DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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DOWN SYNDROME IN ESTONIA

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to Anni and Rebecca,
their families and all their friends

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

I. Reimand T, Õunap K, Zordania R, Ilus T, Uibo O, Sitska M, Talvik T. Descriptive epidemiology of Down syndrome in Estonia. Paediatric and Perinatal Epidemiology 2006; 20: 512–519.

II. Reimand T, Uibo O, Zordania R, Ilus T, Õunap K, Sitska M, Talvik T. Down syndrome in Estonia. Eesti Arst 2006; 85: 78–83 (in Estonian).

III. Reimand T, Uibo O, Zordania R, Palmiste V, Õunap K, Talvik T. Parents'satisfaction with medical and social assistance provided to children with Down syndrome: experience in Estonia. Community Genetics 2003; 6: 166–170.

IV. Reimand T, Grünberg H, Uibo O, Õunap K, Talvik T. Guidelines for the medical managements of children and adults with Down syndrome in Estonia. Eesti Arst 2006; 85: 720–725 (in Estonian).

V. Sitska M, Kuuse K, Ilisson P, Ilus T, Tammur P, Reimand T, Ehrenberg A. Down's syndrome screening in Estonia. Eesti Arst 2003; 82: 192–197 (in Estonian).

MEDICAL ABBREVIATIONS

AC	amniocentesis
AMA	advanced maternal age
AVSD	atrioventricular septal defect
BMI	body mass index
CD	celiac disease
CHD	congenital heart defect
CI	confidence interval
DS	Down syndrome
GTG	G bands by trypsin using Giemsa
ISCN	International System for Human Cytogenetic Nomenclature
MGC-TUH	Medical Genetic Center of Tartu University Hospital
OR	odds ratio
PCR	polymerase chain reaction
PD	prenatal diagnosis
PwDS	Person with Down syndrome
SD-SOE	Statistical Database of the Statistical Office of Estonia
TOP	termination of pregnancy
Tr21	trisomy 21
TSH	thyroid stimulating hormone
VSD	ventricular septal defect

1. INTRODUCTION

Trisomies can be divided into four categories: complete or whole chromosome, trisomies; partial trisomies; microtrisomies and triplications of single genes or single functional genomic elements. Whole-chromosome trisomies result from meiotic or mitotic non-disjunction which is common in humans and accounts for ~0.3–0.5% of live births (Hassold and Chiu, 1985; Benn and Hsu, 2004). The trisomy of human chromosome 21, which results in Down syndrome (DS) is the most frequent event. DS constitutes approximately 8% of cases of registered congenital anomaly in Europe, with over 7 000 affected pregnancies in the 15 member states of EU each year (Dolk *et al.*, 2005).

Down syndrome or trisomy 21 (Tr21) is almost always the whole-chromosome trisomy. It is the most easily clinically recognized single chromosome abnormality, and it is also the most prevalent genetic cause of mental retardation in childhood. John Langdon Down first described this condition in 1866, but it had existed long before that. There are sculptures and paintings from the 15th century of figures that have an appearance of DS (Levitas and Reid, 2003; Kunze and Nippert, 1986). John Langdon Down's essay, which originally appeared in *London Hospital Reports* in England, is generally credited as the earliest detailed clinical description of what he called 'mongoloid idiocy':

> "The face is flat and broad, and destitute of prominence. The cheeks are roundish, and extended laterally. The eyes are obliquely placed, and the internal canthi more than normally distant from one another. The palpebral fissure is very narrow. ... The lips are large and thick with transverse fissures. The tongue is long, thick, and is much roughened. The nose is small. The skin has a slight dirty yellowish tinge, and is deficient in elasticity, giving the appearance of being too large for the body. ... The Mongolian type of idiocy occurs in more than ten per cent of cases which are presented to me. They are always congenital idiots, and never result from accidents after uterine life" (Down, 1866).

Almost a century later and three years after the confirmation of the normal human chromosome number, both Lejeune *et al*. (1959) and Jacobs *et al.* (1959) within one month of one another investigated individuals with DS and found in all of their patients an additional small telocentric chromosome - 47,XX,+21 or 47,XY,+21. The next year, Polani *et al*. (1960) described translocation in a ten-year old girl with DS and the following year, Clarke *et al.* (1961) reported a two-year old girl with the physical stigmata of DS, but near-normal intelligence having both normal diploid and Tr21 cells in skin and blood (mosaicism).

The additional chromosome found by all of them is the smallest human chromosome, numbered 21, which represents around 1–1.5% of the human genome. The 21st chromosome contains 225 genes and 59 pseudogenes. Approximately 41% of the genes that were identified on chromosome 21 have no functional attributes (Hattori, 2000).

Penrose (1933) reported a positive correlation between maternal age and incidence of offspring with DS and since then there has never been any doubt that the main risk factor for DS is advanced maternal age. Current studies indicate that the percentage of pregnant women aged 35 years and older is steadily increasing (Bréart *et al.*, 2003; Resta, 2005; Dolk *et al.* 2005).

The possibility of prenatal diagnosis (PD) has been offered to women with high-risk pregnancies, since the early 1970s, after Steel and Berg had succeeded, in 1966, in culturing and karyotyping amniotic fluid cells (Benn and Hsu, 2004). The procedures for PD were available in Estonia from 1990, and since 1995 PD was implemented as routinely available for every pregnant woman 35 years and older. Second trimester maternal serum screening (double or triple test) for younger women was implemented, in Estonia, from 1999 (Sitska *et al.,* 2003).

Whereas most DS children in Estonia during the Soviet period were institutionalised in nursery homes for disabled people, most of them now live at home. The confirmed live birth prevalence rate must, therefore, be determined in order to permit the planning of health care and rehabilitation services for children and adults with DS as well as their families.

The aims of the present study were to establish the live birth prevalence of DS in Estonia, to investigate the phenotypical features and the prevalence of associated medical problems of DS, and the parents' satisfaction with medical and social care. The practical purpose was to determine and introduce the health care guidelines for individuals with DS to improve the medical care provided to them.

2. LITERATURE REVIEW

2.1. Descriptive epidemiology of Down syndrome

2.1.1. Diagnostic methods and parental origin of extra chromosome 21

Since Lejeune *et al.* and Jacobs *et al.* found the additional small chromosome in patients with DS, in 1959, the cytogenetic analysis of metaphase chromosomes remains the criterion standard practice to confirm the clinical diagnosis of DS. This confirmation is needed in medico-genetic consultation of the families with DS to estimate the recurrence risk.

Tjio and Levan in 1956 and Ford and Hamerton in the same year determined that the correct chromosome number of the human is 46 (McKusick, 2002). Chromosomes can be studied only in dividing cells. Cell culture methods have greatly enlarged the range of tissue and cell types from which dividing cells can be obtained in vitro. These include lymphocytes, fibroblasts from skin and cells from amniotic fluid or chorionic villi. The introduction of a short-term peripheral blood culture technique in 1960 provided a reliable way for obtaining human chromosome preparations of good quality for human cytogenetic investigations and for clinical diagnosis. T-lymphocytes from heparinizated peripheral blood are, in this techinique, stimulated to divide in culture with a mitotic stimulant - phytohemagglutinin. The blood culture is initiated in a suitable culture medium and within three days the stimulated leucocytes provide a very large number of dividing cells. The divisions are blocked in metaphase by adding mitotic spindle poison, such as colchicine, to the culture for a few minutes. Hypotonic solution swells the cells, which allows the spreading of the chromosomes. This enables the setting up of well-spread metaphase chromosome preparation on glass slides suitable for microscopic analysis using chromosome banding methods. A ‘band’ is defined as that part of a chromosome, which is clearly distinguishable from its adjacent segments by appearing darker or lighter (Ferguson-Smith and Smith, 2002). The first of the banding methods was the quinacrine fluorescence method developed in 1970, which was followed by the various methods of Giemsa staining (McKusick, 2002). A Giemsa dye mixture reveals identical patterns of dark and light bands along the chromosomes. This kind of technique is called G-staining method and the resulting bands are G-bands where the dark bands contain mainly A-T rich DNA and the light bands are G-C rich (Ferguson-Smith and Smith, 2002). The picture of normal male chromosomes cultured from peripheral blood as described above G-bands, and the karyogram is shown in Figure 1.

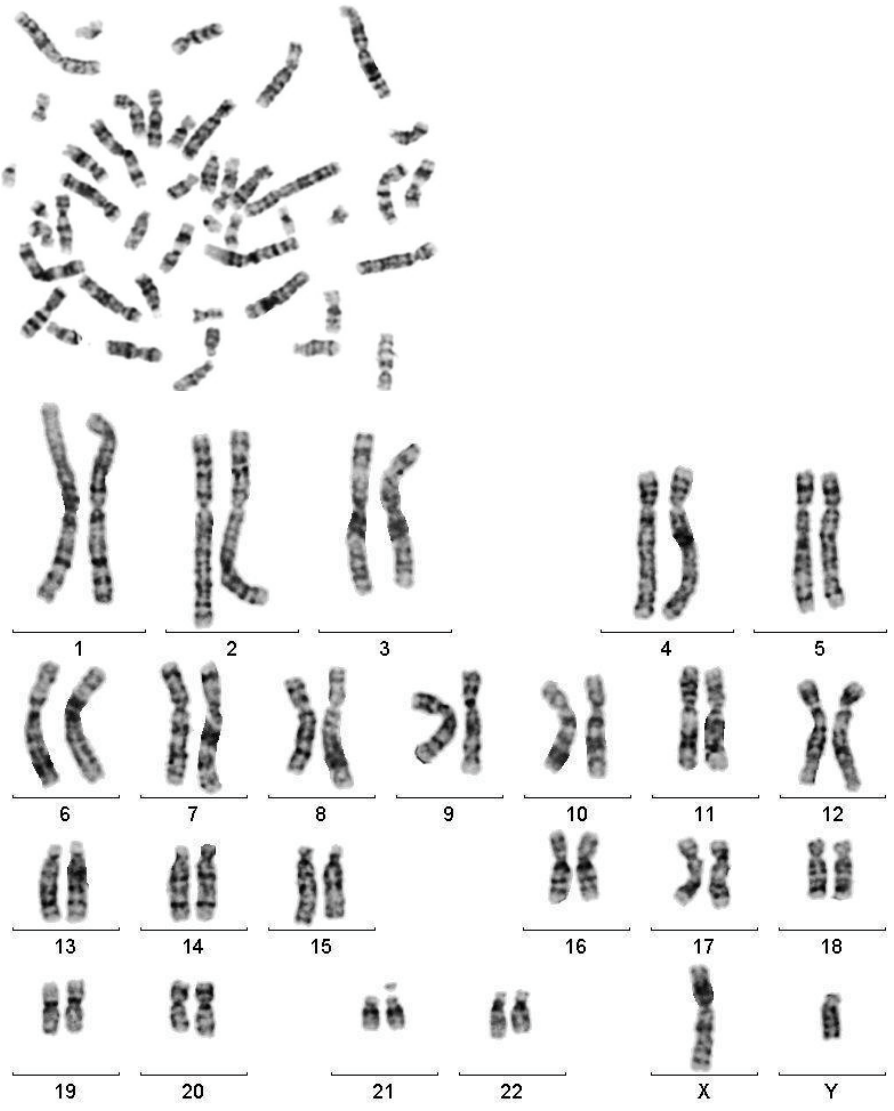

Figure 1. Normal male chromosomes and karyogram (46,XY).

The reporting times of full karyotype analyses have, on average, decreased substantially to, for example in the United Kingdom, a nationwide range of 7–22 days (Mann *et al.,* 2001). There are, however, requirements to get much faster results, especially in prenatal clinics. Several other methods have therefore been

developed and used over the past ten years for the rapid detection of Tr21, either during the foetal life or after birth.

The most widely method used after the cytogenetic analysis of metaphase karyotypes, is fluorescent *in situ* hybridization, which was introduced in the late 1980s (Kuo *et al.*, 1991). Fluorescent *in situ* hybridization involves hybridization of selected chromosome-specific DNA sequences on interphase nuclei, using chromosome 21-specific probes or whole-chromosome 21 painting. This is a labour intensive method, since it requires counting a considerable number of nuclei (25–50) in order to be reliable (Hultén *et al.*, 2003). An alternative method is quantitative fluorescence polymerase chain reaction, in which DNA polymorphic markers (microsatellites) on chromosome 21 are used to determine the presence of three different alleles (Verma *et al.*, 1998; Zheng *et al.*, 2006). This method relies on informative markers and the availability of parental DNA. Additional methods to measure the copy number of DNA sequences include the multiple amplifiable probe hybridization and multiplex probe *ligation* assay (Hollox *et al*., 2002; Schouten *et al.,* 2002). A method, termed paralogous sequence quantification, uses paralogous sequences to quantify the chromosome 21 copy number (Deutsch *et al.*, 2004). PCR based methods (quantitative fluorescence-PCR, multiple amplifiable probe hybridization, multiplex probe *ligation* assay, paralogous sequence quantification), have the advantage of being inexpensive and efficient in terms of labour and high throughput. Quantitative fluorescence PCR is the most established of all the PCR based techniques and despite the problems has been successfully implemented in several diagnostic laboratories (Mann, 2001). Nevertheless not one single molecular method seems to be obviously superior to the rest, since all have advantages and disadvantages. The greatest disadvantage from cytogenetic analysis is that these DNA methods could not discover the structural chromosome rearrangements in balanced form, such as translocations, inversions and insertions, which are common in the general population (Hultén *et al.,* 2003). Detecting a structural chromosome rearrangement is obligatory for offering appropriate counselling regarding the carriers' reproductive risk (Hultén *et al.,* 2003).

The parental origin of the extra chromosome in children with Tr21 has also been investigated with cytogenetic and molecular methods. The cytogenetic method used the detection of inherited morphologic variations of the short arms of chromosomes seen in karyotypes (chromosomal heteromorphism) to determine the parental origin of the extra chromosome (Antonarakis *et al*., 1991). These studies suggested that the origin of the extra chromosome were 16% paternal and 84% maternal in origin (Antonarakis *et al*., 1991). However, the use of chromosomal heteromorphism was initially incomplete because the parental origin of the error in chromosomal non-disjunction could not be determined in approximately 40% of cases and secondly, because the scoring of heteromorphism can be subjective (Antonarakis *et al*., 1991).

The introduction of techniques involving highly polymorphic microsatellite markers has greatly improved the studies of parental origin of the additional

chromosome 21. Furthermore, DNA markers close to the centromeric region indicate the stage of meiosis in which the segregation error had occurred. Homozygosity for all markers throughout 21q, including the pericentromeric markers, is interpreted as the result a mitotic-postzygotic error. Mitotic-postzygotic error incidence occurs in approximately 5% of Tr21 cases (Antonarakis *et al.,* 1993; Antonarakis, 1998). Antonarakis *et al.* (1991) revealed with DNA analysis that the origin of the extra chromosome 21 was 6% paternal and 94% maternal. Machatkova *et al.* (2005) had very similar results in their analysis – the origin of the extra chromosome 21 was 7.8% paternal and 92.2% maternal. These results show considerably less frequent paternal origin than has been reported with cytogenetic methods. Muller *et al.* (2000) tested 110 families with prenatally diagnosed Tr21 and found 10.8% of paternally inherited additional chromosome 21, which is also a significantly higher percentage than reported in live birth data, but they did not find any specific factor for such increased frequency to selective loss of paternal origin foetuses.

Paternal non-disjunction occurs mostly in meiosis II than meiosis I (Antonarakis, 1998), whereas the majority of errors in maternal meiosis occurred in meiosis I. The different studies showed that 77.5–79.4% of non-disjunction was caused by maternal first meiotic non-disjunction and 12.7–22.5% was caused by second meiotic non-disjunction (Antonarakis, 1998; Machtkova *et al*., 2005). The maternal II meiotic non-disjunction is more related with maternal lifetime exposure to lower socioeconomic status (Christianson *et al*., 2004).

2.1.2. Cytogenetic variability of DS and recurrence risks

Down syndrome may be cytogenetically as regular trisosomy 21, translocation of chromosome 21 or show mosaicism for trisomy 21 and normal cell lines.

The most frequent cytogenetical form of DS is regular trisomy – 47,XX,+21 or 47,XY,+21 – which is found in over 90% of cases by most investigators (Mikkelsen, 1977; Stoll *et al.,* 1998; Kim *et al.*, 1999; Schinzel, 2001; Mokhtar *et al.,* 2003, Métneki and Czwizel, 2005), except for instance Jyothy *et al.* (2002), who reported regular trisomy in 87.3% of cases. Regular trisomy 21 is shown in the Figure 2.

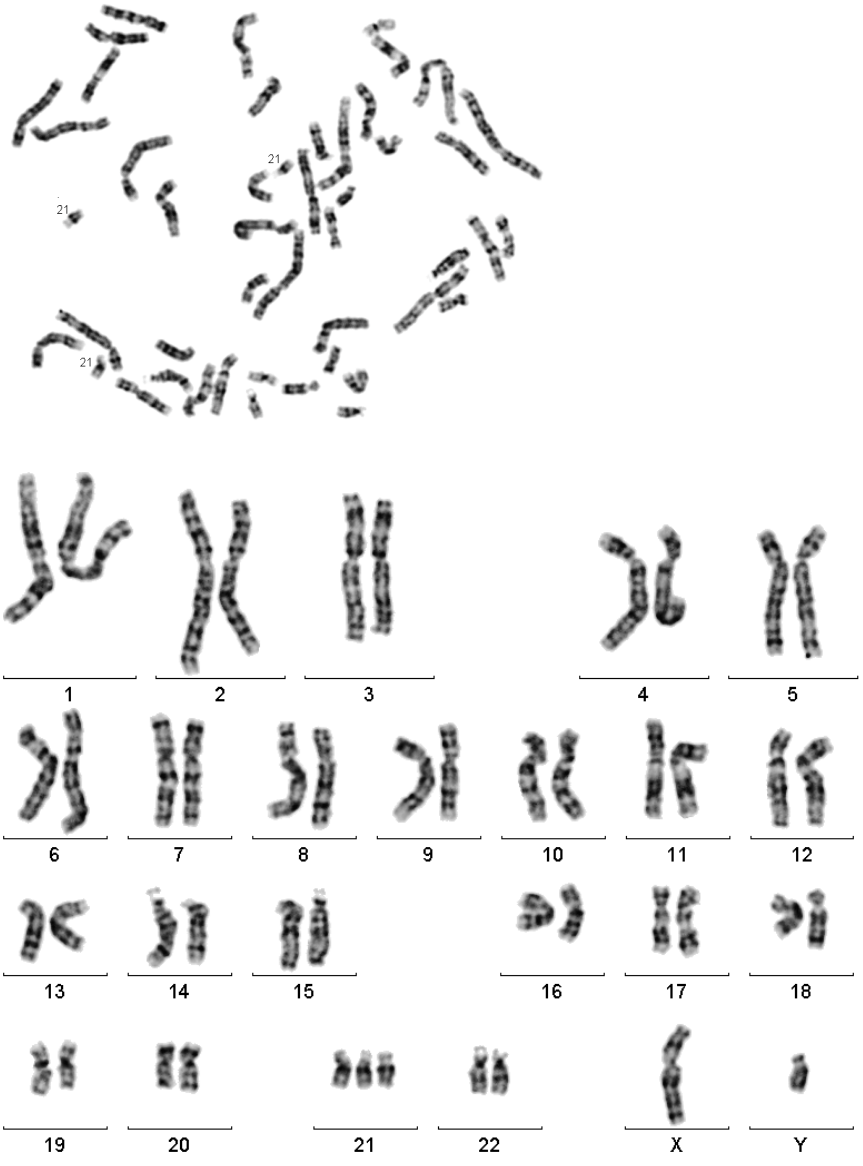

Figure 2. Regular Tr21 in metaphase and karyogram (47, XY,+21).

