

Constitutional aneuploidy and cancer predisposition[†]

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Constitutional aneuploidies are rare syndromes associated with multiple developmental abnormalities and the alterations in the risk for specific cancers. Acquired somatic chromosomal aneuploidies are the most common genetic aberrations in sporadic cancers. Thus studies of these rare constitutional aneuploidy syndromes are important not only for patient counseling and clinical management, but also for deciphering the mechanisms by which chromosomal aneuploidy affect cancer initiation and progression. Here we review the major constitutional aneuploidy syndromes and suggest some general mechanisms for the associated cancer predisposition.

INTRODUCTION

Somatic acquired *genomic instability* is one of the hallmarks of cancer (1). This genomic instability is commonly manifested by structural or numerical chromosomal aberrations. *Structural* genomic aberrations leading to activation of oncogenes or elimination of tumor suppression genes have been studied extensively. However, very little is known about the oncogenic role, if any, of numerical chromosomal aberrations, *aneuploidy*, which are the most common abnormalities in cancer (2).

The association between constitutional aneuploidy and cancer supports a causative role of aneuploidy in cancer. The precise determination of the relative risk of cancer in many of these syndromes is hampered by their rarity and by the short life span of many of the patients. Notwithstanding these difficulties these syndromes provide a unique opportunity to study the neoplastic evolution during ontogeny and the interplay between the developmental abnormalities induced by specific chromosomal. Here we review the major disorders; Table 1 presents a summary and description of additional syndromes.

MOSAIC VARIEGATED ANEUPLOIDY

Unlike the rest of the constitutional aneuploidy disorders, mosaic variegated aneuploidy (MVA) is a rare autosomal recessive *genomic instability* syndrome characterized by

multiple mosaic aneuploidies in somatic cells. Clinical manifestations include cancer predisposition, intrauterine growth retardation, microcephaly, mental retardation and CNS anomalies. Out of 35 cases of MVA syndrome that have been so far reported (3–6), 12 developed cancer: 7 had Wilms' tumor; 4 had rhabdomyosarcoma (one patient had both tumors (7)) and 2 had leukemias.

More than half of the patients have inactivating mutations in the *BUB1B* gene that encodes the mitotic spindle checkpoint protein BUBR1 (4). Mitotic spindle checkpoint proteins (MAD1, MAD2, BUB1, BUB3, BUBR1, MPS1 and other proteins) arrest the metaphase by inhibiting the anaphase-promoting complex/cyclosome until the sister chromatids are correctly attached to the spindle (8,9). This is a central regulatory mechanism ensuring the maintenance of correct chromosome numbers after cell division. Not all patients with MVA have *BUB1B* mutations and these were reported to have a milder phenotype without cancer (10). *Bub1b*^{+/-} mice are developmentally normal but have defective spindle-checkpoint activation and develop lung and colon cancers in response to carcinogens (11). Together, the identification of *BUB1B* mutations as the cause of MVA and the mouse model provide a clear biological causal link between mitotic spindle dysfunction, aneuploidy and cancer development.

What is the extent of similarity between the cancers observed in patients with MVA and sporadic childhood cancers? Hanks *et al.* (12) reported comparative genomic

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Table 1. Summary of major constitutional aneuploidy syndromes and associated cancers

