Common Variants at 9q22.33, 14q13.3, and \textit{ATM} Loci, and Risk of Differentiated Thyroid Cancer in the French Polynesian Population

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Abstract

Background

French Polynesia has one of the highest incidence rates of thyroid cancer worldwide. Relationships with the atmospheric nuclear weapons tests and other environmental, biological, or behavioral factors have already been reported, but genetic susceptibility has yet to be investigated. We assessed the contribution of polymorphisms at the 9q22.33 and 14q13.3 loci identified by GWAS, and within the DNA repair gene \textit{ATM}, to the risk of differentiated thyroid cancer (DTC) in 177 cases and 275 matched controls from the native population.

Principal Findings

For the GWAS SNP rs965513 near \textit{FOXE1}, an association was found between genotypes G/A and A/A, and risk of DTC. A multiplicative effect of allele A was even noted. An excess risk was also observed in individuals carrying two long alleles of the poly-alanine tract expansion in \textit{FOXE1}, while no association was observed with rs1867277 falling in the promoter region of the gene. In contrast, the GWAS SNP rs944289 (\textit{NKX2-1}) did not show any significant association. Although the missense substitution D1853N (rs1801516) in \textit{ATM} was rare in the population, carriers of the minor allele (A) also showed an excess risk. The relationships between these five polymorphisms and the risk of DTC were not contingent on the body surface area, body mass index, ethnicity or dietary iodine intake. However, an interaction was evidenced between the thyroid radiation dose and rs944289.

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Significance

A clear link could not be established between the high incidence in French Polynesia and the studied polymorphisms, involved in susceptibility to DTC in other populations. Important variation in allele frequencies was observed in the Polynesian population as compared to the European populations. For FOXE1 rs965513, the direction of association and the effect size was similar to that observed in other populations, whereas for ATM rs1801516, the minor allele was associated to an increased risk in the Polynesian population and with a decreased risk in the European population.

Introduction

The incidence of differentiated thyroid cancer (DTC) in French Polynesia, and particularly among women, is one of the highest in the world (37.4 per 100000) [1].

It has been hypothesized that repeated exposure to ionizing radiation may play an important role in the particularly high incidence of DTC in this area. Indeed, a total of 41 atmospheric nuclear weapons tests were carried out above the Mururoa and Fangataufa atolls, between 1966 and 1974 [2]. An increased risk of DTC, predominantly of the papillary type, was already observed among the survivors of the two bombings of Hiroshima and Nagasaki in 1945 [2–5], among persons exposed after the Chernobyl accident [6–9], and also in patients treated with radiotherapy [10–12]. To evaluate the impact of the nuclear weapons tests fallout, we recently conducted a large population-based case-control study in French Polynesia. The external and internal radiation exposure was evaluated for 602 native Polynesians, all under the age of 15 in 1974 [13]. The average dose received to the thyroid gland was very low, but the DTC risk was shown to slightly increase with it [14].

However, nuclear fallouts could explain only a small part of the high incidence. Other risk factors such as a family history of thyroid cancer, menstrual and reproductive factors, weight and body mass index (BMI), body surface area (BSA), dietary iodine intake or dietary consumption have also been investigated in the same study and have shown to modify the risk of DTC [15–20]. Genetic factors may also contribute to DTC susceptibility, but they have never been assessed in this population.

Both NKX2-1 (NK2 homeobox 1, also called TTF1 for Thyroid Transcription Factor 1) and FOXE1 (Forkhead factor E1, also called TTF2 for Thyroid Transcription Factor 2) encode thyroid-specific transcription factors. They play a crucial role in the development of the thyroid gland and their expression is modified in thyroid tumors [21–24]. In 2009, a genome-wide association study (GWAS) reported the contribution of two SNPs nearby NKX2-1 and FOXE1 to the risk of developing DTC in Icelandic and European populations [25]. The first SNP, rs944289, is located 337-kb upstream of the NKX2-1 gene and was described to increase the risk of DTC [25–26]. The second SNP, rs965513, is located in an intergenic region, 57-kb upstream of the FOXE1 gene. It has been related to many endocrine and metabolic disorders [27], to changes in the concentration of thyroid hormones [25], and to an increased risk of DTC [25–26,28]. Rs965513 has been reported as a strong genetic factor for both sporadic and radioinduced papillary thyroid cancer (PTC) [29]. Located within the 5’ untranslated region (UTR) of FOXE1, the SNP rs1867277 has been reported as a risk factor for DTC and has been suggested as the causal SNP [28,30]. It has been also proposed that the minor allele A could modulate a transcriptional regulation pathway of the FOXE1 gene, by recruiting USF1/USF2.
transcription factors [31]. Subsequently, association between the poly-alanine expansion within the transcription factor FOXE1 (rs71369530) was reported and transcriptional analyses have also suggested a functional implication of this multi-allelic polymorphism. Indeed, Carré et al. observed different activities and different relations to the risk of thyroid disorder according to the number of alanine repeats [31]. This variable length polymorphism was found in tight linkage disequilibrium with rs1867277 and may be responsible for the strong association that is often observed between FOXE1 and papillary thyroid carcinoma (PTC) [32]. The ATM (Ataxia-Telangiectasia Mutated) gene is involved in the repair of DNA double-strand breaks. Mutations in this gene have been related to ataxia telangiectasia and other disorders, especially characterized by radiation sensitivity and cancer predisposition [33]. Variations in this gene were reported to play a role in hormone cancers [34–35]. An association between the missense substitution D1853N (rs1801516) and a reduced risk of PTC was also reported for both sporadic and radiation-induced thyroid cancer [36]. Recently, we also showed that this coding SNP in ATM contributes to the risk of PTC in Belarusian children exposed to ionizing radiation from the Chernobyl nuclear power plant accident [37].

We thus sought to investigate the contribution of genetic variations at the NKX2-1, FOXE1 and ATM loci to the risk of DTC in the Polynesian population, which is geographically isolated and where residents were repetitively exposed to ionizing radiation during the nuclear tests.

**Results**

The genotypes distribution conformed to Hardy-Weinberg Equilibrium (HWE) for both cases and controls, for all of the investigated SNPs, except the NKX2-1 SNP rs944289 (Table 1). The Minor Allele Frequency (MAF) in cases and in controls is also given for each SNP in Table 1. A 3-fold increased risk of developing DTC was found for subjects carrying the A/A genotype for the GWAS SNP rs965513 near FOXE1, compared to the G/G genotype (OR = 3.32, p = 0.02, under co-dominant model) (Table 2). For the FOXE1 length polymorphism, a 4-fold increased risk of developing DTC was observed for carriers of the L/L genotype, compared to the S/S genotype (OR = 4.16, p = 0.04, under co-dominant model). No association was found with the other proposed functional SNP located in the promoter region of FOXE1 (rs1867277), unlike reported by others in other populations.