Translocation in DS is usually of Robertsonian type, which is the most common chromosomal rearrangement in human, occurring about 1:500–1000 birth (Bandyopadhyay *et al*., 2002; Ferguson-Smith and Smith, 2002). Robertsonian translocation is the whole-arm rearrangement between two acrocentric chromo-

somes and the most common Robertsonian translocations are t(13/14) and t(14/21), which constitute approximately 85% of all Robertsonian translocations (Bandyopadhyay *et al*., 2002). Half of the Robertsonian translocations are *de novo* translocations and in 95% of the *de novo* cases, the translocation originates during maternal meiosis (Bandyopadhyay *et al*., 2002).

The mean frequencies, in DS, of the translocation of chromosome 21 are assumed to be 4–6%, (Mikkelsen, 1977; Kim *et al.*, 1999; Schinzel, 2001; Jyothy *et al.,* 2002; Métneki and Czwizel, 2005). However, there are some investigators, who show much higher or lower frequencies. For example, Mokhtar *et al*. (2003) found in their study group only 2.7% translocation cases and Ahmed *et al.* (2005) in their study found 3.7% of translocation DS cases. Thomas *et al*. (1992), by contrast, found translocation in 7.7% of cases. The most frequent translocation forms are t(21/21) and t(14/21). Jyothy *et al.* (2002) found the t(14/21) form in 43.5% and t(21/21) in 36.9% of translocation cases. The findings of Kim's study group t(21/21) was more prevalent, in 46.6% of translocation DS cases, while t(14/21) type of translocation were found only in 26.6% of cases (Kim *et al.,* 1999). Translocation 14/21 is shown in Figure 3.

Mikkelsen (1977) found that in translocation DS cases the translocated chromosome was inherited from one parent in 54.5% cases. Jyothy *et al.* (2002) found fewer inherited translocation cases, 26.6%, of which 83.3% were maternal in origin, while Pulliam and Huether (1986) found 13.0% inherited translocation cases, of which 91.7% were maternal in origin.

In *de novo* translocation t(14/21), the extra chromosome 21 is maternal in origin in all cases and the most likely mechanism of this event is that the translocation occurs before crossing-over in meiosis I and is followed by normal segregation in meiosis I and II (Antonarakis, 1998). In the majority of *de novo* translocation t(21/21) Tr21, the abnormal chromosome is an isochromosome (duplication 21q) and about half of the isochromosomes were paternal in origin and half of maternal in origin (Antonarakis, 1998). All of the true *de novo* translocations t(21/21) are also maternal in origin like t(14/21) (Antonarakis, 1998).

The mosaicism for Tr21 and normal cell lines is found in 2–4% of patients with DS (Mikkelsen, 1977; Kim *et al.*, 1999; Mikelsaar, 2001; Schinzel, 2001; Métneki and Czwizel, 2005). There are also some investigations, which show a much lower frequency of mosaicism. Ahmed *et al.* (2005) and Mokhtar *et al.* (2003) reported only 0.7% and Mutton *et al*. (1996) 1.0% of mosaicism. Thomas *et al*. (1992) reported a high frequency of mosaicism (5.75%). Pangalos *et al*. (1994) found that 58.8% of mosaicism probably originated from a postzygotic loss of an extra chromosome 21 present in a trisomic zygote and this may lead to uniparental disomy of 21 (UPD21) in the euploid cells. The mosaicism of the remaining 41.2% is the result of a mitotic, postzygotic gain of an extra chromosome 21 in a previously euploid zygote.

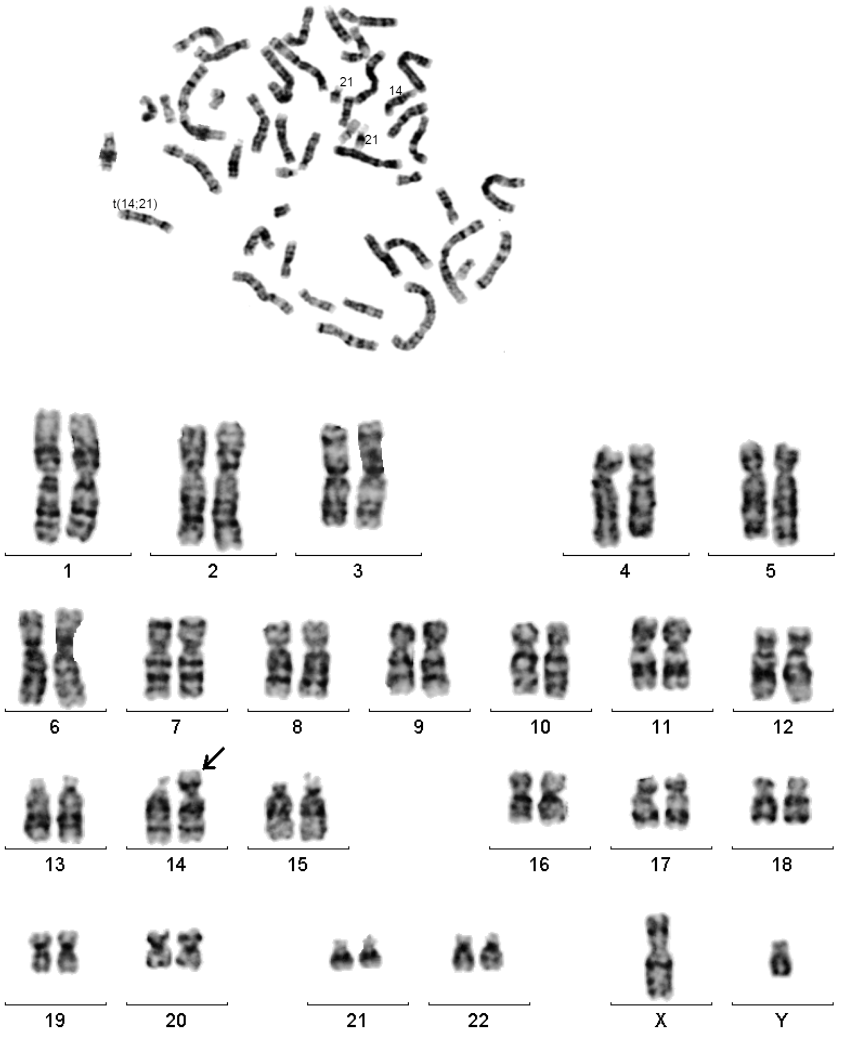

Figure 3. Metaphase and karyogram of DS with translocation 14/21 (46,XY,der(14;21)(q10;q10),+21).

The difference in the variances of cytogenetic results by several authors is shown in Table 1.

The principle task in giving recurrence risk figures to families with a child, who has translocation DS is to determine whether the parents of this child are the carriers of the translocation form. The recurrence risk for pregnancy with a foetus with DS is relatively high in these cases. However, the risk of pregnancy with a foetus with DS is increased also in women, who already have a previous

history for pregnancy with regular Tr21 foetus. Gair *et al.* (2005) described pedigree with four cases of regular Tr21 in three generation whereas the maternal age at the delivery of the child with DS was under 35 years. The possible explanation for this, are the factors that lead to an increased risk of segregation errors at meiosis (Gair *et al.*, 2005). When the mother bearing her first child with DS is younger than 30 years the absolute excess risk (the difference between the risk of the pregnancy of a foetus with DS after a previous affected pregnancy minus the expected age-related risk) is greater than for mothers whose first pregnancy with Tr21 foetus is at an older age (Warburton *et al.*, 2004; Morris *et al.*, 2005b). This indicates that if a younger woman has a pregnancy with Tr21 foetus, it is more likely to be due to factors not known today. Several hypotheses have been proposed for the mechanisms for recurrence of trisomy in the same couple, like parental gonadal mosaicism for trisomy, or other factors that are associated with an increased risk of meiotic error, or only chance, due to the maternal age-associated risk (James *et al.*, 1998; Bruyère *et al.*, 2000; Gair *et al.*, 2005).

Table 1. Cytogenetic variability of DS.

No of cases karyotyped	Trisomy (%)	Translocation (%)	Mosaicism (%)	Study period	Reference
5737	95.0	4.0	1.0	1989–1993	Mutton *et al.,* 1996
3678	94.7	3.5	1.8	1976–1991	Källen *et al.*, 1996
3390	91.0	4.6	4.4	1970–1999	Méteneki and Czeizel, 2005
1021	87.4	4.5	8.1	–	Jyothy *et al., 2002*
673	95.4	2.7	0.7	1992–2001	Mokhtar *et al.,*2003
556	94.6	3.2	2.2	1990–1999	Siffel *et al.,* 2004
518	90.5	6.9	2.5	1968–1982	Iselius and Lindsten, 1986
391	94.2	3.5	2.3	1979–1996	Stoll *et al.,*1998
307	95.6	3.7	0.7	1998–2001	Ahmed *et al.,* 2005
295	92.5	5.1	2.4	1994–1997	Kim *et al.,* 1999
221	95.0	3.2	1.8	1994–2001	Kava *et al.,* 2004
177	90.4	6.2	2.3	1960–1971	Mikkelsen, 1977
120	98.3	1.7	0.0	2000–2004	Wahab *et al.,* 2006
117	94.0	1.7	4.3	–	Muller *et al*., 2000

"–" data was not mentioned

2.1.3. Risk factors that may cause DS and prevalence rates of DS

Many studies, since Penrose's systematic study in 1933, have been conducted with the aim of increasing the awareness of the epidemiology of DS because of the high prevalence of serious health problems in individuals with DS. The reported live birth prevalence of DS may be affected by several factors such as the maternal age distribution of the population, completeness of ascertainment, accuracy of diagnosis, extent of selective prenatal termination of affected pregnancies and unidentified genetic and environmental factors (Carothers *et al.,* 1999).

The prevalence of Tr21 at conception is highly dependent on the maternal age. The advanced maternal age (AMA) is the only well known risk factor for giving birth to a child with DS, since birth prevalence increases 100-fold between the maternal ages of 15 and 45 (Penrose, 1933; Hook, 1976; Jones, 2006). The risk of having a live birth baby with DS for women 45 years of age or over is 1:29 and thereafter the risk remains static with increasing age (Ferguson-Smith and Yates, 1984; Morris *et al.,* 2005a). It is, therefore, important to take maternal age into account in comparing outcomes of pregnancies, since the differences in age distribution cause the difference in Tr21 prevalence rates. Bréart *et al*. (2003) have shown that it is possible to take standardized Tr21 rates of foetuses with DS by age and apply them to age distributions observed in data from Flanders, in Belgium (pregnant women 35 years and over 10.9%) and Ireland (pregnant women 35 years and over 20.8%) to obtain standardized Tr21 rates of foetuses with DS of 19.5 and 26.3 per 10,000 respectively which makes a 20% difference. Källén and Knudsen (1989) found a 60% difference in DS live birth prevalence between the populations of Spain and Czechoslovakia just because of the difference in maternal age distribution.

In spite of advanced maternal age being one of the most known risk factors for giving birth to a baby with DS, about 70% of these babies are born to the mothers younger than 35 years (Binkert *et al.*, 2002; Jou *et al.*, 2005). As the median age of mothers at delivery is increasing, the numbers of women carring for babies with DS are also increasing; for instance, the proportion in United States of AMA group foetuses with DS have increased from 25% in 1980 to 51.1% in 2002 (Dzurova and Pikhart, 2005; Resta, 2005). The same tendency is seen also in Europe where babies with DS born to mothers of a maternal age 35 and over represented 50% or more of all babies with DS (Rösch *et al*., 2000; Niemimaa, 2001; Dolk, 2005).

The possibility of paternal age influence on the Down's syndrome has also been discussed, but not proved statistically (Penrose, 1933; Ferguson-Smith and Yates, 1984; Stoll *et al*., 1998; Kazaura and Lie, 2002).

Despite of many years research to identify the risk factors for DS, the only confirmed risk factor is still AMA. Czarnetzki *et al.* (2003) with colleagues studied 7063 osseous remains from around Europe, dating from 3200 B.C. to 800 A.D., and found the overall frequency of DS lower than expected. Czar-

netzki proposed that one possible reason for this may be due to the absence of some environmental risk factors. There are many factors that have been explored as risk factors of DS. Eskes (2006) conducted a review of the relevant literature, in which folate deficiency that may result in DNA strand breaks, DNA hypomethylation, abnormal gene expression and chromosome segregation are discussed. The review also indicates that an elevated level of homocysteine points towards defective folate metabolism and plasma homocystein concentration is found to be higher in mothers of children with DS than in a control group (Eskes, 2006). Folic acid supplementation and food fortification are associated with a significant reduction in plasma homocystein, but Ray *et al.* (2003) did not find a decline in the prevalence rate of DS following folic acid food fortification.

Several investigators have also examined the effect of some maternal lifetime habits. Stoll *et al.* (1998) did not find any statistical significance with smoking, caffeine or alcohol consumption. The same results concerning smoking (statistically not significant) were found by Torfs and Christianson (2000). The same authors, however, did discover that mothers, who have four or more cups of caffeinated drinks per day or more than four units of alcohol per week during the first months of pregnancy, have a higher risk of miscarrying a foetus with DS (Torfs and Christianson, 2000). At the same time Yang *et al.* (1999) found an increased risk for DS in the children of women, who smoke cigarettes around the time of conception.

Other possible risk factors are maternal low socio-economic status that has been found to give a higher risk for clinically recognised pregnancies of foetuses with DS (Christianson *et al.*, 2004) and advanced maternal 'grandmother' age at the time of birth of the mother of the child with DS (Malini and Ramachandra, 2006). Consanguineous marriages were also analyzed as a risk factor for non-disjunction and there are some reports that are showing a more consanguineous marriage rate in parents of children with DS than in parent of control groups (Roberts *et al.*, 1991; de Braekeleer *et al.*, 1994; Stoll *et al.*, 1998). This may be the result of some recessive trait that leads to non-disjunction or the effect of increased homosygosity, which may prevent the loss of the pregnancy that occurs in the majority of Tr21 conceptions.

A number of studies from the beginning of 1990s have examined the main trends in DS prevalence as there have been changes in demographic factors and in PD possibilities. Some of them are reporting an increase in live birth prevalence (Nicholson and Alberman, 1992; Hoshi *et al.*, 1999), whereas others reported a decrease (Olsen *et al*., 1996; Verloes *et al.*, 2001; Lai *et al*., 2002; Egan *et al.*, 2004; Khoshnood *et al.,* 2004a; Jou *et al.*, 2005) or no change (Rösch *et al*., 2000; Binkert *et al.,* 2002; Bell *et al.*, 2003). These differences are dependent on the proportion of AMA group pregnancies, the policies of antenatal screening methods and the women's uptake of PD possibilities. The prevalence of live birth children with DS has been investigated in different geographic regions and temporal periods. Some of these results are shown in Table 2.

Table 2. Prevalence rate of DS in different countries.

Country	Prevalence rate per 1000 live birth	Expected prevalence rate per 1000 birth without PD	Study period	References
Finland	1.1	2.3	1995–2003	EUROCAT
Finland	–	1.8	1984–1988	Salonen *et al.,* 1993
Sweden	1.4	2.6	2001–2003	EUROCAT
Sweden	1.3	–	1968–1982	Iselius and Lindsten, 1986
Norway	1.1	1.4	1995–2003	EUROCAT
Norway	1.0	–	1967–1998	Kazaura and Lie, 2002
Denmark (Copenhagen)	1.1	–	1960–1971	Mikkelsen, 1977
Denmark (Odense)	1.0	1.6	1995–2003	EUROCAT
Russia	0.9	–	1983–2001	Krikunova *et al.,* 2004
Hungary	1.1	1.2	1970–1999	Metneki and Czeizel, 2005
Czech Republic	0.5	–	1994–1998	Dzurova and Pikhart, 2005
Switzerland	1.0	1.7	1980–1996	Binkert *et al.*, 2002
France (North-eastern part)	1.1	1.7	1979–1996	Stoll *et al.,* 1998
Wallonia (South Belgium)	0.7	1.0	1984–1998	Verloes *et al.,* 2001
England (northern part)	1.0	1.8	1995–1999	Bell *et al.,* 2003
Germany (the eastern part)*	0.8	1.0	1980–1997	Rösch *et al.,*2000
Israel	1.0	2.3	1997	Merrick, 2001
New York state	1.0	1.5	1989–1992	Olsen *et al.,*1996
California	1.1	1.5	1989–1991	Bishop *et al.*, 1997
Atlanta	1.0	1.5	1994–1999	Siffel *et al.,* 2004
Japan	0.6	–	1980–1997	Hoshi *et al.*, 1999
Singapore	1.0	1.5	1993–1998	Lai *et al.,* 2002
Hawaii	0.9	1.3	1986–1997	Forrester and Merz, 2002
India	0.8–1.2	–	–	Malini and Ramachandra, 2006
Quatar	1.9	–	2000–2004	Wahab *et al.,* 2006
South-Africa (Cape Town)	1.4	1.5	1974–1993	Molteno *et al.,*1997

"-" data was not mentioned

2.1.4. Impact of prenatal diagnosis

Prenatal diagnostic procedures for syndromes caused by chromosomal abnormality are a long established part of obstetric care in developed countries. It became possible from the end of the 1960s to screen pregnant women for a number of disabling conditions, including DS. Subsequently in 1973 there was the proposition that complete prevention of DS could be achieved by screening every pregnancy by amniocentesis (AC) and karyotyping of the foetal cells (Carr, 1995). Such a programme has never been established. However, chorionic villus biopsy or amniocentesis is usually offered to pregnant women in all ages with increased risk of having an affected child.

There have been significant advances in the identification of high-risk pregnancies. The main indications for prenatal cytogenetic diagnosis are advanced maternal age, balanced structural rearrangements of parents, previous child with chromosomal abnormality, high-risk results of maternal biochemical serum screening and abnormal ultrasound findings of the foetus (Benn and Hsu, 2004). The using of serum analytic and ultrasound screening methods resulted with an increasing proportion in an indication for PD in women less than 35 years of age. The maternal serum screening results as indication for PD, for instance in England and Wales, increased from 6% in 1989 to 37% in 1996 and ultrasound findings from 13% to 43% (Mutton *et al.*, 1998). This is leading to a higher proportion of prenatally detected pregnancies of foetuses with DS in the younger women group. Mutton *et al.,* (1998) showed that in 1989 only 9% of affected pregnancies were diagnosed prenatally in mothers less than 35 years old, rising to 45% in 1997.

