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Gestational mutations and carcinogenesis

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Abstract

We present a mathematical formulation to evaluate the effects of gestational mutations on cancer risk. The hazard or incidence function of cancer is expressed in terms of the Probability Generating Function (PGF) of the number of normal and mutated cells at birth. Using Filtered Poisson Process Theory, we obtain the PGF for several models for the accumulation of gestational mutations. In particular, we develop expressions for the hazard function when one or two successive mutations could occur during gestation. We also calculate the hazard when the background gestational mutation rates are increased due to exposure to mutagens, such as prenatal radiation. To illustrate the use of our models, we apply them to colorectal cancer in the SEER database. We find that the proportion of cancer risk attributable to developmental mutations depends on age and that it could be quite significant when gestational mutation rates are high. The analysis of the SEER data also shows that gestational mutations could contribute to inter-individual variations in colorectal cancer risk.

Keywords: Gestational mutations; Multistage carcinogenesis; Luria–Delbrück models

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1. Introduction

It is generally recognized that carcinogenesis is a multistage process involving the accumulation of specific mutations. Frank and Nowak [1] suggested that a significant fraction of adult-onset cancers could originate from critical mutations occurring during gestation. Because of rapid cell division, cells mutated early in gestation would give rise to 'jackpots' of mutated cells at birth. The presence of such jackpots of cells possessing a mutation on the pathway to cancer could increase considerably the risk of eventual development of cancer. For example, in the colon, a jackpot of stem cells carrying a mutation of the APC gene could increase the risk of colon cancer. If the mutation were to occur at the very earliest stage of the development of the colon, it is at least theoretically possible that every stem cell in the colon would carry the mutation. The risk of colon cancer imparted by such an early gestational mutation would be equivalent to the risk imparted by inheritance of familial adenomatous polyposis (FAP). In fact, the phenotype in an individual with such an early mutation would be indistinguishable from the phenotype of FAP. At the other extreme, if the gestational mutation occurred late in embryonic life, there would be only a small pool of cells carrying the mutation and the risk of colon cancer would be increased only slightly. Clearly, the risk imparted by a gestational mutation could vary widely, depending on when the mutation occurs. Thus gestational mutations could be one source of the considerable heterogeneity of risks of cancer observed in human populations. In this paper we show how models for the accumulation of critical mutations during gestation can be used in tandem with multistage models of carcinogenesis to derive hazard functions for cancer in specific tissues.

The hazard or incidence function is the standard measure of cancer risk. In Section 2 we develop a general formulation for the hazard function in the presence of both normal and gestationally mutated cells at birth. In Section 3, we evaluate the hazard functions for several models for the accumulation of gestational mutations. In the last section, we discuss the application of our methodology to the case of colorectal cancer using the model proposed by Luebeck and Moolgavkar [2].

2. The Hazard function

2.1. General formulation

The standard measure for cancer risk is the hazard or incidence function. Let T be the time of the appearance of the first malignant cell in a particular tissue. Let $P(t) = P[T \leqslant t]$ be the probability that cancer occurs before age t. Then, the hazard or incidence function is defined as

$$h(t) = \lim_{\Delta t \to 0} \frac{P[t < T \leqslant t + \Delta t | T > t]}{\Delta t}. \tag{1}$$

So, the hazard measures the instantaneous rate of change in cancer probability conditional on $[T > t]$. The quantity $S(t) \equiv P[T > t]$ is called the survival function and

$$h(t) = \frac{-S'(t)}{S(t)} = -\frac{\mathrm{d}}{\mathrm{d}t} \ln(S(t)). \tag{2}$$

Now, during development, stem cell populations divide in an 'almost' exponential way. So the probability of the occurrence of a mutation in a small time interval can be assumed to be directly proportional to the number of stem cells in the tissue. Once a mutation has occurred in a cell, all its descendants will carry the mutation. Thus, if a mutation occurs during early development, then a 'jackpot' of mutated cells will be present at birth. The earlier the first mutation occurs, the larger the number of 'descendants' this cell will have, and the larger the 'jackpot' will be. However, during the early stages of development only a small number of cells exist in any particular tissue, so there is an overall small probability that a mutation occurs. In the later stages there are many cells at risk, so there is a greater probability that a mutation occurs.

We calculate the hazard for the case when some fraction of stem cells could already carry mutations at birth. Let (X, W) be the random variables representing the number of normal and mutated stem cells, respectively, in a particular tissue at the time of birth. At this stage we make no particular assumptions about the process that generates these random variables. We simply assume that stem cell populations grow during gestation and that random mutations can occur during cell divisions, creating a sub-population of mutated stem cells. It is easy to show (see Appendix A) that the hazard function is given by

$$h(t) = \frac{E[-S'(t\,|\,X, W)]}{E[S(t\,|\,X, W)]}, \tag{3}$$

where E denotes the expectation and $S(t|X, W)$ is the survival function conditional on X and W. The expectation is taken with respect to these random variables. It is important to note that the hazard function for a mixed population of normal and mutated cells is not the expected value of the hazard function taken over (X, W).

Now, after birth, cancer could originate from mutated or from normal cells. We will assume from now on that all cells are independent following gestation. Thus, the survival function conditional on the number of normal and mutated cells at birth is given by

$$S(t\,|\,X, W) = (S_{\text{normal}}(t; 1))^X (S_{\text{mut}}(t; 1))^W, \tag{4}$$

where $S_{\text{normal}}(t; 1)$ and $S_{\text{mut}}(t; 1)$ are the survival functions for a single normal cell and a single mutated cell at birth, respectively.

From (4), the denominator of expression (3) for $h(t)$ can be seen to be

$$E[S(t\,|\,X, W)] = E[(S_{\text{normal}}(t; 1))^X (S_{\text{mut}}(t; 1))^W] = G_{X,W}(S_{\text{normal}}(t; 1), S_{\text{mut}}(t; 1)), \tag{5}$$

where $G_{X,W}(s_1, s_2) \equiv E[s_1^X s_2^W]$ is the joint probability generating function (PGF) of (X, W).

Now for the numerator,

$$\begin{aligned} E[S'(t\,|\,X, W)] &= E[XS'_{\text{normal}}(t; 1)(S_{\text{normal}}(t; 1))^{X-1}(S_{\text{mut}}(t; 1))^W] \\ &\quad + E[WS'_{\text{mut}}(t; 1)(S_{\text{mut}}(t; 1))^{W-1}(S_{\text{normal}}(t; 1))^X] \\ &= \frac{S'_{\text{normal}}(t; 1)}{S_{\text{normal}}(t; 1)} E[X(S_{\text{normal}}(t; 1))^X (S_{\text{mut}}(t; 1))^W] \\ &\quad + \frac{S'_{\text{mut}}(t; 1)}{S_{\text{mut}}(t; 1)} E[W(S_{\text{normal}}(t; 1))^X (S_{\text{mut}}(t; 1))^W] \end{aligned}$$

$$= S'_{\text{normal}}(t;1)\frac{\partial G_{X,W}(s_1,s_2)}{\partial s_1}\bigg|_{[S_{\text{normal}}(t;1),S_{\text{mut}}(t;1)]} + S'_{\text{mut}}(t;1)\frac{\partial G_{X,W}(s_1,s_2)}{\partial s_2}\bigg|_{[S_{\text{normal}}(t;1),S_{\text{mut}}(t;1)]}, \tag{6}$$

using the fact that

$$\frac{\partial G_{X,W}(s_1,s_2)}{\partial s_1} = \frac{1}{s_1}E[Xs_1^X s_2^W] \quad \text{and} \quad \frac{\partial G_{X,W}(s_1,s_2)}{\partial s_2} = \frac{1}{s_2}E[Ws_1^X s_2^W].$$

Now, substituting (5) and (6) in (3) we get

$$\begin{aligned} h(t) &= -\frac{S'_{\text{normal}}(t;1)}{G_{X,W}(S_{\text{normal}}(t;1),S_{\text{mut}}(t;1))}\frac{\partial G_{X,W}(s_1,s_2)}{\partial s_1}\bigg|_{[S_{\text{normal}}(t;1),S_{\text{mut}}(t;1)]} \\ &\quad -\frac{S'_{\text{mut}}(t;1)}{G_{X,W}(S_{\text{normal}}(t;1),S_{\text{mut}}(t;1))}\frac{\partial G_{X,W}(s_1,s_2)}{\partial s_2}\bigg|_{[S_{\text{normal}}(t;1),S_{\text{mut}}(t;1)]} \\ &= -\frac{\mathrm{d}}{\mathrm{d}t}\ln[G_{X,W}(S_{\text{normal}}(t;1),S_{\text{mut}}(t;1))]. \end{aligned} \tag{7}$$

So to calculate the hazard function, we need to have expressions for the PGF of the number of normal and gestationally mutated cells at birth and for the survival functions of the two cell types.

