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Role of DNA repair variants and diagnostic radiology exams in differentiated thyroid cancer risk: a pooled analysis of two case-control studies

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Abstract

Background: Given the increased use and diversity of diagnostic procedures, it is important to understand genetic susceptibility to radiation-induced thyroid cancer.

Methods: Based on self-declared diagnostic radiology examination records in addition to existing literature, we estimated the radiation dose delivered to the thyroid gland from diagnostic procedures during childhood and adulthood in two case-control studies conducted in France. A total of 1071 differentiated thyroid cancer (DTC) cases and 1188 controls from the combined studies were genotyped using a custom-made Illumina OncoArray DNA chip. We focused our analysis on variants in genes involved in DNA damage response and repair pathways, representing a total of 5817 single-nucleotide polymorphisms in 571 genes. We estimated the odds ratio per milli-Gray (OR/mGy) of the radiation dose delivered to the thyroid gland using conditional logistic regression. We then used an unconditional logistic regression model to assess the association between DNA repair gene variants and DTC risk. We performed a meta-analysis of the two studies. Results: The OR/mGy was 1.02 (95% CI: 1.00, 1.03). We found significant associations between DTC and rs7164173 in CHD2 ($p = 5.79 \times 10^{-5}$), rs6067822 in NFATc2 ($p = 9.26 \times 10^{-5}$), rs1059394 and rs699517 both in ENOSF1/THYS, rs12702628 in RPA3, and an interaction between rs7068306 in MGMT and thyroid radiation doses ($p = 3.40 \times 10^{-4}$). Conclusions: Our results suggest a role for variants in CDH2, NFATc2, ENOSF1/THYS, RPA3 and MGMT in DTC risk.

Impact: CDH2, NFATc2, ENOSF1/THYS and RPA3 have not previously been shown to be associated with DTC risk.

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1. Introduction

Differentiated thyroid cancer (DTC) is the most common cancer of the endocrine system (1). The registered incidence of DTC has recently been increasing, especially in developed countries mostly due to increased screening (2). Exposure to thyroid ionizing radiation (IR) during childhood was the first risk factor linked with DTC, an association that has been well characterized over several decades (3–8).

In a pooled study performed on doses higher than ≥ 1 Gy (9,10), the excess of DTC relative risk per Gray (ERR/Gy) delivered to the thyroid gland during childhood ranged between 5 and 10. When restricting the pooled analysis to doses lower than 0.2 Gy, however, the ERR/Gy was slightly higher (11). A higher magnitude of excess risk for low dose exposure during childhood was also observed in a cohort study of Japanese atomic bomb survivors (12). Nevertheless, some uncertainties remain about how the recent increase in the use of diagnostic procedures involving irradiation, such as CT (computerized tomography), might affect DTC incidence (13,14).

Despite being more subject to recall bias than cohort studies, case-control studies may be more helpful for improving understanding of thyroid low-dose radiation risk since case-control studies utilize a high number of cases and permit a more detailed investigation of a high number of potential risk cofactors, which are costlier to investigate in cohort studies. Since DTC risk after low radiation dose exposure is also likely modulated by individual susceptibility, investigating the association between DNA variants and DTC risk after low radiation dose could also facilitate an improved understanding of this risk. IR exposure causes DNA damage as well as other physical and chemical biomolecule alterations. DNA double-strand breaks (DSBs) are a characteristic cellular response to IR exposure (15). Exposure to low-dose IR (from levels 50mGy and lower) from X-ray

examinations has been demonstrated to increase the risk of chromosomal translocation (16), suggesting the possibility of long-term adverse health effects.

DSBs trigger the DNA repair system, but the effect of these DSBs varies with cumulative exposures and individual susceptibility. To our knowledge, only one published study has investigated an interaction between genetic background and the radiation-related DTC risk after diagnostic radiation exposure (17). This study identified 24 single-nucleotide polymorphisms (SNPs) with a p-value of variant-RI doses interaction was < 0.05 for all types of DTC cases (papillary and follicular). Currently, few genes including *FOXE1*, *ATM*, and *LIG1* have been associated with DTC risk after IR exposure, but most published studies of these genes were conducted in highly irradiated populations. Moreover, these identified genes were associated with sporadic DTC (18).

Given the increasing use of diagnostic radiology exams and the proliferation of radiology procedure types, it is important to explore the side effects of multiple low-radiation exposures, such as potential effects on DTC development, especially for patients exposed at a young age.

In the current study, we investigated the association between diagnostic low-dose IR exposures and DTC risk. We quantified the doses delivered to the thyroid gland during childhood and adulthood in two French case-control studies. We also investigated the role of genetic variants located in 604 DNA damage signaling and repair genes in modifying the effect of exposure to diagnostic procedure radiation on DTC risk.

2. Materials and methods

2.1. Case-control studies

Our study included cases and controls from two studies conducted in distinct regions from metropolitan France.

The CATHY study (19) was a case-control study conducted in Marne, Ardennes, and Calvados, three French administrative areas covered by a cancer registry. Eligible cases were all DTC patients aged 25 years and older that were diagnosed between 2002 and 2007 and resided in these areas. Controls were recruited by selecting and contacting telephone numbers at random, then frequency-matching the individuals to patients by sex and five-year age groups; controls were randomly selected among healthy individuals residing in the same study areas at the time of the matched cases diagnoses. From the 621 cases and 706 controls initially included in the CATHY study, saliva DNA samples (Oragene® DNA testing kit) were available for 583 cases and 643 controls.

The Young-Thyr study (20) is a case-control study conducted in eastern regions of France: Alsace, Champagne-Ardenne, Corse, Franche-Comté, Lorraine, Rhône-Alpes, and Provence-Alpes-Côte d'Azur. Eligible cases were all DTC patients diagnosed with DTC between January 1st, 2002, and December 31st, 2006 that were younger than 35 years of age with their main residence in a region of eastern France. Controls were selected from the general population and individually matched to a single patient of the same sex, year of birth (within one year), and region of residence during the year when the patient was diagnosed with cancer. From the 805 cases and the 876 controls included in the Young-Thyr study, DNA from saliva (Oragene®) was available for 715 cases and 692 controls.

