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ORIGINAL ARTICLE

Polymorphisms in genes regulating androgen activity among prostate cancer low-risk Inuit men and high-risk Scandinavians

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Summary

In Greenland, with a male population of approximately 30 000 individuals, the incidence of prostate cancer is extremely low with only three cases described during the period 1988–1997. Polymorphisms related to high androgen metabolism and/or response in the 5α-reductase type 2 (SRD5A2) and the androgen receptor (AR) genes, respectively, have been linked to prostate cancer. Our objective was to analyse whether the distribution of these polymorphisms differed between the prostate cancer low-risk population from Greenland and the relatively high-risk Swedish male population. The SRD5A2 polymorphisms A49T, V89L and R227Q, and the CAG and GGN repeats in the AR gene were genotyped in leucocyte DNA from 196 Greenlanders and 305 Swedish military conscripts. All subjects had the wild-type R/R genotype of the R227Q marker. The high-activity variants A49T A/T and V89L V/V occurred less frequently (2% vs. 5%, $p = 0.048$ and 33% vs. 46%, $p = 0.0027$) in Greenland compared with Sweden, whereas the low-activity L/L genotype was more frequent in Greenland (24% vs. 13%, $p = 0.0024$). Greenlanders also had longer AR CAG repeats than the Swedish population (median 24 vs 22, $p < 0.0005$). Greenlanders also had a higher frequency of the GGN = 23 allele (85% vs. 54%, $p < 0.0001$). Our results suggest that Greenlanders are genetically predisposed to a lower activity in testosterone to 5α-dihydrotestosterone turnover and to lower AR activity, which, at least partly, could explain their low incidence of prostate cancer.

Introduction

Prostate cancer (CaP), generally considered as an androgen-dependent disease, is one of the most commonly diagnosed malignancies in men in the western world and is now the leading cause of cancer-related death in men (Kolonel *et al.*, 2004). Ethnic differences in the incidence of clinical CaP have been observed, the highest rates found among African-Americans in the United States and the lowest rates found in Asia while Scandinavia is holding an intermediate position (Crawford, 2003). These differences could be caused by genetic susceptibility, which in family studies on twins and siblings shows a significant risk for siblings of affected individuals (Hemminki, 2001), and in health care and cancer registration, or a combination of these factors. In addition, CaP mortality varies worldwide, with the highest rates reported in Scandinavia and the lowest rates in China, Japan and countries of the former Soviet Union.

In the Inuit population in Greenland, genetically homogenous and related to the Asian (Nielsen & Storm, 1996; Larsen *et al.*, 1999), malignant diseases were virtually nonexistent in the beginning of the 20th century (Friborg *et al.*, 2003). During the second part of the century, the Greenland population underwent dramatic transitions, including changes in diet from mostly sea mammals to a more western diet and lifestyle. In parallel, increased incidences in diseases related to western habits, such as

obesity, diabetes and cardiovascular diseases have been reported (Nielsen *et al.*, 1996). Moreover, an increase in tumours common in western populations has been noted (Friborg *et al.*, 2003). Cancers of the testis and prostate are, however, exceptions, Inuit men still have one of the lowest incidence in the world, with three testicular cancer cases and three CaP cases noted during the period 1988–1997 (Nielsen *et al.*, 1996; Prener *et al.*, 1996; Friborg *et al.*, 2003). Not only manifest tumours, but also latent CaP is rare in these men compared with other populations, including Asians (Dewailly *et al.*, 2003).

Normal prostate function is dependent on maintenance of high 5α-dihydrotestosterone (DHT) concentration. In the prostate gland testosterone is irreversibly converted to DHT by the enzyme 5α-reductase type 2 (SRD5A2), which comprises three gene polymorphisms: A49T, V89L and R227Q, that have been shown to alter enzymatic activity in vitro (Makridakis *et al.*, 2000). The high-activity T and V-alleles were recently shown to be more frequent among men with CaP than among normal controls in ethnically matched study populations (Giwercman *et al.*, 2005b).

The action of testosterone as well as DHT is mediated by the androgen receptor (AR) (Quigley *et al.*, 1995). Within the amino-terminal transactivation domain of the AR gene, two stretches varying in length, one CAG and one GGN repeat, are located. Because of their capability to regulate AR activity in vitro, with short repeats resulting in a more active receptor (Chamberlain *et al.*, 1994; Gao *et al.*, 1996), the CAG repeat in particular has been extensively studied with respect to CaP. In some studies, a low CAG number has been attributed to an increased risk of developing CaP (Hakimi *et al.*, 1997; Stanford *et al.*, 1997; Hsing *et al.*, 2001; Ding *et al.*, 2004), whereas others have failed in finding an association (Coughlin & Hall, 2002; Giwercman *et al.*, 2005b). Less is known regarding the GGN repeat, but it was recently reported that low GGN numbers were associated with death in CaP (Giwercman *et al.*, 2005b). The differences between Scandinavians and Inuit regarding CaP risk make comparisons of these populations an interesting model for studying the impact of the genetic background in androgen turnover and response. Our objective was therefore to compare the frequency of the polymorphisms in the SRD5A2 gene and the AR gene distribution between men from Greenland and men from the general Swedish population.

