**Postnatal screening for Klinefelter syndrome: is there a rationale?**

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**ABSTRACT**

Diagnosis of Klinefelter syndrome (KS) allows for timely beneficial interventions across the lifespan. Most cases currently remain undiagnosed because of low awareness of KS amongst health professionals, the hesitancy of men to seek medical attention and its variable clinical presentation. Given these barriers, population-based genetic screening provides an approach to comprehensive and early detection. We examine current evidence regarding risks and benefits of diagnosing KS at different ages.

**Conclusion:** There is a lack of evidence regarding the influence of age at diagnosis on adult outcomes that can only be obtained through a pilot screening programme.

**INTRODUCTION**

Klinefelter syndrome (KS) is the most common chromosomal disorder affecting males, caused by an additional X chromosome (47,XXY) and characterized by variable clinical features (1). The phenotype almost always includes azoospermia and small testes, but a spectrum of other features are seen including testosterone deficiency, tall stature, gynecomastia, poor virilization and cognitive and behavioural difficulties (2). Testosterone replacement therapy and other interventions are available to at least partly alleviate these difficulties.

The prevalence of KS has been estimated at 1 in 650 (3), with evidence suggesting the prevalence may be increasing (4), and recent estimates as high as 1 in 450 (5). This equates to more than 1.2 million men with KS across Europe – yet up to 75% of KS remains undiagnosed (3). Most cases are diagnosed postnatally, and even then usually in adulthood during fertility investigations, which may be beyond the point for optimal intervention.

Over the past decade, the fate of the undiagnosed majority of men with KS has been the subject of much speculation: Do these individuals experience medical or psychosocial health problems to the same (or lesser) extent than those that are currently identified? Are their lifetime prospects of gainful employment, good quality of life or establishing a stable partnership similar to that of the general population? One can suppose that this undiagnosed majority do indeed experience difficulties across a spectrum of health domains, yet remain undetected because of low awareness of KS amongst health professionals, the hesitancy of men to seek medical attention and the often non-specific manifestation of symptoms (6).

This systemic lack of detection may deprive many men with KS of access to timely and potentially beneficial interventions, both for themselves and for their families, with the possibility of being assigned to a ‘subordinate social niche’, as some have suggested (7). The challenge is to ensure that all KS individuals receive appropriate attention if and when required. What is the optimal strategy for diagnosis and what evidence exists regarding the benefits of treatment to assist these individuals in fulfilling their potential?

Early diagnosis would allow the opportunity for therapy when most effective and in some cases potentially preventing later symptoms or complications (8). Population-based genetic screening for KS would achieve comprehensive and early diagnosis, but has never been formally explored. Screening programmes require consideration of a number of social, legal and ethical issues (9), and the potential consequences of diagnosis, positive and negative, should be examined prior to implementation. Accurate, fast and relatively cheap molecular screening tests are available for KS.

**Abbreviations**

BMD, Bone mineral density; FSH, Follicle-stimulating hormone; FXS, Fragile X syndrome; ICSI, Intracytoplasmic sperm injection; KS, Klinefelter syndrome; LH, Luteinizing hormone; NBS, Newborn screening; TESE, Testicular sperm extraction.
and stages of development: Ages were chosen to represent possible screening points and stages of development:

- Newborn (5 days)
- Infancy (1 year)
- Childhood (5 years)
- Puberty (11 years)

These were compared to being diagnosed in adulthood or never being diagnosed. The ages examined are somewhat arbitrary and could be modified to accommodate the ideal age at diagnosis (e.g., 6 months of age). Whilst impact on individuals is only one consideration that should be examined prior to implementing a screening programme (9), this is an essential first step in deciding whether screening for KS is appropriate.

POPULATION-BASED GENETIC SCREENING

In 1968, Wilson and Junger on behalf of the World Health Organisation published a set of principles to be applied in decision-making for any public health screening programme (14). As technology has advanced, these original screening principles have evolved to include new and potential genetic technologies (15), in addition to considering conditions that do not fulfil the traditional principles (16). For a condition to be considered appropriate for screening from a public health perspective, it should be an important health problem with a latent early symptomatic stage and well-understood natural history, an accepted treatment and facilities for diagnosis and treatment. There should also be a suitable test that is acceptable to the population, a policy on whom to treat and a cost-benefit analysis.

