

The cell-type-specificity of inherited predispositions to tumours:  
review and hypothesis

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## Abstract

Most hereditary predispositions to tumours affect only one particular cell type of the body but the genes bearing the relevant germ-line mutation are not cell-type-specific. Some predisposition syndromes include increased risks of lesions (developmental or tumourous) of unrelated cell types, either in any individual predisposed to the main lesion (e.g. osteosarcoma in patients predisposed to retinoblastoma). Other predispositions to additional lesions occur only in members of some families with the predisposition to the basic lesion (e.g. Gardner's syndrome in some families suffering familial adenomatous polyposis). In yet other predisposition syndromes, different mutations of the same gene are associated with markedly differing family-specific clinical syndromes. For example, identical germline mutations (e.g. in *APC*, *RET* and *PTEN* genes), have been found associated with differing clinical syndromes in different families.

This paper reviews previously suggested mechanisms of the cell-type specificity of inherited predispositions to tumour. Models of tumour formation in predisposition syndromes are discussed, and especially those involving a germline mutation (the first "hit") of a tumour suppressor gene (TSG) and a second (somatic) "hit" on the second allele of the same TSG. A modified model is suggested, such that the second "hit" is a co-mutation of the second allele of the TSG and a regulator which is specific for growth and/or differentiation of the cell type which is susceptible to the tumour predisposition. In some cases of tumour, the second "hit" may be large enough to be associated with a cytogenetically-demonstrable abnormality of the part of the chromosome carrying the TSG, but in other cases, the co-mutation may be of "sub-cytogenetic" size (i.e.  $10^2$ - $10^5$  bases). For the latter, mutational mechanisms of frameshift and impaired fidelity of replication of DNA by DNA polymerases may sometimes be involved. Candidate cell-type-specific regulators may include microRNAs and perhaps transcription factors. It is suggested that searching the introns within  $10^5$ - $10^6$  bases either side of known of exonic mutations of TSGs associated with inherited tumour predisposition might reveal microRNA cell-type-specific regulators. Additional investigations may involve fluorescent in-situ hybridisations on interphase tumour nuclei.

Key words: inherited predispositions; tumours; genes; co-mutation; microRNAs; replicative fidelity of DNA

## 1. Introduction

Inherited predispositions to particular tumour types and their cell-type-specificity have been recognised since the nineteenth century [1]. Various authors have speculated on the mechanism of this specificity as follows:

Cohnheim [2] in 1882, wondered whether inherited predispositions to cancer might involve some inherited "feebleness of connective tissues to resist ingrowth of epithelial cells" (a probable reference to Ribbert's "tissue tension" hypothesis of cancer [3]).

Morgan [4] in 1922, discussed human and experimental predispositions to tumours from a Mendelian genetic standpoint, and observed that "many or all of the factors for susceptibility must be assumed to be dominant". This author went on:

"Other complicating conditions will also suggest themselves ... for, the possibility that the mutation itself is more or less likely to occur in one or another genetic complex must be reckoned with, as well as the likelihood of the mutation showing itself or developing in any tissue or cells, or only in cells of specific tissues, etc."

Little [5] in 1923, reviewed predispositions to tumours in man and experimental animals. He suggested that the fact such cancer predispositions are usually to tumours of specific organs indicates that:

"... subsidiary or modifying factors are influencing the situation".

Bauer [6] in 1928 considered that, in familial polyposis coli, the inherited predisposition is to abnormal hyperplastic responses to environmental stimuli, and that cancers developing in the condition are due to second somatic mutations. On the other hand, Lockhart-Mummery [7] in 1934 considered that the inherited predisposition to human tumours such as familial adenomatous polyps (FAP) must be genetic, and that a second (mutational) event occurs to produce each adenoma:

"... some factor is inherited which renders certain tissue cells of a particular organ unstable, so that mutation takes place, resulting in excessive mitosis of that particular cell."

Numerous subsequent workers [8 – 18] have made suggestions that the basis of inherited tumour predispositions is a germ line mutation, and that tumours arise because additional somatic mutations (of varying numbers) occur in individual cells of the tissue.

The discovery of viral oncogenes and subsequent development of the cellular oncogene theory of cancer [19 – 22], led to attempts to link these genomic elements to inherited predispositions to tumours. For example, it was suggested [23] that the inherited defect in a family (Family G) first described by Warthin [24] in 1913, was of oncogenic viral DNA "integrated" into the germ line of the affected family (see also [25]).

However, by the late 1980s, no inherited mutant oncogenes had been discovered in humans [26] and expression of most oncogenes occurs in a variety of tumours, indicating that their activation is not cell-type-specific [27, 28]. Subsequent interest in the involvement of these genes in inherited tumour predispositions has been limited, except in relation to the suggestion that oncogenes might act as modifiers of the effects of tumour suppressor genes (see below).

## **2. Tumour suppressor genes: genomic location of the additional (somatic) "hit"**

The concept of tumour suppressor genes ("anti-oncogenes") developed directly from both cell-fusion experiments [29-31] and from analyses of the familial incidences of retinoblastoma [15,16]. The history of tumour suppressor genes can be traced through published reviews by Knudson [16, 25, 27, 32-45] and by others [46 - 58]. Only aspects relevant to the general proposal of this paper will be mentioned further.

The genomic location of the additional (somatic) "hit" according to the various versions of the general hypothesis of inherited tumours (see above) has been discussed in the literature as follows. Burch [12] in 1963, describing a model involving four mutations for familial leukaemia,

"... assumed that the other (non-inherited) two 'carcinogenic mutations' affect both homologous genes at this second locus."

Nicholls [59] in 1969, suggested that the tumours of neurofibromatosis arise by a second (somatic) mutation of the already mutant locus (the "n locus") of predisposed cells. Comings [14] elaborated a "general theory" of carcinogenesis, and made the same suggestion that tumours arise by mutation of both copies of "diploid pairs of regulatory genes" (i.e. both alleles of one gene - Comings used the term "gene" for allele in his paper).

Knudson [33, 35] discussed three possible locations of the additional (somatic) "hit" as follows:

Option 1. On the mutant allele, (in current terms: this means a mutation which converts a "hypomorphic" allele into a "null" allele). Although not widely considered in the literature at present, this paper suggests that this option may have a role in tumour development associated with some inherited predispositions.

Option 2. On another site in the genome (in current terms: tumour resulting from additive effects of a new hypomorphic mutation on the effects of an inherited hypomorphic mutation).

Option 3. On the second allele of the mutant gene (i.e. the "both-allele-of-one-gene hit" model, see above).

While "Knudson's hypothesis" is now usually quoted [60-64] in terms of this third possibility, option 2 has not been excluded as a possible basis for some tumours arising in predisposed individuals. This is because the effects of mutant tumour suppressor genes have been shown to be often additive in "double knock-out" mice [65-67].

### **3. Tumour suppressor genes: abnormal function and pleiotrophism of the mutant gene product as the mechanism of cell-type-specificity**

The major notion of the mechanism of the effects of loss of both alleles of a gene on a cell has been that the effects are determined by loss of function of the protein product of that gene (see Knudson's reviews [16, 25, 32, 33, 39] and refs [66, 68-71]). For example, Knudson et al. [32] suggested that:

"... the first (mutational) step involves a tissue specific gene, such as one which is expressed at a cell surface." It was considered likely that there would be one tumour suppressor gene for each inherited tumour predisposition [39].

