Breast cancer risk and possible mechanisms of radiation-induced genomic instability in the Swedish hemangioma cohort after reanalyzed dosimetry

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1. Introduction

Ionizing radiation is known to increase the risk of female breast cancer, one of the most frequent cancers worldwide [1–4]. However, a pooled analysis of radio-epidemiological cohorts showed substantial differences in risk estimates between different cohorts [5]. Considering the increase of exposure in the population by advances in medical diagnostics and radiation therapy, a more consistent description of radiation-induced breast cancer risk is highly relevant.

The current work analyzes breast cancer incidence in the Swedish hemangioma cohort. Children with hemangiomas were treated in Stockholm and Gothenburg by application of 226Ra and X-rays between 1920 and 1965 [6–9]. Since hemangiomas appear at infancy the cohort includes women who had their first treatment before the age of 18 months. In previous analyses [8,10] a relatively low excess relative risk was found. Recently a revision of the dosimetry system resulted in substantially lower dose estimates for the most highly exposed part of the Stockholm cohort [11]. Consequences for the radiation risk are investigated. In addition, the follow-up now includes five additional years until December 2009 and more breast cancer cases.

For radiation protection it is necessary to transfer risk estimates to different populations. This is particularly challenging for breast cancer since Western population incidence rates are about a factor 2–3 higher than in the Japanese population [12]. Therefore the use of either a relative risk transfer or absolute risk transfer from the LSS cohort leads to substantially different risk estimates for Western populations. Since breast cancer risk estimates for different populations seem to agree better for the excess absolute risk, a preference for a transfer of the absolute risk has been suggested [13,14]. A better mechanistic understanding could help to establish risk models that are structurally similar for different cohorts and provide a better basis for risk transfer.

One of the main purposes of this study is to develop mechanistic models of carcinogenesis for radiation-induced breast cancer. A
2. Materials and methods

2.1. The hemangioma cohort

In Sweden a large number of individuals were treated with ionizing radiation for skin hemangioma in childhood during 1920–1965. Two cohorts, one in Stockholm and one in Gothenburg, with more than 25,000 individuals have been established to study late health effects after radiation therapy in infancy. These cohorts have been described in detail elsewhere [6,28]. Two-thirds of the treated children were females – 9,676 and 7,546 persons from both cohorts, respectively – and in this work we study the breast cancer risk of these women. All persons had their first treatment before the age of 18 months. The number of treatments varied between 1 and 37 with a mean of 1.5 treatments. Skin hemangioma is a benign condition and has not been proven to have any correlation to an excess risk for malignancies. In previous studies it was shown that the spontaneous risk for breast cancer in the hemangioma cohort is in accordance to the breast cancer risk for Swedish women [7–9].

The establishment and analysis of the hemangioma cohort have been conducted with the permission of the Swedish Data Inspection board, which is responsible for protection of the privacy of the individuals in the database, and the Swedish Ministry of Justice. This study has also been approved by the ethical review board in Gothenburg.

2.2. Follow-up of the hemangioma cohort

The cohort was matched by record linkage with several national population registers using the unique identification number given to all Swedish residents. The Swedish Cancer Register contains information of new tumor cases since 1958, and breast cancers in the cohort until 2009 were retrieved. Using the Swedish Total Population Register, the Emigration Register and the Swedish Cause of Death Register, the correct follow-up status and person years at risk were estimated. Date of birth and number of children for the women in the cohorts were collected from the Swedish Multi-Generation Register. Four women were not identified in the population registers, 17 had died and 1 had emigrated before 1958.

These 22 women were excluded and 17,200 women were left for the analysis. The follow-up was from January 1, 1958 to the first of the following dates: date of first breast cancer, date of first emigration, date of death or December 31, 2009. There were 829,663 person years at risk, 906 women had a breast cancer and of those 43 had breast cancer in both breasts. Since the follow-up was censored at first emigration and 29 women got their breast cancer after that date only 877 breast cancers were used in the analyses. Compared to a previous study [10] the follow-up has increased by 5 years and has 199 more breast cancer cases. At the end of follow-up there were still 13,952 women alive and their median age was 60.7 years at that date. The number of women that got a child was 14,356 (83%) and totally they got 32,269 children.

2.3. Update of dosimetry system

Retrospective risk assessments of ionizing radiation demand, among other information, high-quality dosimetry data. Previous dose estimations for the Stockholm hemangioma cohort were based on measurements with thermoluminescent dosimeters (TLD) in an anthropomorphic phantom using the original radium sources, and by a simple dose planning system (DPS) [28]. The DPS was used for dose estimations close to the source. The insight of shortcomings of the DPS motivated a reanalysis of the dosimetry of the radium sources. Especially the radiation doses to the female breasts had to be recalculated before this new analysis of radiation-induced breast cancer risk was initiated.

The new dosimetry was performed with Monte Carlo (MC) simulation where the models were created with MCNP5 and GEANT4 [11]. Since the DPS was merely used for treatments close to the breast nipples only these treatments were affected by the new dosimetry. The result for these treatments showed that the highest doses decreased in average by 15% and the mean value by 75%. As only a small part of all treatments was influenced by the reanalizes, the total breast doses for the women, summarizing all treatments, were less affected. The mean total breast dose for all women in the Stockholm cohort decreased by 49%.

The breast doses in the Gothenburg cohort were from the beginning also estimated by a simple DPS. However, some years ago they were recalculated using data from the TLD measurements in Stockholm. This implied that the breast doses in this cohort were only slightly affected by the new MC calculations. After the reanalysis only 3% of the women in the whole cohort had breast doses exceeding 1 Gy (max 32.8 Gy). The mean dose decreased to 0.18 Gy but the median dose remained 0.04 Gy.