Disorder	Neoplasms	Comments
Mosaic variegated aneuploidy (MVA)	Wilms' tumor Rhabdomyosarcoma Leukemia	^a Based on case reports (3-6)
Trisomy 8 mosaicism (cT8M)	Myeloid neoplasia	Based on cases reports (21,23-35,42)
Trisomy 18 (cT18)	Wilms' tumor Hepatoblastoma	<u>Wilms' tumor</u> : based on cases reports (18,19,49-52) and on a Wilms' tumor's study (18) <u>Hepatoblastoma</u> : Only in females, based on cases reports (54-60)
Down's syndrome (DS, cT21)	ALL AML Solid tumors- <u>decreased risk</u>	^b Based on several cohort studies of DS patients (66,132-135) <u>Leukemia</u> : in addition to the DS cohorts, the increased risk of leukemia in DS was also found in acute leukemia's studies (88,136) <u>Solid tumors</u> : in addition to the DS cohorts, the <u>decreased risk</u> of solid tumors in SD was also found in various solid tumors' studies (18,137-139) <u>Testicular germ cell tumors</u> : increased risk of testicular tumors was found in several DS cohort studies (132,133,140), but not in others (66,134)
Del 11p	Wilms' tumor	^c Based on cohort study of 2561 patients with constitutional autosomal chromosome deletions (141)
Del 11q24	Anogenital	
Del 13q	Retinoblastoma	
Del 22q11.2 (Velo-cardio-facial; Digeorge syndrome)	Neoplasms in general	Based on a cohort study of 687 patients with chromosome 22q11 deletion syndrome (142)
Turner syndrome (TS)	Gonadoblastoma CNS bladder and urethra Ocular Breast- <u>decreased risk</u>	^d Based on a cohort study of 3425 women with TS (99) <u>CNS</u> : only in women with 45,X monosomy (not in women with mosaicism) <u>Breast</u> : <u>decreased risk</u> only in women in women with 45,X karyotype (not in women with 45,X/46,XX mosaicism). <u>Colon cancer</u> : increased risk of colon cancer was found in a smaller cohort (597 TS patients), but not in the large cohort (100) <u>Wilms' tumor</u> : increased risk of Wilms' tumor was found in a Wilms' tumor's study (18), but not in TS cohort studies
Klinefelter syndrome (KS)	Breast Lung Non-Hodgkin's lymphoma Prostate- <u>decreased risk</u>	^e Based on a cohort study of 3518 women with KS (110) <u>Breast</u> : only in 47,XXY karyotype (not in men with mosaicism or more than 3 sex chromosomes) <u>Non-Hodgkin's lymphoma</u> : only in men with more than 3 sex chromosomes <u>Germ cell tumors</u> : increased risk of germ cell tumors was found in a smaller cohort (696 KS patients) (112), and in case reports of germ cell tumors (109,119,143,144)

^aThe comment refers to all 3 neoplasms.

^bThe comments refer to DS related neoplasms.

^cThe comment refers to Del 11p, Del 11q24, and Del 13q disorders.

^dThe comments refer to TS related neoplasms.

^eThe comments refer to KS related neoplasms.

hybridization analysis in an embryonal rhabdomyosarcoma (RMS) from an MVA case revealing aneuploidies typical of sporadic embryonal RMS, with gain of chromosomes 3, 8, 13 and loss of chromosomes 9, 14, X (13–15). While somatic *BUB1B* mutations are rare in sporadic childhood malignancies (12), it is possible that the genetic progression in RMS from MVA and non-MVA cases may be similar. Strikingly, however, the pattern of cancers in patients with MVA differs from sporadic childhood cancers. MVA seems to be specifically associated with the relatively rare embryonic myogenic and renal cancers and not with the most common sporadic childhood cancers. Abnormal mitotic checkpoint was described in sporadic neuroblastoma (16) but this tumor was not reported in patients with MVA. The specific association with RMS and Wilms' tumors may be related to frequent predominance of trisomy 8 and trisomy 18 in MVA (17), two trisomies that are associated with RMS and Wilms' tumors, respectively (18–20). Alternatively, the pattern of tumors in MVA possibly suggests a developmental role of *BUBR1* or the mitotic checkpoint in the development of the embryonic kidney and muscle.

CONSTITUTIONAL ANEUPLOIDY OF THE AUTOSOMES AND CANCER

Trisomy 8

Full constitutional trisomy 8 (cT8) is very rare, whereas cT8 mosaicism (cT8M) is more frequent—the prevalence was estimated to be 1:25 000 (21). The full condition presents with physical stigmata, skeletal abnormalities and a mild-to-moderate mental retardation (22).

Trisomy 8 can be seen as a mosaic in the blood or in the skin or both (21). At least 23 cT8M patients were reported to have neoplasms: 18 myeloid malignancies (21,23–35) and five solid tumors (20,36–39). Since cT8M prevalence is very low, this probably represents an increased risk for myeloid neoplasms.

