24]. In all haplotype analyses, the posterior probabilities of pairs of haplotypes per participant, as estimated by Phase, were used as weights in the analyses. Analyses were adjusted for age, sex, compound wealth, and tribe, and in all analyses, an additive model was used. All analyses were performed with STATA version 9 (StataCorp LP) statistical software.

RESULTS

From the total study population of 26 170 people, 4346 were genotyped, of whom 1373 (32%) were males and 2973 (68%) were females. The majority of the selected participants belonged to the Bimoba (72%) and Kusasi (22%) tribes. Ex vivo LPS-induced PTX3 production was determined in 656 people, of whom genotype data was available for 617. This population consisted mainly of women (n = 542; 82.6%) and was mainly Bimoba (n = 506; 77.1%). Table 1 provides the characteristics of the study population. The median (interquartile range) PTX3 production was 31 ng/ml (25–40 ng/ml). The fertility history was obtained for 2379 women. From these women, data on LPS-induced PTX3 production were available

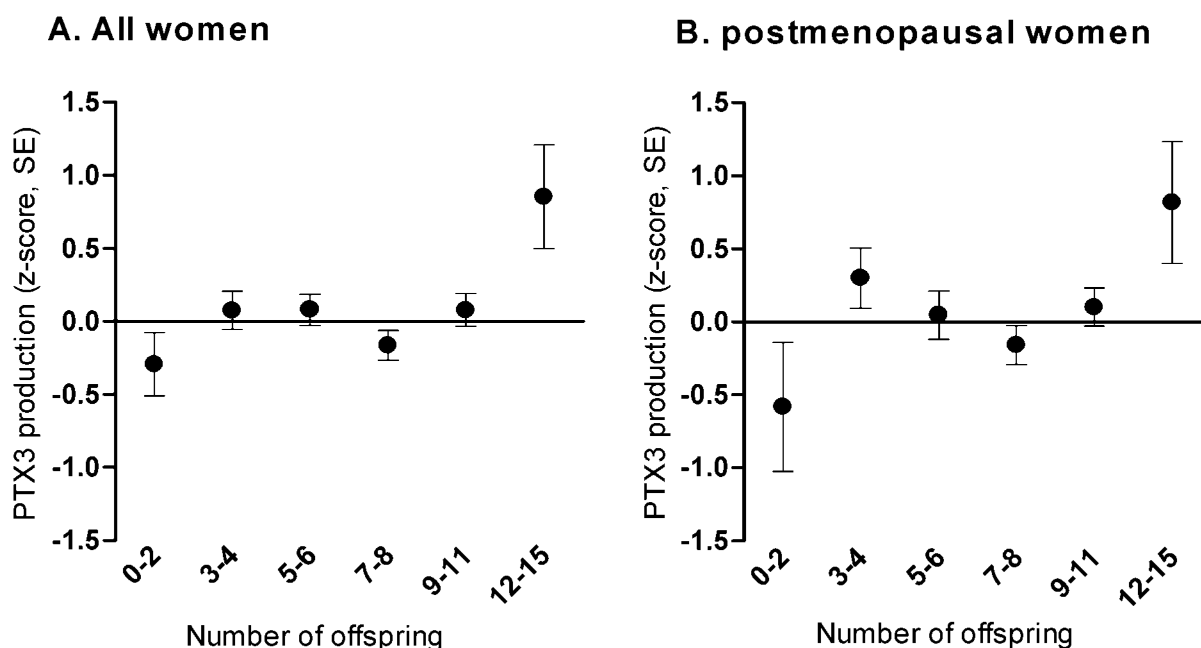

FIG. 1. Association of PTX3 production and female fertility. Data represent mean PTX3 production for all women (**A**; n = 22, 62, 87, 99, 84, and 8, respectively; *df* 5, $P = 0.040$) and postmenopausal women (**B**; n = 5, 24, 38, 60, 66, and 6, respectively; *df* 5; $P = 0.055$) per number of children upon ex vivo stimulation with *E. coli* LPS. Means are calculated based on log-transformed *z* scores and are adjusted for age, compound wealth, and tribe.