Participants from both studies answered similar questionnaires that were collaboratively developed. Information on a personal and familial history of thyroid disease, hormonal and reproductive factors, weight, height, dietary habits, tobacco smoking, and medical history was collected during in-person interviews. Participants from both studies gave their informed written consent before being interviewed by trained interviewers. Both

studies were approved by the Ethics Committee and the French National Commission for Data Protection (CNIL).

Clinical and epidemiological data from both studies were harmonized and aggregated in one database. During this process, 30 subjects were excluded because they were duplicates in the two studies.

2.2. Radiologic procedure history

In the questionnaire, participants were asked to report their history of diagnostic radiology examinations by providing the year and the reason for each examination. The exams considered were conventional radiography, CT scans, and nuclear medicine. Examinations linked to investigating thyroid pathology were excluded and were not considered in the dose estimation or any analysis. Examinations carried out during childhood (before the age of 20 years) and adulthood (over 20 years) were analyzed separately. All procedures reported by participants were classified by period (before 1980, 1980-1989, 1990, and after) to take into account the development of radiologic procedure technology over this time.

2.3. Thyroid radiation dose estimates

To estimate the absorbed radiation doses administered to the thyroid gland for different types of diagnostic radiology examinations, we reviewed papers published up to July 2018 that included absorbed dose estimates to the thyroid. From 1970 to 2002, only one study provided estimates of absorbed doses to organs, including the thyroid gland (21), and was used to calculate dose estimates from conventional radiography.

If sex-specific data were available, a mean dose was used. Since the available data about childhood exams were very limited, specific doses were available only for children under the age of two years old without consideration of the period of exposure. Children

older than two years were assumed to receive the same doses as adults. All doses per exam that were equal to or less than 0.01 mGy were considered to be null because of the magnitude of daily natural irradiation in France. Table 1 summarizes the different values used per diagnostic procedure type (conventional radiography, CT scan, nuclear medicine), per period (before 1980, 1980-1989, 1990 and after), and per age at examination (≤ 2 years old, > 2 years).

2.4. Genotyping and quality control

DNA was extracted from saliva samples using a semi-automated salt precipitation method. All subjects were genotyped using the Infinium OncoArray-500K BeadChip (Illumina). This array has been previously described in detail (22) and contains 499,170 SNPs. We incorporated 13,759 additional custom markers based on prior evidence of association in genes involved in relevant biological pathways, such as thyroid function.

The quality control process was applied to the data of each study separately. A threshold of 5% was applied for a missing call rate per SNP and individual. The Hardy-Weinberg equilibrium (HWE) was also assessed per SNP among controls, using an exact test with a p-value threshold at 10^{-5} . Only SNPs with a minor allele frequency (MAF) above 5% were included in the analyses.

The FastPop R package was used to infer intercontinental ancestry with a principal component-derived method (23). Only individuals identified as European were kept in the genetic analyses. A total of 443 cases and 532 controls from CATHY and 629 cases and 656 controls from Young-Thyr were included in the analysis of SNPs.

SNPs of DNA damage signaling and repair genes according to the Gene Ontology database (24,25) were examined. We initially selected exonic and intronic variants in 604

genes. After the quality control steps, a total of 5817 SNPs in 571 genes were retained for the analysis (all genes are listed in the Supplementary Table S1).

2.5. Statistical methods

We used a conditional logistic regression model to estimate the odds ratio of DTC per mGy (OR/mGy) of radiation dose received by the thyroid gland. Because in our study, thyroid doses were very low and cell killing not likely to play signification, and because our goal was not to define the best model for thyroid cancer risk after low dose but rather the best model to investigate the interaction between thyroid radiation and genetic factors, we only investigate models with one parameter for the thyroid radiation dose. In this way, we compared a linear model ($OR = Cst (1 + \alpha \text{ dose})$) to a “constant” model without a radiation dose ($OR = Cst$) and other models with one parameter, such as quadratic ($OR = 1 + \alpha \text{ dose}^2$) and exponential ($OR = 1 + e (\alpha \text{ dose})$), rather to traditional dose-response models used in radiation epidemiology (26). These comparisons were done using Akaike Information Criterion (AIC) (27). Similarly, we investigated the potential role of a dose-response modifier by introducing a term for interaction in the models. The analysis of the radiation dose was performed by using the PECAN module of Epiwin® software. To assess the association between SNPs and DTC risk, odds ratios (ORs) and 95% confidence intervals (95% CIs) were derived from unconditional logistic regression models, assuming a log-additive model since the stratum was not considered in this step. Analyses were first conducted in the Young-Thyr study to filter our SNPs and limit the number of tests to be performed later; only the 100 SNPs with the lowest p-values from this step were tested in the CATHY study. We also conducted a meta-analysis combining results from both studies; overall z-statistics and p-value for these 100 top SNPs were then calculated from a weighted sum of the individual statistics. Weights were proportional to the square root of the number of individuals examined in

each sample and selected such that the squared weights summed to 1.0, as implemented in the METAL program (28). This step aims to improve signal detection in the genetic analysis by avoiding a large number of tests, and to substantiate homologous signals in both studies since our population is relatively limited.

To correct for multiple tests in the main effect analysis, the FDR procedure (29) was calculated for each result of the meta-analysis. We investigated the interaction between SNPs and radiation doses using the likelihood ratio test, comparing models with and without the interaction term following the same steps described above.

All analyses were adjusted for age (five-year period), sex, thyroid cancer family history (yes/no), goiter history (yes/no), radiotherapy history (yes/no), BMI (continuous), height (continuous), educational level (primary school, secondary school, bachelors or higher), smoking status (never/former smoker/smoker), number of pregnancies longer than seven months in women (three classes), and study (CATHY/Young-Thyr) only in the pooled analysis. Analyses of genetic variants were additionally adjusted for the first three principal components to take into account the population stratification. Subgroup analysis was also performed separately for papillary histological type cases and according to tumor size (microcarcinoma and macrocarcinoma).