Acquired trisomy 8, which is restricted to the malignant cells, is the most common numeric aberration in AML and MDS (40,41). Interestingly, it is also the most common genetic aberration in the myeloid leukemias of Down's syndrome (DS). Maserati *et al.* (42) suggested that some of the myeloid malignancies with 'acquired' trisomy 8 are actually undiagnosed cT8M—they have found 3 such cases out of 14 patients with myeloid malignancies and trisomy 8.

The association of both somatic and constitutional trisomy 8 with myeloid malignancies suggests a cell autonomous leukemogenic role for this trisomy, although the specific oncogenes are presently unknown (43). Interestingly, analysis of bone marrow from a patient with cT8M revealed increased activity of bone marrow stromal cells in supporting hematopoietic progenitor cell expansion (31). In addition, characterization of trisomy 8-positive NK cells from a cT8M patient showed an immunosenescent phenotype that may contribute to the escape and expansion of neoplastic cells as a result of altered immunosurveillance (44). Hence the micro- and macro-environment in patients with cT8M may also play an important role in the pathogenesis of MDS.

Trisomy 18

Constitutional trisomy 18 (Edwards syndrome, cT18) is the most common autosomal abnormality after trisomy 21, occurring in 1:3000 live births (45,46). Renal abnormalities, particularly horseshoe kidney, are common (47). The chances of survival over 1 year are only 10% (48). Therefore, most patients with cT18 do not survive long enough to develop cancer.

There have been nine reports of Wilms' tumor in individuals with cT18, seven of them in females (18,19,49–52). cT18 was also found to occur at higher rates than expected based on chromosome surveys of 5854 newborns with Wilms' tumor (18). Hepatoblastoma, another rare childhood cancer (annual incidence of 1 per million children (53)), was reported in seven patients with cT18, all females (54–60). Thus it appears that there is an increased rate of Wilms' tumor and hepatoblastoma in *female* patients with cT18, especially for long-term survivors.

Hepatoblastoma and Wilms' tumors are embryonal tumors of the liver and kidney, respectively. Acquired trisomy 18 is frequently found in sporadic Wilms' tumors but is rare in hepatoblastoma (<http://cgap.nci.nih.gov/Chromosomes/RecurrentAberrations>). Traditionally the hallmark of cancer predisposition syndromes is an earlier age of cancer occurrence. However, the age of diagnosis of Wilms' tumor with cT18 (7 years) is double than sporadic Wilms' tumor. This and the specific association with the female gender argue for a complex pathogenesis of cancer in patients with cT18.

Trisomy 21—Down's syndrome

DS is reviewed by Weissman *et al.* in this issue. The reader is also referred to comprehensive recent reviews on the leukemias of DS (61–64).

The risk of most solid tumors is reduced in DS (65,66). A mouse model suggested that this is due to a dose-dependent tumor suppressive effect of the Hsa21 transcription factor *Ets2* (67). In contrast, children (not adults) with DS have a X600 increased risk for acute myeloid (ML-DS) and X20 increased risk for B cell precursor lymphoblastic leukemias (ALL) compared with normal children (65).

The myeloid leukemias of DS (ML-DS). These are unique to DS (68). Approximately 5% of children with DS are born with a transient clonal megakaryocytosis syndrome, named 'transient myeloproliferative disorder' (TMD) (69), which usually resolves spontaneously. Approximately 20% of DS patients with TMD will develop, however, a full-blown malignant acute myeloid leukemia with megakaryoblastic phenotype (AMKL) by the age of 4 years, which will not regress without chemotherapy (70). Both TMD and DS-AMKL are characterized by a mutation in the *chromosome X* transcription factor *GATA1* that occurs *in utero* and invariably results in the expression of a shorter isoform *GATA1s* (71–73). *GATA1* regulates normal development of the erythroid, megakaryocytic and basophilic/mast cell lineages. A 'knock-in' experiment in mice and gene expression analysis of DS-AMKL suggest that *GATA1s* enhances the proliferation and block the differentiation of immature embryonic megakaryoblasts (74,75).