Subjects

Greenland

Blood samples of fertile subjects were collected as part of European study (INUENDO) conducted in four regions in Greenland, which represent 62% of the total Greenland population (http://www.statgreen.gl). All subjects born in Greenland and of 18 years of age were included. The men in this study were 18–52 years old (mean: 31, SD 7). Among 257 eligible men, 201 provided a blood sample, resulting in a participation rate of 78%. Of these, one was excluded from the DNA analyses because of consanguinity (cousin to one subject) and four because of poor DNA quality. Thus, 196 blood samples were available for DNA extraction.

Sweden

Blood samples were collected from 305 young Swedish men under medical examination for military service, who agreed to participate in a study of reproductive function. The results have been published elsewhere (Richthoff *et al.*, 2002). Because of insufficient DNA quantity, genotyping was conducted in 291–305 men (numbers differing for the different polymorphisms).

All men participated with written informed consent according to protocols approved by the ethical review boards of Greenland and Lund University.

Methods

Genetic analyses

Genomic DNA was prepared from peripheral leucocytes. Allele-specific PCR for detecting the three SRD5A2 variants, A49T, V89L and R227Q was performed. Exons 1 and 4 of the SRD5A2 gene were first amplified with the flanking primers 5αX1F (TGG GAG GCG GGA TGG AGG) and 5αX1R (CGC CGG GAG CAG GGC AGT) and 5αX4fn (ATT CAG TTG CAA TGA TTG ACC TT) and 5α2x-4r (TCT GCG GGT TAA AAG CCT GTT), respectively, at concentrations of 0.5 μM (Invitrogen, Edinburgh, UK). One microlitre of each PCR product was then used in a subsequent allele-specific nested PCR using two reactions for each subject, each containing one wild-type or one mutant-specific primer: 5αA49 (AAC CAG GCG GCG CGG GC)/5αT49 (AAC CAG GCG GCG CGG GT) (0.5 μM), 5αV89 (ACC TGT GGA AGT AAT GTA C)/5αL89 (ACC TGT GGA AGT AAT GTA G) (0.6 μM) or 5αR227 (CTA TGG TGG TGA AAA GCT C)/5αQ227 (CTA TGG TGG TGA AAA GCT T) (0.5 μM), together with upstream primers 5αX1FN (ATG GAC GGG CGG GAG CCA) (0.2/0.025 μM) or 5αX4fn (0.5 μM) and downstream primers 5αX1RN (AGG GCA GTG CGC TGC ACT) (0.2/0.05 μM) or 5αX2–4r (AGA AGA AAG CTA CGT GAA TGC T) (0.5 μM) (Invitrogen). PCR conditions were established to generate both a short allele-specific and a longer control band in the presence of the variant, and only the longer control band in

its absence. Amplifications were carried out for 35–40 cycles; each cycle including denaturation for 1 min at 96 °C, primer annealing for 15–60 sec at 55–60 °C and primer extension for 3 min at 72 °C, with an initial denaturation step for 3 min at 96 °C and a final extension for 7 min at 72 °C.

Genotyping of the R227Q marker was limited to randomly selected subjects from both Greenland ($n = 102$) and Sweden ($n = 108$) as only the R/R genotype was found. Regarding the A49T and V89L variants, all 196 subjects from Greenland were screened. To verify the results of the allele-specific PCR, sequencing was performed on selected samples representing each genotype.

The CAG and GGN repeats were amplified as previously described (Lundin *et al.*, 2003).

Statistical analyses

The SRD5A2 polymorphisms were divided into categories according to genotype. The inter-population differences in CAG and GGN repeat lengths were tested by use of the Mann–Whitney test. Fisher's exact test was applied for comparing the distribution of the SRD5A2 polymorphisms and for the most common GGN variants (23 and 24). $p < 0.05$ was considered as statistically significant.

Results

SRD5A2 polymorphisms

The distribution of the SRD5A2 polymorphisms A49T and V89L is presented in Table 1. Two per cent of the Inuit population was heterozygous for the A/T genotype, compared with 5% of the Swedish population. This difference was statistically significant ($p = 0.048$). No T/T genotypes were found in any of the groups. Regarding the V89L polymorphism, 33% of the Greenlanders had the V/V genotype, 44% the V/L genotype and 24% the L/L genotype compared with 46%, 41% and 13% among the Swedes (Table 1). This corresponds to a significantly lower proportion of Greenlanders with the high-activity genotype V/V ($p = 0.003$) and a higher proportion with the lower enzymatic activity genotype L/L ($p = 0.002$).