The log-linear model poses fewer convergence problems than the linear model, for that reason the association and interaction tests between radiation doses and DNA variants were performed using a log-linear model. Since we did not apply any cut-off p-values for SNPs selected to be included in the meta-analysis step, in this study we chose to only show our best five results from each analysis.

Association analyses and meta-analyses were performed using R 3.6 and METAL (28). SNPs were annotated using the VEP tool (30), and gene regions were plotted with LocusZoom software (31).

3. Results

Demographic characteristics of the pooled study population (Young-Thyr and CATHY) are described in Table 2. More than 75% of our population were women and non-smokers. Papillary thyroid cancer type was the most frequent type of thyroid cancer observed, with a mean age at diagnosis younger than 40 years old. However, the mean age at diagnosis was lower in the Young-Thyr study than in the CATHY study, as shown in Supplementary Table S2 (27 years *versus* 51 years).

3.1. Diagnostic radiation and thyroid cancer risk

3.1.1. Thyroid doses determined by diagnostic radiology examination estimates

Estimated IR doses administered to the thyroid during childhood were lower than those given after 20 years of age. Among children, the most frequent exam was panoramic dental radiography, which was reported in 971 individuals. Nuclear medicine exams were rarely reported among children; the most frequent exam scan was bone scans reported in 14 children (Table 3). During adulthood, the most frequent conventional radiography exam was chest radiography, which was seen in 30.8% of adults. Skull CT scan was the most common computed tomography exam during adulthood. Nuclear medicine exams were also relatively rarely reported among adults (Table 3). The median thyroid doses delivered during childhood were lower than doses administered during adulthood. The maximum childhood estimated thyroid dose was 194.2 mGy among the whole population, the mean among cases was 2.8 mGy and 2.0 mGy among controls. A total of 1672 subjects reported not having received any thyroid-irradiating diagnostic procedure during childhood. The estimated maximum dose delivered to the thyroid during adulthood in a

diagnostic radiology procedure was 260.0 mGy, and the mean dose was 5.6 mGy and 5.5 mGy among cases and controls, respectively (Supplementary Tables S3 and S4). A total of 1466 subjects did not report experiencing any thyroid-irradiating diagnostic procedures during adulthood. Detailed information on diagnostic radiology exams and thyroid doses in cases and controls in each study are shown in Supplementary Tables S2, S3, and S4. Characteristics and thyroid doses among genotyped subgroups from both studies are presented in Table 2. The DTC risk in relation to the radiation doses in this population is detailed in Supplementary Table S5.

3.1.2. Differentiated thyroid cancer risk in relation to thyroid radiation doses

Only estimated radiation dose received by the thyroid gland during childhood was found to be associated with an increased DTC risk, unlike doses received during adulthood. In the linear model, the DTC OR per mGy administered to the thyroid was equal to 1.02 (95% CI: 1.00, 1.03). When restricting the analysis to papillary thyroid carcinoma, the OR per mGy was very similar. The fit of the model was improved by the addition of a quadratic term to take into account a possible higher effect of high doses, although not significant ($p = 0.06$). No significant difference ($p > 0.05$) was found between the dose responses for men and for women. No interaction was apparent between the thyroid radiation dose and obese status, age at DTC occurrence, study, number of pregnancies, female sex, smoking status, height, or the personal radiotherapy history. In a log-linear model, the odds ratio for a dose of 1 mGy was 1.01 (95% CI: 1.01, 1.04) (Table 4). Results from a sub-analysis by tumor size, shown in Table 4, yielded similar DTC risk in relation to thyroid IR doses to these from the whole population analysis.

3.2. Differentiated thyroid cancer risk in relation to DNA damage repair and signaling gene variants

Association analysis between DTC risk and the 5817 variants in DNA damage response genes from the Young-Thyr study including all histological types yielded only two SNPs with a p-value lower than 5×10^{-4} : rs4962347 (chromosome 10, *C10orf90*) and rs16983787 (chromosome 10, *SMC6*). None of the top 100 variants with the lowest p-values reached the threshold p-value of 5×10^{-4} in the CATHY study. In the meta-analysis, results of the association test with DTC risk showed a significantly reduced risk for carriers of the minor allele [T]; both SNPs, rs6067822 in *NFATc2* and rs7164173 in *CHD2*, had a p-value of 5.79×10^{-5} and 9.26×10^{-5} respectively, under an additive model of inheritance. Associations with both variants were in the same direction in CATHY and Young-Thyr (Table 5A). Other variants in *NFATc2* present among the top 100 SNPs associated with DTC in the Young-Thyr study are described in Supplementary Figure 1. Rs7164173 was the only SNP in *CHD2* among the top 100 SNPs. Rs105939 and rs699517 in *ENOSF1/THYS* were in our best results, but statistical significance was not achieved ($OR_{\text{meta-analysis}} = 1.30$, $p\text{-value}_{\text{meta-analysis}} = 7.81 \times 10^{-4}$). Only one variant in *RPA3* (rs12702628 in 7p21.3) was found to be significantly associated with DTC risk.

When the analysis was restricted to only papillary carcinoma histological types, only rs7164173 was significant ($p\text{-value}_{\text{meta-analysis}} = 9.33 \times 10^{-5}$) (Table 5B). In the *NFATc2* region, two SNPs, rs77971457 and rs6067822, had respective $OR_{\text{meta-analysis}}$ values of 1.45 and 0.77 but did not reach the significance threshold. Rs1290997 and rs1950764 in *RAD51B* had $OR_{\text{meta-analysis}}$ values of 0.74 and 0.60, respectively (Table 5B). The polymorphism rs1950764 had a $p\text{-value}_{\text{meta-analysis}} > 5 \times 10^{-4}$. Detailed results for SNPs in *RAD51B* are shown in Supplementary Figure 2. Results from the best SNPs in the macrocarcinoma analysis (Table 5C) yielded two SNPs in the *MLK7-AS1* gene

(rs72625242, rs16861406) in 2q31.1 in addition to two other SNPs in chromosome 2 (*PMSD1* and *SMC6*) and the *C10orf90* gene in chromosome 10. In the microcarcinoma analysis, a significant result in 2q31.1 was found (rs75361806, *TLK1*) (Table 5D). The best results from this later analysis also yielded three SNPs in the *MACROD2* gene (20p12.1).