Over the last decade, various frameworks have been developed to assist in addressing the many aspects of a screening programme prior to implementation. For example, the ACCE framework (acronym based on the four components of the framework – analytic validity, clinical validity, clinical utility and associated ethical, legal and social implications) was developed by the Centres for Disease Control and Prevention (CDC) as a process for evaluating emerging genetic tests (17). In the United Kingdom, a ‘Gene Dossier’ process has been developed to evaluate genetic tests (18). Many of the outcomes for KS involve morbidity and psychosocial parameters such as quality of life, which are problematic to quantify compared with conditions where mortality is the outcome of non-diagnosis. Before the potential effectiveness of a screening programme for KS can be established, an understanding of the medical and psychosocial risks and benefits of diagnosis with KS at different ages is required.

MEDICAL AND PSYCHOSOCIAL ASPECTS OF KS

We have identified five broad areas of medical and psychosocial considerations relevant to KS (13):

1. testosterone deficiency and treatment,
2. developmental difficulties and available interventions,
3. infertility and reproductive options,
4. comorbidities and related prevention and management strategies and
5. psychosocial impacts of diagnosis and non-diagnosis itself (as distinct from those related to medical symptoms and treatments).

We consider the potential impacts on individuals of being diagnosed with KS at different age points in relation to these domains. For each age, domains are addressed in order of relevance.

NEWBORN SCREENING

Genetic screening of newborns is a convenient age for testing, as up to 99% of newborns are currently tested (19). Newborn screening (NBS) is carried out in most developed countries for conditions such as phenylketonuria, for which early diagnosis may prevent severe morbidity, or cystic fibrosis, for which early intervention decreases morbidity and increases life span (9). Recently, there has been discussion of including genetic conditions for which the outcome of NBS may be an increase in quality of life, such as FXS, or where there is reproductive benefit to the parents, such as Duchenne muscular dystrophy. Like FXS, the presentation of KS can be highly variable, with few clinical indications in newborns (1).

Testosterone deficiency and treatment

Whilst there are no early clinical features of testosterone deficiency in males with KS, there is some evidence to suggest the postnatal testosterone surge normally seen in between 2 and 4 months of age is attenuated in newborns with KS (20). It is thought that early testosterone deficiency impacts on penile growth and testicular size (21) and possibly brain development. With further investigation, testosterone treatment may one day play a role prior to puberty (when it is usually instigated) (22). Ensuring a normal testosterone profile across the ‘mini-puberty’ in early life is an attractive notion, but evidence of benefit is lacking and further research is required.

Psychosocial impacts of diagnosis and non-diagnosis

Diagnosis of a genetic condition in a newborn may have a number of psychosocial risks. It has been suggested that this
may impact on initial bonding between parent and child (23). Concern and anxiety about future health issues (which may never become apparent) could lead to parental hypersensitivity to child behaviour and overprotection (11), known as ‘vulnerable child syndrome’ or the ‘making of the presymptomatic individual’ (24). However, parents and families also develop unique ways of adapting to the uncertainty of a genetic diagnosis such as KS, and an emphasis on such uncertainty may assist in removing the deterministic value a genetic label can carry (25).

Developmental difficulties and available interventions
Decreased muscle tone may be evident in the first year of life (26). This is discussed further at the next age point (infancy).

Infertility and reproductive options
Comorbidities and related prevention and management strategies
There is future benefit of diagnosing newborns, as early knowledge of infertility and comorbidities allows for timely intervention at a later age; however, there is no immediate benefit.

Screening in infancy
Developmental difficulties and available interventions
Delayed speech development is often evident by 12 months of age, as is delayed ambulation (27). Recently collated results from unbiased longitudinal studies showed delays in the earliest stages of language development, with up to 53% of boys requiring later speech and language therapy (28). Difficulties with fine motor skills and strength together with increased clumsiness may also be observed, possibly as a result of decreased muscle tone seen in the first year of life (26). It has been suggested that this may contribute to later avoidance of sporting activities, noted by some studies.

The first few years of development, during which children are extremely receptive to learning, provide a vital opportunity for effective intervention, particularly in regard to brain development (24). Early speech and physical therapies are available to assist with the aforementioned problems and may reduce the need for more extensive interventions later in childhood. Whilst language and motor symptoms may not be severe for many infants with KS, early intervention may prevent secondary problems from developing later (29). In addition, commencing intervention at this age may be preferable to primary or high school, where children receiving special assistance may be more prone to stigmatization by peers (see childhood) (30).