Essential to this notion is that each wildtype allele is "haplosufficient" for adequate normal function, so that the inherited loss of one allele has no phenotypic effect. In a small number of inherited predispositions, the mutant allele has "gain of function" effects, but this change is "haploinsufficient" for the phenotypic change because cells having one wildtype allele have normal phenotype. According to Eng [72], if the function of the gene product is "gain of function", the gene should be referred to as an "(proto)oncogene".

During the 1980s, however, more evidence accumulated of multiple phenotypic abnormalities associated with inherited tumour predispositions. Pleiotrophism of

original gene product, or "gain of function" pleiotrophism of the mutant gene product, was occasionally suggested as the basis of multiple effects of one mutation, for example, the development of osteogenic sarcomas in patients with inherited *RB* gene defect [73]. Hansen and co-workers [74] in 1985 suggested that:

"the molecular basis of mixed cancer families may be the differential expression of a single pleiotropic recessive mutation by tissue specific mitotic segregation abnormalities."

The pleiotrophism of effects transcription factors is well recognised, and the possibility that the product of the gene affected by the inherited mutation might have functions as transcription factors has been emphasised by many authors [71, 75-80].

As further examples of suggestions of gene products having multiple functions: *RB* product has been suggested to have a major role in preserving genetic stability [81, 82]. Similarly, *BRCA1* and *BRCA2* products have been suggested to have roles of preservation of genetic integrity, as well as on checkpoint functions, and act as transcriptional regulators [83, 84].

Nevertheless, all gene products so far identified are not expressed only in the cell type in which the tumour arises, and, even if they function as transcription factors, do not have cell-type specific functions. In contrast, they are almost always related to some general cell function, such as cell signalling or faithful replication of DNA.

#### **4. "Modifier" genes and cell-type-specificity of inherited tumour predispositions**

The molecular biologic discoveries concerning inherited predispositions to tumours of the last 20 years include the variable genotype-phenotype correlations of exonic mutations of particular genes associated with tumour predispositions, and also the striking discoveries that families carrying identical exonic mutations in a gene can have differing clinical syndromes (see below).

These observations appear to be inconsistent with the idea that the effect of the mutant gene product is the only mechanism influencing the clinical outcome associated with the exonic mutation. To overcome this difficulty, many authors have invoked mutations of "modifier genes". This term was used by T H Morgan and co-authors [85] as early as 1915 to provide explanations of variable genotype-phenotype correlations.

Knudson [33] briefly mentioned the possible need for a third mutation in some inherited disorders. Weinberg [86] in 1989 suggested in relation to retinoblastoma that "... inactivation of both *RB* alleles may yield a benign localised lesion, but something else is needed to push cells further into the fully malignant state."

By 1993, Knudson [39] believed that other genes might require mutation for a retinoblastoma to develop:

"... other controls on growth must be overcome; *RB 1* is not the only gene which must mutate."

Eng and Ponder [87] suggested that "Allelism, existence of a multigene complex, or modulation of expression by modifier genes may explain the phenomena."

Knudson [41] in 1997 stated:

"Somatic mutations in second alleles at the relevant loci are necessary, but generally not sufficient for carcinogenesis ...." while Fearon [88] referred to "allelic

variation and modifier genes" in relation to specific mutations being correlated with specific diseases.

Knudson [42] in a paper on tuberous sclerosis and related inherited diseases, concluded: "Understanding the molecular actions of the phakomatoses genes does not explain their tumour biology".

There have been several attempts to identify particular modifying genes for these syndromes in terms of known oncogenes, especially *p53*, by many authors for examples [81, 89, 90]. (This area of investigation does not include the Li-Fraumeni Syndrome, in which mutation of a *p53* allele is the germline "hit").

Hormonally-affected gene modifiers may play a role in the great variability which occurs both between families of patients with inherited *BRCA1* and *BRCA2* mutations [83, 91], a variety of possible modifying factors in inherited tumour predispositions have been discussed [92].

The data from the various experimental studies with double knockout mice (see next section) support the notion that additional modifying genomic alteration(s) may be required for the development of some inherited tumour types.

## **5. Position effects and linkage of genes in relation to the cell-type-specificity of inherited predispositions to tumours**

The genes associated with inherited predispositions to tumours are scattered through the chromosomes, and are not known to be linked to any particular genes or cell-type-specific genes (defined here as genomic elements which encode a protein product).

However, studies of tumour susceptibility in mice demonstrated loci with specific significance for inherited predispositions to tumours in in-bred strains of mice [93, 94]. Furthermore some tumour susceptibility loci in mice may show interactions [95].

Experimental studies involving transgenic mice have shown that the location of the abnormal growth factor/oncogene in relation to cell-type-specific genes is an important factor in cell-type specificity of inherited tumour susceptibilities. Detailed studies in mice with an introduced oncogene under the control of a breast-specific promoter showed that breast tumours, but not other tumours, developed [28, 96]. Similarly, *ras* genes inserted under control of pyruvate kinase genes in the genomes of mice caused tumours in some of the organs in which this enzyme is active [97].

Positional effects have been little considered in studies of human inherited predispositions. However, Knudson [16] discussing the association of aniridia and Wilm's tumour, wrote:

"The simplest explanation of this coincidence would be that, while most cases of aniridia and of Wilm's tumour may be point mutations, some may involve a chromosomal deletion. If the two genes are located near each other, then some deletions will include both loci and so produce aniridia, associated severe defects, and Wilm's tumour."

Godbout and co-workers [98] invoked the possibility of necessary modifier mutations being localised adjacent to the exonic mutation of the wild-type *RB* allele.

"We tentatively conclude that induction of a retinoblastoma tumour requires the somatic inactivation of genes near the ESD locus including the remaining normal gene at the retinoblastoma (*RB*) locus."

The concept of a role of linkage of genes in inherited predispositions to tumours was mentioned by in a later review by Knudson [35] but has been little noted since.

## 6. Flanking regions and flanking genes

The term "flanking region" usually refers to regions of the genome up to  $10^3$  –  $10^4$  bases on either side of the terminal exons of a gene, and which especially contain promoter and other regulatory sequences [99, 100]. Some authors have used the term more widely for larger segments which can include genes are located nearby on the same chromosomal arm [101]. In relation to tumour suppressor genes, *RB-1* was initially located by its proximity to the gene for esterase D [46], and also *BRCA-2* is localised near or in 13q13 [102]. The 5' flanking region of human *Rb2/p130* gene has structural organization characteristic of promoters of "housekeeping" and growth control-related genes [103].

There has been little reported systematic study of the flanking regions up to  $10^5$ - $10^6$  bases of tumour suppressor regions for cell type specific regulators (see below), such as transcription factors, microRNAs or other genomic elements not related directly to control of the nearby gene.

## 7. Other suggested mechanisms of cell-type-specificity of inherited tumour predispositions

Several other suggestions of the mechanisms of cell-type specificity have appeared in the last twenty years as follows.

Huebner and co-workers [104] suggested that some lineage-specificity of mechanisms of gene rearrangement or deletion might be involved in the cell-type specificity of hereditary predispositions to tumours.

