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Genotyping concordance in DNA extracted from formalin-fixed paraffin embedded (FFPE) breast tumor and whole blood for pharmacogenetic analyses

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ABSTRACT

Background: Cancer pharmacogenetic studies use archival tumor samples as a DNA source when germline DNA is unavailable. Genotyping DNA from formalin-fixed paraffin embedded tumors (FFPE-T) may be inaccurate due to FFPE storage, genetic aberrations, and/or insufficient DNA extraction. Our objective was to assess the extent and source of genotyping inaccuracy from FFPE-T DNA and demonstrate analytical validity of FFPE-T genotyping of candidate single nucleotide polymorphisms (SNPs) for pharmacogenetic analyses.

Methods: Cancer pharmacogenetics SNPs were genotyped by Sequenom MassARRAYs in DNA harvested from matched FFPE-T, FFPE lymph node (FFPE-LN), and whole blood leukocyte samples obtained from breast cancer patients. No- and discordant-call rates were calculated for each tissue type and SNP. Analytical validity was defined as any SNP with <5% discordance between FFPE-T and blood and <10% discordance plus no-calls.

Results: Matched samples from 114 patients were genotyped for 247 SNPs. No-call rate in FFPE-T was greater than FFPE-LN and blood (4.3% vs. 3.0% vs. 0.5%, p < 0.001). Discordant-call rate between FFPE-T and blood was very low, but greater than that between FFPE-LN and blood (1.1% vs. 0.3%, p < 0.001). Samples with heterozygous genotypes were

more likely to be no- or discordantly-called in either tissue (p < 0.001). Analytical validity of FFPE-T genotyping was demonstrated for 218 (88%) SNPs.

Conclusions: No- and discordant-call rates were below concerning thresholds, confirming that most SNPs can be accurately genotyped from FFPE-T on our Sequenom platform. FFPE-T is a viable DNA source for prospective–retrospective pharmacogenetic analyses of clinical trial cohorts.

1. Introduction

In cancer there are two relevant genomes, that of the tumor (somatic) and the patient (germline) (McLeod, 2013). The somatic genome has been the area of intense basic and clinical-translational research. Analyses of the somatic genome across tumor types have cataloged oncogenic drivers, many of which are now targets for precision medicine (Kandoth et al., 2013). The germline genome may be less directly involved in predicting cancer treatment effectiveness compared to tumor de novo and acquired resistance mechanisms, but highly relevant for predicting cancer susceptibility and drug exposure, which can have critical downstream effects on treatment efficacy and toxicity (Hertz and McLeod, 2013; Hertz and Rae, 2015). There is great interest in discovering pharmacogenetic predictors for personalizing cancer therapy. In order to do so, DNA from cohorts of patients with long-term clinical outcomes data are required for definitive "prospective–retrospective" pharmacogenetic analyses (Patterson et al., 2011).

Since tumor assessment is ubiquitous in cancer treatment, there are hundreds of thousands of stored specimens available for retrospective biomarker research. The ability to use the DNA extracted from these tumors, particularly those formalin-fixed paraffin embedded (FFPE), for germline genetic association testing would dramatically increase the availability of cohorts for prospective–retrospective studies. However, germline genotyping requires demonstration of analytic validity; that the results obtained from genotyping DNA isolated from FFPE tumors (FFPE-T) are concordant with those obtained from genotyping germline DNA. There is concern that analytic validity may be compromised due to somatic genetic alteration, DNA degradation during FFPE processing and storage, and/or low DNA yield during extraction from stored tumor specimens.