2.2. Accounting for two or more mutations during gestation

Although unlikely, there is a small probability that some cells could acquire two or more distinct mutations on the pathway to cancer during gestation. However, if the mutation rates were high enough, this probability could become significant; for example, in the case of exposure to a mutagen, such as radiation, during gestation. We now calculate the hazard function accounting for this possibility.

Let W_1 and W_2 be the number of cells at birth carrying 1 and 2 mutations, respectively. Following the same argument as before, it is easy to show that the hazard function in this case is given by

$$h(t) = -\frac{\mathrm{d}}{\mathrm{d}t}\ln[G_{X,W_1,W_2}(S_{\text{normal}}(t;1),S_{\text{mut1}}(t;1),S_{\text{mut2}}(t;1))], \tag{8}$$

where $G_{X,W_1,W_2}(s_1,s_2,s_3)$ is the joint PGF of (X, W_1, W_2) and $S_{\text{mut}j}(t;1)$ denotes the probability that a cell carrying j mutations at birth has not originated cancer by age t ($j = 1,2$).

In general, if we assume k different types of stem cells (including normal) in a tissue at birth and that we know the joint PGF (G) of the number of cells of each type, then the hazard is given by

$$h(t) = -\sum_{j=1}^{k}\frac{S'_{\text{type}_j}(t;1)}{G(S_{\text{type}_1}(t;1),\ldots,S_{\text{type}_k}(t;1))}\frac{\partial G(s_1,\ldots,s_k)}{\partial s_j}\bigg|_{[S_{\text{type1}}(t;1),\ldots,S_{\text{type}_k}(t;1)]}, \tag{9}$$

where $S_{\text{type}j}(t;1)$ is the probability that a cell of type j has not originated cancer by age t.

2.3. The Hazard when normal cells are assumed to grow deterministically during gestation

If we assume that normal stem cells grow deterministically during gestation and that the number of normal stem cells at birth is X, then expression (7) takes the form:

$$h(t) = h_{\text{normal}}(t; X) - \frac{\mathrm{d}}{\mathrm{d}t} \ln[G_W(S_{\text{mut}}(t; 1))], \tag{10}$$

where $h_{\text{normal}}(t; X)$ is the hazard function given there are X susceptible normal cells at birth and $G_W(s)$ is the PGF of the number of mutated cells at birth (W). So in this case, the hazard can be decomposed as the sum of the hazard of normal cells and the developmental mutations hazard:

$$h(t) = h_{\text{normal}}(t; X) + H_{\text{mut}}(t)$$

with,

$$H_{\text{mut}}(t) = -\frac{\mathrm{d}}{\mathrm{d}t} \ln[G_W(S_{\text{mut}}(t; 1))]. \tag{11}$$

3. Specific examples

In this section we show how to construct the incidence function under different models for the accumulation of mutations during gestation. We assume that normal stem cell populations have deterministic growth before birth. We use the theory of Filtered Poisson Processes to obtain the PGF of the number of mutated cells at birth for a large class of models. We first calculate the hazard under the assumptions that one or two mutations could occur during gestation and then consider exposure to mutagens during gestation.

3.1. A general PGF when the normal cells growth is deterministic

If we assume that the growth of normal cells during gestation is deterministic, the theory of Filtered Poisson Processes helps us to obtain a general form for the PGF of the number of mutated cells at birth.

Let $X(z)$ be the growth function of normal cells and let T be the time of birth. Then $X(T)$ is the number of normal susceptible cells at birth. Let $\psi(\mathbf{s}; T, z)$ be the PGF of the number of descendants of a mutated cell at time T, given that the mutation occurred at time z. If we assume that mutations occur according to a Non-homogeneous Poisson Process ($N(z)$) with instantaneous rate $\mu(z)X(z)$, then the number of mutated cells at birth W is given by

$$W = \sum_{j=1}^{N(T)} U_j(T - t_j), \tag{12}$$

where t_j is the time of occurrence and $U_j(T - t_j)$ is the number of descendants at time T of the jth mutation event. W has PGF given by [3]:

$$G_W(s) = \exp\left\{\int_0^T \mu(z)X(z)[\psi(\mathbf{s};T,z) - 1]\mathrm{d}z\right\}. \tag{13}$$

This integral can be evaluated analytically for some cases. If it is not possible, it can be evaluated numerically or through a simulation approach.

3.1.1. The hazard under the Lea–Coulson model for accumulation of gestational mutations

First we assume that the normal stem cell population grows exponentially with rate β, i.e., $X(z) = \mathrm{e}^{\beta z}$ and that the mutation rate is constant, $\mu(z) = \mu$. Once a cell is mutated, we assume it divides according to a Yule process also with rate β. This is the Lea–Coulson version of the Luria–Delbrück process [4]. Fig. 1 shows a schematic representation of this model. The PGF of the number descendants of a mutated cell at birth (T), given the mutation occurred at time z is given by [5]:

$$\psi(s;T,z) = \frac{s\mathrm{e}^{-\beta(T-z)}}{1 - s(1 - \mathrm{e}^{-\beta(T-z)})}, \tag{14}$$

substituting this formula in (13) and evaluating the integral we obtain the PGF of the number of mutated cells at birth under the Lea–Coulson model:

$$G^{\mathrm{LC}}(s) = (1 - \phi s)^{\theta\frac{1-s}{s}}, \tag{15}$$

where $\theta = \frac{\mu}{\beta}\mathrm{e}^{T\beta}$ and $\phi = 1 - \mathrm{e}^{-T\beta}$.

Using this expression and substituting it in (11) we get that the incidence function under this model is given by

$$h(t) = h_{\mathrm{normal}}(t;X) + H_{\mathrm{mut}}(t)$$

with

$$H_{\mathrm{mut}}(t) = h_{\mathrm{mut}}(t;1)\left[-\frac{\theta\ln[1 - \phi S_{\mathrm{mut}}(t;1)]}{S_{\mathrm{mut}}(t;1)} - \frac{\theta\phi(1 - S_{\mathrm{mut}}(t;1))}{(1 - \phi S_{\mathrm{mut}}(t;1))}\right], \tag{16}$$

where $h_{\mathrm{normal}}(t;X)$ and $h_{\mathrm{mut}}(t;1)$ are the hazards given there are X normal and 1 mutated susceptible stem cells at birth, respectively, and $S_{\mathrm{mut}}(t;1)$ is the survival function for one susceptible mutated cell.

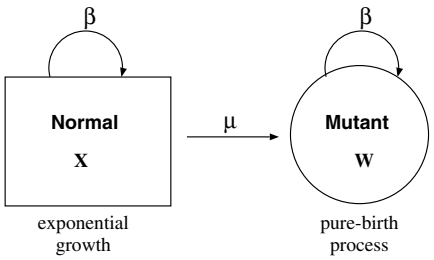

Fig. 1. Lea–Coulson model for gestational mutations.

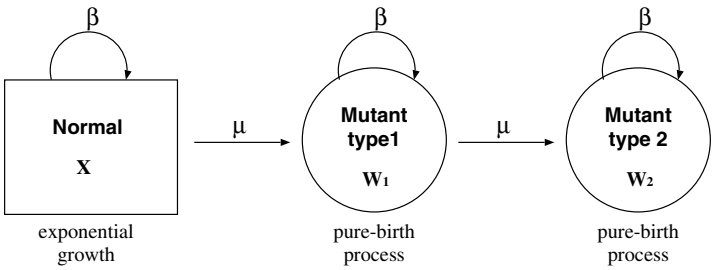

Fig. 2. Model for two successive mutations during gestation.

3.1.2. Hazard when accounting for two successive mutations

If we account for the probability that a cell could suffer two gestational mutations, then we need to know the joint PGF of the number of cells with one and two mutations at birth. We assume again that normal cells divide exponentially ($X(z) = e^{\beta z}$) and that first mutations occur according to a non-homogeneous Poisson process with instantaneous rate $\mu e^{\beta z}$. Once a cell is mutated, it starts a Bartlett process [4]. This means that mutated cells undergo a Yule process with rate β and they may acquire a second mutation with rate μ. Once a cell suffers a second mutation, then it also divides according to a Yule process with rate β. Fig. 2 shows an schematic representation of the model.

The PGF of the number of once and twice mutated cells at birth, given that the first mutation occurred at time z is given by [6]:

$$\psi(s_1, s_2; T, z) = \frac{e^{-\beta(T-z)}}{\left[(s_2 e^{-\beta(T-z)} + 1 - s_2)^{\frac{\mu}{\beta}}\left(\frac{1}{s_1} - \frac{1}{s_2}\right) + \frac{s_2 e^{-\beta(T-z)} + 1 - s_2}{s_2}\right]}. \tag{17}$$

Substituting this formula in expression (13) we obtain the PGF for the total number of once and twice mutated cells at birth. However, it is not possible to evaluate the integral analytically. The integral can be evaluated numerically.

3.2. The effects of an exposure to mutagens during gestation

There is considerable interest in the cancer risk posed by exposures to mutagens, such as radiation, during gestation. For example, the effects of prenatal X-rays on childhood cancer risk is the topic of much debate [7]. Our formulation allows us to evaluate the effects of mutagens on cancer risk.