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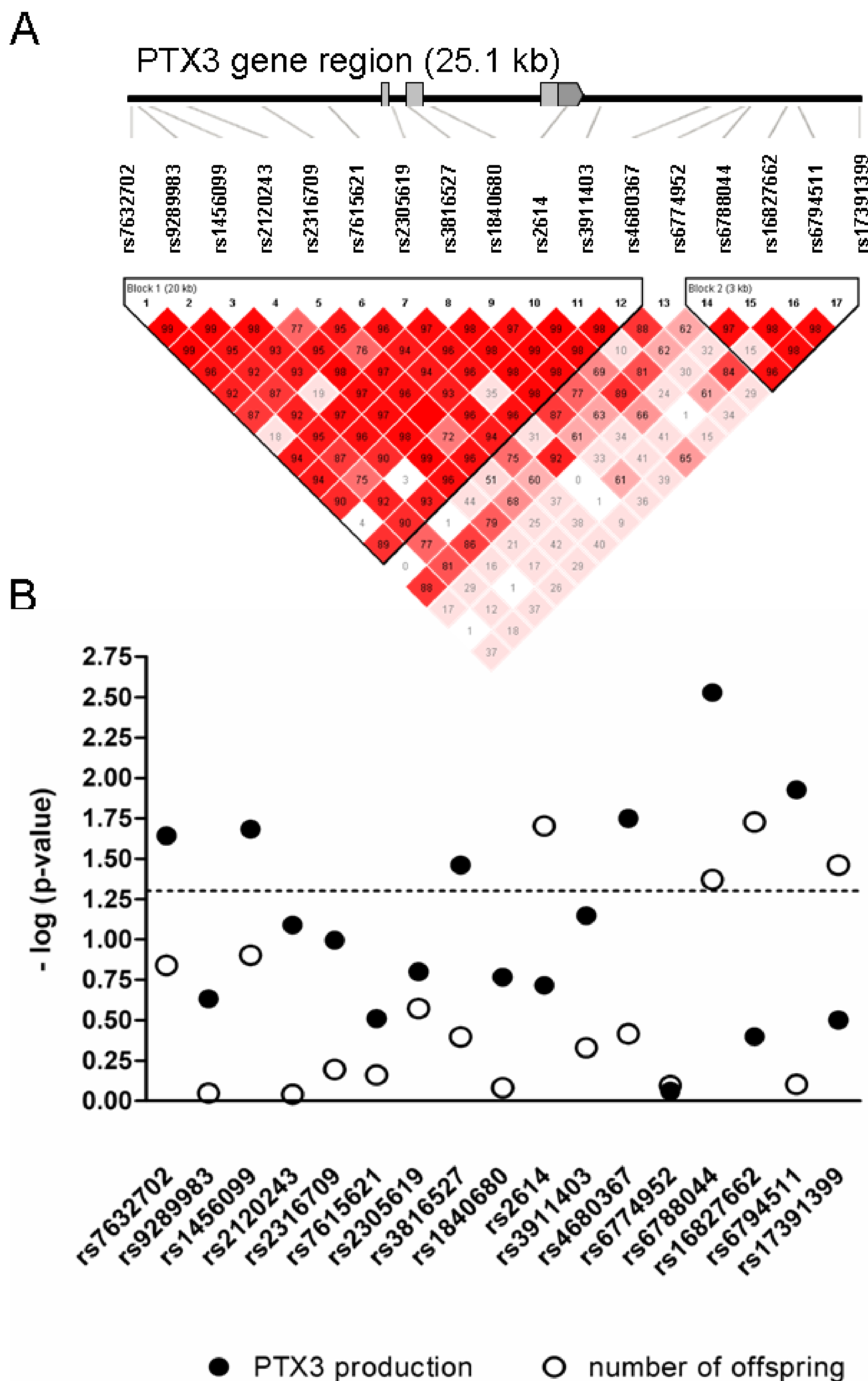