2.4. Risk models

The analysis is performed with a standard excess relative risk (ERR) model, the TSCE model, and a multitarget model with a separate path of genomic instability (GI model). The use of various models with a different baseline parameterisation allows an estimation of the model uncertainty. Since the ERR and TSCE model have been described previously [10], only the GI model is explained in more detail. The models were fitted with the individual maximum likelihood method [18] using the MINUIT2 C++ program from the CERN library [29]. Uncertainty bounds are estimated from the percentiles of 10,000 simulated Monte Carlo realizations based on the parameter distributions, taking into account the full correlation matrix.

2.4.1. Excess relative risk model

A standard parametric form is used to describe the hazard function \( \lambda(a) \) in the excess relative risk model

\[
\lambda(a) = a^{\beta_0}\cdot \left[ 1 + \ln(\alpha/50)\cdot f_1(a)\cdot \ln(\gamma/50)\cdot f_2(a)\cdot \ln(\delta/50)\cdot f_3(a)\cdot \ln(\epsilon/50)\cdot f_4(a)\cdot \ln(\zeta/50)\cdot f_5(a)\right]
\]

\[
\cdot \left(1 + ERR_{cal}(a)\cdot D(a - t_{agg})\right),
\]
where \( a \) is attained age, \( n_{\text{ch}} \) the number of children, \( D \) the total dose and \( \text{ERR}_{pd} \) the excess relative risk per dose. The \( \psi_r, f_s, \psi_{Hs} \) and \( \text{ERR}_{pd} \) are fit parameters. Previously both the number of children and age at first child birth were significant confounders. With the updated follow-up, however, age at first child birth is not significant (based on likelihood ratio test with 95% level) any more in addition to the number of children, though there is still an indication that breast cancer risk is reduced by early child birth. Different age dependencies of the \( \text{ERR}_{pd} \) are tested. For consistency with other work on breast cancer we include a lag time of 5 years although it is irrelevant for this cohort as exposure was in early childhood.

2.4.2. Two-stage clonal expansion model for carcinogenesis

In the TSCE model (Fig. 1) the complex process leading to cancer is reduced to two basic time-limiting steps.

The parameters \( \nu \) and \( \mu \) represent the rates of transition between the stages, \( \sigma \) is the division rate, and \( \beta \) the differentiation or apoptosis rate. After some lag time a malignant cell will lead to cancer [15,16]. Radiation changes the transition rates of the model. Since exposure was at early childhood, only a radiation effect at an early stage of carcinogenesis, the initiation, is relevant since at such an early age almost no initiated cells are present. The parameter change in the mechanistic models usually depends on the dose rate \( d \), i.e. the parameters change during exposure and return to their normal value afterwards, and a standard model is

\[
\text{TSCE-direct: } \nu(a) = \nu_0(1 + r_v \cdot d(a)).
\]

(2)

As discussed in Refs. [10,30], within the framework of the TSCE model a potential radiation-induced genomic instability can be tested by a lifelong increase of the transition rates. This effectively parametrizes the long-term increased accumulation rate of genetic changes and can be described by

\[
\text{TSCE-lifelong: } \nu(a) = \nu_0(1 + r_{\text{Gt}} \cdot D(a)).
\]

(3)

which results in a permanent increase of \( \nu \) after exposure proportional to the total accumulated dose \( D \). The radiation strength is given by the fit parameters \( r_v \) and \( r_{\text{Gt}} \). As in Ref. [10] a lag time of 5 years was assumed, and the division rate was fixed to a monthly cycle \( \alpha = 12.0 \text{yr}^{-1} \). The risk results are independent of these assumptions. \( \beta \) depends on the (age-dependent) number of children \( n_{\text{ch}}(a) \), \( \beta_{\text{bare}}(a) = \beta_0(1 + f_s \cdot n_{\text{ch}}(a)), f_s \) is a fit parameter.

In Ref. [10] the TSCE-lifelong model included an additional direct effect. This effect is not significant any more. Nevertheless, both lifelong models give structurally very similar results, e.g. a similar dependence of the excess relative risk on attained age.

2.4.3. Models of genomic instability

Genomic instability (GI) generally describes an increased rate of alterations in the genome. Radiation-induced GI is observed in cells at delayed times after irradiation and manifests in the progeny of exposed cells multiple generations after the initial exposure [25–27,31]. An important question, especially after exposure to ionizing radiation, is whether GI promotes the development of cancer, i.e., appears at a relatively early time of cancer development, or is a consequence of later stages [32]. Evidence has been presented that mutations in genes controlling genome stability appear at an early stage of carcinogenesis [33–37].

In molecular analyses of 825 breast cancer patients it was found that the mutations patterns depend strongly on the subtype of breast cancer [21]. Only three genes, the oncogene PIK3CA and the two tumor suppressor (TSP) genes TP53 and GATA3 [23] occurred at >10% incidence across all breast cancers. It has been found that radiation promotes breast cancer through effects on the microenvironment and cell interactions [38]. TGFβ was identified as a critical signal, accelerating carcinogenesis. Such cellular and tissue responses to ionizing radiation can have non-targeted effects on non-irradiated cells, such as induction of genomic instability [39].

