2   INTRODUCTION

Down syndrome (DS) due to trisomy 21 is a complex human developmental and neurodegenerative disease, which represents a central issue in medical genetics. It is the most frequent chromosomal abnormality of live born and the most common clinical cause of mental retardation.

2.1   Incidence

With a median incidence rate of 1: 700, it is ten times more frequent than AIDS in Europe. Maternal age influences significantly the risk of conceiving a baby with the syndrome. The risk of occurence is once in every 1,500 births when the mother's age is below 25; one in every 400 births when the mother's age is over 35; once in every 40 births when the mother's age is over 45. Strikingly, approximately 65 to 80% of all fetuses with Trisomy 21 are lost spontaneously (Byrne and Ward, 1994). Currently there are more than 2,000,000 people with Down syndrome worldwide.

2.2   History of Down Syndrome

Almost certainly, there have always been people with Down syndrome in all ethnic groups. While the painting in Figure 2-1 where two DS individuals are depicted is dated approximately 1515, children with Down syndrome are already seen in pictures from 1505. Martinez-Frias (Martinez-Frias, 2005) reported what seems to be the earliest evidence of DS in a terra-cotta head dated from ~500 AD belonging to the Tolteca culture of Mexico. Edouard Séguin, a French physician, was the first person to describe the condition in a book published in 1846 in Paris. However, the first person to recognize Down syndrome as an entity was Dr. John Langdon Down (1828-1896), an English physician working in Surrey. In 1866 he published an essay in the Journal of Mental Science reprinted in Mental retardation (Down, 1995) in which he describes a set of children with common features who are distinct from other children with mental retardation. Early in the 1960s, the condition became called "Down's syndrome." In the 1970s, an American revision of scientific terms changed it simply to "Down syndrome," while it is still called "Down's" in the

UK and some places in Europe. DS had become the most recognizable form of mental retardation and most people affected were institutionalized. Few of the associated medical problems were treated, and most died in infancy or early adult life. Until the middle of the 20th century, the cause of Down syndrome remained unknown, although the occurrence in all ethnic groups, the association with older maternal age, and the rarity of recurrence had been noticed. Standard medical texts assumed it was due to a combination of inheritable factors, which had not been identified. There was some expert opinion that it might result from trauma occurring during pregnancy.

Figure 2-1: The Adoration of the Christ Child (1515). By a follower of Jan Joest of Kalkar. Oil on wood. The Jack and Belle Linsky Collection, Metropolitan Museum of Art, New York, NY.

Three years after that Ford and Hamerton (1956) had established the diploid human chromosome number of 46[1] (Figure 2-2.A) (Ford and Hamerton, 1956), Jerôme Lejeune in France and Patricia Jacobs in Scotland simultaneously

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[1] *46 chromosomes per cell*: 22 pair of autosomes or non-sex chromosomes and one pair of sex chromosomes, xx in females, xy in males.

determined the cause of Down syndrome (Jacobs *et al.*, 1959; Lejeune *et al.*, 1959). They found that individuals with Down syndrome carried additional genetic material in their cells. Instead of having 46 chromosomes in each cell, individuals with Down syndrome most commonly have 47 chromosomes with the extra copy of human chromosome number 21 (HSA21 or chr21) (Figure 2-2.B). Therefore, the term trisomy 21 was used to describe this configuration of three chromosome 21. It was the first time that a chromosomal abnormality was found associated with human pathology.
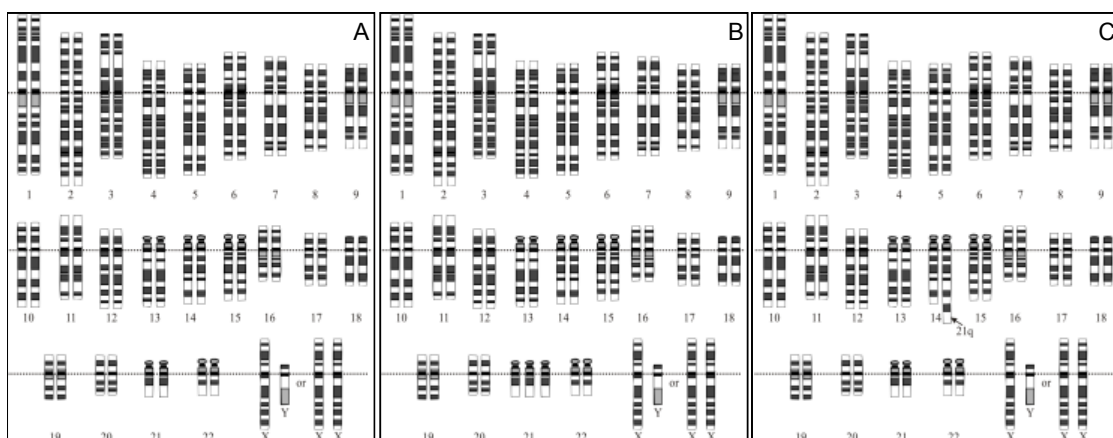

Figure 2-2: Karyotypes. A) Normal human Karyotype. B) Karyotype for trisomy Down syndrome. Notice the three copies of chromosome 21. C) Translocation karyotype for Down syndrome with 14/21 Robertsonian translocation. Notice the three copies of 21q (the long arm of chromosome 21).

2.3 Genetics of Trisomy 21

Aneuploidy is the condition in which the number of chromosomes is abnormal due to gain or loss of chromosomes. In other words, it is a chromosomal state where the number of chromosomes is not a multiple of the haploid set. Aneuploidies can be acquired (clonal) or constitutive (congenital). Clonal chromosome disorders occurring or acquired at any postnatal age are often closely related with the origin of tumours. On the other hand, constitutive, inherited chromosome anomalies conveyed from the zygote to all tissues of the organism may cause a higher risk for the origin of tumours. Constitutive aneuploidies are a frequent cause of early or late abortion or of abnormal development and malformation. The most commonly observed forms of aneuploidies are:

Monosomies are due to the presence of only one copy of a whole chromosome or a portion of it (partial monosomy) instead of two. Examples of human genetic disorders arising from monosomy are: *Turner syndrome*, where there is only one X chromosome instead of two for females or XY for males; *Cri du chat* syndrome that is a partial monosomy due to the deletion of the short arm (p) of chromosome 5. Another partial monosomy is the *1p36 deletion syndrome* due to the deletion of the end of the short arm of chromosome 1. Several Monosomies of chromosome 21 have also been repoterd (Delabar *et al.*, 1992).

Trisomies are due to the presence of three, instead of two, chromosomes of a particular numbered type in an organism. As for monosomies, trisomies can be partial. Most trisomies result in spontaneous abortion, the most common types that survive to birth in humans are: *Trisomy 21* (Down syndrome), *trisomy 18* (Edwards syndrome), *trisomy 13* (Patau syndrome), *trisomy 9*, *trisomy 8* (Warkany syndrome 2), *Triple X syndrome* (XXX), *Klinefelter's syndrome* (XXY) and *XYY syndrome*.

Despite the predominating principle of selective fetal elimination, a few anomalies such as Down syndrome are making it to longer survival due to the relatively mild effects of chromosome 21 triplication. Down syndrome is a congenital disorder characterized by the presence of an extra copy of genetic material namely human chromosome 21, either as a full or a segmental trisomy 21. The resulting effects vary greatly from individual to individual, depending on the extent of the extra copy, on the genetic background, environmental factors, etc. The extra chromosomal material in DS can arise in several distinct forms, all of them a form of partial or complete aneuploidy.

Full or free trisomy 21 is by far the most common form, in which there is an additional copy of chromosome 21 (Figure 2-2.B); this accounts for 95% of all cases of DS. Trisomy 21 is caused by a process called chromosome non-disjunction, a form of faulty cell division (generally occurring during meiosis) prior to or at conception that results in an embryo with three copies of chromosome 21. As the embryo develops, the extra chromosome is replicated in every cell of the body. The parental and the meiotic/mitotic origin of the additional chromosome 21 could be determined in the early 1990's with the help of DNA markers (Antonarakis, 1991, 1992, 1993; Sherman, 1992; Sherman *et al.*, 1991). The main findings were that in 95% of the cases the errors in meiosis leading to trisomy 21 are of maternal origin,

whereas only 5% occur during spermatogenesis. The majority of maternal meiotic errors occurs during meiosis I, accounting for ~70% of all free trisomy cases. About 20% of the cases are due to maternal meiosis II errors. When the non-disjunction is of paternal origin most of the errors occur in meiosis II. Finally in 5% of free trisomy cases the additional chromosome results from an error in mitosis. A seldom form of DS is a condition known as mosaicism, which accounts for only 1-2% of all cases of full trisomy 21 DS (Mikkelsen, 1977). Mosaicism occurs when non-disjunction of chromosome 21 takes place in one of the initial cell divisions *after* fertilization, resulting in a mixture of two types of cells in the body, some with 46 and some with 47 chromosomes. The physical features of Down's may be milder in individuals with mosaicism trisomy 21, especially if the proportion of normal cells is large.

Partial trisomies represent rare events accounting for a maximum of 5% of all reported cases of DS. These are often due to Robertsonian translocations[2] or to very rare de *novo* intrachromosomal duplications.