The proportion of the AMA group is increasing in many countries (Raeburn, 2000; Rösch *et al*., 2000; Lai *et al.*, 2002; Bréart *et al*., 2003; Khoshnood *et al.*, 2004a; Jou *et al.*, 2005; Resta, 2005; Métneki and Czeizel, 2005). This expectation also holds true in the European Union (EU) where, since 1980, the proportion of births to mothers of 35 years of age and over has risen from 8% to 14% throughout the EU, and varied from 10% to 25% between regions (Dolk *et al.,* 2005). The annual age-distribution of pregnancies in England and Wales over the period 1988–1997 shows that delayed child-bearing has led to a 24% increase in the prevalence of PwDS without PD and selective abortion (Cuckle, 1999b).

The use of PD by women of the high-risk group and the decision of termination of pregnancy (TOP) after the foetus with DS was confirmed differs by different geographic regions and by different time periods. It depends on several factors like socioeconomic status, religion, educational level and the race/ethnicity of the pregnant women (Khoshnood *et al*., 2004b; Siffel *et al*., 2004; Dolk *et al*., 2005; Dormandy *et al.*, 2005; Saucier *et al.*, 2005). The uptake of PD and the incidence of prenatally diagnosed foetuses with DS is increasing in several countries (Olsen *et al*., 1996; Mutton *et al.*, 1998; Bell *et al.*, 2003; Khoshnood *et al.*, 2004a; Jou *et al.*, 2005). Women with higher levels of education are more likely to use PD (Khoshnood *et al*., 2004b; Saucier *et al.*,

2005). Also women of the AMA group use more PD possibilities (Olsen *et al.*, 1996; Binkert *et al.*, 2002) and with a higher uptake of PD possibilities the detection rate is also increasing. Seventeen percent of pregnancies with DS in Wallonia, South Belgium were detected during the period 1984–1989, but by 1993 to 1998 this rate had increased to 56% (Verloes *et al*., 2001), whereas in France 31.4% of DS diagnoses were performed prenatally and 29.4% of all DS cases were electively terminated during 1979–1996 (Stoll *et al*., 1998). Siffel *et al.* (2004) found a higher proportion of TOP among pregnancies to older age women and a lower proportion of elective termination among black women compared to white women. There is according to Wahab *et al*. (2006), a total lack of PD and TOP in Qatar for religious reasons. Overall, Mansfield *et al.* (1999) conducted a systematic literature review, where they found that TOP of foetuses with DS is more frequent compared with some other prenatally diagnosed foetal conditions.

Many investigators have shown that high maternal age is a significant risk factor for spontaneous abortion irrespective of reproductive history (Nybo Andersen *et al*., 2000; Wyatt *et al*., 2005). It is known that pregnancies affected by the foetuses with DS have a greater risk of spontaneous foetal loss than those that are unaffected (Hassold and Chiu, 1985; Torfs and Christianson, 2000). Current estimates for foetal loss rate are about 40% between the time of chorionic villus sampling and birth and about 25% between amniocentesis and birth (Bray and Wright, 1998; Cuckle, 1999a; Morris *et al*., 1999). Savva *et al.* (2006) showed in their study that the spontaneous foetal loss rate between chorionic villus sampling and delivery or between the time of AC and delivery in pregnancies of foetuses with DS increases with increasing maternal age. It is important, therefore, to estimate the total prevalence rate to recalculate the prenatally diagnosed cases. Some total or expected prevalence rates e.g. live born children with prenatally diagnosed and thereafter terminated cases in different states are shown in Table 2.

2.1.5. Accuracy of clinical diagnosis and timing of cytogenetical diagnosis of DS

Ideally, birth prevalence rate should be determined from cytogenetic examination of all or a representative sample of live births. Systematic clinical examination with cytogenetic investigation of suspected cases may be almost as effective in achieving complete ascertainment. DS is diagnosed clinically by its characteristic features and associated systemic malformations. There is, however, a wide variation among individuals in the clinical manifestation and not all phenotypic features may be present in every person. Also the occurrence of systemic malformations is variable. So the accuracy of clinical suspicions varies from 64% in United Kingdom (Hindley and Medakkar, 2002; Sivacumar and Larkins, 2004), 69% in Northern Ireland (Delvin and Morrison, 2004) to 90.8% in Rawalpindi (Ahmed *et al.*, 2005). Birth certificate reports extracted by clerks from hospital records in the USA include

5–10% false positives (Carothers, 1999). About half of these are detectable clerical errors in coding diagnoses and the remainder result from mistaken medical judgements before newborn discharge from hospital. Delvin and Morrison (2004) showed that there is a highly significant difference between the mosaic DS group and the regular or translocation DS group, in that mosaic DS is more difficult to detect clinically.

Cytogenetic diagnosis of DS may be done during pregnancy or during lifetime. Stoll *et al*. (1998) showed that from 238,942 consecutive births, diagnosis of DS was performed prenatally in 31.4%, at birth 56.3% and during the first week of life in 12.3% of the patients. By contrast, Heuterman *et al.* (2004) found from 313 live born children with DS that 5.7% were diagnosed prenatally, most (70.8%) were diagnosed on the day of birth, 20.5% were diagnosed during the first month of life and 1.7% were diagnosed after 1 year of age. Delvin and Morrison (2004) showed that in postnatal cases, 89.4% were diagnosed before the 7th day of life, 3% were diagnosed during childhood and 7.6% were diagnosed during adulthood. A study in Sweden states that 75% of the families of a child with DS were told the news within 24 hours of birth and 12% on or after the third day (Hedov *et al.*, 2002b).

2.2. Clinical symptoms of DS

2.2.1. Microanomalies

John Langdon Down (1866) first described the main features of this condition that was subsequently given his name. No single phenotypic feature is pathognomic, but the combination of facial dysmorphisms is recognizable and specific. There are two types of phenotypical features that are observed in Tr21: those seen in every patient like cognitive impairment and those, like congenital heart defects that are present in approximately 40% of the children with DS (Antonarakis *et al*., 2004). There is considerable variability in expression for any given phenotypical features. The extent of cognitive impairment, for example, varies widely in individuals with DS. There are several hypotheses that attempt to explain the phenotypic variability in Tr21. The study of patients with partial Tr21 and phenotype of DS suggested that there is a region of chromosome 21 band q22 that, if triplicated, is associated with numerous features of DS like flat nasal bridge, protruding tongue, high narrow palate, folded ears, short and broad hands, clinodactyly of fifth finger, large gap between first and second toes, joint hyperlaxity, muscular hypotonia, short stature and mental retardation (Delabar *et al.*, 1993; Korenberg *et al.*, 1994). This region was termed as the Down syndrome critical region (DSCR). However, there are also those observations that argue against a single chromosomal region being responsible for most of the DS phenotypic features (Korenberg *et al.*, 1994; Crombez *et al.*, 2005; Kondo *et al.*, 2006).

It has been hypothesized that genes that are present in three copies are overexpressed 1.5-fold relative to the euploid state (Antonarakis *et al.*, 2004). Kahlem *et al*. (2004) investigated a mouse model of DS and found that 82% of genes overexpressed in at least one of the nine tissues tested in control mice, whereas only 11% were detected in all nine tissues. They found that the global overexpression of the trisomic genes was close to 1.5-fold in cortex, heart, testis, and liver, whereas overexpression was collectively less pronounced in skeletal muscle, but was still higher in trisomic than in euploid mice. The cerebral cortex showed the highest molecular complexity, indicating that chromosome 21 genes play an important role in the maintenance of this tissue (Kahlem *et al*., 2004).

Ten common signs in the newborn period are muscular hypotonia, poor Moro reflex, hyperflexibility of joints, loose skin on the neck, flat facial profile, upslanting palpebral fissures, anomalous auricles, dysplastic pelvis, clinodactyly of fifth finger and single palmar crease (Gorlin *et al.*, 2001). Jackson *et al*. (1976) worked out ten of the most helpful signs for infants under two years, as shown in Table 3. Common phenotypical findings in children and adults are listed in Table 3 and shown in Figures 4 and 5.

Table 3. Phenotypical findings (%) in patients with DS by different authors.

References / Microanomalies (%)	Gorlin *et al.*, 2001	Kava *et al.*, 2004	Ahmed *et al.*, 2005	Jones, 2006
No of cases	–	524	295	–
Mean age of patients (months)	–	19	16	–
Palpebral fissures slant up*	79	84	83	80
Epichantal folds	48	57	63	–
Hypertelorism	–	34	62	–
Flat nasal bridge*	61	–	61	–
Ear abnormalities*	70	67	46	60
Protruding tongue	42	30	–	–
Narrow palate*	67	–	–	–
Retrognathia	–	2	–	–
Flat faces	–	51	–	90
Brachycephaly*	–	–	40	–
Excessive skin folds on neck or short neck*	53	37	–	80

References Microanomalies (%)	Gorlin *et al.*, 2001	Kava *et al.*, 2004	Ahmed *et al.*, 2005	Jones, 2006
Simian crease/single palmar crease	52	33	65	45
Brachydactyly	67	11	24	–
Clinodactyly*	59	36	25	50
Sandal gap sign*	50	46	46	–
Hypotonia*	–	76	56	80
Hyperflexibility of joints	62	10	–	80
Strabismus	22	–	6	45
Nystagmus*	11	3	6	35
CHD	–	18	35	40
Gastrointestinal anomalies	–	1	5	12

* Ten most informative signs by Jackson *et al*. (1976)
"–" not mentioned

A. B.

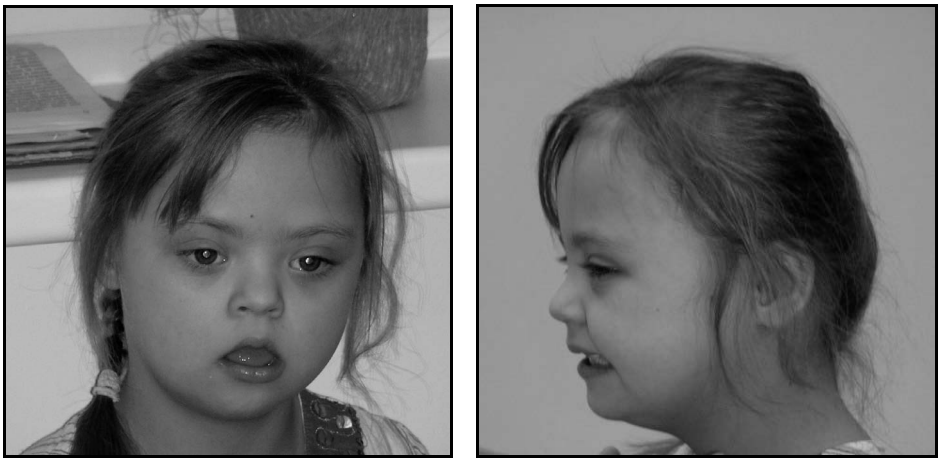

Figure 4. DS facial phenotype of patient A.L.
A. Front view (note: palpebral fissures slant up, epicanthal folds, protruding tongue, hypertelorism, short neck)
B. Profile view (note: flat profile, small ears, short neck)

2.2.2. Congenital malformations

It is generally known that infants with DS have a high frequency of certain major malformations like congenital heart defects (CHD) and gastrointestinal defects. Stoll *et al.* (1998) showed that 61.8% of DS cases had at least one major visceral or limb malformation and the most common types of associated malformations were cardiac anomalies and digestive system malformations. In their study the two following malformations were limb defects, like syndactyly, club foot and polydactyly, and urinary malformations. Bell *et al.* (2003) found fewer congenital anomalies – 45% of live birth PwDS and Lai *et al.* (2002) found birth anomalies among DS live births, stillbirths and abortions only 29.8%.

CHD refers to structural or functional heart disease present at birth, even if initially discovered much later. The most common cardiac defect in DS is endocardial cushion defects; the risk for this anomaly is increased 1000-fold among DS births (Torfs and Christianson, 1998). Although in DS the endocardial cushion defects are found in 50% of all CHD, 70% of individuals with endocardial cushion defects also have DS (Korenberg *et al.*, 1992). Paladini *et al.* (2000) showed that 44% of the foetuses with DS bear a CHD detectable with foetal echocardiography or conversely, 43% of foetuses with atrio-ventricular septal defect (AVSD) have DS. Molecular studies of individuals with CHD and partial duplication of chromosome 21q with features of DS led to the concept that a candidate region responsible for cardiac defects characteristic of DS might be 21q22.2–22.3 (Korenberg *et al.*, 1992; Barlow *et al.*, 2001; Kosaki *et al.*, 2005).

The frequency of CHD differs in different studies from 26% up to 61% (Tubman *et al.,* 1991; Källen *et al.*, 1996; Freeman, *et al.*, 1998; Stoll *et al.*, 1998; Torfs and Christianson, 1998; Venugopalan and Agarwal, 2003; Vida *et al.*, 2005). Källen *et al.* (1996) found difference in frequencies of CHD between three national registries (from 23% to 32%). They found the highest frequency in Sweden's register (32%) which may be due to follow up program up to one year's age of infant. The highest frequency of CHD (60%-61.3%) was found in Oman's population in which consanguinity is widely prevalent (Venugopalan and Agarwal, 2003) and in Saudi-Arabia (Abbag, 2006). Stoll *et al.* (1998) found CHD in 46.2% of DS cases where 42.9% of them were AVSD and 31.5% ventricular septal defect (VSD). Bell *et al.* (2003) found a cardiac anomaly only in 39.1% of live born patients with DS and 43.3% of them were AVSD, 28.7% were VSD. Frid *et al.* (2004) studied children with DS born in two periods 1973–1980 and 1995–1998 and found that the frequency of CHD among the children with DS increased over that period from 47.5% to 61.3%. The same tendency was noticed by Källen *et al.* (1996) where a strong increasing trend in the rate of cardiac defects were found in three programs; this is probably due to better diagnostic possibilities. Tubman *et al*. (1991) showed that echocardiography performed early in life could detect CHD that might otherwise be missed. McElhinney *et al.* (2002) showed also that 13% of patients with normal

cardiac physical examination had an abnormal echocardiogram and in 27%, the physical examination findings did not correctly predict the echocardiographic findings. So it is important to do all neonates with DS echocardiographic investigation. This will lead to prevention of complications that may affect the outcome of cardiac surgery and the survival of PwDS will be improved. This is also recommended by all medical guidelines for patients with DS (Cohen, 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Roizen, 2002; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005). The frequency of CHD types among the PwDS by several authors is shown in Table 4.

Table 4. Frequency of CHD in DS and percent of CHD types by different authors.

References / Type of CHD (%)	Tubman *et al.*, 1991	Källen *et al.*, 1996	Freeman *et al.*, 1998	Stoll *et al.*, 1998	Torfs and Christianson, 1998	Paladini *et al.*, 2000*	Venugopalan and Agarwal, 2003	Vida *et al.*, 2005	Abbag, 2006
Frequency of CHD in DS (%)	**41.9**	**26**	**44**	**46.2**	**55.9**	**56.1**	**60**	**54**	**61.3**
AVSD	38.2	39.0	45.0	42.9	30.6	43.4	27.7	9.5	22.8
VSD	14.7	28.0	35.0	31.5	11.4	47.8	25.9	27.6	33.3
Atrial septal defect	20.5	7.0	8.0	–	11.2	–	33.3	12.7	21.1
Tetralogy of Fallot	–	3.0	4.0	2.7	4.5	4.3	1.9	–	5.3
Patent ductus arteriosus	17.6	4.0	7.0	5.4	43.5	–	9.3	28.6	14.0

* Foetal DS cases

Torfs and Christianson (1998) showed in their study that the occurrence of gastrointestinal defects is about 20 times more common in patients with DS than in patients without DS. Gastrointestinal defects were found in 4.8% of DS live births, stillbirths and abortions (Lai *et al*., 2002). Bell *et al.* (2003) found gastrointestinal defects in 6.6% of live birth patients with DS. The most frequent malformations of gastrointestinal tract are duodenal atresia and Hirshprung's disease. Duodenal stenosis is seen in 4–7% of individuals with DS, but this accounts for 30–50% of all congenital duodenal stenosis (Korenberg *et al.*, 1992). About 1–2% of live birth children with DS have Hirschsprung's disease

and 10–15% of patients with Hirshprung's disease have Tr21 (Stoll *et al.*, 1998; Torfs and Christianson, 1998; Tolmie, 2002). The other findings that may occur more frequently were omphalocele, tracheoesophageal fistula, pyloric stenosis, ileal and jejunal atresia, imperforated anus (Källen *et al.*, 1996; Stoll *et al.*, 1998; Torfs and Christianson, 1998).

Renal malformations such as hypoplasia, dysplasia or obstruction were found in autopsy studies in 3% to 21% of PwDS (Mercer *et al.*, 2004). Lai *et al.* (2002) found only 0.8% of urinary system anomalies among DS live births, stillbirths and abortions. Torfs and Christianson (1998) found obstructive urinary system defects in 1.8% of live birth patients with DS and the risk ratio for obstructive urinary diseases is 14 fold greater than non-DS births. Cryptorchism has been reported to occur in instances from 7% to 27% (Torfs and Christianson, 1998; Mercer *et al.*, 2004) and Torfs and Christianson, (1998) found the risk ratio increasing 37.7 fold in DS births. Källen *et al.* (1996) did not find any increased risk to hypospadia in PwDS, but Torfs and Christianson (1998) found a 5.3 fold increased risk for hypospadia in DS births compared to non-DS births.

2.2.3. Growth problems

The prenatal and postnatal growth retardation is a well-known feature of DS. At birth they show mild growth deficiency. Mean birth weight, length and head circumference is located between the 10^{th} and 15^{th} centiles calculated for general population (Cronk, 1978; Clementi *et al.*, 1990). Cronk (1978) and Cronk *et al.* (1988) found that growth patterns for children with DS differ significantly from those for normal children. During the first three years of life the most marked length deficiency was seen between 6 and 24 months when it was 24% less than that for controls, but greatest deficiency in weight increase velocity occurred between 6 and 18 months of age when increments were 22% less than those in the control population (Cronk, 1978). The reduction in growth rate for girls from the age of 10 to 17 years for girls and for boys from 12 to 17 years 27% and 50% respectively, whereas expected adult heights were 127–158 cm for females and 135–170 cm for males (Cronk *et al*., 1988). Myrelid *et al.* (2002) found that European boys with DS are taller than corresponding American boys, whereas European girls with DS, although being lighter, have a similar height to corresponding American girls.

Body mass index (BMI) is in use as a measure of weight status. The negative impact of obesity on the health of every individual and also on the health of individuals with intellectual disabilities has been paid much attention because the prevalence rate of obesity has tripled in many countries since 1980 (Wold Health Organization, 2006). BMI is calculated kg/m^2 and World Health Organisation according to the classification of weight status (Wold Health Organization, 2006).