Comorbidities and related prevention and management strategies
There is no known early treatment for the prevention of comorbidities associated with KS. However, early diagnosis provides the opportunity for health promotion interventions regarding lifestyle factors that may significantly alter adult outcomes (31). For example, even prior to puberty, boys with KS may exhibit a higher body fat composition than their peers (32), and encouragement of physical activity and good nutrition may reduce the increased risk of conditions such as the metabolic syndrome (33).

Psychosocial impacts of diagnosis and non-diagnosis
Diagnosis in infancy would prevent the ‘diagnostic odyssey’ many parents experience in searching for an explanation for the child who does present with symptoms (24). This age at diagnosis allows for early parent–child bonding to have occurred and initial development of the child’s identity, so damage to the parent–child relationship seems less likely. As for NBS, risks remain regarding heightened parental anxiety and possible hypersensitivity and the consequences of these on the developing child.

Testosterone deficiency and treatment
Infertility and reproductive options
Early knowledge of testosterone deficiency and infertility allows for timely intervention at a later age; however, there are currently no medical interventions available for these domains at this age.

Screening in childhood
Developmental difficulties and available interventions
Diagnosis at school entry is an appealing option because of potential long-term benefits to be achieved through assistance with learning difficulties (34). Boys with KS have an average lower verbal IQ than comparison groups; however, mean scores generally remain within the normal population range (KS weighted mean 95) (28). Performance IQ has not been found to be significantly altered in comparison with control groups, overall indicating a vulnerability to problems with verbal comprehension and working memory, but relatively unimpaired perceptual organization and processing speed.

These specific deficits often manifest as difficulties with word-finding, reading and writing, concentration and memory, learning and comprehension (35,36), and up to 80% of boys with KS may experience these (28). This may lead to frustration and confusion, creating a vulnerability to secondary behavioural and social problems, potentially more so if left unrecognized. Such behaviours include anger outbursts or those associated with autism or attention deficit disorder, which are often diagnosed in place of, or in conjunction with, KS (37). Similarly, learning and behavioural problems may be detected and treated, yet a diagnosis of KS is still not made. This hinders application of the most appropriate interventions based on an understanding of the underlying condition.

It is again important to highlight the variability of KS; it cannot be assumed that learning problems will be present in all individuals. Some men with KS will achieve a university education level (38). Even if learning difficulties are present, others will continue undiagnosed to adulthood, enter productive employment and form stable partnerships, only to be recognized when presenting with infertility. However,
although these may be highly socially functioning, it is possible that they may not be functioning as highly as they might have, had they been diagnosed and treated at an earlier age.

There is a range of interventions available to address these cognitive difficulties, from speech and physical therapy, to targeted learning strategies for teachers and parents (29). Psychological interventions for behavioral difficulties such as counselling, anger management and family therapy may also assist with social integration. Aside from tangible interventions and their outcomes, there is likely to be significant psychosocial benefits to receiving appropriate understanding and assistance with developmental, learning and behavioral problems. A positive emphasis on the individual’s abilities, strengths and learning style can aid their development and help improve confidence (25).

Comorbidities and related prevention and management strategies
See Screening in infancy.

Psychosocial impacts of diagnosis and non-diagnosis
Genetic testing in children is a controversial area, especially as for some boys with KS, the test may be ‘predictive’, with no immediate benefits of diagnosis (39). One argument in favour of a childhood diagnosis is that a child is better able to integrate this knowledge into their developing identity and provides more opportunity for normalization, although there is a lack of evidence for this (40).

Despite the potential benefits of diagnosis at this age, there is the possibility that stigmatization, discrimination or bullying may result (41), particularly if a supportive and understanding environment is not available. This may well be the experience of undiagnosed boys with KS anyway and is currently a poorly understood aspect of the condition. Overall, it is difficult to draw any firm conclusions regarding the psychosocial impact of diagnosis in early childhood.

Testosterone deficiency and treatment
Infertility and reproductive options
See Screening in infancy.