Weinberg [105] in 1989 suggested that all cell types may have multiple tumour suppressor mechanisms, and that in the predisposed cell types, loss of the mechanism affected by the inherited mutation has cell-type specific criticality.

Levine [52] suggested that age-associated hormonal differences or differences in the pathways regulating the cell cycle in different tissues, and Levine and co-workers [53] proposed tissue specific mutagenesis as the basis of specific tumour formation.

Hamel and co-workers [106] suggested that *RB* product has a role in cells which follow a linear process from undifferentiated to fully differentiated, while the same process in continually-renewing cells leads to cell death. (How neurons, which are another type of non-renewing cell, escape this process was not explained.)

Guilford [56] mentioned "unknown interactions between the susceptibility genes and proliferative or survival pathways that are largely tissue specific."

Venkitaraman [107] proposed tissue-specific increases in rate of loss of second allele occur in inherited predispositions.

Tucker and Friedman [108] in 2002 proposed four models of carcinogenesis in inherited predispositions: the first a sequential multihit model similar to that proposed by Vogelstein and Kinzler [17] (see also Introduction), the second a model involving epithelial-stromal interactions for progression, third a model involving a second "hit" at another locus similar to Knudson's [33] option 2, and the fourth, a model involving "greater sensitivity to proliferating (sic) stimuli".

Roskelley and Bissell [109] considered that the specific features of the microenvironment of the breast and ovarian epithelial cells acting through epigenetic mechanisms including adhesion molecules might be the basis of related epithelial malignancies in these organs.

Monteiro [110] noted that tissue specificity of *BRCA1* effect might be due to a tissue-specific-greater importance of the affected pathways in these epithelia compared to all other cells in the body. Other tissues were suggested to have active alternative pathways and the particular tissues are more exposed to damage which requires *BRCA1* repair. It was also suggested that and that the tissue specific function of *BRCA1* might not be in DNA repair but in some other function, such as delayed apoptotic responses. This author suggested that different rates of loss of heterozygosity (effectively rates of lengthy mutations) occur at different loci in different tissues, and result in the tissue-type specificity of effect of *BRCA1*.

Modification of "splicing" of RNA transcripts, or other post transcriptional phenomena have been suggested by Kaufmann and co-workers [111] as the mechanism of tissue-specificity of effect of the inherited genes.

**8. Hypothesis and genomic models for the inherited predispositions to tumours: the second mutational event may be a co-mutation of an exon of the second allele of the relevant TSG with cell-type-specific genomic element(s), by either cytogenetically-demonstrable-length mutations, or "sub-cytogenetic" ( $10^2$ - $10^5$  kb) length-mutations.**

From the foregoing, it is apparent that previous proposals concerning mechanisms of cell-type specificity of inherited predispositions to tumours may not explain all the clinical features (especially identical mutations within one gene causing markedly different syndromes) of the syndromes of inherited tumour predisposition [95].

A particular problem has been the data from cytogenetic studies of tumour cells. Such studies of tumours arising in inherited predispositions have not yielded evidence of consistent chromosomal damage demonstrable by cytogenetic methods. Thus for retinoblastomas, while loss of both *RBI* alleles is demonstrated, damage to 13q at the karyotypic level (i.e. greater than approximately  $10^6$  bases) is uncommon [26, 82, 112-114]. Karyotypic studies have shown various other chromosomal lesions in such tumours, but whether or not these could arise by way of genetic instability in the tumour is unclear [115].

Here, it is proposed that cell-type-specific regulatory genomic structures and perhaps other relevant genomic elements, including insulator sequences are co-mutated as one event to give rise to the tumour. Furthermore, to explain the well-known lack of cytogenetic evidence of such a mutation (see above), it is proposed that cell-type-specific regulatory genomic structures exist within  $10^2$ - $10^5$  bases of tumour-relevant alleles. As a result, loss by co-mutation by any mutational mechanism which can inflict damage of at least  $10^2$ - $10^5$  bases length can provide the tumourous genome for the cell.

The mutational mechanisms which may be involved in this co-mutation process because they can inflict damage of at least  $10^2$ - $10^5$  bases length, include frameshift mutation, and impairment of DNA polymerases during local stem cell mitosis. Both mechanisms can inflict subcytogenetic-length damage to the genome, because each event affects only half a replication "bubble" (15-150 kb) during S phase. On the other hand, chromosomal deletions and inversions tend to be longer, and cytogenetically demonstrable.

The cell-type specific genomic element could be localised in an intron of the tumour enhancing allele, or overlap, or be merely in an adjacent flanking region. This concept is dealt with in previous publications by the present author [116, 117].

The basis of the cell-type specificity of action of the inherited predispositions is suggested to lie, therefore, not only in the biologic actions of the product of the affected gene, but also in the nature of the genomic elements which are within the "co-mutation range" of these  $10^2$ - $10^5$  base or longer, mutational mechanisms.

### **9. Natures of the putative cell-type specific regulators**

Because it appears to be established that the second "hit" for tumour formation in predisposed individuals can be of sub-cytogenetic size (less than approximately  $1 \times 10^6$  bases), and the second hit must include at least part of the exon of the second allele, then the cell-type specific regulator must be (according to the hypothesis here), relatively small, and relatively close to the vulnerable exon of the second allele.

There are three known possible structures:

1. Regulatory genes. These, however, are often over  $10^4$  bases in total length, and separated from the vulnerable exon by the remainder of the tumour suppressor gene and intergenic sequences.
2. Transcription factors. These are usually shorter than full genes, and often located in flanking regions of genes (see section 7, above).
3. RNA regulators. These have been discovered only in the last few years and study of them is in its infancy. However, it is known that these are short (micro RNAs are often 21-23 bases), can have vital roles in differentiation of eukaryotic cells, and are often located in the introns of genes [118-127]. Further, they appear to be transposable in the genome [125], which would provide a basis for the syndrome of one gene, multiple syndromes, no apparent dependence on position of exonic mutation: including different syndromes with identical germline mutations (see section 10.5, below, and Figure 5).

At this time it is premature to speculate on the precise nature of the cell-type-specific regulator, especially for all inherited tumour types, because the nature of the particular regulator may be different from disorder to disorder. The present hypothesis goes only so far as to suggest that

- 1, a cell-type-specificity of a tumour predisposition indicates that, in the lesional tumour cell, there may be a cell-type-specific genomic lesion in conjunction with a linked, but possibly distinct pro-proliferative genomic lesion,
- 2, because studies of the chromosomes of lesional cells have not shown any constant cytogenetic lesion, then the size of the mutation which involves both genomic elements may be of "sub-cytogenetic" size.

### **10. Genomic models of inherited predispositions to tumours**

In all of the following models, the incidence of the tumour or other lesions is proposed to be related to distance between the relevant genomic elements. This is because shorter segments of mutation by most mechanisms are likely to be commoner than longer ones, and hence the incident of co-mutation of two genomic elements is likely to be inversely proportional to the distance between the elements.

*10.1. Possible genomic model for predispositions involving one gene for one tumour type of one cell type or closely related cell types without family-specific differences (Figs 1A, 1B)*

Inherited predispositions apparently involving only one gene, one tumour type and one cell type include papillary renal cell carcinoma (*MET*), familial melanoma (*CDK4*), hereditary diffuse gastric carcinoma (*CDH1*) and familial gastrointestinal stromal tumours (*KIT*) [56, 57].