Since the mechanisms and timing of breast cancer development are complex and not well understood, most efforts to develop mechanistic cancer models including GI concentrated on colon cancer, including additional pathways for genomic instability [40–44]. To investigate whether (radiation-induced) GI can be a driver in breast cancer development, here we use structurally similar types of models. In the model with GI, Fig. 2, a cell may undergo an event of genomic destabilization at an early (\( \sigma_0 \)) of later (\( \sigma_1 \)) stage of carcinogenesis. Such cells will accumulate genetic changes at an increased rate and transform towards cancer through the lower path at a faster rate than cells without GI through the upper path. This model contains more parameters than can be determined from epidemiological data alone. However, the number of parameters can be reduced by fixing practically redundant parameters and considering the biological interpretation, i.e., that the lower pathway contains cells with mutations leading to GI: (i) cells that are not yet ‘initiated’ have no growth advantage, \( \alpha_{0,GI} = \beta_{0,GI} \); (ii) risk depends primarily on the clonal expansion rates \( \gamma_1 = \alpha_1 - \beta_1 \) and \( \gamma_{1,GI} = \alpha_{1,GI} - \beta_{1,GI} (\mu < \alpha, \beta) \), but not on absolute values of \( \alpha \) or \( \beta \).

Since the breast has a monthly cycle we assume \( \alpha_1 = \alpha_{0,GI} = \alpha_{1,GI} = 12 \)
yr$^{-1}$ as for the TSCE model. The final results for the model and radiation risk are shown to be independent of this assumption.

The eight remaining free parameters are $v_0$, $\beta_1$, $v_1$, $\sigma_0$, $\sigma_1$, $\gamma_0$,$\gamma_1$, and $\gamma_{1,GI}$. The baseline path (i.e. without radiation) towards cancer can proceed via the three different pathways $v_0 - v_1$ (without GI), $\sigma_0 - v_0$,$\gamma_{0,GI}$,$\gamma_1$,$\gamma_{1,GI}$ (early GI), or $v_0 - \sigma_1 - v_1$,$\gamma_{1,GI}$ (late GI). All pathways are checked for their quality of fit.

In principle radiation could act at any stage of the carcinogenic process. However, in the hemangioma cohort exposure was at very young ages. At such ages almost no initiated cells are present that can clonally expand. As a consequence, only radiation effects on $v_0$ or $\sigma_0$ can be observed in this cohort. This is later confirmed by the analysis. In the TSCE model a lifelong dose response was implemented to account for GI in an effective way. Since here GI is an inherent structure of the model, there is no reason to implement lifelong effects. A potential GI should manifest itself by a (radiation-induced) transition to the lower path. Radiation increases the transition rates only when radiation is present, afterwards the parameters take again their baseline values, i.e. the parameters should be a function of the dose rate $d$. The two main models are

$$v_0(d) = v_{0,\text{base}} + r \cdot d$$

which corresponds to a radiation-induced transition on the upper path, and

$$\sigma_0(d) = \sigma_{0,\text{base}} + r \cdot d$$

with a radiation-induced transition to GI. $r$ is a fit parameter for the strength of the radiation action. Different functional dependencies on $d$, in addition to the linear one, are investigated as well. Since cells with GI accumulate genetic changes at a faster rate than normal cells, the transition rates of the lower path with GI should be larger than the rates of the upper path. Therefore one or several of the following conditions should be fulfilled: $v_{0,\text{GI}} > v_0$, $v_{1,\text{GI}} > v_1$, or $\gamma_{1,\text{GI}} > \gamma_1$. The last condition, assuming the $\alpha$'s fixed, implies $\beta_{1,\text{GI}} < \beta_1$. The different conditions are tested w.r.t. quality of fit and biological plausibility.

3. Results

Results from the ERR and TSCE model are shortly presented. The model with GI is discussed in more detail, and the significance of the different pathways is investigated. Finally, changes of the radiation risk due to the update of the dosimetry system are described.

3.1. ERR model

In the ERR model the number of children is a significant confounder ($\Delta$Dev = $-31.5$, $p = 2 \times 10^{-8}$), the risk decreases proportional to the number of children. For each child the breast cancer risk decreases by 15%. There is an indication that the breast cancer risk decreases for young age at first childbirth, but it is not significant in addition to the number of children ($\Delta$Dev = $-2.1$, $p = 0.15$). Note that for comparison of different models only the difference in deviance is important, the absolute value of the deviance (or the Akaike information criterion) is meaningless.

A comparison of different ERR models can be found in Table 1. There is no indication for a quadratic dose dependence, and no

| Table 1 | Comparison of different ERR models, the best model is emphasized. p-Values are given relative to the best model with one parameter less. |
| --- | --- | --- | --- | --- |
| ERR model | No. of parameters | Deviance | $p$-Value | AIC weight |
| Pure baseline | 4 | 13583.8 |  |  |
| ERR linear in dose | 5 | 13536.1 | $5.0 \times 10^{-12}$ |  |
| ERR linear-quadratic in dose | 6 | 13360.0 | >0.5 |  |
| ERR linear/log-linear in age | 6 | 13360.0 | >0.5 |  |

indications for a change of excess relative risk per dose (ERR$_{pd}$) with attained age:

$$\text{ERR}_{pd} = 0.48 \text{ Gy}^{-1} \quad (95\% \ CI \ 0.28; 0.69).$$

(6)

The model predicts 72.5 radiation-induced breast cancer cases of the total of 877 cases. In the previous analysis [10] the same linear dose response was found, however, the risk estimates increased substantially with the new DS. A comparison of risk estimates from different models, including mechanistic models, and to the results from the old DS, is presented below.

3.2. TSCE model

In the TSCE model the dependence of the breast cancer rate on the number of children is significant and of similar size as in the ERR model. Also there is an indication that the breast cancer risk decreases for young age at first childbirth, but again not significant.

A comparison of the different TSCE models can be found in Table 2. A radiation effect is highly significant. Both initiation models are non-nested and have the same number of parameters. A weighting according to Akaike information criterion (AIC), e.g. Refs. [45,44], results in a preference for the lifelong model with more than 95% weight, thus the lifelong model is chosen as preferred model.

