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Ethnic differences in occurrence of TDS – genetics and/or environment?

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Summary (243 words)

It has been hypothesized that poor semen quality, testis cancer, undescended testis and hypospadias are symptoms of one underlying entity, the so called testicular dysgenesis syndrome (TDS). TDS was suggested to be a result of disruption of embryonal programming and gonadal development during foetal life and as aetiological factor, an impact of adverse environmental factors such as hormone disrupters, probably acting upon a susceptible genetic background, was suggested.

Extensive studies considering the risk of TDS related diseases in Denmark compared to Finland, showed higher sperm counts and lower risk of cryptorchidism and testicular cancer among Finns. However, when comparing these two populations the question arises, to which degree this difference might be due to discrepancy in genetic background. A more obvious example of the impact of genetic factors on the risk of TDS concerns Afro-Americans having significantly lower incidence of testicular cancer as compared to Caucasians living in the USA.

A yet unexplored scenario is a possible interaction between genetic and environmental/life-style-related factors, certain genotypes making individuals more susceptible to adverse exogenous exposures. Studying such interactions has biological, epidemiological and public health related implications. It will help us to understand the background for the defects in male reproductive organs, facilitate proper design of epidemiological studies and add to identifying individuals susceptible to certain environmental and life-style related hazards. Such ‘susceptibility genes´ need to be identified, those involved in the synthesis, action and metabolism of sex steroids being strong candidates.

Testicular Dysgenesis Syndrome

The Carlsen *et al* paper (Carlsen et al. 1992) published in the British Medical Journal in 1992, was a starting point for a still ongoing discussion on whether male reproductive function has deteriorated with time or not. The question of falling sperm counts was and is still debatable. In order to show that there is a real reduction in sperm numbers, properly designed prospective studies are needed. However, such data are not available and even presuming an 1 % rate of decline yearly, as suggested in the Carlsen *et al* study, it is realistic to assume that it will at least take 10-20 years before such a trend can be documented. Therefore, the focus has switched from 'secular trend' to 'geographical differences'

In 2001 Skakkebaek *et al* introduced the concept of TDS, suggesting that poor semen quality, testicular germ cell cancer, undescended testis and hypospadias are symptoms of one underlying entity (Skakkebæk et al. 2001). TDS **w**as suggested to be a result of disruption of embryonal programming and gonadal development during foetal life and concluded that "…the aetiological impact of adverse environmental factors such as hormone disrupters, probably acting upon a susceptible genetic background, must be considered." One of the implications of the "TDS concept" is that in future human and experimental studies any of the TDS components might be used as a general marker of the male reproductive system. Furthermore, it clearly pointed to the foetal period as a critical time window for abnormalities of the male reproductive organs and to the importance of genetic susceptibility. However, although recent data might indicate that even life-style related factors, as e.g. mothers smoking during the pregnancy, should be considered as a possible factor behind impairment of sperm production (Storgaard et al. 2003; Jensen et al. 2004) and perhaps also the risk of testicular cancer (Kaijser et al. 2003), there are virtually no data showing that certain genotypes predispose to detrimental effects of environment and/or life style on male reproductive function. The evidence of existence of such phenomenon, if any, is rather

indirectly based on association studies, linking certain genetic variants in a given population to its reproductive performance measured as semen quality or risk of testicular cancer or congenital genital malformations. In the following part of this review, some examples will be given and the impact of possible genetically determined susceptibility will be discussed.

Semen quality

During the past few years several studies have addressed the issue of geographical differences in sperm concentration, motility and morphology. However, a significant part of these studies has been based on cohorts of partners to pregnant women (Jorgensen et al. 2001; Swan et al. 2003), recruitment implying a significant selection bias based on the reproductive capability of the study subjects. Surveys based on military conscripts seem to a higher degree to mirror semen quality in 'general population' at least in adolescents. Most of the available data comes from the Baltic area, the biggest study including men from Denmark, Estonia, Finland and Norway, showing clearly higher sperm concentration in Finland and Estonia as compared to that in Denmark and Norway (Jorgensen et al. 2002). Interestingly, the incidence of testicular cancer in Denmark and Norway is among the highest in the world, this type of malignancy being much less frequent in Finland and in the Baltic countries (Richiardi et al. 2004). Subsequent studies have indicated that also Latvian and Lithuanian conscripts have sperm numbers, at a similar level as in Finland and Estonia (Punab et al. 2002; Tsarev et al. 2005). An East-West 'gradient' was suggested, with higher sperm numbers in the Eastern part of the Baltic area (Fig. 1). Whether such a gradient could be due to differences in environmental exposure, life-style or genetic dissimilarities is still an unresolved question. To date, there are no data on any genetic polymorphisms influencing sperm production, which might differ in frequency between Denmark and Norway on one hand and Finland and the Baltic countries on the other hand. However, it has been shown that the Y chromosome haplotype 16 is more

