Down Syndrome in Oman: Etiology, Prevalence and Potential Risk Factors. A Cytogenetic, Molecular Genetic and Epidemiological Study

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Down Syndrome in Oman: Etiology, Prevalence and Potential Risk Factors. A Cytogenetic, Molecular Genetic and Epidemiological Study

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Abbreviations:

ABI	Applied Biosystem
ALL	Acute Lymphoblastic Leukaemia
AMKL	Acute Megakaryoblastic Leukemia
AML	Acute Myeloid Leukemia
AMOVA	Analysis of Molecular Variance
ATP	Adenosine triphosphate:
AVSD	Atrioventricular septal defects:
bp	Base pair
ddNTPs	Dideoxynucleotides
DNA	Deoxyribonucleic acid
dNTP	Ddeoxynucleotide triphosphatase
DS	Down Syndrome
EDTA	Ethylenediaminetetraacetic acid
Exo1	Exonuclease-1
gms	grams
HDG	Human Genome Database
HVRs	Hypervariable regions
HVS	Hypervariable segment
Kb	Kilo base pair
LB	Live Birth
Max	Maximum
µl	Microlitre
MDS	Multi Dimensional Scaling
mg	Milligram
MI	Meiosis I
MII	Meiosis II
ml	millilitre
MoH	Ministry of Health
mtDNA	Mitochondrial DNA
MTHFR	Methylenetetrahydrofolate reductase
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
NDJ	Non-disjunction
NOR	Nucleolus organizer region heteromorphism
OMIM	Online Mendelian Inheritance in Man
PBS	Phosphate buffer saline
PHA	Phytohaemagglutinin
PND	Prenatal Diagnostics
rCRS	Revised Cambridge Reference Sequence
SAP	Shrimp Alkaline Phosphates
SCC	Sister Chromatid Cohesion
SDS	Sodium dodecyl sulfate
STRs	Short tandem repeat
TAE	Tris base, acetic acid and EDTA
TBE	Tris base, Borate and EDTA
TD-PCR	Touchdown PCR
Tm	Melting temperature
UV	Ultraviolet
YHRD	Y-STR Haplotype Refrence Database

1. INTRODUCTION

1.1. Introduction

Down syndrome (DS) is the most common autosomal aneuploidy in man associated with mental retardation, developmental delay, and characteristic physical findings. In addition, people with DS have an increased risk for leukaemia, gastrointerstitial tract abnormalities, immune defects, and Alzheimer disease. The birth prevalence of Down syndrome is approximately 1 in 700 to 1 in 1000 which makes the syndrome the most frequent cause of mental retardation. According to the latest report of the March of Dimes, Down syndrome is at the fourth position of serious birth defects worldwide, with an estimated >217.000 live births per anno. Therefore, DS is registered in nearly all surveillance programs for birth defects as a paradigm for aneuploid mutations. The conspicuous DS phenotype and the high proportion of new mutants, make surveillance of trisomy 21 particularly suitable for assessing mutagenic hazards and identifying genetic factors influencing non-disjunction. Nonetheless, even despite decades of research, apart from advanced maternal age, germ line mosaicism, and altered recombination, no single exogenous or endogenous factor responsible for trisomy 21 has been unambiguously identified (Sperling, Pelz et al. 1994; Bishop, Dellarco et al. 1996; Yoon, Freeman et al. 1996; Sherman, Freeman et al. 2005).

1.2. Down syndrome: historical aspects

In 1866, the British physician John Langdon Down (1828-1896) published an article which described children with a common phenotype and with intellectual disability (Birch 1973; Ward 1999). He accurately described the features of DS including hypotonia, mental retardation and facial features, and classical pattern of palmar creases of hands. He referred the name of DS people to “mongoloids” because of their upward slanting eyes which give the impression of mongolian people. In 1961, the WHO informally recommended not to use the term mongolism and to name it Down syndrome because some biomedical researcher were calling to stop the term “mongolism” and to describe people with DS as trisomy 21 anomaly (Howard-Jones 1979). Already in 1932, Waardenburg hypothesized that non-disjunction which leads to trisomy or monosomy might be the cause of DS (Allen 1974). Then, in 1959, the French geneticist Jerome Lejeune showed that DS is caused by a trisomy of chromosome 21 and his finding was subsequently confirmed by a publication from Jacobs and her group (Jacobs, Baikie et al. 1959).

1.3. Down syndrome and types of chromosomal aberrations

The presence of an extra copy of chromosome 21 is the main cause of intellectual and physical characteristics of DS. Cytogenetically DS is divided into three types (Giraud and Mattei 1975):

- Regular or free trisomy 21: all cells have an extra chromosome 21. Approximately 90-95% of individuals with DS have a free trisomy for chromosome 21 (Pangalos, Avramopoulos et al. 1994; Mutton, Alberman et al. 1996; Savage, Petersen et al. 1998).

- Translocation trisomy: the extra chromosome 21 is attached to another chromosome. Translocation trisomies account for 2-4 % of the DS cases. In almost all cases of translocation trisomy, one of the parents is carrier of a balanced Robertsonian translocation of the long arm of chromosome 21 to the short arm of a D- or G-group chromosome (Pangalos, Avramopoulos et al. 1994; Mutton, Alberman et al. 1996; Savage, Petersen et al. 1998). De novo Robertsonian translocation are rare, one between chromosome 14 and 21 t(14;21) has been described originating from maternal germ cells (Petersen, Adelsberger et al. 1991). In contrast, most translocations between the long arms of two chromosomes 21, t(21;21), are isochromosomes due to a duplication of (21q) rather than a result of a Robertsonian translocation (Antonarakis, Adelsberger et al. 1990).

- Mosaic trisomy 21: it is a free trisomy 21 but only some cells have an extra chromosome 21. Mosaicism is defined having two or more genetically distinct cell lines. Approximately 2-4% of DS patients are mosaics (Aula, Leisti et al. 1973; Mutton, Alberman et al. 1996; Nguyen, Riess et al. 2009; Papavassiliou, York et al. 2009).

1.4. Clinical diagnosis of Down syndrome

Dr Langdon Down (1828-1896) was the first to describe the clinical features of DS children precisely (Lejeune, Turpin et al. 1959; Lejeune, Turpin et al. 1959; Ward 1999). The knowledge of clinical manifestations of DS by physicians and other health professionals is important for an early diagnosis in order to reduce morbidity and mortality of these children (e.g. early operation of heart defects). Furthermore, proper clinical diagnosis of DS children is important to avoid normal children being investigated for DS based on only few clinical features (Devlin and Morrison 2004). In 1976, Jackson et al. created a checklist of 25 signs of Down syndrome to

predict the presence of trisomy 21 in 291 patients suspected with DS (Jackson, North et al. 1976; Keppler-Noreuil, Welch et al. 2002)(Table 1).

Table 1: Clinical evaluation of DS by Jackson's physical examination(Jackson, North et al. 1976)

25 signs of Down syndrome	
▪ Brachycephaly	▪ Oblique eye fissure
▪ Epicanthic eye fold	▪ Blepharitis, conjunctivitis
▪ Brushfield spots	▪ Nystagmus
▪ Flat nasal bridge	▪ Mouth permanently open
▪ Abnormal teeth	▪ Protruding tongue
▪ Furrowed tongue	▪ High-arched palate
▪ Narrow palate	▪ Folded ear
▪ Short neck	▪ Loose neck of skin
▪ Short and broad hands	▪ Short 5th finger
▪ Incurved 5th finger	▪ Transverse palmar crease
▪ Gap between 1st and 2nd toes	▪ Congenital heart defect
▪ Murmur	▪ Joint hyperflexibility
▪ Muscular hypotonia	

Figure.1: Frontal appearance of Omani DS child with oblique eye, epicantha flat nasal bridge, protruding tongue, short neck, open mouth, folded ear and round face.

The most common characteristic features of DS are facial features, development delay, hearing and visual abnormalities, gastrointestinal anomalies, congenital heart defects, and leukemia particularly acute megakaryoblastic leukemia. As Down syndrome is associated with many congenital abnormalities and health problems molecular mapping of the so called Down-critical region, DCR, of chromosome 21 was undertaken. The mapping provided evidence that the DCR which spans 0.4 to 3 Mb on 21q22.2 is playing a role in pathogenesis of DS (Delabar, Theophile et al. 1993; Sinet, Theophile et al. 1994). This interval is thought to be responsible for the expression of 13 features contributing to mental retardation, short stature, muscular hypotonia, joint hyper-flexibility and nine morphological signs: flat nasal bridge, protruding tongue, highly arched palate, narrow palate, folded ears, short and broad hands, incurved 5th finger, high Cummins index and gap between 1st and 2nd toes (Sinet, Theophile et al. 1994). The locus

D21S55-MX1 which is located in band 21q22.3 is thought to be responsible for the expression of other six morphological features: epicanthus, oblique eye fissure, brushfield spots, transverse palmer crease, short stature and hypotonia (Sinet, Theophile et al. 1994). In addition, DS is associated with many complex clinical features which might be located outside the critical region of chromosome 21 indicating that more than one region is responsible for the pathogenesis of the DS phenotypes (Delabar, Theophile et al. 1993; Sinet, Theophile et al. 1994).
With respect to the clinical features, it is important to emphasize that there is a great variability of the frequencies of phenotypic features in individual DS patients.

1.4.1. Leukemia

The association between DS and leukemia was recognised since 1930 (Mejia-Arangure, Ortega-Alvarez et al. 2005). Children with DS have a 10-20 fold increased incidence of leukemia from newborn period to adulthood and a lower but also increased incidence of solid tumors at all ages (Fong and Brodeur 1987; Boker, Blumstein et al. 2001; Hasle 2001). The most common form of leukemia during childhood is acute lymphoblastic leukemia (ALL) with a subtype acute megakaryoblastic leukemia (AMKL) and a subtype of acute myeloid leukemia (AML) (Hitzler and Zipursky 2005). The mechanism which leads to the increased risk of leukemia in DS is not known, but there are several oncogenes which were identified on the long arm of chromosome 21 (Sacchi 1992; Boker, Blumstein et al. 2001).

1.4.2. Alzheimer's disease

DS is associated with early onset of Alzheimer's disease. A study from (St George-Hyslop, Tanzi et al. 1987) suggested that there must be a gene on chromosome 21 involved in Alzheimer disease.

1.4.3. Congenital heart disease

There is a high frequency of congenital heart disease in children with DS ranging between 40-60% (Marino 1993). The most frequent cardiac anomalies seen in DS patients are atrioventricular septal defects (AVSD) (Freeman, Torfs et al. 2009) which affect the mortality rate of DS. Other congenital heart diseases are patent ductus arteriosus, interventricular communication, tetralogy of Fallot, and valve insufficiency. Many DS children present with more than one type of congenital heart disease.

1.5. Genetic basis of Down syndrome

In DS, approximately 95% of the cases are due to non-disjunction resulting in an extra copy of a chromosome 21 (trisomy 21) as described by Lejeune et al. already in 1959 (Lejeune, Turpin et al. 1959; Lejeune, Turpin et al. 1959). The remaining are due to translocations involving chromosome 21 and somatic mosaicism (Sherman, Freeman et al. 2005). Most trisomy 21 cases are due to an error in maternal meiosis, whereby about 70% originate during maternal meiosis I (MI) and about 20% during maternal meiosis II (MII), defective paternal meiosis is found for up to 8-10% of all cases (Savage, Petersen et al. 1998; Petersen and Mikkelsen 2000; Sherman, Freeman et al. 2005). Even though significant progress has been made in recent years, the causes of the increased non-disjunction rate resulting in trisomy 21 are far from understood. Maternal age, germ line mosaicism, and altered recombination remain the only well-established risk factors for non-disjunction of chromosome 21 (Sherman, Freeman et al. 2005).

In contrast to humans, where up to 50% of all conceptions are aneuploid, non-disjunction in most model organisms is a rare event. For example in *Saccharomyces cerevisiae* chromosome malsegregation is estimated to take place every 10 000 meiotic events (Sears, Hegemann et al. 1992). For recombination events in *Drosophila malanogaster* occytes, it has been demonstrated that recombination on MI non-disjunction takes place at the distal part of the chromosome, while for MII non-disjunction, it takes place in proximal location (Koehler, Boulton et al. 1996).

1.6. Risk factors for trisomy 21

1.6.1. Advanced maternal age

Advanced maternal age at the time of conception is the most established significant risk factor for meiotic non-disjunction of chromosome 21 (Sherman, Petersen et al. 1994; Sherman, Freeman et al. 2005; Oliver, Feingold et al. 2008). Penrose, 1933, was the first who noted the effect of advanced maternal age on the rate of DS (Penrose 2009). About 2% of recognized pregnancies of women under the age of 25 years are trisomic, this increases to 10% for women of 36 years and to 33% by the age of 42 years (Hassold and Sherman 2000). The influence of maternal age has been observed in all population studies in respect to race, geography or socioeconomic factors. However, the basis for the effect of increasing maternal age on the non-disjunction rate is largely unclear. In human female, meiosis starts in the 3rd month of fetal life and is arrested in prophase of MI from 6 months of fetal life onwards until ovulation which takes around 10 to 40 years (Warburton 2005, Sperling 2003, Hassold and Sherman 2000). At the time of ovulation the oocytes complete MI and progress to MII where they remain arrested until they are fertilized and subsequently complete the meiotic stage MII. Warburton (2005) presented two hypotheses for the effect of maternal age on the non-disjunction rate: the first is that different

variables which affect the oocytes overtime such as decreased expression of checkpoint proteins which maintain sister chromatid adhesion or meiotic checkpoint, accumulate with increased maternal age resulting in an increased non-disjunction rate (Jeffreys, Burrage et al. 2003; Vogt, Kirsch-Volders et al. 2008). A second hypothesis is that biological aging of the oocytes is an important factor and that the frequency of trisomic conceptions will depend upon the biological age of the women's oocytes, rather than upon the chronological age.

1.6.2. Maternal recombination

Altered recombination is another important factor after maternal age which is associated with non-disjunction error. Warren et al. 1987 were the first who provided evidence that a proportion of maternal non-disjunction errors were associated with reduced recombination along chromosome 21 (Warren, Chakravarti et al. 1987). Further studies (Antonarakis, Petersen et al. 1992; Antonarakis, Avramopoulos et al. 1993; Sherman, Petersen et al. 1994; Yoon, Freeman et al. 1996; Sherman, Freeman et al. 2005) regarding the etiology of Down syndrome demonstrated a relationship between the non-disjunction event and altered recombination. Most of these studies approved that the location of the recombination is a risk factor for non-disjunction of trisomy 21 (Yoon, Freeman et al. 1996; Savage, Petersen et al. 1998; Sherman, Freeman et al. 2005; Oliver, Feingold et al. 2008).

Concering the location of the recombination associated with non-disjunction, three susceptible exchange patterns have been demonstrated for maternal non-disjoiníng error: (1) no exchange leads to an increased risk of MI error, (2) a single telomeric exchange leads to increased risk of MI error, and finally (3) a pericentromeric exchange leads to increased risk of MII error (Hassold and Sherman 2000; Sherman, Freeman et al. 2005; Oliver, Feingold et al. 2008). The association of maternal MII errors with specific recombination pattern is thought to be initiated in MI, at least for a certain proportion of MII errors (Sherman, Freeman et al. 2005). A study in the USA population aimed to examine the number and location of recombination by age group (Oliver, Feingold et al. 2008). The results suggested that the risk imposed by the absence of exchange or by a single telomeric exchange is the same irrespective of the age of the oocyte, while the risk imposed by a single pericentromeric exchange increases with increasing maternal age. Oliver's findings were supported by a study from an Indian population, where the author suggested that the genetic etiology underlying the occurrence of trisomy 21 may be similar across human populations (Ghosh, Feingold et al. 2009).

1.6.3. Abnormal folate and methyl metabolism in mothers with DS

There are some studies which indicate that alterations in the folate metabolism are risk factors for trisomy 21 (Hobbs, Sherman et al. 2000; James 2004; Takamura, Kondoh et al. 2004; Eskes 2006; Rai, Singh et al. 2006). Genes involved in the maternal folate metabolism have been hypothesized to be candidate genes involved in an elevated non-disjunction rate. It has been shown that the 677C→T polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene increased the risk of having a child with DS (OR = 2.6)(James 2004). *MTHFR* catalyzing the conversion of 5,10-methylenetetrahydrofolate, the methyl donor for the remethylation of homocysteine to methionine. Mutation of the *MTHFR* gene (677C→T) causes the substation of alanine to valine in the *MTHFR* protein and reduces enzyme activity. Activity of *MTHFR* is reduced to 37% for heterozygous *C/T* genotype, and 70% with homozygous *T/T* genotype in relative to normal *C/C* genotype (James 2004). The authors assume that low folate status, whether due to dietary or genetic factors, could induce centromeric DNA hypomethylation and alterations in chromatin structure which adversely affect DNA-protein interactions required for centromeric cohesion and normal meiotic segregation. However, various other studies could not confirm these results. It was suggested that one possible explanation for the inconsistent results among the numerous studies may reflect the complex interaction between effects of genetic variants and nutritional intake (James 2004).

1.6.4. Parental germline mosaicism

Parental gonadal mosaicism has been suggested by many studies as a risk factor for cases in families with multiple trisomy 21 conceptions (Nielsen, Poulsen et al. 1988; Tseng, Chuang et al. 1994; Cozzi, Conn et al. 1999; Bruyere, Rupps et al. 2000). If parental gonadal mosaic is present the recurrence risk will be higher and will depend on the proportion of trisomy 21 cells present in the gonads. Therefore, in families with one affected child with free trisomy 21, it is assumed that the recurrence risk estimates to 1-2% on the basis of live births and prenatal diagnosis (Nielsen, Poulsen et al. 1988; Bruyere, Rupps et al. 2000). Studies of genetic implantation diagnosis indicate that aneuploidy in oocytes and embryos is not a rare event and that it increases with maternal age as a result of trisomic germ line and disruption in meiotic division (Munne, Alikani et al. 1994).

1.6.5. Mutations in nuclear encoded genes

The mechanism of meiosis reveal three specific processes: (1) pairing and synapsis of homologous chromosomes, (2) reciprocal meiotic recombination (crossover) and (3) regulation of sister chromatid cohesion (SCC) (Matsuura, Ito et al. 2000; Champion and Hawley 2002;

Nasmyth 2002). Some mutations which control the above mentioned processes may lead to a defect in chromosome segregation and produce cells that are aneuploid. Some studies reported certain gene mutations in model organisms such as Drosophila, Sacchromyces cerevisiae, and mice (Rockmill and Roeder 1990; Knowles and Hawley 1991; Baudat, Manova et al. 2000; Halverson, Gutkin et al. 2000). In humans, several mutations in genes implicated in chromosome segregation have been identified, that increase the risk of mitotic non-disjunction in somatic cells such as the MAD2 and BUB1 gene (Table 2).

Table 2: Selection of genes affecting mitotic non-disjunction in man

Germline mutations:	**Somatic mutations:**
• Apple-Peel syndrome (OMIM 243605) • Mosaic variegated aneuploidy syndrome (OMIM 257300) • MVA with total premature chromatid separation (OMIM 176430) • Roberts syndrome (OMIM 268300) • RECQ4-deficiency (Rothmund- Thomson S; OMIM 268400)	• defective MAD2 gene (OMIM 601467) • defective BUB1 gene (OMIM 602452)

1.6.6. Mitochondrial (mtDNA) mutations

It has been hypothesised that mtDNA mutations may play a role in the etiology of DS. The number of mitochondrial mutations increases with age in different cells specifically in oocytes (Arbuzova, Hutchin et al. 2002). The authors suggested as a possible explanation that mutations in mtDNA may reduce ATP levels and increase the generation of free radicals, which could in turn affect the synaptonemal complex formation, chromosome segregation, the division spindle, and alter recombination (the enzymes participating in recombination and DNA repair are ATP dependent) leading to aneuploidy (Arbuzova, Cuckle et al. 2001; Arbuzova, Hutchin et al. 2002).

1.6.7. Consanguinity

Consanguineous marriages are traditionally common among Arab countries. This leads to an increased birth prevalence of infants with recessive diseases, congenital anomalies, morbidity and mortality. The Omani society has, as other Arab countries, a long tradition of consanguinity. Rajab and Patton reported that among 60635 Omani couples 24.1% were marriages between first

cousins, 11.8% between second cousins, and 20.4% were within specific tribal groups (Rajab and Patton 2000). Individuals who are closely consanguineous have a higher probability of carrying rare recessive alleles which can be transmitted homozygous to their offsprings. Consequently, it is conceivable that homozygous gene mutations, in a gene influencing non-disjunction, could result in an increased aneuploidy rate of the progeny. Thus, it cannot be excluded that an increased non-disjunction rate could result from a recessive gene in combination with other risk factors, specifically in younger aged mothers. There are some publications which report on a positive association between DS and consanguinity and the possible involvement of recessive genes in non-disjunction (Alfi, Chang et al. 1980; Farag and Teebi 1988). Such observations have been made in Shetland (Roberts, Roberts et al. 1991) and Canada (De Braekeleer and Dao 1994) and are explained by recessive genes, possibly preventing the loss of the trisomy 21 fetus. Some other data did not support the association between consanguinity and DS. Basaran et al. reported a lower consanguinity rate and inbreeding coefficient among parents of DS than in parents without DS children (Basaran, Cenani et al. 1992). Similar findings were reported from Kuwait demonstrating that the frequency of consanguineous marriages among controls was higher than that among DS families, though Kuwait has a highly inbred population with 54.3% of consanguineous marriages (Al-Awadi, Moussa et al. 1985).

1.6.8. Exogenous risk factors

There is increasing evidence that maternal meiosis is an error prone process, that is most sensitive to the effect of exogenous factors at the time of chromosomal segregation, which is around conception. This is supported by two convincing associations in which two local clusters of trisomy 21 were explained by hazards occurring around the time of conception: the ingestion of a chemical, trichlorfon, employed against fish parasites (Czeizel, Elek et al. 1993) and the inhalation of iodine-131 from the Chernobyl reactor accident (Sperling, Pelz et al. 1994). Already in 1976 it has been shown that the DS prevalence is increased in certain regions in Kerala with high background radiation (Kochupillai, Verma et al. 1976). Another two studies, one conducted by EUROCAT, demonstrated a higher risk of chromosomal anomalies in people who lived close to hazardous waste landfill sites (0-3 km) than in those who lived further away (3-7 km). The EUROCAT study investigated 245 cases of chromosomal anomalies and 2412 controls who lived near 23 such sites in Europe (odds ratio 1.41, 95% CI 1.00-1.99)(Geschwind, Stolwijk et al. 1992; Vrijheid, Dolk et al. 2002). Many other exogenous factors such as maternal irradiation, alcohol, fertility drugs, low economic status etc. have been implicated in an increased non-disjunction rate (Boue and Boue 1973; Harlap, Shiono et al. 1979; Uchida 1979; Kaufman

1983; Strigini, Pierluigi et al. 1990; Yang, Sherman et al. 1999; Torfs and Christianson 2003; Christianson, Sherman et al. 2004; Padmanabhan, Sugunan et al. 2004). Thus, it seems certain that environmental factors are involved in the etiology of Down syndrome.

There is increasing evidence that maternal meiosis is the main risk for non-disjunction error due to the lack of checkpoint control during chromosomal segregation (LeMaire-Adkins, Radke et al. 1997) which makes it conceivable that this process is also sensitive to the effect of endogenous and exogenous factors (Table 3) (Sperling 2003).

Table 3 Represent risk factors of meiotic non-disjunction in man

Increasing maternal age: o limited oocyte pool o two hit model susceptible bivalent o abnormal processing of metaphase I o defective spindle formation o defective checkpoint control	**Monogenic risk factors:** o defective folate metabolism o apolipoprotein ε4 allele o presenilin-1 gene polymorphism o impaired function of mitochondria o consanguinity
Chromosomal risk factors: o size of chromosomes o NOR variants o aberrant centromere structure o premature centromere division	**Environmental risk factors:** o parental irradiation o oral contraceptives o fertility drugs o thyroid antibodies o viral infection o ingestion of metriphonate
Others: o reproductive activity o seasonal variation in endocrine factors	

1.7. Study design and aims of the study

The Down syndrome study was designed as Omani – German joint project on the etiology and genetic risk factors of Down syndrome in the Omani population. The study was initiated in 2004 and approved by Ethical Committee of the Ministry of Health, Oman[1] and the Ethical Committee of the Charité University Hospital, Berlin, Germany[2].

[1] Ref. MH/DGHA/DSDC/NCD/R&S/472/02 signed by Dr. Jawad Al-Lawati and MH/DIR/genetics/905 signed by Ms. Najla A Al-Riyami;
[2] Ref. Charité 213/2003 signed by Prof. Dr. H. Eichstädt

The study consisted of four parts:

1. **Cytogenetic study:** the aim was to determine the different types of cytogenetic abnormalities in Omani DS children.

2. **Epidemiological survey:** to determine the birth prevalence of Down syndrome in the population. The data were based on Cytogenetic Registry provided by the genetic unit in the Ministry of Health. The Down syndrome registry provided clinical information, patient's full name, date of birth, sex, and place of birth. Only cases with free trisomy 21 were included.

3. **Molecular genetic study:** is divided into three parts. Using high polymorphic STR markers the parental and meiotic origin of the non-disjunction and the frequency and localization of the crossovers were analyzed. To investigate the maternal ethnic group sequencing of the D-loop of the mtDNA was utilized. The paternal ethnic group was investigated by the analysis of Y-specific STRs (16 markers). DNA samples were obtained from buccal smears of the Down syndrome children and both parents at the time when a detailed questionnaire was completed.

4. **A case control study:** based on the cases with free trisomy 21 identified by the cytogenetic laboratory through all regional hospital in Oman. In addition, controls were recruited matched for the delivery of an unaffected child in the same year and in the same Health Institute or region. A detailed structured questionnaire was designed which covers general information on socio-demographic, family history of DS mother and her husband, health and illnesses, history of women pregnancy together with menstrual history, general medical history including X-rays, lifestyle such as medications, drugs, smoking etc, and occupational history. The questionnaires were carried out by the physicians of the local hospitals as personal interviews.

1.8. Reasons for performing the study on Down syndrome in Oman

Birth defect epidemiology is best acquired from well conducted population based birth defect studies. The Sultanate of Oman is one of the countries most suitable for epidemiological studies on trisomy 21 because of a high birth rate of approximately 40,000 births per anno and the nearly complete population-based ascertainment of DS. Furthermore, prenatal maternal serum screening, prenatal diagnostics (PND) and selective terminations of pregnancies do not play any

role, in contrast to most western countries where PND is common practice. A failure to account accurately for terminations of pregnancies, after prenatal diagnosis of a DS fetus which could result in an apparent increase of the DS prevalence for younger mothers compared to older ones, could be ruled out in Oman.

Furthermore, and most important, Oman has a comprehensive health care system which is provided and financed predominantly by the government: 98% of the hospital beds are governmental (87% MoH, 11% governmental non-MoH, and only 2 % private sector). The health care system of Oman provides 30 local hospitals, 13 regional hospitals in the ten health regions of Oman, and four major tertiary referral hospitals in Muscat. More than 95 % of all newborns are delivered in these hospitals and almost all are examined by pediatricians who are aware of the clinical phenotype of DS and prompt a cytogenetic analysis for confirmation. The cytogenetic service is centralized and free of charge at the National Cytogenetic Service of the MoH in Muscat. Since 1999, all DS cases are cytogenetically confirmed. The cases of DS are registered at the National Genetic Disease Registry of Oman. Thus, all criteria for a meaningful epidemiological study are stringently met in Oman.

2. MATERIALS AND METHODS

2.1 Patients recruitment

Sample collection for cytogenetic analysis: All cytogenetic analyses were performed at the Cytogenetic Laboratory located at the Central Health Laboratories in the capital Muscat, Oman.

2.1.1 Sample collection

Protocol of sample collection:

1. Two ml of peripheral blood lymphocytes (whole blood) were collected from different regional hospitals in Oman in a sterile Lithium Heparin or Sodium Heparin vacationers along with request form for the patient's details including clinical information (Appendix.1)
2. Samples were delivered directly on the same day of collection to the cytogenetic laboratory in an ice box.
3. After receiving the samples they were registered in cytogenetic section. Each sample was given an identification number (ID) before processing.

2.2. Cytogenetic analysis

2.2.1 Peripheral blood lymphocytes culture:

1. Two sterile 15ml centrifuge tubes were labelled for each sample (duplicate cultures).
2. 0.4ml of heparinised blood was inoculated into 5ml of RPMI-1640 medium substituted with 20% Inactivated Fetal Bovine Serum.
3. 100µl of Phytohaemagglutinin (PHA) was added in each culture tube and mixed properly.
4. Cultures were incubated at 37° for 72 hours incubation.

2.2.2 Harvesting the culture:

1. 100µl (10µg/ml) of Colcemid was added into culture tubes and incubated at 37ºC for 50 minutes.
2. Culture tubes were placed in a centrifuge at 500g for 5 minutes, subsequently the supernatant fluid was removed with the aid of 7 ml pasture pipette.
3. The deposit resuspended in 5-6 ml of 0.075 mM prewarmed Potassium Chloride.
4. Step 2 repeated.
5. Using a Pasteur pipette 6ml of a cold fixative (1:3 Acetic acid: Methanol) was slowly added to the pellet while agitating constantly on a vortex mixer.
6. The suspension was then stored at 4ºC for overnight before slide preparation.

Table 4: Reagents, Buffers, Solutions for setting up culture, harvesting, slide preparation and GTG band.

Reagent	**Concentration**	**Manufacture**
RPMI-1640 medium w/25MM HEPES;W/L-Glutamine; W/O NaHCO3	1x liquid	GIBCO
Foetal Bovine Serum Heat inactivated	25%	GIBCO
KaryoMax®Colcemid®Solution	10µg/ml	GIBCO
Potassium Chloride	0.075M (75mM)	BDH
Glacial Acetic Acid AnalaR®	100%	BDH
Methanol AnalaR®	100%	BDH
Trypsin 250	10mg/ml	BD Difco™
Phytohaemagglutinin (PHA)	100X	Remel
Penicillin	5000 IU/ml	Sigma
Streptomycin sulphate	5000 µg/ml	Sigma
Giemsa's stain Powder		BDH
Sodium Chloride (NaCl)	0.99%	Qualigens®
Glycerol	99.5%	WINLAB
di-Sodium hydrogen orthophosphate 2-hydrate GPR™(Na2HPO4)		BDH
Potassium dihydrogen phosphateMonobasic (KH2PO4)		HIMEDIA
Thymidine	$1x10^{-3}$M	Sigma T9250
Ethanol (Spirit)	70%	BDH
DPX mount media		BDH

Table 5: Equipments required for culturing and analysis

Equipment	**Manufacture**
Incubator, 37ºC	Heraeus
Biological safety cabinet class II	Holten
Automatic dispenser	Jencons
Slide warmer, 37ºC	Photax
Phase microscope (BX51)	Olympus
Drying oven, 60ºC	Heraeus
Centrifuge	Mistral 1000
Vortex	Stuart Scientific
Genetic work station (karyotype system.)	Vysis

Table 6: Materials and consumable

Materials	**Manufacture**
Pasteur pipette	
Disposable centrifuge tubes, 15ml	Falcon
10ml sterile pipettes	Falcon
100 ml measuring cylinders	Polylab
Disposable syringe (1ml)	Braun
Gloves	Medix
Frosted microscope slides	Marienfeld
Cover glasses, 22x50mm	Marienfeld
Coplin jars, 100ml	
Measuring cylinders, 500ml	Polylab
Slide holders	
Slides storage box	
Microil immersion	BDH
Water bath	Grant

2.2.3 Preparation of slides:

Before preparation of slides, slides were scrupulously clean; therefore a suitable cleaning procedure was used by soaking the slides in 70% ethanol overnight after which they were washed in running water for at least 15 minutes and stored in distilled water at 4ºC.

Protocol of preparation:

1. The suspension was centrifuged at 500g for 5 minutes and supernant discarded and the pellet resuspended with 6 ml cold fresh fixative (1:3 Acetic acid: Methanol).
2. Step 1 was repeated twice and finally the pellet was resuspended in 0.5ml of fixative solution and the suspension is ready for slide preparation.
3. 1-2 drops of the suspension was dropped onto a very cold wet slide and allowed to spread using a hot steam from a water bath.
4. Then slides were labeled with the ID number of the patient and date of preparation.
5. Using a phase contrasts microscope slides were checked for a proper metaphase index and spreading (chromosome quality).
6. Finally all slides placed in an oven at 65 ºC overnight.

2.2.4 Staining with GTG Banding:

For cytogenetic routine samples GTG banding technique according (Seabright 1971)was used at 400 resolution band for diagnosis of all trisomies including trisomy 21. The following procedure that used for staining:

1. Prepared slides were removed from oven one hour before banding.
2. Then slides were placed in coupling jar containing 0.2mg/ml trypsin solution starting with 10-20 seconds of incubation.
3. Afterwards, slides were dipped into 1% normal saline to arrest trypsin activity.
4. Slides were placed in Giemsa solution for 5-6 minutes.
5. Finally slides were rinsed with double distilled water, dried, and mounted using DPX mounting medium and cover slips (46x46 mm size).
6. Slides were examined for a proper band quality using Olympus microscope BX 50 with 100x objective (Oil immersion).

2.2.5 Karyotyping and chromosome analysis:

Slides were examined, karyotyped and analyzed with an Olympus microscope (BX50). A cytogenetic coordinate sheet is used to document patient's details including first name, second name, and tribe name, date of birth, lab code number, clinical information, number of cells counted and analyzed and finally designation of the karyotype according to the ISCN (1995).

After cases have been analyzed microscopically, 4 images of metaphase cells were captured and karyotyped using Vysis karyotype system which is composed of an Olympus microscope BX50 attached with CCD Cohu camera model #4912-5110/0000 and connected to a computer with a software Smart capture VP (1.4 version specific for karyotyping (Figure 2).

If the karyotype results revealed a Robertsonian translocation with trisomy 21 then parent blood was requested to investigate the origin of the translocation if it is a de novo or familial inherited translocation. For the mosaic cases the total number of metaphases counted was between 50-100.

Figure 2: A Vysis system composed of BX50 Olympus microscope, CCD camera and karyotype software. Cytogenetic laboratory, CPHL, MoH, Oman

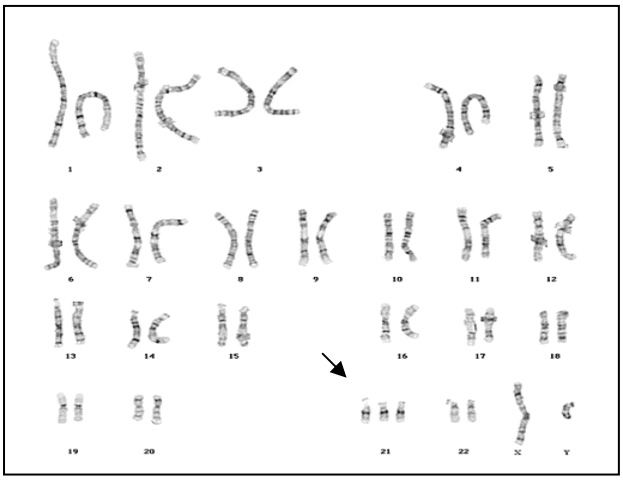

Figure 3: A karyotype showing a male with trisomy 21 after capturing with Vysis system an arrow indicate trisomy 21. Karyotype result is: 47,XY,+21

2.3. Genotyping of chromosome 21 with microsatellite markers

2.3.1. Samples collection and population study

Samples collection was based on a population study through all regions in Oman. All cases of Down's syndrome were diagnosed at the Cytogenetic Laboratories at MoH which is the only cytogenetic laboratory in Oman belong to the Ministry of Health within the years 2000-2005 and the DNA sample was selected from proband and both parents. In January 2004 Prof. Dr. Neitzel visited Oman and started to collect almost all DS cases born between 2000-2004 and the criteria of collection was based on filling a questionnaire structured: Sociodemographics, history of women pregnancies, detailed familial history for two generations, certain events at the conception of the DS Child, health and illnesses, X-ray diagnostic, treatments, and occupational history in addition obtaining DNA buccal smears from the Down's child and both parents. Another 125 samples were collected by the year 2006 by Salma Al-Harrasi from Oman. The total number of samples collected was 400 cases.

2.3.2. Protocol of sample collection:

A buccal sample was collected from the proband and both parents from each family. A sterile OmniSwab(whatman) was used for the collection of cheek buccal cells for DNA extraction and after collection the brush like swab head was easily ejected from the stem of the swab and transferred into Eppendorf tube containing 2ml of lyses buffer (50mM Tris: pH 8.0, 50mM EDTA, 50mM Sucrose, 100mM NaCl,10%SDS). The probes were coded and transfered to the Institute of Human Genetics Charite′ in Berlin.

The DNA extraction was performed by using BioRobort DM48 of Qiagene Company.

2.3.3. DNA extraction

The procedure used for the extraction of genomic DNA from buccal cells was done by using the automated BioRobort®M48, which provides fully automated nucleic acid purification for up to 48 samples. DNA was extracted from 200µl of lyses buffer containing buccal cells using the MagAttract DNA Blood Mini M48 kit from QIAGEN (details of the reagents in the reaction kit are not given by the manufacturer).

In principle, the extraction of genomic DNA by the QIAGEN Kit is based on DNA binding to the silica surface of the magnetic particles in the presence of a chaotropic salt (the flowchart Figure 4). DNA bound to the magnetic particles is then washed with two different buffers followed by a rapid rinse with distilled water which considerably improves the purity of the DNA.

Table 7: MagAttract DNA M48 Kit Content and dilution concentration used

Product name	Volume for 48 samples in millilitres
Buffer ML	36.5
Buffer MW1	47
Buffer MW2	20.6
RNase free water	10.9
Suspension B	4.5
Ethanol	67.4
Deionized H2O	69

Table 8: Equipments and reagents used for automated extraction

Product name	Manufacture
BioRobortM48 workstation	Qiagen
App.Package,M48,Genotyping	Qiagen
Filter-Tips, M48 (1000µl)	Qiagen
Sample tubes, 1.5ml without lid	Sarstedt
Elution tubes, 1.5ml with screw caps	Sarstedt
Sample Prep Plates,42 well,M48	Qiagen
itrile gloves	Ansell
Ethanol (96-100%) (undenatured)	Merck

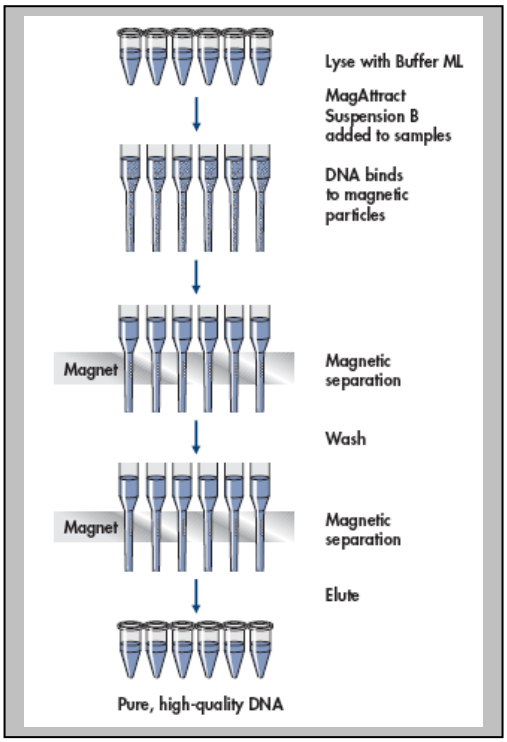

Figure.4: Principle of extraction of DNA by Qiagen kit as described by a manufacture

2.3.4. DNA extraction protocol

The MagAttract DNA M48 kit protocol was followed as described by the Qiagen manual. In brief, 200µl of lyses buffer containing buccal cells were transferred into 1.5ml sample tubes without screw caps. At the beginning the BioRobort M48 and the computer software (Genotyping) were switched on for user. Then all samples tubes plus reagent containers and plastic ware were placed on the worktable according to the software. Next, the door of the workstation was closed and the computer programme started. After the purification protocol started as all steps were fully automated until the software message on the screen indicating the protocol is finished. Finally, elution tubes containing purified DNA were retrieved from the cooling block and the DNA was ready to use.