SCREENING AT PUBERTY
Testosterone deficiency and treatment
The diagnosis of KS at or just prior to puberty represents an important stage in physical development. Aside from small testes and azoospermia, the most common features associated with KS relate to testosterone deficiency, which affects up to 85% of adult cases (42). Despite normal testosterone levels early in puberty, levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) rise relative to healthy controls and testosterone levels are low by mid-puberty (43). The onset of pubertal changes is usually normal, but rather the progression of secondary sexual characteristics may be inadequate. This is relevant as perceived pubertal development is very important for psychosocial adjustment in adolescence, and deviance from peers may be related to increases in delinquent behaviour (44).

If left untreated, the full syndrome of testosterone deficiency may become apparent including poor muscle tone, gynecomastia, decreased facial, body and pubic hair, increased body fat, lethargy, poor concentration and memory, depressed mood and sexual dysfunction. Long-term testosterone deficiency is linked to low bone mineral density (BMD), metabolic syndrome and an increased risk for diabetes and cardiovascular problems (8).

The benefits of commencing testosterone treatment have been assessed in postpubertal boys and include improved muscle, bone and secondary sexual characteristic development (2) and positive effects on mood, concentration, libido, energy, self-esteem and confidence (8). Testosterone treatment may also prevent or reduce gynecomastia, which frequently develops during puberty and is apparent in up to 60% of cases (8).

Treatment is generally recommended for boys with KS from early to mid-puberty, in association with rising LH levels as an endogenous signal of primary testicular failure (45). This ensures full progression through sexual development and reduces the risk of developing related comorbidities later. Treatment is a lifelong commitment that involves regular medical attendance, some inconvenience, cost and discomfort, yet often provides enormous benefits in ensuring full pubertal development and ongoing health into adulthood.

If KS is diagnosed in adulthood, testosterone therapy usually improves physical and psychosocial health but cannot make up for the ‘lost years’ when the condition went unrecognized. In addition, whilst many adults with KS report symptoms consistent with testosterone deficiency, others do not acknowledge these despite clear biochemical evidence (elevated LH and low-normal testosterone). However, when a ‘therapeutic trial’ of testosterone is negotiated with such men, they often recognize the symptoms of deficiency in retrospect, reporting improved energy, sexual function, mood and other benefits and thereby continuing lifelong treatment (46). These non-specific symptoms are often not realized until they are remedied and partly explain the low diagnosis rate of KS.

Although testosterone treatment is a beneficial experience for most men with KS, it should be noted that a minority of men with KS will experience negative side effects such as mood swings, leading to dose reduction or cessation (46).

Comorbidities and related prevention and management strategies
KS is associated with an increased risk for a number of conditions in adulthood including osteoporosis, metabolic syndrome, type 2 diabetes, cancer, autoimmune conditions, thyroid problems, varicose veins and cardiovascular disease (47). It is not clear to what extent these are influenced by genetic and environmental factors in KS, but long-term testosterone deficiency has been suggested to play a role. The advantage of diagnosis at puberty is that testosterone therapy can be implemented as soon as required, potentially
reducing the occurrence of at least some of these comorbidities.

During childhood and until the commencement of puberty, bone density in boys with KS appears normal (32). However, if testosterone is deficient during pubertal bone development, peak bone mass is not achieved, increasing the chance of bone fractures and early osteoporosis (48). BMD is reduced in adults with KS and hypogonadism is a risk factor for osteopenia and osteoporosis, affecting up to 40% and 10% of men with KS, respectively (49). Early testosterone treatment, commencing prior to 20 years, leads to normal BMD in men with KS (50). Even if adolescent testosterone levels are within the normal range, treatment should be considered on the basis of other indicators of hypogonadism, as low BMD has also been observed in adult men with KS whose testosterone levels are within the normal range. This suggests that testosterone levels alone may not be the most accurate indicator of hypogonadism (i.e. LH levels should also be taken into consideration) (45) and that other factors may also predict bone loss in men with KS, such as increased gene dosage because of the presence of an additional X chromosome.

Body composition is altered in men with KS, with increased body fat mass apparent even before puberty and the subsequent onset of hypogonadism (32). In addition, adults with KS have decreased muscle mass and strength. It has been observed that testosterone replacement may reverse the effects of unfavourable body composition and improve insulin resistance and is therefore recommended in those with signs of hypogonadism (51). Although associations exist between low testosterone and body composition, and between the metabolic syndrome and insulin sensitivity, to what extent testosterone treatment can alleviate these conditions in individuals with KS requires further clarification.