common in the East area of the Baltic region as compared to Denmark, Norway and Sweden (Rosser et al. 2000). Although a Danish and a Japanese study indicated some association between Y chromosome haplotypes and sperm numbers, we do not have sufficient information to interpret these haplotypes in terms of function of certain genes involved in the process of spermatogenesis (Kuroki et al. 1999; Krausz et al. 2001).

Baltic countries, due to their ethnic mixture of the native and Russian population – two groups which may differ in genetic terms but live under same conditions – may represent an interesting model for studying the relative influence of genes, life style and environment on sperm numbers. In a recent study of Latvian military conscripts, significantly higher sperm concentration was found in men with both parents born in Latvia (77±60 x 10^6/mL), compared to men with both parents born outside the Baltic area (55±45 x 10^6/mL, p=0.03) (Tsarev et al. 2005). In the latter group the majority being of Russian origin. If such difference is also present in the other Baltic countries has not been shown yet. Furthermore, we do not have any information proving that this difference in sperm concentration between young men of Latvian origin and those having non-Baltic parents is due to genetic differences between these ethnic groups. The men were raised within the same geographical area and life-style related factors as time of abstinence and smoking habits both of the subject himself and of his parents were taken into consideration in the statistical analysis. However it cannot be excluded that other social factors, e.g. dietary habits, may differ between groups with diverging ethnic origin.

It can be speculated which genetic factors, if any, might be responsible for ethnic differences in sperm parameters. Having in mind the role of sex steroids in normal spermatogenesis and the function of epididymis and accessory sex glands as well as the fact that many of the environmental compounds classified as ‘endocrine disrupters’ do have sex hormone mimicking effect, genes involved in androgen and oestrogen synthesis, metabolism

and action belong to strong candidates. One study has shown significantly higher (40.6% vs 21.4%) frequency of the oestrogen receptor (ER) α *XbaI* xx genotype in fertile men as compared to men from infertile couples presenting with with azoospermia or idiopathic severe oligozoospermia (sperm counts of ≤10 x10^6/mL) (Kukuvitis et al. 2002). However, to which extent this polymorphism of ER α affects the function of the receptor and whether the distribution of the *XbaI* xx genotype is dependent on the ethnic origin, is yet unknown.

Another genetic polymorphism which has attracted a lot of attention in relation to semen quality is the glutamine encoding CAG repeat of the androgen receptor (AR) gene. *In vitro* the CAG number was found to be inversely correlated with the receptor function (Tut et al. 1997) and corresponding to the findings, some studies have shown a negative correlation between CAG number and sperm concentration as well as motility (von Eckardstein et al. 2001; Giwercman et al. 2004b). In a German contraceptive study, significantly higher chance of achieving azoospermia, despite incomplete gonadotropin suppression, was found in men with more than 22 CAG as compared to those with a CAG length of 22 or less (Eckardstein et al. 2002). Furthermore, in a group of patients receiving 3 or more cycles of of cytotoxic drugs, due to metastatic testicular cancer, the regeneration of spermatogenesis 1-2 years post treatment was negatively correlated to the AR CAG number (Eberhard et al. 2004). Ethnical differences in the mean CAG number have been reported, with a mean length of 19-20 in Afro-Americans; 21-22 in Caucasians and 23-24 in Asians. However, there are no data showing corresponding differences in sperm concentration between men representing these three ethnic groups.

Although above mentioned examples indicate that there might be a link between genetic factors and ethnic differences in semen parameters any direct evidence is lacking. Furthermore, studies of military conscripts from Denmark and Southern Sweden indicate that even in two genetically similar populations, a significant difference in sperm number –

probably due to yet unknown environmental and/or life-style related factors – may exist (Richthoff *et al*, 2002).