The extracted DNA was checked by running small aliquot in 2% agarose gel. The agarose gel was prepared by adding 2g of agarose (Invitrogen) in 100ml TAE buffer and the mixture was heated in a microwave for 2-3 minutes for boiling. After cooling at room temperature 5µl of Ethidium Bromide (1mg/ml) was added to the agarose, mixed, and poured gently into the electrophoresis tray. After the gel had solidified DNA samples (5µl of extracted DNA and 3µl of loading dye) were loaded and the gel was run in an electrophoresis tank (Life Technologies) at 120 Volts for 40 minutes. Finally, the genomic DNA was visualised by Ultraviolet Transilluminator and photographed.

2.3.5. DNA Quantification

A spectrophotometer (Pharmacia Biotech) was used to determine the concentration and purity of the genomic DNA extracted. The concentration of DNA was determined by measuring the absorbance at 260nm and the absorbance was determined to be accurate when the reading falls between 0.1 and 1.0 unit. An absorbance of 1 unit at 260nm corresponds to 50µg of DNA per millilitre ($A260 = 1 \rightarrow 50$ µg/ml). The DNA purity was determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm. Pure DNA has an $A260/A280$ ratio of 1.7-2.0 and significant protein contamination is indicated by a ratio below 1.7.

Table 9: Equipments and requirements for determining the DNA concentration

Name	**Manufacture**
Spectrophotometer	Pharmacia Biotech
Glass Cuvvette	Hellma
Distilled water	Gibco
Pipette set with different ranges	Gilson
Pipette tips	Sarstedt
Latex Gloves	Charite`

2.3.6. DNA measuring procedure

Sample DNA was diluted at a ratio of 1:100 (10 µl DNA + 99 µl of dist.H_2O, vortexed and left at 37°C in a heating block for at least 10 minutes). Then about 10µl of diluted DNA was added into a glass curette and the absorbance was measured at 260nm and 280 nm using the spectrophotometer.

2.3.7. Microsatellite Analysis

Microsatellites, also known as simple sequence repeats (STR), are highly informative DNA sequences in the human genome. They are commonly used for mapping, population studies, linkage analyses and to trace inheritance patterns. STRs are short tandem repeats which are highly polymorphic due to a variation in the number of repeating units between alleles within a population. The short sequences are repeated in tandem arrays and the length of sequences are most often di, tri, or tetra nucleotides, each repeated 5-50 times at a locus. (Koreth et al,1996). Most microsatellites occur in non-coding or intronic regions of the genomic DNA.

The length of the microsatellites can be determined by PCR using primers that flank both ends of the microsatellite sequence producing DNA fragments which length depends on the number of repeats in the microsatellite. These fragments are analysed by using DNA sequencing instrument utilizing capillary electrophoresis by which the fragment size can be determined. In order to distinguish between fragments varying in length by few bases, the size resolution should be good enough. A size standard is run in each capillary to create a standard curve of sufficient precision. The size standard has to be labelled with a different coloured fluorescent dye from the fragment to be analysed which allows multiplexing of different fragment analysis in each capillary separation run.

The microsatellites for diagnosis of trisomies should be polymorphic with a high level of heterozygosity. Furthermore, different STRs were used for chromosome 21 to insure informative results.

2.3.8. Polymerase chain reaction principle and procedure

The polymerase chain reaction (PCR) is considered to be one of the most advanced technologies in the field of molecular biology developed in 1980's by Kary Mullis (Mullis, Faloona et al. 1986; Mullis 1990). One of the advances of PCR is that only very small amounts of DNA are needed extracted from blood samples, hair roots or tissues. PCR is applied in many research and medical diagnostic fields such as diagnosis of hereditary diseases, identification of infectious diseases and also identification of genetic finger prints in addition to other applications.

PCR is used to amplify specific fragments of a DNA strand and it is based on the enzymatic amplification of a target DNA sequence flanked by a pair of oligonucleotide primers. By PCR it is possible to amplify a single or few DNA copies to millions of copies of this DNA fragment using 20 to 40 PCR cycles. Each PCR cycle requires 3-steps: 1) Denaturation of the DNA at high temperature (94-96ºC) 2) annealing step (54-65ºC) allowing the primers to hybridize to opposite strands of the target DNA 3) elongation (72ºC) or extension of primers by a heat stable DNA polymerase (Taq) which is isolated from thermophilic bacteria.

PCR requires several necessary components and reagents including: DNA template that contains a DNA region to be amplified, a forward and a reverse primer which are complementary to the DNA region at 5´ and 3´ ends of the DNA template, buffer solutions for optimum activity and stability of the DNA polymerase, DNA polymerase such as Taq polymerase or any other head-stable DNA polymerase, deoxynucleotide triphosphatase (dNTP's) and finally Mg^{2+}.

Table 10: Equipments and Consumable for PCR

Name	**Manufacture**
Thermocycler or Mastercycler with gradient	ABI
Centrifuge	Eppendorf
Pipette set with different ranges (10µ1, 20 µ1 and 200 µ1)	Eppendorf
96 PCR plate Full Skirt	Eppendorf
PCR 0.2ml Strips	Biozym Scientific GmbH
Sealing tape Covers	Sarstedt
Pipette tips	Eppendorf
Vortex	IKA®Labortechnik
Minicentrifuge	Fisher Scientific
Sequencing plate (96 PCR plate half Skirt)	Thermo Scientific
PCR 0.5ml tubes	Eppendorf
Multi-rack	New Lab

2.3.9. STR-PCR master mix and conditions

PCR amplification was carried out in a 15 µl reaction volume. The final concentration of the reagents used is presented in the master mix Table 11

Table 11: PCR master mix reactions

Reagent Name	**Concentration**	**Volume Per one Reaction**	**Manufacture**
dH2O	-	10.4µl	Gibco
Buffer with Mg^{2+}	10x	1.50µl	Sigma and Fluka
Primer (For)	10pmol	0.30µl	Operon
Primer (rev)	10pmol	0.30µl	Operon
Taq Polymerase	5U/µl	0.20µl	Solis Biodyne
dNTP's	10pmol	0.15µl	Bioline
DNA	50ng/µl	1.00µl	-
Final volume	-	15.00µl	-

The type of PCR used for the microsatellite study was touchdown (TD) PCR. Touchdown PCR is a method that uses variable annealing temperatures. Annealing temperature started at about 5ºC above the calculated Tm to ensure highly specific amplification of the target sequence. During the following cycles the annealing temperature is gradually reduced by 1-2ºC until it reaches a level of about 5ºC below Tm. Touchdown PCR was performed as follows: After 5 minutes denaturing at 95ºC the PCR was run with each temperature for 30 sec at 5 touchdown PCR steps. The details are shown in Table 12.

Table 12: PCR amplification conditions

	Process	Temperature	Time	Cycles
1	Initial denaturation	95˚C	5min	1
2a	Denaturation	96°C	10sec	5X
2b	Anealing	60°C→54°C (TD)	30sec	
2c	Extention	72°C	30sec	
3a	Denaturation	96°C	10sec	30X
3b	Anealing	55°C	30sec	
3c	Extention	72°C	30sec	
4	Elongation	72°C	10min	1
5	Hold	4°C	∞	

2.3.10. Gel Electrophoresis

The amplified PCR products (5µl) were run by electrophoresis on 2% agarose gel. The agarose gel was prepared by mixing 2 g of agarose in 100 ml of 1x TBE buffer and heated in a microwave for few seconds to boil followed by cooling to room temperature. Afterwards 5µl of Ethidium bromide (1mg/ml) were added and the agarose was poured into the electrophoresis tray with a comb adjusted. As the gel solidified 5µl of PCR product plus 3µl of loading dye were loaded in addition to a negative control in which no DNA is present as a template and a positive control using a known a mount of added template. The gel was run at 120 volts for 30-50 minutes in an electrophoresis tank and visualised by the use of an ultraviolet transilluminator and photographed.

Table 13: 2% Agarose Gel reagents and equipments

Reagents	**Concentration**	**Manufacture**
Agarose	2 gms	Invitrogen
1x TBE buffer from 10x TBE (10x TBE: 108 g Tris + 55g Boric acid + 9.3g Na2 EDTA-H_2O Triplex)	100 ml	Merck
Ethidium Bromide	1 mg/ml	
Loading Dye : 20g Saccharose 0.125g Bromphenol Blue 50ml sterile dst.H_2O (37°C water bath)		Merck
DNA molecular weight marker (100 bp)	0.5 µg/lane	Invitrogen
DNA molecular weight marker (1kb Plus)	0.7 µg/lane	Invitrogen
Balance		Sartorius
Pipette set with different ranges		Gilson
Pipette tips		Sarstedt
Parafilm		Pechiney

Table 14: Gel Electrophoresis requirements

Name	**Manufacture**
Horizontal Gel casting tray	Renner GmbH
Fity Combs	Renner GmbH
Electrophoresis power supply	GIBCO BRL
Transilluminator, UV	UV, INC
Camera	Polaroid
Microwave	Ordinary type

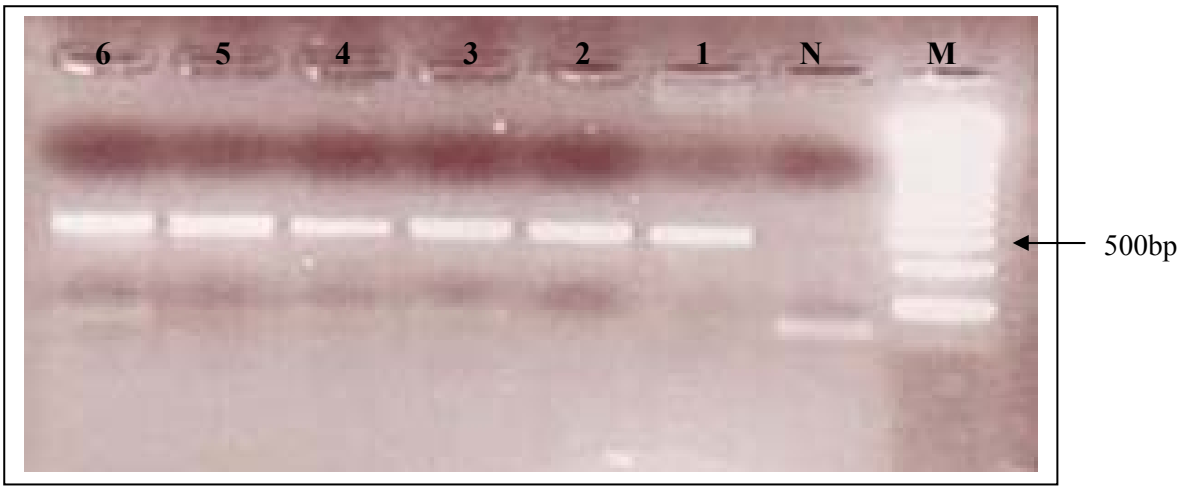

Figure 5: PCR result for the amplification of D21S1120 (300bp). 5µl of each PCR product were loaded into 2% Agarose gel. M: 1kb marker:, N:negative control, 1,2,3: DS family-1 and 4,5,6: DS family-2.

2.3.11. Primer used

Primers used in this research were specifically for microsatellite markers on chromosome 21. Primers were oligonucleotides complementary to the 5' and 3' sequences flanking microsatellites as shown in table 15 below and are fluorescence-tagged at the 5'-end with HEX or FAM.

2.3.12. Primer design

Primers used were designed from in Human Genome Database (HDG), National Centre for Biotechnology Information (NCBI) and Marshfield Centre. Primer pairs for the detection of STR markers on chromosome 21 were obtained from Operon. About 70% of the STRs were di-nucleotide repeats and the remaining 30% were tetra-nucleotide repeats. The level of heterozygosity ranged between 0.7 and 0.8 (Table 15).

Table 15: Microsatellite Primers used in Trisomy 21 for microsatellite typing

Marker name and labeled type	Primers sequence 5′→3′ direction	Max Heterozygosity (%)	Nucleotide Repeats	Fragments Size (bp)	Physical location		
					Cytogenetic	Marshfield (cm)	NCBI (kbp)
D21S215 (FAM)	GCTGACGTGACAGTTGTGAG (F) TCTAAAACAGTGTGTCTAGC (R)	82.0	Di (GT)	168-180	21q11.2		13719
D21S120 (FAM)	GTGTGTCTGCCATTTCTGGGTGTAG (F) GATCCTGGGACAAAGTAGTCTCTAA (R)	75.00	Di (GT)	318-330	21q11.2	2.99	14684
D21S1432 (HEX)	CTTAGAGGGACAGAACTAATAGGC (F) AGCCTATTGTGGGTTTGTGA (R)	74.0	Tetra (GATA)	127-155	21q11	2.99	16265
D21S1414 (FAM)	GGCACCCAGTAAAAAATTACT (F) CTGTCTGTCTGTCTGTCTATC (R)	87.50	Tetra (GATA))	310-390	21q21	9.72	19476
D21S1258 (FAM)	CGTTTCAATATAGACCAGATAAAGG (F) AGGTCAACTGCCAAAATCTAAG (R)	73.34	Di (CA)	139-157	21q21	24.73	27741
D21S1445 (FAM)	TTGTGAGAAGCAAACTGTGG (F) ATAATAGATGGCAAACAAATAGTTG (R)	70.0	Di	266-312	21q22	31.26	34297
D21S1252 (HEX)	TGTTTGTCTCTCTCTGTCTTTG (F) CATCTTACATCTCCTAGGGTGA (R)	80.36	Di (CA)	146-247	21q22	35.45	36748
D21S1890 (HEX)	GGTCTGACCACAGATTTCC (F) AAAAACACTCTGAACGATTAAGG (R)	88.00	Di (CA)	143-173	21q22	52.50	43672

2.3.13. Fragment Analysis

Fragment analysis was carried out using the ABI Prism Genetic analyser 3730 with polymer type POP 7. The instrument is composed of a capillary electrophoresis, a computer workstation for instrument control and data analysis, a software for sample ID import, instrument control, and data collection and finally the GeneScan analysis software version 3.5 for fragment sizing.

Table 16: Equipments and consumables for fragments analysis

Name	Manufacture
3730 genetic analyser (48 capillaries)	ABI model: 3730
Genescan analysis software	ABI version 3.5
Thermocycler	ABI
Centrifuge	Eppendorf
96 well plate septa	ABI
96 well plate retainer	ABI
96 well plate base	ABI
Multichanel Pippeters	Finn pipette
Pippeter tips	Sarstedt

2.3.14. Preparing PCR products for capillaries electrophoresis

After gel electrophoresis the remaining PCR product was used for fragment analysis. It was important to pool the PCR products at the correct ratios in order to get similar fluorescence intensities across all loci. A dilution series of PCR amplification product were carried out before adding them to the sample plate and sending them for capillary separation on the ABI PRISM®3730 Genetic Analyser. The dilution series ranged between 1:20-1:100 (PCR product: deionised H_2O) depending on PCR product as shown on Figure 5. Then 1 µl of diluted amplified samples were combined with 9 µl 400HD Size Standard/ Formamide solution in a MicroAmp optical 96-well reaction plate. The Micro-Amp plate was placed in a thermocycler at 96°C for 2 minutes for denaturation, and immediately placed on ice. Finally, samples were loaded into ABI 3730 machine and subjected to capillary electrophoresis, before loading the plate were fast centrifuged to ensure that all samples were at the bottom of the well and no air bubble present.

Table 17: Reagents required for loading sample

ROX Size Standard	Genescan® 400HD	ABI
Hi-Di™ Formamide	99.5%	ABI

Table 18: Master Mix for loading sample

Reagents	**1 Reaction**	**Master Mix X 96**
Amplified pooled PCR Product	1 µl	-
Hi-Di™ Formamide	9 µl	864
400HD Size Standard	0.2 µl	19.2
Final Volume		9 µl/well

2.3.15. Capillaries Electrophoresis and detection

In the DNA analyser system a Performance Optimised Polymer (POP-7; 10s injection time, 7 KV, 30ºC, 20 minutes) is injected into each capillary before starting the electrophoresis procedure. Then the capillary and electrode are dived into the sample applying voltage inducing negatively charged DNA molecules to move into the capillary using running buffer. In the capillary the labelled DNA fragments (PCR products) are separated and then exposed to a window with a laser beam which separates the fragments depend on the different fluorescent dye-label used for each sample and their wave length. Finally, the light from the array is collected by a spectrograph system with CCD camera detector. This provides a full spectrum data from all 48 capillaries and transfer to special software for analysis.

Table 19: Reagents required for capillary running

10x 3730 Running buffer with EDTA	Applied Biosystem
POP-7™ Polymer (ABI PRISM®3730)	Applied Biosystem
Dye set DS-30(6FAM™,HEX™,NED™,ROX™)	Applied Biosystem

2.3.16. Data analysis

The GeneScan software version 3.5 automatically analyses the fragment size and quantitation data by converting it into user defined results which are transferred to a database storage and analysis.

To size the fragments an internal size standard is used which consist of a set of fragments with known length that are run in each capillary. The size standard 400HD was used as an internal marker for sizing DNA fragments in the oligonucleotide bases 50-500bp range and provides 21 single-strandard labelled fragments of: 50,60,90,100,120,150,160,180,190,200,220,240,260,280, 290,300,320,340,380,400 bp. Analysis of the alleles depend on nucleotides repeats size and the fluorescent dye used. Each fluorescence dye has different absorption and emission spectra. Maximum absorption for HEX is 537 and maximum emission is 556, for FAM the absorption is 495 and maximum emission is 521.

Examples of microsatellite raw data results:

The Figures below are showing some of the STR-markers used to analyse the alleles of chromosome 21 from two different families.

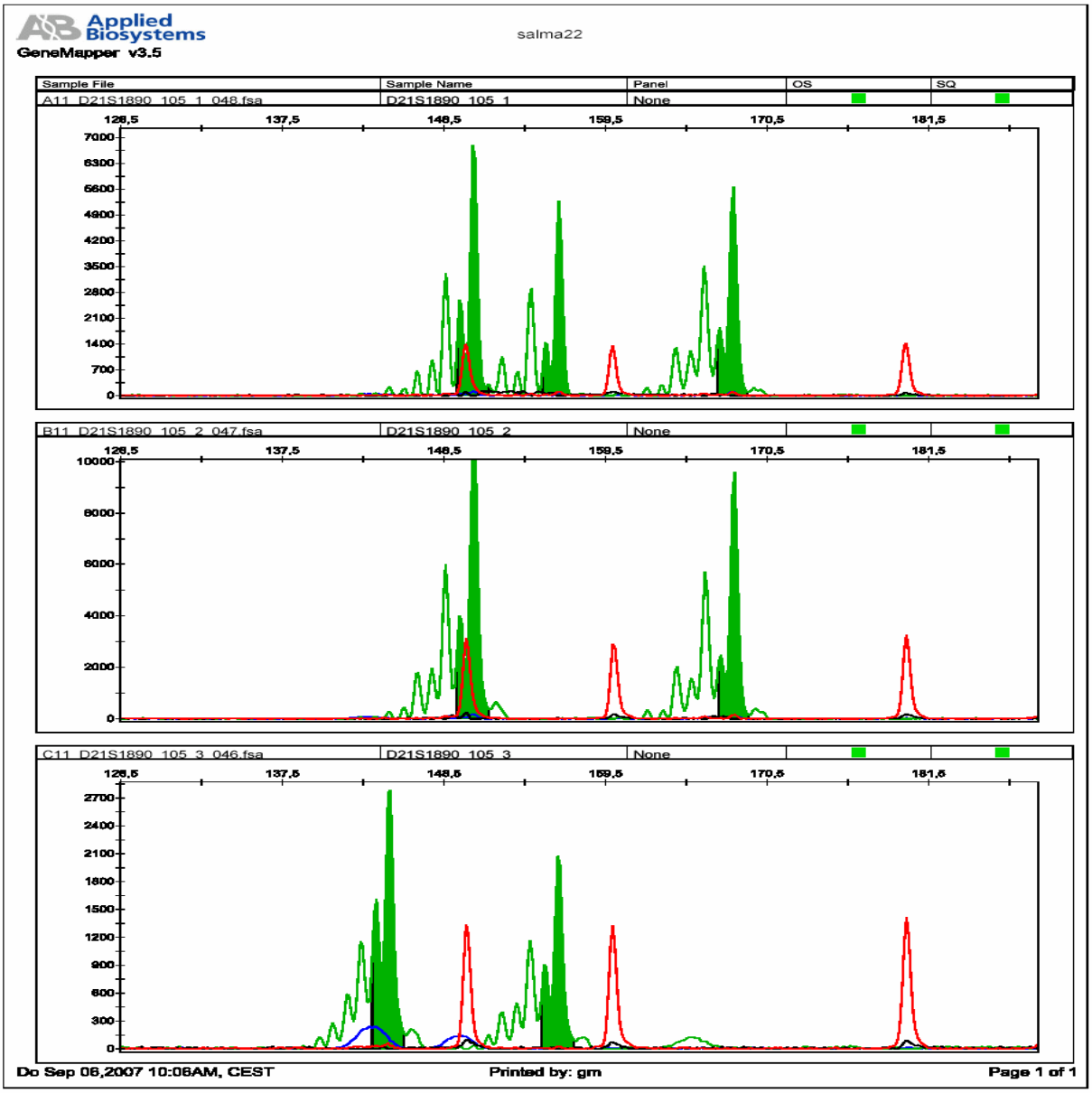

Figure 6: D21S1890 STR-marker (labelled with HEX) for chromosome 21 in the index child and the parents. The STR used is D21S1890 and the fluorescence dye was HEX which is indicted by the green colour. Both parents of the index child are heterozygous, the mother has the alleles with 152-169 bp and the father has the alleles 145-157 bp. The index child has the alleles of 151-157-169 indicating that he inherited the alleles 151- 169 from his mother and the allele 157 from his father.

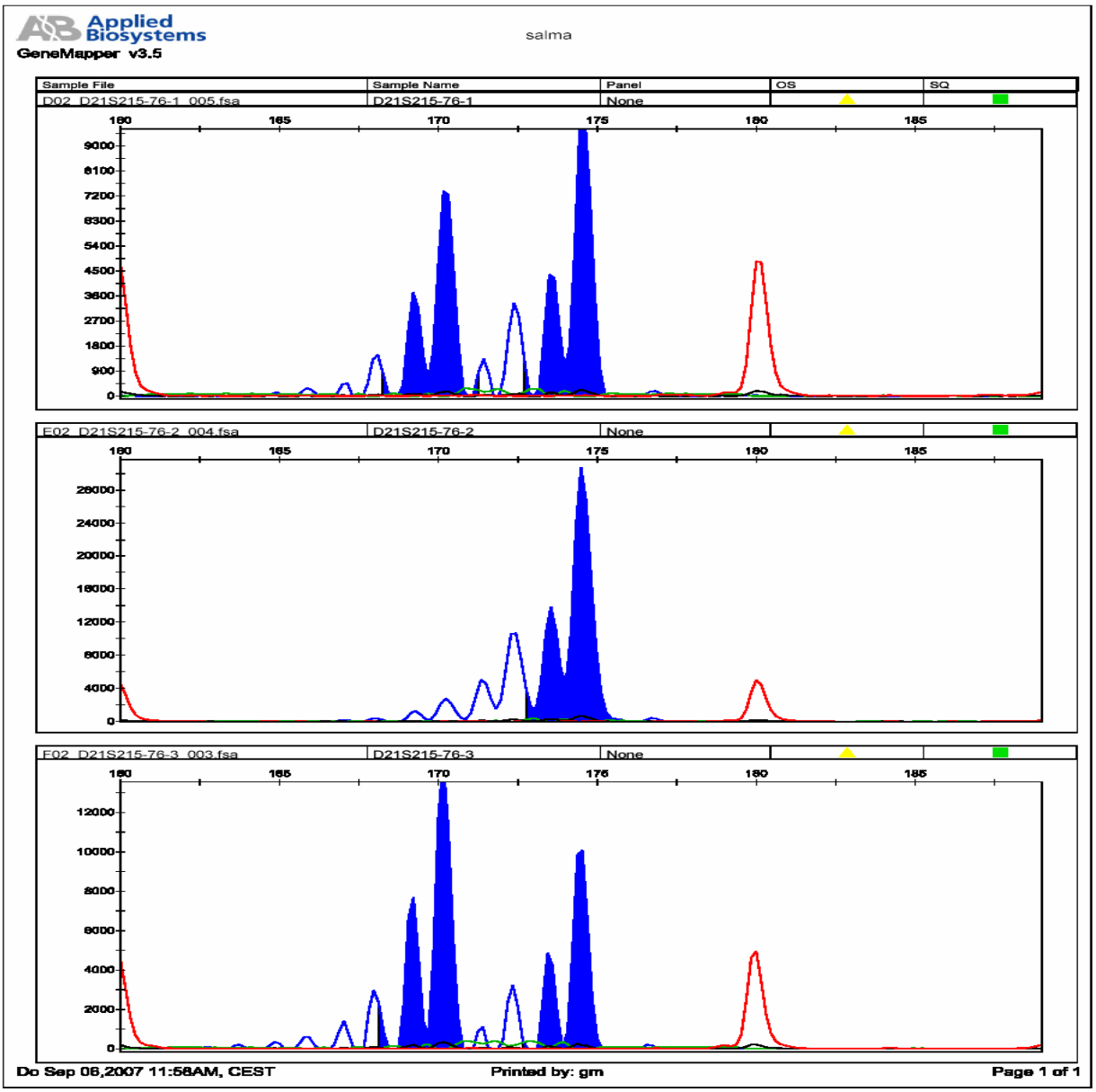

Figure 7: D21S1252 STR-marker (labelled with FAM) for chromosome 21 in the index child and the parents. In this case the mother carries homozygous alleles band at 174 and the father has heterozygote alleles of 168 and 174bp. The index child received allele 168 from the father and two alleles 174 which can be traced by the height of the second peak, however, the additional allele 174 can be from either parent.

2.4. Genotyping of Y chromosomal STRs

2.4.1. Method of Y chromosome STRs study

Y-chromosomal STRs are widely used in the forensic identification of male DNA. In addition other applications for Y-STRs include tracing of paternal lineages, historical studies and genetic genealogy (Jobling and Tyler-Smith 1995; Jobling, Pandya et al. 1997).

Y STR haplotyping is used for typing of male DNA and can trace the paternal lineages into the past which is useful for genealogical study. The Y chromosome carries a non-recombining

segment (Pericic, Barac Lauc et al. 2005), thus once a mutation occurs on the Y chromosome it is a slow process for it to spread into the population. Therefore, Y-chromosomal haplotypes provide a rich source of information about male history. In addition, it is a good tool to study the genetic structure and regional differences among geographical distribution of certain populations and can provide evidence for migration from haplotype structure.

To investigate the Y chromosomal genetic structure in DS fathers in the Omani population seventeen Y-chromosomal short tandem repeats (STRs): DYS19, DYS385 a/b, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (YGATA C4), and YGATA H4 were typed in DNA samples of fathers of Down syndrome families (n= 186). Individuals belong to the Omani nationality and are linguistic of Omani affiliation. All are residents of different geographical regions in the Sultanate of Oman. Table 20 represents each locus designation; dye labeled used, DNA genotype and alleles included in Y filer kit from ABI.

Table 20: AmpFLSTR Yfiler kit loci and alleles

Locus Designation	Alleles Included in Yfiler kit allelic ladder	Dye Label	DNA 007 Genotype
DYS456	13-18	6-FAM™	15
DYS3891	10-15		13
DYS390	18-27		24
DYS389II	24-34		29
DYS458	14-20	VIC®	17
DYS19	10-19		15
DYS385 a/b	7-25		11,14
DYS393	8-16	NED™	13
DYS391	7-13		11
DYS439	8-15		12
DYS635	20-26		24
DYS392	7-18		13
Y GATA H4	8-13	PET®	13
DYS437	13-17		15
DYS438	8-13		12
DYS448	17-24		19

2.4.2. DNA Quantification

DNA samples from the fathers of the DS index children have been extracted for the molecular study of non-disjunction. The final concentration of DNA used was 10ng/µl for all samples. In total 186 samples have been used for Y-STR's study.

Table 21: Yfiler PCR amplification ready kit content

Reagent	Content	Quantity
AmpFℓSTR®Yfiler™Reaction mix	PCR reaction mix containing : MgCl2,dATP, dGTP, dCTP, and dTTP, Bovine Serum Albumin, and 0.05% sodium azide in buffer and salt	1.1 ml/tube
AmpFℓSTR®Yfiler™Primer set	6-FAM™, VIC®, NED™, and PET® dye labeled and unlabeled primers in buffer that amplify the Y-STR loci DYS19, DYS385 a/b, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (YGATA C4), and YGATA H4	0.55ml/tube
Ampli Taq Gold® DNA Polymerase	DNA Polymerase 5U/µl	50 µl/tuve
AmpFℓSTR®Yfiler™ Allelic ladder	Allelic ladder containing the following amplified alleles: 6-FAM™ dye (blue): DYS3891 alleles 10-15; DYS389II alleles 24-34; DYS390 alleles 18-27; DYS456 alleles 13-18. VIC® dye (green): DYS19 alleles 10-19; DYS385 alleles 7-25; DYS458 alleles 14-20. NED™ dye (yellow): DYS391 alleles 7-13; DYS392 alleles 7-18; DYS393 alleles 8-16; DYS439 alleles 8-15; DYS635 (Y GATA C4) alleles 20-26. PET® dye (red): DYS437 alleles 13-14; DYS438 alleles 8-13; DYS448 alleles 17-24; Y GATA H4 alleles 8-13.	50 µl/tuve
AmpFℓSTR® Control DNA 007	0.10 ng/ µl human male genomic DNA in 0.05% sodium azide and buffer	0.3 ml
AmpFℓSTR® Control DNA 0047A	0.10 ng/ µl human female cell line 9947A DNA in 0.05% sodium azide and buffer	0.25 ml

Table 22: Equipments and Consumable for PCR

Name	Manufacture
Thermocycler with gradient	ABI
Microcentrifuge	Eppendorf
Pipette set with different ranges (10µ1, 20 µ1 and 200 µ1)	Eppendorf
96 PCR plate Full Skirt	Eppendorf
Sealing tape Covers	Sarstedt
Pipette tips, sterile, disposable hydrophobic filter-plugged	Eppendorf
Vortex	IKA®Labortechnik
Minicentrifuge	Fisher Scientific
Sequencing plate (96 PCR plate half Skirt)	Thermo Scientific
PCR 0.5ml tubes (for master mix preparation)	Eppendorf
Multi-rack	New Lab
Gloves, disposable	Charite`
Microcentrifuge tube rack	New Lab
Genescan analysis software	ABI
POP-7™ Polymer	ABI

Table 23: PCR master mix reactions

Reagent Name	Concentration	Volume Per one Reaction
dH2O	-	
Primer-Mix	-	5.0 µl
PCR Reaction Mix	-	9.2 µl
Ampli Taq Gold DNA Polymerase	5U/µl	0.8 µl
DNA	10-20ng/µl	10.0µl
Final volume	-	25.0µl

Table 24: PCR amplification conditions

	Process	Temperature	Time	Cycles
1	Initial denaturation	95˚C	5 min	1
2a	Denaturation	94°C	1 min	30X
2b	Annealing	61°C	1 min	
2c	Extension	72°C	1 min	
4	Final Extension	60°C	80min	1
5	Hold	4°C	∞	

2.4.3. PCR master mix and conditions

DNA was amplified according to the AmpFLSTR Yfiler PCR amplification Kit User Manual that allows single tube co-amplification and 5 colour detection of 17-loci multiplex PCR.

10.0µl (10-20 ng/µl) of genomic DNA were amplified in a total reaction mix of 25µl consisting of 9.2 µl AmpFLSTR Yfiler PCR reaction mix, 5.0 µl of AmpFLSTR Yfiler primer set, and 0.8 µl of Ampli Taq Gold DNA Polymerase. PCR amplification conditions were carried out according to the conditions described in the user manual of the AmpFLSTR Yfiler PCR amplification kit Table 24

2.4.4. Gel Electrophoresis

The amplified PCR products (5µl) were identified by electrophoresis on 2% Agarose gel (details described in section 2.3.10. Gel Electrophoresis).

2.4.5. Y-STR Typing and genotyping

1.5µl of PCR products were then mixed with 24.5 µl of Hi-Di formamide and 0.5 µl of 500 LIZ as an internal standard and analyzed by capillary electrophoresis with the ABI 310 genetic analyzer, polymer POP4 and using G5 matrix filter sets (ABI Prism 310, Applied Biosystem) to detect the five dyes 6-FAM, VIC, NED, PET and LIZ.

Table 26: Reagents required for loading sample

LIZ Size Standard	Genescan® 500 LIZ	ABI
Hi-Di™ Formamide	99.5%	ABI

Table 27: Master Mix for loading sample

Reagent Name	1 Reaction
Amplified pooled PCR Product	1.5 µl
Hi-Di™ Formamide	24.5 µl
LIZ Size Standard	0.5 µl
Final Volume	

Finally, data collection of samples and genotyping was carried out automatically with the allelic ladder provided with the AmpFLSTR filer kit template 9 macro (ABI). The Genotyper software (Genotyper® 3.7 NT and GeneScan® 3.7 softwares Appplied Biosystems) assigns the genotypes to the sample alleles by comparing their sizes with those obtained from the known alleles in the AmpFLSTR Y filer allelic ladder by comparison the base pair size obtained for each sample allele peak to the sizes obtained from the allelic ladder peaks.
Allele nomenclature according to the Y-STR Haplotype Refrence Database, YHRD (http://www.yhrd.org.).

2.5. Sequencing of the D-loop of the mtDNA

Mitochondrial DNA (mtDNA) is a circular DNA molecule of 16.5 kb in length. Somatic and germ cells contain up to 8000 copies of the mitochondrial genome and in each mitochondrium there are about 10 copies.

The mitochondrial DNA contains 13 protein coding genes which are components of the respiratory chain enzyme complex in the inner membrane of the mitochondria. In addition, it contains 22 tRNA and 2 rRNA (Anderson, Bankier et al. 1981)coding sequences required for the intramitochondrial translation. Mitochondria have a displacement loop (D-loop) known as control region, which contains the heavy strand promotor and the light strand promotor regions and the initiation site for heavy strand replication, thus the structure form a triple strand DNA where the displaced strand formed the loop of the letter “D”. The D-Loop region in mitochondria is a non-coding region and large parts are highly variable (hypervariable regions = HVRs). The control region concentrated in two hypervariable segments known as HVI (nucleotide positions 16,024-16,383) and HVII (66-370)(Aquadro and Greenberg 1983) and are thus, useful for study of evolutionary history.

The number of mitochondria in a cell varies with depending on the cell type: those which require a lot of energy contain thousands of mitochondria each with 2-10 copies of mtDNA while other cells type contains only few hundred. Female oocytes contain about 100,000 mtDNA molecules, while sperm contain only 50-75 mitochondria. mtDNA is maternally inherited and escapes

recombination because sperm fail to contribute paternal mitochondria to the zygote. Absence of paternal mtDNA inheritance can be due to the low number of mitochondria in the sperm. Furthermore, there is an active system which eliminates paternal mitochondria from the fertilized oocyte. Therefore, maternal mtDNA haplotypes are shared by all individuals within a maternal family line.

mtDNA has unique features that makes it reliable to evolutionationary study: a high copy number, maternal inheritance, lack of recombination and higher mutation rate than found in nuclear DNA(Pakendorf and Stoneking 2005)

2.5.1. Method

In this project, mtDNA sequencing was performed in 244 DS families. The samples were collected in a wide geographical range that spans all 10 health regions in Oman and their tribes.
The study is based on the hypervariable segment (HVS-1) of the control region in order to provide some insights into the genetic structure of the population in this area. Two pairs of primers were used to amplify DNA samples then the fragments were sequenced on a 3730 ABI genetic analyser. The sequence results were analysed with the GeneMapper v4.0. software. Mutations were scored relative to the revised Cambridge Reference Sequence (rCRS) Genbank AC 000021.2 gi:115315570).

2.5.2. DNA extraction

DNA samples were from the mothers of the DS index children. The final concentration of DNA used was 50ng/µl for all samples. Method of extraction is described in microsatellite protocol. Total number of cases used for the mtDNA study was 244 samples.

2.5.3. Primer design

Primers for D-loop of mtDNA sequence were from Revised Cambridge Reference Sequence (rCRS) of human mitochondrial DNA with Reference sequence AC_000021.2 were designed using Primer 3 software as described in Table 28

Table 28: Primers used for PCR and sequence reaction

	Primer type	**Primer sequence (5' to 3')**	**PCR product size**	**Region**
mtDNA human	Forward S1329	5'ctccaccattagcacccaaa3'	S1329-S1330 = 465bp	15975-15994
mtDNA human	Reverse S1330	5'gcactcttgtgcgggatatt3'	S1329-S1330 = 465bp	16439-16420

2.5.4. Polymerase chain reaction (PCR) procedure

DNA samples were amplified by the Polymerase Chain Reaction (PCR) using ABI 9600 Mastercycle and the PCR conditions presented in Table 30. PCR amplification was carried out in a 25 µl reaction volume using 50ng/µl of DNA template.The final concentrations of the reagents and total volume used is presented in a master mix Table 29

The PCR products were identified by electrophoresis on a 2% agarose gel

Table 29: PCR Master mix

Reagent Name	Concentration	Volume Per one Reaction (1x)	Manufacture
dH_2O	-	15.85µl	
Buffer without Mg^{2+}	10x	5.0 µl	Promega
$MgCl_2$	25 mM	2,5 µl	Promega
Primer (for)	10pmol	0.025µl	TIB Molbiol
Primer (rev)	10pmol	0.025µl	TIB Molbiol
Taq Polymerase	5U/µl	0.1 µl	Promega
dNTP's	10pmol/ each n.	0.5 µl	Promega
DNA	50ng/µl	1.00µl	
Final volume	-	25,00µl	

Table 30 : PCR amplification conditions

	Process	Temperature	Time	Cycles
1	Initial denaturation	96˚C	3min	1
2	Denaturation	96°C	30sec	35X
3	Anealing	58°C	30sec	
4	Extention	72°C	30sec	
5	Elongation	72°C	10min	1
6	Hold	16°C	∞	

Table 31: Primers used for PCR and sequence reaction

S1329	5'ctccaccattagcacccaaa3'	mtDNA	15975-15994	human	S1329-S1330=465bp, amplify the HVR1 of DLOOPS
S1330	5'gcactcttgtgcgggatatt3'	mtDNA	16439-16420	human	S1329-S1330=465bp, amplify the HVR1 of DLOOPS

2.5.5. Gel Electrophoresis:

The amplified PCR products (5µl) were identified by electrophoresis on 2% agarose gel. The agarose gel was prepared by mixing 2 g of agarose in 100 ml of 1x TBE buffer and heated at a microwave for few seconds to boil then cooling at room temperature. After 5µl of Ethidium bromide (1mg/ml) were added to agarose mixed and poured into electrophoresis tray with a comb adjusted. As the gel solidified 5µl of PCR product plus 3µl of loading dye were loaded in addition to a negative control in which no DNA is present as a template and a positive control using a known amount of added template. The gel was run at 120 volts for 30-50 minutes in an

electrophoresis tank and visualised by the use of an Ultraviolet Tran illuminator and photographed.