We use the general filtered Poisson process formulation, to calculate the PGF of the number of mutated cells at birth, given exposure to a mutagen during gestation. We analyze and compare the effects of acute and constant mutagen exposure. For simplicity, we adopt the Lea–Coulson model for the accumulation of mutations during gestation.

3.2.1. Acute exposure

Let τ be the time of an acute exposure to a mutagen during gestation. Suppose that the gestational mutation rate as a function of time (z) is given by

$$\mu(z) = \mu + v_a \delta(z - \tau), \tag{18}$$

where $\delta(z-\tau)$ is the Dirac delta function and v_a is a constant that depends on the radiation dose and its effectiveness (See Section 4.4). Under this assumptions, and using (13), the probability generating function of the total number of mutated cells at birth as a function of τ is given by

$$G_W(s;\tau) = \exp\left\{\int_0^T (\mu + v_a\delta(z-\tau))e^{\beta z}[\psi(s;T,z)-1]\,dz\right\}, \tag{19}$$

where $\psi(s;T,z)$ is the same as in (14).

Evaluating the integral we find that the PGF reduces to:

$$G_W(s;\tau) = G^{LC}(s)\exp\left\{-v_a e^{\beta\tau}\frac{1-s}{1-s(1-e^{-\beta(T-\tau)})}\right\}, \tag{20}$$

where $G^{LC}(s)$ is the Lea–Coulson PGF for the number of mutated cells at birth (15). Formula (20) is also equivalent to the PGF of the number of mutated cells at birth when assuming that a Poisson number of mutations with mean $v_a e^{\beta\tau}$ occurs at the time of the acute exposure. Now using (10) we see that in this case the hazard is given by

$$\begin{aligned} h(t) &= h_{normal}(t;X) - \frac{\partial}{\partial t}\ln[G_W(S_{mut}(t;1);\tau)] \\ &= h_{normal}(t;X) + H_{mut}(t) + h_{mut}(t;1)S_{mut}(t;1)\frac{v_a e^{-\beta T+2\beta\tau}}{[1-S_{mut}(t;1)(1-e^{-\beta(T-\tau)})]^2}, \end{aligned} \tag{21}$$

where we recall $h_{normal}(t;X)$ and $h_{mut}(t;1)$ are the hazards given there are X normal and 1 mutated susceptible stem cells at birth, respectively and $H_{mut}(t)$ is the hazard from non-radiation induced mutated cells at birth given in (16).

3.2.2. Constant exposure

Now we calculate the hazard in the case of a constant exposure to a mutagen over a fixed period of time during gestation. Let T_0 and T_1 be the initial and final times of exposure, respectively. Assume that the mutation rate as a function of time (z) is given by

$$\mu(z) = \mu + v_c I_{[T_0,T_1]}(z), \tag{22}$$

where $I_{[T_0,T_1]}(z)$ is the indicator function for the interval $[T_0,T_1]$ and v_c is the increment in the gestational mutation rate due to the exposure. From (13), the PGF of the number of mutated cells at birth as a function of the exposure period $[T_0,T_1]$ is given by

$$G_W(s;T_0,T_1) = G^{LC}(s)\exp\left\{v_c\int_{T_0}^{T_1} e^{\beta z}\left[\frac{se^{-\beta(T-z)}}{1-s(1-e^{-\beta(T-z)})}-1\right]dz\right\}, \tag{23}$$

where again $G^{LC}(s)$ is the Lea–Coulson PGF for the number of mutated cells at birth (15).

Substituting this last expression in (10) we find that the hazard with constant exposure to a mutagen is given by

$$h(t) = h_{normal}(t;X) + H_{mut}(t) + h_{mut}(t;1)S_{mut}(t;1)v_c\int_{T_0}^{T_1} e^{\beta z}\frac{e^{-\beta(T-z)}}{(1-S_{mut}(t;1)(1-e^{-\beta(T-z)}))^2}dz, \tag{24}$$

where the integral is equal to:

$$\left[\frac{e^{\beta T}}{\beta S_{\text{mut}}^2(t;1)}\left(\frac{1-S_{\text{mut}}(t;1)}{1-S_{\text{mut}}(t;1)(1-e^{-\beta(T-z)})}+\ln\left\{e^{\beta T}\left[1-S_{\text{mut}}(t;1)(1-e^{-\beta(T-z)})\right]\right\}\right)\right]_{z=T_0}^{z=T_1}.$$

So, both for acute and continuous exposure during gestation, the hazard function can be expressed as a sum of a background hazard and a hazard attributable to exposure to a mutagen.

4. Application to colorectal cancer case

We apply our model to the analysis of colorectal cancer in the SEER database, which is one of the most comprehensive cancer registries covering approximately 10% of the US population. We use the Lea–Coulson model for mutation accumulation during gestation in tandem with the multistage model for colorectal cancer developed by Luebeck and Moolgavkar [2].

4.1. Multistage carcinogenesis model

There is a vast literature on mathematical models for multistage carcinogenesis. We restrict attention here to a class of models arising from recent analysis of colorectal cancer in the SEER database [2]. This class of models explicitly acknowledges three distinct phases in the process of carcinogenesis. In the first phase, that of initiation, a susceptible stem cell acquires one or more mutations resulting in an initiated cell, which has partially escaped growth control. The second phase, that of promotion, is the clonal expansion of initiated cells. Promotion is an extremely efficient way of bringing about carcinogenesis because clonal expansion results in increased populations of cells that have already acquired some of the genetic alterations on the pathway to malignancy. Finally, in the last phase, that of malignant conversion, one of the initiated cells acquires the further genetic changes required to convert it into a malignant cell. There is considerable evidence that most human malignancies go through these three phases. Environmental agents, such as radiation and tobacco smoke, influence carcinogenesis via their effects on one or more phases of the process.

A k-stage model incorporating these ideas is shown in Fig. 3. In this model, it is assumed that normal susceptible stem cells (stage 0) have to go through $k-2$ pre-initiation stages, before being able to expand clonally (initiated stage). Normal cells become pre-initiated according to a Poisson process with intensity μ_0. Each cell in the pre-initiation stage i can divide (with rate μ_i) into one stage i and one stage $i+1$ cell, $i=1,\ldots,k-2$. Once a cell reaches the initiation stage $(k-1)$, it expands clonally via a birth and death process. Each time an initiated cell divides, it can produce two initiated cells (with birth rate α) or one initiated and one malignant cell (with rate μ_{k-1}). Initiated cells can also die or differentiate with rate γ.

For the k-stage model (with $k \geqslant 3$), the expression of the survival function assuming only one susceptible stem cell is [2]:

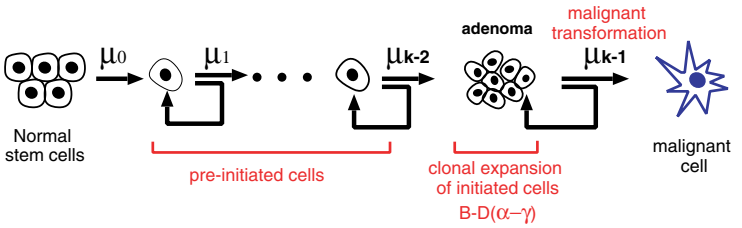

Fig. 3. k-stage carcinogenesis model. Normal susceptible cells (stage 0) have to go through $k-2$ pre-initiation stages, before being able to expand clonally (initiated stage). Normal cells become pre-initiated according to a Poisson process with intensity μ_0. Each cell in the pre-initiation stage i can divide (with rate μ_i) into one stage i and one stage $i+1$ cell, $i=1,\ldots,k-2$. Once a cell reaches the initiation stage $(k-1)$, it expands clonally via a birth and death process (B–D). Each time an initiated cell divides, it can produce two initiated cells (with birth rate α) or one initiated and one malignant cell (with rate μ_{k-1}). Initiated cells can also die or differentiate with rate γ.

$$S_k(t;1)=\exp\left\{\int_0^t \mu_0\left[\exp\left\{\int_{s_1}^t \mu_1\left[\ldots\left[e^{\int_{s_{k-2}}^t \mu_{k-3}[S_2(t-s_{k-2};1)-1]\mathrm{d}s_{k-2}}-1\right]\ldots-1\right]\mathrm{d}s_2\right\}-1\right]\mathrm{d}s_1\right\}, \tag{25}$$

where $S_2(u;1)$ is the survival function of the two-stage clonal expansion model (TSCE) [8,9]:

$$S_2(u;1)=\left[\frac{q-p}{qe^{-pu}-pe^{-qu}}\right]^{\frac{\mu_{k-2}}{\alpha}} \tag{26}$$

with

$$p=\frac{1}{2}\left((-\alpha+\gamma+\mu_{k-1})-\sqrt{(\alpha+\gamma+\mu_{k-1})^2-4\alpha\gamma}\right)$$
$$q=\frac{1}{2}\left((-\alpha+\gamma+\mu_{k-1})+\sqrt{(\alpha+\gamma+\mu_{k-1})^2-4\alpha\gamma}\right).$$