Testicular cancer (TGCC)

Reliable cancer register data clearly show significant geographical and ethnical differences in the incidence of TGCC. This discrepancy is very clear in the Baltic region, where the two countries with the lowest sperm counts – Denmark and Norway – also exhibit equally high risk of this malignancy. Sweden holds an intermediate position, whereas the Baltic countries and Finland have 3-4 times lower incidence (Richiardi et al. 2004) (Fig. 1). Similar considerations regarding the relative impact of environment, life-style and genetic background, as those given with reference to semen studies could be extended to the issue of TGCC. However, until now, no genetic factors associated to the risk of testicular malignancy have been recognised. On the other hand, a recent study based on register data in Denmark, Finland, Norway and Sweden indicated a very strong correlation between the increase in prevalence of maternal smoking during pregnancy and the substantial rise in the incidence of TGCC (Pettersson et al. 2004). The country specific correlations were of the same magnitude in all countries, except Finland, once again indicating the differing genetic origin of this population as a possible cause of diverging panorama of male reproductive disorders.

Comparison of TGCC incidence risk between US men being of Caucasian and African origin is another example of clear ethnic difference in the TDS, the risk of testicular malignancy being very low in the latter group of men (McGlynn et al. 2003). Having in mind the ethnic differences in the AR CAG repeat length, one could speculate that the higher androgen sensitivity among Afro-Americans could protect them against TGCC. However, in two studies, no association between the CAG number and the risk of TGCC was found (Rajpert-De Meyts et al. 2002; Giwercman et al. 2004a).

Cryptorchidism and hypospadias

These two congenital malformations of male genital organs have also been reported to be subjects of time-related increase in incidence and as considers the latter, there are register data indicating a clear ethnic difference (http://www.icbd.org/). However, since the diagnostic criteria for both these conditions differ from centre to centre, such findings should be taken with some caution. However, in a carefully performed prospective study of newborns, the odds ratio for cryptorchidism was found to be 4.4 times higher at birth and 2.2 times higher at 3 months of age among Danish boys as compared to Finnish (Boisen et al. 2004). Notably, these findings parallel semen and TGCC data, showing lower risk of TDS in Finland as compared to Denmark, the reason of such a discrepancy still being unresolved.

Although cryptorchidism and hypospadias are the most common congenital abnormalities their aetiology is not yet completely understood. However, it is known that androgens, including dihydrotestosterone (DHT), play an important role for normal testicular descent and for development of external male genitalia. Mutations in the AR gene or in the gene encoding for the 5-α-reductase type II enzyme, responsible for the conversion of testosterone to DHT are associated with undescended testis(-es) and/or hypospadias (Sultan et al. 2001). However, these mutations are extremely rare and hardly responsible for the ethnic gradient in the occurrence of TDS. No association has been found between the AR CAG repeat length and any of these congenital male genital abnormalities (Muroya et al. 2001). On the other hand, we found a difference between Swedish healthy men and ethnically matched subjects with penile hypospadias or a history of cryptorchidism as considers distribution of lengths of another trinucleotide repeat in the AR gene – the GGN repeat (Aschim et al. 2004). In the control group the dominating GGN length was 23, found in 54% of the subjects, whereas 32% presented with GGN of 24 or more. In patients with penile hypospadias, a statistically significant switch in the GGN repeat lengths was found, with 31% having

GGN=23 and 69% carrying GGN of 24 or more. Interestingly, similar deviation form the control group was found in men with a history of cryptorchidism, the proportions being 35% for GGN=23 and 65% for GGN of 24 or longer (Fig. 2). These data might indicate that those with GGN of 23, which is the most common allele among Caucasians, might be less susceptible to factors causing penile hypospadias and cryptorchidims. Since GGN of 24 or more occurs in more than 30% of healthy subjects, it seems obvious that it not *per se* causes, but rather predisposes to the congenital abnormalities of male genital organs acting in an interplay with other genetic, environmental and/or life-style related factors. Considerable ethnic differences in the distribution of GGN repeat length has been reported. In East Asian men the GGN=23 is the predominating allele, found in 75% of all subjects (Sasaki et al. 2003). Interestingly, Japanese males have one of the lowest incidences of hypospadias in the world (http://www.icbd.org/).

