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ORIGINAL ARTICLE

The impact of androgen receptor polymorphism and parental ethnicity on semen quality in young men from Latvia

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Summary

Recent studies on young men from the general population have demonstrated geographic and ethnic differences in semen quality. The aim of this study was to investigate whether reported ethnic differences in semen quality might be associated with the maternally derived CAG and GGN polymorphisms in the androgen receptor gene or paternal ethnicity. In total 114 military conscripts from Latvia were included in the study. Information on maternal and parental ethnicity was collected by questionnaires. CAG and GGN repeats were analysed by direct sequencing of leukocyte DNA. Men with Latvian mothers ($n = 83$) had marginally shorter CAG repeat length (21.6 ± 2.9) as compared with those with non-Latvian mothers (22.9 ± 3.2, $n = 31$), not reaching statistical significance ($p = 0.053$). Sperm concentration did not differ significantly between these two groups (76 ± 59 and 70 ± 52, $p = 0.9$ respectively). In contrast, significantly higher sperm concentration and total sperm count were found in men with Latvian fathers ($n = 77$) as compared with men with non-Latvian fathers ($n = 37$) (80 ± 61 vs. 62 ± 48, $p = 0.035$, for sperm concentration and 225.7 ± 209 vs. 158.4 ± 134.4, $p = 0.002$, for total sperm count respectively). CAG repeat length did not correlate with any semen parameters in the whole population. However, GGN repeat length correlated with semen volume: men with GGN > 23 presented with higher semen volume (3.2 ± 2.1) as compared with men with GGN = 23 (2.6 ± 1.3, $p = 0.04$) or GGN < 23 (2.0 ± 1.2, $p = 0.006$). We conclude that GGN repeat length has an impact on semen volume, whereas differences in sperm numbers are associated with the paternal ethnicity.

Introduction

During the past few years, several studies addressed the issue of geographic differences in semen quality. A significant amount of data available come from the Baltic area, showing higher sperm concentration in Finland, Estonia, Lithuania and Latvia than in Denmark and Norway (Jorgensen *et al.*, 2002; Punab *et al.*, 2002; Tsarev *et al.*, 2005). Whether these differences could be caused by genetic, environmental and/or lifestyle-related factors is still an unresolved question.

Several studies have addressed a possible association between semen quality and androgen receptor (AR) gene polymorphisms. There are two trinucleotide tracts of polymorphic length in the transactivating domain of the AR, consisting of CAG and GGN repeats respectively (Lubahn *et al.*, 1988). Some reports have indicated associations between longer CAG tracts, within the normal range, and decreased semen quality or male infertility (Tut *et al.*, 1997; Dowsing *et al.*, 1999; Mifsud *et al.*, 2001; Milatiner *et al.*, 2004; Katagiri *et al.*, 2006), whereas others have not found such correlations (Giwercman *et al.*, 1998; Dadze *et al.*, 2000; Rajpert-De Meyts *et al.*, 2002; Van Golde *et al.*, 2002). It has also been suggested that AR polymorphisms per se are not the cause of male infertility, but that certain lengths or combinations of these repeats might increase susceptibility to impairment of sperm production caused by other genetic, environmental or lifestyle-related factors (Ruhayel *et al.*, 2004).

Ethnic differences in CAG repeat numbers in the AR gene have been demonstrated, with a mean length of 19 to 20 in African-Americans, 21 to 22 in Caucasians and 23 to 24 in Asians (Sartor *et al.*, 1999; Hsing *et al.*, 2000; Ruhayel *et al.*, 2004). However, there are no data showing corresponding differences in semen quality in these ethnic groups with a direct link to the AR polymorphisms.