FIG. 2. *PTX3* gene structure and graphical representation of haplotype blocks and associations with PTX3 production and female fertility. **A**) The *PTX3* gene covers 25.1 kb and is located at chromosome 3. In this gene, 17 SNPs were genotyped. The location of the genotyped SNPs is indicated with vertical lines. Pairwise LD (D′) as observed in the Ghanaian research population (n = 4346) is also depicted. **B**) Association between *E. coli* LPS-induced PTX3 production, female fertility, and *PTX3* SNPs in the Ghanaian population. Data presented as −log *P* values for the association between SNPs in the *PTX3* gene and PTX3 production (closed circles; n = 617) and number of offspring (open circles; n = 1999), as obtained by linear regression adjusted for age, sex, compound wealth, and tribe. The PTX3 production was induced by 24-h whole-blood stimulation with *E. coli* LPS. The horizontal dotted line indicates the 0.05 *P* value threshold.

for 362, whereas genotype data were available for 1999 women.

We tested whether LPS-induced PTX3 production is associated with female fertility, as measured by the number of children per woman (Fig. 1A). Most of the women had given birth to 3–11 children (n = 332). There were few women who had given birth to no, one, or only two children (n = 22), and also few women who had given birth to 12 or up to 15 children (n = 8). Women who had more than 11 children had highest PTX3 production, whereas women with fewer than three children had the lowest PTX3 production compared with the population mean (estimate [SEM], 0.046 [0.047]; linear mixed model, *df* 5; $P_{diff} = 0.04$). Because fertility can best be observed when reproduction has terminated, we assessed this also in postmenopausal women. Here, we found that women in the highest fertility category had the highest PTX3 production (estimate [SEM], 0.052 [0.064]; linear mixed model, *df* 5; $P_{diff} = 0.055$; Fig. 1B).

Next, we set out to test whether LPS-induced PTX3 production is associated with genetic variants in the *PTX3*

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TABLE 2. Associations of *PTX3* haplotypes and ex vivo LPS-induced PTX3 production and fertility, and haplotype frequencies in young and old participants.[a]

Haplotype	Frequency	PTX3 [n = 617] (estimate SE)	*P* value	Offspring [n = 1999] (estimate SE)	*P* value	Young MAF [n = 987]	Old MAF [n = 822]	*P* value
Block I[b]								
h1: CGACTTGAGCAA	0.250	−0.05 (0.06)	0.43	0.12 (0.08)	0.17	0.258	0.280	0.14
h2: CGAACAACACTG	0.206	0.13 (0.07)	0.048	0.06 (0.09)	0.52	0.198	0.208	0.48
h3: CGACTTAAGCAA	0.111	−0.08 (0.11)	0.46	0.01 (0.11)	0.90	0.110	0.101	0.41
h4: TATCTTGAGCTA	0.104	−0.11 (0.09)	0.22	0.05 (0.12)	0.69	0.109	0.103	0.59
h5: TGTCTTAAGTAA	0.102	−0.18 (0.08)	0.027	−0.28 (0.13)	0.027	0.102	0.110	0.47
h6: CGAATTAAGCTA	0.070	0.16 (0.11)	0.15	−0.08 (0.14)	0.56	0.075	0.081	0.52
Block II[c]								
h1: TACA	0.454	−0.03 (0.06)	0.57	−0.18 (0.08)	0.021	0.429	0.471	0.012
h2: TGTC	0.226	−0.21 (0.06)	0.001	−0.01 (0.09)	0.95	0.234	0.214	0.15
h3: CGCC	0.145	0.25 (0.07)	<0.001	0.20 (0.10)	0.050	0.146	0.146	1.00
h4: TGCC	0.083	0.26 (0.11)	0.023	0.17 (0.12)	0.17	0.088	0.081	0.46

[a] MAF, minor allele frequency.
[b] Block I haplotypes were estimated from SNPs rs7632702, rs9289983, rs1456099, rs2120243, rs2316709, rs7615621, rs2305619, rs3816527, rs1840680, rs2614, rs3911403, and rs4680367.
[c] Block II haplotypes were estimated from SNPs rs6788044, rs16827662, rs6794511, and rs17391399.

gene. In total, we selected 17 variants from the *PTX3* gene, covering also the flanking regions. The LD structure based on these genotyped SNPs is given in Figure 2. All of the genotyped SNPs were in Hardy-Weinberg equilibrium, except the rs6774952 ($P = 0.024$) and rs6794511 ($P = 0.009$; Supplemental Table S1, all Supplemental Data are available online at www.biolreprod.org). Several of the genotyped SNPs associated with either higher or lower LPS-induced PTX3 production (Fig. 2 and Supplemental Table S2). The strongest association was observed for the rs6788044 SNP, where the variant allele carriers had significantly higher PTX3 production after LPS stimulation than noncarriers (estimate [SEM], 0.21 [0.07]; $P = 0.003$; Supplemental Table S2).

