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# Animal models in the study of the biological function of genes on human chromosome 21 and their role in the pathophysiology of Down syndrome

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**Human chromosome 21 is the smallest human autosome and many important genetic/familial disorders map to this chromosome, e.g., familial amyotrophic lateral sclerosis (FALS), Down syndrome, Alzheimer's disease and some cases of Ewings sarcoma. Hence, the identification of genes localised to this chromosome and studies on their normal biological function and their role in disease is gaining momentum. The use of animal models to generate gain- and loss-of-function mutations is an important element of these studies on functionality/pathology and has yielded powerful insights. However, no animal model has yet been generated that exactly models any of the disorders associated with this chromosome. The major utility of the animal models has been to illuminate the biological functions of genes and the causation of pathophysiology of diseases associated with genes on this chromosome.**

## INTRODUCTION

Human chromosome 21 is of profound medical interest since a large number of genetic/familial disorders that occur at a relatively high frequency in our population map to this chromosome. Genetic/familial disorders involving human chromosome 21 include Down syndrome (1), ~5% of familial amyotrophic lateral sclerosis (FALS or motor neuron disease) (2), and a proportion of familial Alzheimer's disease (3). In addition, chromosomal translocations associated with cancers such as Ewings sarcoma and other primitive neuroectodermal tumours (4), and acute myeloid leukaemia (5), also involve genes on human chromosome 21. Thus, the identification of genes that localise to this chromosome, the biological function of these genes and the manner in which they contribute to disease are important for a thorough understanding of these disorders and the development of treatments for these disorders.

Chromosome 21 is the smallest human autosome. It consists of ~50 Mb of DNA. The short arm is very small and all of the unique genes that have been located to this chromosome have been mapped to the long arm of the chromosome (Table 1). Indeed, even the long arm of the chromosome does not have a uniform concentration of genes or transcriptional units [i.e., the number of genes per unit length of chromosome is not uniform across the chromosome (Fig. 1)]. The region 21q22.2–21q22.3 has a very high concentration of genes compared with the region 21q21, which has been described as a transcriptional desert. This feature of varying gene density per unit length of chromosome is not unique to chromosome 21 but appears to apply to the entire genome (6).

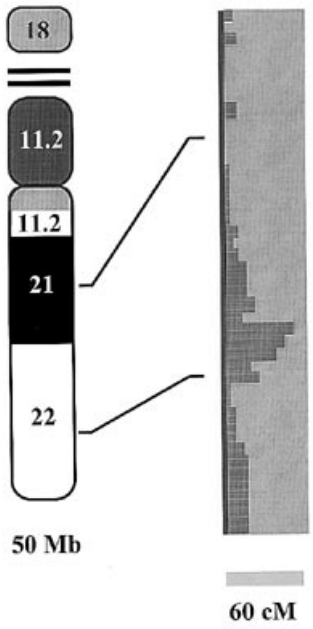


**Figure 1.** Map of the frequency of expressed sequences encoded on different parts of human chromosome 21 (from website, see legend to Table 1).

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**Table 1.** Genes that have been mapped to human chromosome 21 and the homologous location in the mouse

| Gene/protein | Names | GDB Accession no. | Cytogenetic location | Mouse chromosome | Mouse locus |
|---|---|---|---|---|---|
| ACTL5 | ACTFIB | GDB:135326 | 21q-21q | | |
| | actin-like 5 | | | | |
| ADARB1 | adenosine deaminase, RNA-specific, B1 (homolog of rat RED1) | GDB:3929206 | 21q22.3-21q22.3 | 16 | 55.6 |
| APP | Hs.1711 | GDB:119692 | 21q21.2- | | |
| | AD1 | | 21q21.2 | | |
| | amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease) | | | | |
| ASNSL2 | asparagine synthetase-like 2 | GDB:119708 | 21pter-21q21 | | |
| ATP5O | OSCP | GDB:545472 | 21q22.1- | | |
| | ATPO | | 21q22.2 | | |
| | ATP synthesase, $H^+$ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein) | | | | |
| CAF1A-LSB | chromatin assembly factor 1, p60 subunit | GDB:728461 | 21q22.2-21q22.3 | | |
| CBFA2 | AML1 | 128313 | 21q22.1- | 16 | 62.3 |
| | PEBP2A2 | | 21q22.3 | | |
| | Core-binding factor, runt domain, α subunit 2 (acute myeloid leukemia 1; am11 oncogene | | | | |
| CBR | Hs.1346 | GDB:126610 | 21q22.12- | 16 | 66.8 |
| | carbonyl reductase (NADPH) | | 21q22.12 | | |
| CBS | Hs.400 | GDB:119754 | 21q22.3- | 17 | 17.4 |
| | cystathionine-β-synthase | | 21q22.3 | | |
| COL18A1 | Hs.1286 | GDB:138752 | 21q22.3- | 10 | 38.3 |
| | collagen, type XVIII, α1 | | 21q22.3 | | |
| COL6A1 | Hs.3283 | GDB:119065 | 21q22.3- | 10 | 35.5 |
| | collagen, type VI, α1 | | 21q22.3 | | |
| COL6A2 | Hs.3284 | GDB:119793 | 21q22.3- | 10 | 35.5 |
| | collagen, type VI, α2 | | 21q22.3 | | |
| CRFB4 | CRF2-4 | GDB:138168 | 21q22.1- | 16 | |
| | cytokine receptor family II, member 4 | | 21q22.2 | | |
| CRYAA | Hs.64332 | GDB:119074 | 21q22.3- | 17 | 17.4 |
| | CRYA1 | | 21q22.3 | | |
| | crystallin, αA | | | | |
| CSTB | STFB | GDB:5215249 | 21q22.3- | | |
| | EPM1 | | 21q22.3 | | |
| | cystatin B (stefin B) | | | | |
| | CST6 | | | | |
| | Hs.695 | | | | |
| | PME | | | | |
| | epilepsy, progressive myoclonic 1 (Unverricht–Lundborg type) | | | | |
| DCR | DSCR | GDB:125354 | 21q22.2- | | |
| | Down syndrome chromosome region | | 21q22.3 | | |
| DFNB10 | deafness, autosomal recessive 10 | GDB:700061 | 22pter-22qter | | |
| DFNB8 | deafness, autosomal recessive 8 | GDB:636185 | 21q22.3- | | |
| | | | 21q22 | | |
| DYRK | dual-specificity tyrosine-(Y)-phosphorylation regulated kinase | GDB:1297921 | 21q22.2- | | |
| | Hs.83402 | | 21q22.2 | | |