There is no indication for a quadratic or non-linear dose dependence. The radiation fit patterns are $r_1 = 5.1 \pm 1.2$ yr Gy$^{-1}$ and $r_{2,1} = 0.50 \pm 0.11$ Gy$^{-1}$ for Eqs. (2)-(3) respectively, with 1$\sigma$ errors. The results of the lifelong initiation model for the ERR$_{pd}$ and ERR$_{pd}$ are very similar to the ERR model. The model predicts 72.1 excess cases consistent with the ERR model, whereas the TSCE model with direct initiation predicts only 61.8 excess cases with a lower quality of fit.

3.3. Model with path of genomic instability

First the choice of the preferred model is discussed, taking into account quality of fit and biological plausibility. Then the preferred model is presented and implications on transition rates are given. The results for the radiation risk are presented together with the ERR and TSCE model.

3.3.1. Selection of GI model

In principle, the process to cancer without radiation can proceed via the three different pathways $v_0 - v_1$ (without GI), $\sigma_0 - v_0$,$\gamma_{0,GI}$,$\gamma_1$,$\gamma_{1,GI}$ (early GI), or $v_0 - \sigma_1 - v_1$,$\gamma_{1,GI}$ (late GI), whereas for the hemangioma cohort only a radiation action on $v_0$ or $\sigma_0$ is relevant. Since the number of parameters is too large to fit all paths simultaneously, each baseline path is fitted separately by assuming the other two baseline paths vanishing, e.g. setting

| Table 2 | Comparison of different TSCE models, the best model is emphasized. By AIC criteria the model with direct initiation is strongly suppressed compared to lifelong initiation. |
| --- | --- | --- | --- |
| TSCE model | No. of parameters | Deviance | $p$-Value | AIC weight |
| Pure baseline | 4 | 13586.5 |  |  |
| Initiation direct (Eq. (2)) | 5 | 13548.3 | $6.4 \times 10^{-10}$ | 0.013 |
| Initiation lifelong (Eq. (3)) | 5 | 13539.6 | $7.5 \times 10^{-12}$ | 0.987 |
\( \sigma_{0, \text{base}} = \sigma_{1, \text{base}} = 0 \) when testing the path without GI. On the other hand, radiation might induce a transition to another path, and all such possibilities are analyzed. In all models the number of children modifies \( \beta_1 \), except in the model with early GI where it modifies \( \beta_{1, GI} \).

A comparison of the models is presented in Table 3. For the baseline model without GI a radiation action on \( \sigma_0 \) (M12) is a significant improvement compared to \( v_0 \) (M11, \( \Delta \text{AIC} = -6.5 \)). This model has the best AIC of all GI models, all parameters are in a biologically plausible range, and the risk estimates are compatible with the ERR and TSCE model. Therefore it is our preferred GI model. The model with a baseline path of early GI and radiation on \( \sigma_0 \) (M22) corresponds to a three-stage model with clonal expansion in the first stage (\( \sigma_{0, \text{GI}} = \beta_{0, \text{GI}} \)). Although the model has plausible parameter values, the fit quality is not good. The same baseline model but with a radiation action on \( v_0 \) (M21) has a similar deviation, but higher AIC as the best model M12. Furthermore this model has no meaningful biological interpretation since there is no experimental evidence that radiation damage produces much less genomic instability than occurring naturally. In addition, a closer inspection reveals that the observed hazard is poorly approximated by the model for larger ages. Thus this model is discarded. The models with late GI have additional baseline parameters. The quality of fit is not so good, except for the model M33 which has an additional children modifier in \( \beta_{1, GI} \). However, even for this model the AIC is higher than for M12. Furthermore the parameter \( \sigma_1 \approx 0.05 \text{ yr}^{-1} \) is relatively large and casts doubt on the biological plausibility. We will further comment this model after discussing the preferred model in more detail.

In the models described above the three possible pathways were investigated separately. If we assume that the transition probability towards GI is equal for healthy and initiated cells, we have the condition \( \sigma_{0, \text{base}} = \sigma_{1, \text{base}} \). Analyzing the data with this condition the baseline path via \( \sigma_0 \) is very dominant compared to the path via \( \sigma_1 \), so the model reduces essentially to the baseline model with early GI.

### 3.3.2. Results for preferred GI model

In the preferred model M12 the baseline evolution to cancer proceeds via \( v_0 - v_1 \); \( \beta_1 \) is modified by the number of children with \( \beta_{1, \text{base}} = \beta_1 \cdot (1 + f_{\text{ch}} \cdot n_{\text{ch}}) \), where \( n_{\text{ch}} \) is the number of children, and \( \beta_1 \) and \( f_{\text{ch}} \) are free parameters. Radiation acts on \( \sigma_0 \) according to Eq. (5) with a zero baseline rate \( \sigma_{0, \text{base}} \). Since the clonal expansion rate of the non-initiated GI cells is zero (\( \sigma_{0, \text{GI}} = \beta_{0, \text{GI}} \)), effectively only the combination \( \sigma_1 \cdot v_{0, \text{GI}} \) can be determined. In addition, the GI path has the free parameters \( \beta_{1, GI} \) and \( v_{1, GI} \). Due to the limited statistical power not all of these parameters in the GI path can be fitted simultaneously, however, it is possible to analyze them separately.

As shown in the appendix, the M12 model with \( v_{0, \text{GI}} \gg v_0 \) gives by far the best quality of fit. Thus the main effect of GI in the best model is to increase the rate of normal cells with GI towards initiated cells with a proliferative advantage. For the two other parameters in the GI path, \( v_{1, \text{GI}} \) and \( \beta_{1, \text{GI}} \), the model predicts that they are similar to their counterparts in the normal path without GI: \( v_{1, \text{GI}} \approx v_1 \) and \( \beta_{1, \text{GI}} \approx \beta_1 \).