In the case of Robertsonian translocation, chromosome 21 or a portion of it attaches to the centromere of another acrocentric chromosome (usually chromosome 14 and 21) forming a single chromosome, referred to as chromosome t(14;21) (Balkan and Martin, 1983; Hook, 1981; Matalon *et al.*, 1990; Rockman-Greenberg *et al.*, 1982) (Figure 2-2.C). Robertsonian translocations accounts for 3-4% of all cases of DS and may be inherited. The first case of segmental trisomy 21 was reported by Polani *et al.* in 1960 (Polani *et al.*, 1960), but the low resolution of the karyotype did not allow fine mapping of the triplicated region. In all studied cases, de *novo* t(14;21) trisomies originated from a true Robertsonian translocation in maternal germ cells (Petersen *et al.*, 1991; Shaffer *et al.*, 1992), half of which were of maternal and half of paternal origin. In contrast, only 17% of t(21;21) could be attributed to true Robertsonian translocation of maternal origin (Antonarakis

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[2] *Robertsonian translocation* is a common form of chromosomal rearrangement that occurs in the five acrocentric human chromosome pairs, namely 13, 14, 15, 21, and 22. They are named after the American insect geneticist W. R. B. Robertson, who first described a Robertsonian translocation in grasshoppers in 1916 (Robertson, 1916). They are also called whole-arm translocations or centric-fusion translocations and occur when the long arms of two acrocentric chromosomes fuse at the centromere and the two short arms are lost.

et al., 1990; Grasso *et al.*, 1989; Shaffer *et al.*, 1992) The majority of t(21;21) cases seems to be due to a duplication event also called isochromosome[3] (dup21q).

Rarely, a region of chromosome 21 will undergo a *de novo* microduplication event, leading to extra copies of some of the genes on Chr21 within the same chromosome. If the duplicated region has genes that are responsible for Down syndrome physical and mental characteristics, such individuals will show those characteristics. This type of rearrangement is very rare and only few cases have been reported yet (Delabar *et al.*, 1992; Huret *et al.*, 1987).

2.4   The DS Critical Region

While the majority of the DS cases are associated with complete trisomy 21, the occurrence of cases with partial trisomy 21 associated with DS had raised the hopes for narrowing down a DS critical region (DSCR). Using these rare patients for mapping the DS phenotypic components should aid in identifying chromosomal segments associated with the disease. Correlation between cytogenetic findings and clinical phenotypes could only be established in the eighties when the first molecular marker became available together with high resolution banding techniques. Thereafter, several groups have reported that sub-segments located in the 21q22 region were responsible for major clinical components of the DS phenotype (Korenberg *et al.*, 1994; McCormick *et al.*, 1989; Rahmani *et al.*, 1989). These studies were collectively based on the analysis of about 30 patients with partial trisomy 21, but including cases with a karyotype carrying additional chromosomal aberrations potentially blurring formal conclusions. In fact, revisiting initial reports describing these individual cases indicated that only eight of the patients used for defining a DSCR carried a partial trisomy as the sole chromosomal abnormality (Figure 2-3). Much controversy still revolves around the issue of defining the DSCR boundaries, precluding to conclude whether a single critical chromosomal segment could be exclusively causative for the DS phenotype. The smallest critical region was proposed ten years ago from molecular studies of two DS patients (patients 1 and 2 in Figure 2-3) (Rahmani *et al.*, 1989), and was

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[3] *Isochromosome* is a metacentric chromosome produced during cell division when the centromere splits transversely instead of longitudinally; the arms of the chromosome are equal in length and genetically identical.

called DCR. The DCR was defined as the common trisomic segment found in these two patients and was estimated to span 3-4 megabases around marker DS21S55 (Figure 2-3). However, this conclusion was based on the simple assumption that the triplicate segments not shared by these patients do not contribute significantly to the phenotype. Later, by evaluating 33 clinical features of DS in ten patients, the same group reported that about a third of the DS features could be mapped to the DS1S55 region by comparing minimum chromosomal overlaps (Delabar *et al.*, 1993). In another study describing several partial trisomic patients, Korenberg and co-workers attempted to assign individual clinical phenotypes to several regions of the chromosome (Korenberg *et al.*, 1994). Other reports on the subject fail to resolve the DSCR controversy, and it is yet unclear whether or not it is reasonable to assume that a small sub-segment in 21q22 has a key role in DS pathogenesis (Shapiro, 1999). When recapitulating the data from the eight patients shown in Figure 2-3, it becomes difficult to correlate individual signs with a single chromosomal region.
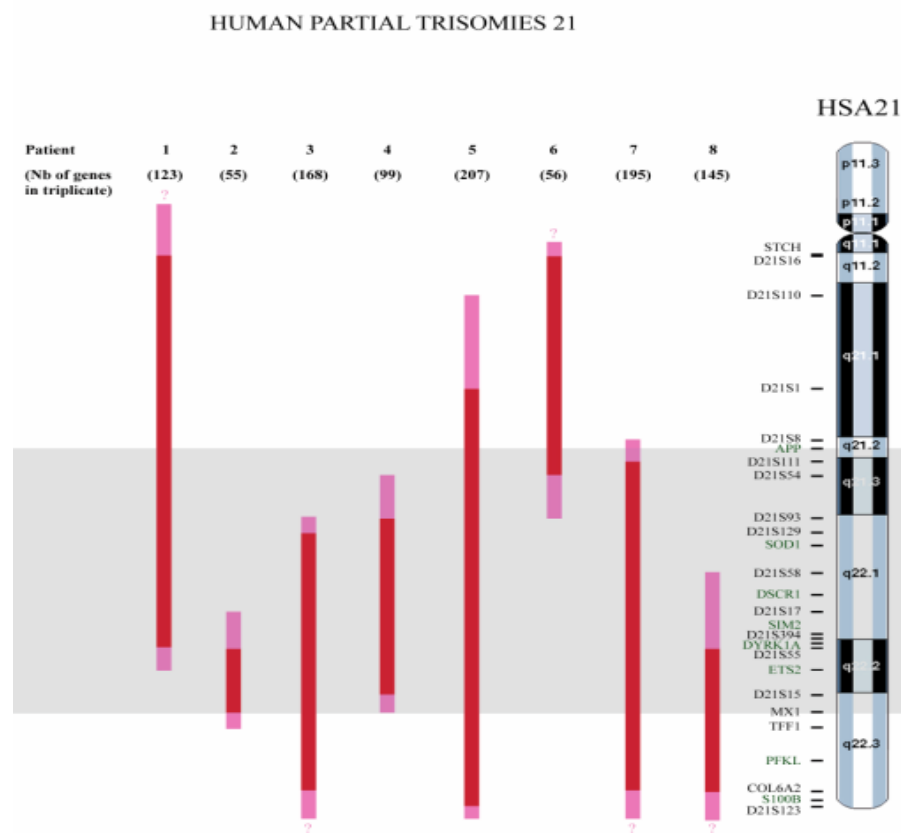

Figure 2-3: Summary of molecular studies on eight patients with partial trisomy 21. *Right:* Schematic representation of human chromosome 21 (HSA21) with the relative location of genes and

genetic markers. *Left:* Patient 1 and 2 correspond to cases FG and IG respectively in Delabar *et al.* (Delabar *et al.*, 1993); Patients 3, 4, 5, and 6 correspond to cases DUP21SOL, DUP21KJ, DUP21DS, and DUP21GY respectively in Korenberg *et al.* (Korenberg *et al.*, 1994); Patients 7 and 8 corresponds to cases GM1413 and MP01 respectively in McCormick *et al.* (McCormick *et al.*, 1989). Red charts represent chromosomal segments containing genetic markers in triplicate. Segments not assessed for gene dosage, located between the triplicated regions and markers with a normal dosage, are in pink. The number of genes located in the triplicated chromosomal segment in each patient is indicated in brackets.

2.5   Other Diseases Associated to HSA21

Other diseases associated to HSA21 are either due to chromosomal rearrangements or to single gene mutations. Constitutive changes in the number or structure of chromosome 21 can have a variety of effects, including mental retardation, delayed development, and characteristic facial features. Changes to chromosome 21 can include a missing segment of the chromosome in each cell (partial monosomy 21) or a circular structure called ring chromosome 21. A ring chromosome occurs when both ends of a broken chromosome are reunited. Apart of DS, somatic clonal rearrangements (translocations) of genetic material between chromosome 21 and other chromosomes have been associated with several types of cancer. For example, acute lymphoblastic leukemia (a type of blood cancer most often diagnosed in childhood; see Chapter 2.6.9) has been associated with a translocation between chromosomes 12 and 21. Another form of leukemia, acute myeloid leukemia, has been associated with a translocation between chromosomes 8 and 21 (Kwong *et al.*, 1993). Several diseases are related to mutations involving genes on chromosome 21:

Alzheimer disease (AD) is a neurodegenerative disease characterized by progressive cognitive deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. The ultimate cause of the disease is unknown. Most cases identified are "sporadic" with no clear family history. Genetic factors are known to be important, and dominant mutations in three different genes have been identified that account for a much smaller number of cases of familial early-onset AD. Mutations in presenilin-1 or presenilin-2 genes have been documented (Murgolo *et al.*, 1996; Rogaev *et al.*, 1995). Notably, mutations of presenilin 1 lead to the most aggressive form of familial Alzheimer's disease (FAD) (Hutton and Hardy, 1997). Mutations in the APP gene on chromosome 21 can also cause early onset disease (Citron *et al.*, 1992). The

presenilins have been identified as essential components of the proteolytic processing machinery that produces beta amyloid peptides through cleavage of APP.