**Table 1.** *continued.*

| Gene/protein | Names | GDB Accession no. | Cytogenetic location | Mouse chromosome | Mouse locus |
|---|---|---|---|---|---|
| ERG | v-ets avian erythroblastosis virus E26 oncogene related | GDB:119884 | 21q22.2-21q22.2 | 16 | 68.6 |
| ETS2 | Hs.1411 | GDB:119888 | 21q22.3- | 16 | 68.8 |
| | v-ets avian erythroblastosis virus E2 oncogene homolog 2 | | 21q22.3 | | |
| EZH2 | EZH1 | GDB:701613 | 21q22.2- | | |
| | enhancer of zeste (*Drosophila*) homolog 2 | | 21q22.2 | | |
| | Hs.73071 | | | | |
| | enhancer of zeste (*Drosophila*) homolog 1 | | | | |
| GABPA | Hs.78 | GDB:138476 | 21q21- | | |
| | E4TF1A | | 21q22.1 | | |
| | E4TF1-60 | | | | |
| | GA-binding protein transcription factor, α subunit (60 kDa) | | | | |
| GART | PGFT | GDB:119487 | 21q22.1- | | |
| | PRGS | | 21q22.1 | | |
| | phosphoribosylglycinamide formyltransferase, phosphoribosyl-glycinamide synthetase, phosphoribosylaminoimidazole synthetase | | | | |
| GRIK1 | GLUR5 | GDB:131462 | 21q22.1- | 16 | 57.9 |
| | glutamate receptor, ionotropic, kainate 1 | | 21q22.2 | | |
| HLCS | Hs.12357 | GDB:392648 | 21q22.1- | | |
| | HCS | | 21q22.1 | | |
| | holocarboxylase synthetase (biotin-[propionyl-Coenzyme A-carboxylase (ATP-hydrolysing)] ligase) | | | | |
| HMG14 | high-mobility group (nonhistone chromosomal) protein 14 | GDB:118809 | 21q22.3 21q22.3 | 16 | 69.7 |
| HPE1 | holoprosencephaly 1, alobar | GDB:136065 | 21q22.3- | | |
| | | | 21q22.3 | | |
| HTOR | 5-hydroxytryptamine (serotonin) oxygenase regulator | GDB:119324 | 21pter-21qter | | |
| IFNAR1 | IFRC | GDB:120078 | 21q22.1- | 16 | 62.8 |
| | IFNAR | | 21q22.1 | | |
| | interferon (α, β and ω) receptor 1 | | | | |
| IFNAR2 | IFNABR | GDB:568494 | 21q22.1- | | |
| | interferon (α, β and ω) receptor 2 | | 21q22.1 | | |
| IFNGR2 | IFNGT1 | GDB:142306 | 21pter-21qter | 16 | * |
| | interferon γ receptor 2 (interferon γ transducer 1) | | | | |
| ITGB2 | MF17 | GDB:120574 | 21q22.3- | 10 | 36.5 |
| | CD18 | | 21q22.3 | | |
| | LFA-1 | | | | |
| | integrin, β2 [antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) β subunit] | | | | |
| KCNE1 | ISK | GDB:127909 | 21q22.1- | | |
| | potassium voltage-gated channel, Isk-related family, member 1 | | 21q22.2 | | |
| KCNJ6 | KCNJ7 | GDB:547949 | 21q22.1- | | |
| | GIRK2 | | 21q22.1 | | |
| | KATP2 | | | | |
| | potassium inwardly-rectifying channel, subfamily J, member 6 | | | | |
| | BIR1 | | | | |
| | Kir3.2 | | | | |

**Table 1.** *continued.*

| Gene/protein | Names | GDB Accession no. | Cytogenetic location | Mouse chromosome | Mouse locus |
|---|---|---|---|---|---|
| KNO | Knobloch syndrome | GDB:4073044 | 21q22.3- | | |
| | KS | | 21q22.3 | | |
| KNP-I | | GDB:5887108 | 21q22.3- | | |
| | | | 21q22.3 | | |
| LSS-PEN | Lanosterol synthase [converts (s)-2,3-oxidosqualene to lanosterol | GDB:642229 | 21q22.3-21q22.3 | | |
| MACSL1 | myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L)- like 1 | GDB:128136 | 21pter-21qter | | |
| MNBH | MNB | GDB:4425789 | 21q22.2- | | |
| | minibrain (*Drosophila*) homolog | | 21q22.2 | | |
| MX1 | IFI-78K | GDB:120206 | 21q22.3- | 16 | 69.7 |
| | myxovirus (influenza) resistance 1, homolog of murine (interferon-inducible protein p78) | | 21q22.3 | | |
| | MxA | | | | |
| MX2 | myxovirus (influenza) resistance 2, homolog of murine | GDB:120207 | 21q22.3-21q22.3 | 16 | 69.7 |
| NF1L1 | neurofibromin 1-like 1 | GDB:216191 | 21pter-21qter | | |
| PCNT | PCN | GDB:555937 | 21q22.3- | | |
| | pericentrin | | 21q22.3 | | |
| PCP4 | Purkinje cell protein 4 | GDB:6233541 | 21q22.2- | 16 | 68.3 |
| | PEP-19 | | 21q22.3 | | |
| PFKL | Hs.26628 | GDB:120276 | 21q22.3- | 10 | 36.5 |
| | phosphofructokinase, liver | | 21q22.3 | | |
| PGA1-LSB | polyglandular autoimmune syndrome 1 | GDB:567198 | 21q22.3- | | |
| | APECED | | 21q22.3 | | |
| PKNOX1 | PBX/knotted 1 homeobox 1 | GDB:5914719 | 21q22.3- | | |
| | | | 21q22.3 | | |
| PNY2 | protein spot in 2-D gels (65 kDa) | GDB:119501 | 21pter-21qter | | |
| PRKCM | Hs.2891 | GDB:330794 | 21pter-21qter | | |
| | PKCM | | | | |
| | protein kinase C, mu | | | | |
| PRSS7 | Hs.3113 | GDB:384083 | 21q21-21q21 | | |
| | protease, serine, 7 (enterokinase) | | | | |
| PWP2H | PWP2 (periodic tryptophan protein, yeast) homolog | GDB:1220218 | 21q22.3- | | |
| | EHOC-17 | | 21q22.3 | | |
| RNR4 | RNA, ribosomal 4 | GDB:119558 | 21p12-21p12 | | |
| S100B | S100 calcium-binding protein, β (neural) | GDB:120360 | 21q22.3-21q22.3 | 10 | 35.5 |
| S14 | surface antigen (chromosome 21) | GDB:119584 | 21pter-21qter | | |
| SIM | SIM2 | GDB:642106 | 21q22.2- | | |
| | single-minded (*Drosophila*) homolog | | 21q22.2 | | |
| SLC19A1 | Hs.25659 | GDB:454112 | 21q22.3- | | |
| | FOLT | | 21q22.3 | | |
| | solute carrier family 19 (folate transporter), member 1 | | | | |
| SOD1 | Hs.26891 | GDB:119596 | 21q22.1- | 16 | 61.2 |
| | ALS | | 21q22.1 | | |
| | ALS1 | | | | |
| | superoxide dismutase 1, soluble [amyotrophic lateral sclerosis 1 (adult)] | | | | |

**Table 1.** *continued.*

| Gene/protein | Names | GDB Accession no. | Cytogenetic location | Mouse chromosome | Mouse locus |
|---|---|---|---|---|---|
| SON | Hs.29140 | GDB:331317 | 21q22.1- | 16 | 64.0 |
| | DBP-5 | | 21q22.2 | | |
| | SON DNA-binding protein | | | | |
| STCH | stress 70 protein chaperone, microsome-associated, 60 kDa | GDB:358960 | 21q11.1-21q11.1 | | |
| TFF1 | D21S21 | GDB:119722 | 21q22.3- | | |
| | BCEI | | 21q22.3 | | |
| | HPS2 | | | | |
| | trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in) | | | | |
| TFF2 | SML1 | GDB:128989 | 21q22.3- | | |
| | spasmolytic protein 1 | | 21q22.3 | | |
| | trefoil factor 2 (spasmolytic protein 1) | | | | |
| TFF3 | trefoil factor 3 (intestinal) | GDB:629964 | 21q22.3- | | |
| | HITF | | 21q22.3 | | |
| TIAM1 | Hs.3205 | GDB:386213 | 21q22.1- | | |
| | T-cell lymphoma invasion and metastasis 1 | | 21q22.1 | | |
| TMEM1 | transmembrane protein 1 | GDB:1320385 | 21q22.3- | | |
| | EHOC-1 | | 21q22.3 | | |
| U2AF1 | U2AF35 | GDB:392758 | 21q22.3- | | |
| | RNU2AF1 | | 21q22.3 | | |
| | U2(RNU2) small nuclear RNA auxillary factor 1 (non-standard symbol) | | | | |
| USH1E | Usher syndrome 1E (autosomal recessive, severe) | GDB:5759584 | 21q21-21q21 | | |
| WHITE1-LSB | white (*Drosophila*) homolog 1, ATP binding cassette transporter superfamily | GDB:717078 | 21q22.3-21q22.3 | | |

Adapted from data available through World Wide Web site at http:www.ncbi.nlm.nih.gov/SCIENCE96/ and related links. We have excluded the HSPA3 gene from this table as we believe that more evidence is required for the localisation of this gene to human chromosome 21 (7). *The IFNGR1 gene is syntenic but a precise location is not known.