It is interesting to give an estimate of the radiation parameter \( r \). The parameter cannot directly be derived from the data since only the combination \( \sigma_0 \cdot v_{0, \text{GI}} = r \cdot v_{0, \text{GI}} \cdot d \) can be determined. Two arguments are presented in the appendix that indicate \( 10^{-6} \text{ Gy}^{-1} < r < 10^{-2} \text{ Gy}^{-1} \) as a plausible range for \( r \). The biological meaning of e.g. the upper limit is that an exposure of 1 Gy about 1% of all healthy cells exhibit genomic instability.

### 3.4. Results for radiation risk

Fig. 3 displays the results for the excess relative risk for the best ERR, TSCE and GI models. For comparison also the results from the disfavored TSCE-direct model, Eq. (2), is shown. All models agree for the risk at central ages of cancer occurrence, around 53 years. Furthermore the dependence of risk on attained age is almost flat in all three best models, even though the baseline and risk parameters are very different among the models. All best models are well within the range of the 1σ errors, which are depicted for the ERR model only since the uncertainties bounds of the other models are very similar. For the TSCE-direct model, however, a strong drop of ERR\(_{1\sigma}\) with increasing age can be observed. This indicates

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Table 3
Comparison of GI models with no radiation and the three different baseline pathways. Only a radiation effect on \( v_0 \) and \( \sigma_0 \) is relevant for the hemangioma cohort due to early exposure. In the GI path effectively only the combination \( \sigma_{0, \text{base}} \cdot v_{0, \text{GI}} \) can be determined. In the model M33 the number of children modifies \( \beta_{1, GI} \) in addition to \( \beta_1 \).

<table>
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<th>No. parameters</th>
<th>Deviance</th>
<th>AIC</th>
<th>Biologically plausible</th>
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<td>4</td>
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<tr>
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<td>13536.2</td>
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</tr>
</tbody>
</table>

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Fig. 3. Results for the ERR\(_{1\sigma}\) with 1σ errors for the ERR model, the uncertainties of the other models are very similar.
that a standard radiation action, without implementing effects of a potential GI, results in a poor description of the radiation risk. This is also manifest in the deviance which is 8.7 points higher than in the TSCE model with lifelong effects.

In Table 4 the ERR<sub>pd</sub> and EAR<sub>pd</sub> of the models are given for different attained age. All risk estimates and the predicted number of excess cases agree well among the different models. For comparison, the TSCE-direct model predicts 62 excess cases of the total of 877 cases.

All presented models are based on a linear dose–response relationship, either with the accumulated dose (ERR model, TSCE-lifelong) or the dose rate (TSCE-direct, GI). All models were tested for different dose–response relationships (linear-quadratic, power, threshold, step function), but no model was superior to the linear one. In particular, there is no indication of a quadratic dose response.

The analyses of the GI model have been performed with fixed number of stem cells N<sub>s</sub> = 10<sup>8</sup> and fixed α<sub>i</sub> = 12 year<sup>-1</sup>. Using different values of N<sub>s</sub>, e.g. 10<sup>9</sup> or 10<sup>10</sup>, the fit is completely unchanged since it can be absorbed by a corresponding parameter change of v<sub>0</sub> and v<sub>0,GI</sub>. Also different values of the α’s leave the risk invariant. Using e.g. α<sub>i</sub> = 1.0 year<sup>-1</sup> or α<sub>i</sub> = 50.0 year<sup>-1</sup>, there is no change in the risk estimates as long as the β’s change accordingly such that the difference α<sub>i</sub> − β<sub>i</sub> remains the same. A more detailed analysis how a change in the α’s affects the estimates of the other parameters can be found in Ref. [10] for the TSCE model.

It is astonishing that the TSCE model with lifelong effects has a similar good quality of fit as the GI model which has a much richer and more detailed structure. The lifelong effects in the TSCE model were introduced to account for possible GI mechanisms in an effective way. It turned out that the lifelong effects in the initiation rate describe precisely the type of GI model that was selected as our best model, i.e., with an accelerated rate of transition from non-initiated GI cells towards initiated cells. It is not clear if different mechanisms, such as e.g. changes in the parameters β<sub>1,GI</sub> or v<sub>1,GI</sub>, could have been well described in the framework of the TSCE model.

### 3.5. Changes from update of dosimetry system

The radiation risk in the current work increased substantially compared to previous analyses of the hemangioma cohort [5,8,10]. Since this increase is a consequence of the recent update of the dosimetry system (DS) [11], we shortly comment on these changes. The ERR model is used since the other models give very similar results.

For the highly exposed women in the Stockholm cohort the new dose estimates are more than a factor of 4 lower than the older ones. For the women in the Gothenburg cohort and the lower exposed women in the Stockholm cohort there was no significant change.

Since the risk depends strongly on the highly exposed people, the risk estimates are substantially higher with the new DS.

Using the same follow-up, but the old DS, the central risk estimates are ERR<sub>pd</sub> = 0.22 Gy<sup>-1</sup> and EAR<sub>pd</sub> = 5.85 × 10<sup>4</sup> PVR Gy<sup>-1</sup>, about a factor of 2 lower than with the new DS. With the old DS no significant change of ERR<sub>pd</sub> with attained age and no quadratic dose dependence is observed, similar to the results with the new DS. The number of predicted excess cases with the old DS is 55 cases, lower than with the new DS. In addition the fit quality with the old DS is slightly worse with a deviance of 2 points higher.