**Table 1. Description of the five studied polymorphisms.**

| Reference | Location | Polymorphism | Minor Allele Frequency | Hardy-Weinberg Equilibrium | Participants (N = 452) |
|-----------|----------|--------------|------------------------|---------------------------|-----------------------|
|           |          | Chromosome   | Allele change          | Residue change            | Cases | Controls | Cases | Controls | p-value |
| rs944289  | Intergenic, 337 kb telomeric of NKX2-1 | 14q13.3 | G>T | - | 0.32 | 0.27 | 0.01 | 0.001 |
| rs965513  | Intergenic, 57 kb upstream to FOXE1 | 9q22.33 | G>A | - | 0.27 | 0.21 | 0.27 | 0.97 |
| rs1867277 | 5'UTR of FOXE1 | 9q22.33 | G>A | - | 0.20 | 0.19 | 0.43 | 0.64 |
| rs71369530 | FOXE1 | 9q22.33 | Poly-alanine tract expansion | - | 0.17 | 0.15 | 0.49 | 0.47 |
| rs1801516 | Missense substitution at codon 1853 of ATM | 11q22-23 | G>A | D[Asp]>N [Asn] | 0.03 | 0.02 | 0.67 | 0.80 |

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The ATM missense substitution D1853N (rs1801516) was much rarer in the Polynesian population than in the European populations (MAF = 0.02). Nonetheless, an excess risk of DTC was detected for carriers of the minor allele (A) (OR = 3.13, p = 0.02).

Finally, no association was found in the Polynesian population for the GWAS SNP rs944289 near NKX2-1.

Compared to controls, the DTC cases had a higher BMI (OR = 2.50, 95%CI = 1.66–3.75, p<0.001) and a higher BSA (OR = 2.70, 95%CI = 1.78–4.08, p<0.001). With regard to

### Table 2. Association results between the five polymorphisms and the risk of developing DTC.

| Genotypes | Genotyped participants | Cases n (%) | Controls n (%) | Crude OR a (95% CI) | p-value | Adjusted OR b (95% CI) | p-value |
|-----------|------------------------|-------------|----------------|---------------------|---------|------------------------|---------|
| rs944289 (near NKX2-1) | n = 168 | n = 262 | | | | |
| C/C | 84 (50) | 149 (56.9) | 1.00 | 1.00 | |
| C/T | 59 (35.1) | 83 (31.7) | 1.27 (0.82–1.95) | 0.9 | 1.33 (0.85–2.08) | 0.9 |
| T/T | 25 (14.9) | 30 (11.4) | 1.50 (0.82–2.73) | 0.3 | 1.66 (0.88–3.12) | 0.2 |
| Risk per T allele c | | | 1.23 (0.94–1.63) | 0.1 | 1.30 (0.97–1.74) | 0.08 |
| C/T+T/T versus C/C d | | | 1.33 (0.89–1.96) | 0.2 | 1.41 (0.93–2.13) | 0.1 |
| T/T versus C/T+C/C e | | | 1.36 (0.76–2.41) | 0.3 | 1.48 (0.81–2.70) | 0.2 |
| rs965513 (near FOXE1) | n = 160 | n = 248 | | | | |
| G/G | 89 (55.6) | 155 (62.5) | 1.00 | 1.00 | |
| G/A | 57 (35.6) | 82 (33.1) | 1.21 (0.79–1.85) | 0.4 | 1.25 (0.80–1.94) | 0.2 |
| A/A | 14 (8.8) | 11 (4.4) | 2.33 (1.01–5.35) | 0.07 | 3.32 (1.34–8.20) | 0.02 |
| Risk per A allele c | | | 1.36 (0.99–1.88) | 0.06 | 1.50 (1.06–2.12) | 0.02 |
| G/A+A/A versus G/G d | | | 1.33 (0.89–2.00) | 0.2 | 1.43 (0.94–2.17) | 0.1 |
| A/A versus G/A+G/G e | | | 2.17 (0.96–4.91) | 0.06 | 3.04 (1.25–7.38) | 0.01 |
| rs1867277 (5'UTR of FOXE1) | n = 137 | n = 222 | | | | |
| G/G | 89 (65.0) | 147 (66.2) | 1.00 | 1.00 | |
| G/A | 41 (29.9) | 66 (29.7) | 1.06 (0.66–1.69) | 0.7 | 1.13 (0.68–1.85) | 0.7 |
| A/A | 7 (5.1) | 9 (4.1) | 1.39 (0.50–3.85) | 0.6 | 1.72 (0.57–5.17) | 0.4 |
| Risk per A allele c | | | 1.11 (0.77–1.61) | 0.6 | 1.20 (0.81–1.79) | 0.4 |
| G/A+A/A versus G/G d | | | 1.10 (0.70–1.71) | 0.7 | 1.19 (0.74–1.91) | 0.5 |
| A/A versus G/A+G/G e | | | 1.37 (0.50–3.76) | 0.6 | 1.66 (0.56–4.94) | 0.4 |
| rs71369530 (length polymorphism in FOXE1) | n = 165 | n = 258 | | | | |
| S/S | 115 (69.7) | 187 (72.5) | 1.00 | 1.00 | |
| S/L | 44 (26.7) | 67 (26.0) | 1.08 (0.69–1.69) | 0.3 | 1.05 (0.66–1.68) | 0.1 |
| L/L | 6 (3.6) | 4 (1.5) | 2.56 (0.71–9.20) | 0.17 | 4.16 (1.07–16.1) | 0.04 |
| Risk per L sequence c | | | 1.23 (0.84–1.79) | 0.29 | 1.29 (0.87–1.92) | 0.2 |
| S/L+L/L versus S/S a,f | | | 1.17 (0.76–1.79) | 0.48 | 1.18 (0.76–1.85) | 0.46 |
| L/L versus S/L+S/S a,f | | | 2.51 (0.70–8.99) | 0.16 | 4.11 (1.07–15.9) | 0.04 |
| rs1801516 (ATM) | n = 175 | n = 270 | | | | |
| G/G | 164 (93.7) | 262 (97.0) | 1.00 | 1.00 | |
| G/A | 11 (6.3) | 8 (3.0) | 2.22 (0.88–5.6) | 0.09 | 3.13 (1.17–8.31) | 0.02 |