It is surprising, given the importance of human chromosome 21 to various pathological conditions and the relatively small size of the chromosome, that currently only ~70 known genes have been mapped to this chromosome (Table 1). The number of functional genes that map to this chromosome is currently unknown but estimates range anywhere from 250 to 1000.

This review mainly addresses the establishment of the biological function of genes on human chromosome 21 and their role in specific pathologies/abnormalities that occur in humans. In particular, this review emphasises mouse models since only this mammal is currently amenable to deliberate and reproducible manipulation of gain- or loss-of-function mutations.

## BIOLOGICAL FUNCTION OF GENES ON HUMAN CHROMOSOME 21

The biological function of a number of genes on human chromosome 21 has been investigated in the mouse using gene knockout technology and these studies have given profound and sometimes surprising insight into the role of such genes.

We have used the knock out of the interferon receptor component IFNAR 1 (8) located at 21q22.1 to investigate the role of the type I Interferon system. IFNAR 1 is involved in binding the >15 (9) interferon (IFN) ligands whose genes are located on human chromosome 9. On the basis of tissue culture experiments, the type I interferon system has been thought to function as antiviral, antiproliferative, differentiation-modulating and natural killer cell-stimulating agents (10). The type I IFN system has also been proposed to function in embryonic development on the basis of its growth regulatory activities and regulated temporal expression of its components during embryonic development (11). Unexpectedly, homozygous null-mutants for IFNAR 1 demonstrate no evidence of abnormalities during embryonic development, since these mice are born at approximately the expected 1:2:1 (+/+:+/–:–/–) ratio according to Mendelian genetics and the mice are by all accounts morphologically normal (12). However, the mice do show a compromised ability to withstand viral infections since IFNAR 1–/– mice die significantly earlier after exposure to viruses such as Semliki Forest virus (SFV) and encepholomyocarditis virus (EMC) compared with +/– and +/+ counterparts. We have shown that known pathways of IFN signalling are inactive in cells from IFNAR 1–/– mice according to measurement of IFN-inducible genes such as 2′-5′ oligoadenylate synthetase and binding of IFN-activated transcription factors to their cognate elements. Primary embryo fibroblasts derived from mice with these respective genotypes similarly show enhanced susceptibility to viral challenge. Fibroblasts from wild-type and heterozygous mice are protected by as

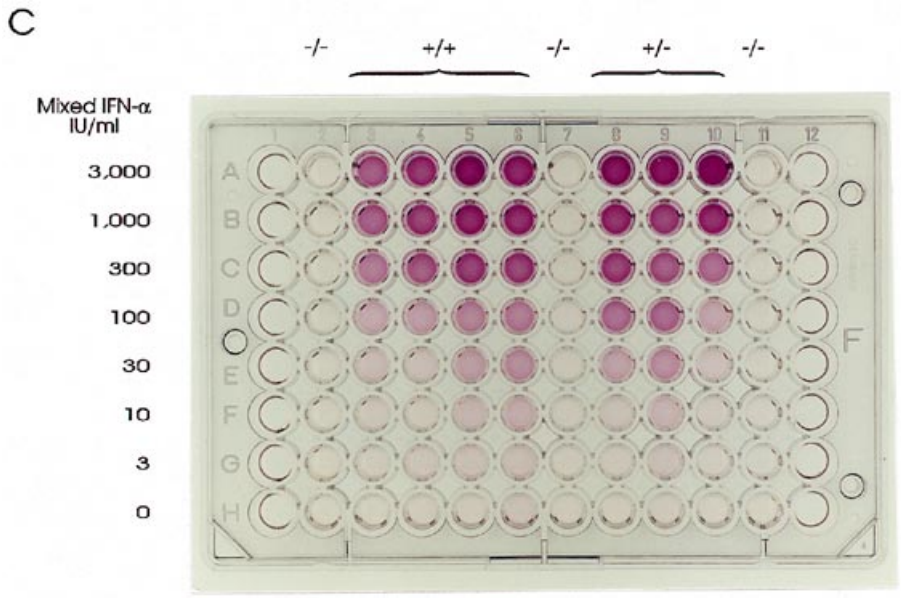


**Figure 2.** Antiviral effects of IFNα on embryo fibroblast cell lines from IFNAR1 +/+, +/– and –/– mice. Each column represents an individual cell line. Colour indicates protection from the cytopathic effect of Semliki Forest Virus.

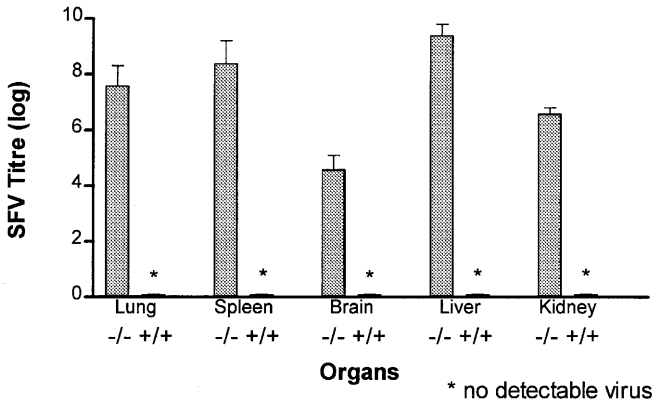


**Figure 3.** Titres of Semliki Forest Virus in organs of IFNAR1 +/+ and –/– mice killed 22 h post infection. *No virus was detectable in organs of +/+ mice.

little as 5 IU/ml IFN, whereas fibroblasts from homozygous mice have no protection from interferon to viral killing at 3000 IU/ml IFN (Fig. 2). Thus, these data prove that the type I interferon system functions in protecting cells against viral infection. The genesis of homozygous null mutant IFNAR –/– mice has also illustrated that type I interferon system acts by inhibiting viral replication. Organs from wild-type mice infected with SFV show no viral titres 24 h post infection, whereas knockout mice show viral titres of $10^4$–$10^{10}$ at the same time point (Fig. 3). These data demonstrate the role of type I interferons in checking or inhibiting viral replication *in vivo*. Our studies have also shown abnormalities in myeloid lineage cells in IFNAR 1–/– mice.

