Genetic factors in human reproduction a trade off between procreation and longevity

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Interleukin-10 promoter polymorphisms in male and female fertility and fecundity

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Genes and Immunity: in press

Abstract

Interleukin-10 (IL10) is assumed beneficial for a successful pregnancy; it may increase fertility and fecundity. Different IL10 promoter polymorphisms were analysed in association with fertility and fecundity in male and female subjects. From 1986 to 1999, all inhabitants of Leiden, The Netherlands reaching the age of 85 years were enrolled in the Leiden 85-Plus Study. Allele frequencies of IL10 polymorphisms at position –2849, -1082 and –592 were analysed in these subjects. The Registry of Births, Deaths and Marriages Leiden provided the dates of birth, marriage and birth(s) of children. Fertility was decreased in association with the –2849 A allele in females; 27% of the AA genotype carriers remained childless compared to 14% of the G allele carriers (OR: 2.2, 95% CI: 1.2-4.2, p=0.01). Effective fecundability was decreased in association with the –2849 A allele in females; 7% of female -2849AA genotype carriers had a child within 371 days of marriage (therefore conceived within 3 months of marriage) compared to 28% of female G allele carriers (OR: 0.2, 95% CI: 0.04-0.7, p=0.01). This suggests that the IL10 –2849 AA genotype is associated with a decreased fertility and fecundity in females; in male subjects no such association was observed.

Introduction

Interleukin-10 (IL10) is a multifunctional anti-inflammatory cytokine that is produced by various cells including monocytes, macrophages, B cells, T cells and mast cells(1;2). IL10 in return modulates the performance of these various cells with important consequences to their ability to activate and sustain immune and inflammatory responses(3). The role IL10 plays in the immune response is in inhibiting the production of various pro-inflammatory cytokines produced by a large number of different cells(2). There is large variation in IL10 production capacity between healthy individuals. These interindividual differences in IL10 production are largely under genetic control; 50-75% of the variation can be explained by genetic factors as demonstrated in twin studies(4-6). In the IL10 promoter region various single nucleotide polymorphisms (SNPs) have been described, in both the distal and proximal promoter region.

The effect of IL10 promoter polymorphisms on IL10 production has not been fully elucidated. The -2849AA genotype has been associated with significantly lower IL10 production upon endotoxin stimulation compared to the G genotype carriers(7). For the IL10 –1082 A allele it seems less clear; both a decreased IL10 production has been described related to the AA genotype(8;9), as well as an increased production, as well as no association(7;10). The IL10–592CC genotype has been related to a low IL10 production capacity after stimulation with S. pneumonia(10).

In pregnancy IL10 is considered one of the major immunoregulatory cytokines important for a successful outcome. Numerous studies have described that at the maternal-fetal interface IL10 production is increased. Moreover, decreased production of IL10 is associated with pregnancy loss and increase in pre-eclampsia(11). The IL10 polymorphisms at positions –2849, –1082 and –592 have been associated with a range of pregnancy-associated phenomena like recurrent miscarriages(12), preterm birth(13) and pre-eclampsia(14). The evidence for the -2849 polymorphism appears to be strongest associated with fertility. The IL10 -2849AA genotype was reported to be two-fold more prevalent among 73 married women who remained childless (RR 2.1, 95% CI 1.2-3.6), which was sustained in a similar study(15). The assumption was made that this genotype may reduce the chance of a successful pregnancy due to a decreased innate IL10 responsiveness.

The current study was initiated to further specify the role of IL10 in relation to two aspects of human reproduction; the ability to have a child (fertility) and the probability of a couple conceiving in a specific period of time (fecundity). This was assessed in a large cohort of subjects born in the late 19th and early 20th century, who were in their childbearing age in a time where modern contraceptive methods were unavailable. The present study is a continuation of earlier studies(15) with the distinction that not only more SNPs were analysed with their respective haplotypes, but also additional specified information on the subjects was obtained. Therefore, the data of both female and male subjects was analysed in relation to fertility, fecundity and the various IL10 polymorphisms.

