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**Non-malignant formalin-fixed paraffin embedded (FFPE) tissues as a source to study germline variants and cancer predisposition: A systematic review**

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**Running title:** Germline variants in non-malignant FFPE tissues

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# 1 **Abstract**

2 **Background:** Archived formalin-fixed paraffin-embedded (FFPE) specimens from non-malignant tissues  
3 derived from cancer patients are a vast and potentially valuable resource for high-quality genotyping analyses  
4 and could have a role in establishing inherited cancer risk.

5 **Method:** We systematically searched PubMed, Ovid Medline, and Scopus databases for all articles that  
6 compared genotyping performance of DNA from non-malignant FFPE tissue with blood DNA derived from  
7 cancer patients irrespective of tumor type. Two independent researchers screened the retrieved studies,  
8 removed duplicates, excluded irrelevant studies, and extracted genotyping data from the eligible studies. .  
9 These studies included, but were not limited to, genotyping technique, reported call rate, and concordance.

10 **Results:** A total of 13 studies were reviewed, in which DNA from non-malignant FFPE tissues derived from  
11 cancer patients was successfully purified and genotyped. All of these studies used different approaches for  
12 genotyping of DNA from non-malignant FFPE tissues to amplify single-nucleotide polymorphisms (SNPs)  
13 and to estimate of loss of heterozygosity (LOH). The concordance between genotypes from non-malignant  
14 FFPE tissues and blood derived from cancer patients was observed to be high, whereas the call rate of the  
15 tested SNPs was not reported in all included studies.

16 **Conclusion:** This review illustrates that DNA from non-malignant FFPE tissues derived from cancer patients  
17 can serve as an alternative and reliable source for assessment of germline DNA for various purposes, including  
18 assessment of cancer predisposition.

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# 1 **Introduction**

2 Polymorphisms in germline DNA have a crucial role in cancer predisposition by acting as oncogenic modifiers  
3 or co-oncogenes that determine the needed complementary subsequent somatic changes for full malignant  
4 transformation <sup>1</sup>. Numerous germline single-nucleotide polymorphisms (SNPs) have been identified as  
5 predisposing to cancer in several genes, including tumor suppressor genes (e.g., *TP53*, *RB*) and genes involved  
6 in DNA repair (e.g., *BRCA1*, *MLH1*), cell proliferation (e.g., *PTEN*, *STK11*), and cell adhesion (e.g., *CDH1*,  
7 *APC*) <sup>2,3</sup>. The usual method for studying inherited cancer risks relies on DNA extracted from blood, saliva, or  
8 oral scrapings. However, collection of prospective cohorts of sufficient size, linked with follow-up  
9 information, requires major investments and typically decades of work. As an alternative or additional source  
10 for these studies, the existing non-malignant formalin-fixed paraffin-embedded (FFPE) samples (normal or  
11 benign) in the pathology archives or biobanks can provide easily accessible material for SNP genotyping and  
12 estimation of polygenic cancer risk <sup>4-6</sup>.

13 The greatest challenge with FFPE DNA analysis is caused by the use of formaldehyde in the fixation process.  
14 Formaldehyde leads to formation of crosslinks between DNA and proteins, which causes DNA fragmentation  
15 and subsequent problems in purification and amplification of FFPE DNA <sup>7,8</sup>. Extraction of DNA from FFPE  
16 specimens has two major technical difficulties, namely dissolving of paraffin and removal of crosslinks <sup>9</sup>. The  
17 feasibility of using DNA extracted from FFPE tissues in various advanced molecular techniques has been  
18 tested. These techniques include whole genome sequencing and high-quality genotyping <sup>10,11</sup>.

19 Although previous studies have examined the genotyping performance of fresh-frozen (FF) tumor tissues and  
20 compared it with matched wild blood genotypes, this approach is still unreliable due to accumulation of  
21 somatic mutations in the tumors <sup>12,13</sup>. In addition, these somatic alterations may appear as genotyping errors in  
22 individual SNPs when comparing tumor DNA with normal DNA <sup>14,15</sup>. While an alternative could be matched  
23 non-malignant FFPE tissue derived from cancer patients, few reports have utilized this source. By comparing  
24 SNP genotypes generated from non-malignant FFPE tissue and blood DNA derived from cancer patients in  
25 large cohorts, it could be possible to evaluate whether non-malignant FFPE tissue is a viable source of DNA  
26 for large-scale genome-wide association studies (GWAS) that aim to identify genetic factors contributing to  
27 cancer risk <sup>16,17</sup>.