2.5.6. DNA Sequencing

DNA sequencing is the process of determining the order of nucleotide bases along a DNA strand. DNA sequencing can be carried out using chemical degradation or chain termination (enzymatic) method. The method used for sequencing of mtDNA was the enzymatic method which is based on the original Sanger's method. This method is based on the in vitro DNA replication in the presence of special differently labeled specific bases called dideoxynucleotides (ddNTPs). ddNTPs are similar to normal dNTPs except that they contain a hydrogen group instead of a hydroxyl group at 3' carbon position. After incorporation of a ddNTP into the newly synthesized DNA strand, the addition of further nucleotides into the sequence is prevented which results in a series of DNA fragments after many running cycles. The DNA fragments are separated according to their size by electrophoresis with DNA analyzer ABI 3730.

The sequencing sample plate will be loaded into ABI automatic sequencing machine which is a capillary electrophoresis with a fluorescent detection camera. The four ddNTPs are labeled with different fluorescent dyes and they will be separated by electrophoresis according to the size of the fragment. Finally the product is detected by a standard multiwave fluorescence detector with ABI Prism 3730 48-capillary system and a computer program will compile the data into a colored graph showing peaks of different colors representing a specific labeled ddNTP.

DNA sequencing required many steps including: purification of PCR products, generation of labelled DNA fragments by a cycle sequencing reaction, purification of the labelled DNA fragments from excess fluorescent dyes and finally analysis of the labelled fragments in an automated DNA analyzer ABI Prism 3730 48-capillary system.

2.5.7. PCR-Purification

The amplified PCR products (1µl) were purified with two enzymes Exo1 (Exonuclease-1) and Shrimp Alkaline Phosphates (SAP) (Exo1 digests the single strand and the SAP catalyzes the dephosphorylation of 5' phosphatase from DNA and dNTP's). The Master Mix was prepared as described in Table 32. The enzyme purification mix was processed in a 96-well MicroAmp plate then placed on ABI 9600 master cycler with the following incubation conditions:

Incubation: Program:

30 min: 37°C

15 min: 86°C for inactivation of the enzymes

Hold: 8 °C

Table 32: PCR-Purification master mix

	x1 Reaction	**Master mix x**
H_2O	4,40 µl	
SAP 1U/µl (FA usb)	0,5 µl	
Exo1 10/µl (Fa. Usb)	0,1 µl	
PCR-Product	1 µl	
Final Volume :	**6 µl**	

Exo1 Exonuclase 1	10 U/µl	usb	70073X
SAP	1 U/µl	usb	70092Z

2.5.8. Sequence Reaction

After PCR product purification the followed step was to prepare a master mix for sequencing reaction (Cycle sequencing). Cycle sequencing was carried out using the ABI PRISM® BigDye® Terminator version 1.1 kit. Preparation of a master mix for sequencing reaction is described in Table 33. The sequencing reaction were added in a 96 well MicroAmp plate then centrifuged for 1 minute and incubated with a standard ABI conditions.

Programme conditions:

Rapid thermal ramp† to 95°C

- 95°C → 30 sec

Rapid thermal ramp to 50-55 °C (depending on template)

- 50–55 °C →10 sec

Rapid thermal ramp to 60 °C

- 60°C → 4 min

Table 33: Sequence reaction master mix

	x1 Reaction	**Master-mix x**
H_2O	2,0	
5xBig Dye Buffer	2,0	
Big Dye Mix V 1.1	1.0	
Per Primer 25ng/µl	1,0	-
PCR-Purification volume	6,0	-
Final Volume:	**12 µl**	

Table 34: Reagents required for sequence reaction

Reagent	Manufacture
5xBig Dye Buffer	ABI
Big Dye® Terminator V1.1	ABI

2.5.9. Sequence reaction-cleaning

Sequence reaction was cleaned up using Sephadexplates (Sephadex G50 from Roche Applied Science). A plate was filled up with sephadex G50 then 300µl dH2O was added. Afterwards the plate incubated for 1h at room temperature or 4°C for overnight, followed by spinning at 2500 U/min 1 min. After, sequence reaction product was added and centrifuged at 2500 U/min 2 min. Finally the plate was covered with septa and heated at 96ºC for 1 minute. Immediately after heating it was placed on ice and it was ready to be loaded in the ABI 3730 for sequencing.

2.5.10. Sequence analysis

Results obtained from ABI 3730 genetic analyser were analysed by the use of ABI sequencing analyisis 5.2 Software and Lasergene6 SeqMan 6.1

Electronic database information**:**

Genbank, http://www.ncbi.nlm.nih.gov/Genbank/ (for the mtDNA complete sequence data AC_000021.2)

2.6. Down syndrome registry

2.6.1. A National register of Down syndrome in the Sultanate of Oman

Oman is administratively divided into 5 Regions and 3 Governorates with 59 Wilayats. These regions are: Ad Dakhliya, Ash Sharqiyah, Al Batinah, Adh Dhahirah, and Al Wusta, and the Governorates are: Muscat, Dhofar and Musandam Governorates. The regions of As Sharqiyah and Al Batinah have each been further subdivided into two (North Al Batinah, South Al Batinah, North As Sharqiyah and South As Sharqiyah) for health administration, giving a total of ten health regions.

Establishing of Down syndrome registry was based on two main registries:

i) Registry initiated by Dr. Anna Rajab in 1993 (A Senior genetic Consultant in Royal hospital) from the department of Paediatric Clinic, Muscat.

ii) A Karyotype registry from Cytogenetic department on 1999-2004 Darsait, Muscat MoH by Salma Al Harrasi (A Senior cytogenetist in cytogenetic department Darsait, Muscat).

Down syndrome registry was first established by Dr. Anna Rajab in 1993 in Royal hospital in the department of paediatric clinic. She started to collect most of the cases which were reported or notified by paediatricians who were working in the neonatal units of inpatient obstetric clinics and various inpatient and outpatient paediatric clinics. In addition, she collected information of Down syndrome from the birth notification form and the notification from neonatal intensive care units form of medical records of Royal Hospital department and from medical records of statistical department in the Ministry of Health. Based on the ten health region divisions, she established a genetic clinic in each health region in Oman which consisted of a trained physician and a staff nurse at each site and abstracted records from birth hospitals and tertiary facilities documenting all information include child full name, date of birth, address, sex, contact number and health region.

Cytogenetic laboratory was established for diagnosis of chromosomal abnormalities in June 1999 and located at Central health laboratories in the capital Muscat and is the only lab in Oman which belongs to the Ministry Of Health. The lab receives samples from all over regional hospitals in Oman. Since its establishment, the laboratory creates a Karyotype registry with all cytogenetic cases received according to the information provided by the physician through a request form received along with patient sample. The request form was designed by cytogenetic laboratory and it contains the following information: referral hospital name, hospital patient number, full name of the patient, age, date of birth, sex, type of sample collected together with date of collection, clinical information or indication for investigation, diagnostic test performed, referral doctor name and his/her contact address and finally the cytogenetic finding results. This information is entered into an excel Karyotype registry with all information in addition to the patients address, date of receiving, cytogenetic results and date of results issue. The Karyotype register included all data's from cytogenetic analysis therefore; it was easy to collect all cases of Down Syndrome analysed and create a register from it. The national Down syndrome cytogenetic register was established based on protocol required documenting of Karyotype for each case included only children with standard trisomy 21 or mosaic trisomy 21 and excluded translocations type. The registry contains the following data's regarding Down syndrome patients: ID code number, 1st name, 2nd name, 3rd name, tribe name, date of birth including month and year, sex, hospital name, health region, mother age, father age, consanguinity, patient address and contact number and finally data source.

2.7. Case control study

2.7.1. Questionnaire design for the case-control study

A pilot study questionnaire of a case control study was designed for the epidemiological and statistical analysis. The questionnaire aimed to identify epidemiological and environmental factors contributing to chromosome non disjunction and to identify potential risk factors for Down syndrome associated with Omani population in different regions of Oman.

Informed consent was obtained from all participating families. Only those families with biological samples from both parents and their children were included. The informed consent form used is presented below (Appendix.3).

In each of the families with a Down syndrome child the mothers with their husbands were interviewed by general medical doctors or staff nurses in the health centers or hospitals belonging to the Ministry of Health. These interviews were carried out personally and the interviewer explains the points below before starting the interview:

1. The interviewer is a medical doctor and will keep all information strictly confidential. No one else will receive any information about you. Your information will be used for statistical/epidemiological purposes only. The persons who will perform the statistical/ epidemiological analysis are physicians too and will get only anonymous data sheets. Your name will not be mentioned in any other report.

2. You may drop out from the interview at any stage.

3. You are free to refuse answering certain questions. I would prefer to receive no answer, than to get a wrong answer for what ever reasons.

4. I will be extremely happy to answer any questions that you may have, it would be easiest if you could save them until the end of the interview.

The questions were mainly concerning a mother of Down syndrome and were divided into main eight sections including:

- General Information and Socio-demographic data.
- Menstrual history.
- History of women's pregnancy.

- Health and illnesses.
- Diagnostic X-rays and X-ray treatment.
- Family History
- Family Health
- Occupational History

Each section containing some questions as mentioned in Table below.

Content of the questionnaire

Sections		**Questions points**
General information and Socio-demographic data	Down syndrome child	Full name Date and place of birth Name of Hospital of birth and region Hospital File # Weight during Birth Gender
	Mother's information	Mothers name of the index child Date and place of birth and country Contact # and Full address Educational level Any relationship with her husband (consanguinity)
	Father's information	Full name of father of the index child Date and place of birth and country Contact # and Full address Educational level
<u>Following questions for mothers of index child:</u>		
Menstrual history		1. Age of first menstrual period, and its interval 2. If medication or surgery required to start a menstrual period 3. If regular or irregular period, reasons, and any medication used. 4. If experienced a large interval without period. 5. If experienced menopause and at which age?
History of women's pregnancy		1. Number of pregnancies and duration of each pregnancy. 2. If each pregnancy end with alive birth or others. 3. Number of abortions if present and the gestational period.

		4. Any detected kind of chromosomal abnormalities in case of abortion or birth defect. 5. Number of alive and dead children and reasons of death. 6. Number of pregnancy with Down syndrome.
Health and illnesses	Any history of certain diseases and medication used for treatment in addition at what age.	**List for some diseases:** Thyroid or hyperthyroidism,diabetes,asthma,leukemia, cancer or malignance tumor,chickenpox,rheumatoid arthritis, tuberculosis, hepatitis or inflammation of liver, hypertension, erythematosis others.
Diagnostic X-rays and X-ray treatment	Diagnostic X-rays and number of times in addition at what age.	Chest X-ray Fluoroscopy. Pelvis X-ray Breast X-ray Esophagus X-ray Gallbladder X-ray Intestine or colon X-ray Heart X-ray Kidney X-ray Thyroid X-ray Vein or arteries X-ray Teeth X-ray MNR (magnetic resonanz) Ultrasound.
	X-ray treatment	1. Cobalt treatment for any disorder and duration of treatment. 2. Any type of X-ray treatment for any disease.
Family History		1. Number of sisters and brothers & age 2. If parents have a still birth children with birth defect or chromosomal abnormalities. 3. Age of father and mother when she was born. 4. If father with any chromosomal rearrangement. 5. Number of her mother's pregnancies and if she experienced miscarriages or induced abortion or other. 6. Any of brother or sister with chromosomal abnormality and is alive now.
Family health		1. Any member of her family with leukemia, or Alzheimer's diseases and the relationship. 2. Any member of her family with Down syndrome and the relationship. 3. Any member of her family with any chromosomal abnormality other than Down syndrome and the relationship.

Occupational history		1. If she is an employee or a house wife. 2. Place of work (company, industry or government sector). 3. Kind of occupation 4. Kind of industry/company 5. If in her regular work or hobby which lasted minimum of 6 month exposed to any of the following metals: lead, mercury, lithium, boron, Manganese, tin, zinc, iron, copper, cadium, aluminum, selenium, nickel, others. 6. From age of 12 until now if she had a regular job or a hobby which lasted not minimum of six moth and exposed to the following chemicals or reagents: Drugs or pharmaceuticals, chemicals used to develop films or dyes such as hair dyes, printing dyes, fabric dyes or others,, grease chemicals for insecticides, pesticides or fungus. Natural gas, stains or paint products, house hold fuel. Chemicals for instruments sterilization or any other chemicals. Also if she frequently used a photocopier or Xerox machines. 7. From age of 12 until now if she had a regular job or hobby where she exposed to X-ray or radiation from any source from her work activity.

Before the completion of interview, the interviewer gave the opportunity to the family to answer any question they would like to ask. In addition, at the end of interview they were asked if they have any extra important information which was not raised during the interview.

Time required to complete the interview was mentioned, the interviewer's ranking the interview from 1 "good"→ 5 "poor" for cooperation of interview partner and reliability of answers obtained.

3. RESULTS

3.1. Results of the cytogenetic analyses

The clinical diagnosis of Down syndrome was confirmed by chromosome analyses and karyotyping after GTG-banding for all children. Subsequently, the frequency of different types of cytogenetic aberrations found in Omani DS children was determined.

3.1.1. Age at diagnosis

Figure 8 shows that 55.5% of the children with DS were diagnosed at less than one month of age and 34.9% when the child was between 1-6 months of age. Thus, almost 90% of DS children born in Oman were diagnosed cytogenetically within 6 months after birth. This is a very good indicator that the paediatricians are aware of the clinical phenotype and initiate cytogenetic analysis for confirmation.

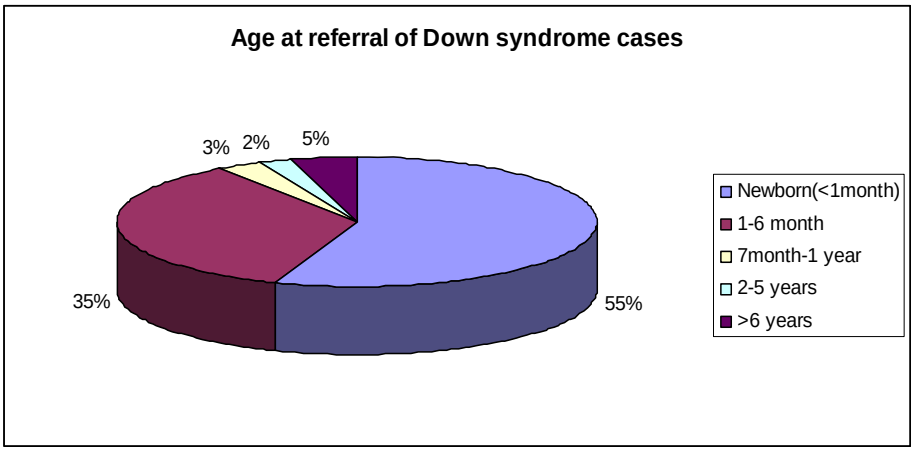

Figure 8: Age at referral of postnatal Down syndrome cases**.**

3.1.2. Results of the chromosome analyses

The cytogenetic results of the analyses of 680 cases of Down syndrome are presented in Table 35. Non-disjunction of trisomy 21 and hence free trisomy 21 was the most common type of abnormality detected in 94.25% (N=640) of the cases. There were 20 cases of translocation trisomy (2.95%) and 19 cases of mosaicism (2.65%). In one case (0.15%) a non classical type of chromosomal abnormalities was detected. Two children had a combination of translocation and mosaicism at the same time: 47,XX,t(21;21)(q10;q10)/46,XX and 47,XY,t(21;21)(q10;q10)/46,XY. One child with regular free trisomy had an additional translocation between chromosome 8 and chromosome 21: 46,XX,t(8;21)(q12;p11),+21. In addition, one more child had a duplication of 5p: 47,XX,+21,5p+ with additional dysmorphic features.

Table 35: Results of karyotype analysis for 680 Down syndrome cases

Karyotype results	Number of cases	%
Free trisomy		
Total	**640**	**94.12**
47,XX,+21	271	
47,XY,+21	369	
Translocation		
Total	**20**	**2.94**
46,XX,t(14;21)(q10;q10),+21	7	
46,XY,t(14;21)(q10;q10),+21	2	
46,XX,t(21;21)(q10;q10),+21	2	
46,XY,t(21;21)(q10;q10)+21	4	
46,XY,t(13;21)(10q;10q),+21	2	
46,XX,t(15;21)(q10;q10),+21	1	
46,XY,t(13;14)(q10;q10),+21	2	
Mosaic		
Total	**19**	**2.79**
47,XX,+21/ 46,XX	8	
47,XY,+21/46,XY	9	
47,XX,t(21;21),+21/ 46,XX	1	
47,XY,+21/47,XY,+t(21;21),+21	1	
Non classical		
Total	**1**	**0.15**
46,XX, der(21),t(18;21) (q12;p11)	1	
Total DS cases	**680**	**100**

3.1.3 Sex ratio of DS cases with different types of trisomy 21

Among karyotyped cases listed in Table 35 there were 389 males and 291 females with a sex ratio of 1.36. Table 36 shows the sex ratio for each karyotype. There was a significant excess of males observed in the group with free trisomy 21 with a sex ratio of 1.36 (χ^2, P=0.0016), while in the groups with translocations and mosaicism the sex ratio was 1.00 and 1.11 respectively which is more close to the sex ratio of 1.06 expected in the general population.

Table 36: Sex ratio of Down syndrome cases among different type of trisomy 21

Karyotype	Males	Females	Male : Female ratio
Free trisomy 21	369	271	1.36
Robertsonian Translocation	10	10	1.00
Mosaic	10	9	1.11
Non classical	0	1	--

3.2. Results from the DS Registry

Oman has a comprehensive health care system which is provided by the government. More than 95 % of all newborns are delivered in governmental hospitals and almost all are examined by

paediatricians who are aware of the clinical phenotype of DS and prompt a cytogenetic analysis for confirmation. The cytogenetic service is centralized and free of charge at the National Cytogenetic Service of the MoH in Muscat. Since 1999 all DS cases are cytogenetically confirmed. All data from the DS registry are restricted to Omani nationals and the study is confined to DS children with free trisomy 21.

We used data from the Omani Down Syndrome Registry to analyse the following:

i. Sex ratio in Down syndrome cases
ii. Down syndrome and maternal age effect
iii. Down syndrome prevalence in Oman
iv. Prevalence of Down syndrome in different regions of Oman
v. Seasonal variation in the Down syndrome birth prevalence

3.2.1. Sex ratio of DS children with free trisomy 21: results from 2000 to 2004

From 2000 to 2004, the DS Registry covers the data of 518 DS children with a free trisomy 21. The sex ratio was calculated for these cases (compare also 3.1.3.; sex ratio of all karyotyped cases with free trisomy 21: N=640, sex ratio: 1.36). Out of the 518 children from the registry 294 were male and 224 female. Thus, the sex ratio was 1.31 (Table 37), accordingly the proportion of males was 56.76% compared to 43.24% in females. The sex ratio of the DS children is significantly different from the sex ratio of the newborns in Oman (N=44116; 22713 males and 21403 females; data all live births 2006) which is 1.06 (χ^2, P=0.00176). The sex ratio of the DS children was also different from their chromosomally normal sibs with a sex ration of 1.09 (N =1760) which is close to the expected ration of 1.06 in the general population.

Table 37: Sex ratio for Down syndrome children between 2000 and 2004 in Oman population.

Sex	**Number of Down syndrome cases**
Female	224
Males	294
Total	**518**
Sex ratio Male : Female	**1.31**

3.2.2. Down syndrome prevalence and maternal age effect

We calculated the parental age at a time of the birth of the DS child. Unfortunately, it was impossible to record the parental age from all parents of the registry. Out of 518 cases there were only 287 cases with identified parental ages (Maternal cases: N=156 and paternal cases: N=131). The mean maternal age was 35 (range from 20 to 53, SD=6.96) and mean paternal age was 41 (range between 23 and 68, SD 9.56) (Table 38).

Table 38: Maternal and paternal age at the time of Down syndrome birth

	DS cases (N)	Mean and SD	CI (95%)	Min	Max
Maternal age	156	35±6.96	33.9;36.1	20	53
Paternal age	131	41±9.56	39.35;42.65	23	68
Total	**287**				

3.2.3. Birth prevalence of Down syndrome in Oman

The total number of live births (LB) in the population is required to calculate the birth prevalence of Down syndrome. Table 39 shows the number of live births (LB) in all institutions of the Ministry of Health (MoH) provided by the statistical department of the MoH, Oman.

Analysis was performed for the whole country by calculating the prevalence of Down syndrome per total live births for each year 2000 to 2004 (Table 40). In total 518 cases were diagnosed over a period of 5 years (2000-2004) out of 200157 live births (LB) (Table 40). The annual total number of live births between 2000 and 2004 is almost in the same range as there was no significant difference among the years. Among Down syndrome cases there was no significant difference ($p = 0.276$) in the number of cases between the years, as there was no specific trend observed. The annual prevalence of trisomy 21 from 2000 to 2004 ranged from 1:423 to 1:358 with an overall prevalence of 1:388 (25.88 per 10.000 live births).

Table 39: Live births in Ministry of Health Institutions (MoH) for the population of Oman.

Year	Month												
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
2000	3407	3017	3173	3258	3366	3327	3430	3615	3407	3401	3228	3365	**39994**
2001	3239	2747	3046	3128	3249	3268	3411	3408	3485	3477	3451	3388	**39297**
2002	3300	3024	3241	3187	3481	3349	3394	3563	3434	3468	3348	3433	**40222**
2003	3403	2966	3208	3272	3366	3384	3493	3659	3383	3471	3217	3240	**40062**
2004	3306	2975	3095	3303	3553	3397	3454	3605	3466	3612	3436	3380	**40582**
2000-2004	16655	14729	15763	16148	17015	16725	17182	17850	17175	17429	16680	16806	**200157**

Table 40: Number of cases and birth prevalence of Down syndrome (DS) (free trisomy 21) between 2000 and 2004 in Oman population.

Year	All LB (N)	DS cases (N)	1DS: LB	Prevalence in 10.000 LB
2000	39994	109	367	27.25
2001	39297	96	409	24.43
2002	40222	105	383	26.11
2003	40062	112	358	27.96
2004	40582	96	423	23.66
2000-2004	**200157**	**518**	**388**	**25.88**

3.2.4. Prevalence of Down syndrome in different regions of Oman

We also analysed the prevalence of DS in different geographical regions of the country based on the number of live births and total live births of Down syndrome born between 2000 and 2004 in each region (Table 41). There are significant differences (compare 3.2.3.) of the prevalence of Down syndrome between the ten health regions (2000-2004) (Table 41). The highest prevalence was found in the South Al Batina region (37.97 per 10,000 live births) followed by Ad Dakhiliya (33.41 per 10,000 live births) and Muscat (30.41 per 10,000 live births). The lowest prevalence is found in South Ash Sharqiya, North Al Batina and Musandam ranging between 15.13 and 16.41 per 10,000 live births. The remaining regions are in the middle range between 25.45 and 26.40 per 10,000 live births. The differences of the DS birth prevalence are statistically significant: South Al Batina (Chi², $p<0.001$) and Ad Dakhiliya (Chi², $p<0.05$). The maternal age among mothers was 34.7 years in high prevalence regions (South Al Batina and Ad Dakhiliya) which is not different from that of the low DS prevalence regions (mean maternal age is 33.9 years) (Figure 9).

Table 41: Number of Down syndrome (DS) live births in different regions between 2000 and 2004. SBAT (South Al Batina), DK (Ad Dakhiliya), MCT (Muscat), DHAH (Adh Dhahira), NSH (North Ash Sharqiya), DF (Dofar), SSH (South Ash Sharqiya), NBAT (North Al Batina), MUSN (Musandam), and WOU (Wousta).

Year	DK	DHAH	DF	MUSN	MCT	NBAT	SBAT	NSH	SSH	WOU	Total
2000	15	11	3	1	36	10	21	5	7	0	**109**
2001	20	7	7	1	19	10	20	7	3	2	**96**
2002	18	9	6	0	28	12	13	13	6	0	**105**
2003	21	9	16	0	23	11	9	15	8	0	**112**
2004	13	8	7	1	34	11	11	3	8	0	**96**
2000-2004	87	44	39	3	140	54	74	43	32	2	**518**

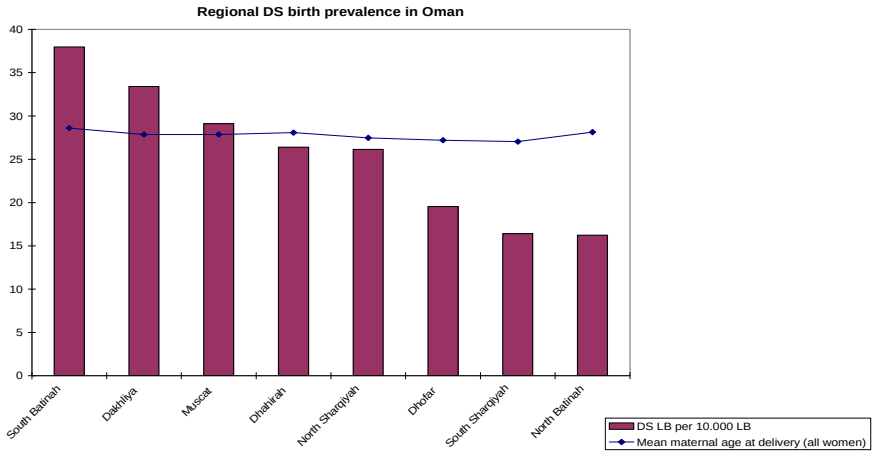

Figure 9: Birth prevalence of Down syndrome in different regions of Oman (red bars) and mean maternal age at delivery of all women in the different regions (blue line).

Table 42: Down syndrome (DS) birth prevalence between 2000 and 2004 in ten regions of Oman

Region (2000-2004)	Total live births (LB)	DS cases (N)	1DS: LB	Prevalence in 10,000 LB
Muscat (MCT)	46033	140	329	30.41
Dhofar (DF)	19950	39	512	19.55
Ad Dakhliya (DK)	26038	87	299	33.41
North Ash Sharqiyah (N SH)	16446	43	382	26.15
South Ash Sharqiyah (SSH)	19500	32	609	16.41
North Al Batinah (N BAT)	33265	54	616	16.23
South Al Batinah (N BAT)	19488	74	263	37.97
Adh Dhahirah (Dhah)	16668	44	379	26.40
Musandam (Musn)	1983	3	661	15.13
Al Wusta (Wousta)	786	2	393	25.45
National wide	200157	518	386	25.88

3.2.5. DS birth prevalence in Oman 2000-2004: highly significant regional differences

To further investigate the regional differences of the occurrence of Down syndrome in Oman, step functions were fitted to the prevalence data by groups of regions[1]. Three distinct clusters within the 10 Omani regions were identified that show highly significant regional differences. A provisional test with four clusters (Table 43), according to the colors in the Table below, reveals that the yellow region (Dhofar) is not significantly different from the blue group. Therefore, it seems reasonable to combine the yellow region with the blue one (Table 44).

Table 43: Provisional test for DS birth prevalence clusters between 2000 and 2004 in ten regions of Oman

Region	LB	DS	DSp	DSpE4	Rank	r4	r3	r2	r1	Color
SBAT	19488	74	0.00380	37.97	4	1	0	0	0	Red
DK	26038	87	0.00334	33.41	4	1	0	0	0	Red
MCT	46033	140	0.00304	30.41	4	1	0	0	0	Red
Dhah	16668	44	0.00264	26.40	3	0	1	0	0	Orange
NSH	16446	43	0.00261	26.15	3	0	1	0	0	Orange
Wousta	786	2	0.00254	25.45	3	0	1	0	0	Orange
DF	19950	39	0.00195	19.55	2	0	0	1	0	Yellow
SSH	19500	32	0.00164	16.41	1	0	0	0	1	Blue
NBAT	33265	54	0.00162	16.23	1	0	0	0	1	Blue
Musn	1983	3	0.00151	15.13	1	0	0	0	1	Blue
Total	**200157**	**518**	**0.00259**	**25.88**						

[1] The statistical cluster analysis was kindly performed by Dr. Hagen Scherb who is from the Institute of Biomathematics and Biometry, Helmholtz Institute Munich.

Table 44: Final test for DS birth prevalence clusters between 2000 and 2004 in ten regions of Oman

Region	LB	DS	DSp	DSpE4	Rank	r4	r3	r2	r1	Color
SBAT	19488	74	0.00380	37.97	4	1	0	0	0	Red
DK	26038	87	0.00334	33.41	4	1	0	0	0	Red
MCT	46033	140	0.00304	30.41	4	1	0	0	0	Red
Dhah	16668	44	0.00264	26.40	3	0	1	0	0	Orange
NSH	16446	43	0.00261	26.15	3	0	1	0	0	Orange
Wousta	786	2	0.00254	25.45	3	0	1	0	0	Orange
DF	19950	39	0.00195	19.55	2	0	0	1	0	Blue
SSH	19500	32	0.00164	16.41	1	0	0	0	1	Blue
NBAT	33265	54	0.00162	16.23	1	0	0	0	1	Blue
Musn	1983	3	0.00151	15.13	1	0	0	0	1	Blue
Total	**200157**	**518**	**0.00259**	**25.88**						

Compared to the new blue region, in the orange region the prevalence is elevated by 53% [27%, 85%], $p<0.0001$, and in the red region the prevalence is elevated by 92% [67%, 122%], $p<0.001$. These differences are highly significant the Chi^2 with 1 degree freedom 20.3 and 81.3 respectively.

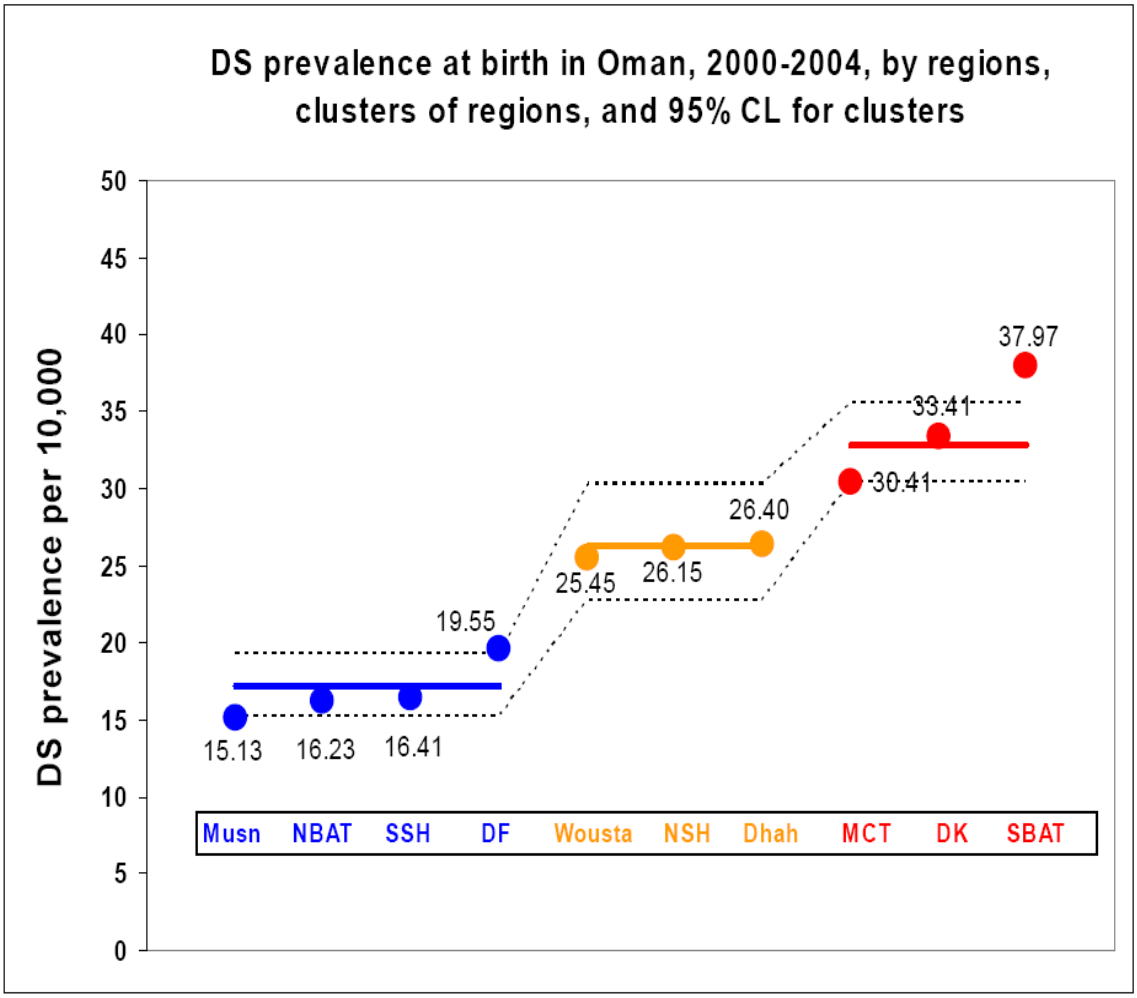

Figure 10: Birth prevalence of DS in Oman, 2000-2004, by groups of regions. Three different clusters identified within 10 Omani regions that reveal highly significant regional differences. The three colors differentiate the regions into three distinct clusters, red: highest DS birth prevalence, orange: middle DS birth prevalence and blue colour: low DS birth prevalence ($p<0.0001$).

3.2.6. Seasonal differences of the Down syndrome birth prevalence in Oman

In our study we also investigated the seasonal variation in the prevalence of Down syndrome at birth. We compared the 12 months trend and the total number of DS cases born per month. These data demonstrate differences in the birth prevalence of DS between months with an increase of the live births of DS between the months of November and December (Figure 11). The highest number of births was in January (N=63) followed by December (N=58). There was only a slight variations in the remaining months (ranged 32-50 cases) (Table 45).

Table 45: Monthly DS birth prevalence 2000 to 2004 in Oman

Month	DS births per month	All births per month	DS birth prevalence per month in 10,000 births
1	63	16655	37.83
2	37	14729	25.12
3	36	15763	22.84
4	48	16148	29.73
5	32	17015	18.81
6	34	16725	20.33
7	42	17182	24.44
8	36	17850	20.17
9	40	17175	23.29
10	41	17429	23.52
11	51	16680	30.58
12	58	16806	34.51
Total	**518**	**200157**	25.88

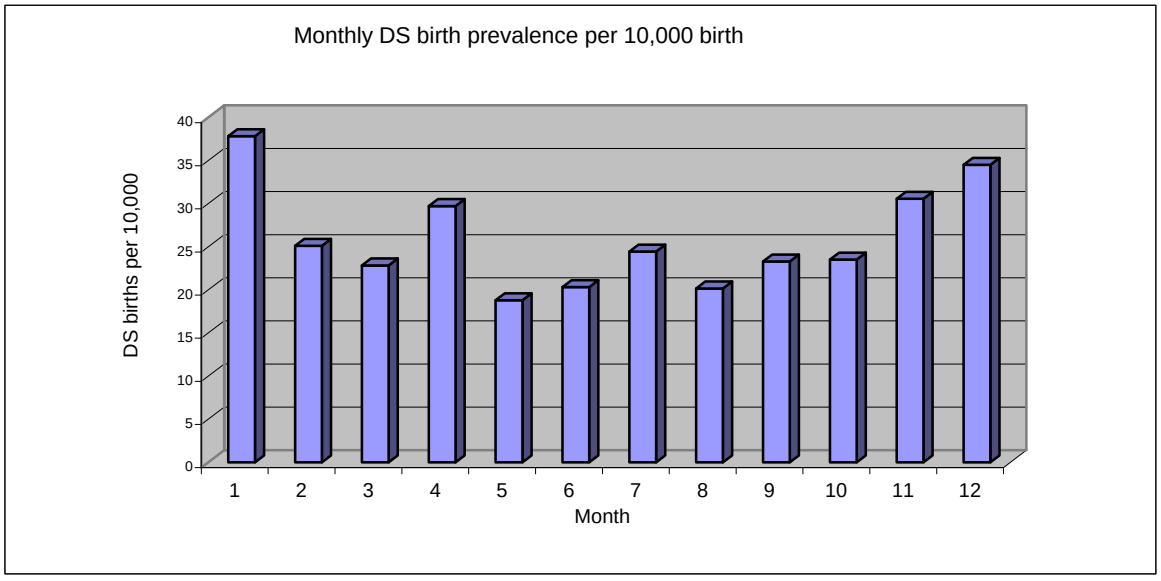

Figure 11: Monthly DS prevalence (N=518) between 2000 and 2004 in the Omani population.

Subsequently, we analysed the seasonal variations of the DS birth prevalence between the three regions which have been identified before by the cluster analysis (Figure 10) having low, medium and high DS prevalence (Table 46).

Table 46: Monthly and regional DS and total live births in the regions with low, middle and high DS prevalence, data 2000 to 2004.

DS births by ranked Omani Regions					All live births by ranked Omani Regions				
Month	**Low**	**Medium**	**High**	**Total**	**Month**	**Low**	**Medium**	**High**	**Total**
1	9	10	44	63	**1**	6216	2821	7619	16655
2	11	6	20	37	**2**	5497	2495	6738	14729
3	12	6	18	36	**3**	5883	2670	7211	15763
4	13	11	24	48	**4**	6026	2735	7387	16148
5	8	9	15	32	**5**	6350	2882	7783	17015
6	3	4	27	34	**6**	6242	2833	7651	16725
7	16	7	19	42	**7**	6412	2910	7860	17182
8	11	5	20	36	**8**	6662	3023	8165	17850
9	9	4	27	40	**9**	6410	2909	7857	17175
10	7	9	25	41	**10**	6505	2952	7973	17429
11	14	10	27	51	**11**	6225	2825	7630	16680
12	15	8	35	58	**12**	6272	2846	7688	16806
	128	**89**	**301**	**518**		**74698**	**33900**	**91559**	**200157**

rank : low = 1

medium = 2

high = 3

ds absolute down syndrome numbers per month and region

1b absolute live birth numbers (assumed) per month and region

sin Sinus: (sin=sin (3.141593*.5)/12;)

rsin Interaction of Sinsus with rank: (rsin=rank*sin;)

The following interactive model is fit to the above data matrix (in SAS notation):

proc logistic date=c;

model ds/1b = rank rsin/scale=d

This model yields an excellent fit to the data (Deviance= 34.7, degree of freedom 33, which means only negligible overdisposition). The following Figure 12 presents the data and the model stratified by the ranked regions[2].

[2] The statistical analysis of the seasonal variation was kindly performed by Dr. Hagen Scherb, Institute of Biomathematics and Biometry, Helmholtz Institute Munich.