Individuals with DS have a tendency to be overweight from early childhood onward. Cronk *et al.* (1988) showed that by nine years of age, the average child with DS is above the 95th percentile on the normal children weight-for-height growth chart. At the same time Al Husain (2003) found that BMI values of children with DS below 5 years were within the normal range. Prasher (1995b) investigated adults with DS and found in his study that 31% of males and 22% of females with DS were overweight, while 48% males and 47% females were obese, whereby overweight and obesity was significantly associated with living in the family home compared to supervised community units or in hospital. Carr (1995) and Melville *et al.* (2005) found a greater prevalence of obesity amongst women with DS than men. Carr (1995) found that 63% of women with DS and 27% of men with DS were overweight at the age of 21 years. Melville *et al.* (2005) showed that 37.6% of women with DS were overweight, 33.3% were obese and 6.8% were very obese. At the same time 20.0% of men with DS were obese and only 1.5% were very obese (Melville *et al.*, 2005).

Syndrome specific growth charts have been developed for several different disorders and also for DS (Cronk, 1978; Cronk *et al.*, 1988; Myrelid *et al.*, 2002; Styles *et al.*, 2002). These charts are important tools in the medical care of these children and growth charts for PwDS using the data from Cronk *et al.* (1988) are also available at http://www.growthcharts.com.

2.2.4. Endocrinological problems

Some of the endocrinological disorders like type 1 diabetes and thyroid dysfunction are more frequent in PwDS. Pubertal development has been reported to be almost normal (Carr, 1995; Arnell *et al.*, 1996). Carr (1995) investigation reported that the mean menarcheal age was thirteen and a half years and 79% of the females with DS had very regular periods. Males also have normal onset of puberty, but the mean testicular volume at final height was reduced and the levels of follicle-stimulating hormone were higher in the boys with DS than in the girls (Arnell *et al.*, 1996).

Thyroid dysfunction means altered levels of thyroxine (T4), triiodothyronine (T3), and/or thyroid stimulating hormone (TSH). Such changes may be present along with other hormonal and biochemical disturbances. The prevalence of hypothyroidism in the general population is approximately 0.8%-1.1% aged 18 and older and the prevalence of hyperthyroidism is 1.1%–1.6% (Prasher, 1999). In PwDS, the prevalence of hypothyroidism is found to be greater than hyperthyroidism, but both conditions are more frequent than in general population. About 1% of newborns with DS are identified as having hypothyroidism due to inadequate development of the thyroid gland (Roizen, 2002). Elderly children have an increasing chance of developing autoimmune hypothyroidism. Ivarsson *et al.* (1997) found autoimmune thyroid antibodies in 39% of patients with DS compared to 16% of the controls and prevalence of thyroid autoantibody posi-

tivity increased with age in patients with DS. Prasher (1994) found among adults with DS 12% of subclinical hypothyroidism, 8% of patients had definite hypothyroidism and 3% had hyperthyroidism. Van Allen *et al*. (1999) reported 28.9% of hypothyroidism in adults with DS and the percent increased with age – patients aged from 30–43 years had hypothyroidism in 22.2% and patients from 50 to 68 years had hypothyroidism in 35%. All clinical guidelines recommend regular screening for thyroid dysfunction (Cohen, 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Roizen, 2002; Roizen and Patterson, 2003; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005).

Diabetes type I develops in approximately 1% of PwDS (Roizen, 2002). Goldacre *et al*. (2004) found that diabetes under the age of 30 years was significantly more frequent in the patients with DS than in the reference cohort (rate ratio 2.8; 95% confidence intervals 1.0 to 6.1). Although regular monitoring for diabetes is not indicated, vigilance is recommended (Roizen, 2002).

2.2.5. Vision problems

Many ophthalmic disorders with high prevalence have been reported in children with DS. These include anomalies like cataract, glaucoma, strabismus, keratoconus, and refractive errors (Tolmie, 2002; Roizen and Patterson, 2003; Yokoyama *et al.*, 2006). Approximately 60% of children with DS have ophthalmic disorder that warranted monitoring and treatment (Carr, 1995, Tolmie, 2002; Roizen, 2002).

Refractive errors are the most frequent ophthalmologic problem in PwDS. Several studies have reported 30–76% of overall frequency of refractive errors whereas myopia is reported in 10.3% to 33% and hyperopia in 46.5% to 67% (Van Allen *et al*., 1999; Bromham *et al*., 2002; Kim *et al*., 2002; Roizen and Patterson, 2003, Mohd-Ali *et al*., 2006). Bromham *et al*. (2002) found that myopia and nystagmus are associated with heart defects in children with DS.

Strabismus and nystagmus are other frequent ophthalmic findings in PwDS. Mohd-Ali *et al*. (2006) found in Malaysian children with DS 30% strabismus and 5% nystagmus under 12 years old. In Korean children, nystagmus was observed in 22% of patients and strabismus was found in 25% of patients, whereas in their study more exotropia than esotropia was found (Kim *et al*., 2002). Yurdakul *et al*. (2006) screened Turkish PwDS for strabismus and found that 19% of patients had strabismus, whereas esotropia was more frequent than exotropia. They also found that the frequency of clinically significant hypermetropia was higher in the group with strabismus than in the group without strabismus.

Strabismus and refractive errors are both treatable conditions, and early diagnosis and treatment improves the quality of life of PwDS. Thus it is important to have an annual control to identify refractive errors that may develop and to screen for other disorders that could appear later in life (Cohen, 1999; American

Academy of Pediatrics, 2001; Marder and Dennis, 2001; Roizen and Patterson, 2003; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005).

2.2.6. Otolaryngologic problems

Otolaryngologic problems are also common in children with DS. This includes problems with stenotic ear canals, chronic ear infections and chronic middle ear effusion with associated hearing loss, airway obstruction, and sleep apnea.

Stenotic ear canals occur in up to 50% of newborns with DS (Shott, 2006). The majority of children show that the stenotic ear canal grows with age and by the age of 2 or 3 years it is not a problem any more (Shott, 2006). However, until that time regular visits to an otolaryngologist are obligatory every three months for cleaning and evaluation of the middle ear space (Shott, 2006).

Chronic ear disease and secondary hearing loss due to chronic ear disease are also three times more frequent in children with DS compared with other children (Shott, 2006). Unilateral or bilateral hearing loss is present in 25% to 78% of children and adults with DS (Van Allen *et al.*, 1999; Tolmie, 2002; Roizen and Patterson, 2003; Shott, 2006). The most frequently seen is sensorineural hearing loss, which is found up to 22% of cases (Roizen, 2002). Conductive and mixed hearing loss is less common. Conductive hearing loss may be mostly due to inadequate treatment of the chronic otitis media, middle ear fluid or structural abnormalities of the middle ear (Roizen, 2002; Tolmie, 2002; Shott, 2006). Full audiological assessments should, therefore, be carried out between six moths and one year and older children may have annual assessments lengthening to two or three yearly intervals in adults (Cohen, 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Tolmie, 2002; Roizen and Patterson, 2003; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005).

Obstructive sleep apnea syndrome is due to upper airway obstruction and includes apnea or hypopnoe episodes with resultant hypercarbia and episodes of sleep fragmentations with increased arousal during sleep. In DS, the predisposing factors are mid-face and mandibular hypoplasia, presence of large tongue, obesity and generalized hypotonia. Sleep-related obstruction has been reported to occur in up to 100% from age 4 weeks to 51 years whereas obstructive sleep apnea was present in 63% (Shott, 2006).

2.2.7. Celiac disease

Celiac disease (CD), also known as gluten-sensitive enteropathy, affects individuals of all ages, both during childhood and adolescence, and is characterized by permanent gluten intolerance. It is an autoimmune disorder, which results from a reaction to gluten, the protein present in cereals. The mean prevalence of CD is 0.3–1.5% in children between 2 and 15 years in general population

(Ivarsson *et al.*, 2000; Mäki *et al*., 2003; Hill *et al.*, 2005). Indeed in Estonia, the incidence of CD is 0.37 per 1000 live births (Uibo *et al.*, 1996).

The first description of a child with DS, retinoblastoma and CD occurred in 1975 (Bentley, 1975). Thereafter a lot of studies have been conducted to investigate the relation between DS and CD and these studies have led to conclusion that CD is significantly more frequent in DS, between 1.6 % to 16.9% of cases, than in the general population (Hilhorst *et al*., 1993; George *et al.*, 1996; Jansson and Johansson, 1995; Pueschel *et al*., 1999; Bonamico *et al*., 2001; Book *et al*., 2001; Carnicer *et al*., 2001; Nisihara *et al*., 2005).

CD, in its classic form, appears with the symptoms and signs of intestinal malobsorption – with chronic diarrhea, abdominal distension and growth retardation with villous atrophy of small-intestinal mucosae. The term "atypical" celiac disease is used if the patients have mostly extraintestinal symptoms like neurological diseases, immunoglobulin-A – nephropathy and villous athrophy. The disease may be also in a "latent" form, which refers to people who have positive serological screening tests, but who do not have a flat mucosa despite a gluten-containing diet and will develop the disease later in life (Holtmeier and Caspary, 2006). An Italian multicenter study showed that 69% of CD patients with DS have a classic form of disease, whereas 11% showed atypical symptoms, like short stature and anemia, and 20% showed a silent form (Bonamico *et al*., 2001).

The diagnostic criteria of CD are based on the findings of small-intestinal mucosal villous atrophy with crypt hyperplasia. Atypical symptoms make the diagnosis difficult, so serological screening tests are used to identify patients for whom the procedure is indicated. The problem for patients with DS is that many clinical symptoms may be attributed to features of DS itself. Commercially available tests include anti-gliadin immunoglobulin-A and immunoglobulin-G, anti-reticulin immunoglobulin-A, anti-endomysium immunoglobulin-A, and anti-tissue transglutaminase immunoglobulin-A antibodies. These tests are widely used in clinical practice. Several studies are shown that anti-endomysium immunoglobulin-A and anti-tissue transglutaminase immunoglobulin-A are highly sensitive and specific tests for identifying individuals with CD also in DS (Rumbo *et al.*, 2002; Collin *et al*., 2005; Hansson *et al.*, 2005). Regular serological testing is recommended for all children with DS (Cohen, 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Roizen, 2002; Suomen Kehitysvammalääkärit, 2004; Hill *et al.*, 2005; Murphy *et al*., 2005).

2.2.8. Alzheimer disease and epilepsy

An increased incidence of Alzheimer disease in people who have DS has been clearly established (Prasher, 1995c; Bush and Beail, 2005; Menéndez, 2005). Alzheimer disease is the major cause of dementia and the prevalence of demen-

tia in institutionalised patients with DS is 8% for the age range 35–49 years, 55% between 50 and 59 years, and for those over 60 years the prevalence rate is 75% (Menéndez, 2005). There is evidence that women with DS have a greater risk of attracting Alzheimer disease than men (Bush and Beail, 2005). Middle-aged women with DS have a more severe form of Alzheimer disease and women with menopause 46 years or younger have an increased risk of Alzheimer disease compared to women with menopause who are 46 years or older (Menéndez, 2005). The diagnosis of Alzheimer disease is quite problematic in individuals with intellectual disabilities. Diagnostic tests, which are available for individuals with normal intellect, are not suitable for people with intellectual disability, because such tests are based on a mean intelligence quotient of 100. This is the reason behind the development of the screening questionnaire Prasher *et al*. (2004) worked on for dementia in Alzheimer disease in adults with DS.

Individuals with DS also show a higher incidence of seizures than individuals without DS; the general prevalence of PwDS with epilepsy is about 5% to 15.9% (Prasher, 1995a; Van Allen *et al*., 1999; Menéndez, 2005), but as with Alzheimer's, the prevalence of epilepsy increases with age reaching 46% in those over 50 years (Prasher, 1995a; Menéndez, 2005). The onset of seizures occur mostly at two widely separated age points: approximately 40% of patients begin having seizures before the age of one year and in 40% the seizure activity begins in the third decade of life (Roisen and Patterson, 2003; Menéndez, 2005). Descriptions of late-onset epilepsy in DS are rare, but as life expectancy of PwDS has increased, this may become more frequent.

2.2.9. Life expectancy of DS

The trend of improved life expectancy for people with all kinds of disabilities and also for persons with DS is global. Increased survival is not only associated with a longer period of care, but is also related to a longer period of more specialized needs. The information of life expectancy of DS is needed in order to facilitate the development of plans for their medical care, education, employment, and integration into the community.

Baird and Sadovnick (1989) calculated the life expectancy in DS up to 68 years and found that 85% of infants survive to one year of age, 80% survive to age 10 years and 60% of PwDS can expect to live longer than 50 years. Although survival beyond 60 years was markedly better than was reported earlier, it was still significantly poorer than survival for the general population. Leonard *et al.* (2000) examined the chances of survival of infants born with DS in Western Australia and found a clear improvement in the probability of survival over time; up to 92% for one year and up to 85% for ten years. These are almost same results that Rasmussen *et al.* (2006) discovered in metropolitan Atlanta, where for the same age ranges the probabilities for survival were 92.9% and

88.6% respectively. Yang compared the median age of PwDS at death in 1983 and in 1997 and discovered an average year on year increase of 1.7 years; the mean age at death of PwDS in 1983 was 25 years, but had increased by 1997 to 49 years (Yang *et al*., 2002). Frid *et al*. (2004) did not find a significant reduction in neonatal mortality in patients with DS born between 1973–1980 and 1995–1998, but found a highly significant reduction in infant mortality from 14.2% to 2.3% between the same two periods.

Many investigators have studied the causes of death in PwDS of which CHD is the major cause. There are significantly higher mortality rates for PwDS with CHD than for those PwDS without CHD (Baird and Sadovnick, 1987; Leonard *et al.,* 2000; Yang *et al*., 2002; Day *et al*., 2005). DS complicated by CHD reduces life expectancy; the chances of survival beyond one year for a child with DS but without CHD is 90.7% but falls to 76.3% for a child with DS and CHD (Baird and Sadovnick, 1987) and while Leonard *et al.* (2000) found a reduction in the chances of survival for the same age period 94.4% falling to 88% the team also indicate that survival of PwDS with CHD is improved with years. The median age at death of those who have CHD, remained low and unchanged in other racial groups, but increased in white people after 1992 (Yang *et al*., 2002).

The second major cause of death, in PwDS, is respiratory infections. Several studies have shown that respiratory infectious diseases are one of the main causes of death in all age groups in PwDS (Yang *et al*., 2002; Day *et al*., 2005; Bittles *et al*., 2006).

Malignancies, except leukaemia and testicular cancer, were much less often than expected the cause of death in people with DS (Yang *et al*., 2002; Day *et al*., 2005). Children with DS younger than 10 years were much more likely to have leukaemia as the cause of death than children without DS (Yang *et al*., 2002).

Females exhibit a longer mean lifespan than males in the general population of all developed and most developing countries. Contrary to this Leonard *et al.* (2000) found that the relative survival of girls with DS was poorer and explained this with a significantly higher CHD rate in girls in the study group. A Western Australian study also showed the increased average survival of males with DS – median age for men was 61.1 years and for women it was 57.8 years (Glasson *et al*., 2003). At the same time several authors have not found any difference by gender in mortality rates in PwDS (Frid *et al*., 2004; Day *et al*., 2005). The median age at death is also lower in black people with DS compared to white population, but there is still no explanation (Yang *et al*., 2002; Rasmussen *et al.*, 2006).

2.3. Parents satisfaction with medical and social benefits

Over the last half-century there have been great changes in how families of disabled children are viewed. They were initially stigmatised as guilt ridden, rejecting, and over-protective (Carr, 1995). Later a greater concern with facts developed, derived from families themselves and the problems they identified. There has in addition been a move away from a search for problems or negative outcomes towards the identification of positive outcomes (Carr, 1995). Consequently more and more children with DS are being seen in every day community situations.

2.3.1. Satisfaction with information

Patients and families react differently to bad news, depending on their preparedness, culture, and coping skills. Parental response in nearly all cases to the news that the baby has Down's syndrome is a traumatic crisis reaction and their need for support at that time is considerable. Quine and Pahl's (1987) research into parental response indicate that 64% were stunned, confused or numb, 4% felt rejection, 8% felt anger, 4% felt relief from anxiety and 19% took the information calmly because they had known that there was something wrong with their child. Ptacek and Eberhardt (1996) concluded in their review of the literature on breaking bad news found that more research should be done on the topic and that medical doctors have to learn more about how to communicate with patients and their families. Parents wish to be informed as soon as possible, both parents together, and in a quiet private room, whereas informing the patient of a negative diagnosis over the telephone is best to be avoided (Cunningham and Sloper, 1977; Shiono and Kadowaki, 1979; Quine and Pahl, 1986; Ptacek and Eberhardt, 1996; Chisholm *et al.*, 1997). The timing of the disclosure and the subsequent effect was researched by Hedov *et al.* (2002b) and showed that the vast majority of the parents were informed within 24 hours after the birth and that 37% of families were satisfied with the timing of the disclosure of the diagnosis; dissatisfaction was most prominent when the disclosure was delayed. Furthermore, 25% of parents were not satisfied with the informant's basic knowledge of DS, with the absence of written information, the preponderance of negative information and the lack of communicative skills in professionals (Hedov *et al*., 2002b). Quine and Pahl (1987) showed that while 64% of parents were informed about the impairment, 21% had been given only the diagnosis and only 27% of parents received advice on how to get help and to care for the child.

2.3.2. Satisfaction with medical benefits

There are to our knowledge only a few publications about the parents' satisfaction with medical care in children with DS. As the life expectancy of PwDS has increased in recent decades, so DS population attending family doctors practice is increasing. However, many medical conditions in children with DS may get less attention than needed by physicians because of the main diagnosis. Mayor (1999) reported that 28% of parents of a child with DS expressed dissatisfaction with the medical care that their child had received. Parents were concerned that the diagnosis of DS affected the treatment decisions made by health professionals. At the same time, Carr (1995) found that more than a half of the mothers of children with DS at age 4 years and 70% of the same mothers at the child's age of 21 years were satisfied with their family doctors and medical care. Many medical guidelines for PwDS in different age groups were developed in different countries to help physicians in their everyday work with patients with DS (Cohen, 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Roizen, 2002; Roizen and Patterson, 2003; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005).

2.3.3. Stress factors and family life

The effect of a child with intellectual disability on the family may be emotional, social or economic or all three together (Turner *et al.,* 1991). Parents of children with disabilities have a more stressful life than do parents without disabled children (Beckman, 1991; Dyson, 1991; Lam and Mackenzie, 2002). They have to accept the fact of losing a 'normal' child and live with reality of having a 'different' child. At the same time they have to integrate the disabled child into both the family and community (Lam and Mackenzie, 2002). They have to spend most of their time, energy and patience in taking care of their child, to manage the child's health, emotional and behaviour problems (Lam and Mackenzie, 2002). Hedov *et al.* (2002a) found that in Sweden 67% of the mothers with a child with DS were stressed by feeling extra demands on their time compared with 46% of the control mothers; fathers of the child with DS also felt more extra demands on their time compared with control fathers. Beckman (1991) found significant difference in the amount and type of stress between mothers and fathers of children with disabilities. Contrary to this, Hedov *et al.* (2002a) found the only difference between mothers of the children with DS and fathers of the children with DS was that mothers were more exhausted compared with fathers.