MATERIALS AND METHODS

Subject recruitment

The Leiden 85-plus Study consists of two separate cohorts. A detailed description of both cohorts has been presented elsewhere(15;16). In short, subjects of the first cohort were enrolled between December 1986 and March 1989. During that period a total of 977 inhabitants of Leiden, The Netherlands, who were aged 85 and over were included. A second cohort of 85-year-old subjects, consisting of 599 subjects, was enrolled between September 1997 and September 1999. There were no selection criteria for health or demographics in either cohort. Of all 1576 subjects a blood sample was obtained. DNA was available for an unselected sample of 1278 subjects. The Leiden University Medical Centre medical ethical committee approved the study protocol for both cohorts.

Date retrievals

The Registry of Births, Deaths, and Marriages of the municipality of Leiden and the Central Bureau of Genealogy (CBG), The Netherlands, provided the date of birth, date of marriage(s), and birth dates of children of all study participants. The CBG is the major documentation and information center for family history and heraldry in The Netherlands. For 42 subjects there was insufficient information available on their marital history or their number or dates of birth of progeny. Hence, complete information was available for 1236 subjects.

Calculation of fecundity

Fecundity was defined as the calculated time interval between the date of (first) marriage and the date of birth of the first-born child. This concept of delay from marriage to the first birth has previously been defined as 'effective fecundability'(17). If the conception had taken place within the first 3 months of marriage, it can be assumed that these children were most likely born within 371 days of the marriage date. This was calculated by adding 3 months (91 days) to the median duration of a term pregnancy (280 days). Subjects with their first child born before marriage were excluded from analysis.

IL10 promoter gene polymorphisms

Participants were genotyped for the IL10 promoter gene at positions –2849, –1082 and –592. The typing of the IL10 G-2849A (rs6703630) polymorphism has been described previously(15). In short, genotypes were obtained using an Assay-by-Design (Applied Biosystems), consisting of PCR primers and TaqMan MGB probes. Amplification reactions were made at standard conditions. Real time PCR was performed on ABI 7900 HT (Applied Biosystems). The IL10 G-1028A (rs1800896) and IL10 C-592A (rs1800872) polymorphisms were genotyped by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS), using the Sequenom MassARRAYtm (Sequenom Inc.) methodology. Amplification reactions and parameters were based on the manufacturer's instructions.

Data analysis

Non-normally distributed data are presented as geometric means and 95% confidence intervals (CI). Differences in prevalence were compared by the Pearson Chi-square test with Fisher's exact test applied when at least one expected frequency was below 5. All tests were 2-tailed. Logistic regression models were applied to correct for age at marriage. P-values < 0.05 were considered statistically significant.

Alleles were considered to be in Hardy-Weinberg equilibrium if the observed genotype frequencies did not differ significantly (P<.05) from those expected when analysed by χ^2 test.

Odds ratios for haplotype comparisons were obtained from THESIAS (available from http://www.genecanvas.org). THESIAS is used for real data analysis, either for a binary, a quantitative or a survival outcome for haplotype-based association studies(18;19).

RESULTS

Baseline characteristics

For 1236 subjects complete information was available; however not all subjects had information for all three SNPs analysed. For 1185 (96%) subjects the IL10 –2849 status was known, for 1132 (92%) subjects the IL10 -1082 status was known and for 1130 (91%) the IL10 –592 status was known. Of 1043 (84%) subjects there was information on all three SNPs available.

Table 1. The fertility characteristics of all 1116 married subjects (759 female and 357 male) of the total 1236 (857 female and 379 male) subjects included in the Leiden 85 Plus Study.

	Married females n = 759	Married males n = 357
Childless	117 (15)	52 (15)
Number of children	2.7 (2.2)	2.8 (2.2)
Age at marriage	25.7 (6.4)	27.3 (4.9)
Age at 1st birth	26.2 (4.5)	28 (5.0)

Values are in n(%) or mean (SD).

Table I shows the general characteristics of all 1236 subjects included in this analysis. No significant differences were found. The cohort comprised of 857 women (69%) and 379 men (31%). The year of birth ranged from 1887 to 1914. Of the 857 female subjects 759 (89%) had been married at least once; of the 379 male subjects 357 (94%) had been married at least once. The year of birth of the first-born children ranged from 1910 to 1954 in female subjects and form 1912 to 1961 in male subjects. The age at marriage for the female subjects was comparable for the various IL10 genotypes analysed (data not shown). All IL10 SNPs were in Hardy-Weinberg equilibrium.