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3 Implementation of GWAS is primarily based on blood samples. In breast cancer patients, GWAS identified  
4 multiple sets of variants with increased susceptibility to cancer, such as prediction of estrogen receptor (ER)-  
5 specific disease. This can facilitate stratification of patients with improved detection and management  
6 strategies <sup>18</sup>. Additionally, combining several genome risk variants may have enhanced predicative ability in  
7 breast cancer <sup>19,20</sup>. Similarly, in lung cancer patients, GWAS-derived polygenic risk scores (PRS) effectively  
8 identified groups with high risk <sup>21</sup>. While PRS evaluation may help to guide preventive measures for patients  
9 with significant cancer risk, more studies with large cohorts are needed. An interesting option for cohort  
10 generation is the use of archived pathology collections, which include large numbers of samples from cancer  
11 patients.

12 We conducted a systematic review of studies that evaluated the performance of the genotyped SNPs in non-  
13 malignant FFPE tissues and matched blood samples derived from cancer patients with different malignancies  
14 along with the observed concordance. The main objective was to confirm whether non-malignant FFPE tissue  
15 derived from cancer patients could be used in the future as a source for polygenic risk assessment.

## 16 **Methods**

### 17 **Search protocol and data sources**

18 We systematically searched for all studies that evaluated genotype concordance between non-malignant FFPE  
19 tissue and blood derived from cancer patients. The systematic search included the PubMed, Ovid Medline, and  
20 Scopus databases from their inception until 17 January 2020. The search included terms ('genotyping' OR  
21 'SNP') AND ('concordance') AND ('formalin fixed paraffin embedded' OR 'FFPE') AND ('blood') AND  
22 ('cancer' OR 'neoplasm' OR 'malignant'). Some search terms were replaced with their synonyms and  
23 abbreviations and the searches were repeated. References of the eligible studies were searched manually to  
24 enhance the inclusion of all relevant studies. The Preferred Reporting Items for Systematic Review and Meta-  
25 Analysis (PRISMA) guidelines were followed <sup>22</sup>.

### 26 **Inclusion and exclusion criteria**

1 All original studies that evaluated the genotype concordance between the non-malignant FFPE tissue and blood  
2 derived from cancer patients were included. The search included only articles published in the English  
3 language. We excluded studies that investigated genotyping of malignant FFPE tissues. Additionally, review  
4 articles, case reports, case series, conference abstracts, editorials, letters to the editor, and commentaries were  
5 excluded.

## 6 **Screening and data extraction**

7 Two independent researchers (OY, AA) screened the systematically retrieved literature at all stages to identify  
8 eligible studies. Disagreements between the two researchers were resolved by discussion to reach a consensus  
9 upon which final studies were included. Extracted data included the following basic information: name of the  
10 first author, publication year, tumor type, and number of matched non-malignant FFPE tissue and blood  
11 samples derived from cancer patients from all eligible studies. Data regarding evaluation of genotyping  
12 included the platform used for both DNA purification and genotyping, sample quality control measures, and  
13 percentage of concordance between non-malignant FFPE tissue and blood genotyping results.

## 14 **Quality assessment**

15 The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system of rating  
16 quality of evidence was used to assess the quality of the eligible studies<sup>23</sup>. The quality of evidence ranged  
17 from high to very low and the factors considered to determine the quality of evidence are summarized in Table  
18 1.

## 19 **Results and Discussion**

20 A total of 152 hits were retrieved, 30 of which were duplicates and 111 were irrelevant studies. Eleven studies  
21 evaluated the genotype concordance between non-malignant FFPE tissue and matched blood samples derived  
22 from cancer patients with various malignancies (Fig. 1). Two additional studies were added manually from  
23 references of the relevant studies; thus, altogether 13 eligible studies were reviewed. The reviewed studies  
24 reported the feasibility of using archived FFPE tissue samples from benign or non-malignant tissues derived  
25 from cancer patients for DNA purification and genotyping approaches using different platforms. In addition,  
26 some of the reviewed studies reported the genotyping call rate and assessed the degree of concordance between  
27 the generated genotypes from the non-malignant FFPE tissue and matched blood samples (Table 2).