In Latvia, 41% of the population is non-Latvian, a majority of them being of Russian origin. In a recent study on Latvian military conscripts, we found significantly higher sperm concentration in men with both parents born in Latvia ($77 \pm 60 \times 10^6$/mL) compared with men with both parents born outside the Baltic area ($55 \pm 45 \times 10^6$/mL, $p = 0.03$) (Tsarev *et al.*, 2005). Because of the ethnic mixture of native and Russian populations that might differ in genetic aspects, but live under the same environmental conditions; Latvia represents an interesting model for studying the relative influence of genetic and environmental factors on semen quality. We aimed to investigate whether differences in the AR polymorphisms could explain the above-mentioned differences in sperm concentration. The association between the paternal ethnicity and semen quality was also assessed.

Materials and methods

A total of 1557 men attending the military service board in Riga from April 2001 until May 2002 were asked to participate. Among them, 133 (participation rate 8.7%) accepted to participate in the study. As all men at the age of 19 must attend the military service board in Latvia, whether or not eligible, they can be considered to represent the general population of young men. Every participant filled in a standardized questionnaire, used in previous studies in the Nordic–Baltic area (Jorgensen *et al.*, 2002). The questionnaire included information about the birthplace of the subject and parental ethnicity. A total of 27% of men had non-Latvian mothers, and 32% had non-Latvian fathers.

All men also delivered a semen sample. The current study was limited to the 114 men who also agreed to provide a blood sample for analysis of the AR polymorphisms.

All subjects signed a written consent form and the study was approved by the local ethical committee.

Semen analysis

Each man provided a semen sample by masturbation. The men were asked to adhere to 48–168 h of abstinence, but in each case, the actual abstinence period from the previous ejaculation time (in hours) was recorded. Samples were allowed to liquefy for 30 min. All semen samples were analysed by the same technician, according to published recommendations (World Health Organization, 1999). Sperm concentration was assessed using positive displacement pipettes and improved Neubauer haemocytometer. Semen volume was determined by estimation from graduated Falcon tubes, whereas the motility of 200 spermatozoa was scored into four categories: rapid progressive, slow progressive, non-progressive motile and immotile.

Genetic analysis

DNA was extracted from leukocytes, and AR gene repeat tracts were amplified using a nested PCR amplification procedure as previously described (Lundin *et al.*, 2003). The numbers of CAG and GGN repeats were obtained by direct sequencing externally on a Beckman Coulter CEQ^{TM} 2000 XL DNA sequencer (Beckman Coulter Inc., Bromma, Sweden). The CAG repeat was sequenced in all men, whereas 99/114 (87%) of the men were analysed regarding their GGN tracts.

Statistical analysis

Results were expressed as mean ± SD. As the AR is located on the X chromosome, the 114 men with available AR gene polymorphism data were divided into two groups according to maternal ethnicity: men with Latvian mothers ($n = 83$) and those with non-Latvian mothers ($n = 31$). Regions outside of Latvia from which the mothers originated included Russia ($n = 23$), Ukraine ($n = 5$) and Byelorussia ($n = 3$). To assess the impact of paternal ethnicity, men were additionally divided into two groups according to their fathers' ethnicity: men with Latvian fathers ($n = 77$) and men with non-Latvian fathers ($n = 37$). Non-Latvian fathers originated from Russia ($n = 27$), Ukraine ($n = 7$) and Byelorussia ($n = 3$).

The influence of ethnic origin and AR polymorphisms on sperm concentration, total sperm count, semen volume and proportion of progressively motile sperms were analysed in general linear regression models, adjusted for the abstinence time. Abstinence time was categorized into four groups: (i) <48 h; (ii) 48–71 h; (iii) 72–96 h and (iv) >96 h. Sperm concentration and total sperm counts were additionally adjusted for the testis volume. Influence of maternal ethnicity was additionally adjusted for paternal ethnicity and vice versa.

The CAG repeat length was treated as continuous variable, whereas the GGN length was categorized into three groups: GGN < 23 ($n = 16$), GGN = 23 ($n = 55$) and GGN > 23 ($n = 28$). Fisher's exact test was applied for testing differences in proportions between different groups.