Table II. Association between IL10 genotype and fertility.

	Genotype*			OR (95% CI)		
	11	12	22	11 versus rest	22 versus rest	22 versus rest adjusted**
Females						
IL10 G-2849A						
Childless	50 (14)	46 (14)	14 (27)	0.9 (0.6-1.4)	2.2 (1.2-4.2)	2.3 (1.1-4.9)
≥ 1 Child	298 (86)	279 (86)	38 (73)			
IL10 G-1082A						
Childless	29 (16)	51 (14)	25 (17)	1.0 (0.6-1.7)	1.1 (0.7-1.9)	1.2 (0.7-2.0))
≥ 1 Child	153 (84)	308 (86)	126 (83)			
IL10 C-592A						
Childless	62 (15)	36 (15)	7 (23)	1.0 (0.6-1.5)	1.7 (0.7-4.0)	2.0 (0.8-5.3)
≥ 1 Child	352 (85)	208 (85)	24 (77)			
Males						
IL10 G-2849A						
Childless	24 (15)	24 (16)	3 (9)	1.1 (0.6-2.3)	0.5 (0.1-1.9)	0.4 (0.1-1.5)
≥ 1 Child	134 (85)	130 (84)	32 (91)			
IL10 G-1082A						
Childless	14 (17)	23 (14)	14 (18)	1.3 (0.6-2.6)	1.2 (0.6-2.3)	1.1 (0.5-2.2)
≥ 1 Child	64 (83)	147 (86)	69 (82)			
IL10 C-592A						
Childless	27 (14)	20 (17)	3 (19)	0.8 (0.4-1.4)	1.3 (0.4-4.8)	1.6 (0.4-6.2)
≥ 1 Child	171 (86)	97 (83)	13 (81)			

Values n (%), OR = Odds Ratio, 95% CI = 95% Confidence Interval.
*The -2849 genotypes are: 11 equals -2849 GG, 12 equals -2849 GA, 22 equals -2849 AA. The -1082 genotypes are: 11 equals -1082 GG, 12 equals -1082 GA, 22 equals -1082 AA. The -592 genotypes are: 11 equals -592 CC, 12 equals -592 CA, 22 equals -592 AA.** Adjusted by logistic regression for age at marriage

IL10 SNPs in association with fertility

Of the 759 married female subjects 117 (15%) of the marriages remained childless. For the 357 married male subjects this was 52 (15%). The total number of children was comparable for all SNPs analysed and was not related to the various SNP genotypes (data not shown). Fertility was classified according to whether the marriage remained childless or not; this was analysed per IL10 SNP at a genotype level and presented in Table II. Female –2849AA genotype carriers had a 2 fold higher likelihood of having a marriage that remained childless (Odds Ratio (OR) 2.2, 95% confidence interval (CI): 1.2-4.2). When adjusting for age at marriage the results remained similar (OR 2.3, 95% CI: 1.1-4.9). There was no such relation found for the other IL10 SNPs. No relation between the IL10 haplotypes and fertility could be found.

In male subjects no clear association between any of the IL10 SNPs and fertility (childlessness) was found. Males carrying the –2849AA genotype did not have a significantly different odds of remaining childless in marriage, (OR 0.5, 95% CI 0.1-1.9).

IL10 SNPs in association with fecundity

Effective fecundity is presented in Table III with the calculated conception time of married subjects analysed dependent on the IL10 polymorphism and their genotypes. Subjects with a child born before the date of marriage were excluded from analysis regarding fecundity; this was the case for 8 (2%) of the 357 married males and 34 (4%) of the 759 married females.

In female subjects the IL10 –2849 AA genotype was found to be associated with a decreased effective fecundability (longer time period between marriage and first-born child) compared to G allele carriers. Of the 31 female subjects carrying the –2849 AA genotype, 2 (7%) had a calculated conception time of 3 months or less, compared to 116 (28%) of the 410 G allele carriers (OR: 0.2, 95% CI: 0.04-0.7). After adjusting for age at marriage the results remained similar (OR 0.2, 95% CI: 0.04-0.8). At a haplotypic level no significant differences for fecundity could be found. If –2849AA was analysed as homozygous factor compared to the other haplotypes, the results were similar to the results done at single SNP level (OR: 0.2, 95% CI: 0.04-0.9).