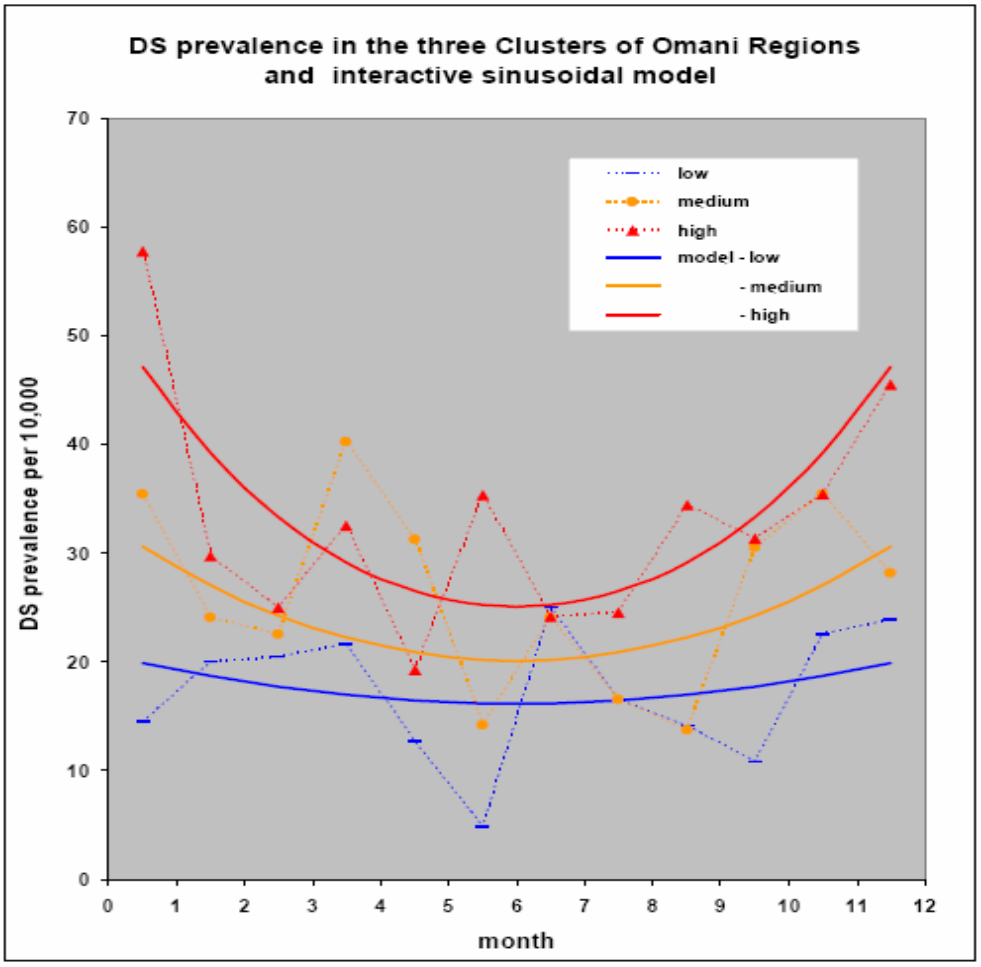

Figure 12: Down syndrome prevalence in the three Clusters of Omani regions, present the data and the model stratified by the rank region.

Figure 12 shows that the distribution and clustering of DS prevalence (prevalence per 10,000) by annual differences (January to December) of the three rank regions (low, middle and high) for the 518 DS cases. The lowest clustering of DS prevalence is between months of April and September, then the curve start to increase steadily between October and December and again between March and January to reach the highest clustering in the month of December and January. On other hand, the significant clustering of the DS prevalence is in the month of January. Analysis of the results with SAS system estimates the odds ratio for the rank is OR: 1.590; 95% Cl: 1.410-1.794 and for the interactive seasonal effect is 0.784; 95% Cl: 0.701-0.878. The rank of the region (rank) and the interactive seasonal effect (rsin) are both highly significant (Chi2>17.0, p<0.0001). These data indicate that there is a seasonal variation in the prevalence of Down syndrome live births in Oman and among the regions between January to December.

3.3. Case control study and results from the questionnaire

3.3.1. Results of the case-control study

As mentioned in the introduction, a case-control study was undertaken covering eight sections: socio-demographic data, menstrual history, history of women pregnancy, health and illnesses, diagnostic X-ray and X-ray treatment, family history, family health and occupational history etc (Appendix.2). The cases were matched with the controls for the birth of a DS child respectively an unaffected child within the same year of birth and from same health region. For each case, the control with the closest date of birth to the DS case was identified. It was difficult to collect more controls as the project covered all regions in Oman and not just one area of the country. The total data consisted of 90 cases and 90 matched controls (total of 180 cases).

From these, we analysed in detail the parental age at the time of DS birth, the history of women's pregnancies and the socio-demographic part. In the remaining sections we did not observe any unusual differences between cases and controls; for example for occupational history approximately 70% of women in both groups were housewives and are therefore considered not to be exposed to any chemicals which can serve as a risk factor. Similarily, the data on X- ray-exposure and health/illness did not show any unusual features as none of the women underwent special X-ray and X-ray treatment during their life apart from normal ultrasound tests during pregnancy.

3.3.2. Parental age at time of DS birth

We have compared the age of the mothers and fathers of the trisomy 21 children with those who gave birth to an unaffected child in the same year and in the same health region (Table 47). The mean maternal age of DS mothers was 33.50 years (age range 14-52 years) compared to 27.5 years of the control mothers (age range 15-56 years). For the DS cases the mean paternal age was 41 compared to 32 years old of control fathers. Thus, the mothers who gave birth to a DS child were significantly older (95% Cl: 32.00; 35.00) than the control mothers (95% Cl: 26.15; 28.91) indicating that there is a clear effect of advanced maternal age of the DS birth prevalence in Oman (T-test, $P<0.0001$).

Table 47: Mean parental age of DS and control cases

	Total cases (N)	Mean	Confidence limit (95% Cl)
Maternal age			
DS families	90	33.50	32.00; 35.00
Control families	90	27.53	26.15; 28.91
Paternal age			
DS families	83	40.84	39.20; 42.88
Control families	67	32.7	29.64; 34.30

3.3.3. Number of pregnancies and spontaneous abortions

The mean number of pregnancies was 9.38 per women (N=85 women) for the mothers of the DS children with a total of 797 pregnancies. Out of these 797 pregnancies, 62 resulted in a spontaneous abortion corresponding to an abortion rate of 7.78% (N=85). In the control mothers, the total number of pregnancy was 538 with a mean of 5.98 pregnancies per woman (N=90). The number of spontaneous abortions was 52 of 538 in the control cases corresponding to a spontaneous abortion rate of 9.67 (N=90). These data demonstrate that the abortion rate of the DS mothers is lower as compared to control mothers even though the DS mothers are on average five years older and had more pregancies.

A comparison between cases and controls for the mean maternal age at the first menstrual shows no differences with 13.59 and 13.56 years, respectively (Table 48).

Table 48: Data of women pregnancy, menstrual and abortions for both cases and controls

	DS families			Control families			T-test
	Total cases (N)	Mean± SD	CI 95 %	Total cases (N)	Mean± SD	CI 95 %	P
Mean number of pregnancies	85	9.38±3.67	8.588;10.17	90	5.98±3.49	5.248; 6.711	<0.0001
Mean pregnancy at birth of index child	83	8.66±3.86	7.817;9.503	87	4.43±3.28	3.731; 5.129	<0.0001
Mean age at first pregnancy	61	18.16±4.58	16.99;19.33	75	20.47±4.56	19.42; 21.52	0.0026
Mean age at first menstruation	81	13.59±1.26	13.31;13.87	89	13.56±1.26	13.29; 13.83	0.8739
Abortions	85	0.73±0.99	0.5164;0.9436	90	0.58±1.03	0.3642;0.7958	0.3226

Based on our observation that the spontaneous abortion rate is lower in the control mothers as compared to the DS mothers, we asked whether there are differences between the regions with the high, middle and low DS prevalence. Therefore, we analysed the data separately for the three regions DS with high (South Batina, Dakhiliiya and Muscat), middle (Wousta, North Sharqiya, Dhahira and Dhofar) and low DS prevalence (Musandam, North Batina and South Sharqiya) for both cases and controls.

Table 49 shows that the rate of abortions of the DS mothers is lower (6.44%) in the high prevalence region as compared to the middle (8.99%) and low (8.51%) prevalence regions. A similar trend can be seen for the control mothers: in the high prevalence region the spontaneous abortion rate is lower with 6.81% than in the middle (13.11%) and the low prevalence region (9.15%). Furthermore, our data show that the abortions rate is lower in DS families as compared to control families between all regions. Thus, there seems to be a trend for a reduced abortion

rate in the high prevalence regions in general, for both DS families and controls families. At this stage of the investigation we can therefore not exclude that an exogenous factor affects the rate of spontaneous abortions in the regions with the higher DS prevalence.

Table 49: Percentage of abortion between cases and controls among regions DS clustering group with high, middle and low DS birth prevalence

Regions	DS families				Control families			
	Total cases (N)	Total pregnancy	Total abortions	% abortions	Total cases (N)	Total pregnancy	Total abortions	% abortions
High	39	326	21	6.44	39	191	13	6.81
Middle	21	189	17	8.99	22	183	24	13.11
Low	30	282	24	8.51	29	164	15	9.15

3.3.4. Consanguinity

It has been speculated that the higher DS prevalence in some Arab countries is associated with the higher rate of consanguinity among parents of DS children (Alfi, Chang et al. 1980; Farag and Teebi 1988; al-Awadi SA 1999). This has been explained by recessive genes, possibly preventing the loss of the trisomy 21 fetus. In our study, information about the degree of relationship was available for 369 couples with a Down child (Table 50). The data clearly demonstrate that the rate of close consanguinity of DS couples (1^{st} and 2^{nd} cousins) is not different from that of the general population in Oman. Figure 13 shows the percentage of consanguineous couples of the DS study. The first cousin marriages were more prevalent with 25.20% compared with second cousin marriages with a rate of 10.84%.

Table 50: Degree of relationship between couples with a DS child and the general Omani population (Rajab and Patton 2000)

	Total cases (N)	1st cousin	2nd cousin	far related	unrelated
DS couples	369	93 (25.20%)	40 (10.84%)	86 (23.31%)	150 (40.65%)
General Omani population	60635	24.1 %	11.8 %	20.4 %	43.7 %

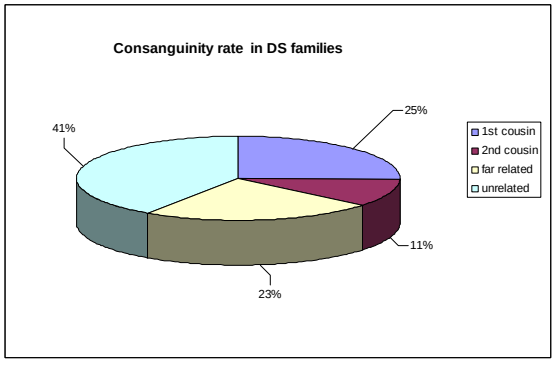

Figure 13: Degree of consanguinity between couples with a DS child in DS family cases

3.4. Genotyping chromosome 21 with microsatellite markers

For the analyses of the segregation of the additional chromosome 21 highly polymorphic STR (short tandem repeat) markers were utilized. In Figure 14 and Table 51 the STRs used their localisation on chromosome 21 are depicted.

Table 51: Interval distances for chromosome 21 markers are taken from Ensembl Genome Browser. The (0 bp) position is at the telomere of the P arm.

Interval	Physical location for Interval (bp)		Markers Genotyped per interval
1	13,719,788 - 16,265,448	13.7Mb- 16.3Mb	D21S215 - D21S1432
2	16,265,317 - 19,476,473	16.3Mb- 19.5Mb	D21S1432 -D21S1414
3	19,476,187 - 27,741,932	19.5Mb- 27.7Mb	D21S1414 -D21S1258
4	27,741,790 - 34,297,829	27.7Mb-34.3Mb	D21S1258 -D21S1445
5	34,297,532 - 36,749,001	34.3Mb- 36.7Mb	D21S1445 -D21S1252
6	36,748,755 - 43,672,758	36.7Mb-43.7Mb	D21S1252 -D21S1890

The description of the interval is similar as Lamb et al.2005 and Oliver et al. 2008.

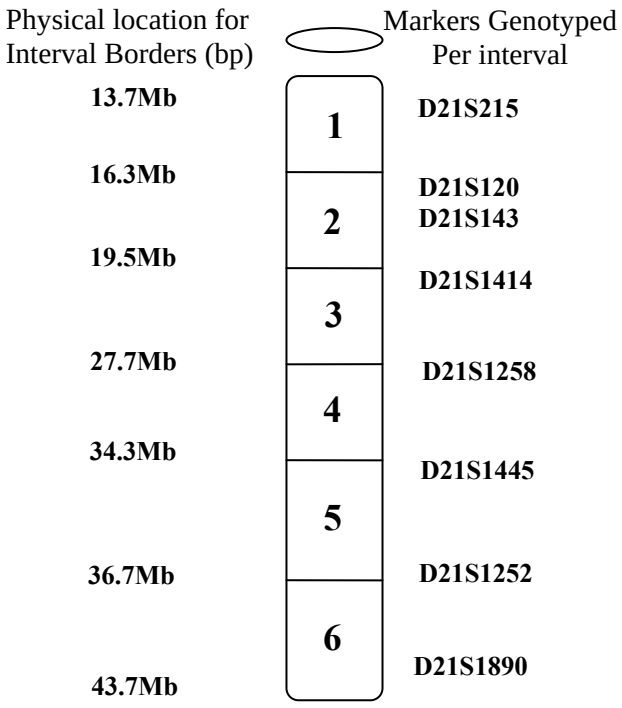

Figure 14: Marker used to define the origin of the meiotic error and determine the recombination profile

3.4.1. Determination of the parental and meiotic non-disjunction error and analyses of the recombination events

The parental origin of the additional chromosome 21 and the meiotic non-disjunction error were determined by analysing the segregation of the parental alleles to the DS child. After establishing

the parental origin, the STR markers allocated along 21q were used to determine the error of non-disjunction in either MI or MII. If parental heterozygosity of the most proximal STRs was preserved of the trisomic children (non-reduced) the non-disjunction was defined as MI error. If the parental most proximal STRs were reduced to homozygosity, non-disjunction was defined as MII error. In addition, if all informative markers in the parent of origin were reduced to homozygosity, then the origin of non-disjunction was referred to be of mitotic origin but another explanation can be that it is an MII error without crossovers or recombination.

For the determination of the recombination profile the long arm of 21q chromosome was divided into three recombination intervals as follows: D21S215-D21S1414 for the proximal interval, D21S1414-D21S1445 for the medial interval, and D21S1445-D21S1890 for the distal interval. The presence of recombination was identified by changes in the status of informative markers from reduced to non-reduced or vice versa. The status of markers was recorded as non-reduced (N), reduced (R), and uninformative (U). Haplotyping was performed by arranging all markers from the most centromeric marker (proximal) of the chromosome 21 in direction to most distal STR of 21q telomere. The recombination events were defined by the change of the status of informative markers either from reduced to non-reduced or from non-reduced to reduced. In cases where a recombination could not be defined to a single interval, the recombination was recorded in both intervals each with a probability of occurrence of 0.5.

A

STR	DS child ID B33			Mother ID B33		Father ID B33	
D21S215	168	170	168	168	170	168	174
D21S120	312	312	312	312	312	312	312
D21S1414	293	297	287	293	297	287	293
D21S1258	139	149	151	139	149	151	147
D21S1445	268	290	288	268	290	288	290
D21S1252	248	248	236	248	248	236	236
D21S1890	158	158	152	150	158	152	152

Figure 15A: Maternal MI non-disjunction: the DS child inherited both homologous chromosomes 21 from the mother; one recombination between the STR markers D21S1445 and D21S1890; D21S1252 is not informative.

B

STR	DS child ID B37			Mother ID B37		Father ID B38	
D21S215	168	168	172	168	174	172	174
D21S120	312	312	312	312	312	312	312
D21S1414	287	293	278	287	293	278	297
D21S1258	147	149	135	147	149	135	139
D21S1445	290	290	268	290	290	268	290
D21S1252	244	246	238	244	246	238	230
D21S1890	146	152	160	146	152	160	166

Figure 15B: Maternal MII non-disjunction: the centromeric marker is homozygous in the DS child, one recombination between the STR markers D21S215 and D21S1414; D21S120 is not informative.

C

STR	DS child ID B93			Mother ID B39		Father ID B93	
D21S215	170	174	168	170	174	174	168
D21S120	304	312	312	304	312	312	312
D21S1414	290	286	296	290	286	286	296
D21S1258	147	147	149	147	139	147	149
D21S1445	268	290	290	268	290	290	290
D21S1252	244	238	246	244	230	238	246

Figure 15C: Paternal MI non-disjunction: the DS child inherited both homologous chromosomes 21 from the father; no recombination.

D

STR	DS child ID A218			Mother ID A218		Father ID A218	
D21S215	170	170	170	174	170	170	168
D21S1432	139	145	139	143	139	145	139
D21S1414	352	344	348	348	352	344	348
D21S1258	141	153	149	149	141	153	149
D21S1445	269	289	299	291	269	289	299
D21S1252	244	240	242	244	244	240	242
D21S1890	153	151	164	149	153	151	164

Figure 15D: Paternal MII non-disjunction: the centromeric marker is homozygous in the DS child and heterozygous in the father, one recombination between the STR markers D21S215 and D21S1432.

E

STR	DS child ID A39			Mother ID A39		Father ID39	
D21S215	174	174	170	170	174	170	174
D21S1414	348	348	362	340	348	362	340
D21S1258	139	139	145	147	139	145	147
D21S1445	291	291	268	291	291	268	292
D21S1252	238	238	240	230	238	240	246
D21S1890	155	155	151	155	155	151	161

Figure 15E: All informative markers in the maternally inherited chromosome 21 are reduced to homozygosity, this non-disjunction error was classified as mitotic, however, another explanation is that it is an MII error without recombination.

Figures 15(A-E): Examples for haplotyping of the STR alleles in children with trisomy 21 and their parents: determination of parental and meiotic non-disjunction error.

3.4.2. The parental origin of the additional chromosome 21 and meiotic stage of the non-disjunction error

The parental origin and the meiotic stage were determined for 346 families with Down syndrome by analysing the segregation of the parental alleles to the probands[3]. The mean maternal age at birth of Down syndrome child was 34 ± 7.10 years and the mean paternal age was 40.85 ± 8.84 years.

The meiotic origin of non-disjunction was determined by one or more informative pericentromeric polymorphic loci D21S215, D21S120, D21S1432. The maintenance of heterozygosity of a given pericentromeric markers was defined to be a result of an error in meiosis MI. The reduction of homozygosity of the pericentromeric marker was interpreted as an error in meiosis MII.

The total numbers of cases included for genotyping were 346, out of these 333 were informative and 13 cases were uninformative. From all informative cases, 298 (88.17%) were of maternal origin and 27 (7.99%) were of paternal origin. In addition, 13 cases (3.85%) were of mitotic non-disjunction (Table 52). Of the maternally derived cases in 72.20% (N=213) the errors occurred during MI and in 27.80% (N=82) the error occurred during MII. For the paternally derived trisomic cases 43.75% were MI errors and 56.25% were MII errors and 11 cases were uninformative concerning the meiotic origin.

[3] The data of 110 Omani DS families were already investigated in a previous thesis (Näthe 2006). I have completed, re-analysed and included these data into the current investigation.

Table 52: Parental and meiotic origin of trisomy 21 cases analysed by microsatellite DNA markers**.**

Parental and meiotic origin	Total cases (N)	Proportion (%)
Maternal origin	298	88.17
MI	213	72.20
MII	82	27.80
Meiotic error unknown	3	
Paternal origin	27	7.99
MI	7	43.75
MII	9	56.25
Meiotic error unknown	11	
Mitotic	13	3.85

3.4.3. Maternal age and meiotic MI and MII errors

We examined parental origin of the non-disjunction error and its relation to the maternal age. The objective was to determine whether the effect of maternal age is different for the risk of MI and MII non-disjunction. Out of 232 cases of maternal origin and with kown maternal age we found no significant difference of the maternal age between MI and MII errors, the mean maternal ages were 34.68 ± 6.57 and 34.21 ± 7.49 years respectively (Table 53). Furthermore, this was similar for the paternal non-disjunction errors as no significance differences were observed for MI and MII cases, the mean maternal ages were 34.1 ± 6.01 and 35.82 ± 5.65 years respectively. The mean maternal age in cases with a mitotic error was 29.84 ± 14.37 years.

Table 53: Origin of non-disjunction and mean maternal ages and standard deviations (SD) in the Omani population

Origin	*N*	Mean maternal age ± SD	CI 95%	Min	Max
Maternal	232				
MI	168	34.68 ± 6.57	33.68;35.68	14	53
MII	64	34.21 ± 7.49	32.34;36.08	14	50
Paternal	12				
MI	5	34.1 ± 6.01	26.64;41.56	26	41
MII	7	35.82 ± 5.65	30.59:41.05	24	42
Mitotic	5	29.84 ± 14.37	11.99;47.69	21	54

3.4.4. The parental and meiotic of origin of trisomy 21 between regions

We determined the parental origin and meiotic stage for the geographical regions with high, middle and low DS prevalence separately and documented the mean maternal age at the time of the DS birth (Table 54). In a high prevalence region, the origin of non-disjunction was in 87.60% (N=113) of maternal origin and 9.30% (N=12) were of paternal origin. In addition 3.03% (N=3) were mitotic non-disjunctions. For the stage of meiotic of maternally derived chromosome 21, there were 68.47% (N=76) with MI error and 37.29% (N=35) with MII error. For paternally derived chromosomes cases 6.67 % (N=4) were MI errors and 33.3 % (N=2) were MII errors.

For the middle prevalence regions: the proportion of maternal origin was 89.39% (N=59) and of paternal origin 9.09% (N=6) and 1.43% (N=1) were mitotic non-disjunctions. For the maternally derived chromosomes, the percentage of MI errors was 62.71% (N=37) and MII errors 37.29% (N=22). For paternally derived chromosomes 60% (N=3) were MI and 40% (N=2) MII non-disjunction errors. For the region with low DS prevalence 89.39% were maternal, 9.09% paternal and 1.43% mitotic. From the maternally derived non-disjunction 62.71% were MI errors, and 18.75% MII errors. Interestingly, the data show that the percentage of maternal MII non-disjunction is higher in the regions with the high and middle prevalence as compared with the low prevalence region.

Table 54: Parental origin and meiotic stage of trisomy 21 cases with mean maternal age, standard deviations (SD) and confidence interval at DS birth among the three prevalence regions. *The asterisk indicates all cases which were informative for the parental origin while the numbers given under N are those cases which were in addition informative for the meiotic origin.

Regions	**Origin of non-disjunction**							
High prevalence	**Maternal* 113 (87.60%)**					**Paternal* 12 (9.30%)**		
		N	%	Maternal age	95% Cl	*N*	%	Maternal age
	MI	76	68.47	34 ± 6.81 (N=60)	32.24;35.76	4	66.66	33 ± 5.39 (N=2)
	MII	35	31.53	34 ± 8.81 (N=30)	30.71;37.29	2	33.33	37 ± 0.37 (N=2)
	Mitotic 3 (3.03%)							36 ± 16.97 (N=3)
Middle prevalence	Maternal* 59 (89.39%)					Paternal* 6 (9.09%)		
		N	%	Maternal age		*N*	%	Maternal age
	MI	37	62.71	35 ± 9.75 (N=28)	31.22;38.78	3	60	26 (N=1)
	MII	22	37.29	33 ± 7.98 (N=18)	29.03;36.97	2	40	34 ± 9.79 (N=3)
	Mitotic 1 (1.43%)							-
Low prevalence	**Maternal* 97 (92.38%)**					**Paternal 6* (5.71%)**		
		N	%	Maternal age		*N*	%	Maternal age
	MI	78	81.25	35 ± 5.58 (N=71)	33.68;36.32	2	50	39 ± 2.59 (N=2)
	MII	18	18.75	36 ± 5.53 (N=16)	33.05;38.95	2	50	38 ± 5.20 (N=2)
	Mitotic 2 (1.80%)							21 ± 0.41 (N=2)

Examining the mean maternal age and the meiotic origin of the non-disjunction we found no significant differences between the regions. In high prevalence regions the mean maternal ages of MI and MII were 34 ± 6.81 and 34 ± 8.81 years respectively, for middle prevalence regions it was 35 ± 9.75 (MI) and 33 ± 7.98 (MII) and 35 ± 5.58 (MI) and 36 ± 5.53 (MII) in the low prevalence regions (Table 54).

3.4.5. Recombination studies

In this part of the study the association between reduced or absent recombination and maternal non-disjunction of chromosome 21 was examined. It has been shown that reduction or absence of recombination is a risk factor for non-disjunction. Sherman et al. (2006) and Lamb et al. (1997) suggested that recombination along maternal non-disjoined chromosome 21 has three susceptible exchange patterns: (i) absence of exchange leads to an increased risk of MI errors, (ii) a single telomeric exchange leads to a risk of MI errors and (iii) a pericentromeric exchange leads to an increased risk of MII errors. Therefore, we analysed the recombination frequencies of maternal MI and maternal MII separately. In addition, we investigated the relationship between maternal age and recombination based on the location of the recombination events in three genetic map intervals of chromosome 21 (Figure 14).

3.4.6. Maternal MI and MII and amount of recombination

The data are based on the analyses of a total of 292 meioses. 211 meiosis were mat MI errors, from which 44.08% (N=93) showed complete absence of recombination (achiasmatic) (Table 55). For those meiotic events with recombination, the association between maternal age and the localisation of recombination were investigated for MI and MII (Table 56). We divided the cases into three maternal age groups: under 29 years, 30-34 years, and above 34 years. For MI we found that 62% of young mothers (<29 years) with no recombination in contrast with 47% for middle age group (29-34 years) and 36% for old age group (>34 years).

For MII cases we found that the proportions of multiple recombinations are increasing with maternal age. The same interesting and unexpected finding was reported in a study of Lamb et al. (Lamb, Yu et al. 2005). But the trend is not linear, as the middle age group posses a higher frequency (0.44) compares to 0.40 and 0.41 in younger and older ages respectively (Table 56). Therefore, we cannot exclude from our data that double exchange events are an age dependent risk factor for MII non-disjunction errors. In contrast, Oliver et al. found that the proportion of cases with multiple recombinations is significantly decreased with increasing maternal age (Oliver, Feingold et al. 2008).

Table 55: Observed number of recombinations for maternally derived non-disjunction for all age groups

	MI		**MII**	
Crossover	Total cases (N)	Proportion %	Total cases (N)	Proportion %
(achiasmate)	93	44.08	-	0
1	58	27.49	50	61.73
2	46	21.80	19	23.46
3 to 4	14	6.64	12	14.81
Total	211		81	

Table 56: Observed frequency of chromosome 21q recombinant patterns for maternally derived NDJ group among different maternal age groups.

Types of NDJ	Maternal age group	Sample size	Frequency of observed number of recombination events		
			0	**1**	**≥ 2**
MI	<29 years	29	0.62	0.17	0.21
	29-34 years	38	0.47	0.32	0.21
	>34 years	97	0.36	0.24	0.40
MII	<29 years	15	-	0.60	0.40
	29-34 years	16	-	0.56	0.44
	>34 year	32	-	0.59	0.41

3.4.7. Location of the recombination events

The analysis of single and double exchange configurations were based on three intervals as described above. We observe that 65.87% (N= 41.5) of single exchanges were shifted to the distal region (telomere) of the chromosome. This is in contrast with MII where the distribution of single exchanges showed a strong shift towards the proximal and medial part of the chromosome with 42.59% (N=23) and 40.74% (N=22) respectively (Figure 16).

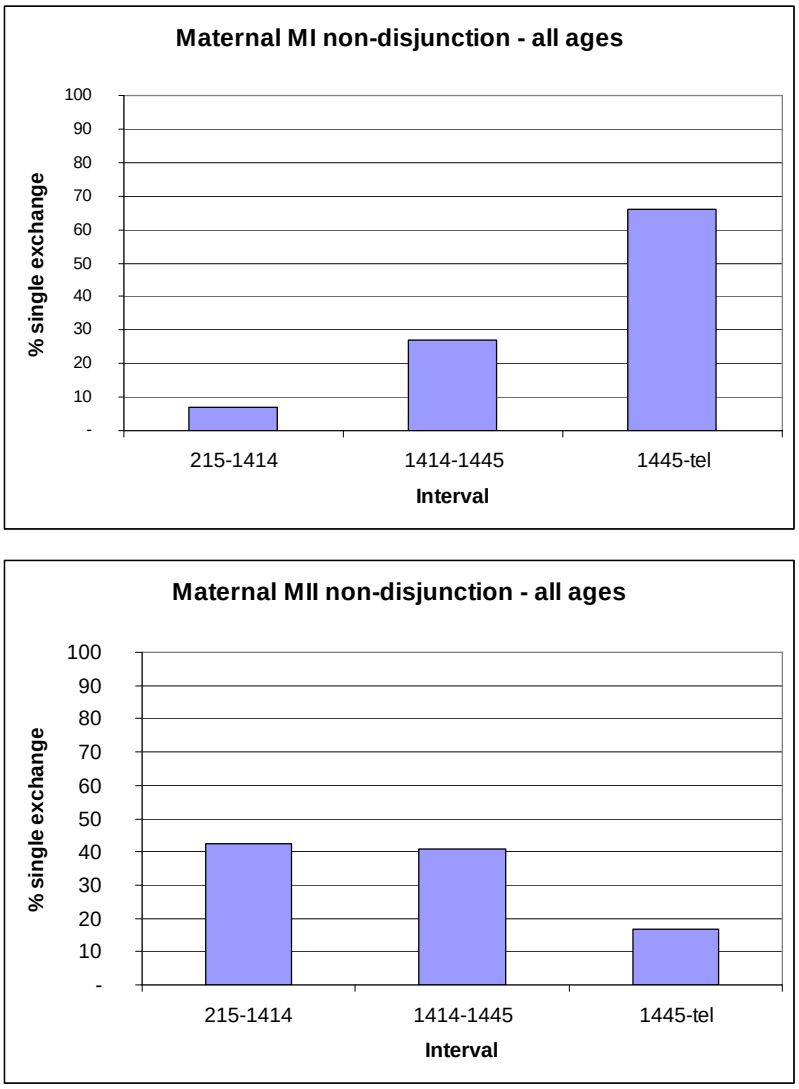

Figure 16. Comparison of chromosome 21 meiotic with single exchange: the percentage of exchanges in each chromosomal interval based on estimations from recombination data for maternally inherited chromosome 21 that have undergo MI non-disjunction or MII non-disjunction.

Then we investigated the localisation of single exchanges in MI with respect to the maternal age. For this investigation we included all cases of MI non-disjunction where the maternal age was available (N= 44) and analysed the location of recombination among the three intervals. We found that in each maternal age group the single exchange was shifting towards the telomere (Figure 17). The proportion of MI errors with telomeric recombinational events is greatest among the youngest group. This finding confirms that the risk of a telomeric exchange for an MI error applies to all age groups as described by other studies such as (Lamb, Yu et al. 2005) and (Oliver, Feingold et al. 2008). As a result we confirmed the hypothesis that a single telomeric recombinant is a risk factor for non-disjunction irrespective of the age of oocyte (a maternal age independent mechanism) (Lamb, Feingold et al. 1997; Lamb, Yu et al. 2005; Oliver, Feingold et al. 2008).

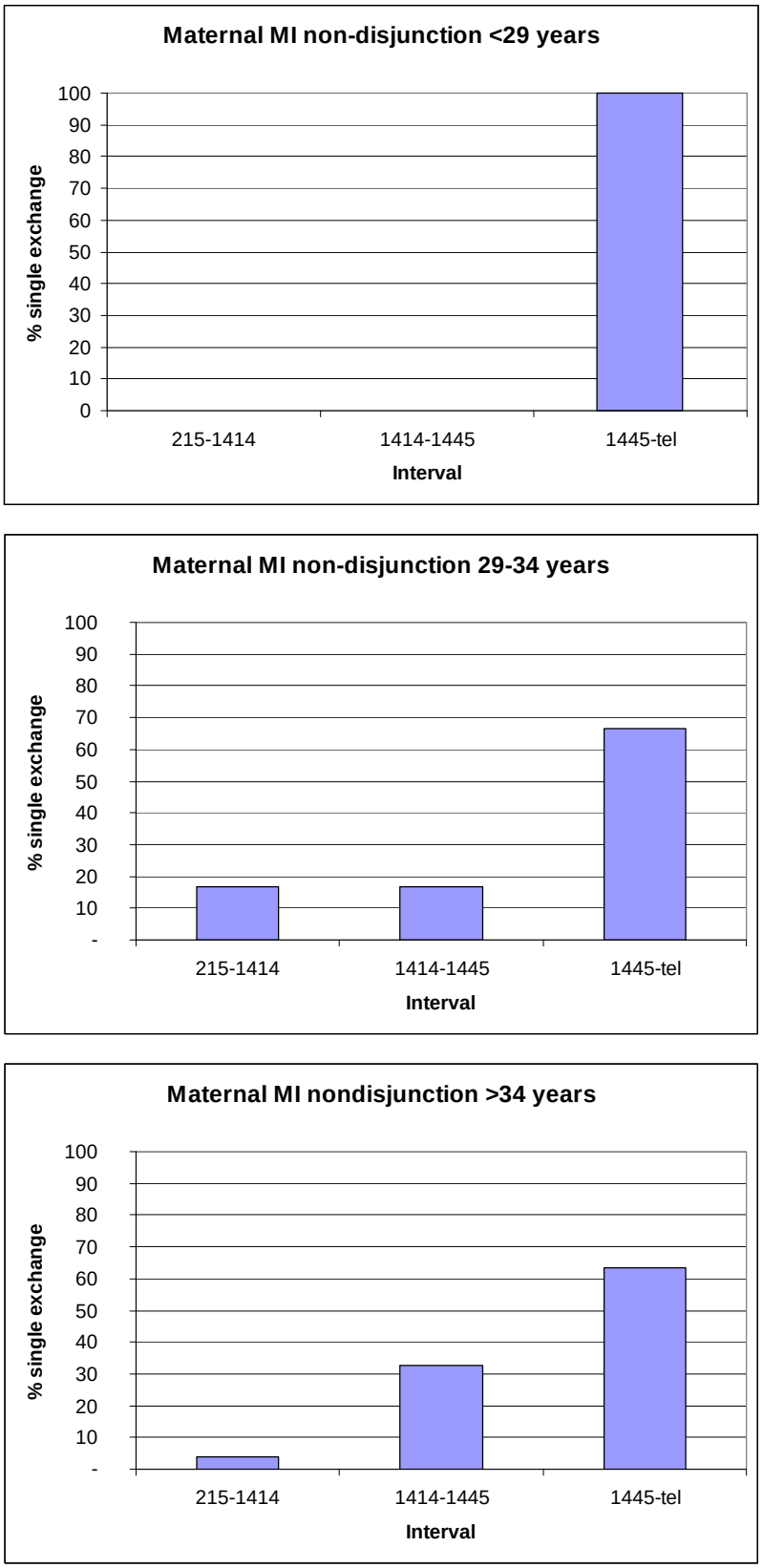

Figure 17: Positional distribution of single exchange events for maternally derived NDJ among different maternal age groups that undergo MI non-disjunction.

Table 57: Positional distribution of single exchange events for maternally derived NDJ among different maternal age groups that undergo MI non-disjunction and MII non-disjunction.

Types of NDJ	Maternal age group	Sample size	Marker intervals along 21q (centromere to telomere)		
			2155-1414	1414-1445	1445-Tel
MI	<29 years	6	0.00	0.00	1.00
	29-34 years	12	0.17	0.17	0.67
	>34 years	26	0.04	0.33	0.63
MII	<29 years	9	0.33	0.56	0.11
	29-34 years	8	0.44	0.44	0.13
	>34 years	25	0.42	0.38	0.20

For MII non-disjunction our results show that the highest percentage of single exchanges are in the medial location of chromosome 21q in the young age group (0.56) while there is a shift to the centromeric interval in the middle and older age groups (0.42 and 0.44, respectively (Table 57) (Figure 18). These findings are comparable to those from Oliver et al. (2008) who suggested that pericentromeric exchanges are an age dependent risk factor for MII non-disjunction errors.

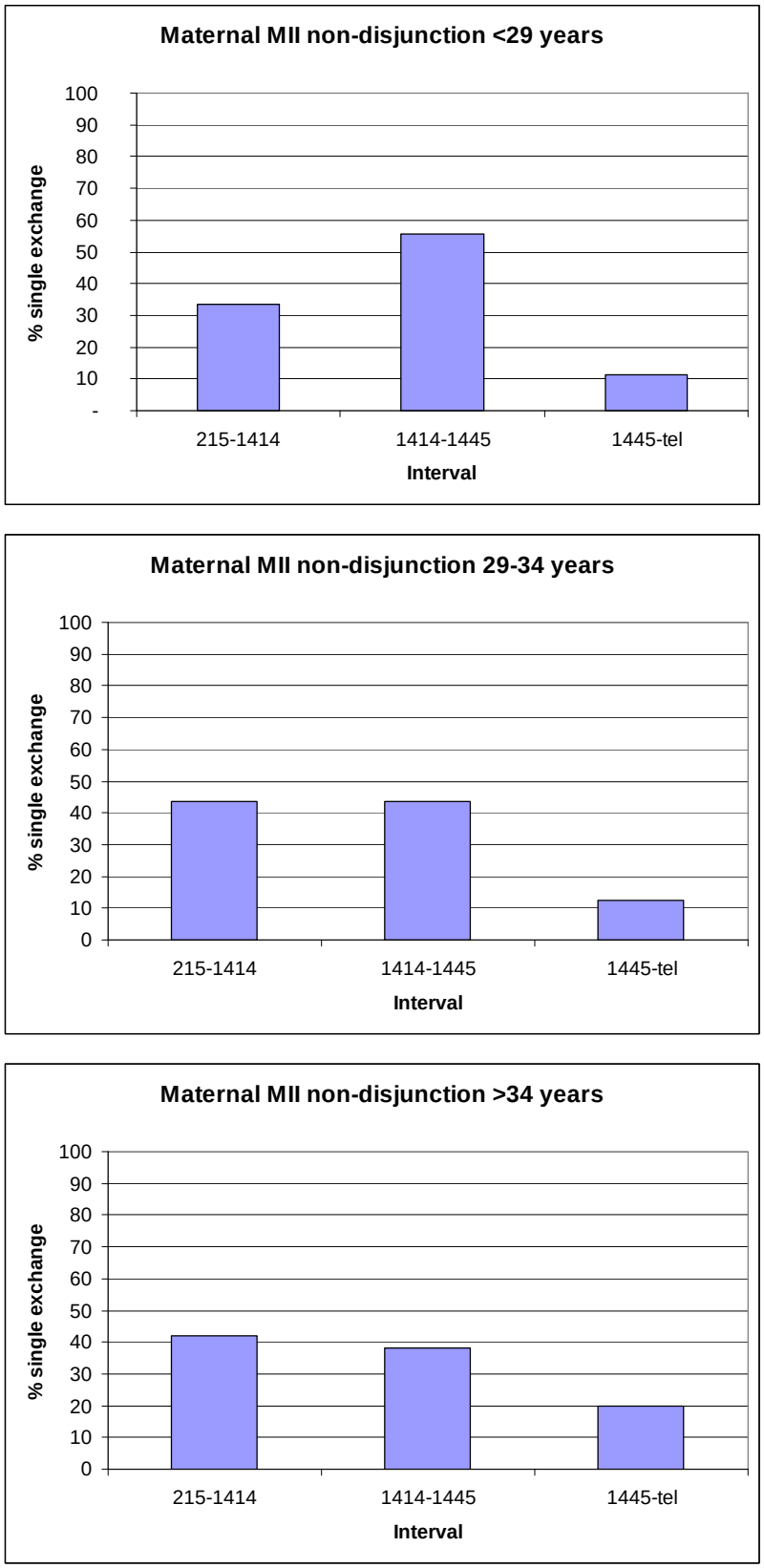

Figure 18: Positional distribution of single exchange events for maternally derived NDJ among different maternal age groups that undergo MII non-disjunction.