All hypotheses testing were two-sided with a probability value of 0.05 considered as significant. Analyses were

conducted with SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

AR polymorphisms and maternal ethnicity

The CAG repeat length in the whole population varied from 16 to 29 with a mean value of 22.0 ± 3.05 (Fig. 1). As previously published, the two most common GGN alleles among Caucasians, 23 and 24, were also the most frequent alleles in our study population (Fig. 2).

When men were divided into two groups according to maternal ethnicity, those with Latvian mothers exhibited marginally shorter CAG repeat length as compared with those with non-Latvian mothers (21.6 ± 2.9 vs. 22.9 ± 3.2), not reaching statistical significance ($p = 0.053$) (Table 1).

The GGN > 23 occurred almost with the identical frequency in subjects with Latvian vs. non-Latvian mothers (22 and 23%, $p = 1.0$) and also GGN = 23 and <23 were not statistically different between these two sub-populations (61% vs. 46%, $p = 0.24$; 16% vs. 31%, $p = 0.15$ respectively) (Fig. 2).

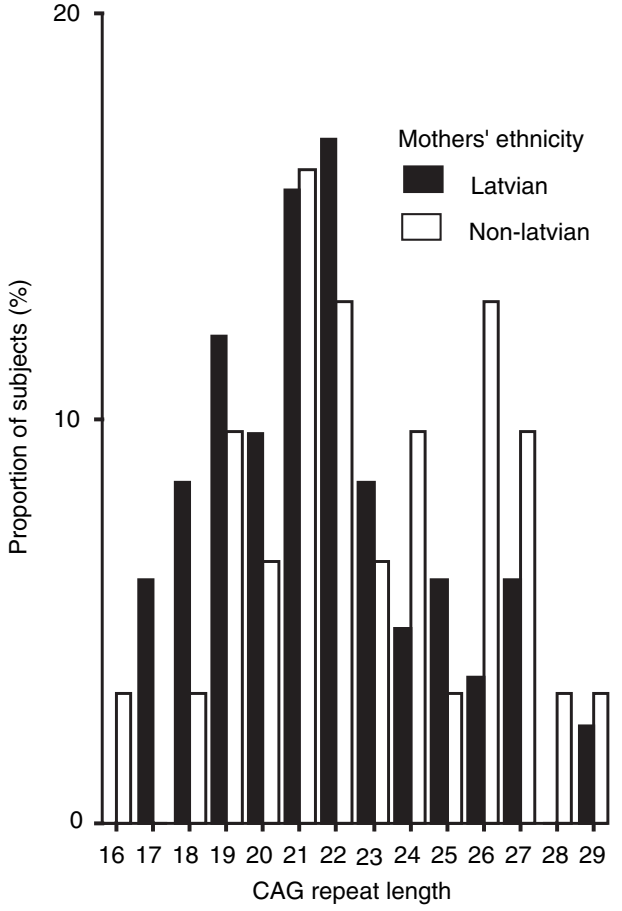

Figure 1 Bar chart displaying distributions of the lengths of the CAG repeat tracts in men with Latvian mothers ($n = 83$, black bars) and men with non-Latvian mothers ($n = 31$, white bars).