Table III. Association between IL10 genotype and effective fecundity.

	Genotype*			OR (95% CI)		
	11	12	22	11 versus rest	22 versus rest	22 versus rest adjusted**
Females						
IL10 G-2849A						
Conception ≤3 months	59 (28)	57 (28)	2 (7)	1.2 (0.8-1.8)	0.2 (0.04-0.7)	0.2 (0.04-0.8)
Conception >3 months	149(72)	145(72)	29 (94)			
IL10 G-1082A						
Conception ≤3 months	28 (23)	61 (28)	30 (33)	0.7 (0.4-1.2)	1.4 (0.8-2.3)	1.4 (0.9-2.4)
Conception >3 months	94 (77)	157(72)	61 (67)			
IL10 C-592A						
Conception ≤3 months	73 (27)	40 (28)	5 (31)	0.9 (0.6-1.5)	1.2 (0.4-3.5)	1.2 (0.4-3.6)
Conception >3 months	196(73)	103(72)	11 (69)			
Males						
IL10 G-2849A						
Conception ≤ 3 months	21 (20)	21 (21)	7 (28)			
Conception > 3months	82 (80)	77 (79)	18 (72)	0.9 (0.4-1.7)	1.5 (0.6-3.8)	1.4 (0.6-3.6)
IL10 G-1082A						
Conception ≤3 months	12 (25)	28 (25)	6 (11)			
Conception >3 months	36 (75)	84 (75)	49 (89)	1.3 (0.6-2.9)	0.4 (0.1-0.9)	0.4 (0.1-0.9)
IL10 C-592A						
Conception ≤3 months	27 (20)	19 (27)	2 (17)			
Conception >3 months	106(80)	52 (73)	10 (83)	0.8 (0.4-1.5)	0.7 (0.1-3.2)	0.7 (0.2-3.4)

Values n (%), OR = Odds Ratio, 95% CI = 95% Confidence Interval.
*The -2849 genotypes are: 11 equals -2849 GG, 12 equals -2849 GA, 22 equals -2849 AA. The -1082 genotypes are: 11 equals -1082 GG, 12 equals -1082 GA, 22 equals -1082 AA. The -592 genotypes are: 11 equals -592 CC, 12 equals -592 CA, 22 equals -592 AA.
** Adjusted by logistic regression for age at marriage.

In male subjects no association between the different IL10 polymorphisms in relation to fecundity could be found. However, in males carrying the -1082AA genotype a decreased

effective fecundability was observed; 6 (11%) of the 55 male –1082 AA genotype carriers had a calculated conception within 3 months of marriage compared to 40 (25%) of the 160 male G allele carriers (OR: 0.4, 95% CI: 0.1-0.9).

DISCUSSION

In the present study we found that female carriers of the IL10 –2849 AA genotype had a significant increase in childlessness and a significant decrease in effective fecundability. Female IL10 –2849 AA carriers were twice as likely to have a marriage that remained childless and were 5 times less likely to have a conception leading to a birth of a child within the first three months of marriage compared to G allele carriers.

Previously the –2849 SNP has been shown to be an important determinant in IL10 responsiveness to endotoxin stimulation with a significantly lower IL10 responsiveness in –2849AA genotype carriers(7). Furthermore an association between reproductive success and a high IL10 responsiveness has been reported previously in addition to a reduced fertility in IL10 –2849 AA genotype carriers(15). The current study confirms and further specifies the influence of the IL10 G–2849A SNP on female fertility. The study was conducted as a continuation on the previous study, comprising a larger cohort with additional information per included subject. It was therefore possible to correct for possible confounders such as age at marriage and age at birth of the first child. Additionally a comparison between males and females was made in relation to fertility, fecundity and the various IL10 polymorphisms.