Amyotrophic Lateral Sclerosis (ALS) is a progressive, almost invariably fatal neurological disease. ALS is marked by gradual degeneration of the nerve cells in the central nervous system that control voluntary muscle movement. The disorder causes muscle weakness and atrophy throughout the body. The cause of ALS is not known, and scientists do not yet know why ALS strikes some people and not others. An important step towards answering that question came in 1993 when scientists discovered that mutations in the gene that produces the SOD1 enzyme were associated with some cases of familial ALS (Gregor *et al.*, 1993; Lafon-Cazal *et al.*, 1993). This enzyme is a powerful antioxidant that protects the body from damage caused by free radicals.

APECED syndrome is a rare autoimmune disease of monogenic background affecting mainly the endocrine glands, but some other organs as well. The acronym APECED stands for Autoimmune Polyendocrinopathy (APE), Candidiasis (C) and Ectodermal Dysplasia (ED). The disease results of mutations in just one gene located on chromosome 21, coding for the AIRE autoimmune regulator protein (Finnish German APECED Consortium, 1997; Nagamine *et al.*, 1997).

Holocarboxylase Synthetase deficiency is an inherited disorder in which the body is unable to use the vitamin biotin effectively. Affected infants often have immunodeficiency diseases, difficulty feeding, breathing problems, a skin rash, hair loss, and a lack of energy. Mutations in the *HLCS* gene cause holocarboxylase synthetase deficiency (Yang *et al.*, 2001). The HLCS gene makes an enzyme, holocarboxylase synthetase that attaches biotin to other molecules. Biotin, a B vitamin, is found in foods such as liver, egg yolks, and milk.

Homocystinuria, also known as *Cystathionine beta synthase deficiency*, is an inherited disorder of the metabolism of the amino acid methionine involving the CBS gene on chromosome 21. This defect leads to a multisystemic disorder of the connective tissue, muscles, CNS, and cardiovascular system (Borota *et al.*, 1997).

Jervell and Lange-Nielsen syndrome is a condition that causes profound hearing loss and arrhythmia, it is a type of long QT syndrome[4]. Mutations in the KCNE1 (HSA21) and KCNQ1 (HSA11) genes cause Jervell and Lange-Nielsen syndrome. The proteins produced by these two genes work together to form a channel that transports positively charged potassium ions out of cells (Tinel *et al.*, 2000). The movement of potassium ions through these channels is critical for maintaining the normal functions of the inner ear and cardiac muscle.

Romano-Ward syndrome is the major variant of long QT syndrome. It is a condition that causes a disruption of the heart's normal rhythm. This disorder is a form of long QT syndrome, which is a heart condition that causes the cardiac muscle to take longer than usual to recharge between beats. Mutations in the ANK2 (HSA4), KCNE1 (HSA21), KCNE2 (HSA21), KCNH2 (HSA7), KCNQ1 (HSA11), and SCN5A (HSA3) genes cause Romano-Ward syndrome (Towbin and Vatta, 2001). The proteins encoded by most of these genes form channels that transport positively-charged ions, such as potassium and sodium, in and out of cells. In cardiac muscle, these ion channels play critical roles in maintaining the heart's normal rhythm. Mutations in any of these genes alter the structure or function of channels, which changes the flow of ions between cells. A disruption in ion transport alters the way the heart beats, leading to the abnormal heart rhythm characteristic of Romano-Ward syndrome

Leukocyte Adhesion Deficiency (LAD) is a rare autosomal recessive disorder characterized by immunodeficiency resulting in recurrent infections. The inherited molecular defect in patients with LAD is a deficiency of the β-2 integrin subunit (ITGB2), of the leukocyte cell adhesion molecule, which is found on chromosome 21 (Hogg and Bates, 2000). This subunit is involved in making three other proteins (LFA-1, CR3, and Mac-1). This basically means that a gene that creates a protein does not function properly resulting in the lack of important molecules which help neutrophils make their way from the blood stream into the infected areas of the body.

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[4] The *long QT syndrome (LQTS)* is a heart disease in which there is an abnormally long delay between the electrical excitation (or depolarization) and relaxation (repolarization) of the ventricles of the heart.

Non-syndromic deafness is a form of hearing loss that is not associated with other signs and symptoms. The causes of nonsyndromic deafness can be complex. Researchers have identified more than 30 genes that, when mutated, may cause nonsyndromic deafness, among those CLDN14 and TMPRSS3 are located on chromosome 21 (Guipponi *et al.*, 2002). Many genes related to deafness are involved in the development and function of the inner ear. Gene mutations interfere with critical steps in processing sound, resulting in hearing loss. Different mutations in the same gene can cause different types of hearing loss, and some genes are associated with both syndromic and nonsyndromic deafness.

2.6   Clinical Characteristics of Down Syndrome

The clinical characteristics of DS have been subjected to extensive investigation resulting in an extremely voluminous accumulation of literature that will not be reviewed in details here. We will describe briefly the main findings based on summaries found in the following books: The Consequence of Chromosome Imbalance (Epstein, 1986), Human Genetics (Vogel and Motulsky, 1986), and The Genetic Basis of Common Diseases (King *et al.*, 2002).

Down syndrome affects most of the organs in the body, and results in a constellation of more than 80 clinical signs affecting almost all body organs (Epstein, 1990). The clinical phenotype of different DS patients is composed of a highly variable combination of all those signs with different degrees of severity. Several signs are constant; this includes some degree of cognitive impairment, the characteristic appearance of the face, reduced size and altered morphology of the brain. Signs that are not always present include musculoskeletal and extremity abnormalities, speech and motor delay, cardiac anomalies (in 40% of DS cases), gastro intestinal problems, conductive type hearing loss (in 65-90% of cases), nystagmus and strabismus (in 40-60% of cases). In addition DS patients have high incidence for health problems like leukemia, early onset Alzheimer's disease, infections and respiratory problems.

Although some of the features can be quantitatively evaluated, others are more subjective. Each single sign taken separately has no diagnostic value as it can be observed in the non-DS population. It is the combination of several clinical

signs in one individual that characterizes DS. The clinical diagnosis of DS generally relies on the collective and simultaneous presence of at least 12 cardinal pathological signs based on a diagnostic index that gives special weight to certain features with discriminative power (Jackson *et al.*, 1976; Rex and Preus, 1982)

2.6.1 Morphology

Down syndrome persons have a distinctive craniofacial appearance (Figure 2-4) including microbrachycephaly with a flat facial profile, a short neck, upslanted palebral fissures with epicanthal fold[5], a depressed nasal bridge and a small nose, small ears, slanting eyes, and a protruding tongue. The hands and feet are broad, with short digits, incurved fifth fingers, transverse palmar creases, and a wide space between the first and second toe. Stature is short, with sloping shoulders, and obesity is often present. Any of these features might be absent in a given affected individual, and the phenotype might be subtle in patients with trisomy 21 mosaicism.

Figure 2-4: Children with DS. Taken from Vogel & Motulsky (Vogel and Motulsky, 1986)

2.6.2 Physiopathology of the Brain in DS

The brain of a child with Down syndrome develops differently, leading to alteration in size and in configuration. Its volume is consistently reduced in DS, with a disproportionately greater reduction in the cerebellum (Crome *et al.*, 1966;

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[5] *Epicanthal fold* is a fold of skin over the top of the inner corner of the eye, which can also be seen less frequently in normal babies

Davidoff, 1928). This reduction is readily apparent on post-mortem analysis, and has been measured quantitatively by magnetic resonance imaging (MRI) studies reporting a total brain volume of 85% of euploid and cerebellar volume further reduced to 73% of euploid (Aylward *et al.*, 1997; Jernigan and Bellugi, 1990; Raz *et al.*, 1995). The improvement in imaging techniques allows nowadays assessing neuroanatomic aberrations in living DS individuals. Using magnetic resonance imaging (MRI) with voxel-based morphometry, gross morphometric abnormalities could be identified in DS neuroanatomy (Teipel *et al.*, 2004; White *et al.*, 2003) such as extensive reduction of grey matter in cerebellum, cingulated gyrus, left medial front lobe, temporal gyrus, and regions of the hippocampus. Histological changes observed in DS brain include cortical dysgenesis (Wisniewski, 1990), loss of choligernic neurons in the basal forebrain, abnormalities in morphology and number of dendritic spine (Becker *et al.*, 1991), poverty of granular cells throughout the cortex, decreased neuronal densities in layers II and IV of the occipital cortex (Wisniewski and Rabe, 1986) . There is also evidence for abnormalities in neuronal differentiation and migration in fetal and infant brain (Epstein, 1986). Neuronal loss is prominent from birth onwards (Cairns, 1999; Colon, 1972; Wisniewski *et al.*, 1984) but a consistent picture of the altered "wiring" of the brain in DS has not yet emerged.

2.6.3 Mental Retardation

Down syndrome is always associated with mental retardation, although the degree of cognitive impairment varies widely. The intellectual handicaps are often the most important and frequent problems but may not be evident in early infancy. On average adults with trisomy 21 have a mean IQ of 45, corresponding to a mental age of 5.5 years. While cognitive dysfunction is usually global, selective deficits have been documented in certain areas, including auditory, visual, mind and language abilities (Epstein, 2001). These development delays tend to become increasingly noticeable later in infancy and during childhood.

2.6.4 Premature Aging

Adults with Down syndrome generally show signs of premature aging together with a decline in thymus integrity and function (thymic involution) (Larocca *et al.*, 1988; Lau and Spain, 2000; Rabinowe *et al.*, 1989; Seger *et al.*, 1977). Individuals with Down syndrome show age-related declines about ten years earlier than the general population (Brown, 1987). Premature aging has been noted in regards to measures of skin elasticity, fenestration of cardiac valves, and premature cataracts (Gilchrest, 1981; Lott, 1982). The premature aging signs are routinely detectable within the brain using MRI (Roth *et al.*, 1996). Some people with Down syndrome begin aging more rapidly once they reach their middle 30s, showing graying hair and physical slowness. Cognitive function often declines with age in adults with Down syndrome, particularly in tasks that require planning and attention. However, auditory processing and comprehension knowledge continue to grow well beyond age 50.