Mice with a null mutation in the CPA 1 gene which encodes β2 integrin (or LFA 1 or CD18) also demonstrate abnormalities in immune function (13). These mice display an impairment in the cytotoxic activity of alloantigen specific CTLs towards allogenic spleen cells as well as cell line targets. Further, con-A stimulated proliferation of CPA1 –/– splenocytes are also improved as compared with wild-type controls. Intriguingly, β2 integrin also seems to be important in tumour rejection since CPA 1 deficient mice are susceptible to metastasis of B16 melanoma cells, even though their *in vitro* NK cell activity appears normal.

Other genes whose products may function in the immune system, in addition to IFNAR-1 and CPA 1, are also located on human chromosome 21, especially those related to the interferon system. Another interferon receptor component, IFNAR 2, is located at 21q22.1 in close proximity to the IFNAR 1 gene (14 and Table 1). We have also identified a gene coding for a novel type interferon signalling molecule designated ISF 21 to human chromosome 21 in the region 21q22.2–21q22.3. The basis for identification of this gene to this region was the elicitation of an antiviral response in somatic cell hybrids containing various parts of human chromosome 21 on a CHO background (15,16). Only hybrids containing the region 21q22.2–21q.22.3 and specifically those that are located between the 10;21 and r21 breakpoints were able to elicit an antiviral response (Fig. 4). The failure to mount an antiviral response was unrelated to the ability to bind the interferon ligand as the vast majority expressed both IFNAR 1 and IFNAR 2 receptor components and Scatchard analyses demonstrated similar binding characteristics in terms of both affinity of binding and numbers of receptors per cell (15). Instead, the available data suggest that ISF 21 functions early in transducing type I interferon signals. It functions upstream of the transactivation of IFN regulated genes such as 2′-5′ oligoadenylate synthetase (2′-5′OAS) (Fig. 5). A similar molecule that functions in the type II (or IFNγ) pathway, IFNGR2, also localises to human chromosome 21 (17). MX1, a 78 kDa interferon inducible protein, and its family member MX2, are located at 21q22.3 (Table 1) and 'natural' mouse mutants which do not produce Mx protein show a susceptibility to influenza virus infection (18). The CRFB4 gene, a member of the class II

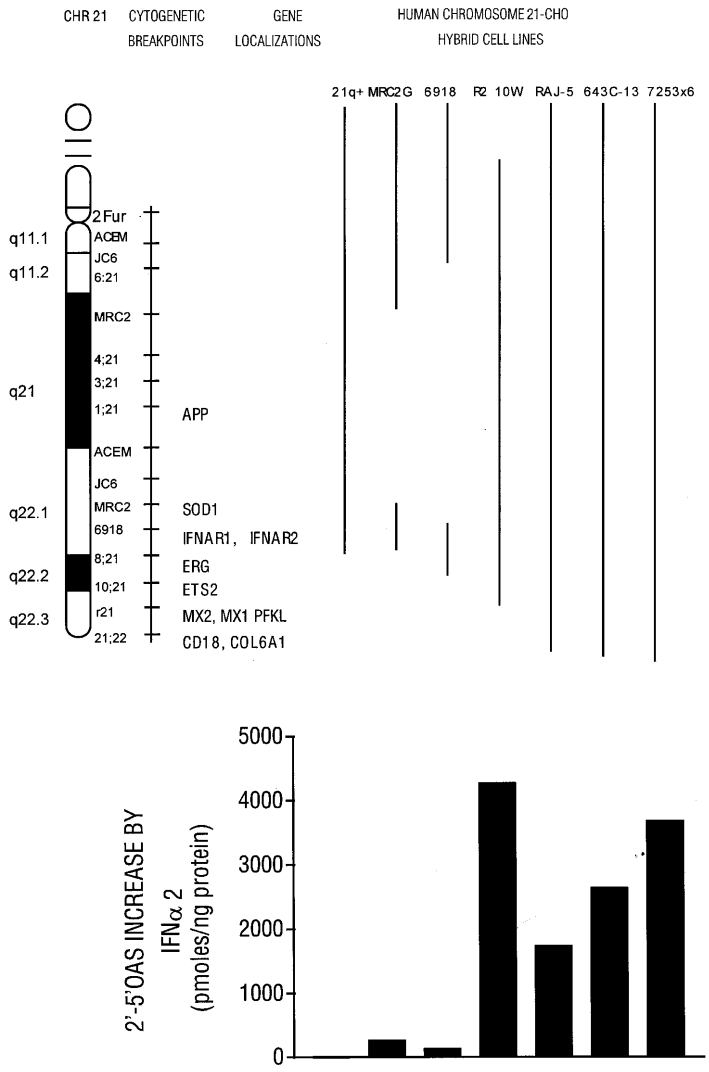


**Figure 4.** Type I IFN signalling in a panel of CHO-human chromosome 21 hybrid cell lines containing different portions of chromosome 21 as indicated in the upper panel. Signalling was determined as IFNα-induced increase in 2′-5′ oligoadenylate synthetase enzyme activity.

cytokine receptor family (which includes the IFN receptors) localises to 21q22.1–21q.2 (19). Thus a large number of genes that function in the interferon signalling pathways and/or are structurally related to interferon receptors localise to human chromosome 21. However, gene knockout studies remain to be carried out on some of these to establish their exact biological functions *in vivo.*

The CBFA2 or AML1 gene located at 21q22.1–21q22.2 is the most frequent target of chromosomal rearrangement in human leukaemia. The gene knockout for CBFA2 has identified this transcription factor as being vital for the regulation of downstream cellular target genes essential for definitive haematopoiesis of all lineages since AML1–/– embryos die between E11.5 and E13.5 and show a lack of fetal liver haematopoiesis (20). This defect appears to be intrinsic to haematopoietic stem cells since AML1–/– ES cells fail to contribute to haematopoiesis in chimaeric animals.

The APP gene has been knocked out and mice show reactive gliosis and decreased locomotor activity (21). The APP gene is also being knocked out using conditional knockouts employing the loxP/CRE system. Germline transmission of ES cells with the APP gene deleted have been obtained and future studies will elucidate the functional consequences of such a deletion. Gene knock in studies are also being employed to humanise the APP gene (by knocking in the human gene harbouring the Swedish mutation). This approach will eliminate confounding variables of: (i) a background of mouse APP gene product; (ii) a separation of the effects of possible phenotypic consequences resulting from over-expression (two copies of the mouse APP gene plus one or more copies of the ectopically expressed mutant APP transgene) versus those resulting from mutation (the ectopically expressed mutant APP gene) (22).

The SOD1 gene has been knocked out and as yet these mice do not display overt phenotypic anomalies; the major phenotype seems to be an inability to cope with oxidative stress (23). Mice with a null mutation in the CRYA gene which encodes alpha crystallin A, develop cataracts, indicating a role for this gene in maintaining eye lens transparency (24).