Morbidity from cardiovascular disease is also associated with KS, but the roles of hypogonadism, genetic and other factors are still not fully elucidated (52), and the degree of risk that can be reduced by optimal intervention remains unknown. Nonetheless, for these conditions, testosterone therapy from puberty could be complemented by earlier behavioural interventions, lifestyle modifications and health education of the individual and their family, as described for previous ages.

In addition to the aforementioned comorbidities thought to be associated with testosterone deficiency, men with KS are also at increased risk for breast cancer (53) and mediastinal germ cell tumours (54). There are no preventative measures for these conditions; however, early diagnosis of KS allows for surveillance and immediate treatment should they occur.

**Infertility and reproductive options**

Almost all men with KS are unable to naturally conceive (>99% cases) (42), and until recently, it was deemed virtually impossible for them to father a biological child. Initial testicular growth in early puberty is quickly followed by extensive and rapid germ cell apoptosis. By adulthood, complete spermatogenic failure is apparent with ‘Sertoli cell only’ or hyalinized seminiferous tubules, <4 mL testicular size and universal azoospermia (55). Infertility is one of the most prominent concerns for men with KS (56) and is an important consideration for parents contemplating termination of a prenatally diagnosed pregnancy (57). Preservation of fertility is identified as an important goal in managing children with disorders of sexual development (58).

Reproductive advances over the past two decades are challenging this notion of complete infertility. In 1998, the first testicular sperm extraction (TESE) was performed on an azoospermic KS man and harvested sperm was successfully used for intracytoplasmic sperm injection (ICSI), resulting in the birth of a healthy child (59). This approach is now well accepted with viable sperm being found in over half non-mosaic KS men, and the birth of over 100 children (60), in addition to unpublished cases. Initial concerns about increased risk for chromosomal abnormalities in offspring have not been substantiated (60), although genetic counseling prior to assisted reproductive treatment is essential. The possibility of men with KS fathering their own biological children has become a standard discussion topic amongst health professionals, men with KS and their families.

This ‘brave new world’ technology has raised questions in regard to the ideal time for males with KS to consider their reproductive options. The presence of spermatogenesis early in puberty may provide a valuable opportunity to obtain and preserve sperm for future fertility of the young man with KS. This could become a common option for diagnosed males with KS and presents a strong argument in favour of diagnosis prior to, or at the commencement of, puberty (and subsequent testosterone supplementation).

Overall, although preservation of fertility represents a favourable outcome of pre-pubertal screening of relevance to all males with KS, it remains an uncertain area requiring thoughtful ethical and social discussion between individuals with KS, their parents, partners and clinicians. There are personal, practical and ethical considerations regarding early preservation of sperm prior to testosterone supplementation, and the accessibility and affordability of this technology varies.

The impact of learning about infertility in adolescence is unclear, and research on the impact of male infertility is still a relatively new area. In adults, diagnosis of infertility has a significant impact on a man, regardless the cause is KS or an alternate condition, often coming as shocking and devastating news to a couple (61). The impact may be less in adolescents, as reproductive issues may not be as relevant as they are to the couple in their 30’s who have been attempting to conceive for several years. Nonetheless, gaining knowledge in adolescence of possible later infertility could play a significant role in planning for the future, identity formation, sexual practices (e.g. unsafe sex) and relationship formation, with both negative and positive consequences.

**Developmental difficulties and available interventions**

Assistance with learning and behavioural difficulties, if not already received, could still be implemented (if needed) for boys diagnosed at puberty. However, this may not be as
effective as interventions commenced earlier in life (34). In our recent study of 87 men with KS diagnosed at different ages, participants diagnosed in adulthood reported experiencing learning, behavioural and communication difficulties in childhood (up to 60%), for which treatment was not received (56). These men commonly reflected on this, e.g. ‘now I understand why I struggled’ and in regard to consequent lack of assistance, ‘I wish my parents and teachers had known, they thought I was just a lazy boy’.

Psychosocial impacts of diagnosis and non-diagnosis
In theory, an adolescent should be able to engage in the decision-making process for testing. On the other hand, a positive diagnosis of KS at what can already be a tumultuous age may have a negative impact on their confidence, self-esteem or sense of identity, and young people at this age may be more prone to bullying, stigmatization or discrimination (39).