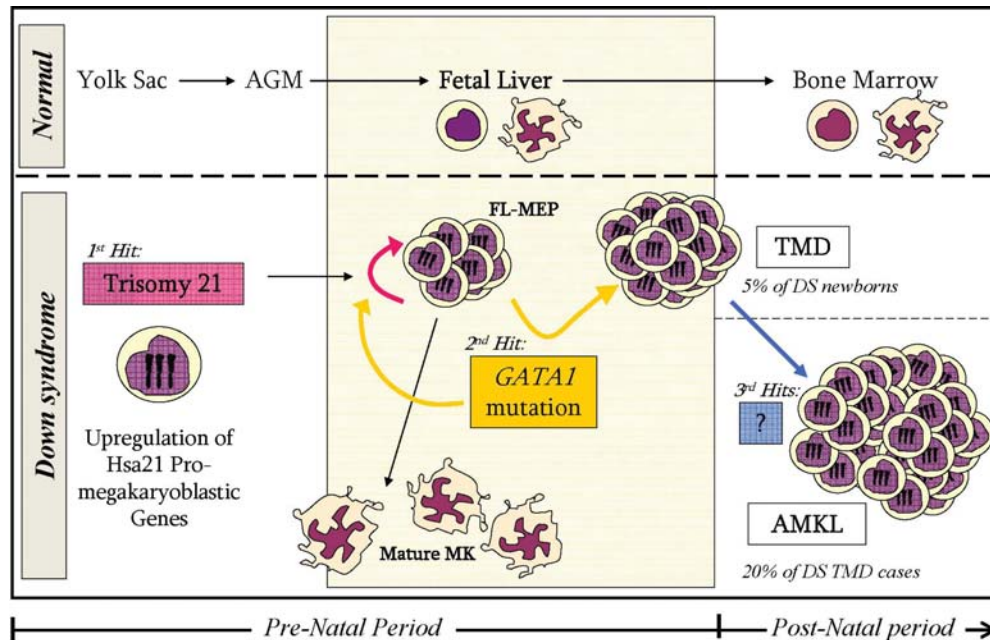


Figure 1. Proposed model for the mechanism underlying DS predisposition to megakaryoblastic leukemias. Increased expression of chromosome Hsa21 genes in fetal liver hematopoietic stem cells leads to increased production of megakaryo-erythroid progenitors (MEPs). The somatically acquired GATA1s mutation further blocks megakaryoblastic differentiation and enhances MEPs proliferation. This can lead to transient myeloproliferative disorder (TMD) in 5% of DS newborns. Acquisition of additional somatic genetic or epigenetic events during infancy will lead to the development of full-blown megakaryoblastic leukemia (AMKL) in one-fifth of TMD patients.

The current model of multi-step myeloid leukemogenesis in DS (Fig. 1) suggests that trisomy 21 enhances the proliferation and self-renewal of fetal liver megakaryo-erythroid progenitors in a cell-autonomous manner (76,77). This 'positive' developmental pressure towards the megakaryo-erythrocytic lineage cooperates with the somatic mutation in *GATA1* that further enhances the clonal proliferation of immature megakaryoblasts diagnosed at birth as TMD. *GATA1* mutations are necessary but insufficient for the development of DS-AMKL. Additional genetic events such as activating mutations of *JAK3* (78), or trisomy 8 (79) have been proposed to mediate the progression from TMD to DS-AMKL.

There are several candidate 'megakaryoblastic oncogenes' on Hsa21 including *RUNX1*, *ETS2*, *ERG* and *miRNA 125* (80–83). Trisomies result in modest elevated expression of multiple genes residing on the trisomic chromosome (84), although the expression of some genes may vary in a more drastic way (85). Thus it is possible that the tilt of normal fetal hematopoiesis towards the megakaryocytic lineage in DS results from the co-expression of several pro-megakaryopoiesis genes from the trisomic Hsa21.

This cooperation between several genes on the same chromosome may represent a general mechanism by which trisomies affect development and cancer.

Acute lymphoblastic leukemia of DS (DS-ALL). This is similar to the 'common' B-cell precursor ALL affecting pre-school children. Indeed it is the most common leukemia in DS, comprising about 1–3% of total children with ALL (86–88). Acquired trisomy or tetrasomy 21 is the most common genetic aberration in sporadic ALL. Hence it is tempting to speculate that constitutional and somatic trisomy 21 may

facilitate leukemogenesis in a similar fashion. Yet recent molecular and cytogenetic studies suggest that at least some of the DS-ALL have unique features.