3.3. Differentiated thyroid cancer risk related to the interaction between thyroid doses and variants of DNA damage repair and signaling genes

We investigated the interaction between variants in DNA damage response genes and thyroid exposure to radiation in DTC risk. In the meta-analysis, the SNP-radiation interaction term yielded a p-value_{meta-analysis} = 3.4×10^{-4} and an OR_{interaction} = 0.65 for rs7068306 in *MGMT* (Table 6). A second *MGMT* SNP, rs7087131, was also among the top 100 SNPs identified in the Young-Thyr study. Results on the modifying effect of rs7068306 genotypes on DTC risk according to diagnostic radiation exposure study separately in CATHY and Young-Thyr are presented in Supplementary Table S6.

4. Discussion

Our main objective was to investigate the potential genetic susceptibility to DTC after exposure to low IR doses from diagnostic procedures performed during childhood. In our study, the estimated OR/mGy for thyroid radiation doses received during childhood (OR/mGy = 1.02 (95% CI: 1.00, 1.03)) was similar to the OR/mGy values of the pooled DTC study by Veiga et al. (10) when considering analyses restricted to thyroid radiation doses lower than 100 mGy (OR/mGy = 1.010, (95% CI: 1.004 - 1.018)) or to thyroid radiation doses lower than 200 mGy (OR/mGy = 1.011, (95% CI: 1.007 - 1.02)) (11). Compared to the latest publication on the US radiologic technologist cohort, including 90,305 radiologic technologists in the U.S. who were followed during 1983–1998, the values of our coefficients for thyroid radiation doses received during childhood are slightly

higher than the coefficients estimated in the general analysis and the papillary carcinoma sub-analysis (32). Our estimates are also slightly higher than estimates in both the most recent analysis among Japanese atomic-bomb survivors(12) and the IARC study of the highly contaminated area of Belarus following the Chernobyl nuclear accident (8).

The genetic analyses showed an association between DTC risk and SNPs: rs6067822 in intron 1 of *NFATc2* (20q11.22), rs7164173 in intron 3 of *CHD2* (15q21.3), rs1059394 (3 prime UTR variant), and rs699517 (3 prime UTR variant) both in *ENOSF1/THYS* (18p11.32), and rs12702628 intron variant in *RPA3* (7p21.3). To our knowledge, none of these genes have previously been reported to be associated with DTC risk. The effect direction of these SNPs was similar in the Young-Thyr and CATHY studies and reached the significance threshold for the meta-analysis; furthermore, *NFATc2* and *CHD2* genes have been implicated in the response to ionizing and ultraviolet radiation *in vitro* studies (33,34). Moreover, cells with mutations in *CHD2* were found to be defective in their ability to repair DNA damage, especially after ionizing and ultraviolet radiation exposure (34). Genes of the *NFAT* family encode for transcription factors, which have a role in cancer progression, and also are important for responding to ionizing and ultraviolet radiation exposure (35–37); these genes were previously found to be associated with other types of cancer risk, such as melanoma, breast, colorectal and oral cancers (38–41). Rs6067822 in *NFATc2* and rs7164173 in *CHD2* showed a similar stabilizing effect in general and papillary subgroup analyses. Our results from the papillary thyroid carcinoma sub-analysis yielded a significant association of rs1290997 in *RAD51B* with DTC risk. Another variant in this gene, rs1950764, was among our top five SNPs but did not reach the significance threshold. These findings suggest a role for *RAD51B* in the etiology of radiation-related DTC. Interestingly, the *RAD51B* gene was involved in DNA damage repair after ultraviolet irradiation exposure (42,43) and associated with breast cancer risk

(44,45). We report this result with great caution because 525 SNPs in this gene were tested in the first step of the analysis (Supplementary Table S1).

An association was found between two SNPs (rs1059394 and rs699517) falling within the *ENOSF1/THYS* gene region. These SNPs are non-coding transcript exon variants, and this region is known to be associated with ovarian and endometrial cancers (46,47).

The polymorphism rs12702628 in replication protein A3 gene (*RPA3*), which was associated with DTC risk in our study, has previously been found to be involved in tumorigenesis, especially in gastric cancer (48).

The macrocarcinoma and microcarcinoma analyses both yielded significant results in 2q31.1. However, since these are subgroups results, they should be taken with caution, especially for the microcarcinoma analysis due to the low number of cases.

The minor allele of *MGMT* rs7068306 was found to interact with thyroid IR doses during childhood in reducing the risk of DTC after exposure. The first study to report an association between an *MGMT* variant and radiation-related DTC risk was performed in Belarusian children exposed to radiation (49). The polymorphism rs2296675, associated with DTC risk in the Belarusian population, is located 94 kb from rs7068306 in intron 4 of the *MGMT* region. A correlation coefficient of $r^2 = 0.005$ was found between rs7068306 and rs2296675. In a study of a Caucasian population, Sandler et al. found an interaction between other *MGMT* variants: rs1762444, rs4750763, and rs12219606, and exposure to radiation from diagnostic procedures (17). These SNPs are located in intron 2 and are 244kb, 26kb, and 15kb from rs7068306, respectively. The r^2 values between these three SNPs and rs7068306 are 0.009 and 0.044 (Supplementary Table S7). The r^2 values between rs7068306 and the SNPs tested in studies by Lonjou and Sandler were estimated in a European reference population of 1000 Genomes project (50). Results

from the present study were obtained after estimation and quantification of thyroid doses, based on a combination of self-reported radiologic procedure history and literature doses separately on childhood and adulthood observations. The Sandler et al. study used a different procedure since no doses were taken into account; only categories of diagnostic procedure exposure based on self-reported history were evaluated. Taken together, results from these different studies highlight the role of *MGMT* in modulating the effect of IR on cells of the thyroid.