1 The eligible studies included patients with the following malignancies: three studies on breast cancer <sup>24-26</sup>, one  
2 study on breast and ovarian cancer <sup>27</sup>, three studies on colorectal cancer <sup>15,28,29</sup>, two studies on osteosarcoma  
3 <sup>30,31</sup>, two studies on prostate carcinoma <sup>32,4</sup>, one study on hepatocellular carcinoma <sup>33</sup>. Additionally, we included  
4 one computational genotyping study that included SNP data from The Cancer Genome Atlas (TCGA) from 12  
5 major types of malignancies (breast adenocarcinoma, gastric adenocarcinoma, colon adenocarcinoma, rectal  
6 adenocarcinoma, pancreatic adenocarcinoma, hepatocellular carcinoma, lung adenocarcinoma, lung squamous  
7 cell carcinoma, prostate adenocarcinoma, ovarian cystadenocarcinoma, head and neck squamous cell  
8 carcinoma, and melanoma) <sup>34</sup>.

9 The number of tested SNPs in the included studies is highly variable, ranging from less than ten SNPs to  
10 hundreds or thousands of tested SNPs according to the genotyping technique used. The tested number of SNPs  
11 was reported to be high with the use of genotyping arrays. The included studies can be classified into small-  
12 scale studies (<10 SNPs) and large-scale studies (>200 SNP) according to the number of tested SNPs.

13 The small-scale studies group included seven studies. By focusing on cancer type, a study by Xie et al analyzed  
14 five variants in genes typically showing LOH in breast cancer (*MTHFR*, *hOGG1*, *DBH*, *DRD2*, *NQO1*) and  
15 reported 100% concordance between non-malignant FFPE tissue and blood derived from breast cancer patients  
16 <sup>24</sup>. Due to the crucial role of the *CYP2D6* enzyme in anti-estrogen hormonal therapy, Rae and colleagues <sup>25</sup>  
17 investigated *CYP2D6* genotypes in FFPE lymph nodes and compared them to blood-derived genotypes and  
18 revealed a concordance of 97.4%. In hereditary breast cancer, a 100% genotype concordance rate was seen  
19 between non-malignant FFPE tissue and blood when investigating the *BRCA1* and *BRCA2* founder mutations  
20 <sup>27</sup>.

21 The results were controversial in colorectal cancer (CRC). When performing LOH amplification assays in  
22 CRC, the data were not always consistent. On one hand, the concordance in the allelic ratio between normal  
23 colonic mucosa and matched blood using PCR was above 85% in 64% of the samples <sup>29</sup>. The authors  
24 recommended normalization of tumor allele ratios with matched normal tissue samples <sup>29</sup>. On the other hand,  
25 a study performed by Marisi et al reported that at the specific marker *VEGF* -1154 G>A the concordance  
26 between non-malignant FFPE and blood derived from CRC was 57% (4 out of 7 samples) <sup>15</sup>. Marisi et al added  
27 that the concordance was much higher (up to 100%) at other *VEGF* markers; (-2578C>A, -1498C>T,  
28 -1154G>A, -634C>G, +936C>T) and *eNOS* markers (+894G>T, -786T>C, VNTR [variable number of

1 tandem repeats] 27bp intron 4) when comparing malignant FFPE tissue with matched blood-derived DNA <sup>15</sup>.  
2 Although the authors could not conclude whether the loss of guanine in *VEGF* -1154 G>A was due to a FFPE  
3 fixation effect or real tumor alterations, they recommended special precautions when analyzing SNPs with C  
4 or G alleles along with optimization of FFPE DNA extraction and genotyping methods <sup>15</sup>.

5 Importantly, C-T or G-A transitions are common events that may occur due to the formalin fixation procedure  
6 to FFPE and can produce sequence artifacts in different amplification procedures such as PCR <sup>35,36</sup>. However,  
7 use of uracil DNA glycosylase (UDG), an enzyme involved in base excision repair greatly enhanced the quality  
8 of purified FFPE DNA with regard to DNA integrity and fragment length <sup>37</sup>. Pretreatment of FFPE tumor  
9 tissues with UDG reduced the amount of artifactual variants when applying next-generation sequencing and  
10 eliminated variants with low allele frequencies <sup>38</sup>.

11 A study on osteosarcoma tested two SNPs in the drug transporter gene *MDR1* and showed a 90% success rate  
12 in genotyped FFPE normal specimens using a PCR TaqMan-based approach <sup>30</sup>. The last study in the group of  
13 small-scale studies was performed on hepatocellular carcinoma (HCC), where the authors performed allele-  
14 specific amplification and TaqMan assays before and after whole-genome amplification. Genotypes were  
15 considered successful when the same results were obtained twice by both gel electrophoresis and TaqMan  
16 assays <sup>33</sup>.