a Stratified by age and sex.
b Stratified by age and sex, and adjusted on BMI, BSA ethnicity, and thyroid radiation dose received before age 15 years.
c Multiplicative model of inheritance.
d Dominant model of inheritance (combined heterozygotes and rare homozygotes versus common homozygotes).
e Recessive model of inheritance (rare homozygotes versus combined heterozygotes and common homozygotes).
f S for alleles coding for 12–14 alanines and L for alleles coding for 16–19 alanines.
exposition to nuclear tests, cases did not receive thyroid radiation dose higher than 2.0 mGy before the age of 15 more frequently than controls (OR = 1.24, 95%CI = 0.71–2.17, p = 0.4). The relationship between the five tested polymorphisms and the risk of DTC was not contingent on BMI, BSA, ethnicity or dietary iodine intake (Table 3). Despite the small number of investigated subjects, a significant interaction was evidenced between the thyroid radiation dose and NKX2-1 rs944289 when considering the T/T genotype (p = 0.04). Hence, among subjects who received 2 mGy or less to the thyroid gland during the nuclear tests, the risk of DTC was similar in subjects with the T/T genotype to others (OR = 0.99, 95%CI 0.52–1.88), whereas among subjects who received more than 2 mGy, subjects with the T/T genotype had a much higher risk than others (OR = 6.13, 95%CI 1.21–31.3). Similarly, a thyroid radiation dose >2mGy had no significant effect on subjects with C/C or C/T genotype (OR = 1.12, 95%CI 0.63–2.00) but was associated with an increased risk in the T/T homozygous subjects (OR = 6.94, 95%CI 1.31–37.0).

**Discussion**

In the present work, we assessed the relationship between five putative or recognised genetic susceptibility markers for DTC in French Polynesia, where the thyroid cancer incidence is among the highest worldwide [1]. To our knowledge, this was the first time that such a study on genetic susceptibility to cancer was undertaken in the population of this French overseas territory, which is unique notably because of a repeated exposure to nuclear weapons tests and a relative geographical isolation.

Because of its very low MAF in controls (2%), the power of our study for evidencing an association between ATM SNP rs1801516, and DTC could reach 80% only for an OR of 3.5 or higher. For the other tested SNPs, MAF in controls was about 20% (range 15% to 27%, Table 1), and our study had a power of 80% for evidencing an association if OR is about 1.7. Hence, our study had a sufficient power (80%) for evidencing gene-environment interactions only for a factor of about 3 or higher, in the best situation (environmental factor frequency = 50%, main OR for environmental factor = 2, MAF = 20%, main OR per minor allele = 1.5).

The association we observed between FOXE1 or ATM and DTC risk in the Polynesian population is not surprising given that we focused on polymorphisms that had been previously found to be associated with DTC in others populations in several studies. Nevertheless, we cannot exclude the possibility of false positive results. Indeed, the p-values of our positive results ranged from 0.01 to 0.04, and did not remain significant when correcting for the multiple (n = 5) tests using Bonferroni correction. Unfortunately, our results could not be compared to others obtained in genetically similar populations, because to our knowledge the SNPs we investigated had not been so far addressed in DTC in other pacific islands populations.

Interestingly, we did not replicate the association with the GWAS SNP rs944289 at the NKX2-1 locus on chromosome 14q13.3, as reported by others in Icelandic and European populations, but a significant interaction was observed between the thyroid radiation dose before the age of 15 and the homozygotes for this polymorphism (p = 0.04). However, it should be noted that the distribution of the rs944289 genotypes deviated from HWE in both case and control groups. Therefore, this result on interaction should be interpreted with caution. Deviations from HWE may point to either a sampling bias, mistyping of genotypes, or spurious gene associations because of population stratification. We ruled out a technical genotyping issue because the same HRM probe and protocol were used in other studies on different populations and HWE proportions were respected in them ([37], Lesueur et al., unpublished data). Moreover, we have re-sequenced a subset of the Polynesian samples to confirm genotypes. Another
Table 3. Results of interaction tests between genetic factors and other putative risk factors for DTC.

| Genetic Variants | rs944289 (near NKX2-1) | rs965513 (near FOXE1) | rs1867277 (5'UTR of FOXE1) | rs71369530 (microsatellite in FOXE1) | rs1801516 (ATM) |
|------------------|-------------------------|------------------------|-----------------------------|--------------------------------------|------------------|
|                  | p-interaction           | p-interaction          | p-interaction               | p-interaction                        | p-interaction    |
| C/C              | G/G                     | G/G                    | G/G                         | G/G                                  | G/G              |
| C/T              | G/A                     | A/A                    | S/S                         | L/S                                  | L/L              |
| T/T              |                         |                        |                             |                                      |                  |
|                  | 0.8                     | 0.9                    | 0.7                         | 0.3                                  | 1.0              |
| Ethnicity        |                         |                        |                             |                                      |                  |
| Participants     |                         |                        |                             |                                      |                  |
| with Polynesian   |                         |                        |                             |                                      |                  |
| parents          |                         |                        |                             |                                      |                  |
| Cases            | 72                      | 50                     | 20                          | 75                                   | 35               |
| Controls         | 132                     | 64                     | 26                          | 127                                  | 71               |
| Participants     |                         |                        |                             |                                      |                  |
| with parents of  |                         |                        |                             |                                      |                  |
| mixed origin     |                         |                        |                             |                                      |                  |
| Cases            | 12                      | 9                      | 5                           | 17                                   | 6               |
| Controls         | 17                      | 19                     | 4                           | 28                                   | 11              |
| Body Mass Index  | 0.4                     | 0.9                    | 0.1                         | 0.9                                  | 0.2              |
| (kg/m²)          |                         |                        |                             |                                      |                  |
| > Median in      |                         |                        |                             |                                      |                  |
| genotyped        |                         |                        |                             |                                      |                  |
| controls         | 63                      | 42                     | 14                          | 64                                   | 42              |
| Controls         | 78                      | 39                     | 14                          | 79                                   | 39              |
| < Median in      |                         |                        |                             |                                      |                  |
| genotyped        |                         |                        |                             |                                      |                  |
| controls         | 21                      | 17                     | 11                          | 25                                   | 15              |
| Controls         | 71                      | 44                     | 16                          | 76                                   | 43              |
| Body Surface     | 0.3                     | 0.8                    | 0.3                         | 0.5                                  | 0.2              |
| Area (m²)        |                         |                        |                             |                                      |                  |
| > Median in      |                         |                        |                             |                                      |                  |
| genotyped        |                         |                        |                             |                                      |                  |
| controls         | 64                      | 44                     | 13                          | 65                                   | 42              |
| Controls         | 82                      | 33                     | 16                          | 75                                   | 43              |
| < Median in      |                         |                        |                             |                                      |                  |
| genotyped        |                         |                        |                             |                                      |                  |
| controls         | 20                      | 15                     | 12                          | 24                                   | 15              |
| Controls         | 67                      | 50                     | 14                          | 80                                   | 39              |
| Dietary Iodine   | 0.3                     | 0.8                    | 0.6                         | 0.6                                  | 0.3              |
| Intake (μg/day)  |                         |                        |                             |                                      |                  |
| > Median in      |                         |                        |                             |                                      |                  |
| genotyped        |                         |                        |                             |                                      |                  |
| controls         | 37                      | 28                     | 10                          | 37                                   | 25              |
| Controls         | 80                      | 41                     | 10                          | 79                                   | 36              |
| < Median in      |                         |                        |                             |                                      |                  |
| genotyped        |                         |                        |                             |                                      |                  |
| controls         | 47                      | 31                     | 15                          | 52                                   | 32              |
| Controls         | 69                      | 42                     | 20                          | 76                                   | 43              |
| Thyroid radiation| 0.1                     | 0.9                    | 0.9                         | 0.9                                  | 0.9              |
| dose received    |                         |                        |                             |                                      |                  |
| before age 15    |                         |                        |                             |                                      |                  |
| years (mGy)      |                         |                        |                             |                                      |                  |
| > 2 mGy          | 17                      | 9                      | 8                           | 17                                   | 9               |
| Controls         | 32                      | 9                      | 2                           | 28                                   | 11              |
| ≤ 2 mGy          | 67                      | 50                     | 17                          | 72                                   | 48              |
| Controls         | 117                     | 74                     | 28                          | 127                                  | 71              |