The socio-economic situation impacts on the whole family and the functioning of the family unit. Mothers in lower social classes are more likely to feel increased levels of stress (Beckman, 1991; Turner *et al.*, 1991). Several mothers who previously had worked full-time had to give up their jobs to take care of

the child (Lam and Mackenzie, 2002). By contrast, Hedov *et al.* (2002a) found that there was no difference in total employment rate between the parents of children with DS and control group, but at the same time the mothers of the children with DS more commonly worked part-time than the control mothers.

Bränholm and Degerman (1992) found that all families of children with DS were satisfied with family life. Dyson (1991) and Van Riper *et al.* (1992) also found that the presence, or not, of a child with DS did not affect the functioning of a family.

3. AIMS OF THE STUDY

There were seven aims of the study:

- to investigate the birth prevalence of DS in Estonia;
- to estimate the influence of antenatal diagnostics on the birth prevalence of DS;
- to determine the accuracy of clinical diagnosis of DS in Estonia;
- to evaluate the cytogenetic findings, clinical features and the incidence of associated medical problems of patients with DS in Estonia;
- to investigate the extent of parents' satisfaction with disclosure of the diagnosis and social services in Estonia provided for DS patients and their families;
- to investigate how the families are coping with having a child with DS;
- to develop the first medical guidelines for DS patients in Estonia.

4. MATERIALS AND METHODS

4.1. Establishment of the prevalence rate of DS and the influence of antenatal diagnostics on the prevalence

This is a population-based descriptive epidemiological study involving the whole of Estonia. The data concerning children with DS born in Estonia between 1 January 1990 and 31 December 2005 were collected from Tallinn Children's Hospital and from the Medical Genetic Center of Tartu University Hospital (MGC-TUH). We upgraded and controlled these data in several ways. First, we personally contacted Down syndrome support groups and requested access to their databases. Second, we visited the institutions of disabled children in Estonia with the aim of finding all children and adolescents with DS. Finally, we contacted all family doctors and/or paediatricians by mail, several times if necessary, and asked about DS individuals in their register.

DS was clinically diagnosed in 264 children during the study period. Six of these children were not included in the epidemiological study as four of them they died before cytogenetic investigation was performed and in one patient the cytogenetical analysis did not confirm the diagnosis of DS. The study group, therefore, consisted of 259 live born children, in all of whom the diagnosis of DS was confirmed by cytogenetic analysis.

As we do not have sufficient information on cases arising from stillbirths and abortions, we have not included these cases in the study.

Amniocentesis for antenatal screening for chromosomal abnormalities has been used in Estonia since 1995 and we used the data of MGC-TUH concerning the prenatally diagnosed DS cases. We have regular follow-up data regarding prenatally diagnosed anomalies and according to this data all prenatally diagnosed foetuses with DS were terminated. Therefore we divided the children with DS into two groups: those born from 1990–1994 (Group I) and from 1995–2005 (Group II). The live birth prevalence of DS per 1000 births was calculated for each group, by dividing the DS incident cases of the period with total live births during the same period. We obtained the data concerning total live births in different maternal age groups in Estonia from the Statistical Database of the Statistical Office of Estonia (SD-SOE), with a final update on 25th of May, 2006 (http://www.stat.ee).

In order to observe the influence of prenatal screening on DS live birth prevalence, we calculated the presumed live birth prevalence of DS. For this we counted the live births babies with DS and prenatally diagnosed foetuses with DS. Before that, we adjusted the prenatal data with late foetal loss rates in pregnancies with DS foetuses by Morris *et al.* (1999), who found that between the time of AC and delivery approximately 23% of pregnancies with DS foetuses end with miscarriage or stillbirth.

The screening of chromosome anomalies for AMA mothers was implemented in Estonia in 1995. Second trimester maternal biochemical serum screening for younger women began in Estonia in 1999 and the markers, which were used were α-fetoprotein (AFP), human chorionic gonadotropin (hCG) and unconjugated estriol-3 (uE3).

We therefore divided Group II into two subgroups to match the chronological implementation of the screening methods: the Group IIa, i.e. children born between 1995 and 1998, when only screening for chromosomal abnormalities of AMA group was possible, and Group IIb, i.e. children born between 1999 and 2005, when second trimester biochemical serum screening and screening for chromosomal abnormalities of AMA group was performed.

Using data about live births and mothers' ages supplied by the SD-SOE, we calculated the estimated live birth prevalence, if no prenatal screening was available. Calculations are based on Wright and Bray (2000) and Bray *et al.* (1998) model. Their model $\pi(m)$ denotes the birth prevalence of Down syndrome for mothers of age-distribution m.

$$\pi(m) = \alpha + \frac{1-\alpha}{1+\exp\{-(\beta_0 + \beta_1 m)\}}$$

where the values of the parameters are:

α=0.000681
β_0=-16.263729
β_1 = 0.2901614

$$\pi(m) = 0.000681 + \frac{1-0.000681}{1+\exp(16.263729 - 0.2901614\, m)}$$

The estimated live birth prevalence from 1990–1994, when there were no possibilities for prenatal screening in Estonia, allows us to compare the applicability of the selected estimation method and test the quality of the available data. At the same time, a comparison with the periods allows us to evaluate the effectiveness of prenatal screening and determine the effect of PD on the prevalence rate.

4.2. Establishment of the accuracy of clinical diagnosis, description of clinical features and incidence of associated medical problems

We investigated 172 patients with clinical diagnosis of DS from institutions of disabled children/adults, Down syndrome support groups and the patients of genetic consulting centres during 1999 to 2003. The diagnosis was confirmed in 170 of the patients by cytogenetic investigations.

The youngest patient in our group was one day old and the oldest patient was 45 years old. The mean age of the patients was 10 years, whereas 90 patients (52.9%) were males and 80 patients (47.1%) were females. There were 15 (8.8%) newborns, 21 (12.3%) were infants, 32 (18.8%) were from one year up to seven years old, school age children were 77 (45.3%) and 25 (14.7%) patients were 18 years or older. Ninety patients (52.9%) lived at home and 80 patients (47.1%) lived in different institutions for disabled persons.

The special study protocol was used for registration of the data based on medical records of the patients or parents' statements and personal examination of the patients.

This study protocol consisted of two parts. In the first part there were general questions about pregnancy, delivery, neonatal period and the official result of chromosomal analysis. Special attention was paid to the presence of congenital anomalies and other medical problems. This data was obtained from medical records or parents' statements. The second part of the study protocol consisted of the description of the phenotype of the patients and this was made during the examination of the patient.

4.3. Cytogenetic investigations of the study groups

Cytogenetic confirmation of the diagnosis of DS was needed for inclusion of the patient in the study and for that we took 2ml heparinized blood sample from the patients for whom we had not yet received an official chromosomal result. The chromosomes were prepared from 72-hour cultures of peripheral blood lymphocyte and banded by using the GTG (G bands by trypsin using Giemsa) banding technique and the chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995). We counted twenty mitosis in each case, and if chromosomal mosaicism was detected, the count was increased to 100 cells per case.

Amniocentesis in prenatal cases was conducted between 15–20 weeks of pregnancy and 15 ml of amniotic fluid was obtained under sterile conditions and collected in a sterile container approved for cell culture. Cells are grown on the inner surface of T-flasks under specific conditions of temperature, humidity and pH until adequate numbers of dividing cells are present. Two parallel cul-

tures were grown from each patient. The cytogenetic analysis was preformed using GTG banding technique and the chromosomes were classified according to the ISCN (1995). We counted twenty cells in each case, and if chromosomal mosaicism was detected, the count was increased to 100 cells per case and the parallel culture was also analysed.

In one case the PD was made by chordocentesis, which was conducted in the 25th week of pregnancy due to abnormal development of the foetus detected in ultrasound investigation. The chromosomal analysis was achieved as with a heparinized routine blood sample. As the child was born in 26th week of pregnancy, we included him in the live born prevalence group and did not count this case as prenatally diagnosed.

4.4. Establishment of the extent of parents' satisfaction with medical and social services provided for DS patients and their families and how families are coping with having a child with DS

The study group consists of 59 families of children with DS, who answered the special questionnaires during the specific research project that focused on autoimmunity in DS. The study period was from 1999 to 2001. The questionnaire was developed for this study and consisted of 31 questions (Appendix I).

One or both of the parents in forty-seven families (79.7%) were Estonians, whereas in eleven families one or both of the parents were of Slavic origin, one mother was of Tartar origin and one father was of German origin. The educational status of mothers and fathers was mostly vocational secondary education (42.4% and 32.2% respectively). 22% of the study parents had higher education. Forty-six percent of mothers and 39% of fathers were between 21–29 years old at the time the child was born.

The youngest person with DS in these families was 5 months old and the oldest was 33 years (mean age 9 years). There were 31 boys/males and 28 girls/females. On average the child with DS was the second child in the family (in 33.9% of cases).

The questionnaire was completed mostly by mothers (in the case of 57 questionnaires). None of those who were asked to fill the questionnaire refused to participate.

4.5. Statistical methods

Statistical analysis for estimating the prevalence of DS was performed using the statistical package R version 2.1.1. (R Development Core Team, 2005). After making assumptions that the relationship between mother's age and DS live

birth prevalence under no prenatal screening is in Estonia similar to the relationship described in Bray *et al.* (1998) and Wright and Bray (2000), we can calculate the population parameter (DS live birth prevalence) using just the age-distribution of Estonian pregnant women. However, the actual number of DS cases in a given year is a random variable. Therefore we cannot predict exactly the actual number of DS cases even if we know exactly the probability of delivering a baby with DS. But, given the DS live birth prevalence as a population parameter, the observed number of DS cases (in a given year) would remain within a certain interval with high probability. Therefore, if the assumptions made (no prenatal screening) are correct, then the number of DS cases (in a given year) should remain within a 95%-tolerance interval with a probability of 0.95 (in 95% of years).

In order to establish the extent of the parents' satisfaction with medical and social services a description of clinical characterization was performed using the statistical package SAS Version 8.02 (Copyright 1999–2001 by SAS Institute Inc., Cary, NC, USA). Continuous variables are presented as mean values with 95% confidence intervals (CI), while quantitative variables are presented as absolute and relative frequencies. The Kolmogorov-Smirnov criterion was used for the assessment of normality. Statistical comparisons between normally distributed continuous variables and categorical were performed with Student's test. In the case of asymmetric continuous variables, the tested hypotheses were based on the calculations of nonparametric tests, such as the Mann-Whitney U-test. To compare proportions (quantitative variables) the Chi-square test and the Fisher's Exact test (when expected values were <5%) were used. Odds ratios (OR) and 95% CI were used to estimate relative risk. To examine the association between the variables, the Pearson's correlation test was used. Statistical significance was set at the 95% level ($p<0.05$).

4.6. Ethics

The Ethics Review Committee on Human Research of the University of Tartu approved the study and informed consent was obtained from the parents or legal guardian of the child with DS for the participation of the study.

5. RESULTS AND DISSCUSSION

5.1. The prevalence of DS in Estonia (Paper I)

There are 259 live birth patients in our study group - 140 boys and 119 girls with DS. The total number of live births in the same period, according to the SD-SOE, was 233,182 (120,066 boys and 113,116 girls). The live birth prevalence of DS was calculated as 1.11 per 1000 live births (1.17 per 1000 live birth boys and 1.05 per 1000 live birth girls). The sex ratio of DS (M:F) is 1.17:1. In all births the sex ratio is 1.06:1. There is no statistically significant difference between the sex ratio of DS and sex ratio of all births. In Figure 5 the live birth DS cases and total live births are shown.

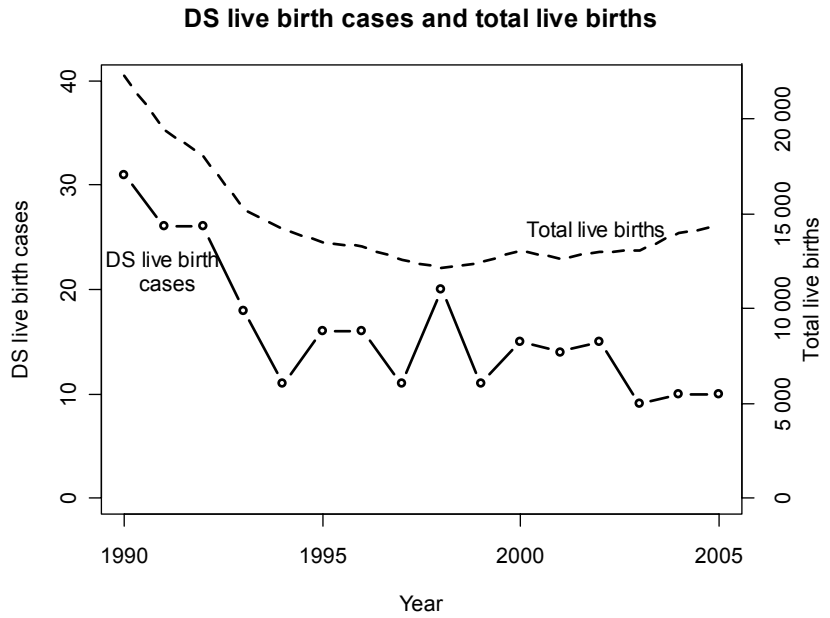

Figure 5 Total live births and DS live births born from 1990 to 2005 in Estonia.

In Group I, 1990–1994, a total of 112 children were born with DS (59 boys and 53 girls; M:F ratio 1.11:1) and according to the SD-SOE, the total number of live births in Estonia was 89,184 (45,870 boys and 43,314 girls; M:F ratio 1.05:1). Based on these data, the live birth prevalence of DS in Estonia was 1.26 per 1000 live births from 1990 to 1994.

In Group II, from 1995 to 2005, DS was diagnosed in 147 live birth patients (81 boys and 66 girls; M:F ratio 1.23:1 and according to the SD-SOE, the total

number of live births in Estonia was during this period 143,998 (74,196 boys and 69,802 girls; M:F ratio 1.06:1). The live birth prevalence of DS in this group was 1.02 per 1000 live births in Estonia.

We divided the second group into two subgroups: IIa for children born between 1995–1998, since up until 1998 there was only screening for the AMA group; and IIb for children born between 1999–2005, since from 1999 onwards the maternal serum screening for mothers less than 35 years was provided.

In Group IIa, DS was diagnosed in 63 patients (38 boys and 25 girls; M:F ratio 1.52:1) and according to the SD-SOE, the total number of births in Estonia for this period was 51,495. The live birth prevalence of DS in this group was 1.22 per 1000 live births. In Group IIb, 84 children with DS were born; 43 boys and 41 girls (M:F ratio 1.05:1). The total number of live births was 92 503 in the same period (M:F ratio 1.06:1). The live birth prevalence of the DS was 0.91 per 1000 live births.

When comparing the children Group I with those in Group IIb, the decrease of prevalence rate of DS live births is statistically significant ($p = 0.024$). Figure 6 shows the estimated DS prevalence with 95% tolerance interval according based on Wright and Bray (2000) and Bray *et al.* (1998) model and in our study observed DS live birth cases.

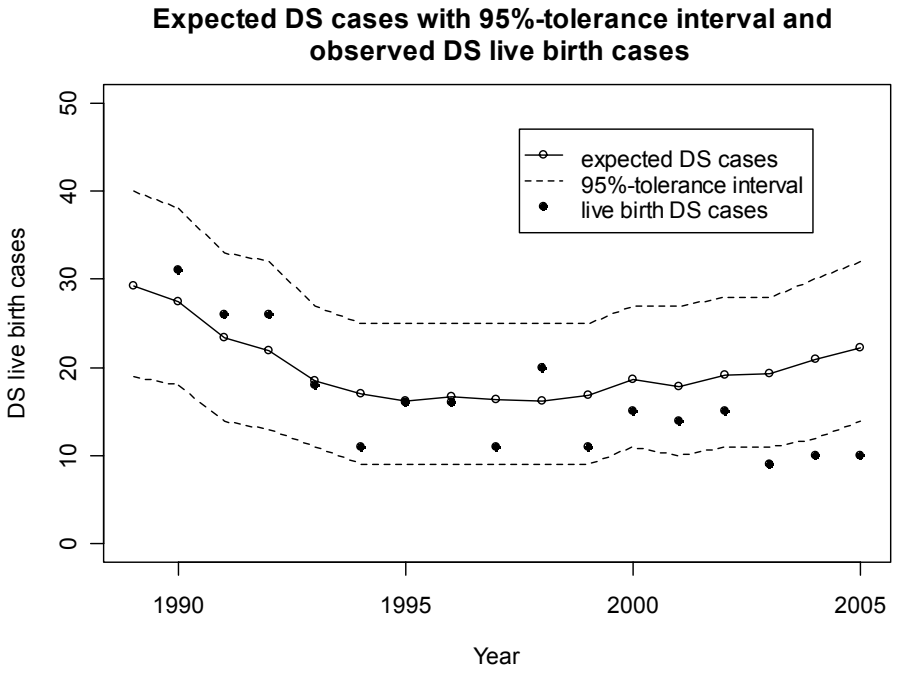

Figure 6. Expected and observed DS live born cases from 1990 to 2005 in Estonia.

The overall life birth prevalence rate in our study is almost the same as the mean prevalence rate in the literature, which is 0.8 to 1.2 per 1000 birth (Mikkelsen, 1977; Olsen *et al.*, 1996; Bishop *et al.*, 1997; Stoll *et al.*, 1998; Rösch *et al.*, 2000; Verloes *et al.*, 2001; Kazaura and Lie, 2002; Lai *et al.*, 2002; Malini and Ramachandra, 2006). Higher prevalence rates were found only in Qatar (1.95), and South-Africa (1.39) (Molteno *et al.*, 1997; Wahab *et al.*, 2006). The provision of PD is not at all possible in Qatar for religious reasons, yet at the same time 48.5% of mothers are above the age of 36 years (Wahab *et al.*, 2006). In South-Africa the number of TOP with foetuses with DS was only 6.1% over 20 years (Molteno *et al.*, 1997). Lower prevalence rates than in our study exist in the Czech Republic and Japan, where the rate is approximately 0.5 DS births per 1000 total live birth (Hoshi *et al.*, 1999; Dzurova and Pikhart, 2005). The low rate in Japan was explained through the estimation method used, in which only hospital-based data and not population based data was analysed (Hoshi *et al.*, 1999). The low prevalence rate of DS in the Czech Republic was explained with the high uptake of PD (almost 1 from every 6 newborns had invasive diagnostic procedures during pregnancy) and also a high ratio of terminated pregnancies (Dzurova and Pikhart, 2005).

The sex ratio (M:F 1.15:1) also bears a close correlation with the data given in the literature (Huether *et al.*, 1996; Tolmie, 2002; Kava *et al*., 2004; Ahmed *et al*. 2005). Even here, however, there are variances. Carothers *et al.* (2001) found a markedly lower sex ratio (0.98:1) and Zahed *et al.* (1998) a more elevated sex ratio (1.66:1) than has been reported in other DS registers. Kovaleva (2002) analysed 55 papers that presented the sex ratio of DS in which the gender was predominantly male. There is no statistically significant difference in sex ratio of DS, in our study, from the whole population sex ratio.

