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

Omar Youssef[1,2*], Alhadi Almangush[1,2,3*], Yossra H.S. Zidi[1,2], Anu Loukola[1,4], Olli Carpén[1,2,4]

[1] Department of Pathology, University of Helsinki, Helsinki, Finland

[2] Research Program in Systems Oncology, Faculty of Medicine, University of Helsinki, Helsinki, Finland.

[3] Institute of Biomedicine, Pathology, University of Turku, Turku, Finland.

[4] Helsinki Biobank, HUS Helsinki University Hospital, Helsinki, Finland

*Equal contribution

Running title: Germline variants in non-malignant FFPE tissues

Omar Youssef, Alhadi Almangush, Yossra H.S. Zidi, Anu Loukola, Olli Carpén, Department of Pathology, University of Helsinki, P.O.Box 21 (Haartmaninkatu 3), 00014 Helsinki, Finland

Email addresses:
Omar Youssef (omar.youssef@helsinki.fi); Alhadi Almangush (alhadi.almangush@helsinki.fi); Yossra H.S. Zidi (yossra.zidi-mouaffak@helsinki.fi); Anu Loukola (anu.loukola@helsinki.fi); Olli Carpén (olli.carpen@helsinki.fi)

Corresponding author: Omar Youssef
Address: Department of Pathology, University of Helsinki, P.O.Box 21 (Haartmaninkatu 3), 00014 Helsinki, Finland
Email address: omar.youssef@helsinki.fi
Phone: +358 449141677
ORCID: 0000-0002-1827-9156

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Abstract

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

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

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

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

Introduction

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

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

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

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

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

Methods

Search protocol and data sources

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

Inclusion and exclusion criteria

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

Screening and data extraction

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

Quality assessment

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system of rating quality of evidence was used to assess the quality of the eligible studies [23]. The quality of evidence ranged from high to very low and the factors considered to determine the quality of evidence are summarized in Table 1.

Results and Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Authors' contributions: OY and AA performed the primary search, filtered the data, revised the inclusion and exclusion criteria, applied the quality criteria, and developed the manuscript. YZ revised the screened reviewed literature and verified the data for the included studies. AL and OC contributed to the development of the manuscript outline and performed the final revisions for the manuscript.

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[a]	None	⊕⊕⊕O Moderate
Matched genotyping concordance	1077	Observational studies (13)	Not serious	Not serious	Not serious	Serious[b]	None	⊕⊕⊕O 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.

[a] 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.

[b] 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.

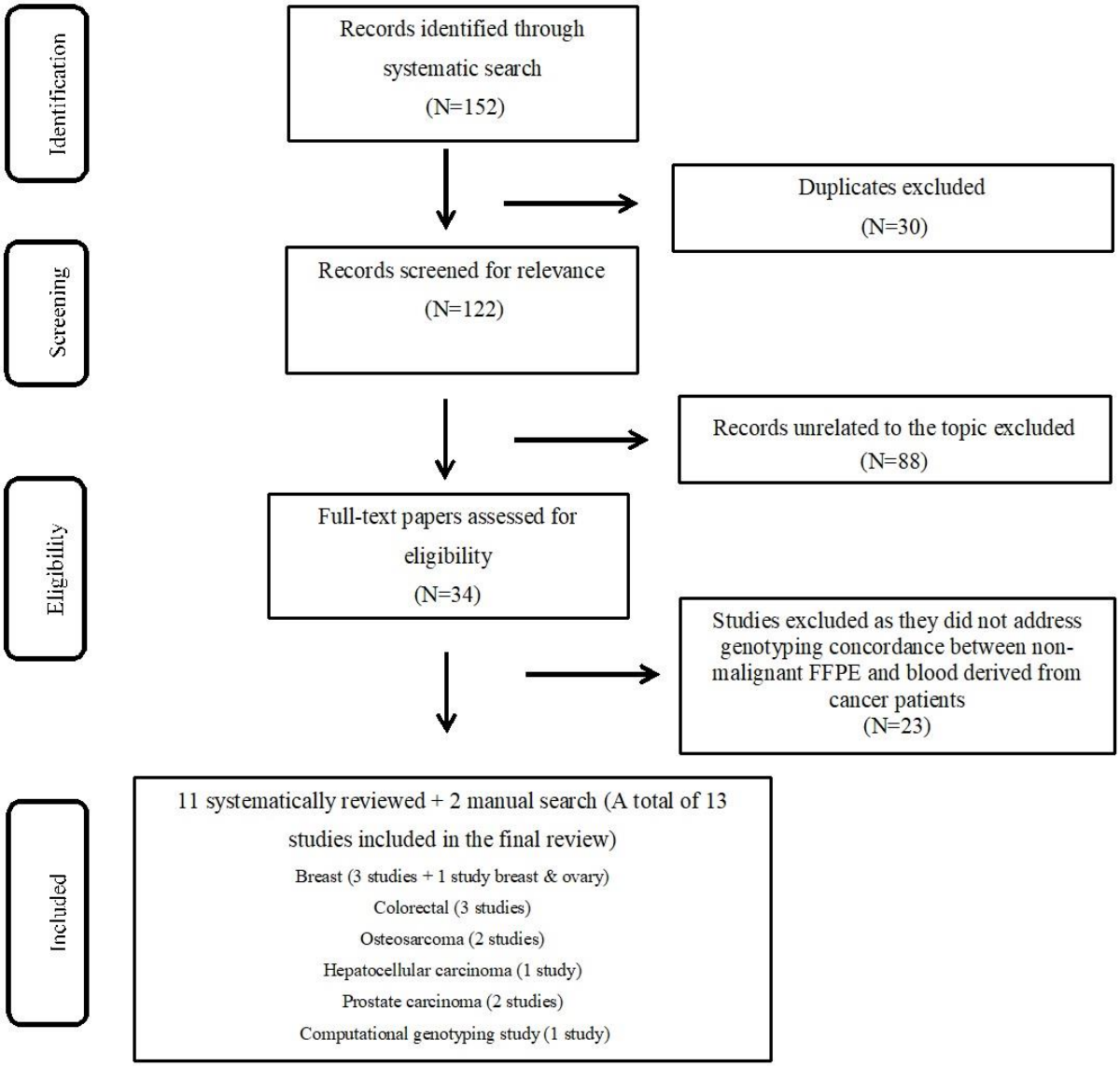

FIG. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart. The chart shows the number of articles identified and the number of articles excluded along with the steps of systematic searching for studies that examined genotyping concordance between DNA derived from non-malignant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