Table 58: Positional distribution of double exchange events for maternally derived non-disjunction among different maternal age groups that undergo MI non-disjunction and MII non-disjunction.

Types of NDJ	Maternal age group	Sample size	Marker intervals along 21q (centromere to telomere)		
			2155-1414	1414-1445	1445-Tel
MI	<29 years	6	0.27	0.47	0.27
	29-34 years	8	0.18	0.47	0.35
	>34 years	27	0.24	0.54	0.22
MII	<29 years	7	0.27	0.40	0.33
	29-34 years	5	0.17	0.17	0.67
	>34 years	7	0.50	0.21	0.29

For double exchanges, our data show that the proportion of double exchange was increased in medial part of 21q with increasing maternal age for MI non-disjunction (Table 58). This was in contrast to MII errors where the distribution of double exchanges events vary among maternal age groups: for younger age, a high proportion of multiple recombination is located at medial part of interval, for middle age group a high proportion is in a distal or telomeric part of the chromosome and for older age group the proportion of multiple recombination is at the centromeric interval. As a result, we can hypothesize that the multiple medial exchanges is a risk factor for MI error among women with different age group (age independent), while it is a maternal age dependent for MII errors and the proportion is increased with increasing maternal age. This is in contrast with Oliver's findings who describes that the maternal age was negatively correlated with the location of recombination (Oliver, Feingold et al. 2008).

Finally, if we combined all maternal ages for both mat MI and MII for the cases of double exchanges, we can see that a high proportion of recombination events is located at the medial interval for MI error with 51.47% (N= 52.5) compared with 23.04% (N= 23.5) in proximal interval and 25.49% (N=26) in a distal part of interval. However, for MII non-disjunction the highest proportion is in both the proximal and distal intervals with 33.78% (N=12.5) and 37.84% (N=14), respectively compared to 28.38% (N=10.5) in a medial interval (Figure 19).

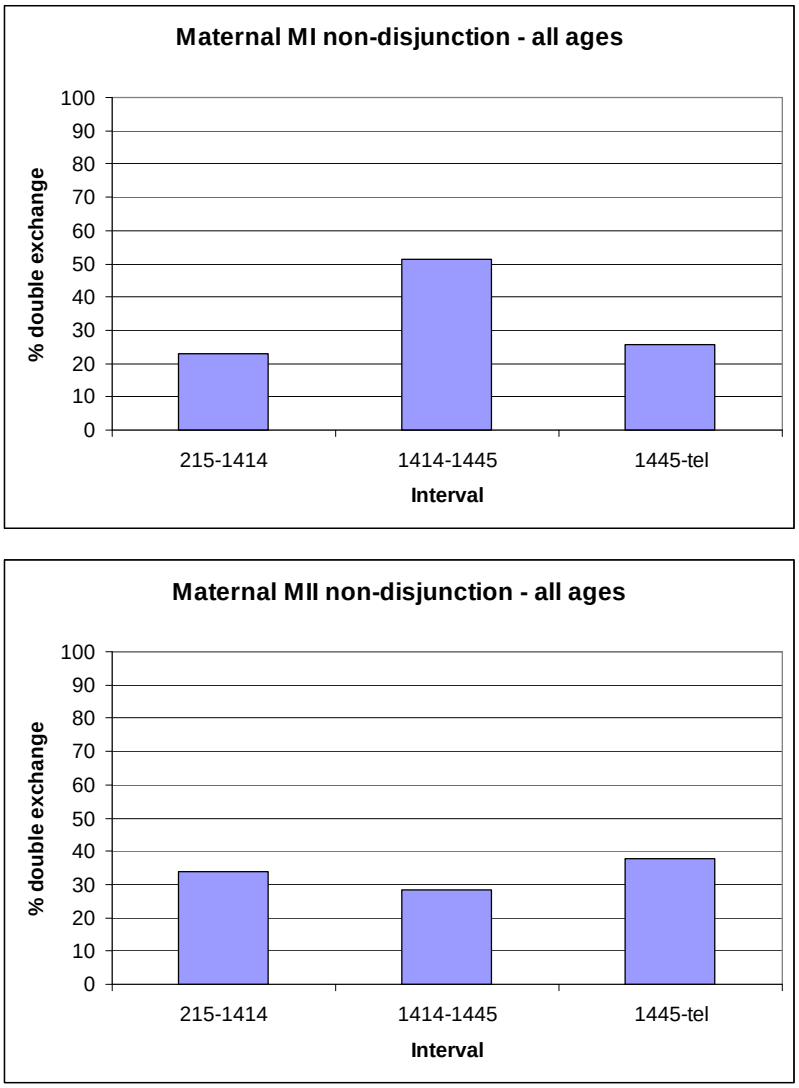

Figure 19: Comparison of chromosome 21 meiotic with double exchange for mat MI and MII errors

3.5 Analyses of the genetic diversity in the Omani population

There are publications which report an association between an increased DS prevalence and the degree of inbreeding in a population. Therefore, I tested this hypothesis by investigating the genetic heterozygosity for (a) autosomal STR loci on chromosome 21 which were used for genotyping the DS families (b) Y chromosomal STRs which are only paternally inherited and (c) the mtDNA sequence of the highly polymorphic mitochondrial D-loop which is only inherited maternally.

3.5.1 Results from genotyping high polymorphic autosomal STR loci of chromosome 21

In our DS study on non-disjunction we selected markers with a high heterozygosity level of > 0.6 as an important factor for a successful genotyping (Table 59). We can see from Table 59 that for maternal cases the percentages of heterozygosity of all markers (range 63-87%) are similar to the percentage of heterozygosity indicated by NCBI. In the paternal cases the percentage of heterozygosity calculated in DS families are quite higher (range 60-91%). Therefore, we concluded that DS families are highly heterogeneous due to a high level of polymorphic molecular satellite markers of chromosome 21q with a high genetic diversity.

Table 59: Comparison of heterozygosity of microsatellite markers in Omani DS families and the distribution of marker heterozygosity in percentage of total markers is shown.

Marker name	Heterozygosity (%) (NCBI)	Total number of cases (N)	Maternal Heterozygosity	Maternal Heterozygosity (%)	Total number of cases (N)	Paternal Heterozygosity	Paternal Heterozygosity (%)
D21S215	76,12	335	255	76,12	145	116	80,00
D21S1432	63,64	33	21	63,64	27	19	70,37
D21S120	63,79	232	148	63,79	109	65	59,63
D21S1414	86,65	352	305	86,65	214	195	91,12
D21S1258	84,86	350	297	84,86	207	185	89,37
D21S1445	80,17	348	279	80,17	212	160	75,47
D21S1252	86,00	350	301	86,00	216	180	83,33
D21S1890	87,28	338	295	87,28	136	121	88,97

3.5.2. Results from genotyping of the Y chromosomal STR markers

A total of 164 samples from fathers of DS children were genotyped using *AmpFlSTR*® *Yfiler*™ kit with 12 Y-STR markers (Appendix 5). The number of cases included in the analyses covered almost all regions in Oman.

We used the YHRD 3.0 biostatistics toolbox (www.yhrd.org/Analyse) to measure the genetic distance between populations (R_{ST} values), to see whether population samples are genetically similar: (i) between 8-regions of Oman (ii) among the three risk DS prevalence regions. For this we used a method called Analysis of Molecular Variance (AMOVA) to calculate the R_{ST} between pairs of populations (Excoffier, Smouse et al. 1992). In addition *P*-values were calculated to check the significance of the values. The results of the AMOVA were illustrated by a Multi Dimensional Scaling (MDS) plot (Sammon 1969).

(i) AMOVA results analysis among 8- geographical regions

Table 60 shows the pair-wise R_{ST} and F_{ST} values among 8-regions. Genetic distances were calculated between pairs of regional populations. Despite high distance values nearly all comparisons turn out to be non-significant on a 0.05 level due to the small sizes per regional sample. This obviates any meaningful interpretation on substructure in Oman.

Table 60: R_{ST} values between different 8-geographical populations. Above diagonal: *P*- values. Below diagonal: R_{ST} values. Figures in bold: $P<0.5$

Regions	SSH	MCT	NSH	DK	DHAH	DF	SBAT	NBAT
SSH	-	0.0439	0.4638	0.0016	0.1948	0.0295	**0.0165**	0.0064
MCT	0.0617	-	0.3262	0.2119	0.0970	0.0312	0.4059	0.1450
NSH	-0.0036	0.0072	-	0.7631	0.9730	0.0116	0.5345	0.4474
DK	0.1771	0.0200	-0.0185	-	0.0578	0.0008	0.7804	0.4487
DHAH	0.0338	0.0488	-0.0470	0.0766	-	0.0045	0.1277	0.2286
DF	**0.0862**	0.0572	0.0767	**0.1571**	**0.1501**	-	0.0344	0.0044
SBAT	**0.1367**	-0.0026	-0.0097	-0.0363	0.0573	0.0821	-	0.8812
NBAT	**0.1314**	0.0249	-0.0022	-0.0041	0.0243	0.1114	-0.0385	-

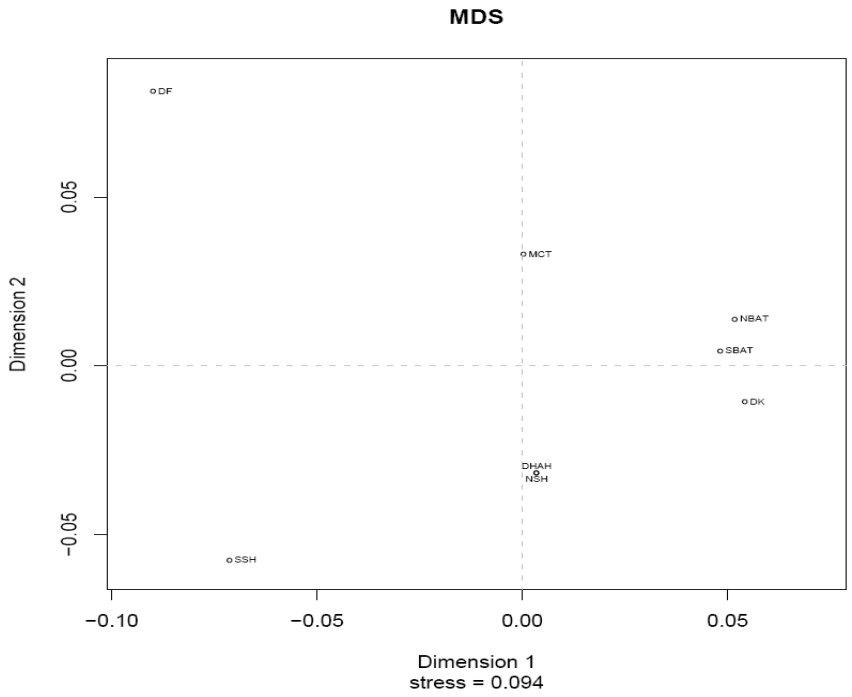

Figure 20: Multi Dimensional Scaling Analysis MDS plot of genetic distances based on population pairwise F_{ST} values from haplotypes data among 8-regions of Omani populations.

(ii) AMOVA results among regions with high, middle, and low DS prevalence:

Table 61 shows the AMOVA results among the three geographical regions with high, middle and low DS prevalence. The R_{ST} values are marginal significant on the 0.05 level only between the high and middle risk regions with a comparably low R_{st} value of 0.0422.

Table 61: R_{ST} values of pair-wise comparisons between 3 risk prevalence geographical population. Above diagonal: *P*- values. Below diagonal: R_{ST} values. Figures in bold: $P<0.05$.

Population	High	Middle	Low
High	**-**	**0.0173**	**0.1864**
Middle	**0.0422**	**-**	**0.2181**
Low	**0.0097**	**0.0092**	**-**

3.5.3. Results from sequencing the D-loop of the mitochondrial DNA

The mtDNA analyses were performed for 244 DNA probes from DS mothers which participated in the DS study. All sequences were analysed by the use of ABI Prism sequence software and Lasergene6 SeqMan 6.1. Sequences were aligned using ClustalW2 at EMBL. In Figure 21 parts of the alignments are displayed in Jalview of ClustalW2. The results of the alignments show a high sequence variation. Thus, also for the maternally inherited mitochondrial DNA similarly high genetic heterogeneity is observed as for the autosomal and Y chromosomal loci.

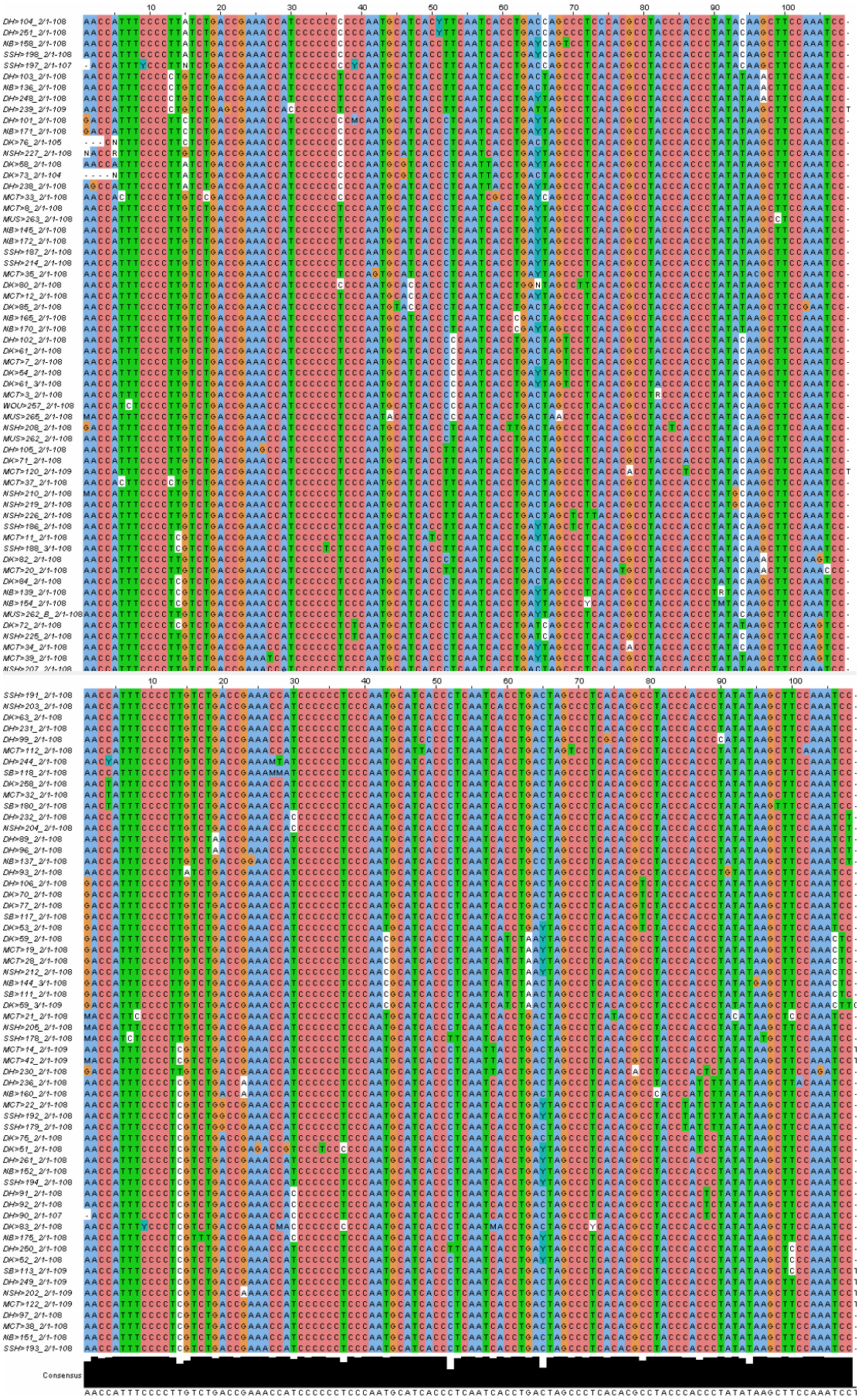

Figure 21: Alignment of the sequences of the D-loop of mtDNA of DS mothers from Oman presented in Jalview of ClustalW2.

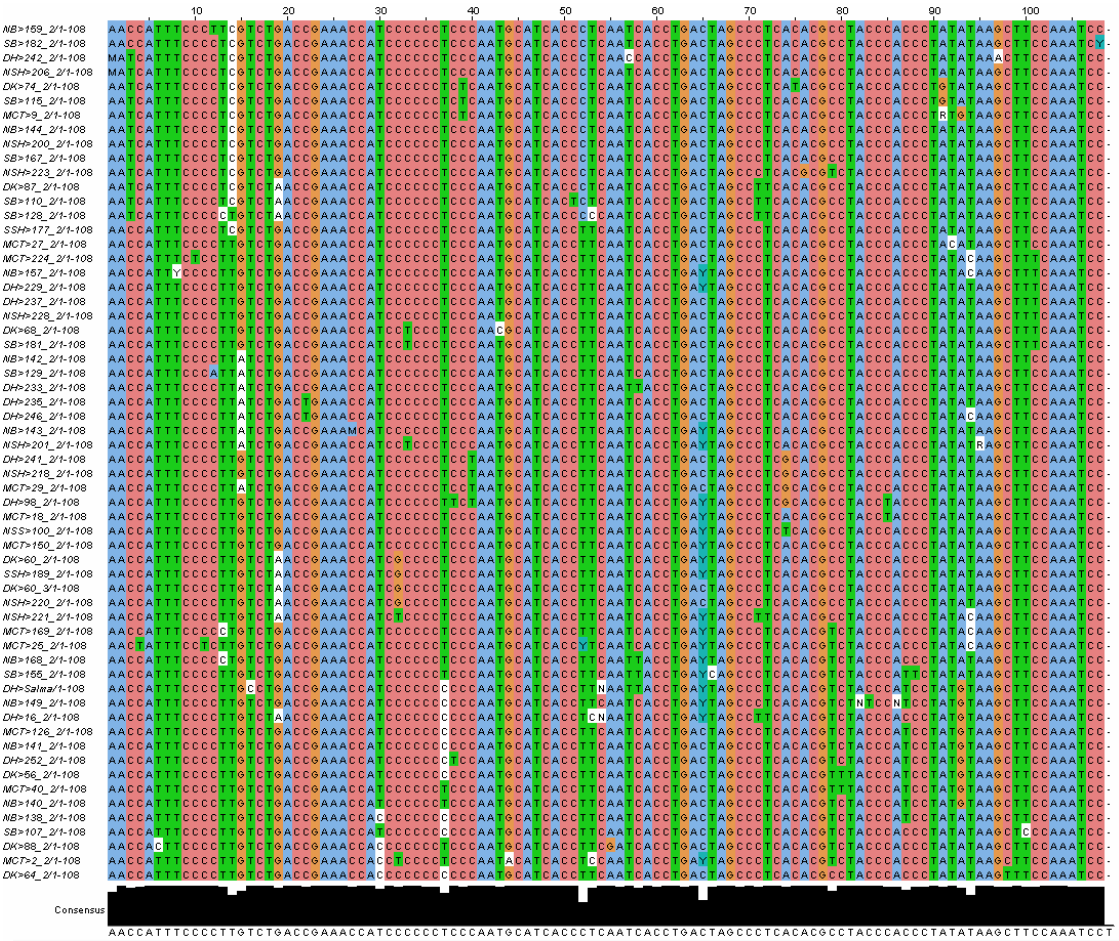

Figure 21 continued: Alignment of the sequences of the D-loop of mtDNA of DS mothers from Oman presented in Jalview of ClustalW2.

4. DISCUSSION

The current investigation is the first comprehensive study of Down syndrome in Oman and consists of four parts:

- A cytogenetic study which includes the karyotypic findings in 680 Omani children with DS who were diagnosed at the Cytogenetic Laboratory of the Ministry of Health from 1999 to 2005.
- The epidemiological data on the DS prevalence in Oman are population-based and cover the period of five years from 2000 to 2004. The ascertainment of DS is considered to be almost complete. During these five years 518 DS children with a free trisomy 21 were born in Oman, the total number of live births was 200,157 resulting in an overall prevalence of 25.88 per 10,000 live births.
- The molecular genetic study is based on the recruitment of 333 Omani families who gave birth to a DS child. DNA samples were analysed with an STR marker set covering chromosome 21 to investigate the parental and meiotic origin of the additional chromosome 21, as well as the frequency and location of recombination events in chromosome 21. To investigate the genetic heterogeneity of DS parents sequencing of the D-loop of the mtDNA analysis and analyses of Y-specific STRs were utilized.
- In addition, we performed a first small case-control study including 90 DS cases and 90 controls, in order to identify potential risk factors responsible for the high birth prevalence of DS in Oman. Controls were matched for the delivery of an unaffected child in the same year and in the same Health Institution or Health Region. The case-control study was carried out by a detailed structured questionnaire.

4.1. Results of the cytogenetic study

The cytogenetic service of Oman is centralized at the National Cytogenetic Service of the MoH in Muscat. This guarantees free access to cytogenetic diagnostics for all decentralized Hospitals of Oman free of charge. In contrast to Oman, the cytogenetic confirmation of DS could be extremely difficult in many other countries where the access to genetic services is limited for financial or various other reasons.

4.1.1. Cytogenetic confirmation of the clinical diagnosis

Since 1999 all DS cases are cytogenetically confirmed. In our study 55.5% of DS children were diagnosed cytogenetically at an age of less than one month and 34.9% were diagnosed at an age between 1-6 months. Thus, more than 90% of DS cases were diagnosed below 6 months of age which indicates that pediatricians and other medical professionals are aware of the clinical phenotype of DS and prompt the cytogenetic confirmation. Similar results were reported from Lebanon (Zahed and Megarbane 1998) and Estonia (Reimand, Ounap et al. 2006) where 47.3% and 48% of DS diagnosis were confirmed cytogenetically during the child's first month of life. In contrast, registries from England and Wales showed that 90% of the DS were confirmed cytogenetically within 10 days after birth (Mutton, Ide et al. 1993).

The early diagnosis of DS is important for providing appropriate treatments for certain common diseases related to DS such as hypothyroidism and cardiac defects. In addition, early diagnosis will allow DS parents to have an access to parents supporting groups and make use of early intervention programmes for special education and training that aims to improve the quality of life for DS children.

4.1.2. Type of the cytogenetic aberration

The aberration type was determined in 680 DS children referred to the Cytogenetic Laboratory of the Ministry of Health from 1999 to 2005. The data show that approximately 94% of the children with DS have a free trisomy 21, while 3% have a translocation trisomy and 3% have mosaicism. In Table 62 the data of the current Omani study are summarized and compared with different other studies. The comparison shows that the percentage of the various aberration types in the Omani DS patients is of the same order of magnitude as reported from other studies. In the context of a high DS prevalence, it is conceivable that e.g. a high frequency of carriers of balanced Robertsonian translocations in a population could result in a higher frequency of cases with translocation trisomies since the risk of having a liveborn child with a translocation trisomy 21 is increased for the carriers. However, in Oman the percentage of children having a translocation trisomy is even lower as compared to one large study from UK (Mutton, Ide et al. 1993). Therefore, it is unlikely that the high DS prevalence in Oman results from any potential chromosomal risk factors present in the Omani population.

Table 62: Numbers and frequencies of different karyotype patterns in DS reported in this study and data from worldwide surveys.

Source	Total No	Free trisomy21		Translocation		Mosaic		Non classical	
		No	%	No	%	No	%	No	%
Current Study (Oman)	680	640	94.12	20	2.94	19	2.79	1	0.15
Lebanon (Zahed and Megarbane 1998)	280	266	95	8	2.0	4	1.4	2	0.7
England and Wales (Mutton, Ide et al. 1993)	5737	5411	94.3	220	3.8	66	1.2	40	0.7
Estonia (Reimand, Ounap et al. 2006)	239	216	90.4	15	6.3	7	2.9	1	0.4
Egypt (Mokhtar, Abd el-Aziz et al. 2003)	673	642	95.4	18	2.7	5	0.7	8	1.2
Gujarat (Sheth, Rao et al. 2007)	382	324	84.8	34	8.9	15	3.9	9	2.4
Dubai (Murthy, Malhotra et al. 2007)	76	75	98.68	1	1.32	0	0	0	0
Kuwait (al-Awadi, Farag et al. 1990)	635	611	96.2	12	1.9	9	1.4	3	0.5
Saudi Arabia (Niazi, al-Mazyad et al. 1995)	38	37	97	1	2.7	0	0	0	0

4.1.3 Sex ratio of DS children in Oman

Out of the 640 liveborn DS children with a free trisomy 21 there were 369 males and 271 females. The resulting sex ratio of 1.36 shows an excess of males compared to the live births sex ratio of 1.07 in the entire world wide population. A similar high sex ratio of 1.31 is found when only the DS cases are included which are recorded in the DS Registry from 2000 to 2004 (294 males, 224 females, total N=518). In contrast, the sex ratio of the live births in Oman is 1.06 (Data source: MoH for 2006: 22713 males, 21403 females). The sex ratio of the chromosomally normal sibs of the DS children was 1.09 (N =1760) in this study which is close to the expected sex ratio of 1.06.

The excess of males among Down syndrome children has been reported from almost all studies from various countries (Petersen, Antonarakis et al. 1993; Griffin, Abruzzo et al. 1996; Huether, Martin et al. 1996; James 1996; Morris, Alberman et al. 1998; Bianca, Bianca et al. 2001). Some of the studies are summarized in Table 63.

However, in spite of decades of research the underlying basis of this skewed sex ratio in DS is far from understood. Several hypotheses have been discussed such as a not optimal timing of insemination in relation to ovulation (James 1996), the joint segregation of chromosome 21 with the Y chromosome in spermatogenesis and paternal non-disjunction (Hook 1989; Soares, Templado et al. 2001; Petersen, Antonarakis et al. 1993) or preferential prenatal selection against female fetuses. Even though few studies showed by molecular cytogenetics that a non-disjoined chromosome 21 segregates preferentially with the Y-chromosome (Petersen, Antonarakis et al. 1993; Morris, Alberman et al. 1998) or that disomy 21 in the spermatozoa

of the father of DS children is not a rare event (Griffin, Abruzzo et al. 1996; Bianca, Bianca et al. 2001), it is highly unlikely that this paternal mechanism is responsible for the shift towards male sex in DS because of the preponderance of maternal non-disjunction (approximately 90%) responsible for the generation of the trisomies 21.

Table 63 Sex ratio of DS patients reported from different worldwide surveys.

Study	**Sex ratio**	**Reference**
Japan	1.23	Toyofuku et al. 1980
Meta study	1.26–1.36	Review by Nielsen et al. 1981
Denmark	1.45	Mikkelsen et al. 1990
South Australia	1.31	Staples et al. 1991
Saudi Arabia	1.2	Niazi 1995
Ireland	1.1	Johnson et al. 1996
California	1.28 (total) 1.66 (Asian origin)	Bishop et al. 1997
England, Wales	1.23	Mutton, Ide et al. 1993
Lebanon	1.66	Zahed and Megarbane 1998
Italy	1.16	Bianca et al. 2001
India	1.37	Thomas IM, Rajangam S et al. 1992

Furthermore, it can be shown that the skewed sex ratio is also present in those cases with proven maternal non-disjunction. The data from Oman demonstrate that the sex ratio is similarly increased when only the cases with maternal non-disjunction errors are taken into account (Table 64).

Table 64: Sex ratio for DS cases with proven maternal non-disjunction errors in Oman.

Sex	**Number of Down syndrome patients**
Female	160
Males	119
Total	**279**
Sex ratio Male: Female	**1.34**

Table 65: Sex ratio of Down syndrome cases among maternal MI and MII cases

Non-disjunction	**Total number of cases (N)**	**Males**	**Females**	**Total (N)**	**Sex ratio Male:female**
Mat MI	213	119	79	198	1.51
Mat MII	82	35	39	74	0.897

Huether et al. (1996) combined the data of several studies and found that the live birth sex ratio was 1.15 (N = 6424) for trisomy 21 being statistically different from controls (1.05) (N = 3660707). They could not detect any effects of maternal age or race on their estimates for trisomy 21. Compared to previous estimates, their results are less extreme, maybe because of larger sample sizes. Their data support the hypothesis that the sex ratio of children with

trisomy 21 is skewed at conception, or become so during embryonic development through differential intrauterine selection (Huether, Martin et al. 1996). Since the sex ratio at the time of conception is considered to be 1:1 (reviewed in Boklage, 2005) one must assume that there is a considerable intrauterine selection against females with trisomy 21. This intrauterine selection must take place in early embryogenesis since the skewed sex ratio is already present in the first trimenon prenatally (Huether et al., 1996) and does not change until birth. We show here that the excess of males is also present in DS in Oman even though the underlying mechanism remains unexplained.

4.2. The prevalence of Down syndrome in Oman based on the DS Registry

4.2.1. Ascertainment and completeness of the data

Criteria for a meaningful epidemiological study are an almost complete population-based ascertainment and a relatively large number of annual births. Both criteria are stringently met in Oman. The annual birth rate in Oman is approximately 40,000. Furthermore, Oman has a comprehensive health care system which is provided and financed predominantly by the government: 98% of the hospital beds are governmental (87% MoH, 11% governmental non-MoH, only 2 % private sector). More than 95 % of all newborns are delivered in these hospitals and almost all are examined by paediatricians who are aware of the conspicuous clinical phenotype of DS and prompt a cytogenetic analysis for confirmation. As mentioned above, 90% of DS children born between 2000 and 2004 were diagnosed cytogenetically within 6 months after birth. Therefore, ascertainment can be considered to be almost complete since 1999. The cases of DS are registered at the National Genetic Disease Registry of Oman. Furthermore, the Sultanate of Oman is one of the countries most suitable for epidemiological studies on trisomy 21 since prenatal maternal serum screening, prenatal diagnostics (PND) and selective terminations of pregnancies do not play any role, in contrast to most western countries where PND is common practice.

4.2.2. Prevalence of DS in Oman compared with the prevalence in other countries

The worldwide birth prevalence of DS ranges from 1: 600 to 1: 1,000 live births (Siffel, Correa et al. 2004). In United States and Europe the live births prevalence of Down syndrome is approximately 10 per 10,000 live births (Forrester and Merz 2002; Thores and Philion 1973; Trimble and Baird 1978). Even though, the prevalence in most Western countries varies considerably depending on the maternal age at the time of child bearing, the availability of prenatal diagnostics and the percentage of subsequent selective termination of the pregnancy, the method of ascertainment and the completeness of the data etc.

The birth prevalence of Down syndrome in the Omani population between 2000 and 2004 was investigated in the current study. In this five-years period a total of 200,157 live births were registered Oman wide, out of which 518 children were diagnosed with DS. In Oman the annual prevalence of trisomy 21 from 2000 to 2004 ranged from 23.60 (1:409) to 27.96 (1:367) per 10,000 live births with an overall prevalence of 25.88 per 10,000 live births (1:388)(Table 40). These results show that Oman has an exceptionally high prevalence rate of Down syndrome. In Table 66 some studies regarding the prevalence of DS in various populations are summarized. It shows that the birth prevalence ranges between approximately 10.00 and 20.90 per 10,000 live births. In the majority of Western countries the prevalence is below 20.00. This is in contrast to most of the Arab countries where the birth prevalence is almost 20.00 per 10,000 births, except in Egypt where they reported the lowest prevalence among all international studies presented in Table 66. Oman has with 25.9 DS births per 10,000 one of the highest live birth prevalence among all countries if not the highest worldwide.

Furthermore, we compared the DS prevalence in Oman with the data of the International Clearinghouse for birth defect where DS prevalences from different countries are summarized including the ascertainment of the prenatal diagnosed cases. This comparison demonstrates that Oman has the third highest Down syndrome prevalence reported worldwide (Figure 22).

Table 66: Prevalence of Down syndrome reported in this study and from some other worldwide studies per 10,000 live births.

Source/Country	**Study duration**	**Birth prevalence per 10,000 live births**
Current study (Oman)	2000-2004	25.9
Libya (Verma, Mathews et al. 1990)	1982-1985	19.0
Malaysia (Azman, Ankathil et al. 2007)	1989-2007	10.44
South Africa (Op't Hof, Venter et al. 1991)	1980-1984	13.4
Eastern part of Germany (Burkart, Grosche et al. 1997)	1980-1997	10.0
West Berlin (Sperling, Pelz et al. 1994)	1980-1989	15.6
Switzerland (Mutter, Binkert et al. 2002)	1980-1996	15.6
Singapore (Lai, Woo et al. 2002)	1974-1993	8.9
Cape Town South Africa (Molteno, Smart et al. 1997)	1974-1993	14.9
South Australia (Staples, Sutherland et al. 1991)	1960-1989	11.86
California (Bishop, Huether et al. 1997)	1989-1991	11.3
Norway (Melve, Lie et al. 2008)	2001-2005	20.0
West Australia (Leonard, Bower et al. 2000)	1980-1996	11.1
England and Wales (Morris, Alberman et al. 1998)	1989-1993	14.0
Estonia (Reimand, Ounap et al. 2006)	1990-2003	11.7
Egypt (Mokhtar and Abdel-Fattah 2002)	1992-2001	10.0
Dubai, UAE (Murthy, Malhotra et al. 2007)	1999-2003	22.2
Kuwait (al-Awadi SA, Kamal KN et al. 2008).	1997-2000	19.9
Saudi Arabia (Niazi, al-Mazyad et al. 1995)	1982-1991	18.1
Qatar (Wahab, Bener et al. 2006)	2000-2004	19.5
Atlanta (Huether, Martin et al. 1996)	1970-1989	9.8
France (Stoll, Alembik et al. 1988)	1979-1987	11.7

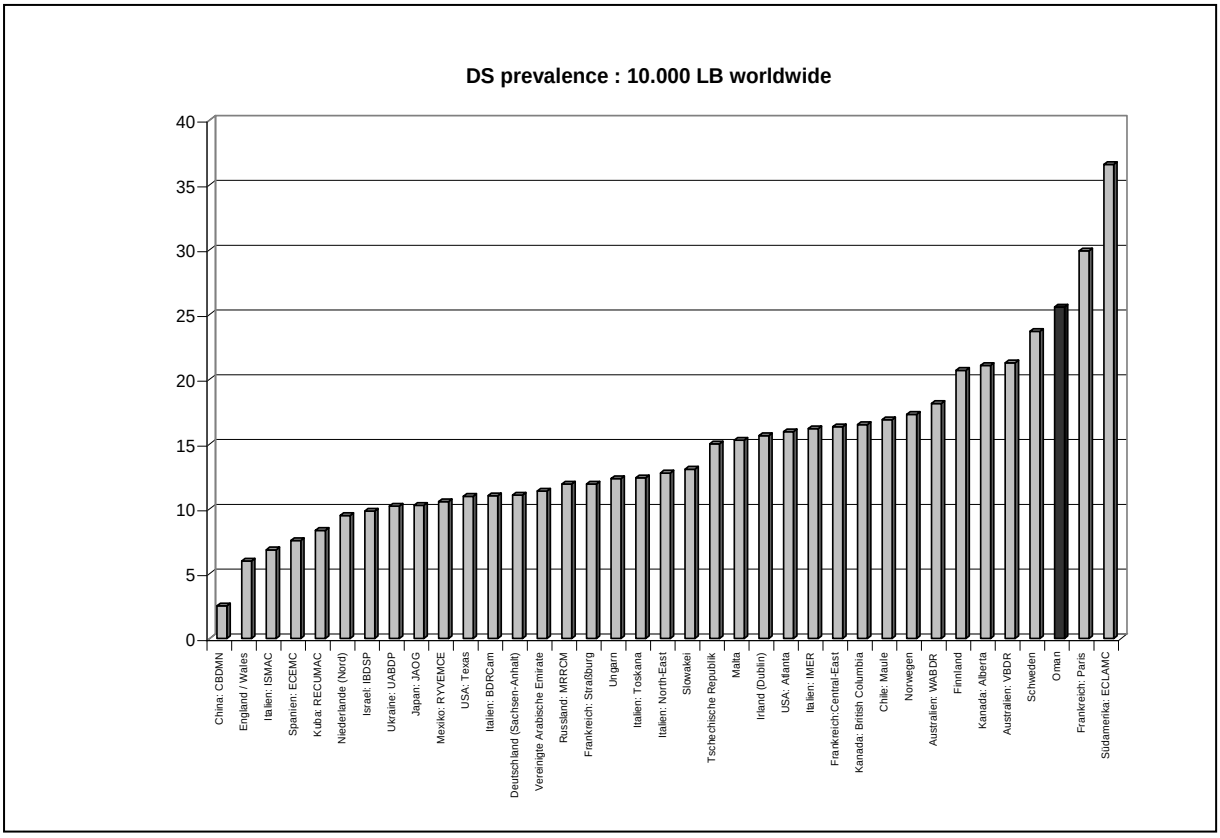

Figure 22: Data are based on International Clearinghouse data 2003; terminations of pregnancy after prenatal diagnostics and selective abortions are adjusted for live births (x 0.7) since approximately 30% of the prenatal detected cases result in a spontaneous abortion until the end of the pregnancy. DS prevalence in Oman is based on the National Registry 2000-2004.

4.2.3. Advanced maternal age and the risk of having a child with Down syndrome

One of the most important risk factors for non-disjunction of chromosome 21 is advanced maternal age. Already in 1933, Lionel Penrose described for the first time the association between increased maternal age and the risk of having a child with Down syndrome (Penrose 2009). Subsequently, many studies approved that maternal age is a very strong confounder for having a child with Down syndrome (Mikkelsen and Stene 1972; Huether, Martin et al. 1996; Forrester and Merz 2003; Allen, Freeman et al. 2009) and confirmed the association between Down syndrome and advanced maternal age as a specific risk factor (Trimble and Baird 1978; Hook 1989; Staples, Sutherland et al. 1991; Czeizel, Elek et al. 1993; Huether, Martin et al. 1996; Yoon, Freeman et al. 1996; Siffel, Correa et al. 2004). A study on the prevalence of DS in Atlanta (Siffel, Correa et al. 2004) during 1994-1999 shows that the prevalence of DS was 55.3 per 10,000 for women above 35 years age compared to 8.5 per 10,000 for women below 35 years. A study of 52 965 amniocentesis to determine the maternal age of some major chromosomal aberrations suggested that the rate of trisomy 21 increases from maternal age of 35 year old (Ferguson-Smith and Yates 1984). A study in England and Wales showed that mother's delayed child-bearing has led to an increase in the prevalence of DS without PND

and selective abortions (De Souza, Alberman et al. 2009). The number of DS births to mothers aged 35 and over increased from 186 in 1989 to 310 in 2003 (Crane and Morris 2006). From 1989 to 2003 the mean maternal age increased from 27 to 29 years and the percentage of all babies born to mothers aged 35 and over increased from 9% in 1989 to 19 % in 2003. However, regardless of the increase in maternal age in these years the total number of births with DS decreased from 770 in 1989 to 603 in 2003 due to prenatal diagnostics and increased numbers of subsequent selective terminations.