Next, we tested whether the genetic variants in *PTX3* gene are associated with female fertility as well. Genotype information as well as fertility histories were available for 1999 women. Genetic variants that were associated with higher LPS-induced PTX3 production were also associated with higher number of offspring and vice versa (Supplemental Table S2). The rs6788044 SNP associated significantly with higher PTX3 production as well as the amount of offspring (estimate [SEM], 0.19 [0.09]; $P = 0.043$; Fig. 2).

Earlier studies by Olesen et al. [14] have indicated a haplotype GAG from SNPs rs2305619, rs3816527, and 1840680 in association with resistance to tuberculosis. Because PTX3 is an important pathogen-recognition receptor, we tested whether in this population, associations could be found for any of the genotyped SNPs with survival as measured by allele frequency differences between newborns and elderly. No clear associations were observed, except for the rs7615621 SNP, which was more frequent in elderly compared with young ($P = 0.038$), and for the rs16827662 SNP, which was less frequent in the elderly than in the young ($P = 0.015$). However, none of these SNPs were associated with PTX3 production. We also constructed haplotypes from the three SNPs analyzed by Olesen et al. [14] and observed that the GAG haplotype associated with increased PTX3 production (0.29 [0.17]; $P = 0.080$) and increased number of offspring (0.73 [0.29]; $P = 0.011$), but not with survival.

Because several SNPs in the *PTX3* gene are in strong LD, falling into two haploblocks, we estimated haplotypes (Table 2). The first haploblock consisted of SNPs rs7632702 to rs4680367. The most remarkable associations were observed for haplotype 5 in block I (frequency, 10.2%) and for haplotype 3 in block II (frequency, 14.5%). Carriers of the block I haplotype 5 had lower PTX3 production (–0.18 [0.08]; $P = 0.027$) and lower fertility (–0.28 [0.13]; $P = 0.027$), whereas carriers of block II haplotype 3 had increased PTX3 production (0.25 [0.07]; $P < 0.001$) and higher fertility (0.20 [0.10]; $P = 0.050$). For these associations, allele dosage-dependent effect was observed (Fig. 3). In addition, we compared the haplotype frequencies of the women of the lowest fertility category (no children to two children) with those of the highest fertility category (12–15 children). We found that haplotype 5 from block I was depleted ($P = 0.019$), whereas haplotype 3 from block II was enriched ($P = 0.046$) among women with the highest fertility. No associations were observed with survival (Table 2).

DISCUSSION

In the present study, we have shown that, in a Ghanaian population, fertility is associated with PTX3 production. In addition, we demonstrated that genetic variants in the *PTX3* gene influence LPS-induced PTX3 production and fertility. These associations were strongest for SNPs in the 3′ untranslated region of the *PTX3* gene.

In the Ghanaian study population, we found that female fertility was associated with ex vivo PTX3 production. In this study, we have chosen to induce PTX3 by LPS because by doing so, disease state or fertility state would not interfere with our measurements. In addition, the LPS-induced PTX3 would best reflect the capacity to induce PTX3 given the genetic background.

Fertility in this population is regarded as natural fertility because the use of contraceptives is virtually absent. We observed that women with more than 12 children had the highest PTX3 production. On the other hand, women who did not conceive or only had one or two children had the lowest PTX3 production. We found no differences in PTX3 production for women with 3–11 children. We realize that the path from ovulation and implantation to actual offspring is long, and therefore the number of offspring is a very crude estimate for success rate of female fertility. Despite that, our observation is in line with studies performed in mouse models. Also, in *Ptx3* knockout mice, reduced litter size was found [3]. Here, we found for the first time in humans a clear association between fertility and PTX3 production.