Regarding the R227Q polymorphism, only the R/R genotype was detected in both groups.

Table 1 Distribution of SRD5A2 polymorphisms among fertile men from Greenland and Swedish military conscripts

	Greenland *n* (%)	Sweden *n* (%)	*p**
A49T			
A/A	193 (98)	276 (95)	0.048
A/T	3 (2)	15 (5)	
T/T	0 (0)	0 (0)	
V89L			
V/V	64 (33)	138 (46)	0.003
V/L	85 (44)	121 (41)	NS
L/L	47 (24)	39 (13)	0.002
R227Q			
R/R	102 (100)	108 (100)	
R/Q	0 (0)	0 (0)	
Q/Q	0 (0)	0 (0)	

NS, not significant. **p*-Value for comparison of frequencies between Greenland and Sweden.

AR polymorphisms

The number of AR CAG repeats in Greenland ranged from 13 to 29 repeats and from 12 to 30 in the Swedish group, with a median number of 24 and 22 respectively (Fig. 1). In the Swedish group, 21 repeats was the most common allele, whereas CAG = 24 was most frequent in Greenland. These distributions were significantly different with higher repeat number in Greenland compared with Sweden ($p < 0.0005$).

Only four different alleles of the GGN repeat (20, 23, 24 and 27) were detected among the Greenland Inuit, with 23 being the most common length, comprising 85% of the population (Fig. 2). Among Swedish males, the

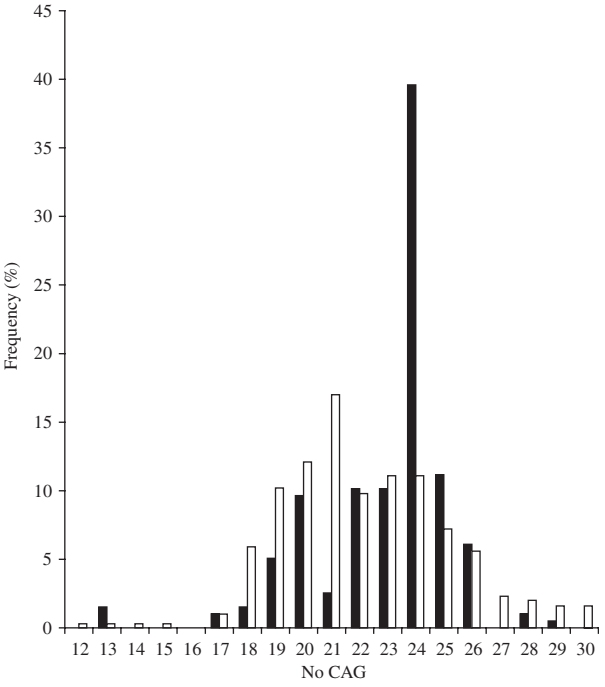

Figure 1 The distribution of CAG repeat lengths in 196 Inuit and 305 Swedish men. Black bars represent the Greenland population and white bars the Swedish population.

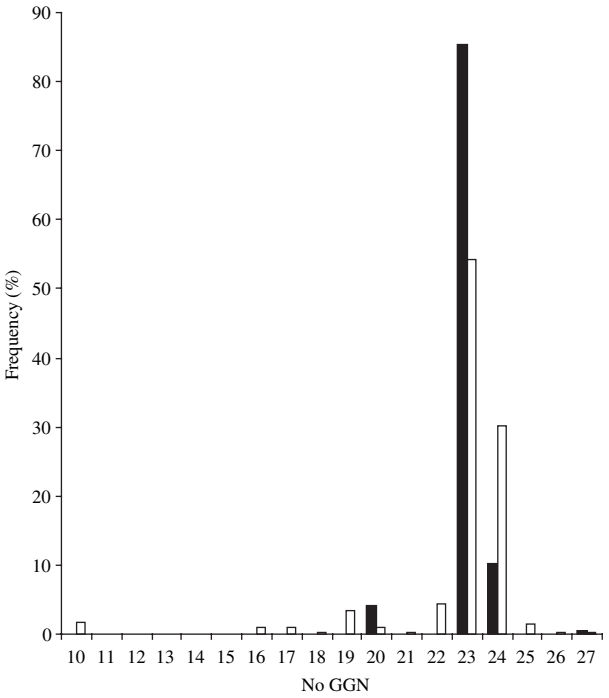

Figure 2 The distribution of GGN repeat lengths in 196 Inuit and 291 Swedish men. Black bars represent the Greenland population and white bars the Swedish population.

number of repeats ranged from 10 to 27, with 23 and 24 as the most frequent alleles (54% and 30% respectively) (Fig. 2). Significantly more Greenlanders than Swedish men had GGN = 23 (85% vs. 54%, $p < 0.0001$), whereas GGN = 24 was less frequent in Greenland (10% vs. 30%, $p < 0.0001$). The median GGN numbers were 23 in both groups. These two distributions also differed significantly ($p = 0.005$).