Cytogenetically DS-ALL is characterized by reduced prevalence of the common aberrations found in childhood leukemia. Rather there are some unique features such as an additional chromosome X as *single cytogenetic abnormality* that suggest a yet unknown, collaborating event between genes on chromosomes 21 and X (79). More recently a mutation in the *JAK2* kinase was identified in about one-fifth of the patients with DS-ALL (89–92). All mutant alleles centered around a highly conserved arginine residue (R683) within the *JAK2* pseudokinase domain. The mutations immortalized primary mouse hematopoietic progenitors, and caused constitutive *JAK/STAT* activation and cytokine independent growth of Ba/F3 cells that was sensitive to pharmacological inhibition of *JAK/STAT* signaling. Mutations in *JAK2* are characteristics of myeloproliferative neoplasms (MPNs). However, similar to the specificity of *GATA1* mutations to DS myeloid malignancies, the novel mutations in R683 of *JAK2* are unique to DS-ALL and are detected neither in MPNs nor in sporadic ALL. Modeling of *JAK2* pseudokinase domain revealed that R683 is situated in an exposed conserved region separated from the one involved in MPNs. These observations not only provide the first molecular specific lesion of DS-ALL but also suggest that these leukemias are candidates for therapy with the novel *JAK2* inhibitors. These findings also raise many interesting questions regarding the nature of this unique collaboration between constitutional (*but not acquired*) trisomy 21 and *JAK2* R683 mutations.

Clinically, DS-AMKL is highly responsive to chemotherapy while the prognosis of DS-ALL is grimmer (93–95). This is in

contrast to sporadic leukemias characterized by a better cure rate of ALL compared with AML. The heightened sensitivity of DS-AMKL blasts may be related to the reduced catabolism of the chemotherapeutic ARA-C caused by genes regulated by the mutated GATA1. In contrast, DS with ALL are highly sensitive to the toxic effects of Methotrexate, a drug that is not used in AML, due to the excess activity of the folate transporter coded by an Hsa21 gene (96). These examples demonstrate the pharmacogenomic effects of a trisomy on drug metabolism and response to therapy.

Aneuploidies of the sex chromosomes

Turner syndrome (45,X). This is a relatively common (1:2000) syndrome associated with decreased adult stature, gonadal dysgenesis, reduced female sex steroids, infertility and other stigmata (97,98).

A cohort study of 3425 women with Turner syndrome (TS) (99) found an increased risk of CNS tumors, ocular cancer, gonadoblastoma and bladder and urethral cancers, while the risk for breast cancer was found to be decreased. An increased risk of colon cancer, which was seen in another study (100), was not noted.

Gonadoblastoma is a rare neoplasm that develops almost exclusively in the dysgenetic gonads of women with Y chromosome mosaicism. Malignant transformation occurs in 60% of these tumors, with 50% developing into dysgerminomas and 10% into other malignant germ cell tumors (101). The predisposition of dysgenetic gonads to develop gonadoblastoma was postulated to be associated with one or more genes on the Y chromosome, with the stimulatory effect of the gonadotropins (101), and with the presence of poorly differentiated XY gonadal tissue in an abnormal (intra-abdominal) environment (102).

The decreased risk for breast cancer was confined to women with 45,X monosomy, whereas women with 45,X/46,XX mosaicism had similar risk to the general population. This may be explained by the fact that women with 45,X/46,XX karyotype have a less severe phenotype and have a relatively high percentage of spontaneous menses and breast development (101).

An interesting finding is the *lack* of significant increased risk for malignant melanoma in TS, since these patients have multiple melanocytic nevi, the strongest established risk factor for melanoma (99). One possible explanation for the absence of an overt increase of incidence of melanoma is the absence of circulating sex hormones as these girls fail to undergo normal pubertal development (103). Another explanation for variation in non-hormonal sensitive tumors may involve a haploinsufficiency of autosomal X linked genes in TS (104–107).

Klinefelter syndrome (47,XXY). Klinefelter syndrome (KS) is a group of chromosomal disorders in which there is at least one extra X chromosome to a normal male karyotype, 46,XY. XXY aneuploidy is the most common disorder of sex chromosomes in humans, with prevalence of one in 500 males (108). KS is characterized by hypergonadotrophic hypogonadism, small testes, infertility, reduced body hair, gynecomastia and tall stature (109).