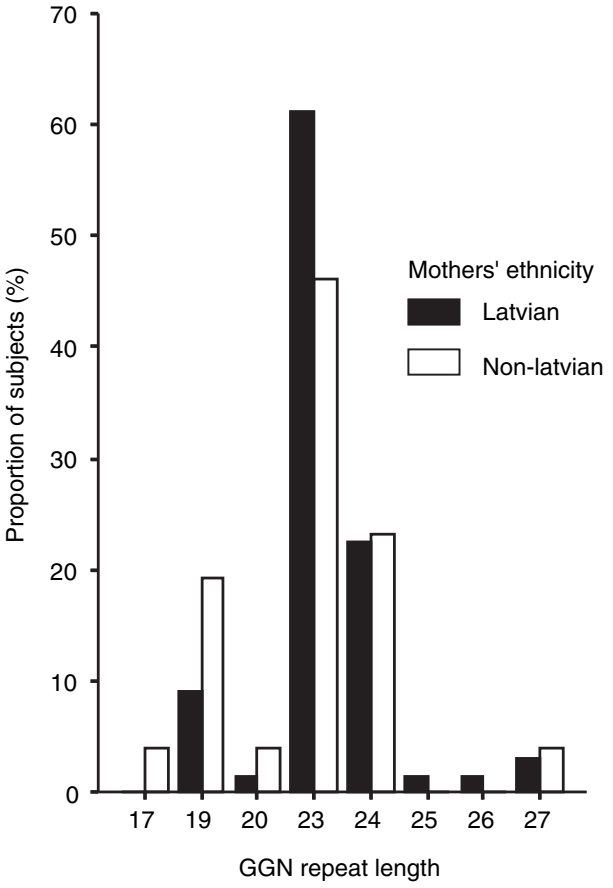

Figure 2 Bar chart displaying distributions of the lengths of the GGN repeat tracts in men with Latvian mothers ($n = 70$, black bars) and men with non-Latvian mothers ($n = 29$, white bars).

Table 1 Association between maternal ethnicity, CAG repeat length and sperm concentration

	CAG repeat l ength (mean ± SD)	Sperm concentration (10^6/mL; mean ± SD)
Men with Latvian mother ($n = 83$)	21.6 ± 2.9	76 ± 59
Men with non-Latvian mother ($n = 31$)	22.9 ± 3.2	70 ± 52
p-value	0.053	0.9

Ethnicity and semen parameters

Sperm concentration was somewhat higher in the group having Latvian mothers as compared with those with non-Latvian mothers. This difference, however, did not

reach the level of statistical significance (Table 1). There was no difference between these two groups regarding the total sperm count, the seminal volume and sperm motility.

Paternal ethnicity was significantly associated with sperm concentration, higher sperm concentrations seen in men with Latvian fathers as compared with men with non-Latvian fathers ($80 \pm 61 \times 10^6$/mL vs. $62 \pm 48 \times 10^6$/mL, $p = 0.035$) (Table 2). Also the total sperm count was higher in the former group as compared with the latter (226 ± 209 vs. 158 ± 134, $p = 0.002$) (Table 2). No association was found between paternal ethnicity and semen volume or sperm motility.

AR polymorphisms and semen parameters

In linear regression analysis, only paternal ethnicity ($p = 0.035$) and abstinence time ($p = 0.04$) were found as significant factors for sperm concentration; CAG repeat length (and maternal ethnicity as well) did not exhibit any significance for sperm numbers. There was no difference in sperm concentration between men with short (<22, $n = 55$) and long (≥22, $n = 59$) CAG repeats: $79 \pm 52 \times 10^6$/mL vs. $69 \pm 62 \times 10^6$/mL, $p = 0.6$, and we did not find any association between CAG repeat length and semen volume, total sperm count or sperm motility.

Regarding the GGN repeat, no association between GGN length and sperm concentration, total sperm count or motility was noted. However, when the relationship between distinct GGN repeat length and semen volume was studied, there was no difference in semen volume between men with GGN < 23 (2.0 ± 1.2, $n = 16$) and GGN = 23 (2.6 ± 1.3, $n = 55$), but seminal volume was significantly higher in men with GGN > 23 (3.2 ± 2.1, $n = 28$) as compared with those with GGN < 23 ($p = 0.006$) and those with GGN = 23 ($p = 0.04$).

Three men (3%), two with both parents of Latvian origin and one with Byelorussian parents, had one additional GGT repeat: $(GGT)_3GGG(GGT)_3(GGC)_n$ instead of $(GGT)_3GGG(GGT)_2(GGC)_n$. Each of the internal $(GGT)_3$ variant was followed by a $(GGC)_{20}$ segment in all three men. These subjects had normal semen parameters and nothing remarkable in their medical history. If these three subjects, all exhibiting GGN = 27 were excluded from the GGN > 23 group, the significant difference in seminal volume between this group and those displaying GGN = 23 disappeared (3.1 ± 2.2, $p = 0.09$). However, exclusion of the three longest alleles did not affect the relation in terms of different semen volume between GGN > 23 and GGN < 23, men with GGN > 23 still having significantly higher seminal volume (3.1 ± 2.2, $p = 0.01$).