The expression of *CLIP2*, a gene located on 7q11.22, has been found to be strongly linked with previous radiation exposure in thyroid cancer (51). In our study, only 22 SNPs located on chromosome 7 were tested (Supplementary Table S1), of which, only two were near the *CLIP2* gene region. None of these were among the 100 best SNPs in our study, since the low numbers of concerned SNPs prevented us from confirming any results about locus 7q11.22.

Limitations of the study

Investigating the thyroid radiation dose-response from self-reported radiation examinations carried out in case-control studies is sensitive to recall bias. This is particularly true when addressing public health issues highly debated by the public, such as the health effects of irradiation. As Hallquist and Jansson previously showed, in case-control studies investigating the impact of self-reported diagnostic radiology procedures on DTC risk, this bias produces under-reporting of examinations by both cases and controls, but significantly more by controls; furthermore, this bias could cause an overestimate of the IR effect (52). We cannot exclude such a bias in our study, which could result in an overestimation of the radiation dose effect. However, in the Young-Thyr study, we previously showed that despite DTC patients being more likely than controls to believe that the consequences of the Chernobyl accident were responsible for DTC

occurrences, self-reported vegetable consumption during the two months after the Chernobyl accident, known to be a major source of radioactive contamination, was correlated with the status of participants, cases or controls, but not with their beliefs (53). Thus, exposure could be over-reported in patients, particularly when they believe that this exposure contributed to their disease. In this type of study, case and control self-declarations are often impacted by recall bias, especially in terms of the number of radio diagnostic exams and images in the present study. Despite these caveats, case-control studies could help to improve understanding of the risks associated with low-dose IR radiation by utilizing a large number of cases and permitting more detailed investigations on several potential risk factors, which are costlier to investigate in cohort studies.

For some types of exams, there are no available thyroid dose estimates available in current literature, especially during childhood. This disadvantage could decrease the precision of our estimates; consequently, to harmonize our analyses we considered the same doses for all children older than 2 years. The simplest way to account for age variation of radiation dose exposure (0-2 years compared to older) in our analysis, has probably a limited effect because the reference doses were the same for cases and controls. Thyroid dose estimates were based on self-declarations of diagnostic exam history. In this data, dates for each exam are not precise, causing the impossibility to impose a lagging period and raising the problem of reverse causation. Despite the absence of specific dates for each procedure, we only kept data from radiological procedures that were not carried out for diagnosing thyroid cancer. There is some heterogeneity in demographic and cancer characteristics between our two studies: patients from the Young-Thyr study were younger at cancer diagnosis and inclusion in the study compared to cases from CATHY. Furthermore, diagnostic radiology technologies that were available in childhood differ substantially between the participants

of the CATHY and Young-Thyr studies; additionally, the availability and frequency of these examinations differ (Supplementary Tables S2, S3, and S4). To better discern signals from the genetic analysis and to increase the statistical power, we chose to perform a meta-analysis to substantiate homologous signals in both studies by keeping a limited number of SNPs in this step to avoid a high number of tests.

Despite the small size of our study populations, our results suggest a role for *NFATc2*, *CHD2*, *RPA3*, and *ENOSF1/THYS* that has not previously been associated with DTC risk and confirm the previously demonstrated role of *RAD51B* and *MGMT*. However, functional and validation studies are needed to better understand and confirm the biological role of these variants.

5. Conclusion

We present evidence that DTC risk is significantly increased in higher thyroid radiation doses received during childhood from radio diagnostic examinations. In addition, we demonstrated an interaction between this thyroid radiation dose and the minor allele of rs706830 located in *MGMT*. Our results also suggest an association between polymorphisms in *NFATc2*, *CHD2*, *ENOSF1/THYS*, *RPA3*, and *RAD51B*. Taken together, despite the limited size of both populations studied, these results underline the role of DNA repair pathways in DTC risk. More studies of larger populations are needed to validate and characterize the interaction between DNA repair genes and exposure to low IR doses.

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674

Table 1 References used for thyroid radiation dose estimates per diagnostic procedure type

Exams types	Doses in mGy				References
	Childhood exposure age < 2	Childhood exposure age > 2 years and adulthood exams			
		Before 1980	1980-1989	1990 and after	
Conventional Radiography					
Skull	- [§]	3.6	3.6	0.42	Chang et al. 2017
Dental (panoramic)	- [§]	0.56	0.07	0.02	Chang et al. 2017
Paranasal sinuses	- [§]	0.02	0.04	0.04	Chang et al. 2017
Intravenous urography	0.38*	0.38	0.21	0.21	Inskip et al. 1995
Chest	0.13	0.16	0.31	0.19	Almén and Mattsson 1995 / Chang et al. 2017
Spine	2.8*	2.8	4.5	0.99	Chang et al. 2017
CT Scan					
Skull	3	0.43	0.49	0.64	Lee et al. 2007 / Chang et al. 2017
Chest	5.9	15	20.5	22	Mabille et al. 2008 / Chang et al. 2017
Spine	13.5	17	24	25.5	Mabille et al. 2008 / Chang et al. 2017
Abdomen	0.17	0.28	0.38	0.41	Mabille et al. 2008 / Chang et al. 2017
Nuclear medicine					
Bone scan	1.1*	1.1	0.96	1.1	Chang et al. 2017
Brain scan	- [§]	1.3	3.6	9	Chang et al. 2017
Lung scan (perfusion/ventilation)	- [§]	0.2	0.25	0.25	Chang et al. 2017
Heart scan	- [§]	11	17	12	Chang et al. 2017
Liver scan	- [§]	0.15	0.15	0.15	Chang et al. 2017
Renal scan	- [§]	0.17	0.31	0.17	Chang et al. 2017

§: No exposed subject at this age

*: No available data for children, adult data used

Table 2 Clinical and demographic description of the population included in DTC risk in relation to thyroid radiation doses analysis and genetic analysis