17 The group of large-scale analysis included six studies. By following the same approach of focusing on cancer  
18 type, Hertz et al analyzed 247 SNPs in FFPE lymph nodes and blood from breast cancer patients using the  
19 Sequenom MassARRAY (the University of Michigan DNA Sequencing core). They reported a genotype  
20 concordance rate of 99.7% and call rate of 97%, indicating that heterozygous genotypes were often not or  
21 discordantly called <sup>26</sup>.

22 In CRC patients, Lips et al performed a comparison of non-malignant FFPE tissue and blood using Illumina  
23 BeadArrays in combination with the linkage mapping panel version 4, revealing 99.4% genotype concordance  
24 with LOH detected on chromosomes 4, 5q, 12q, 14q, 15q, 17p, 18, and 20p, which commonly show LOH in  
25 CRC <sup>28</sup>. In comparison with the LOH analysis in the CRC small-scale studies group, Lips et al illustrated the  
26 different advantages of using genotyping arrays in LOH detection, such as large numbers of tested SNPs in  
27 one experiment, applying quality criteria to remove calls from poorly amplified genotypes, and the ability to  
28 calculate call rate for each SNP individually, and to estimate the concordance with matched blood genotypes.

1 In osteosarcoma patients, non-malignant FFPE tissues were tested on a platform called the drug-metabolizing  
2 enzymes and transporters (DMET) Plus Array (Thermo Fischer Scientific). The array includes 231 genes and  
3 1936 variants covering all pharmacogenetic characteristics such as drug absorption, distribution, metabolism,  
4 excretion, and transport. The reported call rate was  $98.9\% \pm 1.0\%$ , which was comparable to the blood call  
5 rate of  $99.4\% \pm 0.30\%$ .<sup>31</sup> The overall genotyping concordance in all 1936 variants included in the DMET  
6 array was 97.4%. Of note, after removal of no calls and possible rare allele calls, the concordance exceeded  
7 99% except for two samples<sup>31</sup>.

8 In prostate carcinoma patients, the genotyping call rates were high from matched normal tissue FFPE from  
9 both urethra (97%) and seminal vesicles (95.9%)<sup>4</sup>. To resolve the misclustering issue that may affect the  
10 concordance estimates, the authors omitted variants with a minor allele frequency (MAF) less than 5%, which  
11 reduced the number of tested SNPs from 416 047 to 127 847<sup>4</sup>. Similarly, Cannon-Albright used the Illumina  
12 550k SNP data set to select markers that can represent the entire genome with a median heterozygosity of 0.49  
13 and median spacing of 0.14cM. They revealed 99% concordance by testing normal FFPE tissue and matched  
14 blood samples from two patients<sup>32</sup>.

15 Finally, a comprehensive computational approach analyzed a TCGA SNP data platform that used an  
16 Affymetrix 6.0 SNP array in several malignancies and reported significantly higher concordance in blood-  
17 normal tissue pairs (99.17%) than blood-tumor tissue pairs (96.9%) ( $p=1.1 \times 10^{-44}$ )<sup>34</sup>. The authors concluded  
18 that quality control practices are a vital step in the analysis of genotyping array data.

19 Preanalytical variables that affect sample handling (both FFPE and blood) is a crucial issue and should be  
20 considered when dealing with bio-specimens<sup>39</sup>. In the reviewed studies, only five out of 13 reported the age  
21 of the stored blocks (3/5 also reported the storing temperature), and only three out of 13 discussed the details  
22 of their fixation process. The overall quality of the outcomes from the published studies was assessed as  
23 moderate to high (Table 1). Some of the published studies suffered from limitations, such as a small number  
24 of analyzed matched FFPE tissue and blood samples, a small number of tested SNPs, or no reported call rate,  
25 concordance, or both (Table 2). We were not able to perform a meta-analysis because of the heterogeneity  
26 between DNA purification and genotyping techniques in the published studies.

27 Importantly, the reported overall concordance and call rate in the reviewed studies are influenced by three  
28 essential elements, including the number of tested paired blood-normal tissue samples, the number of analysed



1 SNPs, and the genotyping technique used (PCR or a customized array). Considering the earlier classification  
2 of the studies into small-scale and large-scale studies, it is clearly apparent that the former group that applied  
3 PCR assays with specific primers and probes reported a high concordance (usually ranging from 97.4% to  
4 100%). The studies in the latter group used arrays that tested for a large number of SNPs and reported a slightly  
5 lower concordance (ranging from 92.5% to 99.7%). However, in the group of large-scale studies, the overall  
6 genotyping call rate was reported in most of the studies (4 out of 6), thus giving a better idea about the quality  
7 of genotyped SNPs.