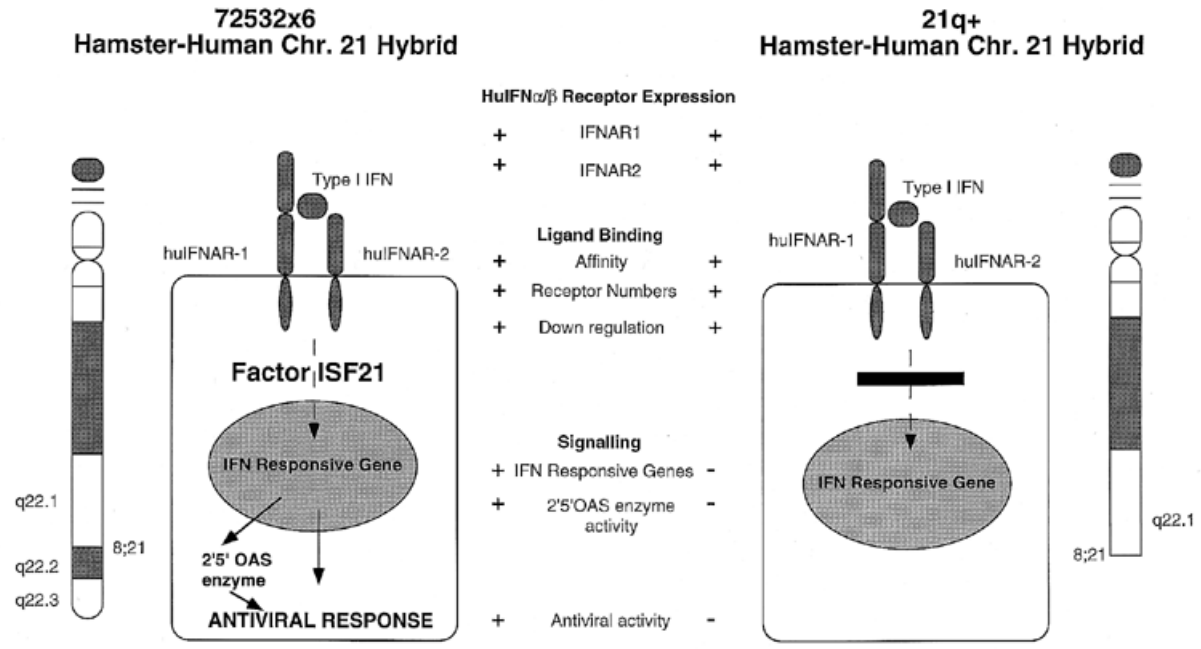


**Figure 5.** Summary of data which characterise the action of ISF21 post receptor binding and pre-transcriptional activation of IFN-responsive genes.

## HUMAN CHROMOSOME 21 AND THE GENETICS OF HUMAN PATHOLOGY

Several human genetic diseases/disorders are related to one or more genes on human chromosome 21. These include several loci related to deafness (DFNB 10, DFNB 8) (25,26) and a severe form of Usher syndrome (IF) (USHIF) (27), familial motor neurone disease (28), polyglandular autoimmune disease (29), Knobloch syndrome (30), holoprosencephaly (31), progressive myoclonic epilepsy (32), acute myeloid leukaemia (5), Alzheimer's disease (3) and Down syndrome (1).

### Down syndrome

Down syndrome (Fig. 6) or trisomy 21 is the most common chromosomal abnormality that comes to term in humans (33). It occurs at a rate of 1 in 700 live births. It is also the most common cause of mental retardation amongst humans. Further, individuals with Down syndrome develop abnormalities of every major organ system, including the deposition of brain amyloid (senile) plaques and neurofibrillary tangles that characterise Alzheimer's disease by the third decade of life; a 50–70 fold higher incidence of childhood leukaemias; characteristic cranofacial features that include brachycephaly, flattened noses with higher bridges; protruding foreheads and epicanthic folds; skeletal defects and bone abnormalities (an osteoporosis-like bone with larger lumens and decreased bone diameter); shorter necks; endocardial cushion defects; congenital cataracts; compromised immune-system or immunodeficiency; some characteristics of autoimmunity; hearing defects and a predisposition to respiratory infections (33). Many of the abnormalities that occur as part of the Down syndrome phenotype also occur in the general population, albeit at a lower frequency and later in life. As a consequence of all of the above, the relationship between individual genes on human chromosome 21 with specific pathophysiological features that occur in Down syndrome has assumed priority. Animal models have been used to define this relationship and to gain insight into Down syndrome and/or its pathophysiologies.

**Figure 6.** A child with Down syndrome.

**Table 2.** Comparison of the phenotype of trisomy 16 mice with that of Down syndrome[a]

| Phenotype | Mouse trisomy 16 (fetal) | Human trisomy 21 (postnatal) |
|---|---|---|
| Survival beyond term | None | <30% |
| Growth *in utero* | Decreased 10–25% | Birth weight reduced ~10% |
| Edema *in utero* | Massive, but transient | Transient edema of neck |
| Brain development | Retarded, with reduced weight | ? Retarded maturation and decreased sulcation |
| | | Head circumference decreased ~ 2% at birth |
| | Reductions in several neuronal neurotransmitter markers | ? Modest reduction in cholinergic cells during infancy (single case) |
| | Structural alteration of the cochlear and vestibular portions of the inner ear | Anomalies of the inner ear |
| Congenital heart disease | Present in 96%, with aortic arch anomalies in >80% and endocardial cushion defect in ~50% | Present in ~45% with endocardial cushion defect in ~32% (of all affected) and aortic lesions in ~15% |
| Immunology and | Severe thymic hypoplasia | Thymic hypoplasia at birth |
| hematology | Delayed maturation of thymic lymphocytes *in vitro* | Reduced T-lymphocyte responses |
| | Reduction in pre-B and B lymphocytes | Decreased antibody responses |
| | Reduced stem-cell populations | ?Decreased circulating CFU-C |
| | Poor lymphoid and erythroid cell survival in radiation and aggregation chimeras | Reduced proportion of trisomic lymphocytes in blood of trisomy 21/2n mosaics |
| Bone/cartilage | Abnormal shaped skull | Thin calvaria |
| | Shorter necks | Delayed closing of sutures |
| | | hypoplasia of basilar facial and nasal bones |
| | Enchondral ossification | Enchondral ossification of vertebrae |
| | Smaller atlas vertebrae | |
| | Cartilaginous hypoplasia | Cartilaginous hypoplasia |

[a]These data are derived from references 34 and 41.

*Trisomy 16 mice.* Human chromosome 21 has significant homology with mouse chromosome 16 (Table 1 and Fig. 7). Therefore, trisomy 16 mice have been generated and investigated as a mouse model for Down syndrome (34). Trisomy 16 mice have significant similarities in some characteristics to human individuals with Down syndrome. These features have been characterised at a morphological, biochemical and immunological level and many striking similarities exist (Table 2).

Mouse chromosome 16 also has homology to other human chromosomes including human chromosome 3, and genes from the more terminal part of human chromosome 21 have rearranged to be located on other mouse chromosomes such as 10 and 17 (Table 1 and Fig. 7) (35). Thus, trisomy of mouse chromosome 16 involves a gene dosage perturbation of many more genes than those that occur in human trisomy 21, as well as a lack of trisomy of some genes which are not syntenic with human chromosome 21. Consequently, trisomy 16 mice do not *totally* model Down syndrome. Among the differences that exist is the fact that trisomy 16 mice never survive to live past the first day of birth and most frequently die in late gestation. It may be that this is due to trisomy of genes located on human chromosome 3 in these mice and hence efforts have been expanded at generating partial trisomy 16 mice as a mouse model that more closely resembles Down syndrome.

*Partial trisomy 16 mice.* Mice that are trisomic for chromosome 16 that have homology to human chromosome 21 from 21q21 to 21q22.3 (36) develop some features reminiscent of Down syndrome. However, gross morphological analysis of liveborn mice did not demonstrate a phenotype as severe as that which occurs in Down syndrome or in trisomy 16 mice. It is possible that mice with the more severe phenotype die *in utero*. Further extensive analysis of morphological, histological, biochemical and immunological abnormalities of these mice should reveal to what extent these mice phenocopy humans with Down syndrome. This should assist the effort of determining genotype/phenotype relationships in Down syndrome. Nevertheless, the genomics of these mice with partial trisomy of mouse 16 that are liveborn is an important advancement, especially as it may now facilitate the evaluation of histopathologies that occur in Down syndrome in later life, e.g., the development of Alzheimer's disease.