2.6.5 DS and Alzheimer

Almost all adults over the age of 40 years with DS display Alzheimer's neuropathology such as characteristic senile plaques, Aβ amyloid deposits, neurofibrillary tangles and granulovascular degeneration in the brain (Mann, 1988; Mann *et al.*, 1990; Wisniewski *et al.*, 1985). The prevalence of dementia in people with DS is 0-4% under the age of 30 years rising to 29-75% at 60-65 years of age, which falls under the category of early-onset Alzheimer's disease (AD) (Zigman *et al.*, 1997). Studies have shown that the prevalence of Alzheimer's disease in people with learning disability, especially in DS, is higher than in those with no learning disability (Patel *et al.*, 1993). DS patients develop the neuropathologic hallmarks of Alzheimer's disease at a much earlier age than persons without DS. Mutations in the APP gene have been correlated previously with early-onset of Alzheimer's disease in families without DS (Goate *et al.*, 1991). The presence of the amyloid precursor protein gene (APP) on chromosome 21 and its triplication in DS has been put forward as the causative agent for the strong association of DS with AD (Lamb *et al.*, 1993).

2.6.6 Cardiovascular Defects

Congenital heart disease is present in approximately 40% of affected individuals. The most common condition is the atrioventricular septal defects (AVSDs). AVSD is a rare congenital heart malformation that occurs in only 2.8% of isolated cardiac lesions. They are the predominant heart defect in children with Down syndrome, making chromosome 21 a candidate for genes involved in atrioventricular septal development (Wilson *et al.*, 1993). AVSDs can result from arrest or interruption of normal endocardial cushion development (Pierpont *et al.*, 2000). But other abnormalities can also be present like patent Ductus arteriosus, ventricular and atrial septal defects, tetralogy of Fallot (Gorlin *et al.*, 1990). DS adults have also an increased risk to develop valvular dysfunction, including mitral valve prolapse (MVP), thickened mitral valve leaflets, and aortic regurgitation (Goldhaber *et al.* 1986, 1987, 1988; Geggel *et al.*, 1993). However, the natural history of cardiac dysfunction in DS remains poorly understood.

2.6.7 Gastrointestinal Aspects

Congenital malformations of the gastrointestinal tract occur in approximately 10% to 18% of DS patients and include tracheo-esophageal fistula, pyloric stenosis, duodenal atresia, annular pancreas, and imperforate anus (Gorlin *et al.*; 1990). Additionally, Hirchsprung disease (colonic agangliosis[6]), that can cause intestinal obstruction, occurs more frequently in children with Down syndrome than in other children (Ryan *et al.*, 1992).

2.6.8 Endocrine Aspects

Thyroid dysfunction, predominantly hypothyroidism, is common in DS, affecting up to 40% of adults (Dinani and Carpenter, 1990; Friedman *et al.*, 1989; Karlsson *et al.*, 1998; Kennedy *et al.*, 1992; Pueschel *et al.*, 1991; Pueschel and Pezzullo, 1985). Although hypothyroidism may be congenital, it is most often acquired from childhood onward. Most cases of acquired hypothyroidism in adults with DS are thought to have an autoimmune basis, since a significant proportion of

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[6] *Colonic aganglionic* is the absence of enteric ganglia along with a variable length of the intestine.

patients have antithyroid and antimicrosomal antibodies. However, many DS patients with these antibodies have normal thyroid function.

2.6.9 Cancer in DS

A clear relationship between human chromosome 21 and cancer is shown by the fact that people with DS are at 20-fold increased risk of childhood leukemia (Fong and Brodeur, 1987; Krivit and Good, 1957). Additionally, trisomy 21 is one of the most frequent non-random clonal change in malignant cells in a variety of hematological malignancies of euploid children and adults (Berger, 1997). An extra one or more chromosomes 21, either as sole cytogenetic anomaly or in combination with other chromosomal aberrations, is gained in 23% of all adult and childhood acute lymphocytic leukemias (ALLs), and it is the most frequently gained chromosome in ALL overall (Berger, 1997). One in every thirty DS children has a chance of being diagnosed with either ALL, myelodysplastic syndrome, acute myeloid leukemia (AML), and/or transient abnormal myelopoiesis during the first 10 years of life. Children with Down syndrome account for approximately 2% of all children diagnosed with ALL and up to 13% of all children diagnosed with AML, representing a 10-20 fold increased risk of either type of leukemia compared to non-DS children (Fong and Brodeur, 1987; Krivit and Good, 1957). The myeloid leukemia, which accounts for nearly 50% of Down syndrome leukemias, is of the acute megakaryoblastic type (AML-M7). It occurs predominantly in the first three years of life, which is very rare in normal children (Lie *et al.*, 1996; Slordahl *et al.*, 1993; Zipursky *et al.*, 1987). Although some individual chromosome 21 genes have been implicated in the pathogenesis of Down syndrome associated AML-M7 leukemia (Ives *et al.*, 1998), very little is known about what triggers abnormal myelopoiesis in DS.

Conversely, constitutive trisomy 21 seems to confer protection from development of solid tumors. Among DS children, neuroblastomas and nephroblastomas, and among DS adults, gynecologic, digestive and breast cancers have very rarely been reported and are significantly under-represented, compared with the age-matched euploid population. This epidemiological data would suggest that chromosome 21 contains genes with suppressive role in solid tumors. This was

reported by three independent studies (Oster *et al.*, 1975; Satge *et al.*, 1998; Yang *et al.*, 2002)

2.6.10 Life Expectancy

Advances in medicine have rendered many of the above mentioned health problems treatable, and the majority of people born with Down syndrome today have a life expectancy of fifty-five years. The life expectancy of the DS patients has nearly doubled in the last 20 years. Yang and co-workers (Yang *et al.*, 2002) showed that the average age at death for Down syndrome patients increased from 25 years in 1983 to 49 years in 1997. This can in part be explained by better treatments for common causes of death and the de-institutionalization of DS patients.

2.6.11 Fertility

Significantly impaired fertility of both sexes is evident in the DS population (Sherzer, 1992). While males have long been assumed to be sterile, Sheridan reports one case of a cytogenetically normal male infant that was fathered by a man with DS (Sheridan *et al.*, 1989). Women have an impaired but still significant fertility: a number of reviews document women with DS carrying pregnancy to term and delivering infants with and without DS (Bovicelli *et al.*, 1982). Infants born from mothers with DS are at increased risk for premature delivery and low birth weight (Bovicelli *et al.*, 1982).

2.7   Prenatal Screening and Diagnosis

There are two types of prenatal tests available to detect DS at a fetal stage: screening tests and diagnostic tests. Screening tests estimate the risk that a fetus has DS; diagnostic tests can tell whether the fetus actually has the condition. Prenatal diagnosis may involve screening and diagnostic tests.

Screening tests are noninvasive and generally painless. They include following tests:

Nuchal translucency testing which is performed between 11 and 14 weeks of pregnancy and uses ultrasound to measure the clear space in the folds of tissue behind the developing neck (fetuses with DS and other chromosomal abnormalities tend to accumulate fluid there, making the space appear larger). This measurement, taken together with the mother's age and the baby's gestational age, can be used to calculate the odds that the baby has DS. Nuchal translucency testing combined with maternal age correctly detects DS in about 80% of the cases (Snijders *et al.*, 1998).

The triple screen (also called the multiple marker test) and *the alpha fetoprotein plus*. These tests measure the levels of three proteins in the mother's blood: the levels of hCG (human chorionic gonadotropin) and Estriol, which are produced by the placenta, and the level of alpha-fetoprotein (AFP), which is produced by the fetus. Together with the woman's age, estimate the likelihood that the fetus has DS. They are typically offered between 15 and 20 weeks of pregnancy and detect DS with about 60% accuracy (Snijders *et al.*, 1998).

Nuchual translucency and triple screen testing are routinely used together. The combined diagnostic value of those tests reach 90% accuracy depending on the center (Schuchter *et al.*, 2001).

Diagnostic tests rely on the establishment of the fetus cell karyotype and are about 99% accurate in detecting DS and other chromosomal abnormalities. However, because they are performed inside the uterus, they are associated with a risk of miscarriage and other complications. For this reason, they are generally recommended only for women with increased risk.

Diagnostic tests include:

Amniocentesis is performed between 16 and 20 weeks of pregnancy involving the removal of a small amount of amniotic fluid through a needle inserted in the abdomen. The cells can then be analyzed for the presence of chromosomal abnormalities.

Chorionic Villus Sampling (CVS) involves a biopsy of the placenta by either transabdominal or transcervical sampling. The advantage of this test is that it can be performed already between 8 and 12 weeks.

Percutaneous Umbilical Blood Sampling (PUBS) is usually performed after 20 weeks; this test uses a needle to retrieve a small sample of blood from the umbilical cord.

Amniocentesis and PUBS carry a 1 in 100 risk of complications, such as preterm labor and miscarriage, whereby the risk of CVS is slightly higher.

2.8   Molecular Analysis of Trisomy 21

In order to investigate the molecular basis of DS it is essential to recapitulate briefly our current knowledge about chromosome 21. The completion of human and mouse genome projects have resulted in vast amounts of sequence information, the identification of thousands of open reading frames and the establishment of gene catalogues for a number of species. This massive increase in the amount of DNA sequence information and the development of technologies to exploit its use has opened avenues for new types of experiments and analysis at an unprecedented scale.