8 Assessment of both sample and SNP quality control is a crucial step when working with arrays. One of the  
9 possible explanations for the observed decreased concordance in arrays can be explained by misclustering,  
10 which makes it difficult for the genotype clustering algorithms to distinguish heterozygotes from major allele  
11 homozygotes, thus causing a false increase in heterozygosity<sup>4</sup>. For a rare variant with MAF below 5%, it is  
12 still challenging for different algorithms to perform accurate clustering. To control the effect of poor genotype  
13 clustering in rare SNPs, Emami et al applied an analytical maneuver that removes SNPs that violate Hardy–  
14 Weinberg equilibrium (HWE), which leads to a significant decrease in heterozygosity in both blood and normal  
15 tissue genotypes to the expected levels. Although more stringent SNP quality control enhances the accuracy  
16 of genotyped calls, it may also eliminate large numbers of accurate genotype calls from the final dataset<sup>4</sup>.  
17 Perreault et al performed a comparison of four different clustering tools (GenCall, GenoSNP, optiCall and  
18 zCall) for analysis of rare variants using the Illumina HumanExome BeadChip, which includes 247 870 SNPs  
19 and uses the 1000 Genomes Project as a reference. The authors also concluded that using multiple clustering  
20 algorithms in a parallel manner enhances identification of discordant SNPs<sup>40</sup>.

21 Other study types (not included in this systematic review) evaluated the genotyping concordance of malignant  
22 FFPE tissue with blood. These studies revealed a widely variable concordance rate, ranging from 54.6% in  
23 early stage non-small cell lung carcinoma<sup>41</sup> to approximately 100% in other tumors, such as CRC and  
24 lymphoma<sup>42,43</sup>. Several factors seem to influence the concordance rate in genotyping of tumor tissues,  
25 including tumor type, tumor stage, and most importantly the influence of somatic alterations and tumor  
26 heterogeneity that occur in cancer<sup>12,13</sup>. Low FFPE DNA quality can cause low SNP genotyping concordance.  
27 Additionally, the rate of no-call genotype is higher in tumor FFPE than in normal tissue FFPE due to the  
28 hypermutation status in tumor tissues<sup>34</sup>.

1 A major factor that affects the SNP genotyping process is the quality of the original FFPE DNA and its yield.  
2 Most of the included studies used Qiagen kits for DNA extraction (9 out of 13), which rely on the same  
3 principle of silica column-based DNA purification methods, resulting in uniform extracted DNA in terms of  
4 purity and quantity. However, only seven out of the total 13 reviewed studies illustrated the methodology used  
5 for FFPE DNA quality assessment such as Nanodrop assay, gel electrophoresis, PicoGreen fluorescence, or  
6 PCR. Only four out of these seven studies mentioned their FFPE DNA quality parameters, such as reporting  
7 A260/280 ratio of 1.8<sup>32</sup>, DNA concentration in the range of 0.9-18.4µg by using the QIAamp method<sup>33</sup>, and  
8 amplicon size range of 100 to 300bp<sup>30,31</sup>.

9 In this sense, the minimum DNA fragment length that can be successfully genotyped by a TaqMan assay was  
10 reported to range from 100 to 400 bp, whereas the DNA quantity required for successful TaqMan genotyping  
11 ranged from 1 pg to 10 ng<sup>10</sup>. For SNP arrays, the starting FFPE DNA input varied from 50 ng/µL (after  
12 normalization) when using an Affymetrix Mendel Nsp 250K chip<sup>44</sup> to 400 ng when using the  
13 HumanOmni5Exome BeadChip<sup>45</sup>. Interestingly, DNA yield from 2 x 10 µm sections and 0.6 mm cores from  
14 the same paraffin blocks was quite comparable; 92.3% to 100% concordant genotypes per given SNP between  
15 the matched cores and sections were observed<sup>10</sup>. To facilitate DNA purification from FFPE tissue samples,  
16 new techniques and methods have been developed to enhance DNA yield for accurate genotyping purposes.  
17 An automated FFPE DNA extraction procedure was reported to successfully extract DNA with adequate  
18 quality and quantity for SNP genotyping in Affymetrix Genome-Wide Human 6.0 arrays and TaqMan SNP  
19 PCR<sup>46</sup>.