* S for Short alleles (12–14 alanines) and L for Long allele (16–19 alanines).

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possible explanation could be a bias due to the poor quality of some DNA samples. Indeed, in our study, 68% of the DNA samples were prepared from buccal swab brushes and 32% from Oragene kits. DNA samples prepared from Oragene kits are usually less degraded than those prepared from buccal swab brushes. We thus re-analysed the SNP rs944289 considering only individuals with DNA samples prepared from Oragene kits. In this subgroup composed of 145 individuals, the genotype distribution conformed to HWE.

We could also hypothesize that deviation from HWE comes from the existence of sub-groups in the Polynesian population, with high rates of homogamy and consanguinity. Indeed, French Polynesia comprises 118 main islands, of which 76 are inhabited. Despite the recent improvement of airlines, French Polynesians remain relatively isolated from each other and a particular structuring of the population could not be excluded, with putative genetic consequences. However, this deviation was not observed with the others four investigated polymorphisms.

Among all of the successfully genotyped participants of the study, we observed MAFs equal to 0.32 for cases and 0.27 for controls for the SNP rs944289. Compared to the frequency reported in the NCBI dbSNP database (MAFNCBI = 0.43) and to those reported in other studies [25–26], the frequency in Polynesian individuals was clearly lower.

The thyroid specific transcription factor FOXE1 plays a major role in the morphogenesis of the thyroid gland and in the regulation of thyroid differentiated state maintenance [22,38]. DNA sequence variations within the FOXE1 gene have been repeatedly associated with susceptibility to DTC but these variations have been so far mostly studied in Icelandic, European and Japanese populations. In the Polynesian population, the MAF of the GWAS SNP rs965513 (0.21) was similar in controls to the observed MAF in European populations [27], that is intermediate to the MAF observed in the Icelandic population (MAF = 0.34) [26] and the MAF observed in the Japanese population (MAF = 0.057) [27]. In French Polynesia, the A/A genotype was also associated with a significant excess risk of developing DTC and the size effect of this SNP was similar to what was observed elsewhere, confirming that rs965513 represents a robust susceptibility marker for DTC.

The length polymorphism rs71369530 is caused by an expansion of a poly-alanine stretch in the FOXE1 protein with consequences on its activity [31–32]. Although the frequency of short alleles (S) in cases was similar to the frequency found in French cases by Carré et al. (0.17 vs. 0.20), the MAF in controls was almost the half of that of the MAF found in the French controls of the same study (0.15 vs. 0.35) [31]. In the Polynesian population, we found a highly increased DTC risk associated with the L/L genotype. No significant interaction with other DTC risk factors was established here. As the intergenic GWAS SNP rs965513, the length polymorphism rs71369530 within FOXE1 represents a susceptibility marker for DTC and reinforces the hypothesis that FOXE1 is the DTC susceptibility gene at locus 9q22.33.

The SNP rs1867277 in the 5’UTR of FOXE1 has been also proposed as the causal SNP involved in the susceptibility to DTC, because it has been related to a modulation of the transcriptional regulation of the gene [28,30]. Interestingly in our study, no association with DTC was found. This result is quite important because it highlights the value of conducting association studies in different populations when performing fine-mapping to identify causal variants. Indeed, in the former published studies, rs1867277 and rs71369530 appeared to be strongly correlated and it was difficult to disentangle the involvement of the respective polymorphisms. In the Polynesian population, the two polymorphisms were clearly not in strong linkage disequilibrium. In French Polynesia, the MAF of rs1867277 was specific of the Polynesian population (0.19 in controls versus 0.39 in Europeans [28], and 0.40 in Spanish controls [30]).

As for NKX2-1 rs944289 and FOXE1 rs1867277, the MAF of ATM D1853N (rs1801516) differed quite a lot from MAF observed in other populations (MAFCaucasians = 0.19 and MAFPolynesians = 0.02). Notwithstanding, the minor allele (A) was significantly associated
with an excess risk of DTC among Polynesians subjects (OR = 3.13, 95%CI = 1.17–8.31). This result was intriguing since a moderate decline in the risk associated with the A allele (OR = 0.69, 95%CI = 0.45–0.86, p-value = 0.03 in 255 Chernobyl radiation-induced or sporadic PTC cases and 596 controls, all from Caucasian origin [36], as well as in a case-control study sampled from children living in the area contaminated by fallout from the Chernobyl power plant accident [37]. The effect of rs1801516 and its role in the modulation of the ionizing radiations effect have long been debated and the results are still conflicting. An association of the minor allele (A) was found with radio-sensitivity in breast cancer patients [39] and this result was supported by a study showing an increased radio-sensitivity of human fibroblasts carrying allele A [40]. By contrast, a protective role of allele A was found on the adverse side effects of radiotherapy [41].