*YAC/BAC/PAC transgenics.* Several transgenic lines of mice have been made with human YACs that span specific loci, e.g., the APP locus (37,38). These mice have been shown to over-express human mRNA and protein at levels comparable to endogenous APP and/or have been shown to appropriately splice primary human APP transcripts in their brains. The exact histopathological lesions that occur in these animals is being awaited to establish

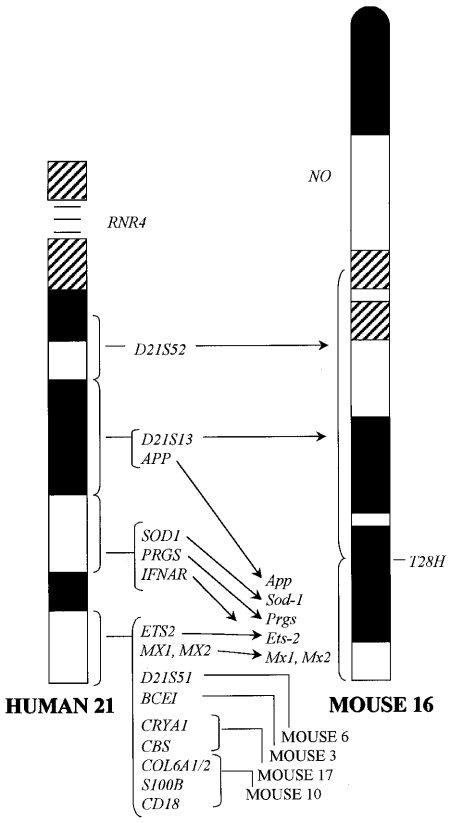


**Figure 7.** A representation of the similarity between genes on mouse chromosome 16 and human chromosome 21.

whether these animals model Alzheimer's disease and/or Down syndrome. Further, the application of YACs to other loci and the genesis of transgenic mice with BACs and PACs still has to occur.

*'In vivo Library' mice.* A recent approach with potentially useful utility for dissecting phenotype/genotype relationships in Down syndrome has been developed (see Smith and Rubin, this issue). The approach entails scanning a large chunk of a desired region of the human genome and sifting through this region to identify phenotype/genotype relationships (39). Overlapping or contiguous YACs spanning up to 2 Mb (4%) of a region of human chromosome 21 (21q21.2) have been used to generate transgenic mice and these were evaluated for learning defects. Mice with such features were obtained from microinjections of two different YACs that were not contiguous. One of these was investigated in detail and resulted in the identification of the human homologue of *Drosophila minibrain* as a candidate for the genesis of these learning defects.

This approach has the advantage that a large region of the human genome can be screened but that phenotypic perturbations can be related to one or a few genes, especially since the YACs can undergo fragmentation during microinjection and the resultant phenotype narrowed to even smaller regions of the genome (Fig. 8) (40).

*Transgenic mice over-expressing single genes.* This approach has thus far received the most widespread application. The utility of this approach lies in evaluating the phenotype/genotype relationship of genes from human chromosome 21. Clearly, this approach will not result in mice that are complete models for Down syndrome, which involves many genes. We have generated

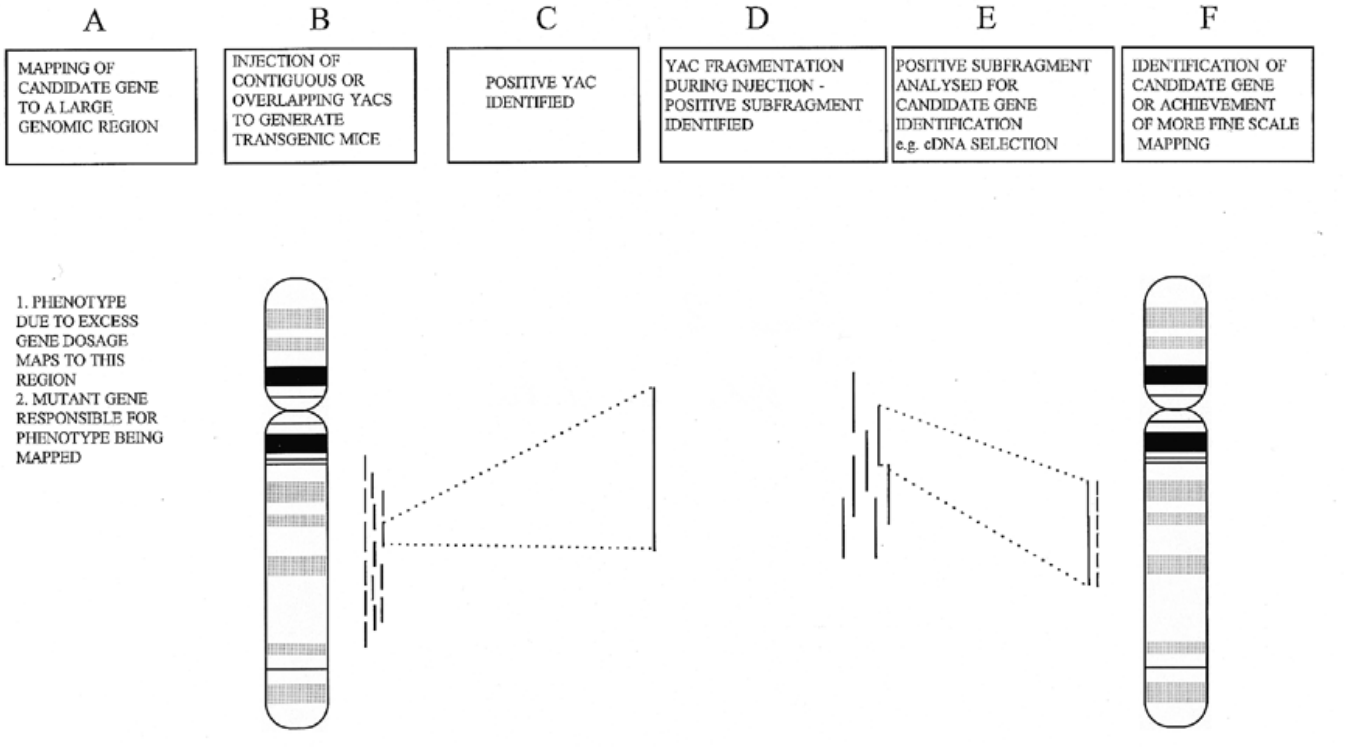


**Figure 8.** The use of the '*in vivo* library' approach for mapping of candidate genes in two potential situations: (i) when a particular phenotype is due to increased gene dosage; or (ii) complementation of a mutation for the identification of a gene responsible for the genesis of a phenotype.