Even though, the association between increasing maternal age and the conception of trisomies has been recognised since many years, there is still a lack of understanding the underlying mechanism behind the maternal age effect. One of the hypotheses is that the increasing rate of meiotic errors is due to the aging process of the ovary (biological aging) (Hunt 2006). The underlying mechanism seems to be due to the long arrest of the oocytes in prophase I of meiosis. In females, the entry of the oocytes into meiosis starts during fetal life. Oocytes are then arrested in a prophase stage of meiosis, the dictyotene, in which they persist from late fetal life until the time of ovulation. The first meiotic division MI is completed in the female just prior to ovulation and the second division MII is completed only if the egg is fertilized. In female the process takes years to complete, which is in contrast to male where cells enter meiosis with the onset of puberty (Hunt 2006). In male, the process of meiosis is not interrupted by an arrest phase and mature gametes are produced continuously throughout adult life. Paternal age has also been concerned as a risk factor of DS birth but the evidence were contradictory (Fabris, Licata et al. 1983; Roecker and Huether 1983; Fisch, Hyun et al. 2003; Kazaura, Lie et al. 2004; Zhu, Madsen et al. 2005; De Souza, Alberman et al. 2009). The study of (Fisch, Hyun et al. 2003) concluded that advanced paternal age combined with maternal age significantly influences the incidence of Down syndrome, and this was the same conclusion of some other studies (Fabris, Licata et al. 1983; Roth, Stoll et al. 1983; Hook 1989; De Souza, Alberman et al. 2009).

The current study in Oman demonstrated a very strong association of advanced maternal age with the birth of a DS child. The mean maternal age for DS families in our case control study was 33.50 which is not different from the 34.4 years age in Western countries (Ferguson-Smith and Yates 1984; Jyothy, Kumar et al. 2001), indicating that there is a clear effect of advanced maternal age on the DS birth prevalence in Oman, compared to control mothers where the maternal age was 27.5 years. Furthermore, our results show that there are no significant differences in the mean maternal age of MI and MII errors which suggests that both types of non-disjunction errors are age dependent. Results from the Atlanta Down syndrome project 1989-2002 also reported that advanced maternal age is a risk factor for both

MI and MII of maternal meiosis non-disjunction errors (Sherman, Freeman et al. 2005). In our case-control study comparison, we found that the mean age of father for DS (40.84 years) is different from the mean age (32.7 years) of control father years, indicating a possible role of paternal age in the etiology of trisomy 21. However, the investigated number of paternal cases in the molecular genetic study on meiotic errors is too small to draw any relevant conclusions. Thus, we can summarize that also in Oman there is strong evidence that the high prevalence of Down syndrome live births in the country is related to advanced age of the mother. However, the maternal age related risk factor alone cannot explain the unusually high DS birth prevalence in Oman. To prove that we calculated the expected number of DS cases in Oman from 2000 to 2004 on the basis of the maternal age specific risk figures surveyed in a Canadian (Huether, Martin et al. 1996) and a Swedish study (Lindsten, Marsk et al. 1981). In both surveys ascertainment of DS among live births was nearly complete and the prenatally diagnosed cases were taken into account. These two studies are believed to be the most precise ascertainments of DS and giving the most precise estimates for the maternal age related risks. The expected birth prevalence of DS was calculated by multiplying the maternal age distribution in the Omani population by the age specific risk of having a pregnancy associated with DS. From these two studies, the expected number of trisomy 21 cases in Oman 2000-2004 would be 398 and 430, respectively, which is significantly less than the observed number of 518 DS cases in Oman (Figure 23).

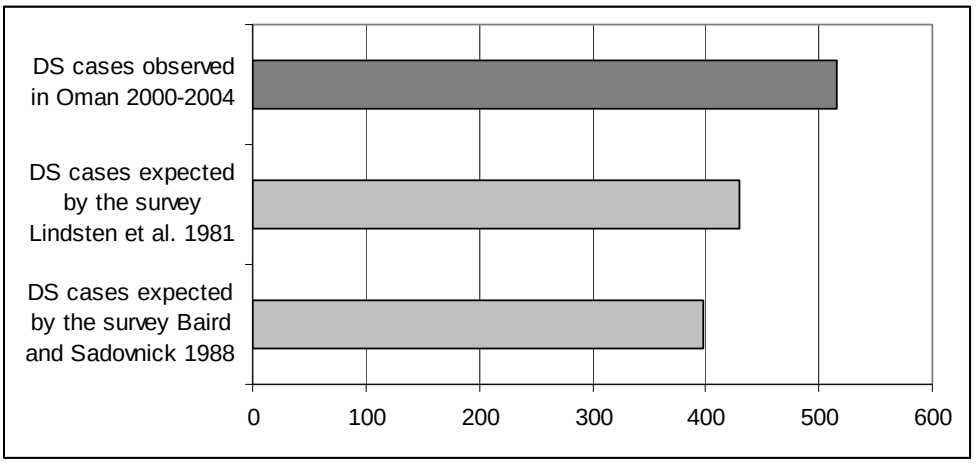

Figure 23: The expected number of DS births in Oman from 2000 to 2004. The observed number of DS live births (518) in Oman is higher than the number expected. DS cases expected by the survey Lindsten et al.1981 is 430 from the survey Baird and Sadovnick and 398 by the survey of Lindsten et al. (Lindsten, Marsk et al. 1981; Baird and Sadovnick 1988).

4.2.4. Regional and seasonal differences in the birth prevalence of Down syndrome in Oman

Oman is divided into ten geographical health regions. We tried to identify if there are any differences in the prevalence among these regions. Our findings indicate significant

differences in the prevalence between these regions. The highest prevalence was found in the South Al Batinah region (37.97 per 10,000 live births) followed by Ad Dakhiliya (33.41 per 10,000 live births) and Muscat (30.41 per 10,000 live births). The lowest prevalence is found in South Ash Sharqiya, North Al Batinah and Musandam ranging between 15.13 and 16.41 per 10,000 live births. The remaining regions are in the middle range between 25.45 and 26.40 per 10,000 live births. The differences of the DS birth prevalence are statistically significant. The maternal age among mothers was 34.7 years in high prevalence regions which is not different from that of the low DS prevalence regions with an age of 33.9 years. Therefore, the maternal age can not be considered as a main risk factor for the different prevalences among the regions.

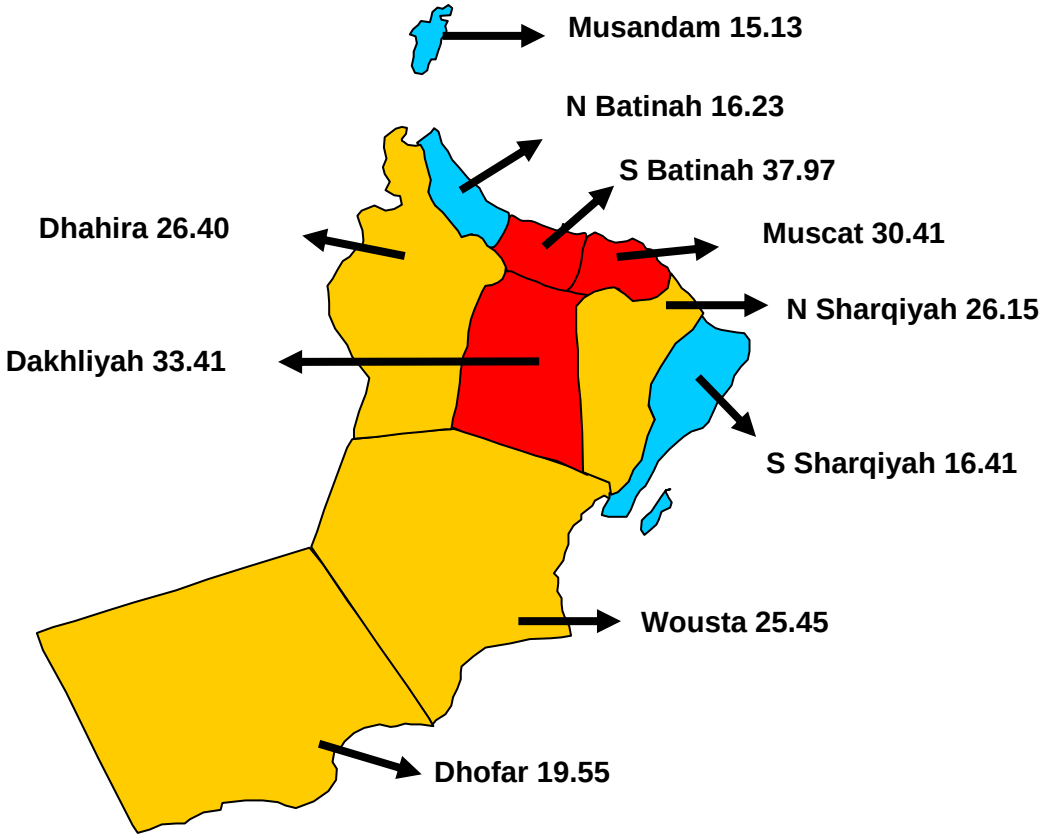

Figure 24: Birth prevalence of Down syndrome in ten regions of Oman between 2000 and 2004

The regions with the highest prevalence span the southern part of the costal region at the Golf of Oman and are extended through the Samail rift of the Hajar mountains to the interior including the big city Nizwa. The whole region lies close together within a cross-section dimension of approximately 200 kilometers and is the most densely populated area in Oman (Figures 24 and 25).

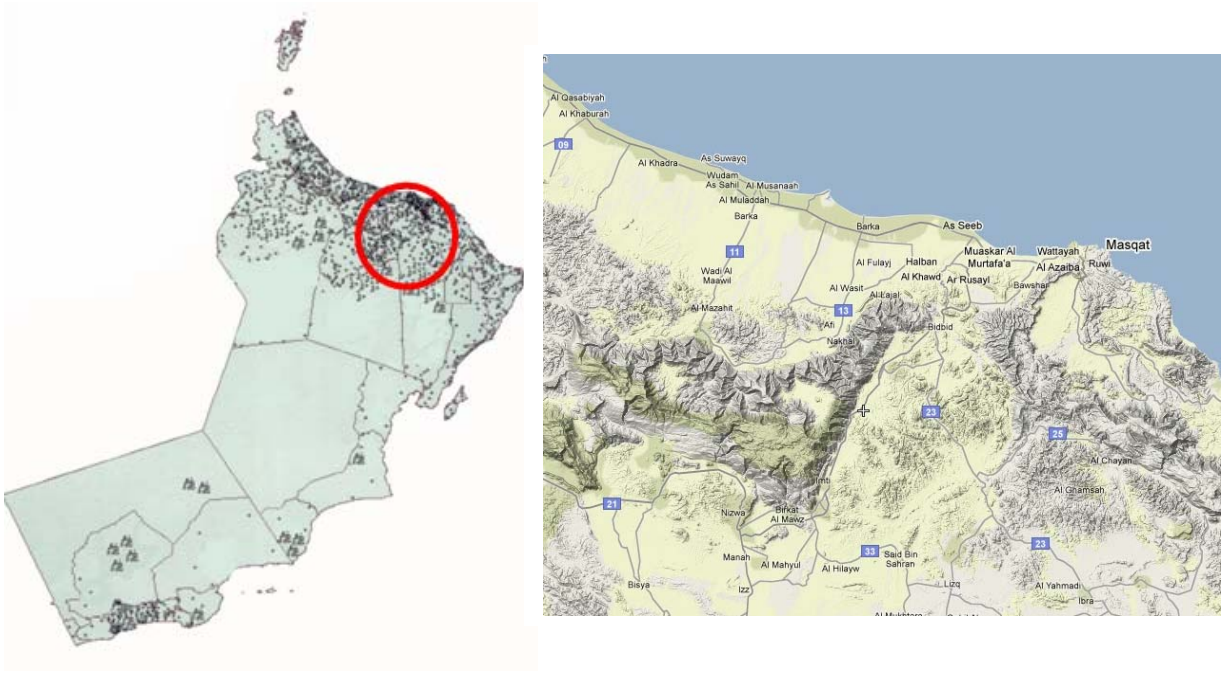

Figure 25: Map of whole Oman (left) showing the population density (black dots), the red circle marks the region with the highest DS prevalence which is enlarged at the right showing the Golf of Oman and the Samail rift connecting the coastal area with the interior.

Seeing the map of Oman with the DS prevalences (Figure 24) it seems that the prevalence is decreased with increasing distance from the high risk reason. At the moment we have no satisfying explanation for this effect. However, a reporting bias can be largely excluded since all pediatricians are equally trained in clinical genetics. We also could exclude a problem of transport of the probes for karyotyping since vehicles from all cities and flights from Salalah (Dhofar) deliver daily probes to the Central Laboratories in Muscat, not only for chromosome analyses. A reporting bias or a problem of transport of the probes is also unlikely when one considers that e.g. two large regional hospitals with a high number of deliveries are in Sohar in a region with low prevalence (North Batinah) and in Nizwa in a region with high prevalence (Ad Dakhiliya) have equal distances to the Central laboratory in Muscat (Figure 25).

In other countries, the significant increase in the prevalence of cases with Down syndrome such as in France, South Australia, and USA (Stoll, Alembik et al. 1988; Krivchenia, Huether et al. 1993; Huether, Martin et al. 1996) can be explained mainly by the increasing proportion of older women at the time of child-bearing. Furthermore, a certain proportion of DS fetuses are detected by prenatal diagnosis which, undetected, might have resulted in a spontaneous abortion while these are nowadays integrated into the statistics. Since prenatal screening for DS is not performed in Oman, neither by serum screening nor by ultrasound, we cannot

consider the above mentioned reasons as causative for the high proportion of Down syndrome live births in the Omani population in general and region wise.

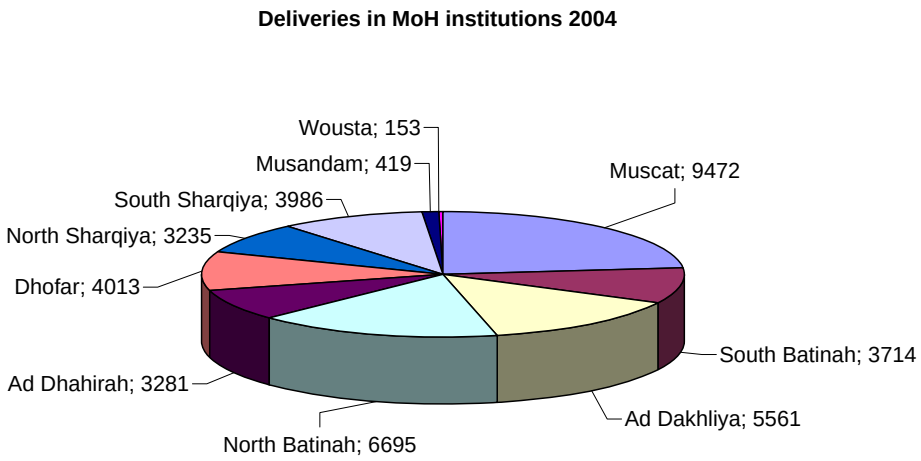

Figure 26: Number of deliveries in MoH institutions in the year 2004

Therefore, we cannot exclude possible environmental causes for the high prevalence of Down syndrome in Oman. In this respect, it is of special interest that we also observed seasonal differences in the DS prevalence. For the period from 2000 to 2004 we observed a significant increase in the birth prevalence of DS children born in January followed by December. A similar observation was made by Al-Awadi et al. 2008 in Kuwait who report on a peak of DS cases in January without any clear conclusion.

In order to focus more intensive on the space-time aspect, we compared the seasonal variations of the DS birth prevalence between the three regions with high, middle, and low DS prevalence which were identified before. The results of this space-time analysis demonstrate that the seasonal deviation is greatest in the regions with the highest DS prevalence (Figure 12). Thus, the seasonal and regional variations of the DS prevalence must be interpreted as a strong indicator for a potential exogenous factor or factors.

It has been shown that clustering of chromosomal aneuploidy can be due to environmental mutagenic effect around the time of conception such as ionising radiations (Harjulehto-Mervaala, Salonen et al. 1992; Sperling, Pelz et al. 1994; Bound, Francis et al. 1995), inhalation of iodine-131 from Chernobyl reactor accident (Sperling, Pelz et al. 1994) and the ingestion of a chemical employed against fish parasites (Czeizel, Elek et al. 1993), or seasonality based on the month of conception (Drugan, Bottoms et al. 1989; Tonelli, Specchia

et al. 2006). Sperling et al. demonstrated such an effect after Chernobyl radiological contamination in West Berlin (Sperling, Pelz et al. 1991; Sperling, Pelz et al. 1994). They found that there was a significant increase in trisomy 21 in Berlin nine months after Chernobyl accident and this was confirmed latter by a study based on prenatal diagnosis where the highest prevalence was found in the most heavily exposed regions. The majority of DS fetuses were conceived during the time of the highest exposure. McNally et al. published 2008 a highly significant space-time relationship of Down syndrome based on population-based data from UK. The effect was confined to a more densely populated area and restricted to maternal age below 40 years. This study suggested an etiological role for transient environmental factors, such as infections. Tonelli et al. 2006 reported the unusual unexplained finding based on prenatal diagnosis: a lower rate of conception on the month of June of all de-novo chromosomal anomalies specifically trisomy 21 which might be related with a higher exposure to daylight hours (summer solstice). In contrast to these studies which report on a space-time effect, there are many other studies which could not find any clustering or where the results were not conclusive (Puri and Singh 1995; Stolwijk, Jongbloet et al. 1997; Morris, Alberman et al. 1998). There is a review article by Stolwijk et al. (1997) on seasonal variation at birth concluding that there is no clear evidence for seasonality in the Down syndrome birth prevalence since all studies did not consider the fetuses which have been lost before term.

At the moment, we can not explain our finding on the space-time relationship of the DS prevalence in Oman. However, there are two additional so far unexplained observations which should be reported in this context. The first observation is the increased percentage of errors in maternal MII in the regions with high and middle DS birth prevalence. This aspect will be discussed in more detail in 4.4.1. of the discussion. The second observation is that the sex ratio of the 518 patients ascertained in the DS registry is highest in the high prevalence region (Table 67). Moreover, for the period from 2000-2004 the highest sex ratio was present in January with 1.95 (N=62: 21 females and 41 males), thus just in this month, where also the DS birth prevalence was highest. Several studies suggest that exposure to environmental factors can influence the sex ratio and that the sex ratio can, therefore, be used as a sentinel indicator (personal communication Prof. Dr. Karl Sperling and Dr. Hagen Scherb). Sperling et al. found the highest sex ratio of 3.67 ever reported for DS in 1987, exactly nine months after the Chernobyl reactor accident where the DS prevalence was significantly increased.

Table 67: Prevalence of Down syndrome reported in this study and from some other worldwide studies per 10,000 live births.

Region	Maternal non-disjunction		Sex ratio
	MI error	MII error	
High prevalence	68.47	31.53	1.351
Middle prevalence	62.71	37.29	1.297
Low prevalence	81.25	18.75	1.237

4.3. Data from the questionnaire and the case control study

There was a highly significant difference in the maternal age between mothers who gave birth to a DS child (33.5 years) and the control mothers (27.5 years) (T-test, $P<0.0001$). Otherwise there were no obvious differences across categories of maternal education, parity, health and illnesses, diagnostic X-ray and X-ray treatment, family health and occupational history. For all we did not observe any unusual.

4.3.1. Abortions

Approximately 4.0% of recognised pregnancies are trisomic and about 0.5% of pregnancies are trisomy 21 and 22 (Hassold and Jacobs 1984). The study of DS in 118,265 in consecutive birth defects found that 5.3% of DS mothers had two spontaneous abortions compared to 3.7% controls (Stoll, Alembik et al. 1988). Recognisable abnormal foetus in younger mothers' uterus is more as compared to older mothers' (Stene, Stene et al. 1981), as a result the number of abortions in younger mothers are higher when compared to older women. Some studies suggest that first child infants may be a high risk of DS to older women than a later born child to women of the same age (Alfi, Chang et al. 1980). This is in contrast to (Stoll, Alembik et al. 1988) as he found that the first born infants were at a lower risk of DS than later born infants.

In our study the mean number of pregnancies at birth of DS was 8.66 (ranged 1- 17 pregnancies) and the mean age at first pregnancy was 18.16 years old. In controls the mean number is 4.43 (ranged 1- 15 pregnancies) and the mean age at first pregnancy is 20.47 years old. This indicates that DS families are with more parity as they start conception at a younger age as compared to controls family and the DS child is mostly not the first infant, in contrast to Alfi's observation.

The fertility rate is higher in DS families as compared to controls, the mean number of pregnancies in DS families was 9.38 as compared to 5.98 in controls. This can be explained by the higher age of the DS mothers. But another explanation for the reduced fertility in controls could be that they have a higher rate of miscarriages, and as they are prone to have a

DS infant also, if it correlated to a high early miscarriage due to chromosomal abnormality. Due to the fact that DS infants are usually born after a shorter gestation than normal infants (Kallen and Masback 1988) and the rate of preterm - either very preterm (24-31 gestation) or moderately preterm (32-36 gestation) are quite high (approximately 1/3) as compared to term (37-41 gestation) (Honein, Kirby et al. 2009), and for infants below 24 weeks gestation there is a high chance to lose them without recognition.

We found that the rate of abortion of DS families (7.78%) was lower than in controls families (9.67%), even though the DS mothers were significantly older and had a higher number of pregnancies (9.38) as compared to controls (5.98).

There are two interesting observations in our results regarding the rate of abortions: first, the percentage of abortions in DS families is lower as compared to control families in a high risk region of DS prevalence. Second, the rate of abortions for both DS families and controls are lower in a high risk prevalence region as compared to other regions groups. This suggests that there might be an exogenous or endogenous factor which affects the rate of abortions and prevents the loss of fetus in high risk regions in general for both DS families and controls families, as well as mainly or specifically for DS families in a high risk region.

4.3.2. Consanguinity

It has been speculated by several authors that the higher DS prevalence reported from some Arab countries is associated with the higher rate of consanguinity among parents of DS children (Alfi, Chang et al. 1980; Farag and Teebi 1988; al-Awadi SA 1999). However, other studies could not confirm this hypothesis (Reference). Consanguineous marriages are favored by the Arab community in the Middle East and first cousin marriages are particularly the father's brother's daughter. The Arab community is in favour of consanguineous marriage for social and economic reasons, and the maintenance of family property. Oman has a high proportion of consanguineous marriages since the past and it continues until the present day. A survey of Omani population by Rajab et al. showed that 35.9% marriages are between first and second cousins and a further 20.4% of marriages are between members of the same tribe (Rajab and Patton 2000). The rate of consanguinity in Oman (56%) is close to the rate reported from Saudi Arabia (57.7%) and Kuwait (54%)(Murthy, Sundareshan et al. 1990; el-Hazmi, al-Swailem et al. 1995; al-Gazali, Bener et al. 1997; al-Awadi SA 1999).

The speculations about an association between consanguinity and increased DS prevalence are mainly based on the increased rate of consanguinity among parents of DS children in Kuwait and its high DS prevalence (Alfi et al. 1980; Farag and Teebi 1988). Similar observations have been made in Shetland (Roberts et al. 1991) and Canada (de Braekeleer et

al. 1994) and are explained by recessive genes, possibly increasing the rate of non-disjunction or by preventing the loss of the trisomy 21 fetus. Alfi et al. reported from Kuwait that the relative risk for DS is approximately four times greater for closely related than for nonrelated parents (Alfi, Chang et al. 1980). However, all these studies are hampered by the low number of cases analysed (<40 cases).

In contrast, our data are based on the investigation of 369 Omani DS families. We did not find any increased consanguinity rate of close degree (1^{st} and 2^{nd} cousins) among DS parents as compared to the general population even though the DS prevalence in Oman is high. Therefore, our findings do not support the hypothesis of an association between consanguinity and increased risk for the occurrence of Down syndrome. These results are in agreement with other studies from Savage et al. and Devoto et al. (Savage et al. 1998; Devoto, Prosperi et al. 1985).

4.3.3. Genetic heterogeneity in the Omani population: evidence from the analyses of autosomal and Y-chromosomal STRs and sequence of the mtDNA-D-loop

The hypothesis of an association between increased DS prevalence and the degree of inbreeding in a population is based on the assumption that recessive alleles can become homozygot in a highly inbred population. If these genes are involved in the execution of correct segregation of the homologous chromosomes and/or the sister chromatids during meiosis, defective alleles of these genes could theoretically influence the non-disjunction rate and, subsequently, lead to the generation of aneuploidy. Potential candidate genes are those implicated in meiotic homolog pairing, assembly of the synaptonemal complex, sister chromatid cohesion and spindle formation. In experimental organisms a number of genes have been identified that affect meiotic non-disjunction. In man, only disease genes have been detected which increase the risk of mitotic non-disjunction in somatic cells (compare Table 2, Introduction) while genes influencing meiotic segregation have not been described so far.

Considering the basic hypothesis, namely the potential increase of homozygosity for alleles involved in meiotic segregation in an inbred population, one would expect that also other loci in the genome show an increased degree of homozygosity. We, therefore, investigated the heterozygosity of autosomal loci in the study population. Since population data for these loci do not exist for the Omani population, we compared the data with those of the European population. For the autosomal loci, eight STRs were analysed which were also used for genotyping of chromosome 21 (Table 68).

Table 68 Heterozygosity of STR marker of chromosome 21 in the present study compared with the data from the National Center for Biotechnology Information (NCBI).

STR	N	Heterozygosity (N) in this study	Heterozygosity (%) in this study	Heterozygosity (%) published at NCBI
D21S215	480	371	77.29	76.12
D21S1432	60	40	66.67	63.64
D21S120	341	213	62.46	63.79
D21S1414	566	500	88.34	86.65
D21S1258	557	482	86.54	84.86
D21S1445	560	439	78.39	80.17
D21S1252	566	481	84.98	86.00
D21S1890	474	416	87.76	87.28

Surprisingly, analysis of the data demonstrates that the degree of heterozygosity of the tested STR loci is not different in the Omani population from that published at NCBI. This implicates that the practice of consanguineous marriages did obviously not lead to an overall increase of the homozygosity for autosomal loci in the Omani population.

Furthermore, we analysed the Y chromosomal STRs of 164 samples from fathers of DS families by genotyping using the AmpFLSTR Yfiler kit with 12 Y-STR markers. The samples analysed cover almost all regions of Oman. Y-chromosomal haplotypes are patrilines and they are more prone to genetic drift than autosomal alleles, therefore the degree of isolated populations can be obtained from Y chromosomal genetic data (Roewer, Kayser et al. 2000). Therefore, Y chromosomal STR markers are used to infer phylogenetic relationships or to obtain indirect estimates of gene flow. Our data demonstrate that the Y-haplotypes show a considerable heterogeneity in the male Omani population (Appendix). Using 11 Y-STR markers, a total of 164 haplotypes were observed, all of which were unique indicating that no specific haplotypes segregate in the population.

The observed heterozygosity for autosomal loci, as well as for paternally inherited Y-chromosomal loci and for maternally inherited mitochondrial sequences indicates that the Omani population seems to be a genetically highly admixtured population which might be explained by extensive migration in ancient times.

4.4. Parental and meiotic origin of the additional chromosome 21

Non-disjunction is a chromosome mis-segregation during either one of the two meiotic or a mitotic cell division. The resulting aneuploidy, either trisomy or monosomy, is the most common abnormality identified in humans. It has been estimated that about 10-30% of fertilised human eggs are aneuploid, either trisomic or monosomic, and that one third of miscarriages reveal aneuploidy which is known to be the main cause for pregnancy loss (Hassold and Hunt 2001). The frequency of chromosome mis-segregation in meiosis in

humans seems to be higher than in any other mammal. In other mammals, such as mice the frequency of aneuploidy in fertilised eggs is only about 1-2% (Hassold and Hunt 2001). Yet, studies of other model organisms revealed an even lower frequency of meiotic mal-segregation e.g. in the yeast Saccharomyces with 1 in 10,000 (Sears, Hegemann et al. 1992).

It is important to determine the parental and meiotic origin of the error in order to investigate the mechanism of non-disjunction and to explain the maternal age effect (Antonarakis, Avramopoulos et al. 1993). The main aim to collect the biological samples of this study was to determine the origin of non-disjunction and patterns of recombination on chromosome 21 and correlate these data with maternal age at Down syndrome birth.

Highly informative polymorphic STR markers allow the determination the parental and meiotic origin of free trisomy 21. STR markers which are close to the centromeric region of chromosome 21 are determining the stage of meiosis in which the mal-segregation error took place either in MI or in MII. If the markers are reduced to homozygosity then the error was interpreted as a result of mitotic postzygotic division (Antonarakis, Petersen et al. 1992; Antonarakis, Avramopoulos et al. 1993; Sherman, Petersen et al. 1994; Lamb, Freeman et al. 1996; Yoon, Freeman et al. 1996).

4.4.1. Determination of the origin of trisomy 21

Data from other studies reported earlier with a high number of analysed cases are presented in Table 56 (Antonarakis et al. 1992, Antonarakis et al. 1993, Sherman et al. 1994, Lamb et al. 1996, Yoon et al. 1996, Sherman et al. 2005). The results of these studies indicate that majority of non-disjunction errors which lead to trisomy 21 are due to errors in the egg as above 85% of cases involve an additional maternal chromosome. We report here a similar result with 88.17% been of maternal origin which is considered not to be different from the above mentioned findings.

Table 69: Origin of chromosome error, mean maternal age and standard deviation (SD) for Trisomy 21 reported in this study and from other studies

Source	**Origin of non-disjunction**						
Oman (present study)	Maternal 298 (88.17%)				Paternal 27 (7,99%)		
		N	%	Maternal age	*N*	%	Maternal age
	MI	213	72.20	34,68 ± 6,57	7	43,75	34,1 ± 6,01
	MII	82	27,80	34,21 ±7,49	9	56,25	35,82 ± 5,65
	Mitotic 13 (3,85%)						29,84 ± 14.37
Antonarakis et al. 1992	Maternal 188 (94%)				Paternal 9 (4.5%)		
		N	%	Maternal age	*N*	%	Maternal age
	MI	128	77.1		2	22.22	
	MII	38	22.9		7	77.78	
	Mitotic						
Antonarakis et al. 1993	Maternal 217 (91.8%)				Paternal 10 (4.20%)		
		N	%	Maternal age	*N*	%	Maternal age
	MI	174	80.18	32.1 ± 6.2	4	1.68	
	MII	43	19.81	34.1 ± 5.7	6	2.52	27.5 ± 2.4
	Mitotic 11 (4.62%)						28.5 ± 4.6
Sherman et al. 1994	Maternal 311 (88%)				Paternal 32 (9%)		
		N	%	Maternal age	*N*	%	Maternal age
	MI	173	74.89	30.3 ± 5.5	9	37.5	26.4 ± 6.7
	MII	58	25.11	31.2 ± 7.1	15	62.5	28.1 ± 4.7
	Mitotic 9 (3%)						28.3 ± 7.1
Lamb et al. 1996	Maternal 571 (88%)				Paternal 51 (8%)		
		N	%	Maternal age	*N*	%	Maternal age
	MI	382	74.17	31.1 ± 6.1	13	38.24	26.0 ± 6.0
	MII	133	25.83	32.2 ± 6.8	21	61.76	26.1 ± 5.7
	Mitotic 20 (3%)						27.7 ± 4.8
Yoon et al. 1996.	Maternal 97 (85.5%)				Paternal 10 (8.8%)		
		N	%	Maternal age	*N*	%	Maternal age
	MI	67	75.3	29.5 ± 6.8	4	50	21.0 ± 5.5
	MII	22	24.7	32.0 ± 7.3	4	50	25.0 ± 4.5
	Mitotic 6 (5.3%)						29.5 ± 6.7
Sherman et al. 2005	Maternal 311 (89.4%)				Paternal 22 (6.3%)		
		N	%	Maternal age	*N*	%	Maternal age
	MI	240	77.2	30.98 ± 6.81	12	54.5	28.50 ± 7.51
	MII	71	22.8	31.44 ± 7.60	10	45.5	26.00 ± 5.39
	Mitotic 15 (13%)						29.73 ± 5.06

A large proportion of maternal non-disjunction errors occur during the first meiotic stage MI. Antonarakis (1991) indicated that almost all non-disjunction errors are initiated during meiosis MI stage (Lamb, Yu et al. 2005). The meiosis MI errors in our study accounted for about 72.2% which is not much different from other reported studies which ranged between 73-77% (Table 69). In all studies maternal meiosis MII errors ranged between 18-25% of maternal origin errors. A small proportion of cases are of paternal non-disjunction which accounted between 4-8%. In paternal non-disjunction errors, there are high proportions of meiosis MII errors compared to MI errors. Finally there are few cases with mitotic errors in

the present study. The proportion of mitotic error in Oman was a total of 13 cases out of 346 (3.85%) families analysed and their mean maternal age was 29.84 (±14.37) years which is lower than the mean maternal age of MI and MII errors.

The present study confirms the report of Peterson et al. (1993) of an excess of MI errors among the paternally derived trisomy 21 cases, however, the number of cases is too small to draw any general conclusions. The finding in paternal cases is in contrast to maternal non-disjunction errors where an MI error is nearly 3 times as likely as an MII error (Savage, Petersen et al. 1998). Hassold and Sherman (2000) estimate that the timing of meiotic errors is differ among egg and sperm, the ratio of errors scored between MI and MII in egg is 3:1 whereas in sperm it is 1:1.

If we compare the different proportions of maternal MI and MII errors in the study from Oman (Table 70) it is conspicuous that the fraction of MII errors is the highest reported from all studies, even though not statistical significant. Therefore, we determined the origin of trisomy 21 among the three Omani regions with significantly different DS birth prevalences. Both, maternal and paternal origins of non-disjunction were in the normal proportions published in other studies (Antonarakis, Petersen et al. 1992; Yoon, Freeman et al. 1996; Lamb, Yu et al. 2005) as well as the proportion of mitotic non-disjunction. However, the proportion of maternal MI errors was lower in the high and middle prevalence regions as compared to the low prevalence region with 68.47%, 62.71%, and 81.25%, respectively.

Table 70: Prevalence of Down syndrome reported in this study and from some other worldwide studies per 10,000 live births.

Maternal non-disjunction					
	Meiosis	*N*	%	Maternal age	95% Cl
High prevalence	MI	76	68.47	34 ± 6.81 (N=60)	32.24;35.76
	MII	35	31.53	34 ± 8.81 (N=30)	30.71;37.29
Middle prevalence	MI	37	62.71	35 ± 9.75 (N=28)	31.22;38.78
	MII	22	37.29	33 ± 7.98 (N=18)	29.03;36.97
Low prevalence	MI	78	81.25	35 ± 5.58 (N=71)	33.68;36.32
	MII	18	18.75	36 ± 5.53 (N=16)	33.05;38.95

If we compare these data with the published data, we conclude that MII errors seem to be higher in regions of higher DS prevalence, even though the maternal age is not different (Table 70). If we furthermore consider the finding that we have evidence for a space-time

relationship of the prevalence of trisomy 21 in Oman and that this could be influenced by exogenous factors, one could speculate that MII errors might be more prone to environmental or exogenous factors than maternal MI errors which are mainly due to endogenous factors depending on the age of the oocytes.

To the best of our knowledge we did not find any data in the literature investigating this interesting aspect of MI or MII non-disjunction in respect to possible exogenous influences. There is only one minor remark in a publication of Czeizel et al. (1992) in Lancet that MII non-disjunction is possibly more prone to exogenous factors.

4.4.2. Recombination

For non-disjunction in human females Lamb et al. 1996 suggested a two hits model:

- Establishment of bivalents with susceptible meiotic configuration (a bivalent with no exchange or exchange in improper location) which occurs during fetal development. This hypothesis is mostly applicable for both, younger and older age groups.
- Degradation of the meiotic process affecting meiotic motor proteins, checkpoint control proteins or spindle components. The probability that such meiotic specific proteins are degraded overtime in oocyte increases with increasing maternal age.

Absence of exchange and altered placement of exchange along chromosome 21 have been identified as risk factors for non-disjunction of chromosome 21 in oocytes (Lamb, Freeman et al. 1996; Lamb, Feingold et al. 1997; Lamb, Sherman et al. 2005; Sherman, Allen et al. 2007; Oliver, Feingold et al. 2008). Warren et al. (1987) were the first who used DNA polymorphic markers and provided evidence of an association between reduced recombination and human trisomy 21 non-disjunction (Warren, Chakravarti et al. 1987). Studies of other human trisomies provided evidence that all are associated with alternations in recombination. For example, reduction of recombination in the proximal region of chromosomes 16 and 18 were described for maternal MI meiosis errors and increased distal (pericentromeric) recombination was reported in maternal MI errors of the trisomy sex chromosome (Fisher, Harvey et al. 1993; MacDonald, Hassold et al. 1994; Fisher, Harvey et al. 1995; Hassold, Merrill et al. 1995).

There are studies from other model organisms such as Drosophila and Yeast to investigate the the relationship between aberrant recombination and meiotic non-disjunction, and the results show a reduced level of recombination which leads to increase level of meiotic non-disjunction (Muris, Vreeken et al. 1997; Hayashi, Ogawa et al. 1998; Pittman and Schimenti 1998; Woltering, Baumgartner et al. 2000; Malmanche, Owen et al. 2007).

In our project, we studied the pattern of recombination in meiosis for our Down syndrome families. Our results confirm the association between altered recombination and non-disjunction. In a normal disjoining maternal meiosis location of recombination occurred at a medial part of chromosome 21q and one single exchange is enough for a proper segregation of homologous chromosomes (Lamb, Feingold et al. 1997; Lamb, Yu et al. 2005). Our aim was to approve findings of other studies regarding the location of recombination as a risk factor for non-disjunction for both maternal MI and MII cases. As described in other studies (Lamb, Feingold et al. 1997; Lamb, Yu et al. 2005; Sherman, Allen et al. 2007; Oliver, Feingold et al. 2008) we investigated whether recombination along maternal non-disjoined chromosome 21 has three susceptible exchange patterns: (i) absence of exchange leads to an increased risk of MI errors, (ii) a single telomeric exchange leads to a risk of MI errors and (iii) a pericentromeric exchange leads to an increased risk of MII errors. Our data are based on the analyses of a total of 292 meioses. 211 meiosis were maternal MI errors, from which 44.1% (N=93) showed complete absence of recombination (achiasmatic) (Table 55) which is nearly one half of the MI derived cases. One recombination in the bivalent is important and responsible for holding meiotic homologous chromosome together and for their proper orientation to the meiotic spindle of metaphase I. Thus, our finding approve that absence of recombination is a risk factor for MI mal-segregation. The percentage of approximately 40% of MI non-disjunction without exchange in DS has also been reported from other groups (reviewed in Oliver et al., 2008). Hassold and Sherman (2000) showed that the presence of a single exchange may be sufficient for proper chromosome segregation, however, this would be more susceptible to non-disjunction when there is a disturbance of meiosis as a result of maternal age or environmental factors.