### 4. Discussion

The Swedish hemangioma cohort is a very important radioepidemiological cohort. A unique property is the very early (first) exposure from birth to the age of 18 months which makes it an ideal cohort for the study of radiation risk after exposure at early childhood. It consists of 17,200 women with 877 breast cancer cases until December 2009, and a mean dose of 182 mGy. Compared to a previous study [10] the follow-up has increased by 5 years and has 199 more breast cancer cases. The re-evaluation of the doses of the most highly exposed part of the Stockholm cohort resulted in increased risk estimates with ERR<sub>pd</sub> = 0.48 Gy<sup>-1</sup> (95 % CI 0.28 : 0.69), about a factor of 2 higher than in previous analyses of the cohort. There was no indication of a change of relative risk with attained age, or a quadratic dose dependence. All different type of models agree well for the risk estimates.

In Ref. [10] it was found that lifelong effects in the mechanistic TSCE model improved significantly the quality of fit, indicating a potential effect of genomic instability. Based on these results the current study extended substantially the mechanistic description, implementing a model with a separate path of GI. It turned out that the evidence for GI, as a consequence of the longer follow-up and more cancer cases, even increased compared to the previous study and was highly significant.

Since the exposure was at infant age, only a radiation effect at an early stage of carcinogenesis, i.e. on the parameters v<sub>0</sub> or α<sub>0</sub>, can be seen in this cohort. A radiation-induced transition on α<sub>0</sub> towards GI was highly significant. Different models of GI were tested. It was found that the parameters for the later stages in the GI path, γ<sub>1,GI</sub> = α<sub>1,GI</sub> − β<sub>1,GI</sub> and v<sub>1,GI</sub>, are relatively similar to the ones in the path without GI, γ<sub>1</sub> and v<sub>1</sub>. The main effect of GI is to increase the rate of transition of a non-initiated cell with GI to an initiated cell that has a proliferative advantage, v<sub>1,GI</sub> ≫ v<sub>0</sub>. The magnitude of this increase cannot be obtained from the epidemiological data, however, since only the combination α<sub>0</sub> · v<sub>0,GI</sub> can be determined. Two arguments have been presented that indicate v<sub>0,GI</sub>/v<sub>0</sub> ≈ 10<sup>−6</sup> as a plausible range. Using a value of v<sub>0,GI</sub>/v<sub>0</sub> = 10<sup>4</sup>, the radiation strength for the transition towards GI is σ<sub>0</sub> = 3.6 × 10<sup>−5</sup> Gy<sup>-1</sup>·d, where d is the dose rate in Gy/year. This number can be directly
translated to the radiation-induced creation of cells with GI: after an exposure of 1 Gy, 3.6 out of 10^2 cells have mutations that make them genomically unstable. Assuming a total of 10^5 stem (or progenitor) cells, 3.6 × 10^8 cells would be genomically unstable. Since these numbers are based on assumptions and cannot be directly obtained from the epidemiological data, they should be confirmed by radiobiological experiments.

For ease of discussion the upper path of the GI model was described as baseline path without GI. This is not necessarily correct. Many spontaneous cancers show genomic instability as one of the hallmarks of cancer. It is possible that GI in the upper path appears at a later stage of carcinogenesis, i.e. in the transition v1 towards a cancer cell, or even later when the cancer cell develops towards a detectable cancer. However, this cannot be inferred from the epidemiological data. Relevant for the analysis is the difference in the time evolution of spontaneous and radiation-induced cancer, manifest in the different transition rates of the two pathways.

The model M33 of Table 3 has an AIC that is only two points higher than the preferred model M12. In this model the spontaneous path propagates through σ1, which indicates a transition towards GI at a later stage. This model has the problem that the parameter σ1 ≈ 0.05 year⁻¹ is relatively large: 5% of all initiated cells per year would become genomically unstable, a very large rate. At the same time σ0 is close to zero, otherwise the spontaneous path would proceed via σ0. Such a large difference in both parameter values towards GI is not likely. Therefore the data do not support that the spontaneous path joins the GI path via σ1. This indicates, at least for exposure at young ages, that radiation-induced cellular changes might activate different molecular pathways than spontaneous changes. If the cellular effects of radiation were similar to the spontaneous ones, the relative risk would show a strong decrease with increasing attained age, as for the TSCE-direct model in Fig. 3.

Whereas the Swedish hemangioma cohort is an excellent cohort for radiation risk of breast cancer after exposure at very young ages, the LSS is the most powerful cohort for more general exposure patterns. In Ref. [4] the LSS cohort with 61,977 women and 1038 breast cancer cases was analyzed for breast cancer incidence with different descriptive ERR and TSCE models. The average person-year weighted age at exposure was 24 years. The best descriptive ERR model found a decrease of ERRpd with attained age, but no indication of a dependence on age at exposure. The best mechanistic TSCE model had a lifelong radiation effect on clonal expansion. In the hemangioma cohort, however, such a potential radiation effect on clonal expansion cannot be seen due to the early exposure. The second best TSCE model for the LSS included a direct radiation effect on the initiation rate, whereas in the hemangioma cohort a lifelong effect on initiation was dominant. The mechanistic differences of both cohorts can be a consequence of the different exposure patterns: for young exposure, lifelong effects on initiation are the ones that remain until later in life. For later exposures as in the LSS, lifelong effects on clonal expansion might become more relevant.

For the risk comparison we use the LSS result of multi-model inference of Ref. [4], which combines the best ERR model and the three best TSCE models. The models are weighted according to the AIC criteria[45,46] with the highest weight (44%) to the ERR model. For the hemangioma cohort the ERR model is used since all models give very similar results. The results are compared for attained age 50, close to the central age of breast cancer for the hemangioma cohort.