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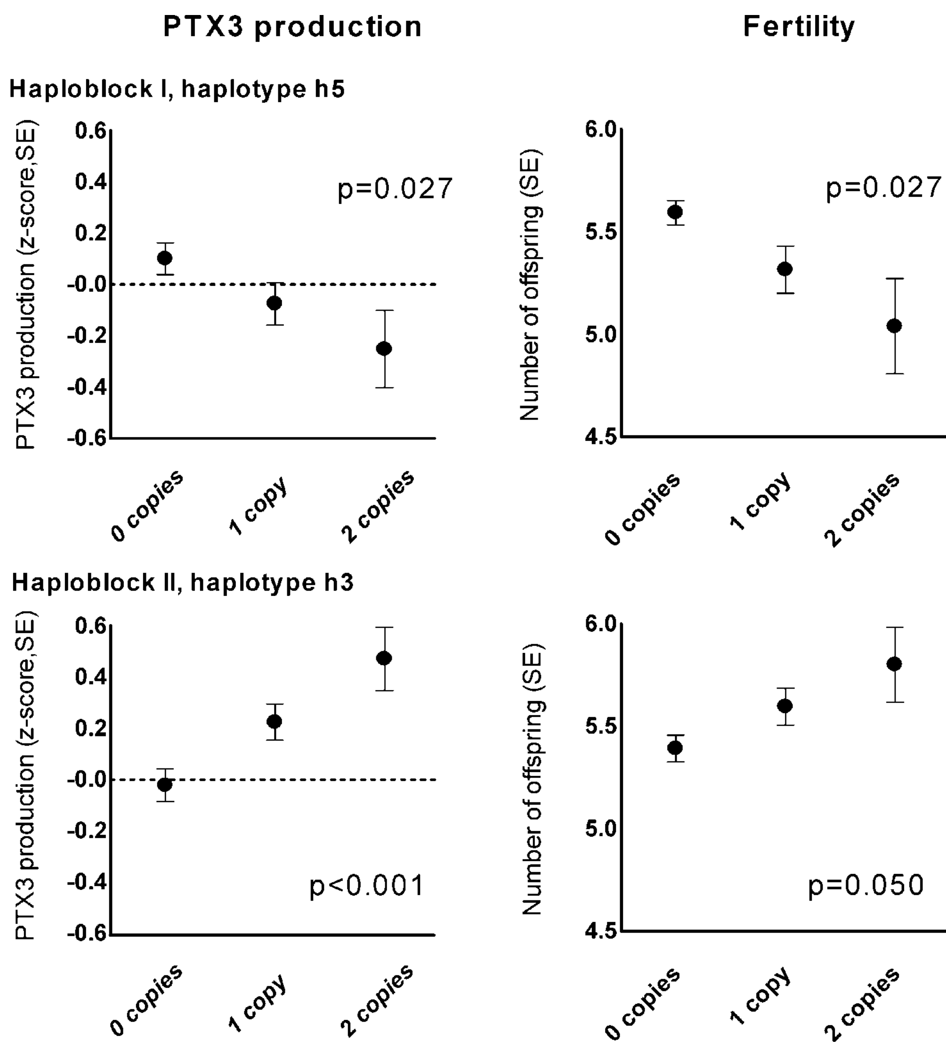

FIG. 3. *PTX3* haplotypes and association with ex vivo PTX3 production and fertility. Data represent the association between *E. coli* LPS-induced PTX3 production for carriers of one and two copies of haplotype alleles h5 in haploblock I and h3 in haploblock II, and the association with number of offspring for these haplotypes.

In this study, we identified several SNPs and haplotypes that showed associations with fertility and PTX3 production. In general, SNPs and haplotypes that associated with higher PTX3 production also associated with higher number of offspring, and vice versa. The rs6788044 SNP that associated the strongest with PTX3 production and fertility is also present in haplotype h3 in block II, and this might explain the associations observed with that haplotype. Because we measured innate PTX3 production, we expect that the associations observed here indicate a causal relation of PTX3 production and fertility.