Female subjects were found to be twice as likely to remain childless when carrying the -2849AA genotype compared to female G allele carriers. The results remained equally significant when correcting for age at marriage. Additionally, it was found that female IL10 -2849AA genotype carriers were 5 times less likely to have a conception within the first 3 months of marriage compared to the G allele carriers, in other words their effective fecundability was significantly lower. The outcome remained equal after correction for age at marriage. These results not only replicate the earlier published results(15;20), but further strengthen the likelihood of a positive link between the IL10 -2849 polymorphism and human reproduction. The relation between non-exon SNPs and gene function is difficult to prove by

biochemical or molecular biological methods because it is not known which stimulus leads to the increased IL10 secretion during pregnancy. Thus methods to prove that a SNP is changing a transcription factor binding site or the rate of transcription of an allele may not be relevant for this specific biological process. Therefore we examined whether IL10 -A2849G was merely a tag of a haplotype or whether it alone was the best predictor of fertility characteristics. Indeed, no relation between the IL10 haplotypes and fertility or fecundity could be found, indicating that the IL10 -A2849G SNP itself is related to affected gene function with regard to fertility. A final conclusion cannot be made as this was generated by a limited number of SNPs. However the low IL10 responsiveness that is particularly found in relation to the IL10 –2849 AA genotype is also compatible with the epidemiological data on low fertility and fecundity associated with the -2849AA genotype. A low IL10 responsiveness may reduce the chance of developing a successful pregnancy.

A low IL10 responsiveness has been reported in relation to recurrent miscarriages. The number of miscarriages or fetal losses could not be analysed with this study design as only births were recorded. The possibility exists therefore, that the effective fecundability (increased interval between marriage and first birth) is reduced due to an increase in the occurrence of miscarriages in -2849 AA carriers. Therefore the exact reason for the decreased fecundity in female IL10–2849 AA genotype carriers remains speculative. No significant association was found when analysing the remaining polymorphisms in association with fertility or fecundity in female subjects.

IL10 has been reported in human semen. Human seminal plasma possesses a generalized immunosuppressive activity(21;22). An important aspect of IL10 in the male genital tract is thought to be the maintaining of the immunological balance and avoid rejection of the spermatozoa(23). Levels of IL10 have been reported lower in semen of infertile men compared to fertile men(24). It has been postulated that a decrease in the presence of IL10 could alter the tolerance to sperm cells in the female genital tract and reduce the favourable condition for fertilisation and implantation. To our knowledge no studies concerning IL10 SNPs and male fertility or fecundity have been published. It would appear likely that a low IL10 responsiveness would decrease male fecundity, with an expectation of finding a relation between male fecundity and the –2849 SNP. The results of the current study however,

revealed no association involving the IL10 –G2849A SNP and the IL10 –C592A SNP in relation to fertility and fecundity in men. An association between the IL10 G-1082A SNP and fecundity in male subjects was seen; men with the IL10 –1082 AA genotype had a decreased effective fecundability compared to G allele carriers. Given the number of comparisons made in this data set and the limited number of subjects, it is most likely a false positive chance finding. If however this finding is a true one, the explanation may lie in the direction of the –1082 SNP interfering with IL10 responsiveness specifically in seminal fluid. No studies have been reported on this topic. Possibly an altered IL10 level in the seminal fluid might interfere with the probability of successful fertilisation, anywhere from the survival of the spermatozoa in the female genital tract to the penetration of the zona pellucidum of the ovum, altering fecundity.

The current study has some limitations. All reproductive information was acquired from registries; therefore all conception times and fecundity rates were calculated. We have no information on pregnancy failures, both miscarriages and stillbirths. The selected cohort was set in a time represented by minimal fertility control and no modern contraceptive methods. We have assumed that starting a family as soon as a marriage was celebrated was desired. The circumstance of the subjects at the time of their marriage is unknown. Any significant illnesses or availability of either partner in the first year after marriage is unknown. Other factors interfering with fecundity as sperm count, regularity of menstrual cycle, frequency of intercourse is unknown.

In conclusion, we found that female IL10 -2849AA genotype carriers were twice as likely to remain childless compared to G allele carriers. Furthermore, female -2849AA genotype carriers were also 5 times less likely to have a conception within the first 3 months of marriage compared to the G allele carriers. These two findings combined clearly confirm that an association between the IL10 –2849 SNP and female fertility exists. Most likely this is due to a decreased IL10 responsiveness found in IL10 –2849 AA genotype carriers, reducing the immunoregulation at the maternal fetal interface decreasing the likelihood of a successful pregnancy.

ACKNOWLEDGEMENTS

The authors would like to thank M. Kuningas and B.A.S. Kurreeman for their valuable help with the analysis at the haplotype level.

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