The corresponding hazard (or incidence function) is easily calculated using (2). As an example, the four-stage survival is given by

$$S_4(t;1)=\exp\left\{\int_0^t \mu_0\left[e^{\int_{s_1}^t \mu_1[S_2(t-s_2;1)-1]\mathrm{d}s_2}-1\right]\mathrm{d}s_1\right\}.$$

If we assume that the first mutation of the process could occur during development and that a k-stage model represents the carcinogenesis of normal stem cells, then a $k-1$ stage model would represent the risk of gestationally mutated stem cells. Thus, under the assumption that stem cells are independent after birth, the survival function conditional on the number of normal (X) and mutated (W) stem cells at birth (4) becomes:

$$S(t\,|\,X,W)=(S_{\text{normal}}(t;1))^X(S_{\text{mut}}(t;1))^W=(S_k(t;1))^X(S_{k-1}(t;1))^W$$

and the unconditional hazard (7) is given by

$$h(t)=-\frac{\mathrm{d}}{\mathrm{d}t}\ln[G_{X,W}(S_k(t;1),S_{k-1}(t;1))].$$

4.2. Data analyses

Luebeck and Moolgavkar did not consider gestational mutations in their analysis of the SEER data [2]. They found that a four-stage model described the data best when the analysis was adjusted simultaneously for birth and calendar year effects. For a biological interpretation of these stages the reader is referred to the original publication. Fig. 4 is a schematic representation of a model for colorectal carcinogenesis when both normal and gestationally mutated stem cells are present at birth. We fit this model to the incidence data in SEER with the accumulation of gestational mutations modeled by the Lea–Coulson process. We account only for cells with one mutation and neglect the possibility of a 2nd mutation in the same cell before birth. We do this because in models accounting for two gestational mutations, the second mutation made no significant contribution to the risk of cancer. So for simplicity, we concentrate on the Lea–Coulson model for our data analysis.

We fit the theoretical hazard (16) to the incidence of colorectal cancer while simultaneously adjusting for birth and calendar year effects by maximizing a likelihood as briefly described below. Incidence data for cancers of the colon and rectum were obtained from the SEER registry for the years 1973–2000 [10]. For our study, we use the reported incidence (number of cases) of colorectal cancers by gender, race, age and calendar year in the nine SEER geographic areas (≈10% of the US population). Populations at risk were obtained from the SEER files (based on data from the US Census Bureau). These data are cross-tabulated by calendar year (1973–2000) and five-year age groups (0–85+). Model fits are restricted to white males and females (including white Hispanics). For all years combined, a total of 149,776 male cases and 149,642 female cases are available for the analysis.

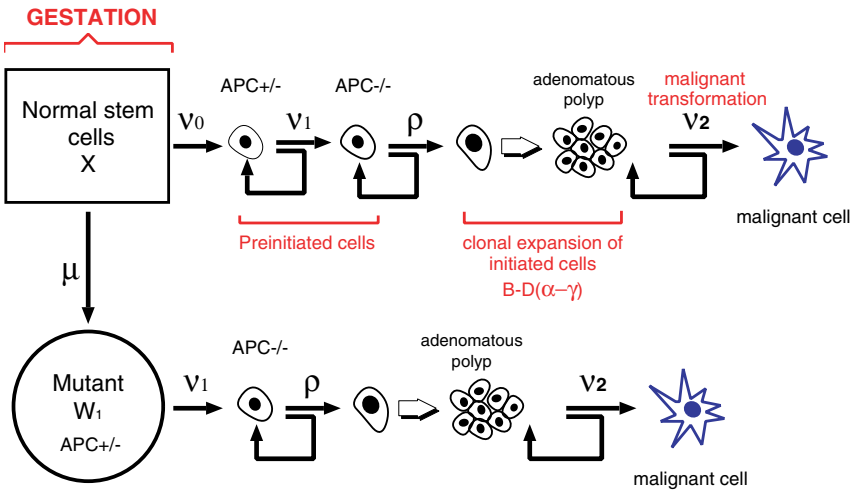

Fig. 4. Colorectal cancer model. This model assumes that the first two mutations that initiate the carcinogenesis lead to the inactivation of both alleles of the APC gene (with rates ν_0 and ν_1). A third event with rate ρ, interpreted to be a positional event, triggers clonal expansion and the formation of an adenoma (B–D process). Finally, another rate-limiting event with rate ν_2, transforms an adenoma cell into a malignant one. If a mutation occurs during gestation, then cells that suffer this mutation are a step ahead in the pathway to cancer.

For each age-group, the number of colorectal cancer cases diagnosed during calendar year j is assumed to follow a Poisson distribution with mean:

$$b_{j-a}c_j PY_{a,j}h(a),$$

where a is the mean age of the group, b_{j-a} and c_j adjust for birth and calendar year effects, $PY_{a,j}$ is the person years at risk and $h(a)$ is our theoretical hazard at age a. Birth years are stratified into 5-yr groups starting in 1885 until 1975, followed by a single open interval thereafter. Thus, we estimated a total of 18 birth-cohort effects and a total of 27 calendar-year coefficients ($b_{1885-1887.5}$ and c_{1990} are set equal to 1).

The overall likelihood $\mathscr{L}$ for the observed incidence in all age-calendar year groups is given by

$$\mathscr{L} = \prod_{a,j} \frac{\Lambda_{a,j}^{o_{a,j}} e^{-\Lambda_{a,j}}}{o_{a,j}},$$

where $o_{a,j}$ is the number of cases in the age group with midpoint a during calendar year j, and $\Lambda_{a,j} = b_{j-a}c_j PY_{a,j}h(a)$. The negative log-likelihood function is minimized via the Davidon–Fletcher–Powell (DFP) algorithm. In particular, we use the implementation found in the BHAT package in R.[1] Please see Appendix of [2] for details of the estimation method.

We assume that the rate of cell division during embryogenesis (β) is approximately 24.6 per cell per year. This yields a colon at birth consisting of 10^8 stem cells, which is approximately the number of crypts in the colon [11]. This is the assumption made by Luebeck and Moolgavkar that the normal colon has approximately 10^8 stem cells and that this population does not expand after birth.

Not all the remaining biological parameters of the model can be identified from incidence data alone. Only certain combinations of the biological parameters are identifiable [2,12,13]. We estimate the following combination of parameters: $\nu_0 = \nu_1$ (the rate of first and second mutations after birth), ρ (the rate of the third event in the four-stage model), $\alpha - \gamma$ (the net proliferation rate of an adenoma) and $\alpha\nu_2$ (the product of the growth rate of an adenoma and the malignant transformation rate). The gestational mutation rate is identifiable in theory, however we could not estimate it with confidence in practice. Thus, we fit the model under different assumptions regarding the value of the gestational mutation rate μ ($\mu = 10^{-6}, 10^{-5}, 10^{-4}$ per cell per year). There is some experimental evidence suggesting an estimated gestational mutation rate of 10^{-5} per cell per year. This is the mean value we use for the analysis, but we are aware of its uncertainty (please see concluding remarks).

When μ is small ($O(10^{-5})$ or less per cell per year), the parameters estimates are quite close to the estimates obtained using only the four-stage model. This is a reflection of the fact that with such small gestational mutational rates, the probability of more than a few thousand mutated cells at birth is exceedingly small (please see Fig. 5(a)). The parameter estimates for males and females are given in Appendix A. The estimates of the period and birth cohort effects are likewise consistent with those reported in [2].