In conclusion, we confirmed the contribution of FOXE1 and ATM genes in the etiology of DTC in French Polynesia. The relevance of the intergenic SNP rs965513 on chromosome 9q22.33 and the poly-alanine tract polymorphism rs71369530 in FOXE1 as robust susceptibility markers for DTC was evidenced in the present study. The role of the missense substitution D1853N in the DNA repair gene ATM in population exposed to radiation is still under debate, since results in the Polynesian population are not consistent with the previously described associations between the ATM coding SNP and a reduced risk of DTC.

Although the studied Polynesian population showed some genetic particularities, in terms of allele frequency, association or interaction, we did not found any major genetic factor likely to explain the high incidence rate observed in the territory. Previous studies in this population reported the important role of anthropomorphic, environmental and behavioral factors as risk factors for DTC. All of these results suggest a cumulative effect. Moreover, we hypothesize that some of these risk factors may interact, in the manner observed between the thyroid radiation dose and ATM missense substitution D1853N. Finally one cannot exclude that other sequence DNA variation such as epigenetic changes may occur after exposure to some environmental factors such as ionizing radiation that could lead to the development of DTC.

**Subjects, Material and Methods**

**Study population**

The study was conducted on a sub-group of subjects from a population-based case-control study carried out in French Polynesia to assess risk factors of DTC potentially involved in this population [18].

Written informed consent was obtained from all participants. The study was carried out with the written agreement of the Ethical committee of French Polynesia and the French “Commission Nationale de l’Informatique et des Libertés” (CNIL), which had previously approved the contact procedure and the consent form, and included the possibility for contacting their medical doctor. All participants were major (> = 18y) at time of interview. All documents, including consent forms and questionnaires, are conserved in a secured cabinet in the premises of the 1018-INSERM Unit U1018.

Six hundred and two subjects participate in the initial case-control study (characteristics of participants were detailed in Clero et al, 2012 [18] and summarized in Table 4). All subjects were born in and residents of French Polynesia. Cases were diagnosed for thyroid carcinoma between 1979 and 2004. Among them, 177 presented the histology of papillary thyroid carcinoma and 52 the histology of follicular thyroid carcinoma. Controls were healthy individuals, selected from the native population and matched with cases, according the date of birth and gender. The selection of controls was directly performed from the registry of births, covering the whole country. The completeness was possible because of the small size of the local
population (264,736 inhabitants in 2007). It allowed us to contact almost all potential controls, even simply with the name, date or place of residence. Five hundred and twenty participants had two parents of Polynesian origin and 82 of them had one Polynesian parent and one non-Polynesian parent (i.e. of Asiatic, European or other ethnic origin).

All study participants were interviewed. Epidemiological and anthropological information were collected, including height, weight, ethnic origin of parents, and dietary habits. A detailed estimation of radiation doses received at different ages during atmospheric nuclear tests has been performed [13], based on the radioactive fallout data published by France at the end of each year of tests, on $^{131}$I and $^{137}$Cs counts in fresh milk and of total g-activity and $^{137}$Cs in vegetables and fish, and on meteorological data 21 days before and seven days after each test. Estimation of the water level in cisterns before each test, size of the cisterns and information on wind and rain were also used. For each study participant, the total thyroid dose was the sum of

Table 4. Characteristics of the 602 participants of the case-control study, born and resident in French Polynesia, and of the sub-group included in the genetic study.

| Characteristics                          | All (N = 602) | Genotyped participants (N = 452) |
|-----------------------------------------|--------------|----------------------------------|
|                                         | Controls N (%) | Cases N (%)                     | Controls N (%) | Cases N (%)                     |
| Gender                                  |               |                                  |               |                                  |
| Male                                    | 47 (12.6)     | 26 (11.4)                        | 31 (11.3)     | 21 (11.9)                        |
| Female                                  | 326 (87.4)    | 203 (88.6)                       | 244 (88.7)    | 156 (88.1)                       |
| Age at diagnosis (years)                |               |                                  |               |                                  |
| ≤25                                      | 38 (10.2)     | 23 (10.0)                        | 28 (10.2)     | 20 (11.3)                        |
| 25–29                                    | 42 (11.3)     | 24 (10.5)                        | 29 (10.6)     | 17 (09.6)                        |
| 30–34                                    | 46 (12.2)     | 30 (13.1)                        | 36 (13.1)     | 22 (12.4)                        |
| 35–39                                    | 76 (20.4)     | 46 (20.1)                        | 58 (21.1)     | 36 (20.3)                        |
| 40–44                                    | 54 (14.5)     | 32 (14.0)                        | 39 (14.2)     | 26 (14.7)                        |
| 45–49                                    | 53 (14.2)     | 31 (13.5)                        | 38 (13.8)     | 22 (12.4)                        |
| ≥50                                      | 64 (17.2)     | 43 (18.8)                        | 47 (17.0)     | 34 (19.3)                        |
| Histology                                |               |                                  |               |                                  |
| Papillary thyroid carcinoma              |               | 177 (77.3)                       |               | 135 (76.3)                       |
| Follicular thyroid carcinoma             |               | 52 (22.7)                        |               | 42 (23.7)                        |
| Ethnicity                                |               |                                  |               |                                  |
| Participants with Polynesian parents     | 320 (85.8)    | 200 (87.3)                       | 234 (85.1)    | 151 (85.3)                       |
| Participants with parents of mixed origin | 53 (14.2)    | 29 (12.7)                        | 41 (14.9)     | 26 (14.7)                        |
| Body Mass Index (kg/m²)                  |               |                                  |               |                                  |
| > Median in genotyped controls           | 193 (51.7)    | 161 (70.3)                       | 137 (49.8)    | 125 (70.6)                       |
| ≤ Median in genotyped controls          | 179 (48.0)    | 68 (29.7)                        | 138 (50.2)    | 52 (29.4)                        |
| Missing data                             | 1 (0.3)       | 0                                | 0             | 0                                |
| Body Surface Area (m²)                   |               |                                  |               |                                  |
| > Median in genotyped controls           | 194 (52.0)    | 162 (70.7)                       | 138 (50.2)    | 127 (71.8)                       |
| ≤ Median in genotyped controls          | 178 (47.7)    | 67 (29.3)                        | 137 (49.8)    | 50 (28.2)                        |
| Missing data                             | 1 (0.3)       | 0                                | 0             | 0                                |
| Dietary iodine intake (µg/day)           |               |                                  |               |                                  |
| > Median in genotyped controls           | 188 (50.4)    | 101 (44.1)                       | 136 (49.5)    | 78 (44.1)                        |
| ≤ Median in genotyped controls          | 185 (49.6)    | 128 (55.9)                       | 139 (50.5)    | 99 (55.9)                        |
| Missing data                             | 1 (0.3)       | 0                                | 0             | 0                                |
| Thyroid radiation dose received before age 15 years (mGy) | | | | |
| > 2                                      | 70 (18.8)     | 47 (20.5)                        | 47 (17.1)     | 34 (19.2)                        |
| ≤ 2                                      | 303 (81.2)    | 182 (79.5)                       | 228 (82.9)    | 143 (80.8)                       |