For the LSS the excess relative risk depends only very slightly on age at exposure. For age at exposure of 10 and 30 years the risk estimates are ERRpd\(10(50) = 1.9 \text{ Gy}^{-1}\) and ERRpd\(30(50) = 1.8 \text{ Gy}^{-1}\), respectively [4]. These risk estimates are substantially higher than for the hemangioma cohort, ERRpd(50) = 0.48 Gy⁻¹, a finding consistent with previous analyses [5]. Western population incidence rates are about a factor 2–3 higher than in the Japanese population [12], i.e. the lower relative risk of the hemangioma cohort is based on a higher background rate.

The absolute risk values are obtained by multiplying the relative risk with the background rate. In the LSS cohort the background rate has a strong birth year dependence [4]. To get a risk value that represents the average risk of the LSS the mean birth cohort of 1915 is used. Since exposure was in 1945 this formally corresponds to an age at exposure of 30 years, however, in fact it is a birth cohort effect since it affects only the background. For this birth cohort the excess absolute risk of the LSS is \(\text{EAR}_\text{pd}(50) = 8.3 \times 10^3 \text{ Gy}^{-1}\) which is in very good agreement with the result from the hemangioma cohort, \(\text{EAR}_\text{pd}(50) = 10.4 \times 10^3 \text{ Gy}^{-1}\). This suggests that for breast cancer an additive transfer of risk estimates between different populations should be preferred compared to a multiplicative one. This is in agreement with the suggestions from BEIR VII and ICRP committees [13,14].

There are few cohorts with women exposed to radiation in childhood, analyzed for the breast cancer risk, other than the Swedish Hemangioma cohorts. The Rochester infant thymic irradiation cohort with 3312 women (mean age at exposure of 0.2 years) and 34 breast cancer cases was analyzed in the pooled study by Preston et al. [5] together with the Swedish hemangioma cohorts, LSS cohort and four other adult cohorts. For the Rochester thymus cohort the risks were \(\text{ERR}_\text{pd} = 1.56 \times 10^3 \text{ Gy}^{-1}\) and \(\text{EAR}_\text{pd}(50) = 30 \times 10^3 \text{ Gy}^{-1}\). These risk estimates are about three times higher than in the Swedish Hemangioma cohorts, but the risk estimates have wide confidence intervals due to few breast cancer cases.

In the pooled Preston study there was no simple model that adequately described radiation-related excess risk in all studied cohorts. The suggestion was to use a basic common EAR model fitted to the LSS cohort, thymus cohort and two American tuberculosis fluoroscopy cohorts as the basis for breast cancer risk estimation in general populations exposed to acute or fractionated exposures. The risk was \(\text{EAR}_{pd}(50) = 9.9 \times 10^3 \text{ Gy}^{-1}\) for the suggested pooled model, mainly driven by the adult cohorts. This estimate is in good agreement with the EAR estimate in the Swedish hemangioma cohorts presented in this study.

Through the unique personal identification number and the national mandatory population registries in Sweden the follow-up of the hemangioma is very complete. Details of the individual given treatments have been retrieved from well-documented clinical records that all have been kept for research purpose. Most of the treatment cards are illustrated with drawings on how the treatment applicators were placed on the children’s skin. The treatment cards have been used and re-examined for the new updated dosimetry system. One strength of the hemangioma cohort for the analysis is the fact that radiation exposure took place during a short age period when the cohort members were new-borns. This makes it possible to differentiate radiation-induced breast cancer development to the spontaneous one. This strength is also a limitation as no age at exposure effect can be examined. Another limitation of the cohort is that there is no information on medical radiation treatments of the cohort members after the hemangioma treatments.

Although mechanistic models with radiation-induced GI were found to be significant, the results should be interpreted with caution: Such models cannot prove the existence of radiation-induced GI, but can only indicate if the data are consistent or not with the GI hypothesis. The estimated parameters describe an average breast cancer development, however, different subtypes of breast cancer might have different dynamics. Nevertheless, a robust finding of these analyses is the different temporal behavior of radiation-induced breast cancer development compared to the spontaneous one.
5. Conclusions

The Swedish hemangioma cohort was analyzed with different descriptive and mechanistic models. Compared to previous analyses, the risk estimates increased by about a factor of 2. This is a consequence of a re-evaluation of the dosimetry system which led to substantially reduced doses for the most highly exposed part of the Stockholm cohort. Comparing the risk estimates to the LSS cohort, the absolute risk values are in much better agreement than the relative ones. This suggests that for breast cancer an additive transfer of risk estimates between different populations should be preferred compared to a multiplicative one.

A mechanistic model with a separate path of GI, induced by radiation, was found to be highly significant. If radiation acted like spontaneous processes, a strong decrease of relative risk with attained age should have been observed, instead of a constant risk. This indicates, at least for exposure at young ages, that radiation-induced cellular changes might activate different molecular pathways than spontaneous mutations, leading to longer-term processes. It would be important to test such hypotheses with molecular biological measurements from samples of radioepidemiological cohorts.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Properties of preferred GI model

In the preferred model M12 the baseline evolution to cancer proceeds via \( v_0 \rightarrow v_1 \). A transition to the GI path is induced by radiation \( \sigma_0(d) = r \cdot d \), according to Eq. (5) with zero baseline rate \( \sigma_{0,\text{base}} = 0 \). Due to the limited statistical power not all of the free parameters in the GI path \( (v_{0,G1}, v_{1,G1}, \beta_{1,G1}) \) can be fitted simultaneously, however, it is possible to analyze them separately.