[1] http://www.r-project.org/

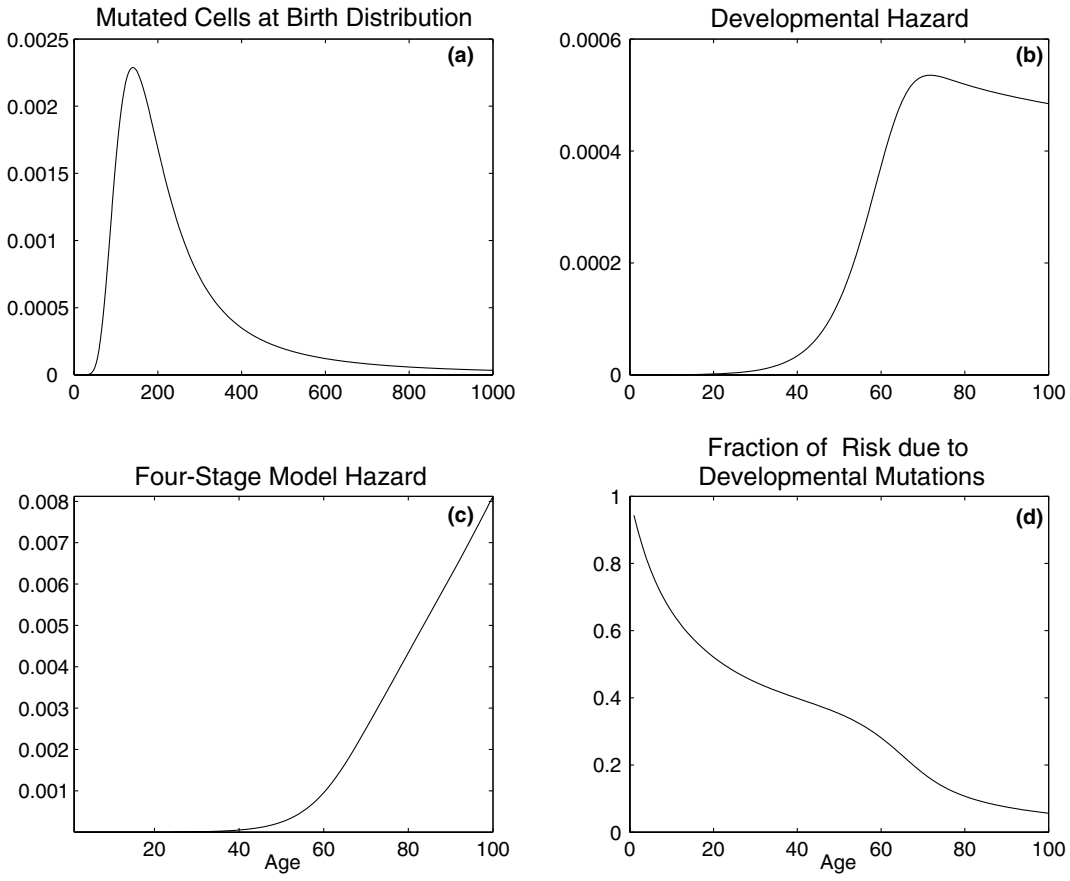

Fig. 5. Results for white males assuming $\mu = 10^{-5}$ per cell per year. (a) Distribution of the number of mutated cells at birth. (b) The hazard functions arising from cells with a gestational mutation. (c) Hazard functions arising from normal cells at birth (computed using the four-stage model). (d) Proportion of cancers by age attributable to gestational mutations.

4.3. Fraction of cancers attributable to developmental mutations

We estimate the fraction of colorectal cancers due to gestational mutations for various values of the gestational mutation rates. The results for white males in the SEER database with an assumed gestational mutation rate of 10^{-5} per cell per year, which corresponds to a rate of approximately 4×10^{-7} per cell division, are shown in Fig. 5. Fig. 5(a) shows the distribution of cells with a gestational mutation as predicted by the LC model. Fig. 5(b) shows the contribution to the incidence (hazard) function arising from cells with a gestational mutation. Note that this function exhibits a distinct downturn after about the age of 60. This downturn is typical of incidence functions in heterogeneous populations and is due to the 'thinning out of susceptibles' at older ages [14]. In this case the downturn is attributable to the population heterogeneity in the number of cells carrying gestational mutations at birth. Individuals in the population with a large number of cells

Table 1
Estimated proportion of cancers due to gestational mutations for the year 2000 with 95% confidence intervals

μ	White females	White males
10^{-6}	2.16% (2.01%, 2.25%)	2.41% (2.39%, 2.42%)
10^{-5}	18.57% (17.76%, 19.29%)	20.47% (20.34%, 20.60%)
10^{-4}	64.66% (60.79%, 68.60%)	78.26% (77.27%, 78.91%)

with gestational mutations are more likely to develop cancer early in life, changing the makeup of the population at risk at older ages. Fig. 5(c) shows the hazard function arising from normal stem cells, which is calculated using the four-stage model. Finally, Fig. 5(d) shows the fraction of cancers attributable to developmental mutations as a function of age.

Using the estimated parameters of the LC model (please see Appendix A, Section A.3), we can also estimate the fraction of colorectal carcinomas in any specific calendar year due to gestational mutations. To do this, we integrate the product of the estimated fraction of cancers due to gestational mutations per age group (Fig. 5(d)) times the number of cases per age group in the calendar year of interest. Then we divide the integral by the total number of cases occurred in the calendar year. Table 1 shows these estimates (with their 95% confidence intervals [2]) for colorectal carcinomas in the SEER database in the calendar year 2000. These estimates are fairly consistent between males and females. The crucial question here is the magnitude of the gestational mutation rate. If it is of the order of 10^{-6} per cell per year (4×10^{-8} per cell division), gestational mutations make only a modest contribution to the cancer risk. If, on the other hand, it is of the order of 10^{-4} per cell per year (4×10^{-6} per cell division), gestational mutations contribute to the majority of colorectal cancers in both males and females. We were unable to find good estimates of gestational mutation rates in the literature. However, according to Cairns [16], μ is probably higher than v_0, the mutation rate in 'established' stem-cells that replicate asymmetrically, as in epithelial renewal tissues such as the crypts in the colon [17].

4.3.1. Heterogeneity in cancer risk arising from the distribution of cells with gestational mutations

Human populations exhibit considerable heterogeneity in cancer risk. At one end of the susceptibility scale are the inherited cancer syndromes that impose enormous risks on the individuals carrying the defective genes [18–21]. Such cancer genes confer risks that are several thousand-fold greater than those in normal individuals [9,22]. More subtle are inter-individual variations in susceptibility imposed by polymorphisms of genes in metabolic pathways, which impose relative risks of the order of 2–4 [23–27]. Similarly inter-individual variations in the efficiency of DNA repair or in rates of cell division would also translate into inter-individual variations in cancer risk [28].

[2] Computed using Markov Chain Monte Carlo with the Metropolis-Hastings algorithm [15]. This method assumes flat priors for the parameters.

Superimposed on the genetic background, environmental factors, such as tobacco smoke and diet, are also involved in inter-individual variation in risk. Genetic and environmental factors however cannot explain entirely the heterogeneity in cancer risks. It is well known that in-bred strains of experimental animals maintained under identical laboratory conditions exhibit substantial variation in the risk of cancer. Gestational mutations could explain part of this heterogeneity of risk. Even with identical gestational mutation rates in all individuals in a population, at birth individuals are at different risks of cancer because of the random variation in the number of mutated cells at birth.

We investigate the implications of gestational mutations for inter-individual variations in the risk of colorectal cancer. The degree of heterogeneity clearly depends on the gestational mutation rate. With a low mutation rate, only a small fraction of cells has the mutation and gestational mutations have only a small impact on the overall risk and this source of heterogeneity of risk is unimportant. With larger mutation rates, however, when a significant fraction of cancers arises from gestational mutations, this source of heterogeneity becomes more important. Fig. 6 shows results for white males with a gestational mutation rate of 10^{-5} per cell per year. Fig. 6(a) shows the developmental incidence function in the white male population as well as the incidence functions in the upper and lower quartiles of the number of gestationally mutated cells (Please see Appendix A, Section A.2). The risk in the highest quartile is considerable higher than in the lowest quartile. Fig. 6(b) shows the (unadjusted for temporal trends) incidence functions from the composite (developmental plus four-stage) model in the population and in the upper and lower quartiles of the number of mutated cells at birth. Fig. 6(c) shows the fraction of cancers attributable to gestational mutations in the same groups. Fig. 7 shows the fraction of cancers attributable to gestational mutations in the three groups in Fig. 6 for different values of the gestational mutation rate.

How does cumulative (life-time) risk compare in the upper and lower quartiles of the gestational mutation distribution? The relative risks for various values of μ are shown in Table 2. These are calculated using the parameter values given in Appendix A, Section A.3. We can see that the degree of heterogeneity imposed by gestational mutations may be similar to that due to polymorphisms in metabolizing enzymes.

4.4. Effects of radiation exposure in colorectal cancer

As a final exercise, we use our methodology to evaluate the effects radiation exposure could have during gestation on colorectal cancer risk. Suppose there is an acute exposure during gestation at time τ and that the mean number of induced mutants due to the exposure is given by

$$Df_r\mu X(\tau) \equiv v_a X(\tau), \tag{27}$$

where D is the total dose (measured in Sievert (Sv)), f_r is the radiation fraction (=effectiveness of acute 1Sv exposure relative to the background mutation rate per year, see [29]), μ is the background gestational mutation rate and $X(\tau)$ is the number of susceptible stem cells at time of exposure. Let $v_a\delta(z-\tau)$ be the increment in the gestational mutation rate due to the radiation exposure in Eq. (18).