Other inherited predispositions involve one gene and one tumour type, but closely related cell types are affected. These syndromes include first, MEN 1 syndrome (benign proliferative disorders of endocrine cells, and occasionally malignant tumours [77, 128-131]. A second example is neurofibromatosis type 1 (benign tumours of neural and neural-crest-derived cells: malignancies may supervene in pre-existing benign lesions, *NF1*; [132, 133]). A third example is neurofibromatosis type 2 (mainly benign tumours of Schwann cells, *NF2*; [134, 135]). Other examples are inherited breast and ovarian cancer syndromes of *BRCA* types 1 and 2 [85, 86, 93, 107, 136].

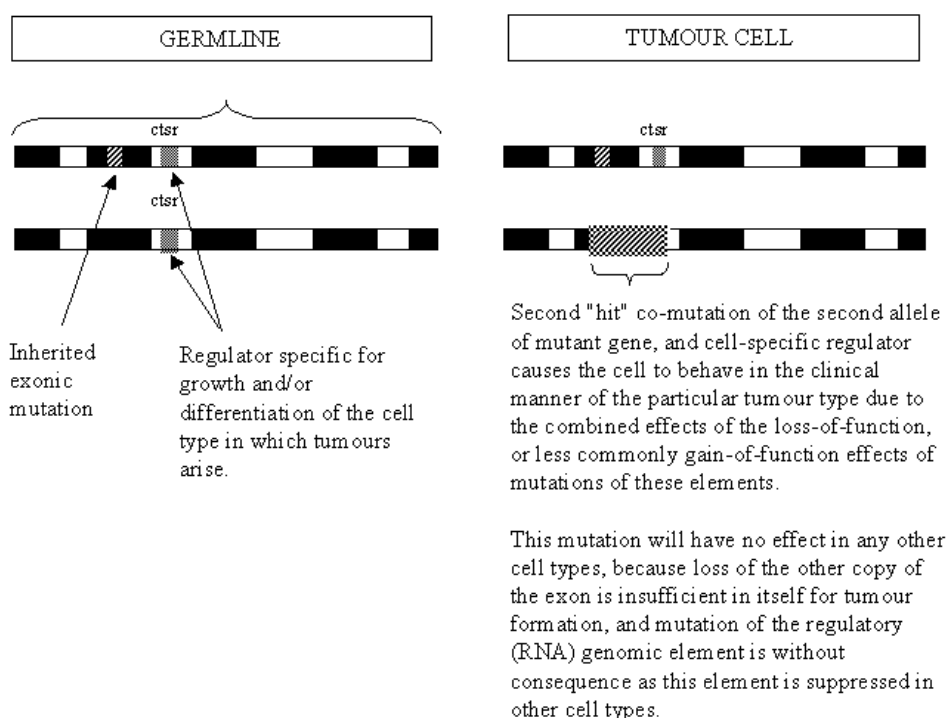


Fig. 1A: A genomic model for inherited predisposition to one tumour type of one (or closely-related) cell type with one gene involved, and no significant differences between families (e.g. inherited renal cell carcinoma) based on the notion of the exonic mutation being within "co-mutation range" of a regulator which is specific for the cell type in which the tumour arises. If the regulator is involved in several cell types, then similar tumours of those cells may arise (e.g. in MEN 1, NF1 and NF2 syndromes). An intronic regulator is drawn, but other types of regulators may be involved (see text). The incidence of tumours in predisposed individuals is likely to be inversely proportional to the distance on the DNA chain between the exonic germline mutation and the intronic regulator, because shorter co-mutations are probably likelier than longer co-mutations.

A simple genomic model for these predispositions is based on the possibility that the relevant genes include intronic regulators which are specific for the one or more of the cell types in which the tumours arise (Fig 1A). In all situations, the combined loss of the exon of the second allele and the loss of one allele of the regulator causes the cell to have the properties of the tumour cells. (See also section on insulators).

It can be noted here that some inherited predispositions are associated with mutations in different genes (hence affecting different families) but cause the same syndrome. This is sometimes referred to as "genetic heterogeneity" of the clinical syndrome [57]. Among this group are first, hereditary non-polyposis colorectal cancer (4 genes). A second example is Turcot's syndrome (some families have mutations of the *APC* gene, and others to mismatch repair gene of hereditary non-polyposis colonic cancer [137]). Additional examples are tuberous sclerosis (2 genes) and Peutz-Jegher's syndrome (only 60% of cases associated with the *LKB1/Strakin* gene [138]). Yet further examples are the Carney complex (3 genes), Li-Fraumeni syndrome (2 genes: *TP53* and *hCHK2*) and xeroderma pigmentosum (7 complementation sites/putative genes) [56, 57].

Furthermore, juvenile polyposis has been associated with mutations of three genes (*PTEN*, *SMAD4*, *BMPRIA*), and inherited Wilm's tumour is associated with *WT1* mutations in many cases, but other genes are involved in the remainder [57, 79].

An explanation for the genetic heterogeneity aspect of these predispositions is that there are multiple sites of the type co-localisation of genomic elements shown in Figure 1A.

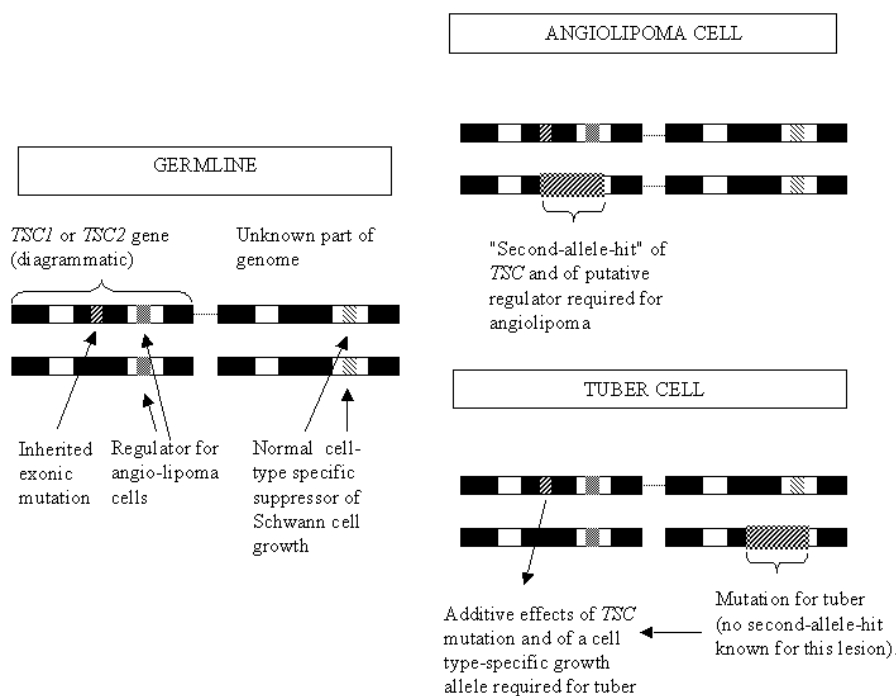


Fig. 1B. A genomic model for syndrome of one known exonic mutation and some tumours with "second-allele hit" mutations, and other tumours without "second-allele-hit" mutations in tumour cell (for example, angiolipomas and brain tubers respectively in tuberous sclerosis). An intronic regulator is drawn, but other types of regulators may be involved (see text).