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the thyroid doses reconstructed for each test, taking into account age, source of drinking water and data collected in the diet questionnaire. The individual consumption of each foodstuff at a given age was deduced from the information on consumption during childhood obtained in the interview, using age-specific scaling factors. Dose reconstruction was conducted without knowledge of the case or control status of the subject.

Four hundred and fifty two participants (177 cases and 275 controls) gave their consent for biological sampling and genetic analyses. For 307 of them, buccal cells were sampled using Epitcentre Biotechnologies MasterAmp buccal swab brushes (Madison, WI, USA), and for the 145 others, a saliva sample was collected using a DNA Genotek Oragene DNA collection kit (Ottawa, Canada). Genomic DNA (gDNA) was extracted from these samples with a Qiagen Autopure LS (Courtaboeuf, France). The gDNA was then quantified with Life Technologies Picogreen (Saint-Aubin, France).

Genotyping

For SNP rs965516 and rs1867277, 10 ng gDNA were analysed using a specific TaqMan Pre-designed SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Fluorescence readings and data analyses were performed with the ABI PRISM 7900HT Sequence detection system. For rs944289 and rs1801516, 25ng gDNA were analysed using High-Resolution Melting curve (HRM), with a specific probe. Some representative samples were re-sequenced by dye-terminator to confirm the genotype [42]. Fluorescence readings and data analyses were done with the Idaho Technology LightScanner Inc. Hi-Res Melting System.

The length polymorphism rs71369530 in \textit{FOXE1} is due to a variable number of alanine repeats. Thirty nanograms of gDNA was amplified by PCR with fluorescently end-labelled forward primers (5'-FAM or 5'-HEX), by using KAPA 2G Fast HotStart ReadyMix (KAPA Biosystems, Woburn, MA, US) in a 10 μl-final reaction volume (0.5 mM MgCl$_2$, 5%DMSO, 0.25 μM primers). The fluorescently-labelled PCR product was loaded on an ABI 3730 capillary sequencer and analysed as a variable length fragment polymorphism using GenScan size standard ROX-500 as internal size standards. Data were collected and visualised with Genotyper Software v3.7. To determine the number of repeats that corresponds to each allele identified in the genotyping assay, the PCR products from 6 homozygous individuals were Sanger sequenced.

The sequences of all PCR primers, HRM probes, TaqMan probes, and all PCR conditions are available from the authors on request.

The proportion of successfully genotyped DNA samples was 95.1% for rs944289 (near \textit{NKX2-1}), 90.3% for rs965513 (near \textit{FOXE1}), 79.4% for rs1867277 (5'UTR of \textit{FOXE1}), 93.6% for rs71369530 (poly-alanine expansion in \textit{FOXE1}) and 98.5% for rs1801516 (\textit{ATM}), respectively. Raw genotyping data are available in S1 Table.

Statistical analyses

Although this case-control study was matched one, each case being matched to 1 to 2 controls on age and sex, we were not able to use conditional regression analysis because DNA was not collected for all cases and controls and this would conduct to a eliminate several strata because of DNA missing either for the case or for each of the controls. The association between these five polymorphisms and the risk of DTC was assessed using multiple logistic regressions, stratified by age and gender, and assuming co-dominant, dominant, and recessive genetic models of inheritance [43–44]. Crude analyses and analyses adjusted for BMI, BSA, ethnicity, and radiation dose were conducted. Tests for interaction were performed to determine whether the putative associations of SNPs with the risk of developing DTC were modified by parameters such as
BSA, BMI, ethnicity, radiation dose, and dietary iodine intake [43]. All statistical analyses were done with the SAS software, version 9.3 (SAS Institute Inc, NC, USA).

Age distribution of study participants, at time of thyroid cancer diagnosis or corresponding age for controls, was ranged from 10 to 62 and was divided into seven homogeneous classes, which were then used along with gender to stratify the cases and controls for statistical analyses. Two groups were designed for ethnic origin: those individuals with two Polynesian parents and others. For BMI, BSA, and dietary iodine intake, the medians of the distributions among the female genotyped controls (25.7 kg/m², 1.8 m², 132.2 μg/day, respectively) and the medians of the distributions among the male genotyped controls (30.1 kg/m², 2.1 m², 146.9 μg/day, respectively) were used as limits to create two groups among the participants in our study. Finally, the individuals were also grouped into two sets for the radiation dose received to the thyroid before the age of 15, according to whether the dose was under or above the median dose estimated among the genotyped controls (0.37 mGy).

Genotype frequencies and Minor Allele Frequencies (MAFs) were calculated in cases and in controls. They were then evaluated for departure from Hardy-Weinberg Equilibrium using a χ² test (see Table 1). The G/G genotype for rs965513, rs1867277, and rs1801516, and the C/C genotype for rs944289 were considered ancestral, because they were the most frequent genotypes in the Polynesian population. For the FOXE1 poly-alanine stretch polymorphism, the different allelic sizes were encoded in two categories: short and long alleles. The short alleles (S) included the alleles coding for a stretch of 12–14 alanines, while the long alleles (L) comprised those alleles coding for a stretch of 16–19 alanines (Table 5). Because the short alleles were more common in the studied population, the S/S genotype was the pattern selected to be the reference.