	Whole population		The population included in the genetic analysis	
	Cases (n = 1393)	Controls (n = 1580)	Cases (n = 1072)	Controls (n = 1188)
Gender (%)				
Men	300 (21.5)	398 (25.2)	228 (21.3)	302 (25.4)
Women	1093 (78.5)	1182 (74.8)	844 (78.7)	886 (74.6)
Age at diagnosis/reference year (years)				
Mean	37.58	37.21	37.4 (15.0)	37.6 (15.2)
Median [Min, Max]	32.0 [9.00, 83.0]	32.0 [9.00, 83.0]	32.0 [11.0, 83.0]	32.0 [9.00, 80.0]
Smoking status (%)				
Non-smoker	739 (53.1)	768 (48.6)	232 (52.4)	271 (50.9)
Former smoker	304 (21.8)	306 (19.4)	140 (31.6)	149 (28.0)
Smoker	350 (25.1)	506 (32.0)	71 (16.0)	112 (21.1)
Alcohol consumption (Number of alcohol glasses per week) (%)				
No consumption	778 (55.9)	763 (48.3)	582 (54.3)	559 (47.1)
Less than 10	504 (36.2)	673 (42.6)	403 (37.6)	521 (43.9)
More than 10	110 (7.9)	144 (9.1)	87 (8.1)	108 (9.1)
BMI (Kg/m²)				
Mean (SD)	24.6 (5.21)	24.1 (4.88)	24.7 (5.36)	24.2 (4.89)
Median [Min, Max]	23.5 [11.0, 51.8]	23.1 [13.4, 49.1]	23.6 [11.0, 51.8]	23.1 [13.4, 48.7]
Goitre history (%)				
No	899 (64.5)	1468 (92.9)	695 (64.8)	1102 (92.8)
Yes	494 (35.5)	112 (7.1)	377 (35.2)	86 (7.2)
Thyroid cancer family history (%)				
No	1542 (97.6)	1322 (94.9)	1023 (95.4)	1158 (97.5)
Yes	38 (2.4)	71 (5.1)	49 (4.6)	30 (2.5)
Radiotherapy history (%)				
No	1558 (98.6)	1354 (97.2)	1043 (97.3)	1168 (98.3)
Yes	29 (2.8)	22 (1.3)	19 (1.7)	20 (1.6)
Educational level (%)				
Bachelor's degree or higher	765 (54.9)	982 (62.2)	618 (57.6)	743 (62.5)
Secondary school	418 (30.0)	406 (25.7)	315 (29.4)	311 (26.2)
Primary school level	210 (15.1)	192 (12.2)	139 (13.0)	134 (11.3)
Childhood thyroid radiation doses in mGy (%)				
0	1021 (73.3)	1161 (73.5)	777 (72.5)	876 (73.7)
]0,1]	112 (8.0)	147 (9.3)	89 (8.3)	104 (8.8)
]1,5]	139 (10.0)	150 (9.5)	116 (10.8)	113 (9.5)
]5,10]	25 (1.8)	45 (2.8)	18 (1.7)	36 (3.0)
]10,20]	49 (3.5)	57 (3.6)	35 (3.3)	42 (3.5)
>20	46 (3.3)	20 (1.3)	37 (3.5)	17 (1.4)
Mean (sd)	2.79 (10.3)	1.98 (6.18)	2.89 (11.1)	2.11 (6.62)
Median [Min, Max]	0 [0, 194]	0 [0, 80.9]	0 [0, 194]	0 [0, 80.9]
Adulthood thyroid radiation doses in mGy (%)				
0	669 (48.1)	797 (50.4)	523 (48.8)	591 (49.7)
]0,1]	247 (17.7)	283 (17.9)	187 (17.4)	220 (18.5)
]1,5]	245 (17.6)	262 (16.6)	182 (17.0)	193 (16.2)
]5,10]	59 (4.2)	61 (3.9)	46 (4.3)	46 (3.9)
]10,20]	53 (3.8)	43 (2.7)	44 (4.1)	35 (2.9)
>20	119 (8.5)	134 (8.5)	90 (8.4)	103 (8.7)
Mean (sd)	5.63 (17.3)	5.50 (17.9)	5.28 (16.6)	5.54 (17.2)
Median [Min, Max]	0 [0, 245]	0.02 [0, 262]	0.02 [0, 245]	0.02 [0, 157]
Number of children (%)				
0 (and men)	666 (47.8)	829 (52.5)	514 (47.9)	622 (52.4)
1-3	648 (46.6)	529 (43.8)	542 (50.6)	542 (45.6)
4-6	67 (4.8)	44 (3.4)	10 (0.9)	20 (1.7)
>6	11 (0.4)	4 (0.3)	6 (0.6)	4 (0.3)
Study				
Young-Thyr (%)	805 (57.8)	876 (55.4)	629 (58.7)	656 (55.2)
Cathy (%)	587 (42.2)	704 (44.6)	443 (41.3)	532 (44.8)
Thyroid cancer histology (%)				
Follicular	140 (10.1)		109 (10.2)	
Papillary	1243 (89.2)		955 (89.1)	
Missing	10 (0.7)		8 (0.7)	
Thyroid cancer size in mm (%)				
< 10	431 (31.0)		341 (31.8)	
> 10	957 (68.8)		729 (68.0)	
Missing	4 (0.28)		2 (0.2)	