Human chromosome 21 (HSA 21) is the smallest human autosome, and its 33.6 Megabase (Mb) long arm represents approximately 1% of the human genome. Since the sixties, chromosome 21 has always attracted an enormous focus of attention mainly triggered by its association with Down syndrome. The high quality sequence of chromosome 21q (long arm) was published in May 2000, 41 years after the discovery of the cause of DS (Hattori *et al.*, 2000). According to the gene catalogue of May 2000, only ~3% of the sequence encodes for proteins, 1.3 % belongs to short sequence repeats, and 38% in total are interspersed repeats (10,8 % are SINE[7] repeats, 15,5 % are LINE[8] repeats and 11,7 % are other DNA repeats). The rest of the sequence is gene-flanking regions, introns[9] and other DNA of unknown function. The total number of genes has not yet been conclusively determined. A total of 225 genes were estimated when the initial sequence of

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[7] *SINE*: Short interspersed nuclear element. A group of retropseudogenes that are found hundreds of thousands times in the human genome and each of which is typically about 300 bases long.

[8] *LINE:* Long interspersed nuclear element. It is a group of retropseudogenes that are found hundreds of thousands times in the human genome and which are typically about 7000 bases long

[9] *Introns* are sections of DNA within a gene that do not encode part of the protein that the gene produces, and are spliced out of the mRNA that is transcribed from the gene before it is exported from the cell nucleus. Introns exist mainly (but not only) in eukaryotic cells. The regions of a gene that remain in the spliced mRNA and encodes the protein are called exons.

HSA21q was published; subsequent ongoing analysis based on computational methods, EST sequencing, laboratory verification and comparative genome analysis, resulted in an estimated 281 protein coding genes (http://chr21.molgen.mpg.de;http://www.ensembl.org/;http://www.ncbi.nlm.nih.gov/). Gene annotation is a dynamic process and the number of estimated genes varies slightly from one gene catalogue or annotation center to the other. The difference in gene density along the long arm of chromosome 21 (21q) is important: The proximal half contains only ~25% of the genes, whereas the distal half contain the remaining ones (Antonarakis, 2001; Hattori *et al.*, 2000). The functional distribution of the genes encoded by chromosome21 (Figure 2-5) shows that more than 40 % of them have not been characterized by now. About 35% of the 281 HSA21 genes have orthologs[10] in *Drosophila melanogaster (D),* 32% in *Caenorhabditis elegans (C)* and 16% in *Saccharomyces cerevisiae (Y).* Moreover, 15% are conserved across the three species (CDY) and 31% of the HSA21 genes have an ortholog in yeast and worm (CD).
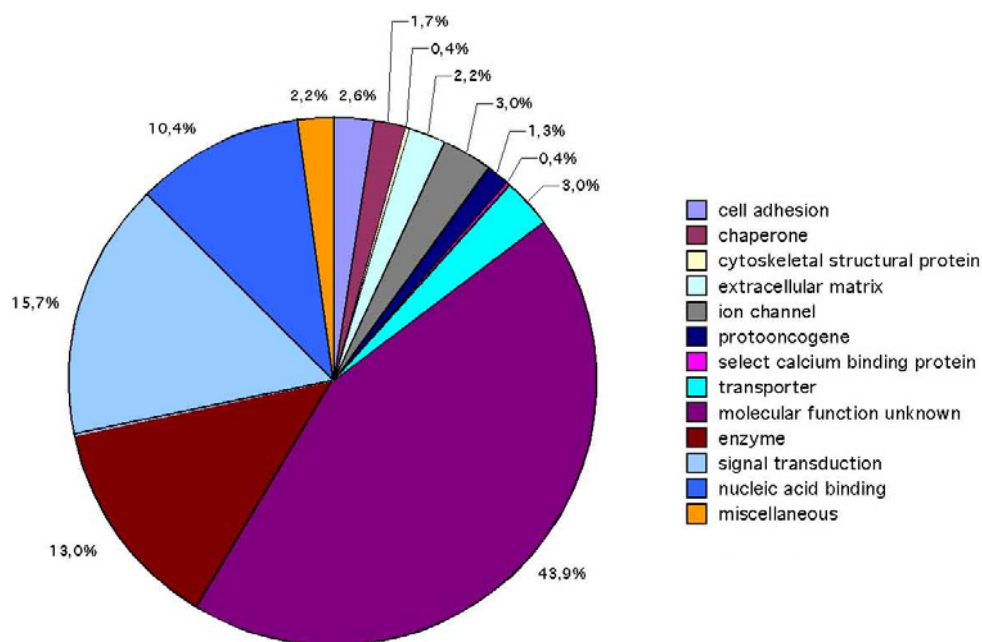
Figure 2-5: Molecular Function of the Genes on HSA 21. The categories are according to the Gene Ontology Consortium [www.geneontology.org]

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[10] *Orthologs* are two genes which share significant homology, and usually identical function, across two or more distinct species. Orthologous genes are assumed to derive from a common evolutionary ancestor.

Unfortunately the billions of bases of DNA sequence do not tell us what all the genes do, how cells works, how cells form organisms, what is their contribution in disease when mutated, etc. This is where the functional genomics comes into play (Lockhart and Winzeler, 2000). Functional genomics is a term englobing a large range of methodological approaches performed either *in vitro* on human genes or more generally in model organisms, attempting to make use of the vast wealth of data produced by genome sequencing projects to describe genome function. Functional genomics uses mostly high-throughput techniques addressing transcriptomics[11], proteomics[12], metabolomics[13], mutant analysis and more recently systems biology[14] to describe the function and interactions of genes. Because of the large quantity of data and the desire to be able to find patterns and predict gene functions and interactions, bioinformatics is crucial to this type of analysis. The development of array technologies, some years ago, opened new possibilities in exploring the genome function. One of the most important applications for arrays so far is the monitoring of gene expression in given cells and tissues (mRNA abundance). The collection of genes that are specifically expressed or transcribed from genomic DNA, referred as the expression profile or the "transcriptome", is a major determinant of cellular phenotype and function.

It was anticipated that the availability of the complete DNA sequence, clone map, and gene catalogue of human chromosome 21 would accelerate and facilitate the discoveries about the disease mechanism of trisomy 21. But it still remains elusive about how an extra copy of a chromosome causes this wide and variable range of clinical features. In fact we know very little about the genes involved in the different phenotypes of DS and the molecular pathophysiology of these phenotypes.One important research goal is to determine which of the HSA21 genes contribute majoritarily to phenotypes of DS and which do not. This requires on one hand to discover the function of the HSA21-encoded proteins, and on the other hand to study the gene dosage effects in affected tissues. Studying the latter will

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[11] *Transcriptomics* examines the expression level of mRNAs in a given cell population, often using high-throughput techniques based on DNA microarray technology.
[12] *Proteomics* is the large-scale study of proteins, particularly their structures and functions.
[13] *Metabolomics* is the study of the metabolic profile of a given cell, tissue, fluid, organ or organism at a given point in time. The metabolome represents the end products of gene expression.
[14] *Systems biology* is the study of the interactions between the components of a biological system, and how these interactions give rise to the function and behavior of that system (for example, the enzymes and metabolites in a metabolic pathway).

enable to identify which genes have a direct or indirect effect on the DS phenotypes and/or which ones have no contribution (Figure 2-6). Moreover, the central hypothesis in Down syndrome research assumes that gene dosage imbalance results in a 50% increase of expression of the HSA21 genes based on tests performed for only a few genes, and that this gene dysregulation may directly or indirectly alters the timing, the pattern or the extent of the developmental processes. It appears thus essential to test systematically the expression of trisomy 21 genes in DS and controls.
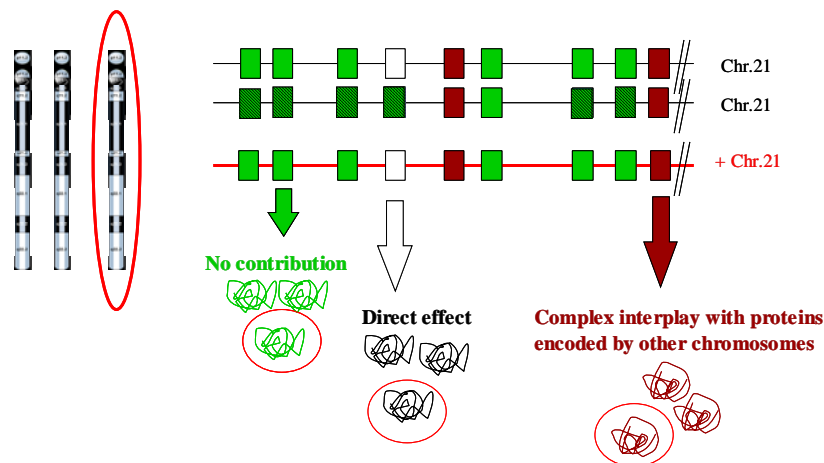
Figure 2-6: Possible gene contribution in Trisomy 21. Determining the roles of multiple genes that contribute to this complex phenotype remains a genetic puzzle. Chromosome 21 is expected to encode 281 protein-coding genes and one of the fundamental issues is to decipher which of those genes contribute to various DS phenotypes

Accessing gene molecular function can make use of so called test tube model organisms (e.g. yeast, worm, fruitfly), whereas modeling human diseases generally make use of mammal organisms like the mouse. Down syndrome can occur in all human populations, and one anecdotical case has been reported in chimpanzees (McClure *et al.*, 1969). Recently, researchers have been able to engineer trisomic mice carrying most of the human chromosome 21 gene orthologs, in addition to their normal chromosomes (see Chapter 2.9).