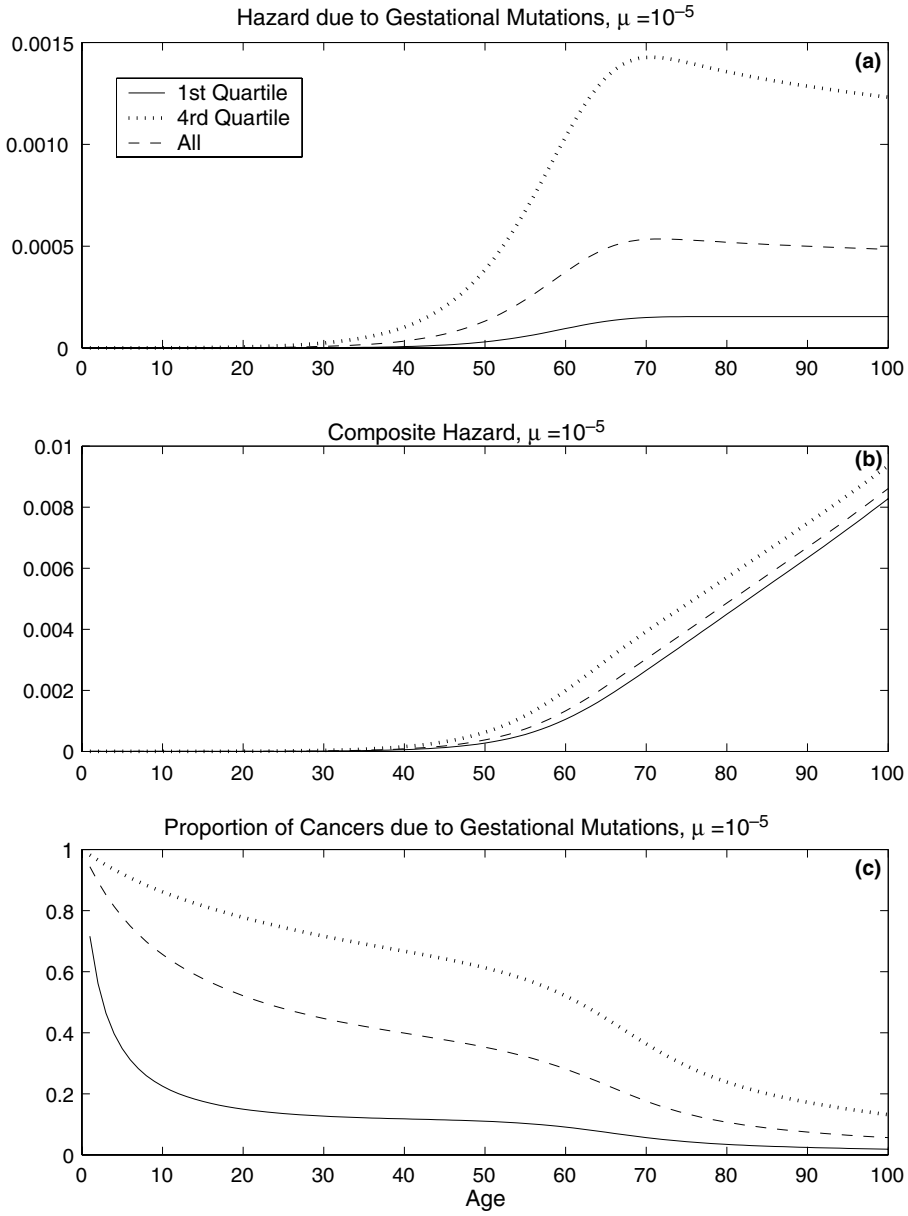

Fig. 6. Heterogeneity in cancer risk among males in SEER arising from developmental mutations when $\mu = 10^{-5}$ per cell per year. (a) The hazard functions arising from gestational mutations for individuals in the upper and lower quartiles of the distribution of mutated cells at birth and for the entire population. (b) The total hazard for each group. (c) The proportion of cancers attributable to gestational mutations for each group.

For this example we use $f_r = 40$ per Sv-year in accordance with the estimations of Kai et al. [29] and $D = 0.01$ Sv, which represents the typical dose for an X-ray exam during the 1950's [30].

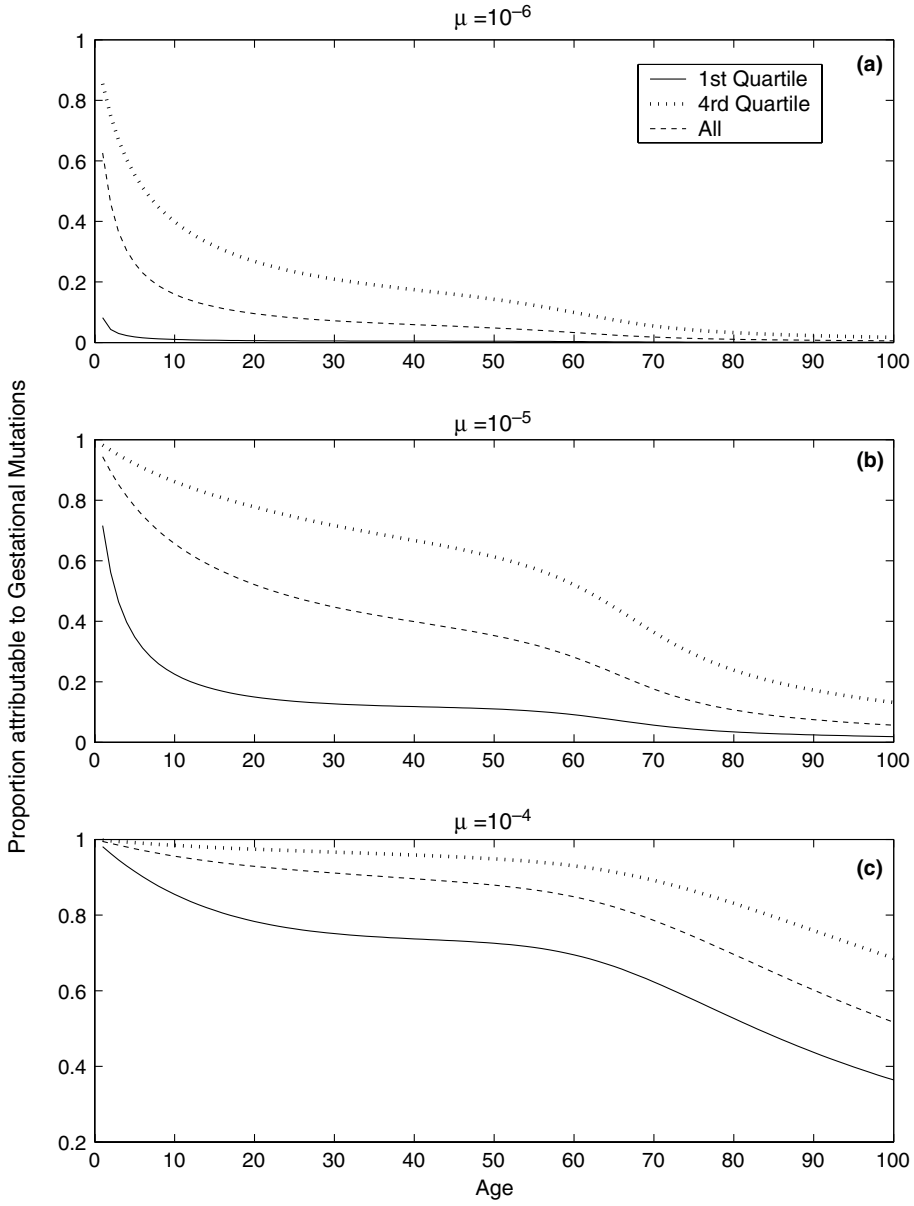

Fig. 7. Heterogeneity in the proportion of cancers attributable to gestational mutations for different values of the gestational mutation rate, μ.

Assuming a background gestational mutation rate of $\mu = 10^{-5}$, the distribution of the number of mutated cells at birth as a function of exposure time is shown in Fig. 8. The mean number of mutated cells is the same for all distributions. What changes dramatically is the variance. For late exposures, the mass of the distribution is concentrated around 400. As the exposure occurs earlier

Table 2
Cumulative (lifetime) risk in the upper quartile relative to that in the lower quartile

μ	White females	White males
10^{-6}	1.04	1.04
10^{-5}	1.3	1.3
10^{-4}	2.4	2.4

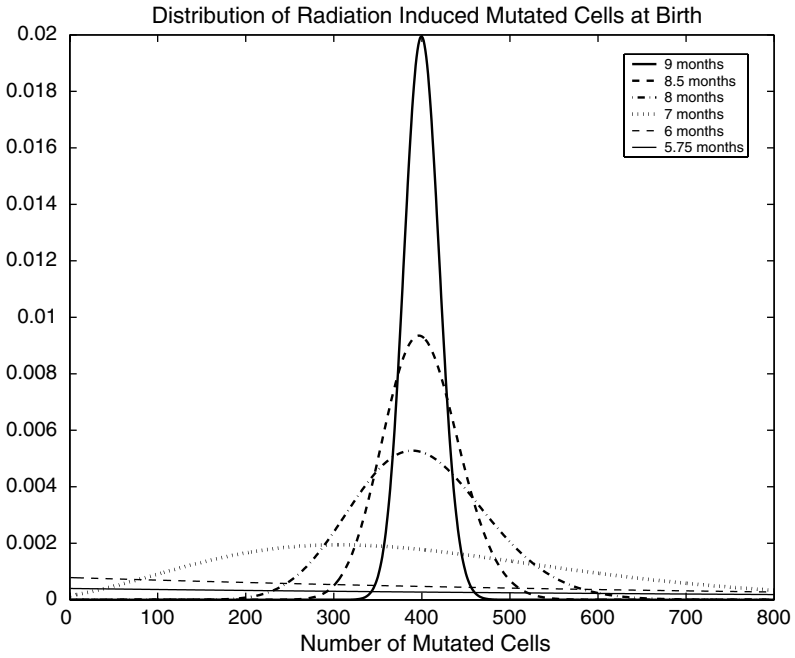

Fig. 8. Distribution of mutated cells for several acute exposure times. The probabilities are calculated by inverting the corresponding PGF's using inverse Fourier transform techniques [32].