A cohort study of 3518 men with KS revealed increased risk of lung cancer, breast cancer and non-Hodgkin lymphoma, while a decreased risk of prostate cancer (110). Contrary to an anecdotal report (111), there was no statistically significant increase in the incidence of leukemia. The lower risk for prostate cancer is in accordance with a Danish cohort (112). The reduced prostate cancer and the increase in breast cancer likely represent the hormonal milieu of these patients characterized by high estradiol and low androgen levels.

There are contradictory reports regarding the frequency of extragonadal (mediastinal or pineal) germ cell tumors (EGGCT) in KS. No such tumors were reported in the British cohort study (110), while increased prevalence was reported in several previous studies (112–115). Studies suggested that 15% of patients with intracranial germ cell tumors and 8% of male patients with primary malignant mediastinal germ cell tumors have KS (109,113,116–119). The increased prevalence of EGGCT in KS may reflect either the role of extra chromosome X in these tumors (120) or a developmental defect in the migration of germ cells in KS (109,115,121,122).

GENERAL PRINCIPLES OF THE PATHOGENESIS OF CANCER BY CONSTITUTIONAL CHROMOSOMAL ANEUPLOIDIES

Cancer as a developmental disease

A fundamental difference between constitutional and acquired aneuploidy is that the former exists in many tissues from the earliest stage of embryonic development, whereas the latter is acquired and exists only in the transformed cells. Thus constitutional aneuploidy can predispose to cancer in variety of ways. It may exert a *direct* oncogenic activity in a *cell autonomous* manner. This may be the situation in cancers in which the same aneuploidy is also common in sporadic cancers such as trisomy 21 in ALL, trisomy 8 in myeloid leukemias and trisomy 18 in Wilms' tumors. Alternatively, cancer may arise because of aberrant effects of the trisomy on immediate microenvironment, for example the enhancement of hematopoietic progenitor proliferation by constitutional trisomy 8. Cancer may also arise because of abnormalities in the 'macro-environment'—for example the hormonal abnormalities in disorders of the sex chromosomes, hormonal replacement therapy (99,123), susceptibility of mal-developed organs to malignant transformation (e.g. horseshoe kidney in cT18) (47) or even altered immunosurveillance (e.g. NK cells in cT8M) (44) (Fig. 2).

What is common to all constitutional aneuploidies is their marked effect on embryonic development. Indeed the types of cancers observed reflect these abnormalities, e.g. the megakaryoblastic malignancies in DS and the gonadoblastomas in TS. Even more striking is the specific association of a general aneuploidy defect, as seen in MVA, with rather specific embryonal tumors (RMS and Wilms' tumors). This type of association is seen also in other inherited cancer-predisposing syndromes. Thus mutations in Rb and p53 tumor suppressor genes are observed in most sporadic cancers, yet germline mutations in these genes are associated with childhood retinoblastoma and soft tissue sarcomas,