DISCUSSION
For KS, a common yet under-diagnosed condition, there is certainly a rationale for further exploration of population-based screening. We have presented here the possible risks and benefits across different ages which reflect varying stages of development for which diagnosis and subsequent intervention may be important. Whilst the various symptoms of KS, both physical and cognitive, have been relatively well documented in children and adolescents, there is little research that empirically demonstrates benefits of diagnosis over non-diagnosis. There is, however, no evidence to suggest that non-diagnosis would be preferable, as many of these men would seem to benefit from speech and educational interventions, in addition to testosterone therapy from puberty.

The least understood aspect of KS is the psychosocial impact of diagnosis, both on the individual and on their parents. How children and adolescents may react to a diagnosis is not clear. Will it have long-lasting detrimental effects? Will it cause increased anxiety that may be carried through life? Or overall will these impacts be less than those experienced through diagnosis in adulthood, or the psychosocial consequences of not being diagnosed at all, even though difficulties may be present.

So which is the most appropriate age for diagnosis of KS, and potentially, for screening of KS? Figure 1 shows a schematic summative evaluation of risks and benefits to individuals of screening at the four age points considered. NBS offers a convenient point for diagnosis, but with no immediate benefits to the child, the potential negative psychosocial consequences to the parent (and inevitably the child) seem to exclude this as the ideal option. It scores lowest on the schematic evaluation. Screening in infancy and childhood seems to be ages at which diagnosis may carry the least potential negative impact and where treatments and interventions can be offered for those who require it. Primary school entry scores higher than infancy on the schematic, because it offers more benefit in the domains of comorbidity and psychosocial. Whilst diagnosis through screening at puberty ensures testosterone therapy is implemented when (if) required, this may be a difficult time for diagnosis given the complexities of adolescence. Although it may be preferable to never being diagnosed, there is little to support that testing in adolescence would be preferential over testing in infancy or childhood, where learning and behavioural interventions are more likely to lead to positive outcomes, but opportunities for future testosterone treatment and reproductive options are still available. It also means that optimal periods for speech and educational assistance may have been passed.

The evaluation and scoring system in the schematic summary is very broad-brush, designed to give only a rough overall rating. At each age point, solid evidence is lacking in one or more domains. Hence, the schematic gives only a tentative indication of the preferable age for KS screening, but this is still useful to guide further research.

DISCUSSION FOLLOWING THE PRESENTATION
At the First International Workshop on KS (Copenhagen, Denmark, May 2010), there was a fruitful discussion around the topic of screening for KS. Indeed, it was noted that KS may be detected as part of potential future screening programmes, e.g. for FXS. However, to fully justify screening,
unequivocal evidence regarding the benefit of early intervention on later health and psychosocial outcomes is needed, and these gaps are exemplified in this paper. On the other hand, we must also consider how much evidence is required before a pilot screening programme can be implemented, as ultimately this may be the only way to obtain the information required to inform decision-making around screening for KS.

Research in the form of a pilot programme, screening children contemporaneously at different ages, for example, in infancy (1 year), childhood (5 years) and adolescence (11 years), with follow-up and comparison of outcomes between groups over a number of years, would provide valuable information. This would certainly have a complex design and methodology, and would need to be a multi-centred, well-coordinated effort. The cost of implementing such a programme is also a consideration. Whilst the molecular test itself may not present a major cost (9), the overall financial commitment is likely to vary greatly between countries depending on current levels of infrastructure, existing supports and services and type of health care system. This information should be collected as part of a pilot screening programme and compared to known costs of health burdens (8).

Inherent in such a programme would be the need to garner the opinions and attitudes towards diagnosis and screening of key stakeholders – including health practitioners, parents and the individuals themselves. The prior development of an international validated tool for measuring phenotype, with associated treatment and intervention recommendations, is essential for comparison between countries and could also be used for provision of information of families. Whilst there are some excellent resources available for individuals with KS and their parents, a collaboration of these materials, which are often located across different countries, organizations and individuals, would also be of great use to the KS community.

CONCLUSION
This paper presents the tip of the ‘screening iceberg’. There is a very plausible case for KS screening, given that so many males remain undiagnosed, and follow-up studies of prenatally diagnosed individuals and newborns suggest that the majority will experience difficulties caused by the condition that can at least partially be alleviated with treatment. Yet the benefit of early intervention remains anecdotal. A pilot screening programme of children at different ages, with subsequent treatment as required and comprehensive follow-up, would provide valuable answers to the questions that remain.