A disorder in which more than one genomic model may occur is tuberous sclerosis. In this disease, the angioliopomas have been shown to have the "both-allele" hit (on the *TSC1*, *TSC2*, genes), but the "tubers" do not [139]. For this disorder, a model involving Knudson's [33] option 3 is postulated for angioliopomas, and Knudson's option 2 for tubers (Figure 1B).

*10.2. A possible genomic model for one basic syndrome with occasional additional features; involving one or a small number of genes, and no significant differences between families e.g. retinoblastoma (Figure 2)*

With more intensive studies of families with inherited dispositions to tumours, this group has become perhaps become the largest. In the considerations of whether or not the association of the particular less common additional tumours with the basic syndrome are statistically significantly (rather than simply chance additional sporadic tumours), it is worth remembering that treatment (radiotherapy and chemotherapy, such as cyclophosphamide) can cause of secondary neoplasms in patients [140]. Secondly, there may be factors of case selection which can lead to the incidence of second neoplasms being overestimated [141]. A third consideration is that malignancy supervening on a benign counterpart may be a spontaneous event, and not dependent on any genetic predisposition. For example, in familial polyposis coli, carcinomas develop in large adenomas caused by the predisposition, but the carcinomas are no more commonly, adenoma-for-adenoma, than carcinoma occurring in sporadic adenomas. This situation must be distinguished from predisposition to malignancy *ab initio*, which, for example, is the manifestation of hereditary nonpolyposis colonic cancer [142].

An example of inherited germline mutations of a single gene which is associated with predisposition to tumours or lesions of multiple cell types is inherited retinoblastoma (*RB*) [33, 35, 69, 74, 82] in which the basic syndrome is retinoblastoma with retinoma, but in which occasional members of all families develop osteogenic sarcomas, and possibly non-treatment-related fibrosarcomas.

For this predisposition, a model is suggested which involves two or more cell-type specific regulatory elements within mutation range of the exonic mutation (Figure 2).

Another example of an inherited predisposition to a tumour with occasional other lesions in members of all families is the naevoid BCC syndrome (*PTCH* gene). In this disorder, the basic syndrome is of basal cell carcinomas, but some individuals in all affected families have developmental abnormalities [143, 144]. For this predisposition, the model shown in Figure 2, could be a sufficient mechanism if one of the second genomic elements was a regulator for the developmental progress (rather than the growth) of the second cell type.

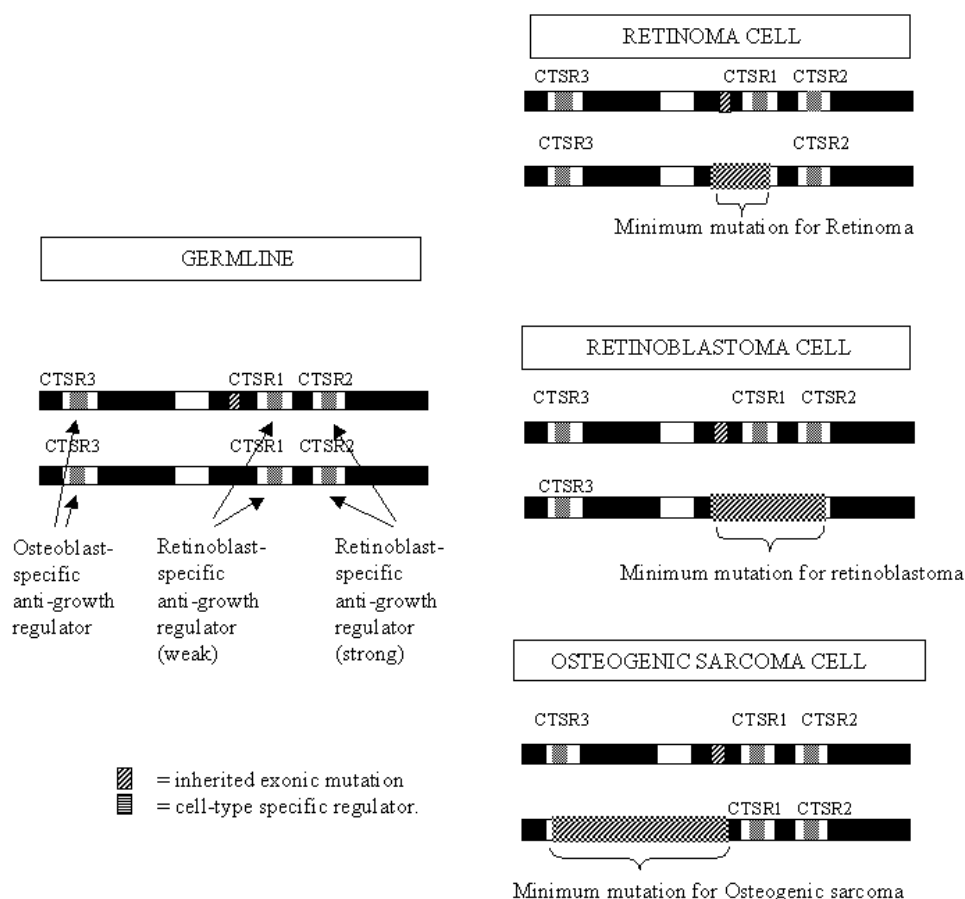


Fig. 2. A genomic model for one basic syndrome with occasional additional features; involving one or a small number of genes, and no significant differences between families (e.g. retinoblastoma, with retinoma and occasional osteogenic sarcoma). Intronic regulators are drawn, but other types of regulators may be involved (see text).

The relative incidences of the tumours is affected inversely by the distance between the genomic elements which are co-mutated, because these distances determine the likelihood that a mutation will involve each particular pair of elements.

### 10.3. Possible genomic model for one gene, one tumour type, some families have additional features e.g. Gardner's and Turcot's syndromes (Figure 3).

In familial polyposis coli, there are germline mutations (mainly in the APC gene [57, 145] the basic syndrome is of polyps of the colon. However, in particular families, some members develop additional lesions, such as soft tissue tumours and cysts (Gardner's syndrome [146-148]) and brain tumours (Turcot's syndrome, [137, 149]).

In the von Hippel-Lindau syndrome (*VHL*) renal epithelial tumours, pheochromocytomas, and vascular tumours of the brain and retina occur. Various family-specific clinical variants (Types 1, 2, 2a) are recognised [150]. A genomic models for these types of disorders could involve family-specific location of the relevant additional genomic element with range of co-mutation with the second allele of the exonic mutant (Figure 3).