Previous publications have assessed genotype concordance between germline DNA and DNA isolated from tissues stored in a variety of conditions (Supplementary Table 1). Very high genotyping concordance (>97%) has been documented comparing germline DNA and with DNA isolated from FFPE non-tumor tissue (Adank et al., 2006; Cannon-Albright et al., 2011; Hagleitner et al., 2011; Vos et al., 2015; Xie et al., 2006; Zhang et al., 2015), non-FFPE tumor tissue (Marsh et al., 2005; A. Thompson et al., 2010) and FFPE-T tissue (Goetz et al., 2005; Horn et al., 2010; Rae et al., 2003, 2013; van Huis-Tanja et al., 2013). Despite these highly consistent findings there continues to be concern raised regarding the use of FFPE-T DNA in retrospective–prospective studies (Nakamura et al., 2012),

perhaps due to isolated reports of inadequate genotyping performance for single SNPs genotyped from FFPE-T (Goetz et al., 2014; Marisi et al., 2014). Given these concerns, it is critical to demonstrate analytic validity of genotyping FFPE-T prior to prospective–retrospective analyses (Greytak et al., 2015). We collected matched FFPE-T, FFPE non-cancer lymph node (FFPE-LN), and germline DNA to document high genotyping concordance in CYP2D6, which we previously reported (Rae et al., 2013). In this study we reused these samples to assess the overall genotyping performance from FFPE-T and document analytic validity of genotyping our target SNPs pertinent to cancer pharmacogenetics on a custom multiplex array in FFPE-T DNA for prospective–retrospective analyses.

2. Methods

2.1. Patient enrollment and sample collection and processing

Details of patient recruitment and enrollment, sample collection and processing, and DNA preparation have been previously published (Rae et al., 2013). Briefly, patients seen consecutively by their University of Michigan breast medical oncology team were offered the opportunity to participate in this study, ClinicalTrial.gov identifier NCT00858585. Eligible patients were over the age of 21, carried the diagnosis of invasive breast cancer and were able to provide access to FFPE archived primary tumor and benign lymph node tissues. Patients who received neo-adjuvant chemotherapy were eligible but patients with metastatic disease were not.

An 8 mL whole blood sample was collected in a 10 mL EDTA vacutainer tube for germline DNA isolation from peripheral leukocytes. Whole blood was stored on ice or at 4 °C. Whole blood was aliquoted within 8 h of collection into one 0.5 mL sample and the remainder into 1 mL aliquots, which were stored at −80 °C. Stored FFPE tumor blocks were obtained from the University of Michigan Pathology Department and stored at room temperature until processing. DNA was extracted from peripheral whole blood samples within 24 h of collection using the Qiagen DNeasy® Blood and Tissue Kit (kit 69506, Valencia, California, USA) and from FFPE-T and unaffected FFPE-LN utilizing the same kit in conjunction with a previously published protocol (Sikora et al., 2011). The amount of useable DNA was quantified in the samples via Nanodrop. DNA was stored at −80 °C until genotyping was performed.

2.2.   Genotyping

Approximately 30 μL of DNA from whole blood, FFPE-T and FFPE-LN was submitted to the University of Michigan DNA Sequencing core. Assays were designed using standard Sequenom software (both online Assay Design Suite tools and desktop Assay Design 4.0) and assays were performed using a Sequenom MassARRAY Compact instrument, according to manufacturer's standard protocols. Results were processed to generate SNP calls automatically, using Sequenom TyperAnalyzer software, and then manually reviewed by the operator to validate the allele calls. Automatic SNP calls that were of concern, based on questionable spectra, were removed. A total of 304 unique SNPs were genotyped on 8 Sequenom MassARRAY plexes with up to 40 SNPs each. These SNPs were not selected specifically for this project, but represent a collection of candidate SNPs relevant to the metabolism, mechanism, or toxicity of breast cancer treatments, for genotyping in prospective–retrospective pharmacogenetic analyses of breast cancer clinical trials. Sample concentration was determined using a Nanodrop spectrophotometer. Ideal sample concentrations for Sequenom are between 50 and 100 ng/μl. DNA concentrations were less than optimal from FFPE-T (median = 10.09, range: 0.78–88.0) and FFPE-LN (median = 57.00, range 2.57–97.29).