Furthermore, it should also be mentioned that the combination of AR CAG length below 21 and the GGN length of 23 was found to be 4 times more common in the general Swedish male population as compared to infertile men with low sperm counts (Ruhayel et al. 2004), this findings linking the AR polymorphisms to three of the four components of the TDS.

TDS – genetics, life-style and environment

Although knowing the importance of several genetically regulated factors for a normal development and function of the male reproductive organs, apart from some few association studies, there is only a limited amount of evidence to prove the impact of genetic variation on the ethnic differences in the occurrence of the TDS. However, there is also a lack of data showing that the geographical variation in the frequency of disorders of male genital organs mirrors regional differences in exposure to major 'endocrine disupters'. Additionally, at least

when considering studies combining postnatal exposure assessment and sperm parameters as outcome variables, the effect of exposure to some of the most common 'endocrine disrupters' – PBC's and phthalates – on male reproductive function seems rather limited (Rignell-Hydbom et al. 2004; Duty et al. 2003a; Duty et al. 2003b; Duty et al. 2004; Jönsson et al. 2005). Only little attention has yet been paid to combining exposure data with genetic predisposition of the subjects in question. However, the advantages of including genetics are obvious from *biological, public health* and *epidemiological* point of view.

Digging into genetic aspects of susceptibility of male reproductive organ functions to external factors is important for understanding the biology of male reproductive dysfunction. Furthermore, it can also teach us about the normal physiology of male reproduction, a knowledge which is of highest importance for developing treatment strategies for such a common disorder as male sub-fertility. From the public health point of view, in order to prevent disorders of male reproductive organs, it seems important to develop tools for identifying individuals being susceptible to adverse effects of environment and life-style. Finally, taking genetically determined susceptibility into account is also important when planning epidemiological studies. This could be illustrated by the following example. We assume that it is only individuals with a 'susceptibility genotype' that are affected by a certain environmental exposure, and that 50 % of the population have the 'susceptibility genotype'. In addition, we assume that 50 % of the individuals in a population have environmental exposure above the necessary threshold level. If we then compare this sensitive group of individuals (exposed individuals with the 'susceptibility genotype', *i.e.* 25 % of the population) with all other individuals, we need 400 subjects for a study to detect a difference in sperm concentration of 20 x 10^6/mL (SD 60 x 10^6/mL) with a power of 80 % (α=0.05). If we do not perform genetic analysis, the difference in mean sperm concentration between the exposed and non-exposed group will be 10 x 10^6/mL, and we will need 1,100 subjects to

detect a difference between environmentally exposed and unexposed individuals. Figure 3 illustrates that the number of individuals necessary to include in the study increases dramatically when the 'susceptibility genotype' is rare. The lesson learned from this example is that a prerequisite for a meaningful evaluation of gene-environment associations is that 'susceptibility' genotypes are relatively prevalent in the study base. Infrequent alleles may from theoretical or experimental points of view be highly relevant for modifying gene-environment interactions, but their impact may be impossible to prove empirically due to the extremely large studies needed. For case-control designs data on minimum sample size estimation to detect gene-environment interaction can be derived from the literature (Hwang et al. 1994).

One of the reasons behind the scarcity of studies on genetically determined susceptibility to environmental/life-style related exposure in relation to TDS may be lack of good 'candidate genes' to be investigated. However, the rapid development of the field of molecular biology and the increasing level of knowledge regarding the human genome open up for rapid and low-cost multi-gene screening. Such an approach should be applied in future studies on the interaction between the environment/life-style and male reproductive function.