In the GI path mutations are accumulated with an accelerated rate. Therefore one or several of the following conditions should be fulfilled: \( v_{0,G1} \gg v_0 \), \( v_{1,G1} \gg v_1 \) or \( \beta_{1,G1} < \beta_1 (\gamma_{1,G1} \gg \gamma_1) \). Each parameter condition is tested separately with the other two parameters fixed: (a) \( \lambda_{0,G1} = v_{0,G1}/v_0 \gg 1 \) with \( v_{1,G1} = v_1 \) and \( \beta_{1,G1} = \beta_1 \); (b) \( \lambda_{1,G1} = v_{1,G1}/v_1 \gg 1 \) with \( v_{0,G1} = v_0 \) and \( \beta_{1,G1} = \beta_1 \); (c) \( \Delta \beta = \beta_{1,G1} - \beta_1 < 0 \) with \( v_{0,G1} = v_0 \) and \( v_{1,G1} = v_1 \). Furthermore a model is tested where \( v_{0,G1} \) and \( v_{1,G1} \) increase simultaneously, (d) \( \lambda_{0,G1} = v_{0,G1}/v_0 \gg 1, \beta_{1,G1} = \beta_1 \). This model implicates that GI enhances \( v_{0,G1} \) and \( v_{1,G1} \) by the same amount relative to baseline.

Before discussing the results of the models it is helpful to give a rough estimate on the biologically plausible range for the radiation parameter \( r \). Using the TSCE model with a direct effect, Eq. (2), or the GI model with a radiation effect on \( v_0 \), the corresponding radiation parameter is about \( 10^{-9} \text{ Gy}^{-1} \). Multiplicated with the dose rate, this results in a probability per cell per year to transform to an initiated cell that has a growth advantage. As shown in Ref. [23], most breast cancers have at least one TSP driver gene mutation. Thus let us assume that (at least) two mutation events are necessary to create an initiated cell, e.g. for the two alleles of a tumor suppressor gene like TP53. To create genomic instability a single event in one of the possible genes that maintain genomic integrity might be sufficient.

The probability for a single hit event should be of the order of magnitude of the square root of the parameter \( r \), and assuming that a couple of genes are the target for disrupting genomic integrity, one gets a rough estimate for \( r \approx 10^{-4} \text{ Gy}^{-1} \). Considering the uncertainties underlying this estimate we allow for a variation of a factor of 100. Thus one can expect the radiation parameter to be in a range of \( 10^{-6} \text{ Gy}^{-1} < r < 10^{-2} \text{ Gy}^{-1} \).

For the four models (a)–(d) the parameters \( \lambda_{0,G1}, \lambda_{1,G1}, \Delta \beta \) or \( \lambda_{0,G1}, \lambda_{1,G1}, \Delta \beta \), that represent the difference between the GI and baseline path, are scanned over a broad range. From a simultaneous fit over the other parameters then the best deviance, the baseline parameters and \( r \) are obtained. It turns out that model (a) with \( \lambda_{0,G1} = v_{0,G1}/v_0 \gg 1 \) gives by far the best quality of fit. Compared to this model, the deviations of models (b) and (c) are higher by about 30–40 points, a very strong difference. Even model (d), that is related to model (a), has a deviance of about 12–40 points higher for biologically plausible values for \( r \). Therefore we now concentrate on the results from model (a).

The main effect of GI in model (a) is to enhance the rate \( v_{0,G1} \) compared to the baseline rate \( v_0 \). The magnitude of enhancement cannot be obtained from the quality of fit, however. Since the clonal expansion rate of the non-initiated cells in the GI path is zero, \( \sigma_{0,G1} = \lambda_{0,G1} \), only the product \( \sigma_0 \cdot v_{0,G1} = r \cdot d \cdot v_{0,G1} \) can be effectively determined, i.e. the combination \( r \cdot v_{0,G1} \). Using e.g. a value of \( \lambda_{0,G1} = 10^4 \), the corresponding value for the radiation action is \( r = (3.64 \pm 0.75) \times 10^{-5} \text{ Gy}^{-1} (1 \sigma \text{ error}) \), well within the estimated plausible range for \( r \). Furthermore, in Ref. [40] it has been estimated, albeit for colon cancer, that the rate of loss of heterozygosity (LOH) in chromosomal instable cells is of order \( 10^9 \) faster than in normal cells. Only a part of the cells with LOH will remain fully functional. If we assume that 10% of these cells will be able to survive and reproduce, we again obtain \( \lambda_{0,G1} = 10^4 \). Although both estimates are based on several assumptions, it is reassuring that they indicate a similar range of values.

Using \( \lambda_{0,G1} = 10^4 \) we now investigate the possible range of values for the other parameters in the GI path, i.e. \( v_{1,G1} \) and \( \beta_{1,G1} \). The best quality of fit is obtained when both parameters are similar to their counterparts in the normal path without GI: \( v_{1,G1} \approx v_1 \) and \( \beta_{1,G1} \approx \beta_1 \). The 95% CI for both parameters is (0.3; 5.3) for \( \lambda_{1,G1} \), and (-0.047; 0.087) year\(^{-1} \) for the difference in the clonal expansion rate \( \Delta \gamma = - \Delta \beta \). The possible range of values for these parameters is therefore quite restricted. These ranges do not change if different values for \( \lambda_{0,G1} \) are used, e.g. \( 10^3 \) or \( 10^5 \).

Table A.5 shows the parameters of the GI model. \( v_0, v_1, \beta_1 \) and \( f_0 \) are the baseline parameters. In addition there is the freedom to choose \( v_{0,G1} \) which also determines the strength of the radiation parameter \( r \).

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