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References


APPENDIX: DISCUSSION FOLLOWING ROBERT MCLACHLAN’S PRESENTATION

Postnatal screening for Klinefelter syndrome – is there a rationale?

Anders Juul (Copenhagen, Denmark):
The importance of screening has been discussed over the last 5 years, and we have received approval to conduct a pilot PCR based screening investigation to see if it is feasible to analyse blood stored on filter paper. The preliminary results should be available next year to assess if it should be extended to a larger study. There is important material stored in a biobank and we shall see if the technique works.

Robert McLachlan:
The larger study will require much more funding and I assume that funding for the pilot study implies that approval for the major programme to be funded if the pilot results are successful.

Anders Juul:
Unfortunately funding for the pilot study and for the screening programme comes from different sources.

Carole Samango-Sprouse (Davidsonville, USA):
Families know from an early stage that their babies are different and bonding occurs because the parents are protective when they perceive that something is wrong. They feel bad for years because they do not have a name for the condition and are relieved when a diagnosis is made. An earlier diagnosis from earlier screening would be very beneficial in that context.

Robert McLachlan:
We have heard that intervention at 12–18 months of age is beneficial, but if the Danish screening programme goes ahead and a diagnosis is made at birth, when is the best time to inform the parents? Should it be immediately, at 1 month, 12 months or 18 months? Should the parents be kept in the dark until treatment is likely to commence?

Carole Samango-Sprouse:
Informing the parents immediately is easier for them. Tonal abnormalities are evident in 90% of KS boys at 4 months and we are planning a treatment programme. The information must be presented to the parents properly. They should be informed that the boy is not different but has an abnormality and is being offered help and support rather than telling the parents that the child has a deficit. Newborn diagnosis is best for the baby.

Rodolfo Rey (Buenos Aires, Argentina):
I am not an expert on screening programmes but I would suggest that for practical reasons the parents should be recalled immediately and given the diagnosis otherwise they might be lost to the system.

Gary Butler (London, UK):
Data from this conference provide an excellent case for screening. Dr Herlihy presented an excellent study on psychosocial deficits and poor function in KS boys. Had any of the boys with these problems been treated with interventional therapy such as speech therapy, behaviour therapy or testosterone at any time as that would have a major influence on the outcome? If there had been any benefit from therapy that would also support the case for screening.

Amy Herlihy:
All these data were collected by questionnaires for each major area of symptomatology in KS. We examined 17 areas including learning difficulties, low testosterone levels and gynaecomastia: we documented when the symptoms first appeared, when KS was diagnosed, and if it had been treated. We asked when treatment was started, what kind of treatment was given and how long it lasted. I am attempting to combine all these factors to produce a composite score. Some individuals had started a testosterone treatment but stopped because of adverse effects. We assume that if therapy is given then adequate doses were used and provided the help they needed. We hope to have an insight into the effect of treatment in quality of life outcomes.

Nicole Tartaglia (Denver, USA):
Did you track from your questionnaire the route of recruitment to the study? It has been suggested that individuals with KS who are involved in support groups are more affected.

Amy Herlihy:
We asked participants how they heard about the study and many had been informed by a support group, GP or endocrinologist, or from multiple sources. We also asked about the indications leading to a diagnosis. Prenatally diagnosed participants were classified as incidental with no phenotype, and were used as a reference group. Some adults were also diagnosed incidentally including one student who was being karyotyped along with others in the science class.

Nicole Tartaglia:
There is newborn screening for fragile X syndrome in US because fragile X is associated with early developmental abnormalities but is often only diagnosed later in
childhood. It has been shown that earlier intervention can improve the outcome of children with fragile X syndrome, justifying that early screening is beneficial, and medications are being developed which target the neurobiological abnormalities resulting from the fragile X mutation. Brad Coffee in Emory University in Atlanta studied all blood spots in a new born screen for fragile X using a methylation method, and their group found 7 cases of fragile X syndrome but 57 cases of KS. Population screening for KS has not yet been studied because there is no solid research showing that early intervention has a beneficial effect on outcomes, even though anecdotally we all believe this to be true. The dilemma is what to do with the KS diagnoses when the patients are being screened for fragile X – should the patients be informed if a different diagnosis like KS is identified? Or, should they screen for fragile X with a molecular method that doesn't identify all cases of KS? So, there is now an urgency to determine if early identification improves health or developmental outcomes in individuals with KS. If early identification is beneficial, the test for fragile X which also picks up cases of KS could also be used to screen for KS.