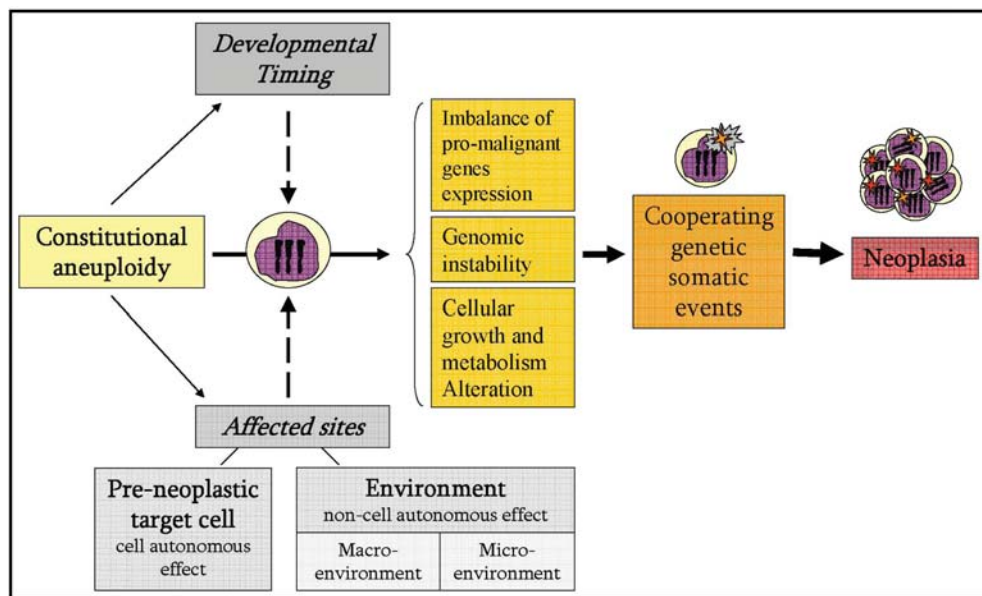


Figure 2. Factors affecting the cancer predisposition by constitutional aneuploidy. Imbalance of pro-malignant genes expression, genomic instability and cellular growth alterations coupled with specific acquired cooperative genetic events are the main mechanisms that underlie the neoplastic predisposition of constitutional aneuploidy. In addition, as the constitutional aneuploidy exists from a very early developmental stage, the developmental timing of the aneuploidic effect may have an influence on the end-point result of the aneuploidy. Moreover, as the constitutional aneuploidy resides in many tissues, it can affect the pre-neoplastic target cell in a cell-autonomous manner, and/or affect the micro- and macro-environment of the pre-neoplastic cell in a non-cell-autonomous manner.

respectively, two rare embryonal childhood cancers (124,125). Indeed, *sporadic* childhood cancer may be viewed as a developmental disorder. While this is obvious for the embryonal cancers, such as RMS, Wilms' tumors, neuro-, medullo- and hepto-blastomas, the basis of the most common childhood cancer, acute lymphoblastic leukemia (ALL), is also developmental (126,127) and is reminiscent of the pattern of the myeloid leukemias of DS. Acquired somatic structural or numerical genetic aberration arising during embryonic lymphoid development leads to a proliferation of a pre-leukemic clone that can be identified by molecular means in 1–5% of normal newborns. One or more, less common, *postnatal* somatic genetic events are needed for transformation of these pre-leukemic cells similarly to the events required for the evolution of TMD to AMKL in DS.

Aneuploidy and cooperating oncogenic genetic aberrations in cancer

Aneuploidy alone is not sufficient for carcinogenesis. Most children with DS do not develop leukemia and even in the presence of widespread aneuploidies in MVA the prevalence of cancer is <50%. In most of the constitutional aneuploidies, the additional oncogenic events that cooperate with the aneuploidy have not been studied. The mutations in GATA1 and the JAK2 cooperating with cT21 in the myeloid and lymphoid leukemias of DS are remarkably specific to DS. They have not been described in sporadic leukemias with acquired trisomy 21. The cause of the specific association between cT21 and these mutations, outside Hsa21, is presently unknown.

A more general model that may explain these specific cooperative events emerges from recent experiments by Williams *et al.* (128). By generating a series of cell lines that carry an

extra copy of one of four mouse chromosomes they showed that aneuploidy reduces cellular fitness by repressing cell proliferation, alters their metabolic properties and influences their immortalizing capabilities. This is consistent with the general growth inhibitory role of constitutional trisomies and with the potential for tumor suppression as observed in DS and in some experimental aneuploidy models in mice (129,130). Overcoming this proliferative block requires specific cooperating mutations. This phenomenon is similar to classical requirement for cooperative oncogenic activity to overcome the senescence or cell death induced by single oncogenes (131). Thus the association of a constitutional trisomy with cancer may depend on a specific 'permissive cell type' (e.g. an embryonic megakaryocytic erythroid progenitor in DS) and a specific cooperating mutation.

With few exceptions, the major challenge is in deciphering the actual genetic elements (including all type of genes) on the aneuploidic chromosomes involved in cancer initiation and/or progression. The difficulties stem from the general imbalance of expression of most genes from the aneuploidic chromosomes and from the lack of appropriate *in vitro* and *in vivo* models. Such models are required for clarification of the shared and unique pathways by which constitutional and acquired aneuploidies affect neoplastic transformation.

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