5.2. Prevalence and maternal age (Paper I)

The ‘delivery day’ age of the mothers participating in our study was known for 255/259 of the patients. During the entire study period 80 (31.4%) mothers were 35 years and older while 60 mothers (23.5%) were between 25 and 29 years old when the DS child was born. During 1990–1994 period, 32 (29.6%) mothers were 35 years or older and 21 (19.4%) mothers were between 25–29 years old. In Group II, children with DS born during the period of 1995–2005, 48 (31.0%) of mothers were 35 years or older while 39 (26.5%) mothers of the children with DS were between 25–29 years old. Maternal age distribution and the rates of live born DS cases are shown in the Table 5.

Maternal age at delivery has changed during the last 14 years according to the SD-SOE. The overall proportion of mothers in the age of 35 years and over giving birth to a child increased twice from 1990 to 2005. Only 7.1% of the mothers were 35 years or older in 1990. This percentage decreased by 1994 to

6.7%, but by 2005 was up to 12.5%. At the same time the number of live births in Estonia dropped from 22,304 (11,482 boys and 10,822 girls) in 1990 to 12,167 (6283 boys and 5884 girls) in 1998 and but has since then gradually increased to 14,350 (7486 boys and 6864 girls) in 2005. A decline in the Estonian population of about 200,000 was caused mainly by a low birth rate (especially during the first half of the decade) and by emigration. Most of the emigration related to the end of the Soviet regime in Estonia, and was caused by the departure of people employed by the Soviet armed forces or in the so-called Soviet enterprises. Emigration had considerably less influence on the decline in the birth rate than the changes in the socio-economical situation. There was hope for freedom in 1989, during the "singing revolution" and the birth rate was almost at its highest level. But by the time Estonia declared its Independence in 1991 the socio-economic was in a poor condition and life was quite stressful. Nevertheless, the proportion of the Estonian mothers giving birth during 1989–2005 remained almost the same (66–70%) according to the data of SD-SOE.

Table 5. Maternal age distribution and prevalence rates of DS.

Maternal age groups	Total live births 1990–1994	No of DS cases 1990–1994	Prevalence rate 1990–1994	Total live births 1995–1998	No of DS cases 1995–1998	Prevalence rate 1995–1998	Total live births 1999–2005	No of DS cases 1999–2005	Prevalence rate 1999–2005	p/value 1990/1994 vs 1999/2005
≤19	12671	6	0.47	6428	3	0.46	8338	5	0.6	**0.94**
20–24	34937	27	0.77	18569	13	0.7	26681	10	0.37	**0.067**
25–29	22729	21	0.92	14869	14	0.94	28734	25	0.87	**0.96**
30–34	12521	23	1.84	7547	11	1.45	18911	17	0.9	**0.034**
35–39	5236	13	2.48	3321	12	3.61	8067	20	2.48	**1**
≥40	1075	18	16.74	751	10	13.31	1763	7	3.97	**0.0009**
Unknown maternal age	15	4		10	0		9	0		
Total	89184	112	1.26	51495	63	1.22	92503	84	0.91	**0.024**

5.3. The impact of prenatal diagnosis on the prevalence of DS (Paper I and V)

Although we prenatally diagnosed 103 cases of DS from 1995 to 2005, one child diagnosis achieved in the 25th week of pregnancy by chordocentesis was not included in the prenatally diagnosed group as the child was born in the following week. So the prenatally diagnosed study group actually numbered 102.

For the study period, 1995–2005, these 102 prenatally diagnosed cases constitute 40.9% of all detected cases of DS. The proportion of prenatally diagnosed cases increased from 12.5% in the Group IIa (1995–1998) to 52.5% in Group IIb group (1999–2005). All prenatally diagnosed foetuses with DS were terminated thereafter.

In Estonia, the uptake of antenatal diagnosis in the AMA group has increased over the years from 10% up to 67%. Also the uptake of serum screening is steadily increasing. Eighty-eight percent of pregnant women younger than 35 years in Estonia were monitored with serum screening in 2005. The increasing uptake of PD is mirrored in several countries (Olsen *et al*., 1996; Mutton *et al.*, 1998; Bell *et al.*, 2003; Khoshnood *et al.*, 2004a; Jou *et al.*, 2005).

The increase in uptake of PD leads to an increasing number of prenatally diagnosed DS cases and this is also evident in several other studies (Mutton *et al.*, 1998; Verloes *et al*., 2001; Binkert *et al.,* 2002; Bell *et al.,* 2003) and consequently may cause the decrease of prevalence rate of DS life birth (Olsen *et al*., 1996; Verloes, 2001; Lai *et al*., 2002; Egan *et al.*, 2004; Khoshnood *et al.* 2004; Jou *et al.*, 2005). However, with an increasing proportion of older women giving birth, the overall prevalence of DS should increase as well or not show any changes (Nicholson and Albrman, 1992; Hoshi *et al.*, 1999; Rösch *et al*., 2000; Binkert *et al.* 2002; Bell *et al.*, 2003). We found in this study the prevalence rates decreasing from 1.26 in 1990–1994 to 0.90 per 1000 live birth n 1999–2005 ($p = 0.024$). The most significant decrease is among the women 40 years old and older, where the prevalence dropped from 16.74 to 3.97 per 1000 live birth ($p = 0.0009$). The second age group of women between the ages of 30–34 years the decrease in prevalence was also statistically significant from 1.83 to 0.9 per 1000 live birth ($p = 0.034$). While there is a visible decrease in the DS prevalence in the age group 20–24 years, this decrease was not statistically significant ($p = 0.067$). This data is also shown in Table 6.

Using SD-SOE data about live births and mothers' ages, we calculated the estimated live birth prevalence if no prenatal screening was available. Estimated live birth cases from 1990–1994, when there was no possibility for prenatal screening in Estonia, would be 88 to 129 children with 95% tolerance interval. One hundred and twelve children with cytogenetically confirmed DS diagnosis were born in this period, so we may say that there is no statistically significant loss in our data. When AMA screening began to be offered, from 1995–1998, the estimated DS live birth prevalence rate would have been 1.27 per 1000 total live births or the birth of 50 to 82 children with a 95% tolerance

interval; in reality 63 children were born in this period. Second trimester screening with AMA screening has been offered since 1999. The estimated live birth prevalence of DS for the period from 1999–2005 would have been 1.46 per 1000 live birth or the birth of 113 to 158 DS children, with a 95% tolerance interval; in reality 84 children were born. Figure 7 shows the estimated DS prevalence based on Wright and Bray (2000) and Bray *et al.* (1998) model with 95% tolerance interval and live born DS cases with prenatally diagnosed DS cases after adjustment by Morris *et al.* (1999).

Table 6. Livebirths, prenatally diagnosed and estimated cases of DS**.**

Period	Total number of live-births in Estonia	No of DS livebirths cases	Livebirth prevalence per 1000 livebirth	Sex Ratio M:F	No of estimated DS live-births 95% tolerance interval [a]	No of prenatally de-tected DS pregnancies	No of DS cases would be born without prenatal diagnosis[b]
1990–1994	89,184	112	1,25	1,05	88–129	–	–
1995–1998	51,495	63	1,22	1,52	50–82	9	70
1999–2005	92,503	84	0,91	1,06	113–158	93	152

[a] calculated by Bray *et al.* (1998)
[b] after the correction for late fetal loss rates in DS pregnancies by Morris *et al.* (1999)

The choices available to women, after a prenatally diagnosed DS, differ very much and depend on the cultural and religious customs of the country. TOP at the woman's request has been legal in Estonia since 1955 under the legislation of the Soviet Union. Pregnancy may be terminated until the 11th week of pregnancy at the woman's request, and until the 21st week of pregnancy on medical grounds only. While Siffel *et al.* (2004) found the higher proportion of TOP among pregnancies to older age women; the proportion of cases of DS which were prenatally diagnosed and followed by TOP, in EU as a whole in 1995–1999, varied from 0% in Ireland and Malta, where the TOP is illegal, to 77% in Paris (Dolk *et al.*, 2005). The highest rates of TOP for prenatally diagnosed DS are the totalities of Estonia where foetuses with DS diagnosed prenatally before week 22 of pregnancy are terminated and Sweden (Saltvedt *et al.* 2005); 97% were terminated in Hungary (Métneki and Czeisel, 2005) and 92% in England and Wales (Mutton, 1998; Savva *et al.,* 2006). There are lower rates of TOP for prenatally diagnosed DS in Israel where the data for 1997 possibly indicates a religious limitation to choice as the rate was 61.2% among Jewish women living in Israel and 35.7% among Arab women living in Israel 35.7% (Merrick, 2001).

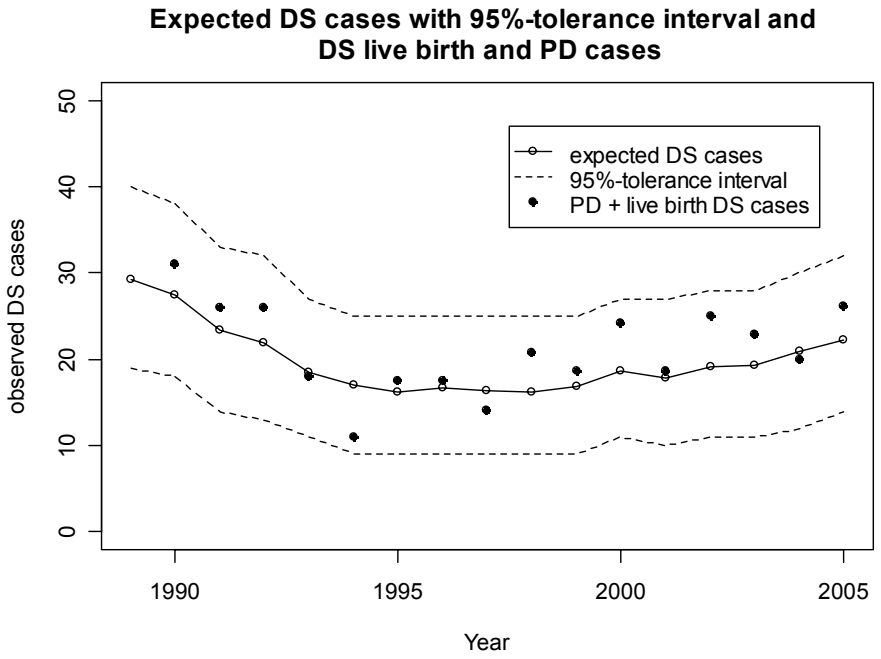

Figure 7. Expected DS cases with 95% tolerance interval and live born DS cases with prenatally diagnosed DS cases.

5.4. The results of cytogenetic analysis and the accuracy of clinical diagnosis of DS (Paper I; II and III)

The clinical features of DS are quite easy to recognize if you can observe them. Cytogenetic analysis confirmed diagnosis in 98.8% (170/172) of clinically diagnosed patients with DS; normal human karyotyping was found in two (2/172) cases in our study. Ahmed *et al.* (2005) and Delvin *et al.* (2004) indicated in their studies that cytogenetic analysis confirmed 69%-90.8% of clinically diagnosed patients with DS. The accuracy, however, in the United Kingdom of clinical suspicion is only 64% (Hindley and Medakkar, 2002; Sivacumar and Larkins, 2004). This comparison indicates that Estonian pediatricians and family doctors are excellent at clinically diagnosing DS.

Ninety-one percent of the clinical DS diagnoses were made at maternity hospitals by paediatricians. The cytogenetic confirmation of 48% was made during the child's first month of life. The diagnosis was confirmed in 10%, 6 from 59, when the child was older than one year. Heuterman *et al.* (2004) showed in their work that there is a suspicion of DS inside the first week of life in 87.2% of patients, that the cytogenetical conformation was received during their first month of life in 91.3% of babies with DS and only 1.7% of patients

were cytogenetically diagnosed later than one year after of age. The relatively low percent of cytogenetic diagnosis during the first month of child's life and the consequential high rate of diagnosis after one year in our study was mostly due to logistical reasons; since for many years it was only possible to perform the cytogenetic analysis at the Hospital of University of Tartu, and therefore the families who lived far away often decided to postpone this investigation. One of the mothers, of these 6 children, stated when the diagnosis was confirmed much later than one year that she had known all along that her 8 years-old daughter had DS, and the cytogenetic confirmation would not give her any new insight. Another mother came to a geneticist when her child was 1.5 years old, only because she was pregnant and afraid of giving birth to a second baby with DS. Heuterman *et al.* (2004) found that the speed of diagnosis of DS was faster for babies born in hospital compared to those born at home. Therefore as in Estonia no possibility of giving birth at home, this is not the reason for under-ascertainment here.

The regular trisomy (47,XX,+21 or 47,XY,+21) was found in 234/259 (90.3%) patients, translocation in 17/259 (6.6%) and mosaicism in 7/259 (2.7%) cases. There was one instance of a combination of translocation and mosaicism at the same time: 46,XY,t(7;21)(p22.2;q11.2),+21(80%)/ 46,XY(20%). One child with a regular Tr21 had an additional translocation between chromosomes 13 and 14 – 46,XY,der(13;14)(q10;q10),+21.

Regular trisomy was found in 97/112 (86.6%) patients in Group I (born 1990–1994), 52 boys and 45 girls. Translocation was found in 11 (9.8%) patients (5 boys and 6 girls) and mosaicism in 4 (3.6%) cases (2 boys and 2 girls). In Group II, regular trisomy was found in 137/147 (93.2%) patients (born 1995–2005), 75 boys and 62 girls and mosaicism in 3 (2.0%) patient (2 boys and 1 girl). There were fewer translocation cases in Group II, only 6 (4.1%) patients (3 boys and 3 girls), but there was one child in this Group with translocation and mosaicism at the same time.

Cytogenetic analysis is essential in order to confirm the clinical diagnosis, and it is needed in order to determine the recurrence risk for relatives. In translocation DS, karyotyping of parents and other relatives is required for proper genetic counselling (Mikelsaar, 2001; Chen, 2006). Our cytogenetic results are in correlation with the mean frequencies of the translocation of chromosome 21, which are assumed to be 4–6%, and mosaicism 2–4% (Métneki and Czeizel, 2005; Schinzel, 2001; Mikkelsen, 1977). At the same time, some investigations show a much lower frequency of mosaicism and translocation cases as explained in Section 2.1.2. There is no specific reason for this variance in the frequency of cytogenetic anomalies. It may be due to differences in the time period or the proportion of mothers 35 years and older. Our study found in Group I (born 1990–1994) translocation in 9.7% of patients and mosaicism in 3.5%, but in Group II (born 1995–2005) these numbers were 4.1% and 2.0% respectively. However, the proportion of mothers of the AMA group is almost the same in

both groups. We can, therefore, explain this variance in translocation cases in our study on the different time periods.

The two main translocation subtypes in our study were 21/21 (58.8%) and 14/21 (23.5%). These are also the main translocation subtypes according to the study literature where the incidence of translocation 21/21 was found to be 28.5%-46.6% and translocation 14/21 was 14%-45.7% (Pulliam and Huether, 1986; Kim *et al.,* 1999; Kava *et al.,* 2004).

Tables 7 and 8 show the cytogenetic results of our study and translocations' subtypes that were found.

Table 7. Results of cytogenetic analysis.

Time period	1990–2005		1990–1994		1995–2005	
	No	%	No	%	No	%
Regular trisomy	234	90.3	97	86.6	137	93.2
Translocation	17	6.6	11	9.8	6	4.1
Mosaicism	7	2.7	4	3.6	3	2
Translocation and mosaicism at the same time	1	0.4			1	0.7

Table 8. Subgroups of translocation cases.

Karyotype	No
46,XY,der(21;21)(q10;q10),+21 or 46,XX,der(21;21)(q10;q10),+21	10
46,XY,der(14;21)(q10;q10),+21 or 46,XX,der(14;21)(q10;q10),+21	4
47,XY,t(8;21)(p21;q21),+21	1
47,XY,t(9;21)(q13;q11),+21	1
46,XY,der(13;21)(q10;q10),+21	1

5.5. The phenotypical features, congenital anomalies and the associated medical problems of patients with DS (Paper II)

We investigated 170 patients with DS and described their phenotypical features, the prevalence of congenital anomalies and the prevalence of associated medical problems. Ninety patients (52.9%) were living at home, 80 patients (47.1%) lived in different institutions for the disabled. The children born in or after 1990 are more probably living at home compared with children born in or before 1989 ($p < 0.0001$; OR = 8.4 with 95% CI 3.8 to 18.6).

Eighty percent of DS patients were born at term; the mean birth weight was 3.1 kg (95% CI 3.0–3.2). Intensive care was needed in 22.1% of the children.

The most typical features of DS were upslanting palpebral fissures (92.9%), muscular hypotonia (91.7%) and hyperflexibility of joints (91.1%). The other most frequent features were protruding tongue (88.2%), and flat facial profile (81.1%). All these features are also the most frequent in the study literature (Gorlin *et al*., 2001; Kava *et al*., 2004; Ahmed *et al*., 2005; Jones, 2006). The features are shown in Table 9.

Table 9. The prevalence of phenotypical features in 170 DS patients.

Microanomalies	**This study**		**Gorlin *et al.*, 2001**	**Kava *et al.*, 2004**	**Ahmed *et al.*, 2005**	**Jones, 2006**
	No	**%**	**%**	**%**	**%**	**%**
Palpebral fissures slant up	158	92.9	79	84	83	80
Hypotonia	156	91.7	–	76	56	80
Hyperflexibility of joints	155	91.1	62	10	–	80
Protruding tongue	150	88.2	42	30	–	–
Flat faces	138	81.1	–	51	–	90
Dry skin	131	77.1	–	–	–	–
Brachydactyly	123	72.3	67	11	24	–
Sandal gap sign	122	71.7	50	46	46	–
Narrow palate	122	71.7	67	–	–	–
Ear abnormalities	121	71.1	70	67	46	60
Epichantal folds	119	70.0	48	57	63	
Short neck	115	67.5	53	37	–	80
Furrowed tongue	105	61.8	–	–	–	–
Flat nasal bridge	104	61.1	61	–	61	–
Abnormal teeth	89	59.3	–	–	–	–
Clinodactyly	95	55.8	59	36	25	50
Hypertelorism	86	52.4	–	34	62	–
Hyperkeratotic skin	87	51.2	–	–	–	–
Excessive skin fold on neck	75	44.1	–	–	–	–
Simian crease	61	35.8	52	33	65	45
Cryptorchidism	9	10.0	–	–	–	–
CHD	51	30.0	–	18	35	40
Gastrointestinal anomalies	8	4.7	–	1	5	12

"–" not mentioned

The existence of CHD was diagnosed in 30% of patients. The lack of the specification of the defect occurred in 22.6% of fixed cases. The most frequent

documented CHD was AVSD, which occurred in 21.5% of the CHD. The same occurrence was documented in VSD. Atrial septal defect was reported in 19.6% of patients. At the same time according to the study literature, the prevalence of CHD in DS is from 26–61%. Källen *et al*. (1996) found the lowest frequency of CHD, 26% of DS patients, but they also noticed the tendency that the frequency of CHD in DS patients is increasing over time. Their explanation for this was the availability of better diagnostic possibilities in recent years. The mean frequency of CHD reported in the study literature is 49%, whereas the most frequent anomalies are AVSD with a mean frequency of 33.2% and VSD with mean frequency of 28.3% (Tubman *et al*., 1991; Källen *et al*., 1996; Freeman *et al*., 1998; Stoll *et al*., 1998; Torfs and Christianson, 1998; Paladini *et al*., 2000; Venugopalan and Agarwal, 2003; Vida *et al*., 2005; Abbag, 2006).