Supporting Information
S1 Table. Genotyping data obtained for the 5 tested polymorphisms.
(XLSX)

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Author Contributions

Conceived and designed the experiments: F. Damiola EC F. Doyon CR FL FDV. Performed the experiments: SM F. Damiola MP NR HB VD FDV. Analyzed the data: SM FL FDV. Contributed reagents/materials/analysis tools: FR JLB JS LS FBB PP. Wrote the paper: SM FL CX HB VD FDV.

References

1. IARC (2007) Cancer Incidence in Five Continents, Vol. IX; Curado MP, Edwards B, Shin HR, Storm H, Ferlay J et al., editors. Lyon, France: IARC Scientific Publications. 896 p.

2. Wood JW, Tamagaki H, Neriishi S, Sato T, Sheldon WF, Archer PG, et al. (1969) Thyroid carcinoma in atomic bomb survivors Hiroshima and Nagasaki. Am J Epidemiol 89: 4–14. PMID: 5762817

3. Beebe GW, Kato H (1975) Review of thirty years study of Hiroshima and Nagasaki atomic bomb survivors. II. Biological effects. E. Cancers other than leukaemia. J Radiat Res 16 Suppl: 93–99. PMID: 1195207

4. Boice JD Jr. (2006) Thyroid disease 60 years after Hiroshima and 20 years after Chernobyl. JAMA 295: 1060–1062. PMID: 16507808

5. Hayashi Y, Lagarde F, Tsuda N, Funamoto S, Preston DL, Koyama K, et al. (2010) Papillary microcarcinoma of the thyroid among atomic bomb survivors: tumour characteristics and radiation risk. Cancer 116: 1646–1655. doi: 10.1002/cncr.24872 PMID: 20120034

6. Brenner AV, Tronko MD, Hatch M, Bogdanova TI, Oliynik VA, Lubin JH, et al. (2011) I-131 dose-response for incident thyroid cancers in Ukraine related to the Chornobyl accident. Environ Health Perspect 119: 933–939. doi:10.1289/ehp.1002674 PMID: 21406336

7. Cardis E, Hatch M (2011) The Chernobyl accident—an epidemiological perspective. Clin Oncol (R Coll Radiol) 23: 251–260. doi:10.1016/j.clon.2011.01.510 PMID: 21396807

8. Cardis E, Kesminiene A, Ivanov V, Malakhova I, Shibata N, Preston DL, et al. (2005) Risk of thyroid cancer after exposure to 131I in childhood. J Natl Cancer Inst 97: 724–732. PMID: 15900042

9. Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, et al. (1995) Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiat Res 141: 259–277. PMID: 7871085

10. Garsi JP, Schlumberger M, Ricard M, Labbe M, Ceccarelli C, Schwartz C, et al. (2009) Health outcomes of children fathered by patients treated with radioiodine for thyroid carcinoma. Clin Endocrinol (Oxf) 71: 880–883. doi: 10.1111/j.1365-2265.2009.03561.x PMID: 19250267

11. de Vathaire F (2008) The carcinogenic effects of radioiodine therapy for thyroid carcinoma. Nat Clin Pract Endocrinol Metab 4: 180–181. doi: 10.1038/ncpendmet0761 PMID: 18283326

12. Drozdovitch V, Bouville A, Doyon F, Brindel P, Cardis E, de Vathaire F (2008) Reconstruction of individual radiation doses for a case-control study of thyroid cancer in French Polynesia. Health Phys 94: 418–433. doi:10.1097/01.HRP.0000299293.06218.88 PMID: 18403963

13. Brindel P, Doyon F, Rachedi F, Boissin JL, Sebbag J, et al. (2010) Thyroid cancer following nuclear tests in French Polynesia. Br J Cancer 103: 1115–1121. doi: 10.1038/sj.bjc.6605862 PMID: 2080813

14. Brindel P, Doyon F, Rachedi F, Boissin JL, Sebbag J, et al. (2008) Menstrual and reproductive factors in the risk of differentiated thyroid carcinoma in native women in French Polynesia: a population-based case-control study. Am J Epidemiol 167: 219–229. PMID: 17965111

15. Brindel P, Doyon F, Rachedi F, Boissin JL, Sebbag J, Shan L, et al. (2008) Menstrual and reproductive factors in the risk of differentiated thyroid carcinoma in native women in French Polynesia: a population-based case-control study. Am J Epidemiol 167: 219–229. PMID: 17965111

16. Brindel P, Doyon F, Bourgain C, Rachedi F, Boissin JL, Sebbag J, et al. (2010) Family history of thyroid cancer and the risk of differentiated thyroid cancer in French Polynesia. Thyroid 20: 393–400. doi: 10.1089/thy.2009.0356 PMID: 20373983

17. Brindel P, Doyon F, Rachedi F, Boissin JL, Sebbag J, Shan L, et al. (2009) Anthropometric factors in differentiated thyroid cancer in French Polynesia: a case-control study. Cancer Causes Control 20: 581–590. doi: 10.1007/s10552-008-9266-y PMID: 19043789

18. Ciero E, Doyon F, Chungue V, Rachedi F, Boissin JL, Sebbag J, et al. (2012) Dietary Iodine and Thyroid Cancer Risk in French Polynesia: A Case-Control Study. Thyroid 22: 422–429. doi: 10.1089/thy.2011.0173 PMID: 22280227
19. Clero E, Leux C, Brindel P, Truong T, Anger A, Teinturier C, et al. (2010) Pooled analysis of two case-control studies in New Caledonia and French Polynesia of body mass index and differentiated thyroid cancer: the importance of body surface area. Thyroid 20: 1285–1293. doi: 10.1089/thy.2009.0456 PubMed: 20932181

20. Clero E, Doyon F, Chunqve V, Rachedi F, Boissin JL, Sebbag J, et al. (2012) Dietary patterns, goitrogenic food, and thyroid cancer: a case-control study in French Polynesia. Nutr Cancer 64: 929–936. doi: 10.1080/01635581.2012.713538 PubMed: 23061901

21. Parlati R, Rosica A, Rodriguez-Mallon A, Affuso A, Postiglione MP, Arra C, et al. (2004) An integrated regulatory network controlling survival and migration in thyroid organogenesis. Dev Biol 276: 464–475. PubMed: 15581879

22. Zanini M, Avantaggiato V, Biffali E, Arnone MI, Sato K, Pischetola M, et al. (1997) TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is consistent with a role in controlling the onset of differentiation. EMBO J 16: 3185–3197. PubMed: 9214635