Discussion

We have compared the distributions of three polymorphisms in the SRD5A2 gene as well as the CAG and GGN repeats in the AR gene in a CaP low-risk population of Inuit men from Greenland and a cohort of Swedish males, having significantly higher risk of the malignancy.

A distinct difference in the distribution of the genotypes was revealed. The prevalence of the SRD5A2 A49T T-allele, which is rare in most healthy populations (Coughlin & Hall, 2002), was only 40% in men from Greenland compared with that in the general Swedish population. In contrast, in 89 Swedish CaP patients, the frequency of the T-allele was found to be more than two times higher than that among the controls (Giwercman *et al.*, 2005b). In experimental studies, the T-allele has been shown to increase the enzymatic activity almost five-fold (Makridakis *et al.*, 2000) and in vivo, the serum concentration of prostate-specific antigen, which is used worldwide as a marker of prostate disease, has been reported to be higher in men heterozygous for the T-variant (Schatzl *et al.*, 2002).

Our data also showed that the L/L genotype of the V89L marker, which in vitro was shown to result in an almost 30% reduction in enzymatic activity compared with the V/V genotype (Makridakis *et al.*, 2000), was approximately twice as common in men from Greenland (24%) compared with Swedish men (13%). Distributions of V89L genotypes reported parallel the patterns of CaP in high- and low-risk populations, with L/L genotype prevalence of 22–25% among Asians and about 4% of Caucasians and African-Americans (Hsing *et al.*, 2001). Keeping in mind that the Greenland population is genetically related to the Asian population, our data are consistent with these results.

With respect to the AR gene and CaP risk, the polymorphic CAG segment has been a matter of debate for more than 10 years in a number of studies without any firm conclusions. Some studies have also reported short GGN alleles to be associated with CaP (Hakimi *et al.*, 1997; Stanford *et al.*, 1997; Edwards *et al.*, 1999), whereas others reported a lack of association (Correa-Cerro *et al.*, 1999). However, both the CAG and the GGN repeats have been found to regulate AR activity in vitro; long CAG repeats resulting in a less active receptor than shorter repeats and a 30% reduction in the AR transactivating capacity when the GGN repeat was deleted (Chamberlain *et al.*, 1994; Gao *et al.*, 1996). Among our Caucasian controls, the most common GGN alleles were 23 and 24 GGN repeats (Lundin *et al.*, 2003), whereas the most common allele on Greenland was 23 GGN. Interestingly, GGN numbers shorter than 23, which have been related to death in CaP, were only found in four individuals. The functional consequences of small differences in GGN lengths are currently unknown. There are no pathophysiological conditions linked to this segment, and to date there is only one in vitro study regarding different lengths. ARs expressing 19–23 GGN triplets were tested in response to 10 nM of the synthetic testosterone R1881. No significant difference in AR transcriptional activity was noted. However, only one concentration of R1881 was tested and no comparisons were made with the second most common GGN length of 24 repeats, or with extremely short or long GGN repeats. Furthermore, all constructs contained a CAG repeat length of 24, which is above the average length (Ding *et al.*, 2005).

In this study, we compared a population of fertile Greenlanders with a group of Swedish military conscripts representing the general population of young males, of

whom some are expected to experience infertility later in life. A recent study of almost 50 000 men with CaP indicated an increased risk of this malignancy in fertile men (Giwercman *et al.*, 2005a). As the Inuit men represent a low-risk population, a slightly increased CaP risk among fertile men should diminish the difference between the two cohorts rather than produce false-positive results. The same is true for a possible mixing of a slight proportion of men with Danish ancestors, genetically similar to Swedes, among the Inuit.

We did not take dietary habits or environmental exposure into consideration and to our knowledge no specific dietary factor or environmental exposure has so far been associated with increased CaP risk. In contrast, studies suggest that monounsaturated fatty acid and *n*-3-polyunsaturated fatty acids of marine origin might have a protective effect (Terry *et al.*, 2001; Dewailly *et al.*, 2003). Although the fat intake now is equivalent to that of western societies, marine mammals and fish are still the traditional diet and important sources of protein and fat in Greenland. Thus, a protective ingredient in the food cannot be excluded.

In summary, our data suggest that the Greenland population, at least partly, is protected against CaP because of a generally lower genetically determined androgenicity compared with the high-risk general Swedish population. It remains to be elucidated whether this is a direct effect of lower rate of conversion of testosterone to DHT and lower AR action, or rather that these genotypes in combination with other genetic or lifestyle-related factors make men from Greenland less susceptible to factors that might have an impact on the risk for prostate malignancy.

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