20 Another crucial element is the possibility of optimizing the FFPE DNA extraction methodology to purify DNA  
21 of high quality and sufficient concentration for downstream molecular reactions, such as sequencing. Recently,  
22 Frazer et al used one FFPE DNA extraction kit (QIAamp DNA FFPE Tissue) to test three different  
23 modifications to the protocol. The authors applied different amounts of proteinase K (20 µl versus 40 µl) and  
24 different incubation periods (24 hours versus 72 hours), and observed that 40 µl proteinase K with a 24-hour  
25 incubation gave the highest DNA yield with good DNA integrity<sup>47</sup>.

26 Furthermore, Bonnet et al performed a comparison of three different FFPE DNA extraction kits (QIAamp  
27 DNA FFPE Tissue kit and GeneRead DNA FFPE kit from Qiagen and Maxwell™ RSC DNA FFPE Kit from

1 Promega) along with their performance on an exome sequencing platform <sup>48</sup>. The authors reported superiority  
2 of both Qiagen kits regarding DNA quality and median coverage of the mapped reads in the sequencing data  
3 <sup>48</sup>. Interestingly, the authors evaluated the FFPE artifacts in the three kits through paired sequencing of the  
4 FFPE samples with the matching fresh-frozen (FF) samples by calculating the difference in the number of  
5 variants for each pair of matched FF/FFPE samples for both single-nucleotide variants (SNVs) and insertions  
6 and deletions (INDELs). The authors observed the lowest variations with the GeneRead kit, which emphasizes  
7 the importance of UDG in FFPE pretreatment and its role in removal of FFPE artifacts <sup>48</sup>.

8 Importantly, almost half of the included studies (6/13) used whole blood-derived DNA as a reference against  
9 which concordance was calculated, and reported concordance (four out of these six studies) from 92.5% to  
10 100%. Two other studies used blood and saliva derived DNA with reported concordance of 97.4% to 100%.  
11 In addition, three studies used leukocyte derived DNA and one study used lymphocyte derived DNA with  
12 reported concordance of 97.4% to 99.7% and 100%, respectively. DNA derived from plasma and serum was  
13 used in one study with no reported concordance rate (Table 2). The reviewed studies did not provide clear  
14 evidence that refers to the difference in the concordance rate to the source of blood derived DNA used as a  
15 reference. Indeed, a crucial and future aim is to incorporate genotyping arrays for DNA extracted from FFPE  
16 tissue into clinical applications. Lyons-Weiler et al optimized the Affymetrix GeneChip 10k 2.0 assay for  
17 assessment of LOH and copy number alterations in routine clinical use from malignant FFPE tissue specimens  
18 <sup>49</sup>. Although Lyons-Weiler and colleagues did not test the protocol for non-malignant FFPE tissues derived  
19 from cancer patients, they reported 96% concordance with the genotyping data from fresh tumor tissues <sup>49</sup>.

20 In conclusion, our systematic review identified only a few studies that compared both normal tissue FFPE and  
21 blood derived DNA derived from cancer patients as a source for large-scale germline genotyping. Importantly,  
22 these studies, and studies that used only few selected SNPs, show that DNA extracted from non-malignant  
23 FFPE tissue specimens derived from cancer patients can be successfully genotyped using different genotyping  
24 methods. Our review confirms the feasibility of using non-malignant FFPE tissue derived from cancer patients  
25 for analysis of germline DNA. However, systematic studies with larger sample sizes that assess qualitative  
26 factors and compare the recently introduced methods for improving FFPE DNA yield and quality are still  
27 needed to demonstrate the applicability of archived normal FFPE specimens in the evaluation of polygenic  
28 risk of cancer predisposition.

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**Compliance with ethical standards**

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Ethical approval:** All studies that were included in this systematic review are stated to be in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent:** The studies included in this systematic review stated that informed consent was obtained from all individual participants included in the respective studies.

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**TABLE 1. GRADE EVIDENCE PROFILE AND QUALITY ASSESSMENT OF THE 13 INCLUDED STUDIES**

Outcome	No. of participants (studies)	Study design (Number of studies)	Limitations (risk of bias)	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality
Optimal FFPE DNA yield	651	Observational studies (12)	Not serious	Not serious	Not serious	Not serious	None	⊕⊕⊕⊕ High
FFPE DNA genotyping call rate	651	Observational studies (12)	Not serious	Not serious	Not serious	Serious <sup>a</sup>	None	⊕⊕⊕○ Moderate
Matched genotyping concordance	1077	Observational studies (13)	Not serious	Not serious	Not serious	Serious <sup>b</sup>	None	⊕⊕⊕○ Moderate

The quality of evidence was assessed using the GRADE approach.