Congenital gastrointestinal anomalies were reported in 4.7% whose medical record contains this data. Two of the children had anal atresia, two had duodenal hernia, two patients had Hirshprung disease, one child had duodenal atresia and in one case there was pyloric stenosis. The overall frequency of gastrointestinal defects found by other investigators is from 1.3% to 6.6% (Lai *et al*., 2002; Bell *et al*., 2003; Kava *et al*., 2004). Although the study literature defines the most frequent gastrointestinal defects as duodenal atresia and Hirshprung's disease (Korenberg *et al*., 1992; Stoll *et al*., 1998; Torfs and Christianson, 1998, Tolmie 2002; Kava *et al*., 2004); Ahmed *et al*. (2005) found the most prevalent gastrointestinal defects in perforate anus, tracheoesophageal fistula and Hirshprung's disease. The most frequent gastrointestinal defect in this study is Hirshprung disease, but the same frequency was found for duodenal hernia and anal atresia.

Hearing impairment was diagnosed in 5.3% of patients. This is much less than is found in other studies, where the frequency of unilateral or bilateral hearing loss is present in 25% to 78% of individuals of all ages with DS (Van Allen *et al.*, 1999; Tolmie 2002; Roizen and Patterson, 2003; Shott, 2006). A great deal more attention must, therefore, be paid for hearing control in our PwDS. A regime of regular check-ups is recommended in all medical guidelines (Cohen, 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Roizen, 2002; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005).

The most frequent visual problems in the study group were refractive errors, which were found in 25.3% of patients. Myopia was found in 14.1%, and hyperopia was found in 11.2%. Nystagmus was found in 18.4% of patients and strabismus in 28.8%. Several studies have reported refractive errors as the most frequent visual problems in PwDS whereas contrary to this study, hyperopia is found to be more frequent than myopia (Van Allen *et al*., 1999; Bromham *et al*., 2002; Kim *et al*., 2002; Roizen and Patterson, 2003; Mohd-Ali *et al*., 2006). The frequency of nystagmus was, in the other studies, found from 3.2% to 22% and strabismus is also found in quite a large scale, from 6.6% to 30% (Kim *et*

al., 2002; Kava *et al*., 2004; Ahmed *et al*., 2005; Mohd-Ali *et al*., 2006; Yurdakul *et al*., 2006).

At the time of the study, celiac disease had already been diagnosed only in one patient (0.6%). The prevalence of celiac disease in persons with DS in the other studies is 1.6% to 16.9% (Hilhorst *et al*., 1993; George *et al.*, 1996; Jansson and Johansson, 1995; Pueschel *et al*., 1999; Bonamico *et al*., 2001; Book *et al*., 2001; Carnicer *et al*., 2001; Nisihara *et al*., 2005). Another study, which was looking just for celiac disease in persons with DS in Estonia, showed that the controlled prevalence of celiac disease in the patients with DS is 3.0% in Estonia (Uibo *et al.,* 2006). There is therefore an argument for the application of regular screening for celiac disease and subsequent diagnostic procedures to patients with DS.

Three patients (1.8%) had the diagnosis of hypothyreosis at the time of the study. The frequency of thyroid dysfunction is also presumed to be much higher. Prasher (1994) found definite hypothyroidism in 8% of individuals with DS and hyperthyroidism in 3%. At the same time, the probability for hypothyroidism increases with age, but the mean age in this study group was 10 years. This may be one reason for the much lower prevalence of hypothyroidism in our patients.

The difference between the results of this study and the results of other studies concerning the frequency of associated medical problems in PwDS might be due to the study design as we only looked at the medical records and asked about medical problems from the parents, but we did not conduct any diagnostic procedure for finding these conditions. Table 10 shows the data about the frequency of congenital anomalies and associated medical problems in our study group.

Table 10. The data about the frequency of congenital anomalies and associated medical problems in 170 DS patients.

Medical problem	**Yes (%)**	**No (%)**	**Not mentioned (%)**
CHD	51 (30.0)	68 (40.0)	51 (30.0)
Congenital gastrointestinal anomalies	8 (4.7)	137 (80.6)	25 (14.7)
Refractive errors	43 (25.3)	78 (45.9)	49 (28.8)
Myopia	24 (14.1)	97 (57.1)	49 (28.8)
Hyperopia	19 (11.2)	102 (60.0)	49 (28.8)
Nystagmus	31 (18.2)	139 (81.8)	0
Strabism	49 (28.8)	121 (71.2)	0
Hearing imairement	9 (2.3)	121 (71.2)	40 (23.5)
Celiac disease	1 (0.6)	0	169 (99.4)
Hypothyreosis	3 (1.8)	0	167 (98.2)

The proportion on the entire group who were breastfed was 64.3% and the mean duration of breastfeeding was four months (95% CI 3.1–4.9). There was no statistically significant difference in breastfeeding between the children living at home and the children living in institutions. However the children, who were not in intensive care had a five-fold greater probability for being breastfed longer than one month ($p = 0.01$; OR = 4.8 with 95% CI 1.3 to 17.1). The mothers of DS children in Estonia are, therefore, more eager to give breast milk to their child than for example in Italy, where Pisacane *et al.* (2003) found that 43% of mothers with a child with DS gave the breast milk to their child and the mean duration was 54 days, which was much less than the duration in the control group. As in this study, the neonates, who were admitted in neonatal unit, were breastfed significantly less frequently than those not admitted to hospital (Pisacane *et al.*, 2003).

The weight for children and adolescents younger than 18 years old with DS, was in 43.6% of cases below 3 percentile and the height was below 3 percentile in 56.1% of patients according to gender and age in growth standards of normal children. Whereas, if comparing their weight and height with DS growth charts produced by Cronk *et al.* (1988) based on United States data, 76.7% of patients' height and 80.9% of patients' weight were between 5 and 95 percentile. The mean BMI for boys/males was 16.5 and for girls/females 17.1. The patients were divided according to their age into three different groups: preschool age from 0 to 7 years, school age from 7 to 18 years and adults 18 years and over. Comparing the BMI of these groups the weight status was worse in persons 18 years and over, where the mean BMI was already a pre-obese group (BMI = 25.2 CI 95% 22.2 to 28.2). This group, however, numbered only 25 patients (15 male and 10 female), which is too few for doing any additional statistical analysis. We looked at the weight for height of DS children in the children and adolescent group against weight for height growth charts for normal children, in two age group – children younger than 7 years and children 7 years to 18 years, and found that 23.2% of the older children were above 90th percentile compared to 5.7% in the younger group ($p = 0.0005$). Cronk *et al.* (1988) found that the average child with DS is above 95th percentile of normal children weight for height growth chart by the age of nine years. BMI values of children below 5 years were found to be in the normal range (Al Husain, 2003). This study shows that it is clinically important to use syndrome specific growth charts that are better for the identification of growth retardation or overweight.

5.6. The parents' satisfaction with disclosure of diagnosis and social services provided for patients with DS and their families (Paper III)

Clinical diagnosis of DS was made at maternity hospitals by paediatricians in fifty-four cases (91.5%) the and therefore the initial information was mostly gathered from them, but also from the geneticist, the family doctors, the doctors from the other hospital and from acquaintances. Forty families (68%) reported that more information and support was needed whereas only 19 (32%) parents were satisfied with the initial information about DS. Quine and Pahl (1987) controversially found in their study, that more than half of parents (55%) of children with DS were satisfied with the way the initial information was given. Also 44% of the parents in a study in Sweden felt that they received the support they needed while a further 30% of parents said that the information they received was sufficient (Hedov *et al.*, 2002b).

Once cytogenetic confirmation of DS had occurred additional information was gathered in several ways – mostly from medical staff, but also from other parents with a child with DS and from publications. Four families said that they had no interest in any additional information because they simply did not need it, and two were also satisfied with the initial information. Thirty-free families (56%) reported satisfaction with the additional information, but only 10 (17%) really knew the precise cytogenetic cause of DS.

Many authors have focused on how to deliver the "bad news". Ptacek and Eberhardt (1996) made a review of the literature on breaking bad news, in which they examined the 13 most consistently mentioned recommendations from 67 English-language articles, and they found that more research should be done on the topic of breaking bad news. Our study shows, there is a need in Estonia to learn to communicate with the parents, especially just after the child's birth, when clinical diagnosis is not yet supported by cytogenetic confirmation. The parents said that they have been given plenty of information about the additional conditions (congenital heart defect or other congenital anomalies and associated medical problems affecting the child in the future), but less information about DS itself. Doctors should not give more information than parents needs or ask for.

Only thirteen families (22%) were fully satisfied with the social benefits. Approximately half of the families of children with DS (31 families) did however state they were more or less satisfied with the social benefits, but 15 of them (25%) were not at all satisfied. The situation regarding possibilities for rehabilitation was more complicated. Only eleven parents (19%) were satisfied with these possibilities, and 28 (48%) were not at all satisfied. Table 11 shows the main results concerning parents' satisfaction with information about the child's disability and the social assistance provided to the family of a child with DS.

Table 11. Parents' satisfaction with information and social help.

Questions	Yes (%)	More or less (%)	No (%)	Not answered (%)
1. Were you satisfied with the initial information you received?	19 (32.2)		40 (67.8)	
2. Are you satisfied with the additional information?	33 (55.9)		24 (40.7)	2 (3.4)
3. Satisfaction with rehabilitation possibilities	11 (18.6)	19 (32.2)	28 (47.5)	1 (1.7)
4. Have you received enough social help for your child?	13 (22.0)	31 (52.5)	15 (25.4)	

5.7. Family coping with having a child with DS in the family (Paper III)

The question of how a child with DS influenced relationships within the family received a response rate of 94.9%. While 29 parents (49%) felt that the atmosphere at home became better or that there were no changes over the years, there were 10 cases (17%) in which the response indicated that the child with DS was the reason for divorce. Carr (1995) indicated that after 21 years with a child with DS, the majority of mothers (96%) deemed the child' effect on the marriage was good or mostly good. She also found that only 7% of marriages in families of child with DS ended with divorce, which was a much lower frequency than in the control group. Unfortunately there was not a control group in our study, so we cannot say if the percentage of divorces in families with a child with DS is higher or not, since the overall percentages of divorces in Estonia is quite high according to SD-SOE (from 1994 to 2004 there was 81,826 legal marriage and 66,207 divorce).

According to this study the family of children with DS was supported mostly by other family members (husband, parents) and less by friends. Carr's study (1995) showed that half of the mothers with an 11 year-old DS child had to manage by themselves haveing a little help from their families, and also from their friends.

However, in spite of their own problems, 41 families (69.5%) agreed to support other families of the child with DS. There is a correlation (the value of Pearson's correlation $r=0.4729$) between a mother's educational status and her agreement to support other families. The more highly educated mothers are more ready to provide support ($p<0.001$). One reason for this is probably the socio-economic situation of the family. Higher educated persons are socio-

economically on a better level and they have less stress factors. Turner *et al.* (1991) showed that mothers in lower social classes are in greater stress.

The most worrying aspects, as perceived by the families, were fears about the future of the child with DS – and their ability to manage without their parents. The second most important topic of concern mentioned was the initial shock experienced after receiving the information about having a child with DS. Nine families said that there were no terrifying aspects at all. Lam and Mackenzie (2002) also showed that the mothers are mostly worried about the child's future in nearest and distant future. They state in their study that some mothers even hoped that their child would die before they did and they were worried about who would take care for their child when they and their spouse died. In Sweden 44% of the mothers of child with DS and 48% of the fathers of the child with DS also felt stressful when thinking of the child's future compared to 11% and 13% of control parents respectively (Hedov *et al.,* 2002a).

The most pleasant aspects perceived by the families in our study are the emotionality and development of the child with DS. Only eight families do not find any pleasant aspect at all. Lam and Mackenzie (2002) also reported that several mothers said that being a mother of a child with DS was not only a negative experience; the positive aspects they related were their child's progress in social and psychomotor skills, other people's acceptance of their child, and also their increased knowledge about social services. The main results of the effect of the child on family life are shown in Table 12.

Table 12. Main results about family life.

	Yes (%)	More or less (%)	No (%)	Not answered (%)
Effect of the child on the marital relationship				
1. Who support you when you have problems?				
a) Husband	38			
b) Parents	20			
c) Parents of the husband	13			
d) Friends	16			
e) Others	17			
2. How did the child influence your home atmosphere?				
Improved	7 (11.9)			
No effect	22 (37.3)			
Deteriorated	27 (45.7)			
Not answered	3 (5.1)			

	Yes (%)	More or less (%)	No (%)	Not answered (%)
Parents' willingness to support other families				
1. Are you willing to be a support family for others?	41 (69.5)		17 (28.8)	1 (1.7)
a) Mothers with elementary education	0		3 (5.0)	
b) Mother with secondary education	9 (15.2)		9 (15.2)	
c) Mothers with vocational education	20 (33.9)		4 (6.8)	
d) Mothers with higher education	12 (20.3)		1 (1.7)	
2. Are you interested in meeting other DS families?	53 (89.8)		6 (10.2)	

5.8. Guidelines for medical management of persons with DS (Paper IV)

Before this project there were no medical guidelines for individuals with DS in Estonia. The development of the guidelines for PwDS was based on the guidelines available in the relevant literature (Cohen, 1999; Van Allen *et al.,* 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Roizen, 2002; Roizen and Patterson, 2003; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005) and the results of our study. These guidelines were approved by the Boards of the Estonian Society of Human Genetics, the Estonian Society of Pediatrics, and were published in "Eesti Arst", the journal that is available to all family doctors and specialists. The guidelines for patients with DS in different age groups are given in Table 13.

Table 13. Medical guidelines for individuals with Down syndrome.

	Heart	Gastrointestinal	Thyroid function	Hearing	Vision	Genitourinary concerns	Others
Neonatal period (birth to one month)	Clinical examination by cardiologist and ultrasound investigation.	To control the congenital malformations.	To check the results of neonatal hypothyreosis screening.		Eye examination by ophtalmologist to exclude congenital catarract, glaucoma, stenotic nasolacrimal duct.		Chromosomal analysis as soon as possible after the clinical diagnosis of DS. Genetic councelling. Complete blood analysis for detecting possible myeloproliferative disorders.
Infancy (1 to 12 months)		Length, weight, head circumference — plot on Down syndrome specific growth charts.	To measure T4 and TSH at 12 month of life.	Full audiological review in 9th month of life.			Neurological examination by child neurologist at 3 and 12 months of life
Childhood (1 year to 12 years)		Height, weight, head circumference — plot on Down syndrome specific growth charts. Screening tests for celiac disease every two years.	To measure TSH at 24 month of life. Afterwards every two years. To consult with endocrinologist in the case of abnormality.	Full audiological review twice a year till 3 years of life. Afterwards once a year.	Ophtalmological refraction examination every year.		Neurological examination to evaluate the signs of spinal cord compression. To give advices for skin care (dry, hyperkeratotic skin, ect)
Adolescence (12 years to 18 years)		Height, weight, head circumference - plot on Down syndrome specific growth charts. Screening tests for celiac disease every two years.	To measure TSH every two years. To consult with endocrinologist in the case of abnormality.	Full audiological review once a year.	Ophtalmological refraction examination every year.	To follow pubertal development. Sexually active women the gynecological follow up every year. To discuss with the PwDS and their parents about contraceptives and abuse possibilities.	To check for diabetes.
Adults (over 18 years)	Clinical examination and in suspicion consult to cardiologist	To observe the overweight signs. Calculate the BMI. Give dietary recommendations.	To measure TSH every two years. To consult with endocrinologist in the case of abnormality.	Full audiological review in every three years if there is no concerning signs. In suspicion for sleep apnea consult with otorhinolaryngologist.	Ophtalmological refraction examination every year.	The gynecological follow up of all (sexually active and not active) women with DS in every year. Ultrasound examination of pelvic region if needed. Annual brest examin of women over 40 years. Control of the testicular cancer.	Annual control of cognitive skills, once in every five years the specialist consulting is obligatory. To check for diabetes.

6. CONCLUSIONS

1. The overall live birth prevalence rate of Down syndrome from 1990 to 2005 was 1.11 per 1000 live births. The prevalence from 1990 to 1994, before prenatal diagnosis was 1.26 per 1000 live birth; from 1995 to 2005, after implementation of prenatal diagnostic procedures, was 1.02 per 1000 live births.

2. The prevalence rate of Down syndrome is significantly lower from 1999 to 2005 compared to 1990–1994 (0.91 and 1.26 per 1000 live birth respectively; p=0.024), when the serum screening of women below 35 years with the advanced maternal age group screening was performed. The decrease is statistically significant in the age groups of women 40 years and over (p=0.00089) and 30 to 34 years (p=0.034). The use of both screening strategies is therefore beneficial for the detection of foetuses with Down syndrome.

3. The clinical diagnosis of Down syndrome is proved by cytogenetical investigations in 98.8% of cases, which is better than in some other regions. Clinical diagnosis was done in a maternity hospital in 91.5% of cases, but only 48% of patients with Down syndrome received the cytogenetical confirmation during the first month of life.

4. The karyotypes of patients with Down syndrome revealed 90.3% regular trisomy, 6.6% translocation, 2.7% mosaicism and 0.4% translocation and mosaicism at the same time, which is in good correlation with other investigations.

5. The prevalence of congenital malformations (30% congenital heart defects and 4.7% gastrointestinal defects) is quite low in our patients with Down syndrome compared to other studies. At the same time it is important to follow up for these malformations just after the birth of the child with Down syndrome, as congenital heart defects for example, may be not noticed only by clinical examination. Among the visual impairments, the most frequent (35.5%) is refractive errors as it is also in the literature. The prevalence of hearing impairment (5.3%), celiac disease (0.6%) and hypothyreosis (1.7%) are lower than in other studies. The follow up procedures for congenital malformations and routine screening for these conditions was suggested in medical guidelines for patients with Down syndrome.

6. A significant majority of parents (68%) feel they would benefit from having more information, especially in the beginning, before cytogenetic confirmation. The additional information was sufficient to more than half of the parents (56%). The medical staffs need more training of breaking bad news and supporting the family. The parental attitude towards the social services benefits available is basically ambivalent with a clear majority (52%) accepting them without clearly defined positive or negative views.

7. Overall almost half of the families (49%) indicate that they are fundamentally positive about the effect a child with DS has on the functioning of their family as a unit and a clear majority of families (69.5%) stated an eagerness share their knowledge and experience with other parents of a child with DS although this eagerness is clearly linked to educational status. The biggest concerns for parents of a child with DS remain the combination of long-term care and financial support should the children outlive them.