One of the aims for our study was to investigate the recombination among three age groups of mothers. We found that the proportion of cases with no exchange are highest among the youngest age group compared with middle and older age groups. Our data revealed that 62% of younger mother (<29 years), 47% in middle (29-34 years) and 36% in higher (>34 years) age group mothers of MI non-disjunction experienced non-recombinant meiosis. Among all three age groups the youngest mothers exhibit the largest proportion with achiasmate meiosis and this was predicted because low age group mothers are not subjected to age related risk factors. Our findings of the differences between younger and older mothers are similar to those reported in the study of Oliver et al. (Oliver, Feingold et al. 2008). In model organisms there are known mutations that lead to an increase of achiasmatic meiosis which in turn increased the non-disjunction such as a mutation in the gene *Nod* in female *Drosophila which* is related to non-disjunction in MI meiosis (Knowles and Hawley 1991).

We also analysed the number of observed recombination events in non-disjunction by maternal age for both MI and MII events. Our results show that the number of crossover with zero, 1 and 2 recombinants for maternal MI is not correlated with maternal age which was also shown in a previous study (Lamb, Feingold et al. 1997).

Study of model organisms such as *Drosophila melanogaster* demonstrate that recombinations of MI non-disjunction are predominantly localized in the distal part of the chromosome, while MII non-disjunction is associated with exchanges in the proximal exchanges part of the chromosome (Koehler, Boulton et al. 1996). Therefore, we analysed if the position of recombination along 21q is a susceptible factor for chromosome 21 non-disjunction, and in addition the association between location of the recombination and maternal age. Our data support the data found in model organisms as we observe that single exchanges were shifted towards the distal region of 21q in MI errors which is in contrast to MII error where the distribution of single exchanges shows a strong shift towards the proximal (pericentromeric) part of 21q.

For MI non-disjunction a high number of telomeric exchanges was demonstrated for all three maternal age groups. In the youngest age group all observed recombinations were found to be in the distal part of the chromosome while for the higher age groups a shifting of the location from the telomere towards the middle part of the chromosome is observed. These findings are consistent with the data described in other studies (Lamb, Freeman et al. 1996; Lamb, Feingold et al. 1997; Oliver, Feingold et al. 2008).

For MII non-disjunction with a single exchange we observed that there is a shift towards the proximal (pericentromeric) part of 21q in older age (42%), in contrast to 33% of younger mothers. These results suggest that a single pericentromeric exchange is an age dependent risk for MII non-disjunction although we do not have any explanation for the occurrence of higher proportion (44%) in middle age group. We conclude that a greater proportion of trisomy 21 cases among older group are related to pericentromeric exchanges at MII. Oliver et al. (2008) explained that a pericentromeric exchange in older age group protects the bivalent segregation from maternal age related risk factors allowing proper segregation of homologous chromosomes.

Robinson et al. (1998) studied the maternal age effect on the level of recombination for chromosome 15. He found that for maternal MI cases the age of mother was significantly increased among cases with multiple recombination compared with one or zero recombinant (Robinson, Kuchinka et al. 1998) and the same was found for trisomy 18 (Fisher, Harvey et al. 1995). In our study a similar pattern was observed for double exchanges in both maternal meiosis MI and MII. The only explanation can be that multiple recombinants might be more

resistent to non-disjunction because of increased stability of the tetrad (Oliver, Feingold et al. 2008). Thus, our finding support the evidence that the number of exchanges might be protective against maternal age related factors (Oliver, Feingold et al. 2008). In conclusion, our data on the association between non-disjunction, number and localisation of recombination events and maternal age confirm earlier studies of trisomy 21 in populations with an other ethnic background.

4.5. Conclusions and Outlook

Considering that the clinical phenotype of Down syndrome was described already in 1866 by John Langdon Down and that the underlying cause of DS, a trisomy of chromosome 21, was reported by Jerome Lejeune more than 50 years back in 1959, there is a remarkable lack of knowledge and many open questions. So far, comprehensive - and particularly population-based studies of Down syndrome in Arab countries are missing, even though several, mainly hospital-based, investigations have emphasized the high prevalence of Down syndrome in Arab countries. Therefore, the current study was undertaken investigating several aspects of Down syndrome in Oman. It reports a high prevalence of trisomy 21 in the Omani population and investigated the parental and meiotic origin of the additional chromosome 21. One the most surprising result of the current study was the detection of a space-time clustering of the Down syndrome prevalence in Oman suggesting that exogenous factors might be involved. In principle, these could act in two ways: either at the time of conception disturbing the normal segregation of the chromosomes in meiosis or they could be involved in implantation and /or survival of the DS fetuses during embryogenesis. Until now we have no satisfying answers concerning the causative factors involved. Further studies on Down syndrome prevalence and possible etiological factors are under the way at the Ministry of Health of the Sultanate of Oman.

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6. SUMMARY

The current investigation is the first comprehensive study of Down syndrome in Oman. Criteria for a meaningful epidemiological study are an almost complete population-based ascertainment and a relatively large number of annual births. Both criteria are stringently met in Oman. The annual birth rate in Oman is approximately 40,000. Oman has a comprehensive health care system which is provided and financed predominantly by the government. More than 95 % of all newborns are delivered in governmental hospitals and almost all are examined by paediatricians who are aware of the conspicuous clinical phenotype of DS and prompt a cytogenetic analysis for confirmation. Therefore, ascertainment can be considered to be complete since 1999. Furthermore, the Sultanate of Oman is one of the countries most suitable for epidemiological studies on trisomy 21 since prenatal maternal serum screening, prenatal diagnostics and selective terminations of pregnancies do not play any role, in contrast to most western countries where prenatal diagnostics is common practice.

The cytogenetic study includes the karyotypic findings in 680 Omani children with DS who were diagnosed at the Cytogenetic Laboratory of the Ministry of Health from 1999 to 2005. The data show that 94% of the children with DS have a free trisomy 21, while 3% have a translocation trisomy, and 3% have mosaicism. These results are similar to the findings of other published studies. Out of the 640 liveborn DS children with a free trisomy 21 there were 369 males and 271 females resulting in a sex ratio of 1.36 which is significantly different from the overall sex ratio of 1.06 of live births in Oman. The skewed sex ratio in Down syndrome has also been reported from other studies, however, the underlying mechanism is still unexplained. Since the sex ratio at the time of conception is considered to be 1:1 one must assume that there is a considerable intrauterine selection against females with trisomy 21 during early embryogenesis.

The epidemiological data on the DS prevalence in Oman are population-based and cover the period of five years from 2000 to 2004. During these five years 518 DS children with a free trisomy 21 were born in Oman, the total number of live births was 200,157 resulting in an overall prevalence of 25.88 per 10,000 live births which is one of the highest if not the highest live birth prevalence world-wide.

One of the most important risk factors for non-disjunction of chromosome 21 is advanced maternal age. The current study in Oman demonstrated a very strong association of advanced maternal age with the birth of a DS child. The case control study showed that the mean maternal age of the mothers of DS children was 33.50 years which is significantly higher than the maternal age of the control mothers with 27.5 years. Furthermore, our results show that there are

no significant differences in the mean maternal age of meiosis MI and meiosis MII errors which suggests that both types of non-disjunction errors are age dependent. However, the maternal age related risk factor alone cannot explain the unusually high DS birth prevalence in Oman. If we calculate the expected number of DS cases in Oman on the basis of the maternal age specific risk figures of two surveys for the maternal age distribution in Oman, the expected number of trisomy 21 cases from 2000 to 2004 would be N=398 and N=430, respectively, which is significantly less than the observed number of N=518 DS cases.

A cluster analysis of the DS prevalence showed statistically significant differences in the prevalence between different geographical regions in Oman. The highest prevalence was found in the South Al Batinah region (37.97 per 10,000 live births) followed by Ad Dakhiliya (33.41 per 10,000 live births) and Muscat (30.41 per 10,000 live births). The lowest prevalence is found in South Ash Sharqiya, North Al Batinah and Musandam ranging between 15.13 and 16.41 per 10,000 live births. The remaining regions are in the middle range between 25.45 and 26.40 per 10,000 live births. The maternal age was not different among the regions and can therefore not be considered as a risk factor. The region with the highest prevalence lies within a cross-section dimension of approximately 200 kilometers and is the most densely populated area in Oman. Furthermore, for the period from 2000 to 2004 there was a significant increase in the birth prevalence of DS children born in January followed by December. When we compared these seasonal variations of the DS birth prevalence between the three regions with high, middle, and low DS prevalence we found that the seasonal deviation is greatest in the regions with the highest DS prevalence. At the moment, we can not explain the finding on the seasonal and regional variations of the DS prevalence in Oman. However, such a space-time relationship of the DS prevalence must be interpreted as a strong indicator for a potential exogenous factor involved in the high birth prevalence.

The molecular genetic study to determine the parental and meiotic origin of the extra chromosome 21 was based on the recruitment of 346 families with Down syndrome by analysing the segregation of the parental alleles to the DS child with a high polymorphic STR marker set of chromosome 21. Out of 333 informative families 298 (88.17%) were of maternal origin and 27 (7.99%) were of paternal origin. In addition, 13 cases (3.85%) were of mitotic non-disjunction. Of the maternally derived cases in 72.2% (N=213) the errors occurred during meiosis MI and in 27.8% (N=82) the error occurred during meiosis MII. The analysis of the recombination profiles confirms the association between altered or absent recombination and non-disjunction which has been reported in earlier studies. Of the 211 meiosis maternal MI errors 44.1% (N=93) showed complete absence of recombination. The occurrence of achiasmatic meiosis was present in 62% of mother aged <29 years, 47% aged 29-34 years, and 36% aged >34 years. In addition to

absence of recombination, the position of single exchanges along 21q is a known susceptible factor for chromosome 21 non-disjunction. The data from the Omani DS families support published data as we observe that single exchanges were shifted towards the distal region of 21q in MI errors while MII errors show a strong shift towards the proximal part of 21q.
It has been speculated that the higher DS prevalence in some Arab countries is associated with the higher rate of consanguinity among parents of DS children. Data of the degree of relationship were available for 369 couples with a Down child demonstrating that the rate of consanguinity of DS couples is not different from that of the general population in Oman. In addition, this hypothesis was tested by investigating the genetic heterozygosity for autosomal STR loci, paternally inherited Y chromosomal STR loci and maternally inherited mtDNA sequence of the highly polymorphic mitochondrial D-loop. A high degree of heterozygosity was observed for all three entities indicating that the Omani population seems to be a genetically highly admixtured population which might be explained by extensive migration in ancient times.

One the most surprising results of the current study was the detection of a space-time clustering of the Down syndrome prevalence in Oman suggesting that exogenous factors might be involved. In principle, these could act in two ways: either at the time of conception disturbing the normal segregation of the chromosomes in meiosis or they could be involved in implantation and survival of the DS fetuses during embryogenesis. Until now we have no satisfying answers concerning the causative factors involved.

7. ZUSAMMENFASSUNG

Die vorliegende Arbeit ist die erste umfassende Studie des Down Syndroms im Oman. Wichtige Kriterien einer aussagekräftigen epidemiologischen Untersuchung sind eine fast komplette Populations-basierte Erfassung und eine relativ große Anzahl jährlicher Geburten. Beide Kriterien sind im Oman erfüllt. Die jährliche Geburtenrate im Oman liegt bei ca. 40 000. Der Oman hat darüber hinaus ein flächendeckendes staatlich finanziertes Gesundheitssystem. Mehr als 95% aller Neugeborenen werden in staatlichen Kliniken geboren, fast alle werden von Pädiatern untersucht, die den auffälligen klinischen Phänotyp kennen und eine zytogenetische Untersuchung zur Bestätigung der Verdachtsdiagnose veranlassen. Deshalb ist davon auszugehen, dass die Erfassung der Down Syndrom Fälle seit 1999 vollständig ist. Das Sultanat des Oman ist auch deshalb für epidemiologische Untersuchungen der Trisomie 21 ideal, weil maternales Serumscreening auf DS, Pränataldiagnostik und selektive Schwangerschaftsabbrüche keine Rolle spielen, im Gegensatz zu vielen westlichen Ländern, in denen Pränataldiagnostik verbreitete Praxis ist.

Die zytogenetische Studie umfasst die Chromosomenbefunde von 680 omanischen Kindern mit Down Syndrom, die im Zytogenetischen Laboratorium des Ministry of Health von 1999 bis 2005 diagnostiziert wurden. Die Daten zeigen, dass 94% der Kinder mit Down Syndrom eine freie Trisomie 21 hatten, 3% hatten eine Translokationtrisomie und 3% wiesen Mosaike auf. Ähnliche Resultate wurden von anderen publizierten Studien berichtet.

Von den 640 lebendgeborenen Kindern mit freier Trisomie 21 waren 369 männlich und 271 weiblich. Daraus ergibt sich ein Geschlechterverhältnis von 1.36, das sich signifikant vom Geschlechterverhältnis aller Lebendgeburten von 1.06 im Oman unterscheidet. Das verschobene Geschlechterverhältnis beim Down Syndrom wurde auch in anderen Studien beschrieben. Der zugrunde liegende Mechanismus kann aber nach wie vor nicht erklärt werden. Da davon auszugehen ist, dass das Geschlechterverhältnis zum Zeitpunkt der Konzeption 1:1 ist, muss man annehmen, dass eine erhebliche intrauterine Selektion gegen weibliche Feten mit Trisomie 21 in der frühen Embryogenese erfolgt.

Der epidemiologischen Daten zur DS Prävalenz im Oman sind Populations-basiert und umfassen den Zeitraum von 2000 bis 2004. Während dieser fünf Jahre wurden 518 Kinder mit einer freien Trisomie 21 im Oman geboren, die Gesamtzahl der Lebendgeburten betrug 200157. Daraus ergibt sich eine Gesamtprävalenz von 25.88 auf 10000 Lebendgeburten, die eine der höchsten, wenn nicht die höchste DS Prävalenz von Lebendgeburten weltweit ist.

Einer der wichtigsten Risikofaktoren für Non-disjnction des Chromosoms 21 ist das erhöhte maternale Alter. Die vorliegende Studie zeigt eine eindeutige Assoziation von erhöhtem

mütterlichen Alter und der Geburt eines Kindes mit Down Syndrom. In der Fall-Kontroll-Studie betrug der Mittelwert des Alters der Mütter mit Down-Kindern 33.5 Jahre, was signifikant höher ist als das Alter der Mütter aus der Kontrollgruppe, die im Mittel 27.5 Jahre alt waren. Des weiteren zeigen unsere Daten, dass der Mittelwert des mütterlichen Alters keine signifikanten Unterschiede zwischen den Frauen mit Meiose MI oder Meiose MII Non-Disjunction aufweist, d.h. dass Fehlverteilungen in beiden meiotischen Teilungen eine Altersabhängigkeit zeigen. Die Daten zeigen außerdem, dass das maternale Altersrisiko als Erklärung für die ungewöhnlich hohe DS Prävalenz im Oman nicht ausreicht. Wenn man die Zahl der erwarteten DS Fälle auf der Basis von Alters-spezifischen Risikozahlen, die auf zwei zuverlässigen Erhebungen basieren, für die Altersverteilung der Mütter im Oman berechnet, ergibt sich für die Zeit von 2000 bis 2004 eine erwartete Zahl von DS Fällen von N=398 bzw. N=430, was signifikant geringer ist, als die Zahl der beobachteten Fälle mit N=518 DS.

Eine Cluster-Analyse zur DS Prävalenz in unterschiedlichen geographischen Regionen des Omans zeigte statistisch signifikante Unterschiede in der Geburtsprävalenz zwischen den Regionen. Die höchste Prävalenz wurde in South Al Batinah nachgewiesen (37.97 auf 10000 Lebendgeburten) gefolgt von Ad Dakhiliya (33.41 auf 10000 Lebendgeburten) und Muscat (30.41 auf 10000 Lebendgeburten). Die niedrigste Prävalenz lag in South Ash Sharqiya, North Al Batinah und Musandam vor mit einer Prävalenz zwischen 15.13 und 16.41 auf 10000 Lebendgeburten. Die verbleibenden Regionen lagen zwischen 25.45 und 26.40 auf 10000 Lebendgeburten. Das mütterliche Alter in den verschiedenen Regionen war nicht unterschiedlich, so dass das Alter als Risikofaktor zur Erklärung der unterschiedlichen regionalen Prävalenzen nicht in Betracht gezogen werden kann. Die Region mit der höchsten DS Prävalenz umfasst ein benachbartes Gebiet mit einem Durchmesser von ca. 200 Km und gehört zu den Gebieten mit der höchsten Bevölkerungsdichte.

Die Analyse der epidemiologischen Daten zeigte außerdem, dass für den Zeitraum von 2000 bis 2004 die Geburtsprävalenz in den Monaten Januar und Dezember am höchsten war. Wenn man diese saisonalen Unterschiede in den einzelnen Regionen mit der hohen, mittleren und niedrigen Prävalenz untersucht, so zeigt sich, dass die saisonale Abweichung in der Region mit der höchsten Prävalenz am größten ist. Momentan haben wir keine Erklärung für die regionalen und saisonalen Unterschiede der Prävalenz des Down Syndroms im Oman. Allerdings ist eine derartige Raum-Zeit-Beziehung ein starker Indikator für das Vorhandensein von exogenen Faktoren, die für die hohe Prävalenz mitverantwortlich sind.

Die molekulargenetische Untersuchung des parentalen und meiotischen Ursprungs des zusätzlichen Chromosoms 21 basierte auf der Analyse der Segregation der parentalen Allele auf das Kind mit DS. Insgesamt wurden dafür 346 Familien rekrutiert und deren DNA mit einem Set

von hoch polymorphen STR-Markern von Chromosom 21 untersucht. Insgesamt waren 333 Familien informativ, von denen 298 (88.17%) auf maternales Non-Disjunktion zurückgingen und 27 (7.99%) auf paternales Non-Disjunktion. In 13 Fällen (3.85%) lag ein mitotisches Non-Disjunktion vor. Von den maternalen Fällen waren 72.2% (N=213) Fehlverteilungen in der ersten Reifeteilung MI und 27.8% (N=82) Fehlverteilungen in der 2. Reifeteilung MII.

Die Analyse der Rekombinationsprofile bestätigte in der Literatur publizierte Daten, nach denen veränderte oder fehlende Rekombination mit einem erhöhten Risiko für Non-Disjunktion einhergeht. Von 211 Non-disjunktion, die in der ersten maternalen Meiose MI auftraten, zeigten 44.1% (N=93) das komplette Fehlen von Rekombination. Das Auftreten von achiasmatischen Meiosen wurde bei 62% der Mütter mit einem Alter von <29 Jahren, bei 47% mit einem Alter von 29-34 Jahren, und bei 36% mit einem Alter von >34 Jahren nachgewiesen.

Neben dem Fehlen von Rekombination, besteht ein weiterer Risikofaktor in der Lokalisation eines einzelnen Rekombinationsereignisses. Die Daten der omanischen Familien zeigen, dass bei Vorliegen einer einzelnen Rekombination deren Lokalisation bei MI Non-Disjunktion nach distal auf dem Chromosom 21 verschoben ist, während bei MII Fehlern gehäuft Verschiebung in den proximalen Bereich nachweisbar war.

Es wurde spekuliert, dass die höhere Down Syndrom Prävalenz, die in arabischen Ländern beobachtet wurde, mit der erhöhten Rate an konsanguinen Verbindungen korreliert ist. Die Angaben über den Verwandtschaftsgrad lagen in dieser Studie von 369 Paaren mit einem Down Syndrom Kind vor. Sie unterscheiden sich nicht von den Konsanguinitätsraten, die für die omanische Gesamtbevölkerung angegeben werden. Außerdem untersuchten wir den Grad der genetischen Heterogenität durch die Analyse der Heterozygotie von autosomalen STR-Loci, von nur paternal vererbten Y-chromosomalen STR-Loci und von nur maternal vererbten mtDNA-Sequenzen. Für alle drei Entitäten konnte ein hoher Grad an Heterozygotie nachgewiesen werden, was zeigt, dass die omanische Population eine genetisch stark durchmischte Population ist, was durch extensive Migration in historischer Zeit erklärt werden kann.

Das überraschendste Ergebnis der vorliegenden Arbeit war der Nachweis eines Raum-Zeit-Clusters der DS Prävalenz im Oman. Dies macht wahrscheinlich, dass exogene Faktoren an der hohen Prävalenz mitbeteiligt sind. Dabei sind grundsätzlich zwei Möglichkeiten denkbar: zum einen könnten die Faktoren störenden Einfluss auf die normale Chromosomensegregation zum Zeitpunkt der Konzeption nehmen, zum anderen könnten sie einen Einfluss auf die Implantation oder das Überleben der Feten mit Trisomie 21 während der Embryogenese haben. Bislang haben wir keine befriedigende Antwort bezüglich der möglichen ursächlichen Faktoren, die involviert sein könnten, gefunden.

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Last but not least, I would like to acknowledge and recognize the moral support from my respective family members, my husband Mohamed, my children Sarah, Ahmed and my newborn baby, Alyaa, all of whom I missed a lot during the course of this work and whose personal sacrifice made this possible.

Confirmation

According to § 7 Abs. 4 of the rules for the doctoral degree procedure of 4th of September 2004 in the Department of Biology, Chemistry, Pharmacy

Herewith I confirm that, I have done my doctoral degree thesis with the title:

Down syndrome in Oman: Etiology, prevalence and potential risk factors. A cytogenetic, molecular genetic and epidemiological study.

Completely on my own and without impermissible help.

And I confirm that I won´t submit my doctoral degree thesis for another examination, in case of rejection.

Date: March 30, 2010 .....
Signature

Curriculum Vitae

Das curriculum vitae wurde aus datenschutzrechtlichen Gründen in der elektronischen Form nicht publiziert.

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APPENDIX

Appendix 1: Cytogenetic request form for the patient' with clinical information

Sultanate of Oman
Ministry of Health
Directorate General of Health Affairs
Central Health Laboratories
Darsait

Lab No.

FORM FOR CONSTITUTIONAL KARYOTYPE AND CYTOGENETIC ANALYSIS

Hospital Hospital No.: Name of Patient: Age: Sex: Nationality:	Ref. Doctor's Name: Address: Signature: Tel. No.: Date:

Request:

Type of Specimen (Specify)
Blood Culture ☐ Bone Marrow Aspirate ☐
Peripheral Blood Lymphocyte ☐ Others (Specify) .....

Clinical Information (Symptoms, Signs & Diagnosis)

Whether the patient is on any drug or treatment

Previous Report: Yes/No	Specimen Collection Time & Date:
Specimen Receiving (Cytogenetic Laboratory) Time: Date:	**Specimen Status:** Good ☐ Insufficient ☐ Clotted ☐ Haemolysed ☐ Other (Specify).....

Cytogenetic Findings:

Technician's Name & Signature
Tele Fax: 24788250

Signature & Stamp
Head of Cytogenetics Section

Appendix 2 Questionnaire of the case-control study

CASE-CONTROL-STUDY

Introduction

Before I ask you any questions, I want to mention a few things:

1. The interviewer is a medical doctor and will keep any information confidentially. No third party will receive any information about you. The information you will give will be used for statistical/epidemiological purposes only. The persons who will do the statistical/ epidemio-logical analysis are physicians too and will get only anonymised data sheets. Your name will never be mentioned in any report.

2. You may stop the interview at any point.

3. You should know that you are free to refuse to answer any question. I would prefer to receive no answer, than to get a wrong answer for what ever reasons.

4. I will be glad to try to answer any questions that you may have, but, if possible, it would be easiest if you could save them until the end of the interview.

This first page will be kept by the interviewer and will be separated from the rest of the questionnaire. [If the interview is done with a proxy of a ceased person, skip Q5 - Q6]

1. ID Number [must be unique for the study popülation] 1._____

2. Pedigree number 2._____

3. Name:_____, _____, _____
first maiden last

4. Initials to be used in the pedigree 4._____

5 Present address:_____
Street Number

_____
City

6 Phone: Day:_____

Evening:_____

Sometimes new questions may come up after interviews have been done, and there is a small chance that we may ask to talk with you in the future. It would be helpful if you could give me an address and phone number of a person who will always know where you can be reached. Who would that be and what is their address and phone number?

Name:_____

Relationship:_____

Address:_____

_____

Phone:_____

General

1 ID Number 1_____

2 Pedigree number 2_____

3 Initials to be used in the pedigree 3_____

4 Gender: 4_____

man	1
woman	2
'??'	99

5 Date of birth 5____/____/____
dd mm yy

6 Vital status: 6_____

alive	1
dead	2
'??'	99

[if dead Q7 - Q9; skip if alive, instead of you/yours use he/his or she/her]

7 Date of dead or last contact 7____/____/____
dd mm yy

8 Cause of death:_____
verbatim

ICD 10th revison:_____

9 Who gave the interview (explain relationship to ceased):

_____

10 What is the highest year of regular school or college that you completed 10_____
years

[if applicable]
11 How many years did you visit university 11_____

years

Pedigree:

Menstrual history

1 How old were you when you had your first menstrual period? 1_____ years

2 Did your periods start by themselves, or was a shot, medication, or surgery necessary to start them? 2_____

By themselves	1
Injection, medication	2
Surgery	3
'??'	99

3 Many women have very irregular periods when they first start. How old were you when your periods became regular, that is, the time between periods did not vary by more than about 10 days. 3_____ years

'??'.....99

4 Do you consider yourself to have regular, somewhat regular, or very irregular periods? 4_____

regular	1
somewhat regular	2
very irregular	3
'??'	99

5 After your periods became regular or somewhat regular, how long was your cycle on average, that is the time from the start of one period to the start of the next? 5_____ days

'??'.....99

6 Until now, has there ever been a time since your periods started when you had no periods for four consecutive months or more. Do not include times during which you may have been pregnant, nursing or using birth control pills, shots, or implants? [Do not include menopause.] 6_____

yes	1
no	2
'??'	99

6a How often did such intervalls without periods occurr? 6a_____

6b If possible specify the years of this/these events:

_____['??' .. 99]

[following questions [Q7 - Q8], when applicable]

7 Did your menstruation cease, did you experience menopause? This should be understood as no periods for six consecutive months. 7_____

yes	1
no	2
'??'	99

8 How old were you, when you experienced menopause? 8_____ years

'??'.....99

9 Until now, did you ever have one or both of your ovaries removed? 6_____

yes, one	1
yes, both	2
yes, numer unknown	3
no	4
'??'	99

10 How old were you, when your first ovary was removed? 10_____ years

'??'.....99

11 How old were you, when your second ovary was removed? 11_____ years

'??'.....99

History of woman's pregnancies

The following questions are about your pregnancies:

1 How many times altogether have you ever been pregnant? Please be sure to include any pregnancies that ended in a livebirth, a miscarriage, a stillbirth, an induced abortion, a tubal pregnancy, or anything else. 1_____

Now I'd like to ask you a few questions about (this pregnancy/ these pregnancies). As we go through these questions, if you remember any other pregnancies, please be sure to tell me about them. [Complete all questions concerning the 1st pregnancy, then move on to the 2nd, etc.]

2 Thinking about your (1st / 2nd/ etc.) pregnancy, in what month and year did it end?

1____/ ____ 2____/ ____ 3____/ ____ 4____/ ____ 5____/ ____ 6____/ ____

7____/ ____ 8____/ ____ 9____/ ____ 10____/ ____ 11____/ ____ 12____/ ____

3 How long had you been pregnant at the time this pregnancy ended? [weeks]

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

4 Did this pregnancy end in a

livebirth	1
stillbirth	2
miscarriage	3
induced abortion	4
tubal pregnancy	5
molar pregnancy	6
'??'	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

5 How many [babies or fetuses] were you pregnant with this time?

one	1
two	2
three	3
'??'	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

6 What was the outcome for the (1st, 2nd,...) baby?

livebirth	1
stillbirth	2
'??'	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

7 Was this [baby or fetus] a male or a female?

male	1
female	2
'??'	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

8 How much did (he/she) weigh at birth? [gram]

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

9 Did you become pregnant spontaneously, or did you receive medical treatment to become pregnant like hormonal treatment, artificial insemination or in vitro fertilization [if spontaneously Q 9a]

spontaneously	1
hormonal treatment	2
artificial insemination	3
in vitro fertilization	4
'??'	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

9a You became pregnant spontaneously. Which of the following alternatives describes best how this pregnancy occurred?

I wanted to become pregnant at that time [Q 9b]	1
I did not care whether I would become pregnant at that time [Q 9b]	2
I became pregnant unwillingly though I did not use any anticonception [Q 10]	3
I became pregnant unwillingly despite using anticonception [Q 10]	4
'??' [Q 10]	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

9b [use one of the alternatives according to the answer to Q 9a]
- After you decided to become pregnant, how long did it take until you conceived?
- After you started not to take care about becoming pregnant, how long did it take until you conceived?
[give the time interval in months]

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

10 Did (he/she/this fetus) have a birth defect or chromosome abnormality that was diagnosed by a doctor?

yes	1
no	2
'??'	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

11 [if yes], what kind of birth defect or chromosome abnormality was that?

1_____ 7_____

2_____ 8_____

3_____ 9_____

4_____ 10_____

5_____ 11_____

6_____ 12_____

[only if live birth]

12 Is (he/she) alive now?

yes	1
no	2
'??'	99

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

13 [if not alive now], how old was (he/she) when (he/she) died?

SCALE:	days	d
	weeks	w
	months	m
	years	y

1_____ 2_____ 3_____ 4_____ 5_____ 6_____

7_____ 8_____ 9_____ 10_____ 11_____ 12_____

14 what did (he/she) die from?

1_____ 7_____

2_____ 8_____

3_____ 9_____

4_____ 10_____

5_____ 11_____

6_____ 12_____

Health and illnesses

The next set of questions concerns your health and illnesses that you may have had. Did a doctor ever tell you that you had.....

1A an underactive thyroid, or hypothyroidism? 1A_____

Yes..... 1
No..... 2
'??'..... 99

1B [If yes], how old were you when this was first diagnosed? 1B_____

1C [If yes], were you taking any medications for this disease? 1C_____

Yes..... 1
No..... 2
'??'..... 99

1D [If yes], name of medication(s)? 1D_____, _____

[generic name to be provided by responsible expert:]

_____

1E [If yes], for how long did take this medication? 1E_____

2A an overactive thyroid, or hyperthyroidism? 2A_____

Yes..... 1
No..... 2
'??'..... 99

2B [If yes], how old were you when this was first diagnosed? 2B_____

2C [If yes], were you taking any medications for this disease? 2C_____

Yes..... 1
No..... 2
'??'..... 99

2D [If yes], name of medication(s)? 2D_____, _____

[generic name to be provided by responsible expert:]

_____

2E [If yes], for how long did take this medication? 2E_____

3A diabetes or sugar diabetes? 3A_____

Yes..... 1
No..... 2
'??'..... 99

3B [If yes], how old were you when this was first diagnosed? 3B_____

3C [If yes], were you taking any medications for this disease? 3C_____

Yes..... 1
No..... 2
'??'..... 99

3D [If yes], name of medication(s)? 3D_____, _____

[generic name to be provided by responsible expert:]

_____

3E [If yes], for how long did take this medication? 3E_____

4A high blood pressure or hypertension? 4A_____

Yes..... 1
No..... 2
'??'..... 99

4B [If yes], how old were you when this was first diagnosed? 4B_____

4C [If yes], were you taking any medications for this disease? 4C_____

Yes..... 1
No..... 2
'??'..... 99

4D [If yes], name of medication(s)? 4D_____, _____

[generic name to be provided by responsible expert:]

_____

4E [If yes], for how long did take this medication? 4E_____

5A epilepsy or seizures? 5A_____

Yes..... 1
No..... 2

'??'..... 99

5B [If yes], how old were you when this was first diagnosed? 5B_____

5C [If yes], were you taking any medications for this disease? 5C_____

Yes..... 1
No..... 2
'??'..... 99

5D [If yes], name of medication(s)? 5D_____, _____

[generic name to be provided by responsible expert:]

_____

5E [If yes], for how long did take this medication? 5E_____

6A asthma? 6A_____

Yes..... 1
No..... 2
'??'..... 99

6B [If yes], how old were you when this was first diagnosed? 6B_____

7A leukemia? 7A_____

Yes..... 1
No..... 2
'??'..... 99

7B [If yes], how old were you when this was first diagnosed? 7B_____

7C [If yes], were you taking any medications for this disease? 7C_____

Yes..... 1
No..... 2
'??'..... 99

7D [If yes], name of medication(s)? 7D_____, _____

[generic name to be provided by responsible expert:]

_____

7E [If yes], for how long did take this medication? 7E_____

7F [If yes], specify time period(s) of treatment: 7F_____

7G [If yes], did you ever have a relapse of leukemia? 7G_____

Yes..... 1
No..... 2
'??'..... 99

8A cancer or a malignant tumor other than leukemia? 8A_____

Yes..... 1
No..... 2
'??'..... 99

8B which kind of cancer or malignant tumor:

8B_____

8C [If yes], how old were you when this was first diagnosed? 8C_____

8D [If yes], did you receive any kind of chemotherapy for this disease? 8D_____

Yes..... 1
No..... 2
'??'..... 99

8E [If yes], name of medication(s)? 8E_____, _____

[generic name to be provided by responsible expert:]

_____

8F [If yes], for how long did take this medication? 8F_____

8G. Did you ever receive X-ray or cobalt treatment for this cancer or malignant tumor? 8G_____

Yes..... 1
No..... 2
'??'..... 99

8H How many times did you have this type of treatment? 8H_____

8I How old were you when you first had this treatment? 8I_____

8J How old were you when you last had this treatment? 8J_____

8K. Did you ever receive a surgical treatment for this cancer or malignant tumor? 8K_____

Yes..... 1
No..... 2

'??'..... 99

8L. What did the surgeons do? 8L _____

8M Did you ever have a relapse of the cancer or tumor? 8M_____

Yes..... 1
No..... 2
'??'..... 99

9A a second cancer a malignant tumor other than leukemia? 9A_____

Yes..... 1
No..... 2
'??'..... 99

9B which kind of cancer or malignant tumor:

9B_____

9C [If yes], how old were you when this was first diagnosed? 9C_____

9D [If yes], did you receive any kind of chemotherapy for this disease? 9D_____

Yes..... 1
No..... 2
'??'..... 99

9E [If yes], name of medication(s)? 9E_____, _____

[generic name to be provided by responsible expert:]

_____

9F [If yes], for how long did take this medication? 9F_____

9G. Did you ever receive X-ray or cobalt treatment for this cancer or malignant tumor? 9G_____

Yes..... 1
No..... 2
'??'..... 99

9H How many times did you have this type of treatment? 9H_____

9I How old were you when you first had this treatment? 9I_____

9J How old were you when you last had this treatment? 9J_____

9K. Did you ever receive a surgical treatment for this cancer or malignant tumor? 9K_____

Yes..... 1
No..... 2
'??'..... 99

9L. What did the surgeons do? 9L _____

9M Did you ever have a relapse of the cancer or tumor? 9M_____

Yes..... 1
No..... 2
'??'..... 99

10A chickenpox? 10A_____

Yes..... 1
No..... 2
'??'..... 99

10B [If yes], how old were you when this was first diagnosed? 10B_____

11A lupus or lupus erythematosis? 11A_____

Yes..... 1
No..... 2
'??'..... 99

11B [If yes], how old were you when this was first diagnosed? 11B_____

12A rheumatoid arthritis? 12A_____

Yes..... 1
No..... 2
'??'..... 99

12B [If yes], how old were you when this was first diagnosed? 12B_____

13A ankylosing spondylitis? 13A_____

Yes..... 1
No..... 2
'??'..... 99

13B [If yes], how old were you when this was first diagnosed? 13B_____

14A tuberculosis? 14A_____

Yes..... 1
No..... 2
'??'..... 99

14B [If yes], how old were you when this was first diagnosed? 14B_____

15A hepatitis or inflammation of the liver? 15A_____

Yes..... 1
No..... 2
'??'..... 99

15B [If yes], how old were you when this was first diagnosed? 15B_____

Diagnostic X-rays and X-ray treatments

The next part of questions is about diagnostic or therapeutic X-rays or rays of any kind you may have had during your lifetime; this includes your childhood as far as you can remember, your adolescence and your adulthooD

The first set of questions is about diagnostic X-rays.

1A Did you ever have diagnostic X-rays to check any kind of injury, lower back problems, or other pains in a muscle or joint? This includes X-rays for a broken or fractured bone (e.g. extremities or head) or an injury to a ligament or tendon, a sprain, a whiplash, or arthritis? Do not include magnetic resonance imaging (MRI). 1A_____

Yes..... 1
No..... 2
'??'..... 99

1B How many times did you have this type of X-ray? 1B_____

1C How old were you when you first had this X-ray? 1C_____

1D How old were you when you last had this X-ray? 1D_____

2A Did you ever have a fluoroscopy, that is a type of chest X-ray commonly used for TB patients or during surgery? This includes an X-ray machine used to watch you breath. 2A_____

Yes..... 1
No..... 2
'??'..... 99

2B How many times did you have this type of X-ray? 2B_____

2C How old were you when you first had this X-ray? 2C_____

2D How old were you when you last had this X-ray? 2D_____

3A Did you ever have a regular chest X-ray? For this you stand behind an X-ray plate and pictures of your chest are taken while you hold your breath. This procedure is performed routinely during school-time and often when you start a new employment. 3A_____

Yes..... 1
No..... 2
'??'..... 99

3B How many times did you have this type of X-ray? 3B_____

3C How old were you when you first had this X-ray? 3C_____

3D How old were you when you last had this X-ray? 3D_____

4A Did you ever have an X-ray of your pelvis? This is an X-ray of the stomach or hip areA Do not include ultrasound or magnetic resonance imaging (MRI). 4A_____

Yes..... 1
No..... 2
'??'..... 99

4B How many times did you have this type of X-ray? 4B_____

4C How old were you when you first had this X-ray? 4C_____

4D How old were you when you last had this X-ray? 4D_____

5A Did you ever have an X-ray of your breasts? This is also called a mammogram or a mammography. You place the breasts on an X-ray plate while a picture is taken. 5A_____

Yes..... 1
No..... 2
'??'..... 99

5B How many times did you have this type of X-ray? 5B_____

5C How old were you when you first had this X-ray? 5C_____

5D How old were you when you last had this X-ray? 5D_____

6A Did you ever have an X-ray of your esophagus, stomache and/or small intestine (the duodenum, jejunum, and illeum)? This is also called an upper GI. You drink a cup of chalky material called barium and pictures are taken. 6A_____

Yes..... 1
No..... 2
'??'..... 99

6B How many times did you have this type of X-ray? 6B_____

6C How old were you when you first had this X-ray? 6C_____

6D How old were you when you last had this X-ray? 6D_____

7A Did you ever have an X-ray of your gallbladder? This is also called a cholangiogram. As a preparation for this kind of diagnostics you have to swallow some pills. 7A_____

Yes..... 1
No..... 2
'??'..... 99

7B How many times did you have this type of X-ray? 7B_____

7C How old were you when you first had this X-ray? 7C_____

7D How old were you when you last had this X-ray? 7D_____

8A Did you ever have an X-ray of your large intestine or colon? This is also called a lower GI. You are given an enema of barium and pictures of your colon are taken. 8A_____

Yes..... 1
No..... 2
'??'..... 99

8B How many times did you have this type of X-ray? 8B_____

8C How old were you when you first had this X-ray? 8C_____

8D How old were you when you last had this X-ray? 8D_____

9A Did you ever have an X-ray of your heart? This is also called an angiogram. A catheter is placed in an artery or vein, passed to the heart and dye is released while pictures of the heart are taken. 9A_____

Yes..... 1
No..... 2
'??'..... 99

9B How many times did you have this type of X-ray? 9B_____

9C How old were you when you first had this X-ray? 9C_____

9D How old were you when you last had this X-ray? 9D_____

10A Did you ever have an X-ray of your kidneys? This is also called an IVP or an intravenous pyelogram. For this kind of diagnostic dye is injected into a vein in the arm and then pictures of the kidneys are taken. 10A_____

Yes..... 1
No..... 2
'??'..... 99

10B How many times did you have this type of X-ray? 10B_____

10C How old were you when you first had this X-ray? 10C_____

10D How old were you when you last had this X-ray? 10D_____

11A Did you ever have an X-ray of your thyroid? This is also called a thyroid scan. You are given an injection or swallow a special fluid and pictures of your throat and neck are taken. 11A_____

Yes..... 1
No..... 2
'??'..... 99

11B How many times did you have this type of X-ray? 11B_____

11C How old were you when you first had this X-ray? 11C_____

11D How old were you when you last had this X-ray? 11D_____

12A Did you ever have an X-ray of your veins or arteries? This is also called a venogram or arteriogram. Dye is injected into your vein or artery and pictures of that area are taken. 12A_____

Yes..... 1
No..... 2
'??'..... 99

12B How many times did you have this type of X-ray? 12B_____

12C How old were you when you first had this X-ray? 12C_____

12D How old were you when you last had this X-ray? 12D_____

13A Did you ever have X-rays of teeth? 13A_____

Yes..... 1
No..... 2
'??'..... 99

13B How many times did you have this type of X-ray? 13B_____

13C How old were you when you first had this X-ray? 13C_____

13D How old were you when you last had this X-ray? 13D_____

14. Did you have at any time during your life a nuclear spin MNR (magnetic resonanz)? 14._____

Yes..... 1
No..... 2
'??'..... 99

15. Which organ or which part of the body has been investigated by MNR [subdivide the regions head / chest / abdomen including pelvis / genitalia / extremities]? How often?