Table 3 Number of subjects by age and exam type

	Cases n (%) having at least 1 listed exam		Controls n (%) having at least 1 listed exam	
	<20 years	>=20 years	<20 years	>=20 years
Conventional radiography				
Skull	89 (6.4)	93 (6.7)	94 (5.9)	82 (5.2)
Sinus	67 (4.8)	124 (8.9)	77 (4.9)	133 (8.4)
Dental	416 (29.9)	260 (18.7)	555 (35.1)	309 (19.6)
Spine	180 (12.9)	277 (19.9)	199 (12.6)	296 (18.7)
Chest	159 (11.4)	451 (32.4)	198 (12.5)	465 (29.4)
Intravenous urography	27 (1.9)	94 (6.7)	31 (2.0)	74 (4.7)
CT scan				
Abdomen	7 (0.5)	47 (3.4)	2 (0.1)	33 (2.1)
Chest	8 (0.6)	46 (3.3)	0 (0)	33 (2.1)
Skull	53 (3.8)	137 (9.8)	47 (3.0)	117 (7.4)
Spine	31 (2.22)	122 (8.75)	29 (1.83)	160 (10.12)
Nuclear medicine				
Cerebral	1 (0.1)	3 (0.2)	0 (0)	9 (0.6)
Heart	1 (0.1)	2 (0.1)	1 (0.1)	7 (0.4)
Lung	1 (0.1)	3 (0.2)	0 (0)	7 (0.4)
Bones	8 (0.6)	43 (3.1)	6 (0.4)	52 (3.3)
Hepatic	0 (0)	2 (0.1)	0 (0)	2 (0.1)
Renal	4 (0.3)	5 (0.4)	5 (0.3)	13 (0.8)

Table 4: Dose-response analysis for thyroid radiation dose: – Whole population and subgroups

	Whole population: Adjusted analysis				Only micro-carcinomas cases (431 cases and 1580 controls)				Excluding micro-carcinomas cases (962 cases and 1580 controls)			
	Coefficient (95%CI) for 1 mGy	OR ^a (95%CI) at 1 mGy	Deviance	P-value	Coefficient (95%CI) for 1 mGy	OR ^a (95%CI) at 1 mGy	Deviance	P-value	Coefficient (95%CI) for 1 mGy	OR ^a (95%CI) at 1 mGy	Deviance	P-value
No radiation dose-												
			3966.7				2370.9				3029.4	
The radiation dose received during childhood												
Linear	0.017 (0.00060, 0.035)	1.02 (1.00, 1.03)	3959.5	0.007	0.024 (-0.0024, 0.051)	1.02 (1.00, 1.05)	2363.6	0.008	0.015 (-0.0041, 0.035)	1.01 (0.99, 1.04)	3025.2	0.04
Quadratic	0.00053 (-0.000047-0.0011)	1 (1.00, 1.00)	3957.9	0.003	0.00078 (-0.00011, 0.0017)	1 (1.00, 1.00)	2361.8	0.003	0.00048 (-0.00015, 0.00079)	1 (1.00, 1.00)	3024	0.02
Exponential	0.022 (0.0081-0.036)	1.01 (1.01, 1.04)	3058.4	0.004	0.026 (0.010, 0.042)	1.03 (1.01, 1.04)	2362	0.003	0.021 (0.0049-0.037)	1.02 (0.99, 1.05)	3024.6	0.03
The radiation dose received during adulthood												
Linear	8.7 10 ⁻⁵ (-6.0.10 ⁻⁴ , 7.7.10 ⁻⁴)	1 (1.00, 1.00)	3966.7	>0.9	-8.6 10 ⁻⁴ (-5.8.10 ⁻³ , 4.0.10 ⁻³)	1 (0.99, 1.01)	2370.8	> 0.9	-2.7 10 ⁻⁵ (-5.4 10 ⁻³ , 5.3.10 ⁻³)	1 (1.0, 1.00)	3029.4	> 0.9
Quadratic	-9.1 10 ⁻⁶ (-2.610 ⁻⁵ , 7.4 10 ⁻⁶)	1 (1.00, 1.00)	3966.2	>0.5	-6.0 10 ⁻⁶ (-3.1 10 ⁻⁵ , 1.9 10 ⁻⁵)	1 (1.00, 1.00)	2370.7	> 0.9	-1.5 10 ⁻⁵ (-1.5 10 ⁻⁵ , 1.5 10 ⁻⁵)	1 (1.00, 1.00)	3028.5	0.4
Exponential	1.2 10 ⁻⁴ (-0.0089, 0.0091)	1 (0.99, 1.01)	3966.7	>0.9	-1.6 10 ⁻³ (-0.014, 0.011)	1 (0.99, 1.01)	2370.8	> 0.9	-3.4 10 ⁻⁵ (-0.011, 0.011)	0.99 (0.99, 1.01)	3029.4	> 0.9

^a Stratified on age, sex, and study, and adjusted on thyroid cancer family history, radiotherapy history, BMI, height, smoking habits, and number of pregnancies

Table 5 SNPs with the lowest p-values associated with DTC risk in the meta-analysis among all DTC types cases (A), among only papillary DTC cases (B), macrocarcinoma cases (C), and microcarcinoma cases (D)