Table 3
White males parameter estimates

μ	$\nu_0 = \nu_1$	ρ	$\alpha - \gamma$	$\alpha\nu_2$	Log-likelihood
0	1.36E–006	5.07E+001	1.52E–001	7.00E–007	807048.5
1E–6	1.36E–006	4.68E+001	1.54E–001	6.96E–007	807048.0
1E–5	1.37E–006	2.51E+001	1.59E–001	7.04E–007	807044.6
1E–4	1.22E–006	3.30E+000	1.64E–001	1.08E–006	807057.8

during gestation, the distribution is flattened and the variance increases. How does this affect cancer risk?

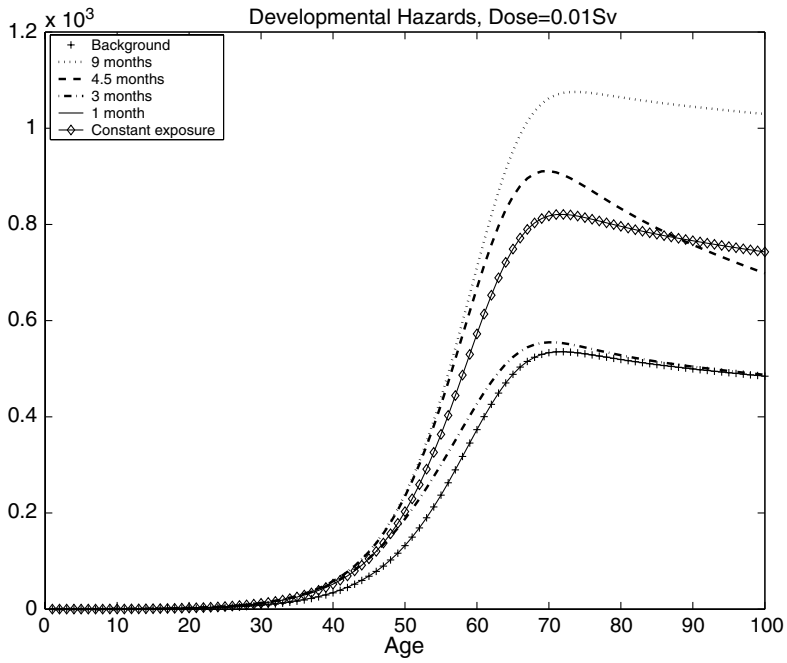

Fig. 9. Hazard functions arising from gestationally mutated cells (spontaneous plus radiation-induced) for exposure to radiation at various times during gestation.

Using the estimated parameters for males from the LC model when $\mu = 10^{-5}$ (Table 3) and Eq. (21), we calculate the hazard associated with acute exposures at various exposure times during gestation. Fig. 9 shows the 'Developmental' part of the incidence function for different exposure time points. We can see that the later the exposure, the larger the effect on cancer risk. The figure also shows the 'Developmental' part of the hazard when a constant (protracted) radiation exposure is present during gestation (Eq. (24)), assuming that the total amount of radiation dose is the same as for the acute exposures ($\nu_c = \nu_a/0.75$ year).

Fig. 10 shows the risk of exposure to radiation during gestation relative to the background incidence (with no radiation exposure). We note that the risk early in life, before about the age of 15, appears to be independent of the period during gestation when radiation exposure occurs. Since colorectal cancer is an adult onset tumor, very few cancers occur before the age of 15 and thus radiation exposure during gestation increases what is a very small risk to begin with. However, as age increases, we find that the relative risk decreases to 1 faster with early exposures. Thus exposure at full term imposes the highest risk and exposure during the last trimester of pregnancy imposes a higher risk than earlier exposures. There is limited experimental data supporting these conclusions [7]. Epidemiological interest has focused on the effects of gestational radiation on childhood cancers. However, our results suggest that gestational radiation could increase the risk of adult onset tumors as well.

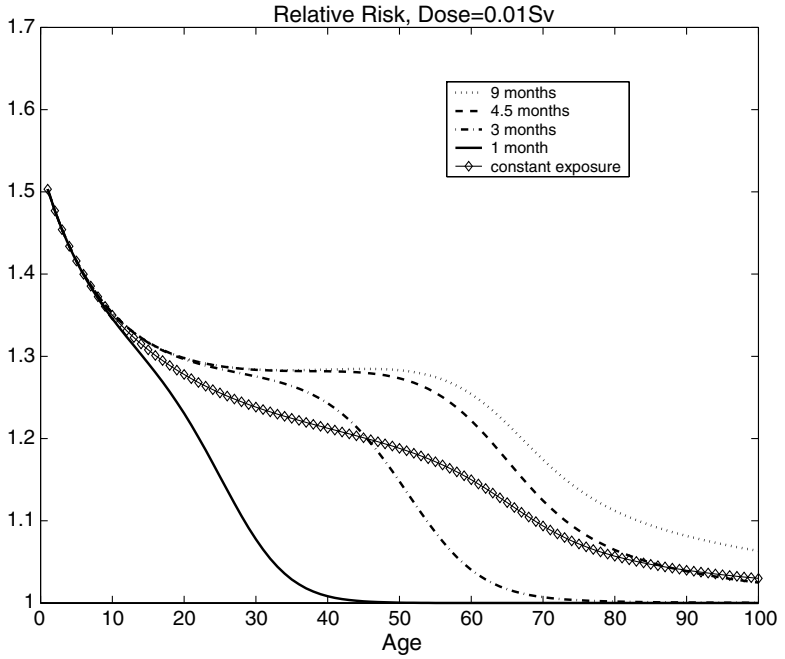

Fig. 10. Relative risk associated with radiation exposure at various times during gestation. The initial relative risk is independent of the exposure time. The relative risk of a constant exposure lies between the relative risks of early and late acute exposures.

5. Concluding remarks

In this article we present a mathematical framework to explore the effects of gestational mutations, whether spontaneous or induced by exposure to mutagens, on cancer risk. Gestational mutations could have substantial impact on cancer risk if the mutation rate is large enough. From experimental data, gestational mutation rates have been shown to reach values up to 10^{-4} per cell per year in mice [31]. We were unable to find estimates for gestational mutation rates in humans.

Spontaneous mutations during gestation may be responsible for some of the heterogeneity of cancer risk in human populations. Individuals in the highest quartile of the number of mutated cells at birth have relative risks of about 1.5–2.0 when compared to individuals in the lowest quartile, which is approximately the magnitude of risk conferred by polymorphisms in metabolizing enzymes. We could, of course, have chosen other quantiles of the distribution of mutant cells at birth for this comparison. For example, the risk in the highest relative to the lowest decile would be larger.

Our methodology can be used to estimate the effects of mutagen exposure during gestation on cancer risk. For adult onset cancers the largest risk of gestational exposure to mutagens appears to be conferred by exposure late during pregnancy.

In this paper we do explicit computations for the effects of gestational pre-initiation mutations in colorectal cancer risk. However, it is possible that gestational mutations occur in genes

associated with late events in colorectal carcinogenesis. We ignore this possibility, because the risk associated with mutational events occurring after clonal expansion would be expected to be smaller than the risk associated with pre-initiation events.