We used herein two models, a mouse model of Down syndome (Ts65Dn) to study gene dosage effects on a trisomic organism and *Caenorhabditis elegans* as a tool to get insight into gene function.

2.9 Mouse Models of Trisomy

Depending on the context It has become routine to use transgenic mice or knock-out mice to model human genetic disorders, since the development of methods to introduce targeted mutations by homologous recombination. Although a number of excellent mouse models exist for many human single-gene disorders such as hemophilia[15] or Zellweger[16] syndrome, mouse models may only partially mimic or sometimes fail to recapitulate any aspect of the human syndrome. It is in a way surprising that some mouse models of human conditions that are caused by chromosome-scale anomalies have proved to be valuable. Perhaps the most ambitious of these efforts is the creation of mouse models for Down syndrome (Figure 2-7). A basic assumption of using animal models to study DS is that although the phenotypes will vary in ways that reflect species differences, basic genetic processes disrupted by gene-dosage imbalance will frequently be conserved. The ability to study phenotypes in a viable mouse model allows verification of phenotypic parallels with DS to provide a catalog of traits that can be compared across species (Ramirez-Solis *et al.*, 1995). Whereas some differences between mouse and human gene content have been reported for HSA21 predicted genes (Gardiner *et al.*, 2003), well-annotated genes are nearly perfectly conserved in content and order between the two species (syntheny). HSA21 is orthologous to mouse chromosomal regions mapping to three different chromosomes. From the centromere of HSA 21 to 21q terminal, about 23.2 Mb are homologous to mouse chromosome 16 (MMU16), 1.1 Mb to MMU17, and 2.3 Mb to MMU10 (Figure 2-7) (Toyoda *et al.*, 2002). The largest region of genetic homology of human chromosome 21 is thus shared with MMU16. This observation has led scientists in the 1980's to the use of mice trisomic for MMU16 as an animal model of DS (Ts16) (Epstein *et al.*, 1985). The Ts16 mouse has three full copies of MMU16, which contains not only the region orthologous to HSA21 but also ones orthologous to HSA3, 8, 16 and 22. It was the first model used for DS studies (Epstein *et al.*,

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[15] *Hemophilia* or *Haemophilia* is the name of any of several hereditary genetic illnesses that impair the body's ability to control bleeding.
[16] *Zellweger syndrome* is a rare, congenital disorder (present at birth), characterized by the reduction or absence of peroxisomes (cell structures that rid the body of toxic substances) in the cells of the liver, kidneys, and brain. The most common features of Zellweger syndrome include an enlarged liver, high level of iron and copper in the blood, and vision disturbances. There is no cure for Zellweger syndrome and death usually occurs within 6 months after onset.

1985). Although trisomy 16 mice have several phenotypic features suggestive of DS, their value as a DS model is limited by several factors: 1) Ts16 mice are not viable postnatally, precluding many types of analysis, 2) Ts16 are trisomic for many genes located on human chromosomes other than 21 and therefore not implicated in the pathogenesis of DS, and 3) they do not include all orthologs of HSA 21 in 3 copies. To circumvent these problems several segmental trisomy mouse models have been generated since (Figure 2-7) and proved to be valuable for gaining insights in the molecular basis of trisomy. Ts1Cje, Ms1Ts65, Ts1Rhr, Ms1Rhr/Ts65Dn and Ts65Dn mice only contain trisomic regions orthologous to HSA21. Chimeric mice in which a large percentage of cells contain all or part of human chromosome 21 have also been reported (Inoue *et al.*, 2000; Shinohara *et al.*, 2001). Recently O'Doherty and colleagues (O'Doherty *et al.*, 2005) generated a transpecies aneuploid mouse line that stably transmits a freely segregating, almost complete human chromosome 21. But to date still none of the existing trisomic mouse models perfectly mimic the chromosomal abnormality seen in DS. Existing segmental trisomy models are briefly described below.
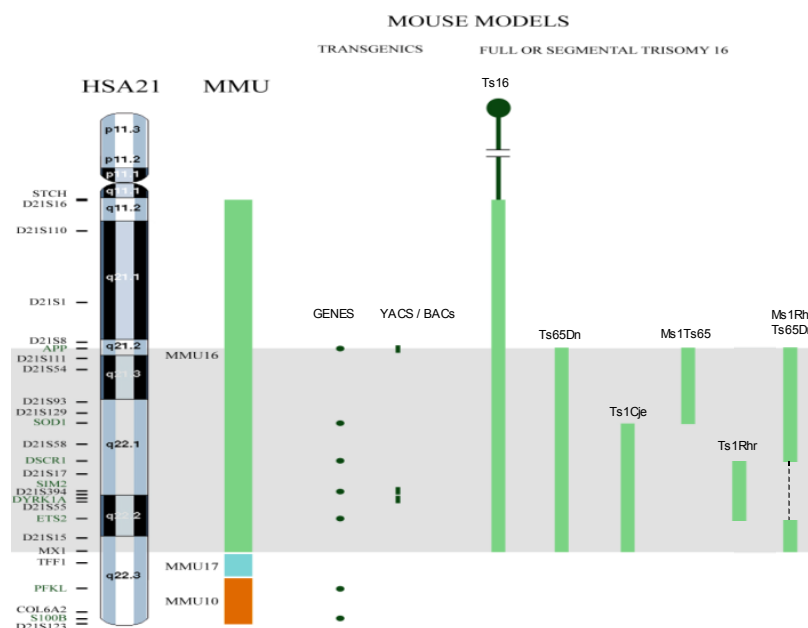
Figure 2-7: Segmental trisomy mouse models. *From left to right* : Human chromosome 21 (HSA21) | Mouse synteny : MMU 16 (green), 17 (blue), and 10 (orange) | Transgenic mice are represented by a green dot or a line at the position of the involved genes: *Sod1, Pfkl, S100b, Ets2, App, Dscr1, Sim2, Mnb*. Green bars represent partial or full trisomy 16 mouse models. The chromosomal region triplicated in the mouse Ts65Dn is represented as a gray horizontal shadow.

The Ts65Dn mouse (Figure 2-8) is by now the most studied model for DS and was the model used herein. It is derived by Davisson and colleagues (Davisson *et al.*, 1990) using translocation chromosomes; Synonyms are also T(16C3-4;17A2)65Dn, T(16;17)65Dn, or T65Dn. These mice are an advanced C57BL/6Jei X C3H/HeJ intercross, meaning that each individual contains on average 50% B6 alleles and 50% C3H alleles such that an average 25% of loci are homozygous B6, 25% are homozygous C3H, 50% are heterozygous, and the actual loci in each class differ among individuals. It exhibits a segmental trisomy for orthologs of about 128 human HSA21 linked genes (from *App* to *Znf295*), which is nearly half of the genes on HSA21. This mouse remains viable into adulthood (Reeves *et al.*, 1995) that facilitates studies of features occurring at postnatal stages of development. Using trisomic and euploid littermates for relative comparisons further reduces genetic variation between trisomic and euploid mice. Ts65Dn mice have been used widely in studies relevant to DS and display a variety of phenotypes that recapitulate those seen in DS like those shown in Table 2-1.

Figure 2-8: The Ts65Dn mouse. The Ts65Dn mouse (left) is smaller than its euploid littermate (right).The picture was taken from the Jacksons laboratory web page.

Among these parallels, cerebellar phenotypes are very relevant characteristics as they occur universally among DS and represent "the end result of developmental pathways that are highly conserved across vertebrate evolution" (Reeves *et al.*, 2001). They encompass craniofacial abnormalities (Figure 2-9), reduced cerebellar (Figure 2-10) volume and decreased density in granule and Purkinje cells; related developmental delay and deficits in spatial learning and memory in DS are as well observed in Ts65Dn mice (Baxter *et al.*, 2000; Demas *et al.*, 1996; Reeves *et al.*, 1995). Even if this model does not perfectly mimic DS,

it correctly predicts many analogous clinical signs in DS and it therefore represent a highly valuable tool for establishing genotype-phenotype correlation in trisomy.

Figure 2-9: Dysmorphology of the craniofacial skeleton in Ts65Dn mice parallels that in DS. Euclidean distance matrix analysis was used to compare form in euploid and Ts65Dn mice. Bones that were most profoundly affected in Ts65Dn mice (left) and corresponding human bones (right) are colored. Lines on the mouse skull indicate some of the distances that are significantly reduced as a result of segmental trisomy when compared statistically with normal littermates. The affected craniofacial bones include: nasal, darker green; maxillae and premaxillae (fused into one element in humans, separate bones in mice), light green and blue; jugal/zygomatic, cerise; frontal, orange; mandible, indigo; occiput, lavender. Figure & Legend were taken from Reeves *et al.*, Trends in Genetics, 2001.
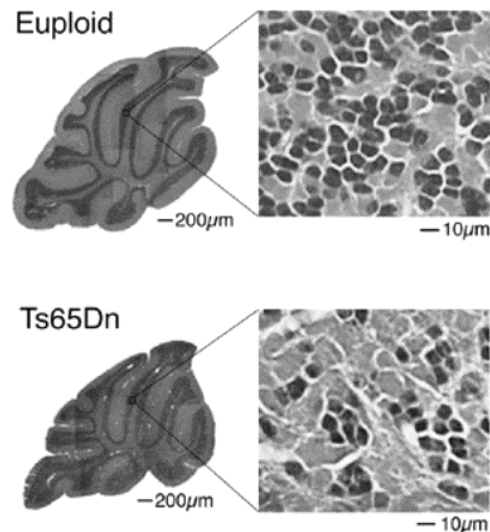
Figure 2-10: Cerebellar phenotype in Ts65Dn mice. Cerebellar area and granule cell density are reduced in Ts65Dn mice. Images of midline sagittal sections from a Ts65Dn and euploid cerebellum are shown on the left, illustrating the smaller area of cerebellum seen in trisomic mice. The higher magnification views of the granule cell layer on the right show the reduced cellular density in Ts65Dn. Figure and legend are taken from Baxter *et al.*, Human. Mol. Genet. 2000.