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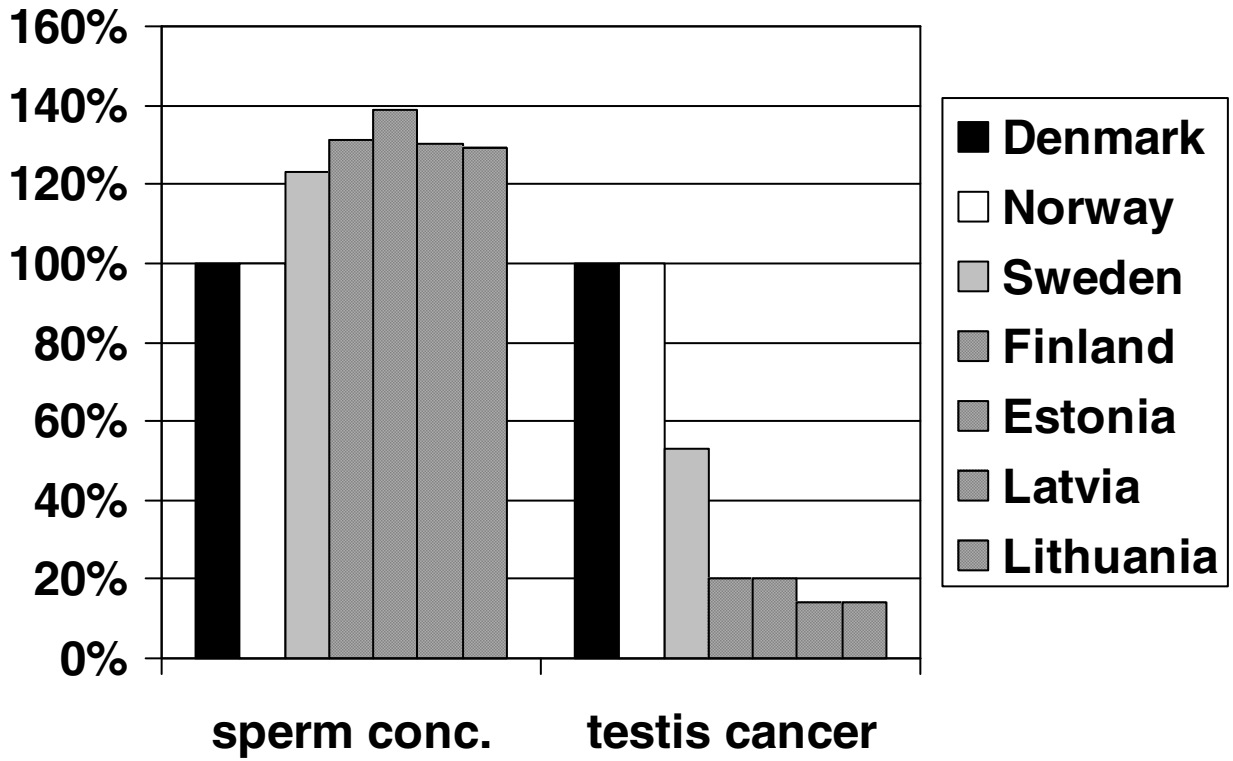
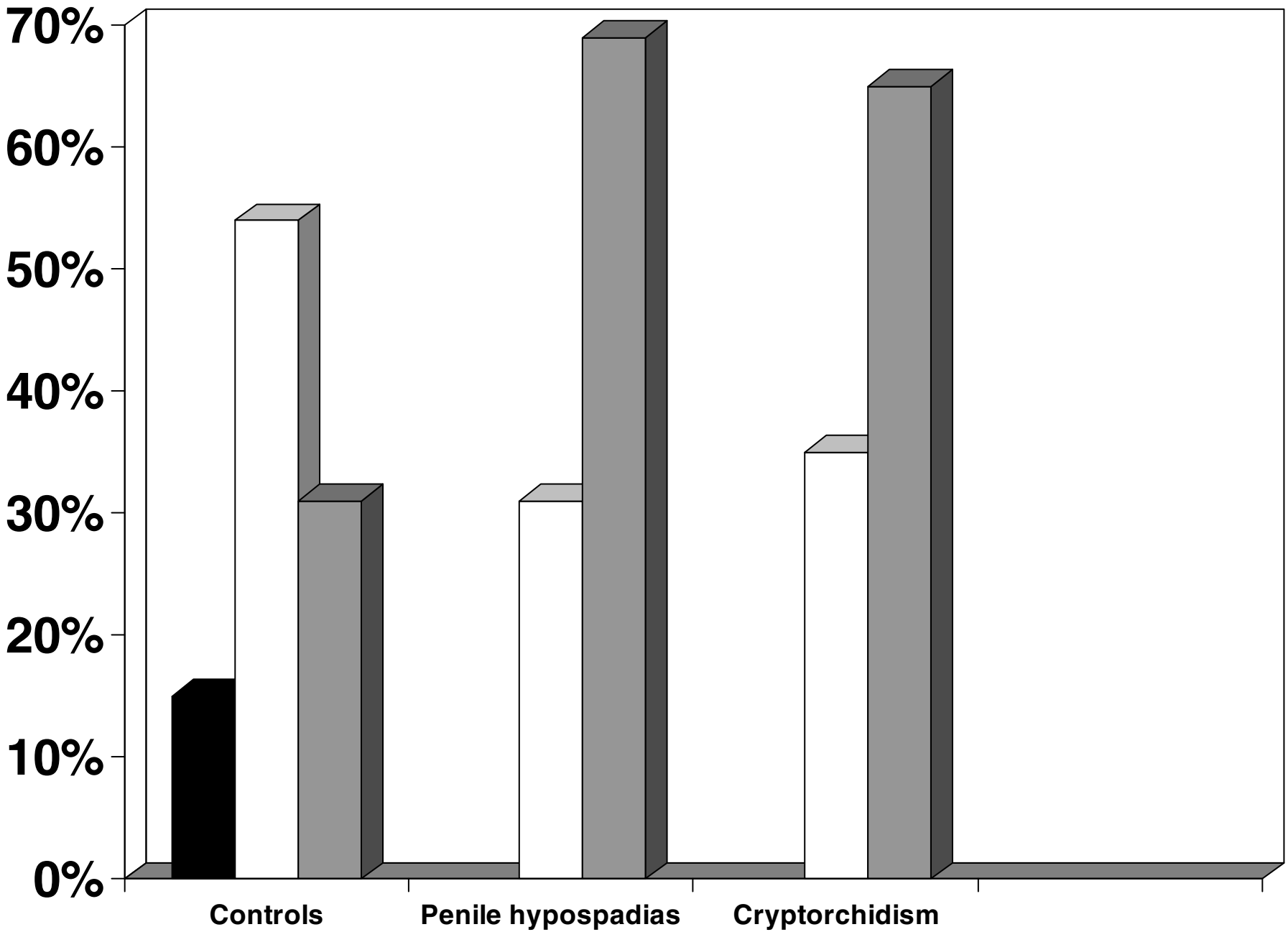
Legends to figures