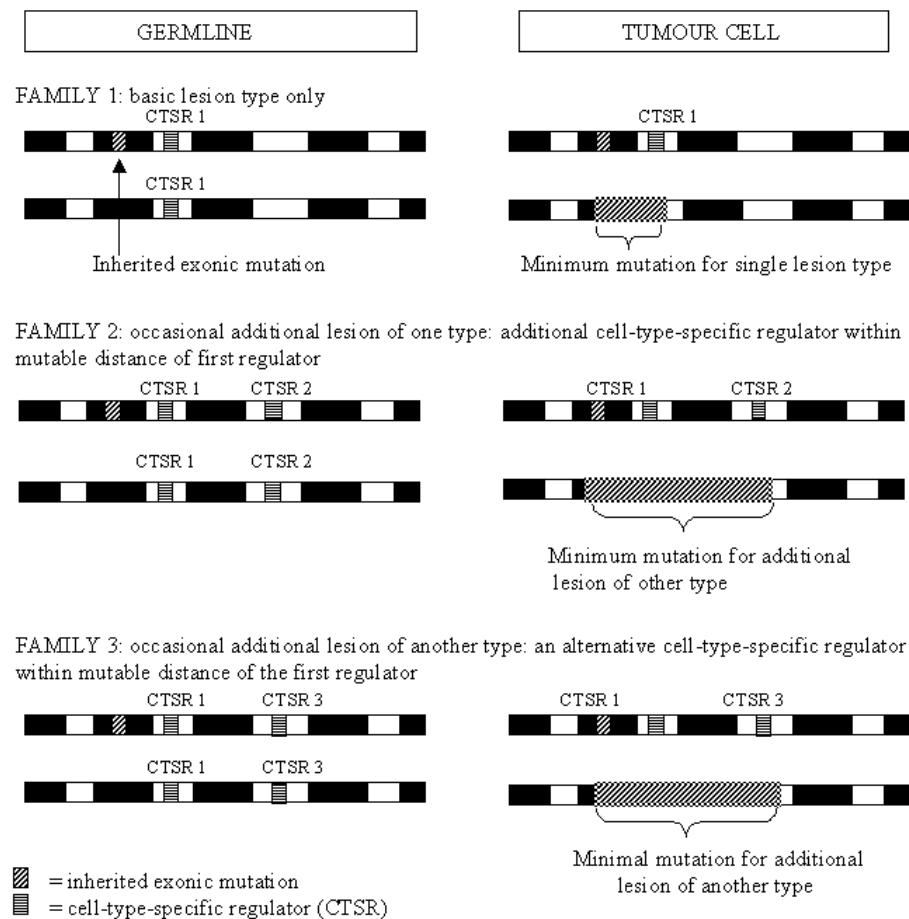


Fig. 3. A genomic model for one gene, one tumour type, some families having additional features e.g. Turcot's, Gardner's syndromes. The type of family-specific additional lesion depends on the particular cell-type-specific intronic regulator being within co-mutation range of the inherited exonic mutation. Intronic regulators are drawn, but other types of regulators may be involved (see text).

The frequency of the additional feature will vary inversely with the distance between the inherited exonic mutation and the intronic regulator.

*10.4. Possible genomic model for exonic mutations of one gene being associated with completely different syndromes according to different positions of the exonic mutation. e.g. RET, PTEN (Figure 4).*

This phenomenon essentially indicates a high level of genotype-phenotype correlation as is currently understood, and is recognised in *RET* and *PTEN* genes.

*RET* gene [57, 72, 151-153] is named after the function of the *RET* product (receptor tyrosine kinase) and germline mutations give rise to several variants: familial medullary carcinoma of thyroid; medullary carcinoma with other epithelial and non-epithelial tumours (MEN 2A, 2B), and Hirschprung's disease. In genetic-phenotypic analyses, MEN 2A associated with external part of protein, and MEN 2B with intracytoplasmic tyrosine kinase parts of protein [153]. However, familial medullary carcinoma (FMTC) seems to be associated with mutations along the whole length of the protein, and may represent a minor form, possibly due to less severe mutations.

The same gene is mutated in Hirschprung's disease (congenital aganglionosis of the colon), which is not associated with any tumour. Eng [72] points out that the

germline mutations of Hirschprung's are more often truncating, and hence loss of function in type, while those of MEN2 are missense, and possibly gain of function type.

Mutations of *PTEN* gene are associated with Cowden's syndrome and Bannayan-Riley-Ruvalcaba syndrome and identical germline mutations have been found associated with different syndromes in different families [57]. However, in most families, the syndrome correlates with the position of the mutation in the gene [154]. A model which could account for multiple syndromes from one gene is that the exonic mutation of the gene is located differently for each syndrome so that it lies within co-mutation range of different disorder-specifying intronic regulators for each family-specific syndrome (Figure 4).

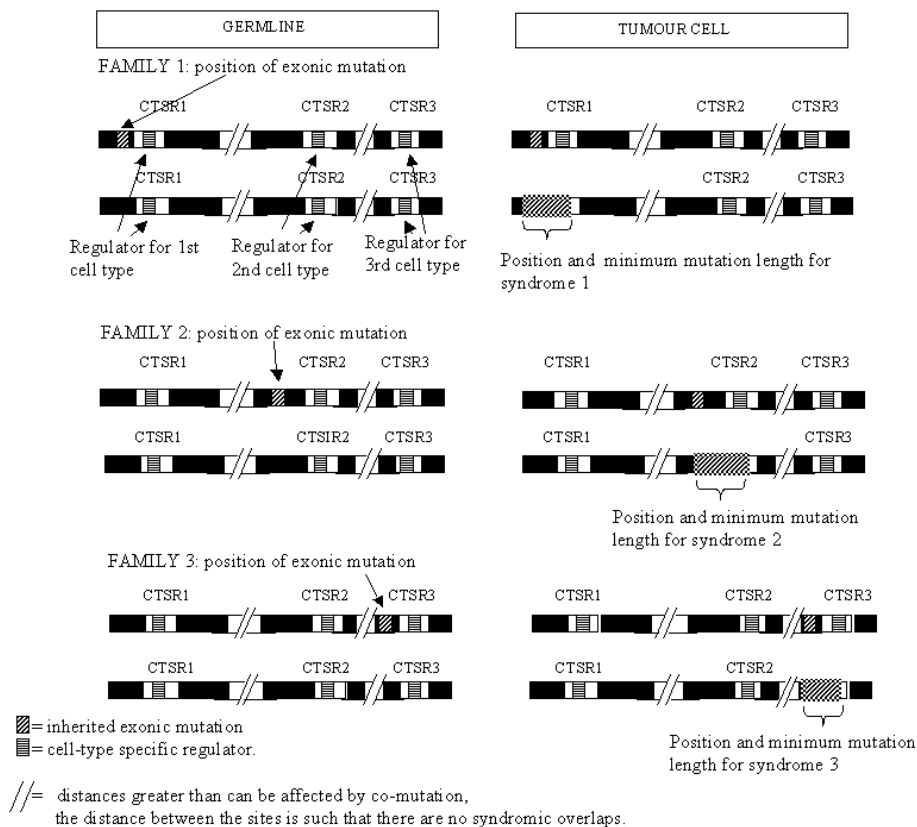


Fig. 4. A genomic model for exonic mutations of one gene being associated with completely different syndromes according to different positions of the exonic mutation, e.g. *RET*, *PTEN*. Intronic regulators are drawn, but other types of regulators may be involved (see text).