Table 2 Association between paternal ethnicity and sperm counts

	Sperm concentration (10^6/mL; mean ± SD)	Total sperm count (10^6) (mean ± SD)
Men with Latvian father ($n = 77$)	80 ± 61	226 ± 209
Men with non-Latvian father ($n = 37$)	62 ± 48	158 ± 134
p-value	0.035	0.002

Discussion

This is, to our knowledge, the first report investigating whether reported ethnic differences in semen quality (sperm concentration) can be associated with differences in maternally derived AR gene polymorphisms or paternal ethnicity. With respect to the AR, we found a borderline significant difference for CAG tract in men with Latvian mothers as compared with men with non-Latvian mothers, and also no significant difference in sperm concentration between these two groups. It cannot be excluded that it may be attributed to the limited size of the study; therefore, these results should be interpreted cautiously especially taking into account the borderline level of significance.

There was no difference in GGN repeat length distributions between men with Latvian and non-Latvian mothers, and no association between GGN repeat numbers and sperm concentration. However, a correlation was found between GGN length and semen volume. This is in line with previous observations (Lundin *et al.*, 2006). Because secretions from prostate and seminal vesicles are the main contributors to the seminal volume, this observation is confirming previously published data that GGN repeat length is associated with accessory sex gland secretory function (Ruhayel *et al.*, 2004). Similarly to the previous findings in Swedish men (Lundin *et al.*, 2006), we found the most pronounced difference in semen volume between those with GGN < 23 and those having GGN ≥ 24. In a recently published paper (Lundin *et al.*, 2007), we found that GGN lengths below 23 are, in vitro, associated with the lowest AR activity, which fits well with the results of this study. On the other hand, lengths above 23 in vitro were associated with slightly lower AR activity, as compared with GGN = 23, something which does not fit with our in vivo results of this study. This contradiction might be because of the influence of other genetic factors in an in vivo situation.

A polymorphism in the AR GGT region in 3% of the men was observed, which is a higher fraction than earlier reported (Lundin *et al.*, 2003).

Contrary to maternal ethnicity, paternal ethnicity was demonstrated as a significant factor for both sperm concentration and total sperm count, with higher sperm

numbers in men with Latvian fathers as compared with those with non-Latvian fathers. The design of the study does not allow any identification of possible paternal factors, which might imply higher sperm counts in sons of Latvian men. Genetic differences in Y chromosome haplo groups between Latvians and Eastern/Central European nations like Russians have been shown (Rosser *et al.*, 2000; Tambets *et al.*, 2004), which might be one of the possible explanations for the observed association between sperm number and the ethnicity of the father. Unfortunately, we were not able to run Y chromosome haplo types because we were out of both DNA and blood samples in more than half of study subjects.

Participation rate in this study was quite low (8.7%). However, low participation rate is typical for the studies of the military conscripts, also in the other countries (Richthoff *et al.*, 2002). It can be expected that men at the age of 19 have no knowledge of their reproductive capability. The low participation rate should, therefore, not imply any selection bias with respect to fertility. In addition, 27% of men in this study had non-Latvian mothers and 32% had non-Latvian fathers, and these figures correspond to the figures of the ethnical background of young men in Latvia from the general population. We, therefore, assume that the ethnincity as such did not also play any role for the participation in the study.

In conclusion, our study demonstrated that the differences in sperm numbers found among young men from Latvia were associated with paternal ethnicity. With respect to the AR, higher seminal volume was found in men with long GGN tracts, but no other associations with seminal parameters were observed.

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