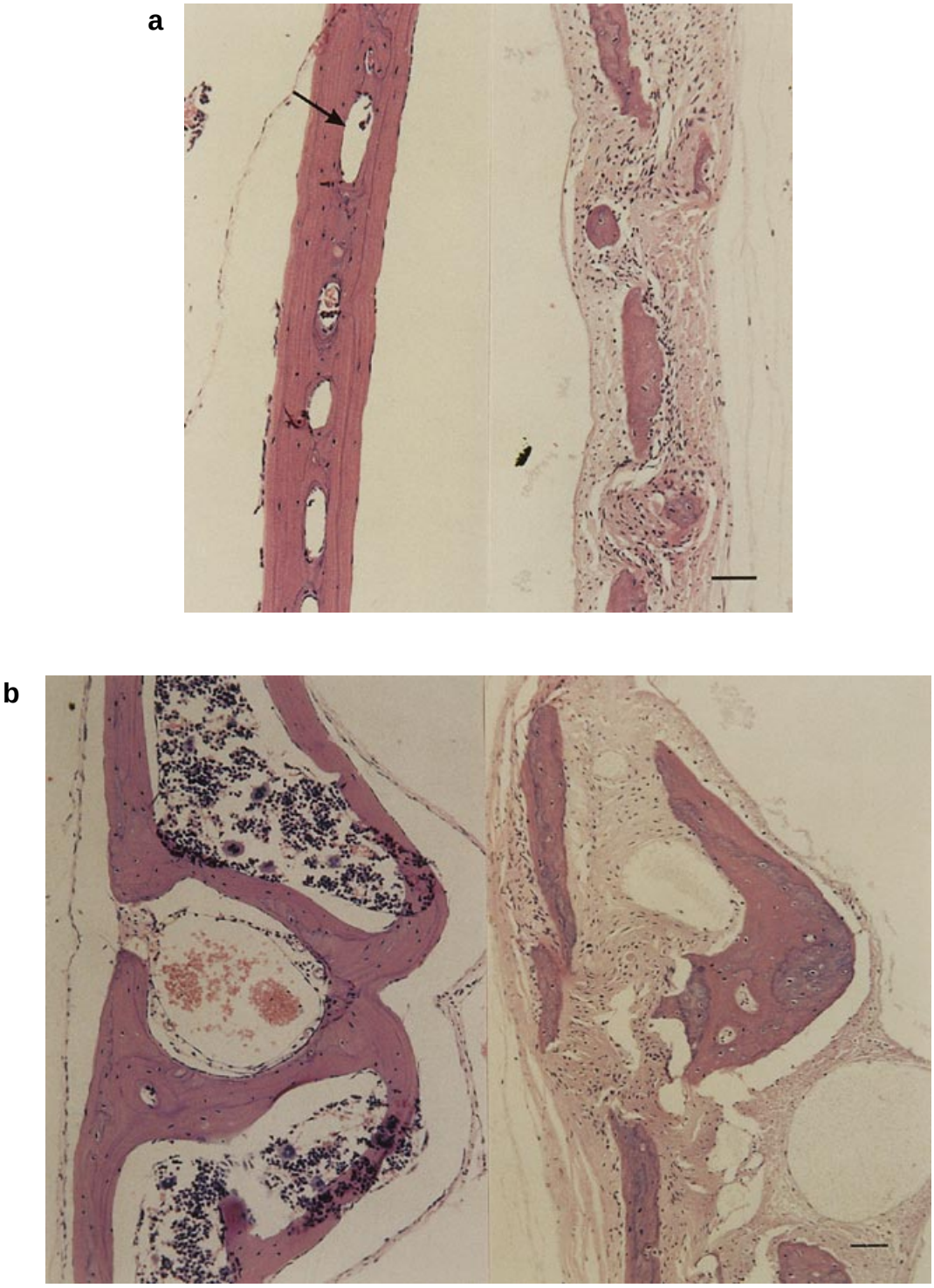


**Figure 9.** (**a**) Photomicrograph of a section of calvaria from control (left) and ets2 transgenic (right) mouse stained with haemotoxylin and eosin. Scale bar = 100 μm. (**b**) Photomicrograph of a section of the sutures of control (left) and an ets2 transgenic mouse stained as in (a).

transgenic mice that over-express the Ets2 transcription factor at levels roughly similar to those seen in human individuals with Down syndrome (41). These mice develop skeletal/bone defects reminiscent of those seen in Down syndrome and particularly those that involve the cranofacial region (Fig. 9a,b). The mice have altered head-shape, brachycephaly, with thin calvaria that lack diploid structure. Further, the intramembranous cartilage deposition in these mice are absent and the sutures are under-developed. The bones in these mice are thinner, almost osteoporosis-like, with larger lumens and less bone. The mice are also shorter

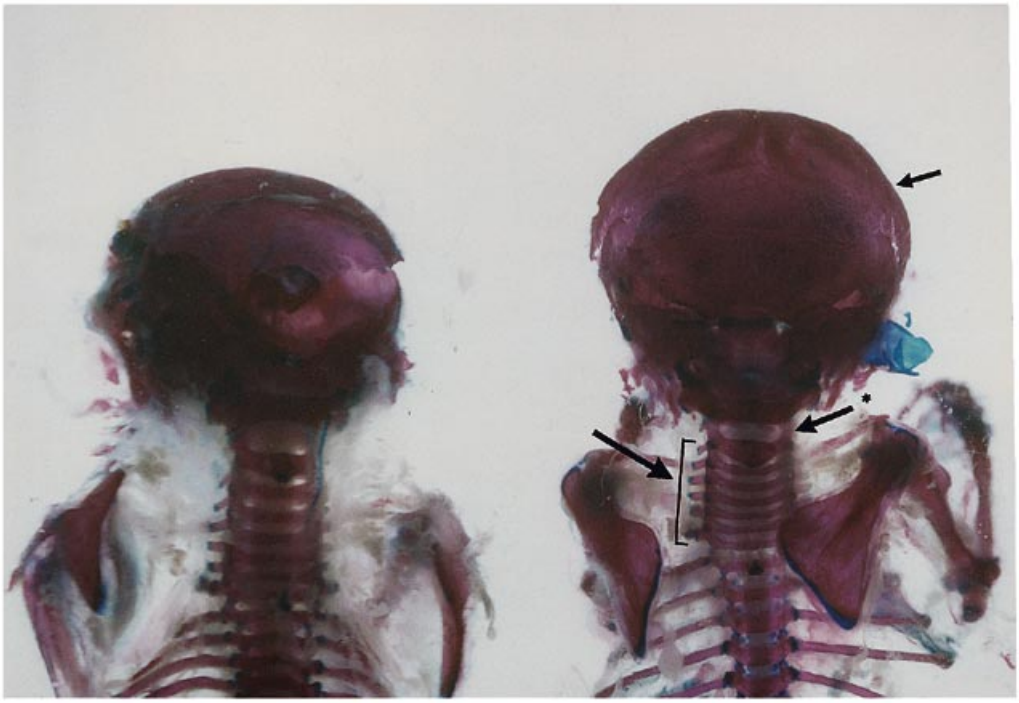

**Figure 10.** Staining of skull and cervical skeleton of control (left) and an ets2 transgenic (right) mouse, stained with alizarin red –S/alcian blue. Note abnormally shaped skulls (small arrow), abnormal atlas-axis vertebrae (large arrow and asterisk) and shorter neck (bracket, large arrow).

in stature with smaller necks (Fig. 10). All of these features occur in human individuals with Down syndrome and in trisomy 16 mice, thus establishing that Ets2 over-expression may be responsible for the genesis of these pathophysiological features. These data also give some insight into the role of this transcription factor in skeletal/bone development, especially since the levels of Ets2 are elevated during embryonic development in the cells/tissues (cartilagenous precursor cell and vertebral primordia) that give rise to the structures that are defective in mice that over-express Ets2. Conditional gene knockouts that test this hypothesis are currently in progress.

Transgenic mice overexpressing other genes on human chromosome 21 have been generated. Those over-expressing HMG14 develop thymus abnormalities and epithelial cysts (42). The S100β overexpressing mice have astrocytosis and axonal proliferation (43).