#### 10.5. Genomic model for one gene, multiple syndromes, no apparent dependence on position of exonic mutation: including different syndromes with identical germline mutations (Figure 5)

These circumstances amount to extremely poor correlation between exonic mutations and phenotype, and the extreme situation, of different syndromes being associated with identical germline mutations has been described to occur in *APC*, *RET* and *PTEN* genes [57]. An explanation of these is that the position of the intronic regulator for the disease type is variable from family to family (Figure 5).

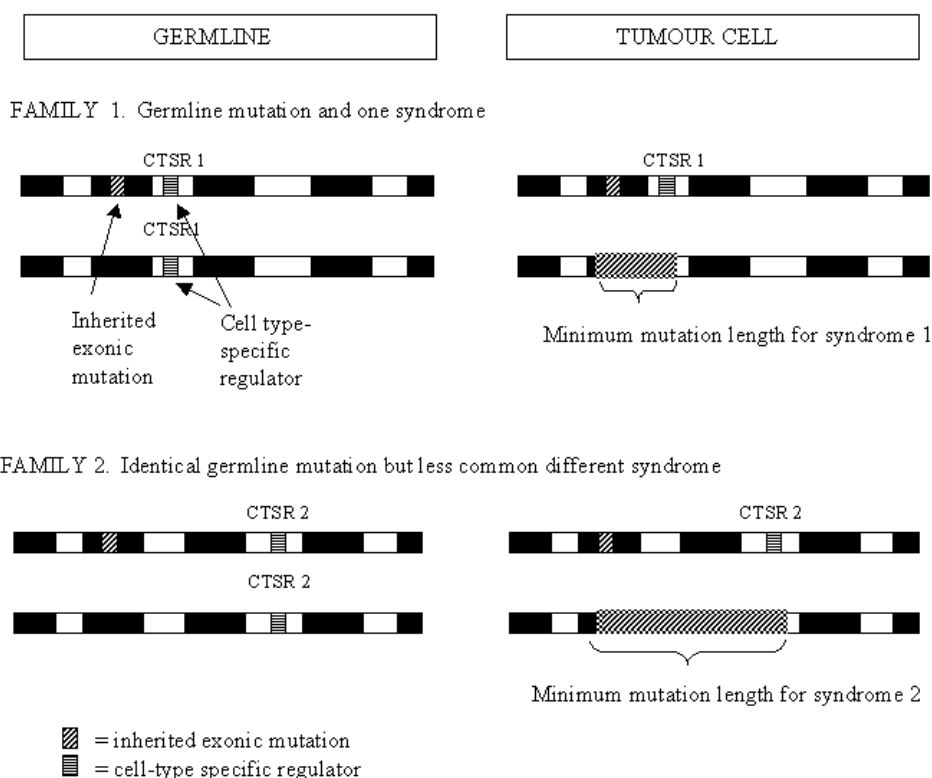


Fig. 5. A genomic model for identical exonic mutations, providing for different diseases in different families. e.g. APC, RET, PTEN. Intronic regulators are drawn, but other types of regulators may be involved (see text).

The syndrome-determining genomic condition is a family-specific localisation of cell-type specific regulator within "mutation range" of an appropriate mutational mechanism (see text).

In this arrangement of genomic elements, individual lesions in syndrome 1 are likely to be commoner than the individual lesions of syndrome 2, because the regulator of 2 is closer to the inherited exonic mutation than is regulator 1.

#### 10.6. Genomic model involving inherited mutations of insulators (Figure 6).

Many cell types including adipose cells and chondrocytes give rise to corresponding benign tumours which show little tendency to cytological abnormality genetic instability or malignant change. These would biologically appear to involve simply an acquired increased growth rate of cells. Insulators are genomic elements which separate genes from one another so that transcriptional activity of one gene does not flow to its successor in the DNA chain [155, 156]. Loss of an insulator between cell-type specific gene and a growth-promoting gene could well have this effect.

Although inheritance of mutations of insulators have not been described in mammals, a simple mechanism of predisposition to increased growth in a cell population would be an inherited hypomorphic mutation of an insulator between a normally suppressed growth factor and a cell-type specific gene of the cell type (Figure 6). The "second-hit" would be a second mutation on the same insulator, leading to its loss of function, and hence over-proliferation of the cell affected. This would correspond to Knudson's [33] option 1 (see above).

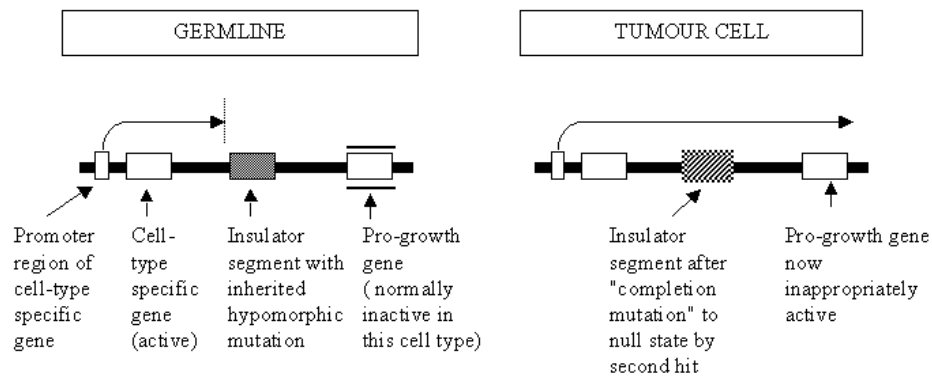


Fig. 6. A genomic model for inherited predisposition to tumour formation involving insulator segments.

The pro-growth gene in this model could be alternatively a developmental growth gene, an oncogene, or a transcription factor for such genes, to provide for different syndromes.

### 10.7. Genomic model for autosomal recessive disorders (Figure 7)

In the inherited tumour syndromes which are autosomal recessive (e. g. xeroderma pigmentosum [157-159], the predisposition only occurs after two inherited "hits". More "hits" are required to turn the particular "target" cell into a tumorous one.

A simple model for autosomal recessive tumour predispositions is that tumour formation requires co-mutation of one of the hypomorphic alleles to a "null" allele, together with loss of a cell-type-specific pro-growth genomic element (Figure 7).