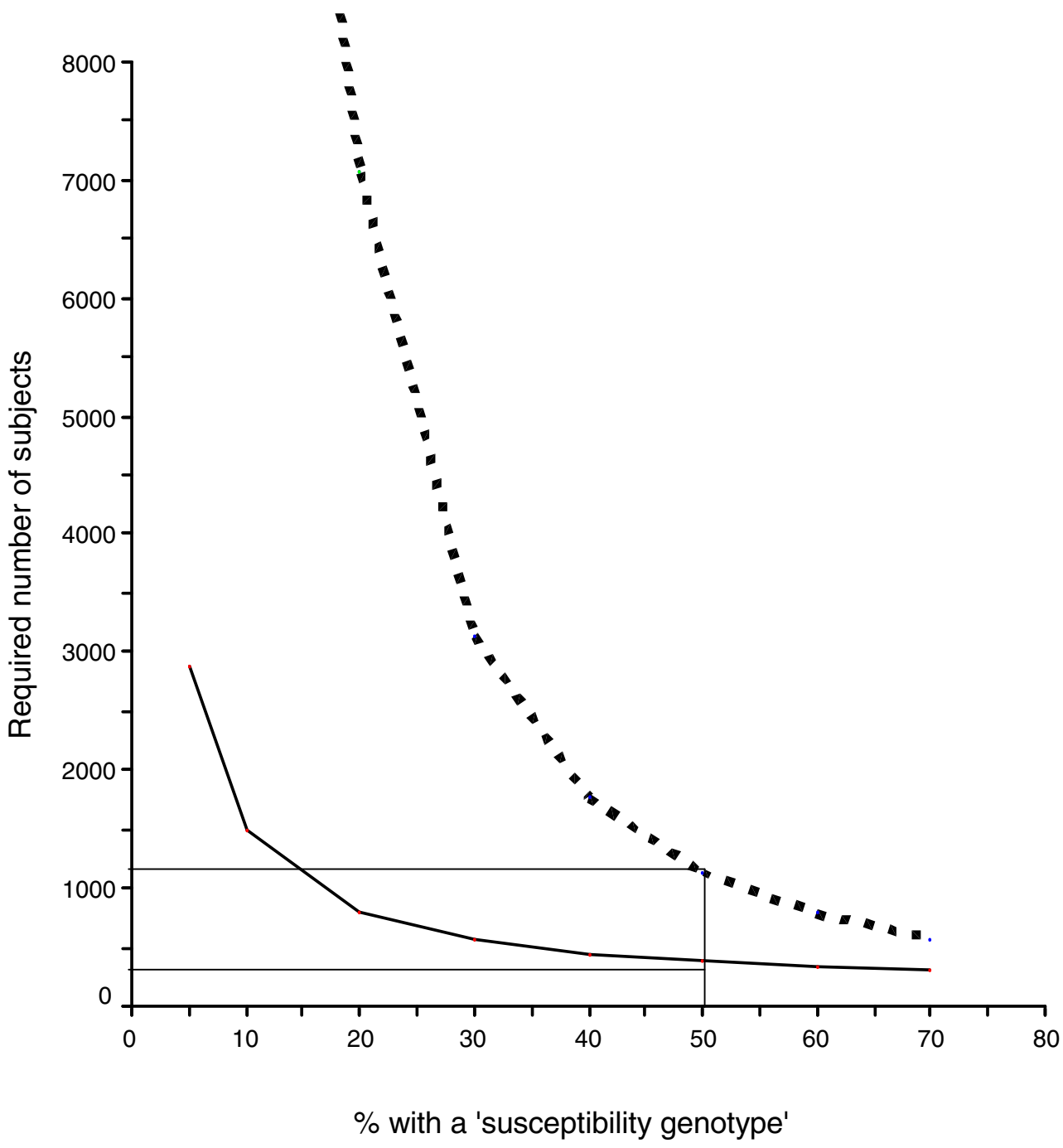
Fig 1: Relative levels sperm concentration among military conscript and incidence of testicular cancer in Denmark, Estonia, Finland, Latvia, Lithuania, Norway and Sweden. These figures were based on data from literature (Jorgensen *et al* 2002; Punab *et al*, 2002; Richthoff *et al*, 2002; Richiardi *et al*, 2004; Tsarev *et al*, 2005). Denmark was set as 'reference country' (=100%);