	Organ, part of the body	How often?
15A	_____	15a1. _____
15B	_____	15b1. _____
15C	_____	15c1. _____
15D	_____	15d1. _____
15E	_____	15e1. _____

16. Did you have at any time during your life an echography or an ultrasonic of the abdomen, pelvis, hip, genitalia or thigh? 16._____

Yes..... 1
No..... 2
'??'..... 99

17. Which organ or which part of the body has been investigated by an ultrasound? How often?

	Organ, part of the body	How often?
17A	_____	17a1. _____
17B	_____	17b1. _____
17C	_____	17c1. _____

The following set of questions is about **X-ray treatments** you may have received as a child, an adolescent or as adult. This does not include ultraviolet treatments, such as the use of a sunlamp.

18A Did you ever receive X-ray treatment for a skin condition such as acne, psoriasis, a birthmark or a mole? 18A_____

Yes..... 1
No..... 2
'??'..... 99

18B How many times did you have this treatment? 18B_____

18C How old were you when you first had this treatment? 18C_____

18D How old were you when you last had this treatment? 18D_____

19A Did you ever receive X-ray treatment for enlarged adenoids, tonsils, or thymus, or a hearing difficulty? 19A_____

Yes..... 1
No..... 2
'??'..... 99

19B How many times did you have this type of treatment? 19B_____

19C How old were you when you first had this treatment? 19C_____

19D How old were you when you last had this treatment? 19D_____

20A Did you ever receive X-ray or cobalt treatment for any other disorder that we have not already talked about? 20A_____

Yes..... 1
No..... 2
'??'..... 99

20B [If yes], specify the disorder:_____

20B How many times did you have this type of treatment? 20B_____

20C How old were you when you first had this treatment? 20C_____

20D How old were you when you last had this treatment? 20D_____

21A Do you often (i.e. once or more per month) stay in high altitudes (e.g. frequent flights, mountain-climbing etc.)? 21A_____

Yes..... 1
No..... 2
'??'..... 99

21B [If yes], how often do you practice this habit? 21B_____ per month

21C How old were you when you started this habit? 21C_____

21D How old were you when you last practiced this habit? 21D_____

21E During this time period [21C - 21D], for how many years did you practice this habit?

Smoking history

The following set of questions is about smoking.

1 Have you ever smoked a total of 100 cigarettes or more over your lifetime? 1_____

Yes..... 1
No..... 2
'??'..... 99

2A Did you ever smoke cigarettes regularly, that is, at least one per day for six months or longer? 2_____

Yes..... 1
No..... 2
'??'..... 99

3 How old were you when you started smoking at least one cigarette per day? 3_____ years

4 Are you still smoking now? 4_____

Yes..... 1
No..... 2
'??'..... 99

5 For how many years between [age stated in Q. 3] and now did you smoke cigarettes regularly, that is at least one cigarette per day? 5_____ years

6 For this question, please think about only the years from []age stated in Q.3] and now. During the years that you smoked regularly, how many cigarettes per day did you usually smoke? 6_____ years

7 Did you ever smoke at least one cigar per week at least six months or longer? 7_____

Yes..... 1
No..... 2
'??'..... 99

8 How old were you when you started smoking at least one cigar per week? 8_____ years

9 Are you still smoking at least one cigar per week now? 4_____

Yes..... 1
No..... 2
'??'..... 99

10 For how many years between [age stated in Q. 8] and now did you smoke cigarettes regularly, that is at least one cigar per week? 10_____ years

11 For this question, please think about only the years from []age stated in Q. 8] and now. During the years that you smoked regularly cigars, how many cigars per week did you usually smoke? 11_____ years

12 Did you ever smoke at least one pipe of tobaco per week at least six months or longer? 12_____

Yes..... 1
No..... 2
'??'..... 99

13 How old were you when you started smoking at least one pipe of tobaco per week? 13_____ years

14 Are you still smoking at least one pipe of tobaco per week now? 14_____

Yes..... 1
No..... 2
'??'..... 99

15 For how many years between [age stated in Q. 8] and now did you smoke pipes of tobaco regularly, that is at least one pipe of tobaco per week? 15_____ years

16 For this question, please think about only the years from []age stated in Q. 8] and now. During the years that you smoked regularly pipes of tobaco, how many pipes of tobaco per week did you usually smoke? 16_____ years

Use the following table for an overview about the smoking history. Mark the years (if possible months) during which smoking has been practiced. Provide the average number of cigarettes per day or cigars or pipes of tobaco per week. [Try to be as complete as possible, since smoking is an important confounder.]

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug	Sep.	Oct.	Nov.	Dec.
1960												
1961												
1962												
1963												
1964												
1965												
1966												
1967												
1968												
1969												
1970												
1971												
1972												
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1987												
1988												
1989												
1990												
1991												
1992												
1093												
1994												
1995												
1996												
1997												
1998												
1999												
2000												
2001												

Occupational history

1 Up to now, hat was your usual job? That is, what job did you hold longest?

[verbatim] _____

2 What kind of company was it? What industry?

[verbatim] _____

3 Please give now all occupations you hold during your lifetime for at least half a year. Please start with your first job and give begin and end of occupation

	kind of occupation	from		until	
		month	year	month	year
3.A					
3.B					
3.C					
3.D					
3.E					
3.F					
3.G					
3.H					

4 In what kind of industry/company were you occupied?

	kind of industry/company	from		until	
		month	year	month	year
4.A					
4.B					
4.C					
4.D					
4.E					
4.F					
4.G					

4.H

From age 12 until now, did you ever have a regular job or a hobby which lasted 6 months or longer where you were exposed to any ot the following metals (either from your work activities or from another worker's activities; regular means at least weekly)?

5A	Lead or lead compounds?	(yes..1;	no..2;	'??'..99)	5A____
5B	Mercury or mercury compounds?	(yes..1;	no..2;	'??'..99)	5B____
5C	Arsenic or arsenic compounds?	(yes..1;	no..2;	'??'..99)	5C____
5D	Lithium or lithium compounds?	(yes..1;	no..2;	'??'..99)	5D____
5E	Boron or boron compounds?	(yes..1;	no..2;	'??'..99)	5E____
5F	Manganese or manganese compounds?	(yes..1;	no..2;	'??'..99)	5F____
5G	Tin or tin compounds?	(yes..1;	no..2;	'??'..99)	5G____
5H	Zinc or zinc compounds?	(yes..1;	no..2;	'??'..99)	5H____
5I	Iron or iron compounds?	(yes..1;	no..2;	'??'..99)	5I ____
5J	Copper or copper compounds?	(yes..1;	no..2;	'??'..99)	5J____
5K	Chromic or chrom compounds?	(yes..1;	no..2;	'??'..99)	5K____
5L	Cadmium or cadmium compounds?	(yes..1;	no..2;	'??'..99)	5L____
5M	Aluminium or aluminium compounds?	(yes..1;	no..2;	'??'..99)	5M____
5N	Selenium or selenium compounds?	(yes..1;	no..2;	'??'..99)	5N____
5O	Nickel or nickel compounds?	(yes..1;	no..2;	'??'..99)	5O____
5P	Other	(yes..1;	no..2;	'??'..99)	5P____

[verbatim]:_____

From age 12 until now, did you ever have a regular job or a hobby which lasted 6 months or longer where you were exposed to any ot the following metals (either from your work activities or from another worker's activities; regular means at least weekly)?

6A	drugs or pharmaceuticals?	(yes..1;	no..2;	'??'..99)	6A____
6B	chemicals used to develop photographic films?	(yes..1;	no..2;	'??'..99)	6B____
6C	dyes?	(yes..1;	no..2;	'??'..99)	6C____
6D	if dyes, were they: hair dyes?	(yes..1;	no..2;	'??'..99)	6D____

6E	printing dyes?	(yes..1;	no..2;	'??'..99)	6E____
6F	fabric/yarn (textile) dyes?	(yes..1;	no..2;	'??'..99)	6F____
6G	other? specify:_____	(yes..1;	no..2;	'??'..99)	6G____
6H	grease or oil?	(yes..1;	no..2;	'??'..99)	6H____
6I	solvents (chemicals which dissolve grease, oil, paint or other materials?	(yes..1;	no..2;	'??'..99)	6I ____
6J	if yes, specify 6J_____				
6K	chemicals used to make rubber?	(yes..1;	no..2;	'??'..99)	6K____
6L	chemicals used to make plastics?	(yes..1;	no..2;	'??'..99)	6L____
6M	chemicals used to control insects (insecticides)?	(yes..1;	no..2;	'??'..99)	6M____
6N	chemicals used to control fungi (fungicides)?	(yes..1;	no..2;	'??'..99)	6N____
6O	chemicals used to control rodents (rodenticides)?	(yes..1;	no..2;	'??'..99)	6O____
6P	chemicals used to control weeds (herbicides)?	(yes..1;	no..2;	'??'..99)	6P____
6Q	chemical fertilizers?	(yes..1;	no..2;	'??'..99)	6Q____
6R	stains, varnishes or other wood finishes?	(yes..1;	no..2;	'??'..99)	6R____
6S	paints or paint products or thinner?	(yes..1;	no..2;	'??'..99)	6S____
7A	natural gas, gasoline, or fuel products?	(yes..1;	no..2;	'??'..99)	7A____
	if yes, was it:				
7B	motor vehicle fuel? [excluding pumping gas for personal auto]	(yes..1;	no..2;	'??'..99)	7B____
7C	aircraft fuel?	(yes..1;	no..2;	'??'..99)	7C____
7D	household fuel oil?	(yes..1;	no..2;	'??'..99)	7D____
8A	chemicals used to sterilize instruments?	(yes..1;	no..2;	'??'..99)	8A____
	if yes, was it:				
8B	phenols?	(yes..1;	no..2;	'??'..99)	8B____
8C	ethylene oxide?	(yes..1;	no..2;	'??'..99)	8C____
8D	propylene oxide?	(yes..1;	no..2;	'??'..99)	8D____
8E	formaldehyde?	(yes..1;	no..2;	'??'..99)	8E____
9A	anesthetic gases?	(yes..1;	no..2;	'??'..99)	9A____
	if yes, was it:				

9B	ether?	(yes..1;	no..2;	'??'..99)	9B____
9C	halothane?	(yes..1;	no..2;	'??'..99)	9C____
9D	methoxyflurane (penthrane)?	(yes..1;	no..2;	'??'..99)	9D____
9E	trichlorethylene (trilene)?				9E____
9F	enflurane (ethrane)?	(yes..1;	no..2;	'??'..99)	9F____

10. Photocopy or Xerox machines? (yes..1; no..2; '??'..99) 10_____

11. From age 12 until now, did you ever have a regular job or a hobby which lasted 6 months or longer where you were exposed to X-rays or radiation from any source (either from your work activities or from another worker's activities; regular means at least weekly)?

(yes..1; no..2; '??'..99) 11_____

We have now completed the interview. Is there anything about you, your health or your lifestyle that I did not mention, but that you think would be important for me to know?

Time needed to complete the interview: _____ minutes

Interviewer's ranking of the interview:

cooperation of interview partner (1 good, ... , 5 poor) _____

reliability of answers obtained (1 good, ... , 5 poor) _____

Appendix 3: A design of informed consent for Down syndrome family

سلطنة عمان
وزارة الصحة
دائرة خدمات الدم
Sultanate of Oman
Ministry of Health
Department of Blood Services

HOSPITAL NO:……..
NAME:……
AGE/DOB:…….. SEX :……
ATIONALITY:……
Word/ Dept:…… Unit:…….

استمارة موافقة كتابية على اجراء فحوصات وراثية
WRITING EXPRESSED GENETIC TESTING CONSENT FORM

A) I _____
_____ Age _____
Resident of _____
Hereby give this written consent/ permission for the genetic testing.
Relative _____
Relationship _____

I had been given adequate information about nature effects, potential harms and benefits of genetic testing, about its limitations, possibilities of informative results and chances of exact prediction.

I had been informed that the results will be kept strictly confidential and should not be disclosed to anybody without my permission.

Patient/ Relative _____
Signature _____
Date _____ Time _____

B) I also agree to be conducted at home/ office.

Name. Staff No. and signature of attending doctor _____
_____

Date: / /

Time: / /

_____ أنا
_____ عما البالغ من العمر

والمقيم في_____
أمنح هذه الموافقة والتفويض الكتابي لأجراء الفحوصات الوراثية لشخصي_____
أو ل _____

(صلة القرابة _____) ويجريها الأطباء العالمين، لقد تم اعطائي معلومات كافية عن احتمالات فوائد ومخاطر الفحوصات الوراثية وكذلك عن احتمالات النتائج الايجابية وعدم القدرة على التنبئ بماعية الفحوصات.

كما أنه قد تم ابلاغي بأن نتائج هذه الفحوصات سوف تكون سرية ولن يتم افشاؤها لأي شخص بدون اذن من.

المريض/ولي الأمر _____
التوقيع _____
التاريخ_____الوقت _____

ب) كما أني أوافق على الاتصال بي في المنزل أو في العمل .

اسم الطبيب المعالج ورقمه الظيفي وتوقيعه
_____
_____

التاريخ: / /

الوقت:

Appendix 4: Parental and meiotic origin analysed by STR analyses.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
	Mat MI	Mat MII	Mat ni	Pat MI	Pat MII	Pat ni	Mit.	Ni	#1	#2	#3	#4
A1	X											
A2	X								215-1414			
A3	X								1445-1252			
A4	X											
A5	X											
A6	X											
A7	X								1445-1252	1252-1890		
A8		X							1445-1890			
A9	X								1432-1258	1258-1252		
A10	X								215-1414	1258-1445		
A11	X								1414-1258	1258-1445		
A12	X											
A13	X											
A14			X									
A15								X				
A16	X								1252-1890			
A17		X							215-1414	1252-1890		
A18	X								1252-1890			
A19	X											
A20	X								1445-1252			
A21	X								1414-1258			
A22	X											
A23	X								1252-1890			
A24	X								215-1414			
A25	X								1414-1258	1258-1445		
A26		X							1414-1258			
A27		X							120-1258			
A28		X							1414-1258			
A29	X								120-1414	1414-1258	1258-1445	1445-1252
A30		X							215-1414	1252-1890		
A31	X											
A32	X								1252-1890			
A33	X								120-1414			
A34	X								1445-1252			
A35						X						
A36						X						
A37		X							1414-1258			
A38	X											
A39							X					
A40							X					
A41	X								215-1414	1258-1445		
A42						X						
A43	X											
A45							X					
A46		X							1414-1258	1445-1252		
A47						X						

Appendix 4: Parental and meiotic origin analysed by STR analyses continued.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
A48	X								1445-1252			
A50			X									
A51	X											
A52	X								1252-1890			
A53	X								1258-1252			
A54		X							215-1432	1432-1414		
A55	X								1414-1258	1258-1445		
A56	X								1258-1445			
A58						X						
A59	X								215-1414	1414-1258		
A60	X											
A61	X											
A62	X											
A63		X							1258-1445			
A64		X							1414-1258			
A65		X							215-120	1445-1252	1252-1890	
A66	X								1414-1258	1258-1445		
A67		X							1414-1258			
A68		X							1414-1258			
A69		X							215-1258	1445-1252	1252-1890	
A70				X								
A71	X								1252-1890			
A72	X								120-1414	1258-1445	1252-1890	
A73						X						
A74	X											
A75	X								120-1414	1258-1445		
A76	X											
A77	X								1252-1890			
A78	X											
A79	X											
A80		X							120-1414	1252-1890		
A81	X											
A82	X											
A83		X							215-1414			
A84	X								215-1258			
A85	X											
A86		X							120-1414			
A87	X								215-1414	1414-1258		
A88		X							1445-1252			
A89	X											
A90		X							215-1432	1414-1258	1445-1890	
A91	X											
A92							X					
A93	X											
A94		X							1414-1258	1445-1252	1252-1890	
A95	X											
A96		X							1252-1890			
A97		X							1432-1414			

Appendix 4: Parental and meiotic origin analysed by STR analyses continued.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
A98	X								215-1414	1414-1445	1445-1252	
A99	X											
A100	X											
A101	X											
A102	X								1258-1445	1252-1890		
A103	X								1258-1445			
A104	X											
A105		X							120-1414			
A106						X						
A107		X							1258-1445	1445-1252		
A108	X								1258-1445			
A110	X											
A111	X								215-1414	1414-1258	1258-1445	
A112		X							1414-1258			
A113		X							120-1258			
A115	X								1252-1890			
A117	X											
A118	X								1414-1258	1258-1890		
A119	X											
A120						X						
A121						X						
A122	X								1414-1258	1445-1252		
A123	X								1445-1252			
A124		X							120-1414			
A125	X											
A126		X							215-1445			
A127		X							1414-1258			
A128							X					
A129	X								120-1414	1414-1258		
A130		X							1252-1890			
A131	X											
A132	X											
A133		X							1414-1258			
A134	X								1252-1890			
A136	X											
A137						X						
A138		X							120-1414	1252-1890		
A139a		X							215-1414	1445-1252		
A139b	X											
A140		X							1414-1258			
A141	X											
A142	X								1252-1890			
A143	X											
A144	X								1258-1445	1252-1890		
A145		X							1414-1258			
A146				X								
A147	X											
A148	X											

Appendix 4: Parental and meiotic origin analysed by STR analyses continued.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
A150	X											
A151	X											
A152		X							215-1414			
A153	X								120-1414	1414-1258	1252-1890	
A154		X							215-1414			
A155								X				
A156	X											
A157	X											
A158							X					
A159		X							120-1414	1414-1258	1445-1252	
A160		X							120-1414			
A161	X											
A162		X							1414-1258			
A163				X					215-1258			
A164	X								1414-1258	1445-1252	1252-1890	
A165	X								1414-1258	1258-1445		
A166		X							1252-1890			
A167	X											
A168								X				
A169	X								215-1432	1432-1414		
A170		X							1414-1258			
A171	X								1252-1890			
A172	X								120-1414	1414-1258		
A173	X								1445-1252			
A174		X							1414-1258			
A175	X											
A176	X											
A177	X								1252-1890			
A178						X						
A179	X								215-1414	1414-1258	1258-1445	1252-1890
A180					X				1258-1445			
A181	X								215-1432	1258-1445		
A182	X								1252-1890			
A183	X											
A184	X								1252-1890			
A185		X							120-1414			
A186		X							120-1414	1414-1258		
A187					X							
A188	X								1258-1445	1445-1252		
A189	X											
A190	X											
A191								X				
A192	X								1445-1252	1252-1890		
A193	X								1258-1445	1445-1252	1252-1890	
A194	X								1252-1890			
A195				X								
A196	X								1445-1252			

Appendix 4: Parental and meiotic origin analysed by STR analyses continued.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
A197	X								1414-1258	1258-1445		
A198		X							1445-1252	1252-1890		
A199					X							
A200	X											
A201	X								215-1414	1414-1258		
A202								X				
A203	X											
A204								X				
A205		X							120-1414			
A206		X							120-1414	1445-1252		
A207		X							1414-1258	1445-1252	1252-1890	
A208		X							120-1414	1258-1445		
A209	X								215-120	120-1890		
A210		X							1445-1252			
A211					X				215-1432	1445-1252	1252-1890	
A212	X											
A213					X							
A214							X					
A215		X							1252-1890			
A216	X											
A217	X								215-1258	1445-1890		
A218				X								
A219	X								120-1414	1414-1258	1252-1890	
A220	X								120-1414	1414-1258		
A221		X							215-1414	1252-1890		
A222	X								1252-1890			
A223		X							1414-1258			
A224	X								1252-1890			
A225	X								1445-1252	1252-1890		
A226		X							120-1414	1414-1258	1258-1445	1252-1890
A227	X											
A228		X							215-120	1258-1445		
A229		X							120-1414	1252-1890		
A231	X											
A232	X								1252-1890			
A233	X											
A234			X									
A235	X								1258-1445	1252-1890		
A236								X				
A238	X								1445-1890			
A239	X								1445-1890			
A240	X								215-120	120-1414	1252-1890	
A241	X								1445-1252			
A242		X							120-1414			
A242							X					
A243	X								1414-1258			
A244	X								215-1414	1414-1258		
A246	X								1252-1890			

Appendix 4: Parental and meiotic origin analysed by STR analyses continued.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
A247	X											
A248	X											
A249	X											
A250	X											
A251	X											
A252	X								1252-1890			
A253	X											
A254	X											
A255		X										
A256	X								1258-1445	1445-1252		
A257	X								1258-1445			
A258	X											
A259	X											
A261		X							1258-1445	1252-1890		
A262	X								1414-1258	1258-1445		
A263	X								1252-1890			
A264	X											
A265		X							1414-1258	1252-1890		
A266	X											
A149A	X											
A149	X								1258-1445	1445-1252		
AN1	X								1414-1258			
AN2		X							1414-1258			
AN3	X											
AN4	X								1252-1890			
B2	X											
B3							X					
B5		X							215-120	1414-1445	1445-1252	
B7					X				120-1414	1258-1445	1445-1252	
B8	X								1414-1258	1258-1445	1445-1252	1252-1890
B9	X								1445-1252			
B13								X				
B14	X								1252-1890			
B15		X							1258-1445	1252-1890		
B17	X											
B18	X											
B19								X				
B20	X								1258-1445			
B21		X							120-1414			
B22	X								1445-1252	1252-1890		
B23	X											
B25	X								1445-1252	1252-1890		
B26								X				
B27	X								215-120	1414-1445	1445-1252	
B28		X							120-1414			
B29	X								1414-1258	1252-1890		
B30	X								120-1414	1414-1258		
B31								X				
B33	X								1445-1252			

Appendix 4: Parental and meiotic origin analysed by STR analyses continued.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
B37		X							215-120			
B38	X								1252-1890			
B39					X				1258-1445	1445-1252		
B42	X								1252-1890			
B44	X											
B46								X				
B47	X											
B48		X							120-1414	1414-1258	1445-1252	1252-1890
B49		X							120-1414	1414-1258		
B51		X							1252-1890			
B53		X							215-120			
B54		X							120-1414			
B55	X								120-1414	1252-1890		
B56	X								120-1414	1258-1445		
B60	X								1414-1258	1258-1445		
B63	X								120-1414	1414-1258		
B64	X								1414-1258			
B65		X							215-120			
B67	X											
B68		X							120-1414			
B70	X											
B71	X								215-120	120-1414		
B73	X											
B74								X				
B75	X											
B76	X											
B78	X								1445-1252			
B79					X				120-1414			
B80		X							215-120			
B82		X							1252-1890			
B83	X								1445-1252	1252-1890		
B84		X							215-120			
B85	X											
B87	X								1414-1258	1258-1445		
B88		X							120-1258			
B90		X							120-1414			
B91	X											
B92		X							120-1414	1414-1258	1252-1890	
B93				X								
B94	X											
B95					X				1414-1445			
B97	X								1414-1258	1252-1890		
B98	X											
B99	X								1252-1890			
B100	X											
B103	X								1252-1890			
B105	X											
B106	X											
B107	X								1258-1445			
B108	X								120-1414	1414-1258	1252-1890	

Appendix 4: Parental and meiotic origin analysed by STR analyses continued.

Family ID	Parental and meiotic origin of the non-disjunction								Crossover between STR markers D21S			
B110	X								215-1414			
B111	X											
B112	X											
B113	X											
B114	X								215-120	120-1414		
B115	X								215-120	120-1414	1252-1890	
B116								X				
B118				X								
B119		X							215-1414			
B120		X							120-1258	1252-1890		
B121		X							120-1414	1414-1258	1258-1445	1445-1252
B122	X								1252-1890			
B123		X							1414-1445	1445-1252	1252-1890	
B124	X											
B125	X											

Appendix 5: Y-STR Haplotypes in 164 fathers of Down syndrome families from Oman population. n = number of individuals observed for each haplotype.

Haplotype ID	DYS 19	DYS 389I	DYS 39II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385ab	DYS 438	DYS 439	DYS 437	n
2/3	13	13	32	24	10	11	12	16,17	10	12	14	1
4/3	14	13	30	23	11	11	12	13,18	10	11	14	1
5/1	15	13	31	23	11	11	12	13,16	10	11	14	1
6/3	15	13	30	23	10	11	12	14,20	9	14	15	1
9/3	15	13	30	23	10	11	12	13,18	11	12	14	1
10/3	15	14	31	23	10	11	12	14,21	9	11	16	1
11/3	14	13	30	23	10	11	12	13,18	10	11	14	1
12/3	15	14	32	25	10	11	13	11,14	11	10	14	1
15/3	14	13	30	24	10	11	12	13,18	10	11	15	1
16/3	15	13	30	23	10	11	12	14,14	9	13	15	1
17/3	13	13	32	24	10	11	12	17,17	10	11	14	1
18/3	14	13	31	23	10	11	12	13,17	10	11	14	1
19/3	14	13	30	25	10	11	12	13,17	9	13	15	1
20/1	14	14	30	23	9	11	13	14,17	9	11	15	1
22/3	13	13	32	24	10	11	12	15,21	10	12	14	1
23/3	14	13	31	23	10	11	12	13,15	10	12	14	1
24/3	16	13	31	25	11	11	13	11,15	11	10	15	1
25/3	15	13	30	24	10	14	13	14,15	9	12	14	1
28/3	14	13	31	24	10	11	12	14,17	10	11	14	1
29/1	14	13	31	23	10	11	12	13,17	10	11	14	1
30/3	15	13	30	23	10	11	12	13,19	9	13	15	1
31/1	15	13	29	23	10	11	12	16,17	9	12	16	1
33/3	15	13	30	25	11	11	13	11,14	11	10	14	1
34/3	14	13	30	23	10	11	12	13,18	10	11	14	1
38/1	15	13	29	23	10	11	12	16,18	9	11	14	1
39/3	15	12	29	24	9	11	12	18,20	9	11	14	1
40/1	14	14	30	23	10	11	13	14,17	9	10.2	15	1
41/3	14	13	31	23	11	11	12	13,18	10	11	14	1
42/1	14	13	31	23	11	11	12	12,18	10	11	14	1
49/3	16	14	31	24	11	11	13	11,14	11	10	14	1
50/3	14	13	31	23	10	11	12	13,14	10	11	14	1
51/3	14	13	31	23	11	11	12	13,15	10	11	14	1
52/3	14	13	29	23	10	13	13	16,16	9	11	14	1
53/3	14	13	29	24	10	13	13	14,16	9	11	14	1
54/3	14	13	31	23	10	11	12	13,17	10	11	14	1
59/3	14	13	31	24	10	11	12	14,17	10	11	14	1
60/3	15	13	30	22	11	12	12	15,18	9	11	14	1
61/3	14	13	31	23	10	11	12	13,17	10	11	14	1
62/1	14	13	31	24	10	11	12	14,17	10	12	14	1
63/3	14	13	31	23	10	11	12	12,17	10	11	14	1
64/1	14	13	29	23	10	11	12	13,17	10	11	14	1
66/3	14	13	31	23	10	11	12	14,17	10	11	14	1
69/1	14	13	30	23	10	11	12	13,16	10	11	14	1
70/1	14	13	30	23	11	11	12	14,18	10	11	14	1
72/1	13	13	32	24	10	11	12	16,17	10	13	14	1
74/1	15	13	30	24	10	11	13	14,16	10	13	14	1
77/1	14	13	29	23	10	13	13	14,16	9	11	14	1
78/3	13	10	27	24	11	11	14	15,16	10	12	14	1
81/3	14	13	31	23	10	11	12	13,17	10	12	14	1
83/3	13	13	32	24	10	11	12	16,18	10	13	14	1
85/3	14	13	31	23	10	11	12	13,17	10	11	14	1
87/3	14	12	28	23	10	11	12	14,15	9	13	15	1
88/3	14	13	31	23	10	11	12	13,17	10	12	14	1
90/3	13	13	32	24	10	11	12	16,17	10	12	14	1
91/1	14	12	28	22	10	14	11	14,17	11	12	15	1
92/3	14	14	32	23	11	11	12	13,19	10	11	14	1
95/1	15	13	30	21	10	11	14	16,17	11	12	14	1
98/1	13	13	32	25	10	11	12	16,17	10	12	14	1
99/3	14	13	30	23	10	11	12	14,18	10	12	14	1
100/1	13	13	30	25	10	11	13	16,17	10	13	14	1

Appendix 5 continued: Y-STR Haplotypes in 164 fathers of Down syndrome families from Oman population. n = number of individuals observed for each haplotype.

Haplotype ID	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385ab	DYS 438	DYS 439	DYS 437	n
101/3	14	14	31	23	11	11	12	13,19	10	12	14	1
102/1	13	13	32	24	10	11	12	16,16	10	13	14	1
104/1	14	13	31	23	10	11	12	14,18	10	13	14	1
106/1	14	14	30	23	10	11	13	14,17	9	12	14	1
107/1	14	13	30	23	11	11	12	13,19	10	13	14	1
110/3	14	13	31	23	10	11	12	13,18	10	12	14	1
112/3	13	13	30	25	10	11	13	16,17	10	11	14	1
115/3	13	13	30	24	10	11	13	16,17	10	13	14	1
118/1	13	13	30	24	10	11	12	16,16	10	12	14	1
121/3	14	13	30	24	10	13	13	14,16	9	11	14	1
122/1	14	13	30	23	10	11	11	12,14	10	11	14	1
124/1	14	13	29	23	10	13	13	14,16	9	11	14	1
126/1	13	13	32	24	10	11	12	16,17	10	12	14	1
128/3	15	13	30	25	11	11	13	11,14	11	10	14	1
129/1	15	13	29	25	11	11	14	11,15	11	10	14	1
130/3	15	14	31	23	9	11	13	16,17	10	11	14	1
131/1	15	13	30	25	11	11	13	11,14	11	10	14	1
133/3	15	13	32	21	10	11	13	15,21	11	12	14	1
136/1	14	13	30	23	10	11	12	13,17	10	11	14	1
138/1	15	13	31	23	11	11	12	13,18	10	11	14	1
139/3	14	13	30	23	10	11	12	13,17	10	11	14	1
142/1	15	13	30	24	10	11	13	14,16	10	12	14	1
143/3	15	12	29	21	10	11	16	17,17	11	12	14	1
144/3	14	13	31	23	10	11	12	13,17	10	11	14	1
145/3	14	12	28	22	10	11	12	14,16	10	13	14	1
147/3	14	14	30	23	11	10	13	13,21	11	11	16	1
148/3	13	13	32	24	10	11	12	16,17	10	12	14	1
150/3	14	13	30	24	10	13	13	14,16	9	11	14	1
151/1	16	13	31	24	11	11	13	11,14	11	10	14	1
152/3	15	13	30	24	10	12	13	14,16	10	12	14	1
153/1	14	13	30	23	10	11	12	13,17	10	11	14	1
154/3	14	13	29	23	11	15	12	11,15	12	12	15	1
155/3	14	12	28	23	10	11	12	13,17	9	12	15	1
156/3	14	14	30	23	10	10	14	14,18	11	12	15	1
157/3	12	13	30	21	10	11	14	18,18	11	12	14	1
158/3	15	13	29	24	10	11	13	14,16	10	12	14	1
160/1	15	13	31	21	10	11	13	15,17	11	12	14	1
162/3	15	12	29	21	11	11	13	17,18	11	11	14	1
163/3	14	13	31	23	10	11	12	13,14	10	11	14	1
166/3	13	31	32	24	10	11	12	16,18	10	12	14	1
167/3	14	13	30	23	11	11	12	13,19	10	12	14	1
168/3	13	13	32	24	10	11	11	16,18	10	12	14	1
169/3	14	13	30	23	10	11	12	13,18	10	11	14	1
170/3	15	13	31	25	11	11	13	12,14	11	10	14	1
173/3	16	14	32	24	11	11	13	11,14	11	10	14	1
176/1	14	12	28	23	10	11	12	14,15	9	12	14	1
177/1	14	12	28	23	10	11	12	14,15	9	12	14	1
180/3	12	13	32	24	10	11	12	16,20	10	12	14	1
181/3	13	13	32	24	10	11	12	16,18	10	12	14	1
183/1	13	13	32	24	10	11	12	16,18	10	11	14	1
184/3	14	13	29	24	10	11	9	18,19	10	12	14	1
187/1	14	13	29	24	10	11	9	18,19	10	12	14	1
189/1	13	13	32	24	10	11	12	16,18	10	12	14	1
190/3	14	13	30	23	10	11	12	13,19	10	12	14	1
191/3	14	13	30	23	11	11	12	13,18	10	12	14	1
192/1	15	13	29	24	9	11	12	18,19	9	12	14	1
194/1	14	14	31	24	10	11	12	12,19	10	13	14	1
195/3	14	13	30	23	11	11	12	13,18	10	12	14	1
196/3	14	13	30	23	11	11	12	13,18	10	12	14	1
197/3	12	14	32	24	11	11	12	13,19	10	11	14	1

Appendix 5 continued: Y-STR Haplotypes in 164 fathers of Down syndrome families from Oman population. n = number of individuals observed for each haplotype.

Haplotype ID	DYS 19	DYS 389I	DYS 39II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385ab	DYS 438	DYS 439	DYS 437	n
198/3	14	13	30	23	11	11	12	13,17	10	12	14	1
199/3	14	13	30	23	12	11	12	13,18	10	12	14	1
200/3	13	13	32	24	10	11	12	16,19	10	12	14	1
201/3	13	13	32	24	10	11	12	16,18	10	12	14	1
202/3	14	13	30	23	11	11	12	13,17	10	12	14	1
203/3	13	13	32	24	10	11	12	15,18	10	12	14	1
209/1	15	13	30	23	14	11	12	13,17	10	11	14	1
210/3	14	13	29	23	10	13	13	14,16	10	13	14	1
211/3	14	13	31	24	11	11	12	17,18	10	12	14	1
213/3	13	13	32	24	10	11	12	16,17	10	12	14	1
215/3	14	14	30	23	10	10	14	13,19	11	10	15	1
217/3	14	13	30	23	13	11	12	16,16	10	11	14	1
218/3	15	13	30	23	10	11	12	14,20	9	14	15	1
219/1	16	13	30	24	10	11	13	11,14	11	10	14	1
220/3	--	13	32	24	10	11	12	16,17	10	12	14	1
221/3	14	13	29	23	10	12	12	15,18	9	11	15	1
222/1	14	13	31	25	10	11	13	14,17	10	11	14	1
224/1	15	13	30	23	10	11	12	14,14	9	13	15	1
227/3	14	12	29	22	10	14	11	13,13	10	13	15	1
229/3	14	14	30	24	10	11	12	12,20	10	12	14	1
230/3	14	13	29	21	10	11	15	13,15	10	12	16	1
231/3	16	12	28	24	9	11	12	17,19	9	12	14	1
233/3	14	13	30	23	11	11	12	12,18	10	11	14	1
234/3	14	14	30	24	10	11	12	13,19	10	12	14	1
235/3	15	13	31	23	10	12	12	13,19	10	12	14	1
237/3	14	13	29	22	11	11	12	13,19	10	11	14	1
238/3	14	13	30	23	11	11	12	13,18	10	11	14	1
239/1	14	13	30	23	11	11	12	13,18	10	11	14	1
240/1	14	14	30	24	10	11	12	12,20	10	12	14	1
241/3	17	12	28	24	9	13	12	18,19	9	12	14	1
243/3	15	12	28	24	9	11	10	19,19	9	11	14	1
244/3	14	14	30	24	10	11	12	12,21	10	12	14	1
246/3	14	14	30	24	10	11	12	12,20	10	12	14	1
248/1	14	13	30	23	10	11	12	13,18	10	11	14	1
251/3	14	13	30	23	9	11	12	12,19	10	11	14	1
252/1	14	13	29	21	10	11	15	13,15	10	12	16	1
253/3	14	14	30	24	8	11	12	14,18	9	11	14	1
254/3	14	14	30	25	10	13	13	11,15	12	12	15	1
260/1	14	15	31	24	10	11	12	13,18	10	12	14	1
261/1	14	13	30	23	11	11	12	12,18	10	12	14	1
264/3	14	13	29	23	10	11	12	15,18	9	11	14	1
266/3	14	12	28	25	10	11	13	14,20	11	11	14	1
267/3	14	12	27	24	11	15	12	11,15	12	12	15	1

Appendix 6 Calculation of the expected number of DS cases in Oman based on the age specific rates published by the authors given in the table

Age distribution		Lindsten et al. 1981	Lindsten et al. 1981
Oman 2000-2004	N	age specific rate	DS expected
< 20	11360	0,000626611	7,1
20 - 24	59079	0,000707272	41,8
25 - 29	60031	0,00091771	55,1
30 - 34	37827	0,001570074	59,4
35 - 39	22570	0,004097399	92,5
40 - 44	7995	0,015161898	121,2
44 and More	1158	0,046043165	53,3
Total	200020		**430**
Age distribution		Cuckle et al. 1987	Cuckle et al. 1987
Oman 2000-2004	N	age specific rate	DS expected
< 20	11360	0,000640	7,3
20 - 24	59079	0,000676	39,9
25 - 29	60031	0,000844	50,7
30 - 34	37827	0,001532	58,0
35 - 39	22570	0,004410	99,5
40 - 44	7995	0,016440	131,4
44 and More	1158	0,004090	4,7
Total	200020		**392**