Factors that reduce the quality of the evidence: risk of bias (limitations in the study design), inconsistency of results, indirectness of evidence, imprecision, and publication bias.

Factors that increase the quality of the evidence: large effect size, dose-response gradient, all plausible confounding would reduce a demonstrated effect, all possible confounding would suggest a spurious effect when the actual results show no effect.

High quality: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate quality: We are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is markedly different.

Low quality: Our confidence in the effect estimate is limited; the true effect may be markedly different from the estimate of the effect.

Very low quality: We have very little confidence in the effect estimate; the true effect is likely to be markedly different from the estimate of effect.

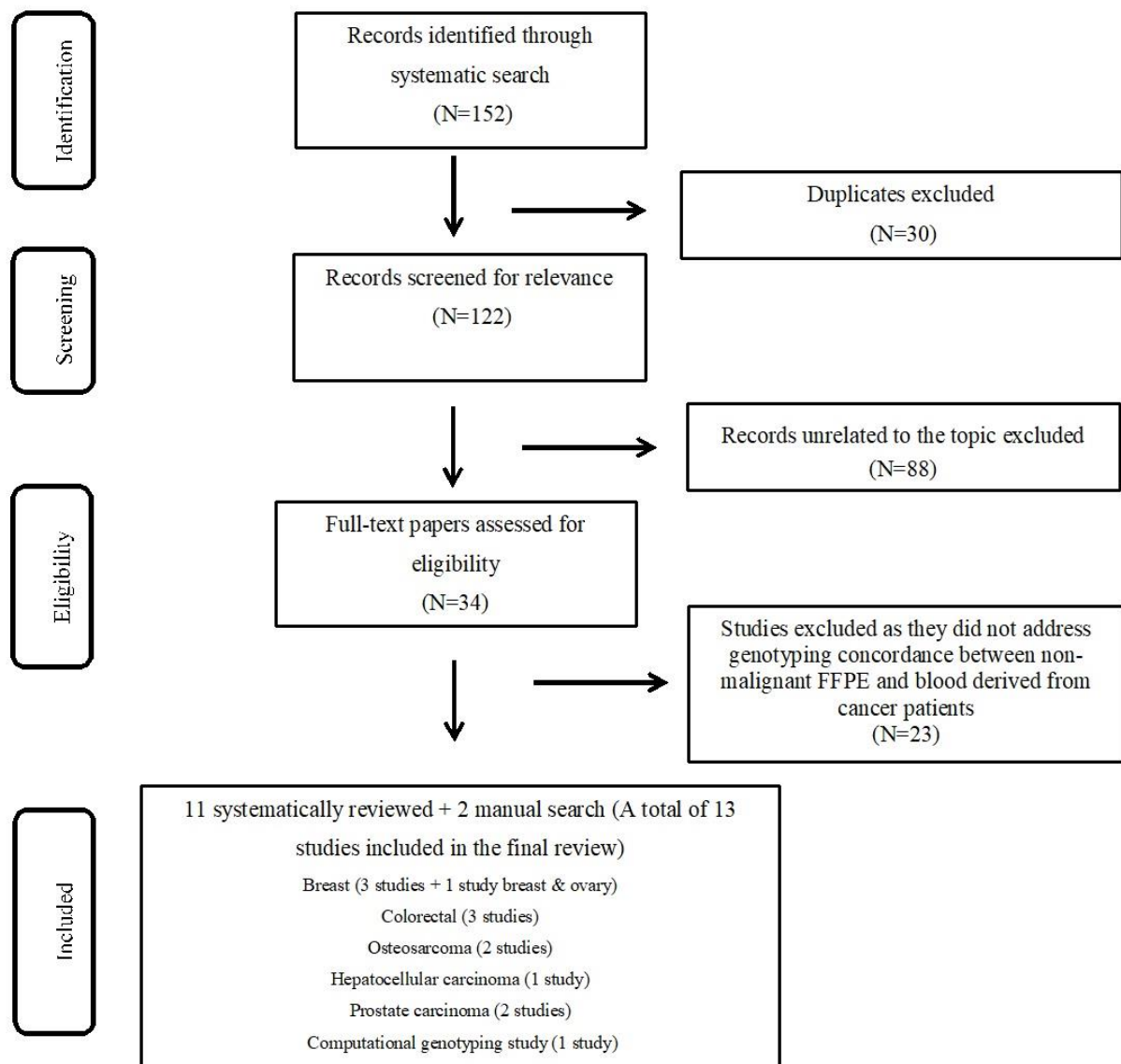
<sup>a</sup> Different methods for DNA genotyping and some studies have no reported call rate; this could have a potential effect on the quality of the results.