The previously reported haplotype GAG of Olesen et al. [14] that was associated with resistance to tuberculosis fell into haplotypes h1 and h4 of block I. These haplotypes were not associated with PTX3 production or fertility. However, when we constructed haplotypes from only the three SNPs analyzed by Olesen et al., we observed that the GAG carriers had increased PTX3 production and increased number of offspring. This observation suggests that the GAG haplotype tags a functional variant, but that this variant is not in complete LD with the GAG haplotype.

Because PTX3 is needed for recognition of several pathogens and for fertility, from an evolutionary point of view, we would have expected that genetic tendency to produce high PTX3 would be associated with survival. This was not the case. We have found no strong enrichment or depletion of *PTX3* alleles in the elderly, indicating no survival benefit for either high or low PTX3 production. In this population, malaria is the major cause of death, which might suggest that PTX3 does not play a role in this disease. On the other hand, because high production of PTX3 has also been associated with pathology [25–27], suggesting that high PTX3 responsiveness might have a downside as well.

The strengths of this study include the unique environment, the large number of participants, and genotyped polymorphisms that were selected to capture the majority of the common variants present in the PTX3 gene. However, the large number of SNPs analyzed in this study is also a limitation. Considering the number of tests performed, adjustment for multiple testing would eliminate many statistically significant associations observed. In addition, we did not take into account abortions, spacing between pregnancies, as well as time to first pregnancy, as such data were not available. Also, sexually transmitted diseases, which might have interfered with fertility rates, have not been taken in account for the same reason. In addition, the *PTX3* gene might be in LD with genes nearby. The closest genes are *VEPH1* and *SHOX2*, and it might be that these variants in these genes also influence fertility. Further research has to point out whether these genes are involved in fertility.

The results reported here provide the first evidence, based on protein production and analysis of polymorphisms, that the long pentraxin PTX3 plays a role in female fertility in humans. These data raise a number of questions to be addressed in

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further studies, including the relative importance of the action of this molecule at the level of the cumulus oophorus versus implantation, and the functional significance of polymorphisms discussed here.