23. Zhang P, Zuo H, Nakamura Y, Nakamura M, Wakasa T, Kakudo K (2006) Immunohistochemical analysis of thyroid-specific transcription factors in thyroid tumours. Pathol Int 56: 240–245. PubMed: 16669872

24. Perna MG, Civitareale D, De Filippis V, Sacco M, Cisternino C, Tassi V (1997) Absence of mutations in the gene encoding thyroid transcription factor-1 (TTF-1) in patients with thyroid dysgenesis. Thyroid 7: 377–381. PubMed: 9226206

25. Gudmundsson J, Sulem P, Guddbjartsson DF, Jonasson JG, Gudbjartsson DF, Jonsdottir I, et al. (2009) Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. Nat Genet 41: 460–464. doi: 10.1038/ng.339 PubMed: 19196613

26. Matsuse M, Takahashi M, Mitsutake N, Nishihara E, Hirokawa M, Kagawuchi T, et al. (2011) The FOXE1 and NKX2-1 loci are associated with susceptibility to papillary thyroid carcinoma in the Japanese population. J Med Genet 48: 645–648. doi: 10.1136/jmedgenet-2011-100063 PubMed: 21730105

27. Denny JC, Crawford DC, Ritchie MD, Bielinski SJ, Basford MA, Bradford Y, et al. (2011) Variants near the gene encoding thyroid transcription factor-1 (TTF-1) are associated with thyroid dysfunction: findings from the METASTABLE study. J Hum Genet 89: 529–542. doi: 10.1016/j.jhg.2011.09.008 PubMed: 21981779

28. Jones AM, Howarth KM, Martin L, Gorman M, Mihai R, Moss L, et al. (2012) Thyroid cancer susceptibility polymorphisms: confirmation of loci on chromosomes 9q22 and 14q13, validation of a recessive 9q24 locus and failure to replicate a locus on 5q22. J Med Genet 49: 158–163. doi: 10.1136/jmedgenet-2011-100586 PubMed: 22282540

29. Takahashi M, Saenko VA, Rogounovitch TI, Kagawuchi T, Drozd VM, Takigawa-Imamura H, et al. (2010) The FOXE1 locus is a major genetic determinant for radiation-related thyroid carcinoma in Chernobyl. Hum Mol Genet 19: 2516–2523. doi: 10.1093/hmg/ddq123 PubMed: 20950937

30. Landa I, Ruiz-Llorente S, Montero-Conde C, Inglada-Perez L, Sura-Trueba S, Szinnai G, et al. (2009) The variant rs1867277 in FOXE1 gene confers thyroid cancer susceptibility through the recruitment of USF1/USF2 transcription factors. PLoS Genet 5: e1000637. doi: 10.1371/journal.pgen.1000637 PubMed: 19730683

31. Carre A, Castanet M, Sura-Trueba S, Szinnai G, Van Vliet G, Tocchet D, et al. (2007) Polymorphic length of FOXE1 alanine stretch: evidence for genetic susceptibility to thyroid dysgenesis. Hum Genet 122: 467–476. PubMed: 17717707

32. Bullock M, Duncan EL, O’Neil C, Tacon L, Sywak M, Sidhu S, et al. (2012) Association of FOXE1 Polymorphisms with Papillary Thyroid Cancer. J Clin Endocrinol Metab 97: E1814. doi: 10.1210/jc.2012-1456 PubMed: 22736773

33. Canman CE, Lim DS (1998) The role of ATM in DNA damage responses and cancer. Oncogene 17: 3301–3308. PMID: 9916992

34. Schrauder M, Frank S, Strissel PL, Lux MP, Bani MR, Rauh C, et al. (2008) Single nucleotide polymorphism D18S53 of the ATM gene may alter the risk for breast cancer. J Cancer Res Clin Oncol 134: 339–342. doi: 10.1007/s00432-007-0355-9 PubMed: 18264724

35. Barrett JH, Iles MM, Harland M, Taylor JC, Atkin JF, Andresen PA, et al. (2011) Genome-wide association study identifies three new melanoma susceptibility loci. Nat Genet 43: 1108–1113. doi: 10.1038/ng.955 PubMed: 21983787

36. Akulevich NM, Saenko VA, Rogounovitch TI, Drozd VM, Lushnikov EF, Ivanov VK, et al. (2009) Polymorphisms of DNA damage response genes in radiation-related and sporadic papillary thyroid carcinoma. Endocr Relat Cancer 16: 491–503. doi: 10.1677/ERC-08-0336 PubMed: 19286843

37. Damiola F, Byrnes G, Moissonnier M, Pertesi M, Deltour I, Fillon A, et al. (2013) Contribution of ATM and FOXE1 (TTF2) to risk of papillary thyroid carcinoma in Belarusian children exposed to radiation. Int J Cancer.
38. Fagman H, Nilsson M (2011) Morphogenetics of early thyroid development. J Mol Endocrinol 46: R33–42. PMID: 21322126

39. Angele S, Romestaing P, Moullan N, Vuillaume M, Chapot B, Friesen M, et al. (2003) ATM haplotypes and cellular response to DNA damage: association with breast cancer risk and clinical radiosensitivity. Cancer Res 63: 8717–8725. PMID: 14695186

40. Alsbeih G, El-Sebaie M, Al-Harbi N, Al-Buhairi M, Al-Hadyan K, Al-Rajhi N (2007) Radiosensitivity of human fibroblasts is associated with amino acid substitution variants in susceptible genes and correlates with the number of risk alleles. Int J Radiat Oncol Biol Phys 68: 229–235. PMID: 17331670

41. Edvardsen H, Tefre T, Jansen L, Vu P, Haffty BG, Fossa SD, et al. (2007) Linkage disequilibrium pattern of the ATM gene in breast cancer patients and controls; association of SNPs and haplotypes to radio-sensitivity and post-lumpectomy local recurrence. Radiat Oncol 2: 25. PMID: 17623063

42. Nguyen-Dumont T, Calvez-Kelm FL, Forey N, McKay-Chopin S, Garritano S, Gioia-Patricola L, et al. (2009) Description and validation of high-throughput simultaneous genotyping and mutation scanning by high-resolution melting curve analysis. Hum Mutat 30: 884–890. doi: 10.1002/humu.20949 PMID: 19347964

43. Breslow NE, Day NE (1980) Statistical methods in cancer research. Volume I—The analysis of case-control studies. IARC Sci Publ: 5–338.

44. Lewis CM (2002) Genetic association studies: design, analysis and interpretation. Brief Bioinform 3: 146–153. PMID: 12139434