Mice that over-express the SOD1 gene develop several features that occur as part of the Down syndrome phenotype. These mice have thickened tongues, abnormalities at the neuromuscular junctions seen in younger transgenic pups that are characteristically seen in older animals and decreased thymocytes with ill-defined corticomedullary junctions in the thymuses (44). The abnormalities at the neuromuscular junctions are thought to constitute at least part of the 'premature ageing' phenotype seen in Down syndrome.

SOD1 over-expression is thought to contribute to premature aging by producing elevated levels of $H_20_2$ and a similar mechanism is thought to occur during the normal ageing process *per se*. It has been shown that SOD1 levels/activity increase in all the organs of mice as a function of age (45). All the organs, with the exception of the brain, show a concomitant increase in Gpx1 and/or catalase activity such that the ratio of the enzyme, SOD, catalysing the first phase ($0_2$ to $H_20_2$) to another enzyme, GPX, catalysing the second phase ($H_20_2$ to $H_20$) (Fig. 11) is not significantly changed. In the brain, however, this ratio is elevated during ageing and is associated with lipid peroxidation (46). Similar changes in enzyme ratios in cell-lines result in the acquisition of a senescent phenotype at early passages according to morphological, biochemical and genetic criteria (47). These effects can also be recapitulated by treatment of cells with low doses of $H_20_2$ (up to 200 μM) although apoptosis occurs at higher concentrations (48). Intriguingly, cortical neurons from aborted Down syndrome conceptuses undergo neurodegeneration due to apoptosis at accelerated rates compared with non-Down syndrome aborted conceptuses and these effects appear to be mediated via increased levels of $H_20_2$; compounds such as *N*-acetylcysteine, vitamin E and catalase (49) inhibit the apoptosis and consequent degeneration of neurons from aborted Down syndrome conceptuses.

Transgenic mice that over-express SOD1 also develop features of motor neurone disease after exposure to elevated levels of peroxide (50). The SOD1 gene is mutated in familial cases of motor neurone disease and the mutation is thought to produce a gain-of-function in that it appears that the $H_20_2$ generated has the additional ability to oxidise other substrates such as SOD1 itself. Transgenic mice overexpressing a mutant form of SOD1 develop features of motor neurone disease, thus proving that this mutation is aetiological in the genesis of this disease (51). There have been no reports in the literature that indicate that individuals with Down syndrome develop motor neurone-like disease and the over-expression data emanating from the transgenic mice suggest that at least SOD1 over-expression predisposes to the development

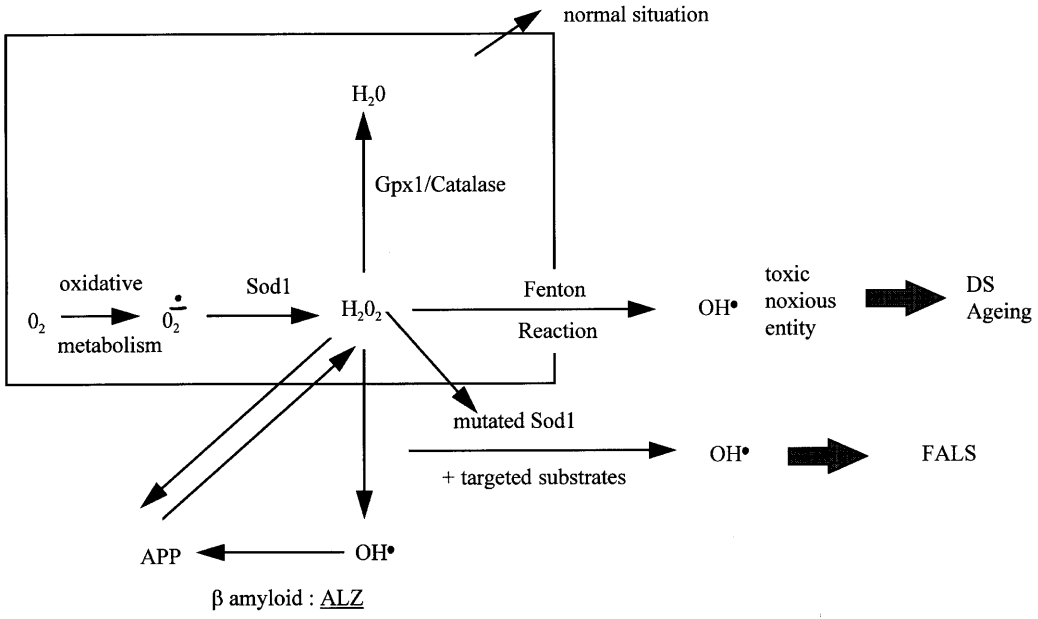


**Figure 11.** Oxygen metabolism and possible abnormalities in ageing and disease.

of motor neurone disease and that other oxidative stress is required to elicit mild forms of motor neurone disease.

There have also been some reports that implicate elevated levels of $H_20_2$ in mediating the toxicity of the amyloid protein. Cells treated with the β-amyloid protein display toxicities that are reversible by *N*-acetylcysteine and vitamin E, thus implicating $H_20_2$ as the mediator of these toxicities (52). If such a situation was correct, then it could be exacerbated since oxidative stress can activate gene transcription via the heat shock element (53) and the APP gene promoter is known to contain a functional HSE (54); this in turn could result in elevated levels of APP and β amyloid leading to further elevated $H_20_2$ and the cascade could continue. However, these experiments need to be carried out *in vivo* via transgenic mice. In general, transgenic mice over-expressing APP or related peptides show some but not all of the features of Alzheimer's disease (55,56).

## CONCLUSIONS

Gene knockout studies in mice are increasingly being employed to identify the function of genes on human chromosome 21 and thus far important data have been derived from such studies. These studies have the potential to illuminate the possible mechanisms involved in diseases/disorders that map to human chromosome 21; for example, the role of CBFA2 (AML1) in the genesis of haematopoietic lineages suggests that deregulation of the expression of this gene can result in the abnormalities in lineage development of haematopoietic cells that occur in leukaemias associated with translocations of this gene. However, only a few of the genes on human chromosome 21 have been knocked out. The genesis of other gene knockouts will illustrate the value/utility of these studies in illuminating the relationship of genes on human chromosome 21 with pathophysiologies associated with this chromosome.

Gene 'knock in' studies in mice have thus far only been developed for limited studies on genes located on human chromosome 21. However, this is a powerful approach that will become more widely used to create mouse models of human disease, especially those that arise out of single gene mutations and/or the formation of chimaeric fusion transcripts resulting from chromosomal translocations. Further, this approach will be increasingly employed to 'humanise' the mouse system.

Various approaches have been used to study the consequences of increased gene dosage in Down syndrome and particularly investigate phenotype/genotype relationships of genes. Also, many of these have been used to model Down syndrome. Thus far, no mouse exists that *exactly* models Down syndrome. However, many lines of mice exist that model one or more aspects/features of Down syndrome. The greatest value that has been derived from these studies has been the identification of genes related to specific phenotypic or pathophysiological features that occur in Down syndrome. Further, powerful insights into biochemical mechanisms operational in the genesis of these features have been derived. Importantly, many of these findings will have more widespread relevance since many of the individual parts that constitute the Down syndrome phenotype also occur in the general population. Thus, these types of studies may identify gene targets for drug therapy of these individual pathologies in the general population and the animal models generated may prove useful in the validation of such targets.

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