Acknowledgment

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Appendix A

A.1. The hazard

Let (X, W) be the random variables corresponding to the number of normal and mutated cells at the time of birth. Let us assume that the probability of the event $[X = m, W = n]$ is known. Let $h(t|m,n)$ and $S(t|m,n)$ be the hazard and survival functions, given that there are m normal and n mutated cells at birth. Then, the unconditional hazard or incidence function is given by

$$\begin{aligned}
h(t) &= \lim_{\Delta t\to 0}\frac{1}{\Delta t}P[t < T \leqslant t+\Delta t | T > t] \\
&= \lim_{\Delta t\to 0}\frac{1}{\Delta t}\sum_{m,n}\frac{P[t < T \leqslant t+\Delta t | X = m, W = n]P[X = m, W = n]}{P[T > t]} \\
&= \sum_{m,n}\frac{P[T > t | X = m, W = n]P[X = m, W = n]}{P[T > t]} \\
&\quad \times \lim_{\Delta t\to 0}\frac{\mathrm{Prob}[t < T \leqslant t+\Delta t | X = m, W = n]}{\Delta t P[T > t | X = m, W = n]} \text{ by Dini's theorem} \\
&= \sum_{m,n}\frac{P[T > t | X = m, W = n]P[X = m, W = n]}{P[T > t]}h(t|m,n) \\
&= \sum_{m,n}\frac{P[T > t | X = m, W = n]P[X = m, W = n]}{\sum_{i,j}P[T > t | X = i, W = j]P[X = i, W = j]}h(t|m,n) \\
&= \sum_{m,n}\frac{S(t|m,n)h(t|m,n)P[X = m, W = n]}{\sum_{i,j}S(t|i,j)P[X = i, W = j]} \\
&= \frac{E[-S'(t|X,W)]}{E[S(t|X,W)]}.
\end{aligned}$$

A.2. Quartile hazards

Of particular interest are the incidence functions, conditional on the number of mutated cells at birth (W) being in the upper or lower tail of its distribution. These functions allow us to investigate the heterogeneity in cancer risk introduced by gestational mutations. In the case that normal

Table 4
White females parameter estimates

μ	$\nu_0 = \nu_1$	ρ	$\alpha - \gamma$	$\alpha\nu_2$	Log-likelihood
0	1.27E–006	5.28E+000	1.37E–001	1.09E–005	809028.4
1E–6	1.28E–006	5.03E+000	1.39E–001	1.03E–005	809028.8
1E–5	1.34E–006	3.58E+000	1.47E–001	7.09E–006	809026.0
1E–4	2.11E–006	5.44E–001	1.49E–001	5.01E–006	809014.8

stem cells, growth is assumed to be deterministic, the hazards conditional on W being in the lower or upper quartile of its distribution are:

Lower quartile:

$$h_{q_1}(t) = h_{\text{normal}}(t;X) + \frac{\sum_{n=1}^{q_1} S_{\text{mut}}(t;n)h_{\text{mut}}(t;n)P_n}{\sum_{j=1}^{q_1} S_{\text{mut}}(t;j)P_j}. \tag{28}$$

Upper quartile:

$$h_{q_3}(t) = h_{\text{normal}}(t;X) + \frac{H_{\text{mut}}(t)G_W(S_{\text{mut}}(t;1)) - \sum_{n=1}^{q_3-1} S_{\text{mut}}(t;n)h_{\text{mut}}(t;n)P_n}{G_W(S_{+\text{mut}}(t;1)) - \sum_{j=1}^{q_3-1} S_{\text{mut}}(t;j)P_j}, \tag{29}$$

where P_n is the probability that the number of mutated cells at birth is equal to n and q_1 and q_3 are the first and third quartiles of this distribution, respectively. Also, $h_{\text{normal}}(t;X)$ is the hazard for normal stem cells given there are X at birth and $h_{\text{mut}}(t,n)$ and $S_{\text{mut}}(t;n)$ are the hazard and survival functions for mutated stem cells, given there are n at birth. Finally $G_W(s)$ is the PGF for the number of mutated stem cells at birth and $H_{\text{mut}}(t)$ is the developmental hazard given in (11).

A.3. Parameter estimates

Tables 3 and 4 show the estimated model parameters for white males and females in the SEER database. Different values of the gestational mutation rate (μ) are assumed. For a definition of each parameter, please see Section 4.1 and Fig. 3.

References

[1] S.A. Frank, M.A. Nowak, Developmental predisposition to cancer, Nature 422 (2003) 494.
[2] E.G. Luebeck, S.H. Moolgavkar, Multistage carcinogenesis and the incidence of colorectal cancer, Proc. Natl. Acad. Sci. USA 99 (2002) 15095.
[3] D.L. Snyder, Random Point Processes, Wiley-Interscience [John Wiley & Sons], New York, 1975.
[4] Q. Zheng, Progress of a half century in the study of the Luria–Delbrück distribution, Math. Biosci. 162 (1999) 1.
[5] S. Karlin, H.M. Taylor, A First Course in Stochastic Processes, Academic, London, 1975.
[6] M.S. Bartlett, An Introduction to Stochastic Processes with Special References to Methods and Applications, Cambridge University, London, 1978.
[7] J. Valentin (Ed.), Biological Effects After Prenatal Irradiation (Embryo and Fetus), Annals of the ICRP 33 (2003) 1–206.
[8] S.H. Moolgavkar, D.J. Venzon, Two-event models for carcinogenesis: incidence curves for childhood and adult tumors, Math. Biosci. 47 (1979) 55.

[9] S.H. Moolgavkar, A. Knudson, Mutation and cancer: a model for human carcinogenesis, J. Natl. Cancer Inst. 66 (1981) 1037.
[10] L.A.G. Ries, M.P. Eisner, C.L. Kosary, B.F. Hankey, B.A. Miller, L. Clegg, A. Mariotto, M.P. Fay, E.J. Feuer, B.K. Edwards (Eds.), SEER Cancer Statistics Review, 1975–2000, National Cancer Institute, Bethesda, Available from: <http://seer.cancer.gov/csr/1975_2000>, 2003.
[11] Y. Yatabe, S. Tavare, D. Shibata, Investigating stem cells in human colon by using methylation patterns, Proc. Natl. Acad. Sci. USA 98 (2001) 10839.
[12] L.G. Hanin, A.Yu. Yakovlev, A nonidentifiability aspect of the two-stage model of carcinogenesis, Risk Anal. 16 (1996) 711.
[13] W.F. Heidenreich, E.G. Luebeck, S.H. Moolgavkar, Some properties of the hazard function of the two-mutation clonal expansion model, Risk Anal. 17 (1997) 391.
[14] P. Herrero-Jimenez, G. Thilly, P.J. Southam, A. Tomita-Mitchell, S. Morgenthaler, E.E. Furth, W.G. Thilly, Mutation, cell kinetics, and subpopulations at risk for colon cancer in the United States, Mut. Res. 400 (1998) 553.
[15] W.R. Gilks, S. Richardson, D.J. Spiegelhalter, Markov Chain Monte Carlo in Practice, Chapman Hall, London, 1996.
[16] J. Cairns, Somatic stem cells and the kinetics of mutagenesis and carcinogenesis, Proc. Natl. Acad. Sci. USA 99 (2002) 10567.
[17] S.P. Bach, A.G. Renehan, C.S. Potten, Stem cells: the intestinal stem cell as a paradigm, Carcinogenesis 21 (2000) 469.
[18] A.G. Knudson, A.G. Mutation, cancer: statistical study of retinoblastoma, Proc. Natl. Acad. Sci. USA 68 (1971) 820.
[19] F.P. Li, Familial aggregation, in: D. Schottenfeld, J.F. Fraumeni (Eds.), Cancer Epidemiology and Prevention, Oxford University, Berlin, 1996, p. 546.
[20] L.C. Strong, C.I. Amos, Inherited susceptibility, in: D. Schottenfeld, J.F. Fraumeni (Eds.), Cancer Epidemiology and Prevention, Oxford University, Berlin, 1996, p. 559.
[21] P. Peltomäki, A. De la Chapelle, Mutations predisposing to hereditary nonpolyposis colorectal cancer, Adv. Cancer Res. 71 (1997) 93.
[22] S.H. Moolgavkar, E.G. Luebeck, Multistage carcinogenesis: population-based model for colon cancer, J. Natl. Cancer Inst. 84 (1992) 610.
[23] R. Ayesh, J. Idle, J. Richie, M. Crothers, M. Hetzel, Metabolic oxidation phenotypes as markers for susceptibility to lung cancer, Nature 312 (1984) 169.
[24] N.E. Caporaso, M.A. Tucker, R.N. Hoover, R.B. Hayes, L.W. Pickle, H.J. Issaq, G.M. Muschik, Lung cancer and the debrisoquine metabolic phenotype, J. Natl. Cancer Inst. 82 (1990) 1264.
[25] C.C. Harris, Interindividual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair, Carcinogenesis 10 (1989) 1563.
[26] C.C. Harris, Chemical and physical carcinogenesis: advances and perspectives for the 1990s, Cancer Res. 51 (1991) 5023s.
[27] J.D. Potter, Colorectal cancer: molecules and populations, J. Natl. Cancer Inst. 91 (1999) 916.
[28] K.R. Loeb, L.A. Loeb, Significance of multiple mutations in cancer, Carcinogenesis 21 (2000) 379.
[29] M. Kai, E.G. Luebeck, S.H. Moolgavkar, Analysis of the incidence of solid cancer among atomic bomb survivors using a two-stage model of carcinogenesis, Radiat. Res. 148 (1997) 348.
[30] R. Wakeford, M.P. Little, Risk coefficients for childhood cancer after intrauterine irradiation: a review, Int. J. Radiat. Biol. 79 (2003) 293.
[31] Y.R. Paashuis-Lew, J.A. Heddle, Spontaneous mutation during fetal development and post-natal growth, Mutagenesis 13 (1998) 613.
[32] J. Abate, W. Whitt, Numerical inversion of Laplace transforms of probability distributions, ORSA J. Comput. 7 (1995) 36.