APPENDIX I

QUESTIONNAIRE FOR PARENTS OF CHILDREN WITH DOWN SYNDROME

1. Child's date of birth:
2. Child's sex: □girl □ boy
3. Number of children in family:
4. Age of mother at birth of child:
5. Age of father at birth of child:
6. Mother's nationality:
7. Father's nationality:
8. Place of residence (Province/city)
9. Mother's education: □ elementary school □ secondary school
   □ vocational secondary education □ higher education
10. Father's education: □ elementary school □ secondary school
    □ vocational secondary education □ higher education
11. Mother's profession:
12. Father's profession:
13. Your marital status at the birth of your child: □ married
    □ common law □ single
14. During pregnancy, did you have any fears in connection with your child?
    □yes □no If yes, what fears were they:
15. How old was your child when you were told that he or she may have Down syndrome?
16. How old was your child when the diagnosis of Down syndrome was confirmed?
17. From whom did you obtain the initial information about your child diagnosis?
18. Was this information sufficient for you?
19. Did you obtain later sufficient information about your child?
20. From whom did you receive the information later?
21. Do you know the precise cause of Down syndrome?
22. Are you satisfied with the opportunities for rehabilitation in the place where you live? □yes □no □ more or less
23. Do you feel that you have received sufficient help for your child?
    □yes □no □ more or less
24. Who supports you most in problems with your child? □ spouse
    □parents □ husband's parents □ friends □ other (please specify)
25. How did the birth of a Down syndrome child influence your family situation?
26. From your point of view, what has been the most terrible thing about your child?

27. What has been the most pleasant thing?
28. Has what you have experienced in some way influenced your desire to have more children? □ no □ yes (if yes, please specify how):
29. Do you have any recommendations, advice, warnings (for doctors or for other parents):
30. Would you be willing to act as a support person for other families with children suffering from Down syndrome? If so, please provide your contact information (address, telephone). If you wish to remain anonymous, but would still be willing to assist other parents, please write your contact information on a separate piece of paper.
31. Would you be interested in gatherings and training events that would be held once a year (or more often)?

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SUMMARY IN ESTONIAN

DOWNI SÜNDROOM EESTIS

Trisoomia 21 (Tr21) ehk Downi sündroom (DS) on inimese sagedamaid kromosoomihaigusi. Esmakordselt kirjeldas seda sündroomi John Langdon Haydon Down 1866 aastal (Down, 1866). Kolm aastat pärast seda, kui Tjio ja Levan ning Ford ja Hamerton 1956 aastal olid kindlaks teinud, et normaalselt on inimesel 46 kromosoomi (McKusick, 2002), leidsid Lejeune kaasautoritega ja kuu aega hiljem Jacobs kaasautoritega, et Downi sündroomiga patsientidel on üks kromosoom rohkem (47,XX,+21 või 47,XY,+21). Aastase vahega kirjeldasid Polani kaasautoritega (1960) translokatsiooniga DS-iga patsienti ning Clarke kaasautoritega (1961) DS-i mosaiikset vormi. 21. kromosoom on inimese väikseim kromosoom, see sisaldab umbes 1–1,5% inimese genoomist, koosnedes 225 geenist ja 59 pseudogeenist (Hattori, 2000).

DS-i esinemissagedust on uuritud erinevates geograafilistes piirkondades erinevatel perioodidel ning saadud esinemissageduseks 0,8 kuni 1,2:1000 elusalt sündinud vastsündinu kohta (Mikkelsen, 1977; Olsen *et al*., 1996; Bishop *et al*., 1997; Stoll *et al.,* 1998; Rösch *et al.,* 2000; Verloes *et al.,* 2001; Kazaura ja Lie, 2002; Lai *et al.,* 2002). Üheks ammu teadaolevaks riskifaktoriks Tr21 tekkel on ema vanus (Penrose, 1933). Tänapäeval on igal pool maailmas tendents sünnitada hilisemas eas (Beart *et al.,* 2003; Resta, 2005; Dolk *et al.,* 2005). Seega peaks olema tõenäoline ka DS-iga laste sünni sagenemine. Samas on alates 1970ndatest, pärast seda kui Steel ja Berg 1966. aastal näitasid, et amnionivedeliku rakkudest on võimalik uurida tulevase lapse kromosoome, pakutud kõrge riskiga rasedatele sünnieelseid diagnostilisi protseduure (Ben ja Hsu, 2004). Mitmeid uuringuid on tehtud selgitamaks demograafiliste faktorite muutusi ja sünnieelse diagnostika mõju DS-iga laste esinemissagedusele (Nicholson and Albrman, 1992; Olsen *et al.,* 1996; Hoshi *et al.,* 1999; Rösch *et al.,* 2000; Verloes *et al.,* 2001; Lai *et al.,* 2002; Bell *et al.,* 2003; Egan *et al.,* 2004; Jou *et al.,* 2005).

DS-i on kliinilise sümptomatoloogia alusel suhteliselt lihtne ära tunda. Samas on kliinilise diagnoosi täpsus erinevates riikides erinev. DS-i diagnoosi kinnitab tsütogeneetiline analüüs. Näiteks Inglismaal leiab DS-i diagnoos tsütogeneetiliselt kinnitust 64%-l juhtudest (Hindley ja Medakkar, 2002; Sivacumar ja Larkins, 2004), Põhja Iirimaal on see 69% (Delvin ja Morrison, 2004) ning Rawalpindis 90,8% (Ahmed *et al.,* 2005). Täpne tsütogeneetiline diagnoos - regulaarne 21. kromosoomi trisoomia, mosaiiksus (kus esineb ka normaalse karüotüübiga rakuliin või translokatsioon) - määrab kordusriski suuruse ning on oluline perekonna nõustamisel (Mikelsaar, 2001; Chen, 2006).

Iseloomulikud mikroanomaaliad ning kaasnevad väärarengud esinevad DS-iga isikutel erineva sagedusega. On tehtud mitmeid uuringuid selgitamaks, millised fenotüübi tunnused on selle haiguse korral kõige iseloomulikumad (Jacson

et al., 1976; Gorlin *et al.,* 2001; Ahmed *et al.,* 2005). Stoll jt. (1998) on leidnud, et 61,8%-l DS-iga lastest esineb mõni kaasasündinud väärareng, samas Bell kaasautoritega (2003) leidis 45%-l DS-iga vastsündinutel kaasasündinud arenguanomaaliaid. Kõige sagedamaks arenguanomaaliaks on südamerikked, mida erinevad autorid on leidnud 26% kuni 61.3% (Tubman *et al.,* 1991; Källen *et al.,* 1996; Freeman *et al.,* 1998; Stoll *et al.,* 1998; Ttorfs ja Christianson, 1998; Paladini *et al.,* 2000; Venugopalan ja Agarwal, 2003; Vida *et al.,* 2005; Abbag, 2006). Samuti on DS-iga patsientidel leitud 4,8%-6,6% seedetrakti väärarenguid (Lai *et al.,* 2002; Bell *et al.,* 2003).

Kirjanduses on rõhutatud, et DS-iga isikud ei saa sageli oma põhidiagnoosi (DS) tõttu piisavalt meditsiiniabi (Mayor, 1999). Samas esineb neil mitmeid haigusi, nagu näiteks nägemisprobleemid, kuulmisprobleemid, hüpotüreoos, tsöliaakia ja epilepsia, ülekaalulisus sagedamini kui üldpopulatsioonis (Tolmie, 2002; Roizen ja Patterson, 2003). Seetõttu on mitmete riikide arstid välja töötanud DS-iga isikute meditsiinilise jälgimise juhendid (Cohen, 1999; American Academy of Pediatrics, 2001; Marder and Dennis, 2001; Tolmie, 2002; Roizen and Patterson, 2003; Suomen Kehitysvammalääkärit, 2004; Murphy *et al*., 2005).

Eestis pole varem süstemaatiliselt DS-i esinemissagedust uuritud.

Töö eesmärgid

1. Määrata DS-i esinemissagedus Eestis.
2. Uurida sünnieelse diagnostika mõju DS-i esinemissagedusele.
3. Hinnata DS-i kliinilise diagnoosi täpsust Eestis.
4. Hinnata tsütogeneetilise analüüsi tulemusi, fenotüübi kliinilist kirjeldust ning kaasnevate haiguste esinemissagedust DS-iga patsientidel Eestis.
5. Uurida vanemate rahulolu neile antud informatsiooniga haigusest ja neile osutatava sotsiaalse abiga.
6. Uurida, kuidas mõjutab DS-iga laps pere toimetulekut.
7. Töötada välja DS-iga isikute meditsiinilise jälgimise juhend.

Uurimisgrupid ja uurimismeetodid

DS-i esinemissageduse määramine ja sünnieelse diagnostika mõju hindamine esinemissagedusele

Ajavahemikul 1. jaanuar 1990 – 31. detsember 2005 sündinud ja tsütogeneetiliselt diagnoositud DS-iga laste andmed kogusime Meditsiinigeneetika Keskusest ja Tallinna Lastehaiglast. Lisaks kontrollisime neid andmeid mitmel moel: kontakteerusime Downi sündroomi tugigruppidega; külastasime väikelastekodusid, koolkodusid ning hooldekodusid DS-iga isikute leidmiseks; kontak-

teerusime kõikide perearstidega kirja teel, paludes andmeid nende nimistus olevate DS-iga patsientide kohta.

Ajavahemikul 1. jaanuar 1990 – 31. detsember 2005 diagnoositi DS kliiniliselt 265 lapsel. Neli last surid enne tsütogeneetilist uuringut. Kahel lapsel oli tegemist normaalse inimese karüotüübiga. Seega jäi uuringugruppi 259 last, kellel DS-i diagnoos oli tsütogeneetilisel analüüsil kinnitust leidnud.

Alates 1995. aastast on pakutud Eestis sünnieelse diagnostika protseduure kõrgenenud riskiga rasedatele. 1995–1999 olid riskigrupiks rasedad vanuses 35 aastat ja enam. 1999–2005 kuulusid riskigruppi rasedad, kellel vereseerumi sõeluuring näitas kõrgemat riski sünnitada DS-iga laps, ning rasedad vanuses 35 aastat ja enam. Seega jagasime uuringugrupi kaheks: I grupi moodustasid lapsed, kes olid sündinud 1990–1994, mil Eestis ei pakutud rutiinselt sünnieelseid diagnostikaprotseduure, ning II grupi moodustasid lapsed, kes olid sündinud 1995–2005. Viimase grupi jagasime omakorda kaheks: IIa gruppi kuulusid DS-iga lapsed, kes olid sündinud 1995–1999, mil peeti kõrgenenud riskiks vaid raseda vanust, ning IIb gruppi moodustasid lapsed, kes olid sündinud ajavahemikul 1999–2005, mil pakuti riski hinnangut ka raseda vereseerumi markerite alusel. Kõigi gruppide kohta leidsime esinemissageduse 1000 elusalt sündinud vastsündinu kohta, jagades DS-iga sündinud laste arvu elusalt sündinud vastsündinute arvuga. Elusalt sündinud vastsündinute arvu saime Eesti Statistikaameti koduleheküljelt (http://www.stat.ee).

Selleks et hinnata sünnieelse diagnostika mõju elusalt sündinud DS-iga laste esinemissagedusele, arvutasime DS-iga laste potentsiaalselt võimaliku sünnisageduse. Selleks liitsime elusalt sündinud DS-iga lapsed ja sünnieelselt diagnoositud DS-iga looted, olles viimaste arvu eelnevalt korrigeerinud vastavalt Morrise jt (1999) järgi.

Kasutades Eesti Statistikaameti andmeid, arvutasime oletatava DS-i esinemissageduse arvestades sünnitajate vanuselist koosseisu. Arvutusteks kasutasime Wrighti ja Bray (2000) ja Bray jt. (1998) poolt antud valemeid. Samas võimaldas see ka hinnata andmete täpsust ajavahemikul 1990–1994, mil ei pakutud kõrgenenud riskiga rasedatele sünnieelse diagnostika protseduure.

Kliinilise diagnostika täpsus, fenotüübi kirjeldus ning kaasnevate haiguste esinemissagedus

Ajavahemikul 1999–2003 uurisime 172 patsienti, kes elasid erinevates hooldekodudes (väikelaste-, kool- või hooldekodudes), pöördusid geneetiku konsultatsioonile Tartu Meditsiinigeneetika Keskusesse, Tallinna Lastehaigla geneetika kabinetti või kuulusid Downi sündroomiga isikute tugigruppidesse. Uuringusse lülitamise eelduseks oli DS-i diagnoosi kinnitus tsütogeneetilisel analüüsil. Kahel isikul diagnoos tsütogeneetiliselt kinnitust ei leidnud ja seega moodustas uuringugrupi 170 isikut.

Iga patsiendi kohta täideti uuringu protokoll. Uuringu protokoll koosnes kahest osast. Esimeses osas olid üldandmed: kromosoomianalüüsi vastus, andmed raseduse, sünnituse ja sünnijärgse perioodi kohta. Oluline tähelepanu oli

pööratud kaasasündinud väärarengute ja DS-iga sagedamini kaasnevate haiguste esinemisele. Vastavad andmed saime hooldekodude meditsiinilisest dokumentatsioonist ja/või vestlusest lapsevanematega. Teine osa uuringu protokollist koosnes patsiendi fenotüübi kirjeldusest ning see täideti DS-iga isikute läbivaatusel, mida teostas protokolli täitja.

Uuringugrupi tsütogeneetiline analüüs

Uuringugruppi kuulusid isikud, kellel oli tsütogeneetiliselt kinnitatud DS-i diagnoos. Tsütogeneetiline analüüs tehti perifeerse vere lümfotsüütide kultuurist. Sünnieelselt diagnoositud DS-iga loodete kromosoome uuriti amnionirakkude kultuurist. Mõlemal juhul värviti kromosoomipreparaadid Giemsa värvinguga, analüüsiti valgusmikroskoobis 550-bandi tasemel ning karüotüüpide kirjeldused anti vastavalt rahvusvahelisele inimese tsütogeneetika nomenklatuurile (ISCN, 1995).

Vanemate rahulolu neile antud informatsiooniga haigusest ja DS-iga lapse mõju perele

Küsitlesime 59 perekonda, kus elas DS-iga isik. Töötati välja spetsiaalne küsimustik, mis koosnes 31 küsimusest (Appendix I). Küsimustiku täitis üks vanematest, enamikul juhtudel (57/59) ema.

Uuringu peamised tulemused

1. DS-i esinemissagedus Eestis ajavahemikul 1990–2005 oli 1,11:1000 elusalt sündinud vastsündinu kohta. Ajavahemikul 1990–1994, mil Eestis ei pakutud kõrgriski rasedatele rutiinselt diagnostilisi protseduure, oli esinemissagedus 1,26:1000 elusalt sündinud vastsündinu kohta. Ajavahemikul 1995–2005, mil Eestis hakati tegema sünnieelseid protseduure kõrgriski rasedatele (raseda vanus 35 a ja enam ning rasedate vereseerumi sõeluuringu alusel sõelale jäänud kõrgenenud riskiga rasedad), on esinemissageduseks 1,02:1000 elusalt sündinud vastsündinu kohta. Need sagedused on sarnased kirjanduses esitatud andmetega.
2. DS-i esinemissagedus on märkimisväärselt langenud ajavahemikul 1999–2005 võrreldes ajavahemikuga 1990–1994 (0,91:1000 elusalt sündinud vastsündinu kohta *vs* 1,26:1000; p = 0,024). Sel perioodil oli võimalik hinnata riski sünnitada kromosoomianomaaliaga laps nii raseda vanuse kui vereseerumi markerite muutuste alusel ning pakkuda kõrgriskiga rasedatele loote kromosoomide uurimise võimalust. Statistiliselt oluline DS-i esinemissageduse vähenemine on toimunud vanusegruppides 40 aastat ning enam (p = 0,00089) ja 30–34 eluaastat (p = 0,034). Seega on DS-iga loodete sünnieelsel avastamisel oluline nii raseda vanuse riskihinnang kui ka seerumiskriiningu riskikalkulatsioon.
3. DS oli kliiniliselt õigesti diagnoositud 98,8%-l patsientidest. See tulemus on oluliselt parem kui kirjanduses toodud ning viitab Eesti arstide heale kliinilisele DS-i äratundmisele. Enamasti (91,5%) pandi kliiniline diagnoos sünnitusmajas,

kuid vaid 48%-l patsientidest kinnitati diagnoos tsütogeneetiliselt esimese elukuu vältel.

4. Tsütogeneetilise analüüsi tulemusel oli 90,3%-l DS-iga isikutest regulaarne trisoomia, 6,6%-l translokatsioon, 2,7%-l esines mosaiiksus ning ühel patsiendil (0,4%) oli tegemist translokatsiooni ja mosaiiksuse koosesinemisega.

5. Samas on meie uuringugrupi DS-iga isikutel suhteliselt vähe kaasasündinud arenguanomaaliaid. Vaid 30%-l esines kaasasündinud südamerikkeid ning 5%-l seedetrakti väärarenguid. Nende arenguanomaaliate esinemist/mitteesinemist tuleks kindlasti kontrollida kohe peale sündi, sest erinevad uuringud on näidanud, et vaid kliinilisel läbivaatlusel võivad kaasasündinud südamerikked jääda avastamata. Sagedaim nägemisprobleem meie DS-iga patsientidel oli nägemisteravuse langus (35,5%), nagu väidab ka sellealane kirjandus. Seejuures oli sagedasemaks probleemiks lühinägelikkus (20%). Kuulmislangust, tsöliaakiat ja hüpotüreoosi esines meie patsientidel vastavalt 5%, 0,6% ja 2%, mis on oluliselt vähem kui kirjanduses toodud. DS-iga isikute tervisliku seisundi paremaks jälgimiseks oleme koostanud meditsiinilise käsitlemise juhendi.

6. Enamik DS-iga laste vanematest (68%) ei ole rahul esmase informatsiooniga, mida nad on saanud kliinilise diagnoosi selgumisel. Edasise informatsiooniga oli rahul 56% vanematest. Samas vaid 17% vastanutest teadis DS-i etioloogilist põhjust. See näitab, et meditsiiniline personal vajab paremat informeerimise ja nõustamise alast koolitust krooniliste haigusseisundite osas. Sotsiaalabiga oli enamik vanemaid (52%) enam-vähem rahul.

7. Enamasti on DS-iga laste emasid toetanud nende abikaasad ning vanavanemad ja peaaegu pooled vastanuist (49%) leidsid, et nende pere läbisaamine läks paremaks pärast DS-iga lapse sündi. Samas tunnistas 10 ema (17%), et DS-iga laps oli perekonna lahkumineku põhjuseks. Vaatamata igapäevaprobleemidele on 69,5% peredest, kus kasvab DS-iga laps, nõus olema toeks teistele DS-iga lapsi omavatele peredele. Selleks olid enam valmis kõrgema haridustasemega vanemad ($p<0,001$), mis võib olla tingitud majanduslikust toimetulekust – kõrgem haridustase tagab enamasti ka parema toimetuleku.

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