Amy Herlihy:
It is essential to determine quickly if early intervention in KS is beneficial. In Australia, the newborn screening card only includes conditions which have been proven to respond to therapy. It is only recently that cystic fibrosis was included, and that is controversial.

Robert McLachlan:
A cohort of several hundred KS infants would yield a great deal of information and we would see in 5-6 years if the boys had a demonstrable improvement at that age as a result of intervention compared to those who were not treated. A further important outcome would be seen after a further 5-10 years when the boys entered puberty, and they could be followed up thereafter in the long term. Denmark is an ideal place to track these KS individuals because of their ideal health system.

Ronald Swerdloff:
Universal screening is not required for such a study as only a subpopulation is required.

Robert McLachlan:
This could be a staged investigation starting with a small non-biased cohort then applying it to a broader population.

Niels E Shakkæbek (Copenhagen, Denmark):
There are strict criteria for screening and these criteria may not be fulfilled in the small study. This would generate data but on a research rather than a screening basis.

Hilgo Bruining (Utrecht, Netherlands):
Genetic copy number variants are increasingly being identified in the field of psychiatric genetics. Many individual copy number variants show pleiotropic phenotypes. Diagnostic subtyping is becoming more feasible on the basis of such single genotypes. I think it is also important to draw attention to KS as an example of a highly penetrant CNV with great prospects for genotype-phenotype efforts.

Martin Ritzén (Stockholm, Sweden):
Is it possible to detect X and Y chromosomes on dried filtered blood spots? In Sweden we have DNA in cold storage from almost all newborn babies since 1966 and we might be able to pick up KS patients if the technology is available. The true incidence could easily be determined by analyzing a suitable number of unidentified samples. However, the ethical aspects of identifying these individuals as adults need careful consideration. If we tested, say, 2 million blood samples, we would identify large numbers of KS patients up to the age of 40 years.

Jacques Giltay (Utrecht, Netherlands):
I am a clinical geneticist, and it is technically feasible to check the dried blood for the number of X and Y chromosomes. However, we would also identify XXX, XO and possibly XYY patients and you would have to decide how to act on that information.

Robert McLachlan:
Confirmatory karyotyping would be necessary because these methods may not be able to differentiate with certainty the more complicated chromosomal abnormalities.

Jacques Giltay:
If we detect 3 copies of the X chromosome we can assume the individual is XXX female. If the results indicated KS phenotypic confirmation? Having detected an abnormality we cannot withhold the information, and the patient should be informed.

Niels E Shakkæbek:
Many years ago in Eastern USA a karyotype analysis detected many men with XYY genotype and there was much debate about what to do with the information. Similar problems may arise if stored blood is tested and KS phenotype identified. A previously undiagnosed KS patient may be surprised if a clinical geneticist knocked on his door and told him he had an extra X chromosome. This type of screening must involve the patient from the outset. In a screening study, the parents should be informed before testing and the benefits of a positive diagnosis explained to them.

Robert McLachlan:
These are two different types of experiments and both meritorious. Examining stored blood retrospectively will allow assessment of a life history of unintervened KS because most of the individuals will be unaware of their diagnosis. It would provide interesting real health hard outcomes in a representative population. The prospective study will notify
the diagnosis at an early stage allowing intervention and recruitment to a longitudinal study.

**Claus Gravholt (Aarhus, Denmark):**
In Denmark we also have stored dried blood on PKU cards going back for a long time stored in large freezers. It is technically possible to use these for chromosomal analysis but there are practical problems. Each card has to be identified and taken out of the freezer. There are many millions stored and our largest study to date has examined 3,000 cards because of the difficulty working at such low temperatures. Large studies would require greater funding and manpower. A prospective study is more feasible.

**Niels E Skakkebæk:**
Who in this room agree that a large study in Sweden as proposed by Dr Martin Ritzén should be undertaken. I see by your show of hands that the vast majority of the delegates at this conference are in favour of conducting this experiment.