Fig 2: Distribution of androgen receptor GGN repeat lengths among controls, subjects with penile hypospadias and men with a history of cryptorchidism. Based on data from Aschim *et al* (2004);

Fig 3: Minimum numbers of subjects required for 80% power at 5% Type I error in a study on effect of environmental exposure/genotype on sperm concentration. The calculations were based on a hypothetical scenario indicated in the inserted table. Fifty percent of a given population is exposed above a certain level necessary to decrease sperm concentration of the exposed individuals. However, this effect is only seen in subjects having certain 'susceptibility genotype'. In this subgroup the mean sperm concentration decreases from 60 x 10^6/mL to 40 x 10^6/mL, whereas it remains unchanged in the remaining subgroups. The two curves indicate the size of study population – set in relation to the frequency of 'the susceptibility genotype' - necessary to show statistically significant difference between two groups in case genotyping is performed (continuous line: exposed and having 'the susceptibility genotype' against others) or not (dotted line: exposed vs. non exposed). The difference in mean sperm concentration between the exposed and non-exposed group is 10 x 10^6/mL, in case the frequency of the 'susceptibility genotype is 50%, 5 x 10^6/mL if it is 25% etc. The standard deviation for inter-individual variation in sperm concentration was set to 60 x 10^6/mL.


160%
140%
120%
100%
80%
60%
40%
20%
0%
sperm conc.
testis cancer
Denmark
Norway
Sweden
Finland
Estonia
Latvia
Lithuania


70%
60%
50%
40%
30%
20%
10%
0%
Controls
Penile hypospadias
Cryptorchidism


Required number of subjects
8000
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
% with a 'susceptibility genotype'