Ts1Cje mouse, was developed in 1998 (Sago *et al.*, 1998) and carries a triplicated segment of MMU16 containing 95 genes orthologous HSA21 (from *Sod1*

to *Znf295*). Ts1Cje mouse is genetically very similar to Ts65Dn but does not have the triplication of the region from *App* to *Sod1* (Figure 2-7). Although this mouse does not perfectly model human trisomy 21, there are substantial similarities in phenotype, notably craniofacial, cerebellar phenotypes (Olson *et al.*, 2004; Richtsmeier *et al.*, 2002), behavioral and learning abnormalities (Sago *et al.*, 1998).

Ms1Ts65 mouse is trisomic for the region of difference between Ts65Dn (see below) and Ts1Cje mice and harbors 33 triplicated genes (from *App* to *Sod1*). This model was generated by Sago and colleagues (Sago *et al.*, 2000) in order to compare it to the two original partial trisomies (Ts65Dn and Ts1Cje) and to assess the contributions of the regions above and below *Sod1* to the phenotype. The behavioral deficits of Ms1Ts65 mice are significantly less severe than those of Ts65Dn. However the gain of knowledge on genotype-phenotype correlations out of the comparison of the three strains remained limited.

Olson and coworkers (Olson *et al.*, 2004) engineered mice to carry either a duplication (*Ts1Rhr*) or deletion (Ms1Rhr) of a 3.9 Mb segment of mouse chromosome 16 containing the 33 orthologs of genes (between *Cbr1* and *Mx2*) found in the human DSCR. By crossing the Ms1Rhr deletion onto the Ts65Dn background they further engineered mice that carried 75% of the triplicated genes found in Ts65Dn but had normal two copies of genes located in the critical region (*Ms1Rhr/Ts65Dn*) (Figure 2-7). By comparing the craniofacial phenotypes of these mice the authors showed that the DSCR is not sufficient (and to some extent not necessary) to produce the cranofacial phenotype (Olson *et al.*, 2004).

Recently a promising mouse model was built carrying an extra human chromosome 21 (O'Doherty *et al.*, 2005). Trisomy in Tc1 triggers essentially three main anatomical phenotypes: reduction of cerebellar granule neurons, atrioventricular septal defects of the heart and reduced mandible size, the later two features being shared with the Ts65Dn mice. It is striking that, as a mirror situation to DS, cerebellar defect affects all tested individuals, whereas heart malformations occur only in 60% of the cases. To generate transchromosomic chimeras, the authors injected ES cells into host blastocysts and mated the resulting chimeras to mice from the C57BL/6J strain. Usually when chimeric mice are engineered to carry human chromosome fragments, the human fragment is differentially retained in different organs and on different genetic backgrounds. Therefore mosaicism in this

mouse could be one explanation for the phenotypic observations. In fact, the percentage of nuclei carrying Hsa21 is the highest in the brain. It could also be that the damage occurs very early during the development of the central nervous system. Behavioral studies indicate that the Tc1 mice have deficits in both hippocampal synaptic plasticity and hippocampus-dependant learning and memory, reminiscent of typical mental retardation in DS. Even if Tc1 mice recapitulate only part of the complex DS phenotype (and we cannot expect more from a mouse for modelling a complex syndrome compounded by the genetic background and allelic differences between individuals) Tc1 brings undoubtedly a boost in DS research in complementing the existing mouse models, in addition to representing an impressive technical advance.

	TISSUES		CELL TYPE	Ts65Dn mouse	Human DS
MORPHOLOGICAL PHENOTYPES	GENERAL			Male infertility, female subfertility, live into adulthood.[1]	Infertility (mostly in man).
				Lower fasted body weights (in average aged 2,13 and 1,8 months mice),[10,11] and lower body weight (in average aged 2,75 months mice).[11]	High prevalence for obesity in children.[46]
	CRANIOFACIAL			Small size, mild hydrocephalus (in 1 to 14 months aged mice), early onset obesity (before 5 months of age).[1]	Craniofacial dysmorphology (in 4 to 7 months aged mice).[36-37]
		Whole brain		No volume difference for average volume of most of total brain and brain excluding cerebellum (in 3 to 12 months aged mice).[1]	Overall reduction in head dimensions, microcephaly.[48]
					Reduced to 85% of Euploid (in 5-23 years aged patients).[24.51]
	BRAIN	Basal forebrain	Medial Septal Nucleus / Ventral Diagonal Band	Cholinergic, serotonergic and catecholaminergic neurons are normal in young mice (3 months old).[22] Atrophy and loss of BFCNs after 6 months (study on PD2 to 20 months aged mice).[1]	Normal BFCNs in infancy and young adult.[50]
				Age related (from 6 months up) loss of 30% of ChAT-immunoreactive neurons (study on 4 to 10 months aged mice).[1-6]	Age related BFCNs degenerate with increased age (in 27-57 years old patients).[39-40]
			Neurons	Age related (from 6 months up) shrinkage in main cell body size, decrease in density of cholinergic neurons (BFCN). At all time point: Loss of trkA (High affinity NGF), an ChAT immunoreactivity (studies on 4 to 10 months aged mice).[6,10,29]	Reduced to 73% of Euploid (in 5-23 years aged patients).[24.51]
		Cerebellum	Global	Reduced volume (88,5% of Euploid), (in 3 to 12 months aged mice).[1]	
			Whole cerebellum	Reduced volume (88,5% of Euploid), (in 3 to 12 months aged mice).[1]	
			Internal Granule Layer	Reduced (80,6% of Euploid), (in 3 to 12 months aged mice).[1]	Reduced to 73% of Euploid (Age 2-12 years), 67% of Euploid (Age 19-33 years), 69% (Age 58-59 years).[1]
			Molecular Layer	Reduced (92,3% of Euploid), (in 3 to 12 months aged mice).[1]	
			Total granule cells	Reduced (78% of Euploid), (in 3 to 12 months aged mice).[1]	
		Cortex	Neurons	30% less asymmetric synapses (excitatory synapses), compensated by an increase of the contact zone area of the existing synapses, (in 16 to 23 months aged mice).[7]	Smaller hippocampus.[37]
			Global	Astrocytic hypertrophy (BFCNs degeneration and astrocytic hypertrophy are markers of the Alzheimer disease pathology), (in 3 months aged mice).[6]	Aged related astrocytic hypertrophy and increasing number of astrocytes (study in 1 from 34 gestational week to 57 years old patients).[31-32] Neuropathology of Alzheimer disease by age 30-40 years.[34-35]
		Hippocampus	Global	Loss of cholinergic input after 6 months[22] / regressive changes of cholinergic neurons in the hippocampal terminal fields/ impaired retrograde transport of nerve growth factor from hippocampus to basal forebrain (in 6 to 18 months aged mice).[14]	
			Dentate gyrus	Lower number of neurons (in 5 to 7 months aged mice).[15]	
			CA2	Smaller volume (in 5 to 7 months aged mice).[15]	
			CA3	Higher number of neurons (in 5 to 7 months aged mice).[15]	
	HEART			No characteristic defects (in live born mice).[16]	Cardiac anomalies in 30-50% of live born individuals.[47,54]
	INTESTINE	Small intestine	Jejunum	Shorter villus height, decreased planar circumference (in average aged 2,75 months mice).[11]	Decreased nutrient absorption (in 29 to 46 years old patients).[20] (i.e. Xylose malabsorption in 25-61 age ranged patients).[24]
			Global	Shorter length, but no difference in weight (in average aged 2,13 and 1,8 months mice).[10]	
		Large intestine	Global	Shorter length, but no difference in weight (in average aged 2,13 and 1,8 months mice).[10]	
	EPIDIDYME			Epidydimal fat pad weight is larger (in average aged 2,13 and 1,8 months mice).[11]	
METABOLISM	GENERAL			Anomalies in digest/ive function, and amino acid metabolism (less O₂ consumption per gram of fasted body weight (in average aged 2,75 months mice).[11]	50% increase of myo inositol levels (in 28-39 age ranged patients), even higher levels were observed in 42-62 years old patients (elevated myo-inositol levels, and in 1 case decreased N-acetyl aspartate concentration (adult).[30]
	BRAIN	Whole brain		The ratio of myo inositol over total creatine is increased (+38%), the ratio N-acetyl aspartate over total creatine is decreased (-18%), (in >3 months aged mice).[8]	
		Cerebellum		Increased aluminium concentrations (in average aged 2,13 months mice).[10]	
		Cortex	Global	Normal levels of cAMP (in 5-7 months aged mice).[9]	
			Frontal cortex	Decreased affinity of b-adrenoceptors, lower level of basal cAMP.[9]	
		Hippocampus		Elevated myo-inositol level (+30%), (in average aged 2,17 months mice).[9]	
				Elevated myo-inositol level (+28%), (in average aged 2,17 months mice).[9]	
				Lower levels of basal cAMP (in 5-7 months aged mice).[9]	
		Brain stem		Elevated myo-inositol level (+21%), (in average aged 2,17 months mice).[9]	
	PLASMA			Higher concentrations of tyrosine, phenylalanine, valine, leucine, isoleucine and citrulline (in average aged 2,75 months mice).[11]	Higher concentration of leucine, isoleucine, phenylalanine, and cysteine in adults.[28]
				Lower concentration of hydroxyproline (in average aged 2,75 months mice).[11]	
				Increased ammonia, levels, decreased glucose levels (in average aged 2,13 and 1,8 months mice).[10]	Elevated concentrations of mannitol, ribitol and arabitol (in 22-63 age ranged patients).[29]
	INTESTINE	Small intestine	Jejunum	Higher apparent energetic of fiduciary of jejunal active glucose uptake (in average aged 2,75, 2,13 and 1,8 months mice).[10,11]	Increased intestinal absorption of Aluminium (in 35-46 years aged patients).[45]
	LIVER			Elevated myo-inositol level (+44%), (in average aged 2,17 months mice).[9]	
	SKELETAL MUSCLE			Elevated myo-inositol level (+63%), (in average aged 2,17 months mice).[9]	
IMMUNOLOGY	THYMUS	General		Enhanced apoptosis of the thymus/ enhanced generation of reactive oxygen intermediate (i.e. H₂O₂). In correlation with SOD-1 overexpression (in 5 to 15 weeks aged mice).[12]	Abnormal lies in thymus (in children under 2 years of age).[47]
		Thymic cortex		Lower number of subcapsular lymphocyte/ cellular intermediate/ intracellular edema/ hypocellularity/ hyperchromatic apoptotic-like clusters (in 5 to 15 weeks aged mice).[12]	Subclinical hypothyroidism in children.[54]
BEHAVIOUR		Behavioral phenotype		Developmental delay and hyperactivity during early development (studied during post-natal and adult periods of mice).[5]	Developmental delay and hyperactivity in children with DS.[41-44]
				Abnormal behaviour, cognitive impairment (in 6-8 months mice).[5]	Mental retardation.[41-42-43]
				Memory loss after 6 months, initial slowness in learning (in average aged 4,7 and 8,1 months mice).[5]	Hyperactivity in childhood.[42-63]
				Hyperactivity (~4 times higher), early onset tremors (after 6 weeks).[18,19]	
				Decreased long-term potentiation, increased long term depression (in 2 months aged mice).[16,18]	
				The male is more aggressive in neutral areas, but less in home areas (in 4-6 months aged mice).[23]	
				Reduced responsiveness to pain.[17]	
		Spatial disorimination		Decrease (in average aged 4,7 and 8,1 months mice).[20]	
		learning and reversal		More lateralized, no difference in visual reversal learning (in average aged 4,7 and 8,1 months mice).[5]	
				Learning deficits in a hippocampal depending task.[13]	