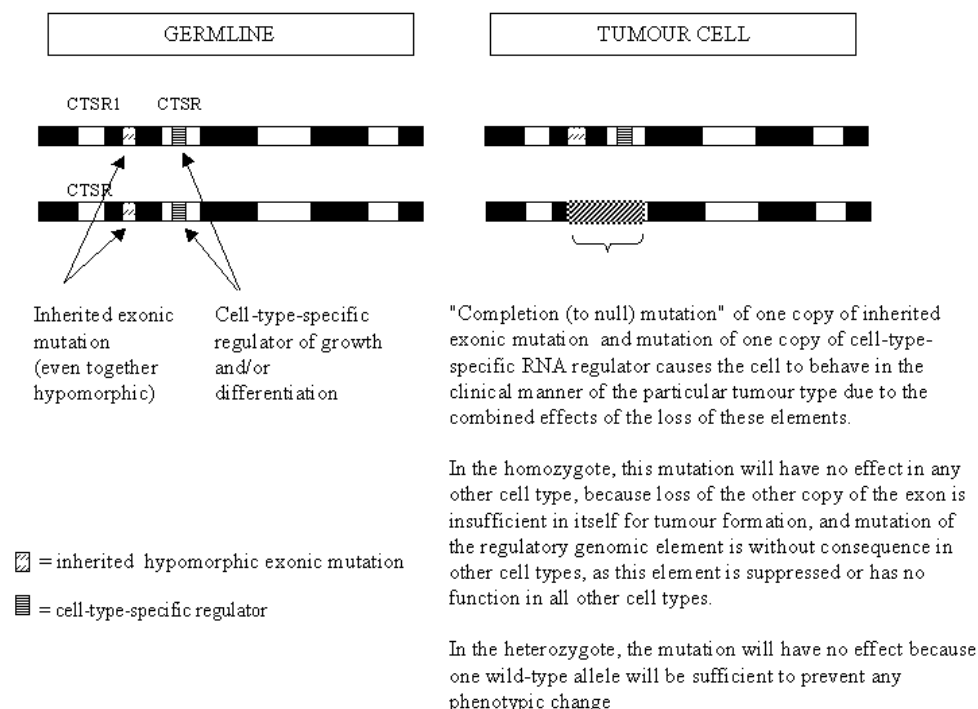


Fig 7. A genomic model for autosomal recessive cell-type specific inherited predisposition to specific tumour type.

One wild-type allele can prevent the syndrome entirely. Two hypomorphic alleles can prevent tumour formation, but not other features (especially reduced growth) of the syndrome. Complete (to "null") loss of one hypomorphic allele and loss of a cell type specific regulator by co-mutation leads to tumour formation only in the specific cell type. Intronic regulators are drawn, but other types of regulators may be involved (see text).

## 11. Discussion

The current proposal for a mechanism of cell-type-specificity of inherited tumour predispositions is based on the underlying principle of the possible importance of co-mutation as a single second "hit" of cell-type specific regulatory elements and pro-tumour genetic elements (signalling mechanism, pro-growth mechanisms, genetic stability preserving genes) in the development of tumours in syndromes of inherited predisposition. The emphasis here is that the occurrence of tumours may require the combined effects of the mutation of the regulator, and of the altered gene product.

The models mentioned are only intended as examples. Several additional genomic mechanisms may be relevant in some disorders.

1. Only "loss-of-function" of alleles have been included in the models. However, "gain of function" mutation of alleles (see Eng [72]) and other post-translational mechanisms may well play roles.

2. Altered alternative splicing during transcription [111] is not excluded as a mechanism of some manifestations of inherited tumour predispositions.

3. The " $10^2$ - $10^5$  base or longer " second hit mutation may not be necessary at all if the germline mutation is of an equivalent length, and affects the other allele of the cell type-specific regulator. Thus in the "D-deletion syndrome" there is an inherited loss of a large part of p13 [160]. In such individuals, only relatively short mutation of the *RB* gene may be sufficient for a second "hit".

4. It is possible that the tumorigenicity of mutations of a particular sequence of DNA may be reduced by the presence of a "lethal-if-mutated" genomic element in the region. Thus, in some families carrying the exonic mutation, such a lethalising element may be present which protects the individual from the tumour because any cell dies which suffers a long mutation in the region. This hypothetical arrangement of genomic elements was mentioned by Knudson [37].

5. The possibility of "gain of function" mutations and "alteration of function" mutations of RNA regulators has not been explored here. Being short sequences, point mutations of some RNAs could possibly change their function from a suppressor of proliferation to a growth enhancer. It is conceivable that some RNAs, if responsible for cellular specialisation as suggested by Herbert [125], could be altered by mutation from a differentiation controller for one type of cell, (say a squamous cell) to a differentiation controller for another type of cell (say a glandular cell). Thus the mutant form of the regulator may result in "aberrant" differentiation in the tumour type with respect to the original cell type. Such a phenomenon occurs in uncommon human tumours, such as squamous cell carcinoma arising in the mammary gland [161].

## 12. Testing the hypothesis

The hypothesis presented here involves well-established genetic principles. First it involves lengthy mutations, in terms of either abnormal chromosomes in tumour cells or "subcytogenetic"-length mutational mechanisms (such as frameshift

mutations, and the mutations caused by impaired fidelity of replication of DNA by DNA polymerases [116, 117]. Second, genetic linkage, which was discovered in 1906 by Bateson [cited in ref 85]. Third the concept that more than one mutation is involved in making a cancer cell, which dates back at least to Morgan [4] in 1922. The "missing elements" of previous hypotheses have been first, any genomic element which could provide for the cell-type specificity aspect of inherited tumours (now provided possibly by RNA regulators), and second, the perception that sub-cytogenetic-length mutational mechanisms may have a role to play in Knudson's "second hit".

Further search of the human genome for transcribed and non-transcribed regulatory elements within the range  $10^2$ - $10^5$  bases or longer, of the known germline exonic mutations for each syndrome would be necessary evidence for the hypothesis. In the absence of these linkages of exonic mutations and mutant regulators in tumours arising in predisposed individuals, the present hypothesis fails. Whether or not these elements can be transposed within the human genome is another important issue for the part of the hypothesis which concerns family-to-family variability of incidence of second lesions (see section 10.5. and Figure 5).

In addition, further study of insulator elements, especially in inherited tumour syndromes may be helpful in relation to simple tumour types.

Fluorescence in-situ hybridisation (FISH) studies [162, 163], preferably on interphase nuclei of minimally fixed tumour cells may be a particularly useful technique for investigating this hypothesis. Mutations may be demonstrable by this technique using labelled DNA probes to regions flanking the known germline exonic mutations in disorders such as retinoblastoma. Minimal fixation of tissues provides better quality DNA after denaturation, and the avoidance of *in vitro* culture prevents exaggeration of karyotypic instability by the tumour cells. If the hypothesis is correct, the cells of such a tumour, excepting only those which lose the relevant chromosome by *in vivo* karyotypic instability (i.e. prior to surgical resection), should show a consistent mutational lesion of the second allele and adjacent DNA for  $10^2$ - $10^5$  bases or longer. Nevertheless, the intensity of loss of hybridisation may depend on the type of mutation present. If the mutation of the flanking length of DNA was entirely frame-shift in type, then most reduced hybridisation would be expected. On the other hand, if the mutation was due to reduced fidelity of replication of DNA, then only variable reductions of hybridisation of the DNA probe might be expected, because this mutational mechanism causes only scattered point mutations in the affected length of DNA [116, 117].

### 13. Conclusions

Inherited predispositions to tumours represent a difficult problem for genetics and oncology. The most complex aspect of these disorders has been the cell-type specificity of the major lesions. In additional complex aspect has been the variability of the associations of features, and particularly that some associations occur only in some families (as syndromes), while others can occur in any individual with the predisposition. Furthermore, for many inherited predispositions, there is a lack of exonic genotype-phenotype correlation. That is to say, there is no obvious relationship between particular exonic mutations of single genes and their clinical effects. These complexities are difficult to explain in terms of the dominant/recessive concepts of Mendelian genetics, in association with the effects of loss of function of the protein-

products of mutant genes. The long-established concepts of linkage of genomic elements and co-mutation together with the newer concept of cell-type specificity on the basis of non-translated genomic elements (regulatory RNAs), may provide a basis for further investigation of the genomic bases of these disorders.

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