<sup>b</sup> Some studies have no reported concordance ratio; this could have a potential effect on the quality of the results.

**TABLE 2. SUMMARY OF STUDIES THAT EXAMINED GENOTYPING CONCORDANCE BETWEEN NON-MALIGNANT FFPE AND BLOOD-DERIVED DNA FROM CANCER PATIENTS.**

First author, year	Cancer type	Number of matched non-malignant FFPE and blood samples	FFPE DNA isolation method	FFPE pretreatment with UDG	Source of blood derived DNA	Genotyping technique	Number of tested SNPs	Call rate (%)	Genotyping concordance (%)
Zauber et al, 1999	CRC	28	QIAmp Tissue Kit	Not used	Whole blood	PCR assay	1	NA	NA
Sjöholm et al, 2005	HCC	31	QIAquick and QIAamp	Not used	Plasma and serum	Restriction fragment length polymorphism (RFLP) and TaqMan PCR assay	4	NA	NA
Lips et al, 2005	CRC	2	Chelex extraction	Not used	Leukocytes	SNP array genotyping (Illumina Bead Arrays)	5861	NA	99.4
Xie et al, 2006	Breast	106	MagAttract DNA Mini M48 Kit	Not used	Whole blood	TaqMan PCR assay	5	NA	100
Adank et al, 2006	Breast and ovary	161	NA	Not used	Lymphocytes	PCR assay	3	NA	100
Hagleitner et al, 2011	Osteosarcoma	18	DNeasy Tissue kit	Not used	Blood and saliva	TaqMan PCR assay	2	NA	100
Cannon-Albright et al, 2011	Prostate	2	QIAamp DNA FFPE Tissue Kit	Not used	Whole blood	SNP array genotyping	27 157	88-98	99
Rae et al, 2013	Breast	122	NA	Not used	Leukocytes	TaqMan PCR assay	1	NA	97.4
Marisi et al, 2014	CRC	20	QIAamp DNA Micro kit	Not used	Whole blood	TaqMan PCR assay	7	NA	NA
Vos et al, 2015	Osteosarcoma	16	QIAamp DNA Micro Kit	Not used	Blood and saliva	SNP array genotyping (DMET)	1931	98.9	97.4
Hertz et al, 2015	Breast	114	Qiagen DNeasy Blood and Tissue	Not used	Leukocytes	SNP array genotyping (Sequenom Mass arrays)	247	97	99.7
Emami et al, 2017	Prostate	31	QIAamp DNA FFPE Tissue Kit	Not used	Whole blood	SNP array genotyping (Affymetrix Axiom 2.0)	416 047	Urethra 97 and seminal vesicle 95.9	Urethra 94.1 and seminal vesicle 92.5
Guo et al, 2018a	BRCA, COAD, HNSC, LIHC, LUAD, LUSC, OV, PAAD, PRAD, READ, SKCM, STAD	426	NA	NA	Whole blood	SNP array genotyping (Affymetrix 6.0 SNP data set)	>906 600	NA	99.17

NA not available, HCC hepatocellular carcinoma, CRC colorectal carcinoma, BRCA breast adenocarcinoma, COAD colon adenocarcinoma, HNSC head and neck squamous cell carcinoma, LIHC liver hepatocellular carcinoma, LUAD lung adenocarcinoma, LUSC lung squamous cell carcinoma, OV ovarian cystadenocarcinoma, PAAD pancreatic adenocarcinoma, PRAD prostate adenocarcinoma, READ rectal adenocarcinoma, SKCM skin cutaneous melanoma, STAD stomach adenocarcinoma, UDG uracil DNA glycosylase enzyme, DMET drug metabolizing enzymes and transporters plus array.



**FIG. 1.** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart. The chart shows the number of articles identified and the number of articles excluded along with the steps of systematic searching for studies that examined genotyping concordance between DNA derived from non-malignant FFPE and blood from cancer patients.