Table 2-1: Parallels between Ts65Dn and DS phenotypes

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2.10 The Nematode *Caenorhabditis Elegans*

In 1965, Sydney Brenner chose the free-living nematode *Caenorhabditis elegans* as a promising model for a concerted genetic, ultrastructure, and behavioral investigation of development and function in a simple nervous system (Brenner, 1974). We used the worm to functionally investigate a set of candidate genes that have been selected throughout the study (see Chapter 2.11). Since then knowledge about the biology of the worm has accumulated to the extent that *C. elegans* is now probably the most completely understood metazoan in terms of anatomy, genetics, development and behavior. The model organism *Caenorhabditis elegans* is a small, free-living soil nematode found commonly in many parts of the world. It feeds primarily on bacteria and reaches about 1 mm in length and 65-µm in diameter. The two sexes, hermaphrodites and males differ in appearance as adults. The hermaphrodite lays during its life about 300 eggs that hatch as L1 Larvae. Three more larvae stages (L2, L3, L4) are following until it reaches the adult state after about 3 days (Figure 2-11). The transition from one stage to the next is defined by the molt. Under unfavorable conditions like starvation or overcrowding, the L2 larva goes into a dauer larva state. The dauer larvae state can survive up to 3 months, and develops to an L4 larva when conditions turn better. *C. elegans* is a simple organism, both genetically and anatomically featuring defined specific cell types such as muscle cells, nerve cells of similar organization to the equivalent cells of vertebrates. The adult hermaphrodite has only 959 somatic cells, and the adult male 1031. The developmental fate of every single somatic cell has been mapped out. These patterns of cell lineage are largely invariant between individuals, in contrast to mammals where cell development from the embryo is more largely dependent on cellular cues. *C. .elegans* was the first metazoan to be fully sequenced in 1998 (The *C. elegans* Sequencing Consortium, 1998). The haploid genome size is 8x107 base pairs, which is about eight times that of the yeast *Saccharomyces* or one-half that of the fruit fly *Drosophila*. It is organized in five pairs of autosomes and one pair of sex chromosomes harboring about 19000 genes. Hermaphrodites have a matched pair of sex chromosomes (XX); the rare males have only one sex chromosome (X0). *C. elegans* is one of the simplest organisms with a nervous system. In the hermaphrodite, this comprises 302 neurons whose pattern of connectivity has been

completely mapped out, and proven to be a small-world network (Chalfie and White, 1988). The whole is controlled by a sort of brain called circumpharyngeal nerve ring. Interestingly, the neurons fire no action potentials.
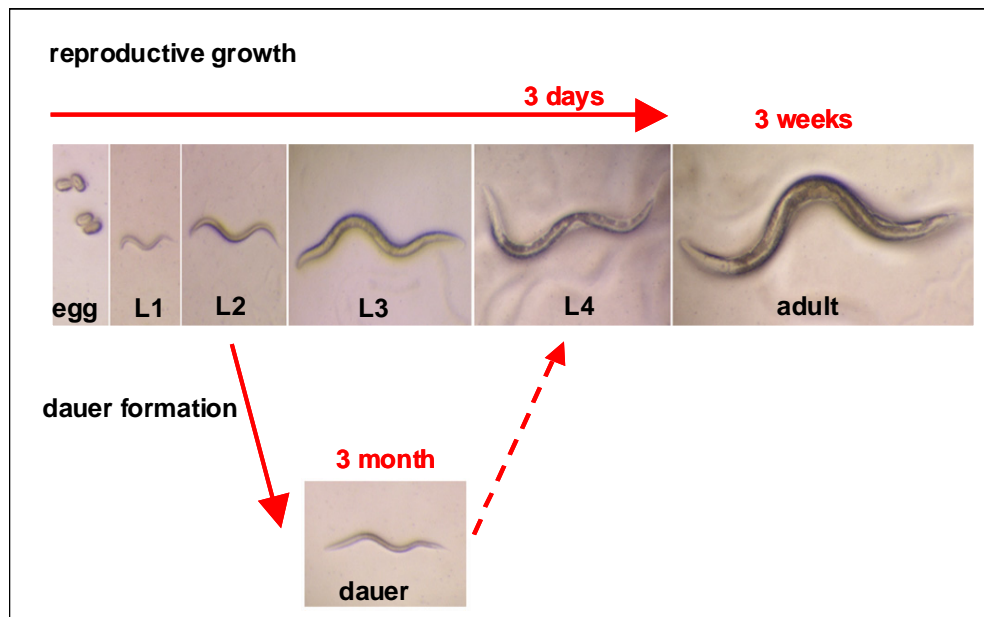

Figure 2-11: *C. elegans* **life cycle with the optional dauer formation choice**

From a research perspective, the worm has the advantage of being a multicellular eukaryotic organism that is simple enough to be studied in great detail. The total transparency of the animal at all stages of development, the relatively small number of cells making up the fully formed individual, its short life cycle and hermaphrodite mode of reproduction, make *C. elegans* the ideal model to test candidate genes and to dissect some aspects of the molecular mechanisms involved in developmental pathways.

2.11 Aims of this Study

We tried here to contribute answering following essential questions in DS research: Are all trisomic genes over-expressed by 50% in all tissues? Do some genes never follow this trend? Are there genes that do not follow this trend at a certain time point and/or in a certain tissue? How variable is the level of expression from one tissue to another, and/or from one individual to another?

Regarding the fact that the availability of human trisomic sample is very limited and difficult to obtain, we choose the Ts65Dn mouse model for studying the gene dosage effect of the HSA21 orhologs in a trisomic context. We compared here the transcriptome of trisomic and normal adult mice tissue samples, which provided us first elements for answering these questions and led us to the identification of interesting candidate genes that could be directly involved in specific DS phenotypes. After gaining insights into the general expression pattern of the trisomic genes, we furthermore investigated the relation between gene dosage effects and variation in gene expression among individuals. In a parallel work, we studied more in details the molecular function of selected genes in *C. elegans.*

In summary the strategy described in this study encompasses:

-Functional genomics of Chr21 genes integrating cDNA chip-based profiling of trisomic samples in an animal model in order to determine which genes are relevant to the phenotype.

-Gene expression studies using quantitative Real Time PCR in order to establish a relation between gene dosage effects and the variation of gene expression among individuals.

-Selection and investigation of potential candidate genes that could play a role in the pathology of the syndrome. The orthologous genes from the selected candidates were investigated for spatial and temporal expression pattern at all stage points in *C. elegans* by GFP-monitoring. To further understand their molecular function we attempted to investigate the effects of loss of function (RNAi technique) experiments of the candidates in the worm.