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REFERENCES

1. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annu Rev Immunol 2005; 23:337–366.
2. Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, Nota R, Maccagno A, Riva F, Bottazzi B, Peri G, Doni A, Vago L, et al. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. Nature 2002; 420:182–186.
3. Varani S, Elvin JA, Yan C, DeMayo J, DeMayo FJ, Horton HF, Byrne MC, Matzuk MM. Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. Mol Endocrinol 2002; 16:1154–1167.
4. Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, Doni A, Bastone A, Mantovani G, Beck PP, Salvatori G, Mahoney DJ, et al. PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in in vivo fertilization. Development 2004; 131: 1577–1586.
5. Reading PC, Bozza S, Gilbertson B, Tate M, Moretti S, Job ER, Crouch EC, Brooks AG, Brown LE, Bottazzi B, Romani L, Mantovani A. Antiviral activity of the long chain pentraxin PTX3 against influenza viruses. J Immunol 2008; 180:3391–3398.
6. Bozza S, Bistoni F, Gaziano R, Pitzurra L, Zelante T, Bonifazi P, Perruccio K, Bellocchio S, Neri M, Iorio AM, Salvatori G, De SR, et al. Pentraxin 3 protects from MCMV infection and reactivation through TLR sensing pathways leading to IRF3 activation. Blood 2006; 108:3387–3396.
7. Bottazzi B, Garlanda C, Cotena A, Moalli F, Jaillon S, Deban L, Mantovani A. The long pentraxin PTX3 as a prototypic humoral pattern recognition receptor: interplay with cellular innate immunity. Immunol Rev 2009; 227:9–18.
8. Tranguch S, Chakrabarty A, Guo Y, Wang H, Dey SK. Maternal pentraxin 3 deficiency compromises implantation in mice. Biol Reprod 2007; 77: 425–432.
9. Reese J, Das SK, Paria BC, Lim H, Song H, Matsumoto H, Knudtson KL, DuBois RN, Dey SK. Global gene expression analysis to identify molecular markers of uterine receptivity and embryo implantation. J Biol Chem 2001; 276:44137–44145.
10. Popovici RM, Betzler NK, Krause MS, Luo M, Jauckus J, Germeyer A, Bloethner S, Schlotterer A, Kumar R, Strowitzki T, von Wolff M. Gene expression profiling of human endometrial-trophoblast interaction in a coculture model. Endocrinology 2006; 147:5662–5675.
11. Hess AP, Hamilton AE, Talbi S, Dosiou C, Nyegaard M, Nayak N, Genbecev-Krtolica O, Mavrogianis P, Ferrer K, Kruessel J, Fazleabas AT, Fisher SJ, et al. Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. Biol Reprod 2007; 76:102–117.
12. Popovici RM, Krause MS, Jauckus J, Germeyer A, Brum IS, Garlanda C, Strowitzki T, von Wolff M. The long pentraxin PTX3 in human endometrium: regulation by steroids and trophoblast products. Endocrinology 2008; 149:1136–1143.
13. Zhang X, Jafari N, Barnes RB, Confino E, Milad M, Kazer RR. Studies of gene expression in human cumulus cells indicate pentraxin 3 as a possible marker for oocyte quality. Fertil Steril 2005; 83(suppl 1):1169–1179.
14. Olesen R, Wejse C, Velez DR, Bisseye C, Sodemann M, Aaby P, Rabna P, Worwui A, Chapman H, Diatta M, Adegbola RA, Hill PC, et al. DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. Genes Immun 2007; 8: 456–467.
15. Meij JJ, Van Bodegom D, Ziem JB, Amankwa J, Polderman AM, Kirkwood TB, de Craen AJ, Zwaan BJ, Westendorp RGJ. Quality-quantity trade-off of human offspring under adverse environmental conditions. J Evol Biol 2009; 22:1014–1023.
16. Van Bodegom D, May L, Kuningas M, Kaptijn R, Thomese F, Meij HJ, Amankwa J, Westendorp RGJ. Socio-economic status by rapid appraisal is highly correlated with mortality risks in rural Africa. Trans R Soc Trop Med Hyg 2009; 103:795–800.
17. Meij JJ, de Craen AJ, Agana J, Plug D, Westendorp RGJ. Low-cost interventions accelerate epidemiological transition in Upper East Ghana. Trans R Soc Trop Med Hyg 2009; 103:173–178.
18. van der Linden MW, Huizinga TW, Stoeken DJ, Sturk A, Westendorp RGJ. Determination of tumour necrosis factor-alpha and interleukin-10 production in a whole blood stimulation system: assessment of laboratory error and individual variation. J Immunol Methods 1998; 218:63–71.
19. May L, Van Bodegom D, Kuningas M, Meij JJ, de Craen AJ, Frolich M, Westendorp RGJ. Performance of the whole-blood stimulation assay for assessing innate immune activation under field conditions. Cytokine 2009; 45:184–189.
20. Westenbrink BD, Stienstra Y, Huitema MG, Thompson WA, Klutse EO, Ampadu EO, Boezen HM, Limburg PC, van der Werf TS. Cytokine responses to stimulation of whole blood from patients with Buruli ulcer disease in Ghana. Clin Diagn Lab Immunol 2005; 12:125–129.
21. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005; 37: 1217–1223.
22. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263–265.
23. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559–575.
24. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001; 68:978–989.
25. Muller B, Peri G, Doni A, Torri V, Landmann R, Bottazzi B, Mantovani A. Circulating levels of the long pentraxin PTX3 correlate with severity of infection in critically ill patients. Crit Care Med 2001; 29:1404–1407.
26. Latini R, Maggioni AP, Peri G, Gonzini L, Lucci D, Mocarelli P, Vago L, Pasqualini F, Signorini S, Soldateschi D, Tarli L, Schweiger C, et al. Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. Circulation 2004; 110:2349–2354.
27. Fazzini F, Peri G, Doni A, Dell'Antonio G, Dal CE, Bozzolo E, D'Auria F, Praderio L, Ciboddo G, Sabbadini MG, Manfredi AA, Mantovani A, et al. PTX3 in small-vessel vasculitides: an independent indicator of disease activity produced at sites of inflammation. Arthritis Rheum 2001; 44: 2841–2850.

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