A/ SNPs with lowest p-values associated with DTC risk in the meta-analysis including all histological types												
SNP	Locus	RA	EA	EAF	Gene	Young-Thyr		CATHY		Meta-analysis		
						OR ^a	P-value	OR ^a	P-value	OR ^a	P-value	FDR p-value
rs12702628	7p21.3	A	G	0.4	RPA3	1.25	1.03 10 ⁻²	1.22	4.37 10 ⁻²	1.24	1.12 10 ⁻⁰³	0.02
rs7164173	15q26.1	G	T	0.1	CHD2	0.7	1.05 10 ⁻³	0.69	1.06 10 ⁻²	0.68	9.26 10 ⁻⁰⁵	0.004
rs1059394	18p11.32	C	T	0.3	ENOSF1/THYS	1.34	2.91 10 ⁻³	1.19	8.97 10 ⁻²	1.3	7.81 10 ⁻⁰⁴	0.01
rs699517	18p11.32	C	T	0.3	ENOSF1/THYS	1.34	2.91 10 ⁻³	1.19	8.97 10 ⁻²	1.3	7.81 10 ⁻⁰⁴	0.01
rs6067822	20q13.2	G	T	0.3	NFATc2	0.64	1.6 10 ⁻⁴	0.7	1.13 10 ⁻⁴	0.64	5.79 10 ⁻⁰⁶	0.005
B/ SNPs with lowest p-values associated with papillary thyroid carcinoma risk in the meta-analysis (563 cases from Young-Thyr and 392 cases from CATHY)												
SNP	Locus	RA	EA	EAF	Gene	Young-Thyr		CATHY		Meta-analysis		
						OR ^a	P-value	OR ^a	P-value	OR ^a	P-value	FDR p-value
rs1950764	14q24.1	G	A	0.2	RAD51B	0.56	2.87 10 ⁻⁰³	0.66	7.27 10 ⁻⁰²	0.6	6.08 10 ⁻⁰⁴	0.01
rs1290997	14q24.1	G	T	0.2	RAD51B	0.77	2.01 10 ⁻⁰³	0.7	7.18 10 ⁻⁰²	0.74	4.36 10 ⁻⁰⁴	0.01
rs7164173	15q26.1	G	T	0.3	CHD2	0.69	3.24 10 ⁻⁰³	0.68	1.02 10 ⁻⁰²	0.68	9.33 10 ⁻⁰⁵	0.009
rs77971457	20q13.2	G	T	0.1	NFATc2	1.44	1.09 10 ⁻⁰³	1.47	1.76 10 ⁻⁰²	1.45	5.06 10 ⁻⁰⁴	0.01
rs6067822	20q13.2	G	T	0.3	NFATc2	0.81	3.47 10 ⁻⁰³	0.72	4.63 10 ⁻⁰²	0.77	5.59 10 ⁻⁰⁴	0.01
C/ SNPs with lowest p-values associated with large thyroid carcinoma (>10mm) risk in the meta-analysis (501 cases from Young-Thyr and 237 cases from CATHY)												
SNP	Locus	RA	EA	EAF	Gene	Young-Thyr		CATHY		Meta-analysis		
						OR ^a	P-value	OR ^a	P-value	OR ^a	P-value	FDR p-value
rs72625242	2q31.1	C	T	0.22	MLK7-AS1	1.927	6.27 10 ⁻⁴	0.9934	1	1.927	6.25 10 ⁻⁴	0.004
rs17619600	2q37.1	T	C	0.15	PMSD1	1.855	1.20 10 ⁻³	1.01	0.9999	1.855	1.20 10 ⁻³	0.004
rs7578070	2p24.2	A	G	0.41	SMC6	1.538	1.86 10 ⁻³	1.03	0.9998	1.538	1.86 10 ⁻³	0.004
rs16861406	2q31.1	A	G	0.21	MLK7-AS1	1.912	2.56 10 ⁻³	1.009	1	1.912	2.56 10 ⁻³	0.005
rs5023821	10q26.2	T	C	0.25	C10orf90	0.6249	1.14 10 ⁻³	1.026	0.9998	0.6249	1.14 10 ⁻³	0.004
D/ SNPs with lowest p-values associated with small thyroid carcinoma (<10mm) risk in the meta-analysis (128 cases from Young-Thyr and 206 cases from CATHY)												
SNP	Locus	RA	EA	EAF	Gene	Young-Thyr		CATHY		Meta-analysis		
						OR ^a	P-value	OR ^a	P-value	OR ^a	P-value	FDR p-value
rs75361806	2q31.1	A	G	0.05	TLK1	25.55	1.12 10 ⁻⁴	1.02	1	25.5494	1.12 10 ⁻⁴	0.03
rs73234091	20p12.1	T	C	0.05	MACROD2	30.89	6.99 10 ⁻⁵	1.007	1	30.8891	6.99 10 ⁻⁵	0.03
rs17757541	18q21.33	C	G	0.06	BCL2	7.188	1.22 10 ⁻²	1.005	1	7.1877	1.12 10 ⁻²	0.01
rs116851051	20p12.1	T	C	0.1	MACROD2	28.39	1.12 10 ⁻²	0.9935	1	28.3897	1.12 10 ⁻²	0.035
rs2327965	20p12.1	A	G	0.05	MACROD2	27.11	1.12 10 ⁻²	1.016	1	27.1096	1.12 10 ⁻²	0.03

RA=Reference allele

EA= Effect allele

EAF= Effect allele frequency

^a: Adjusted on age, sex, childhood IR thyroid doses, thyroid cancer family history, radiotherapy history, goiter history, BMI, height, educational level, smoking status, number of pregnancies, and three first genetic components

Table 6 Five SNPs with the lowest p-values in SNPs - diagnostic radiation thyroid doses interaction term from the meta-analysis

SNP	Locus	Reference allele	Effect allele	EAF	Gene	Young-Thyr		CATHY		Meta-analysis		
						OR ^a	P value	OR ^a	P value	OR	P value	FDR correction
rs10779613	1q41	T	C	0.4	<i>SMYD2</i>	0.60	1.19 10 ⁻⁰³	0.94	6.08 10 ⁻⁰¹	0.80	5.42 10 ⁻⁰³	0.1
rs17514740	7p11.2	G	C	0.4	<i>EGFR</i>	0.71	9.39 10 ⁻⁰³	1.19	1.84 10 ⁻⁰¹	1.30	4.64 10 ⁻⁰³	0.1
rs7068306	10q26.3	C	G	0.3	<i>MGMT</i>	0.63	1.91 10 ⁻⁰³	0.70	5.85 10 ⁻⁰²	0.65	3.40 10 ⁻⁰⁴	0.03
rs10402248	19p13.3	C	T	0.4	<i>MUM1</i>	0.65	4.79 10 ⁻⁰³	0.85	2.54 10 ⁻⁰¹	0.75	4.03 10 ⁻⁰³	0.1
rs6066138	20q13.12	G	A	0.2	<i>EYA2</i>	1.52	9.73 10 ⁻⁰³	1.22	9.01 10 ⁻⁰²	1.32	2.20 10 ⁻⁰³	0.1

EAF= Effect allele frequency

OR = Odds Ratio for the interaction between Effect Allele and radiation dose, considered as a continuous variable in an exponential dose-response model

^a: Adjusted on age, sex, thyroid cancer family history, radiotherapy history, goiter history, BMI, height, educational level, smoking status, number of pregnancies, and three first genetic components

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