2.3.   Sample and SNP quality control

Samples with whole blood call rate <95% or tumor call rate <80% were eliminated prior to analysis. SNPs were eliminated for blood sample genotype call rate <95%, minor allele frequency <2%, or if the p-value for Hardy–Weinberg Equilibrium (HWE) was <0.0002 (0.05/304), to remove weak or failed SNP assays.

## 2.4.   Genotyping performance within sample types

Patients with all three matched tissue types (blood, FFPE-T, and FFPE-LN) that passed quality control were included in the analysis. Genotyping performance in each tissue type was compared in several ways. The overall no-call rate was calculated for all three tissue types. The no-call rates in FFPE-T and FFPE-LN were also calculated by genotype (heterozygous or homozygous), assuming the genotype call in blood was correct. Discordance was defined in two ways: strict discordance and possible discordance. Strict discordance is a different genotype call made in the tissue of interest (FFPE-T or FFPE-LN) compared with blood. Possible discordance combines strict discordance and instances in which a genotype call was not made in either blood or the tissue of interest (FFPE-T or FFPE-LN). Strict and possible discordance rates overall were calculated similarly to no-call rates. Strict discordance rates were also calculated by genotype, and each discordant instance was categorized into the 3 possible transitions between homozygous and heterozygous, again assuming the blood genotype call was correct.

2.5.   Analytical validation of SNPs

FFPE-T genotyping results for individual SNPs were interrogated to identify SNPs with unacceptable rates of no-calls and/or discordant calls. SNPs failed analytical validation if they had FFPE-T strict discordance rate >5% or possible
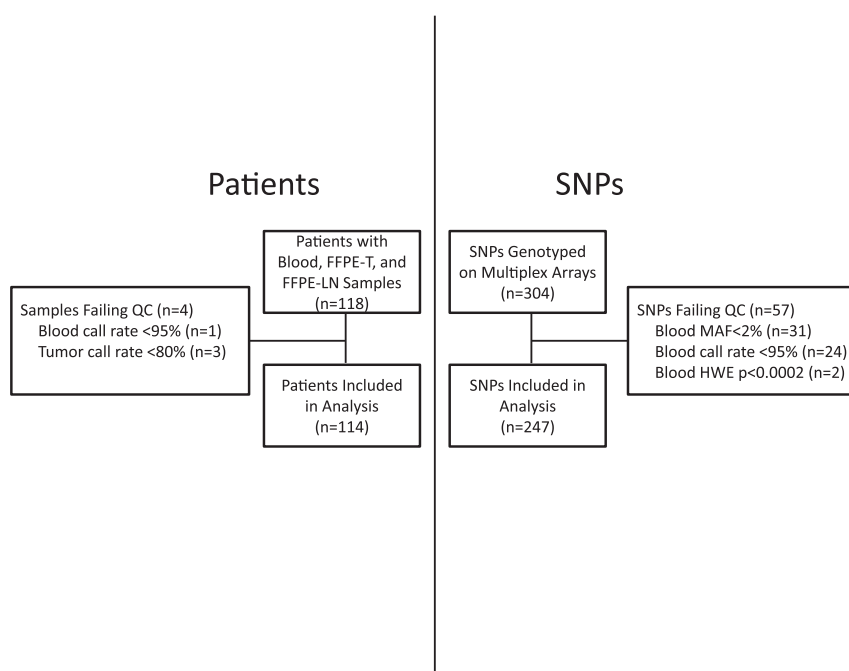
Figure 1 – Consort Diagram for Patients and SNPs. Quality control filtering of patients whose samples failed genotyping in blood or tumor (left) and SNPs that failed genotyping in blood (right). After quality control 114 patients with matched blood, FFPE-T and FFPE-LN samples genotyped for 247 independent SNPs were included in the analysis. Footnote: Abbreviations: MAF: Minor allele frequency, HWE: Hardy–Weinberg Equilibrium.
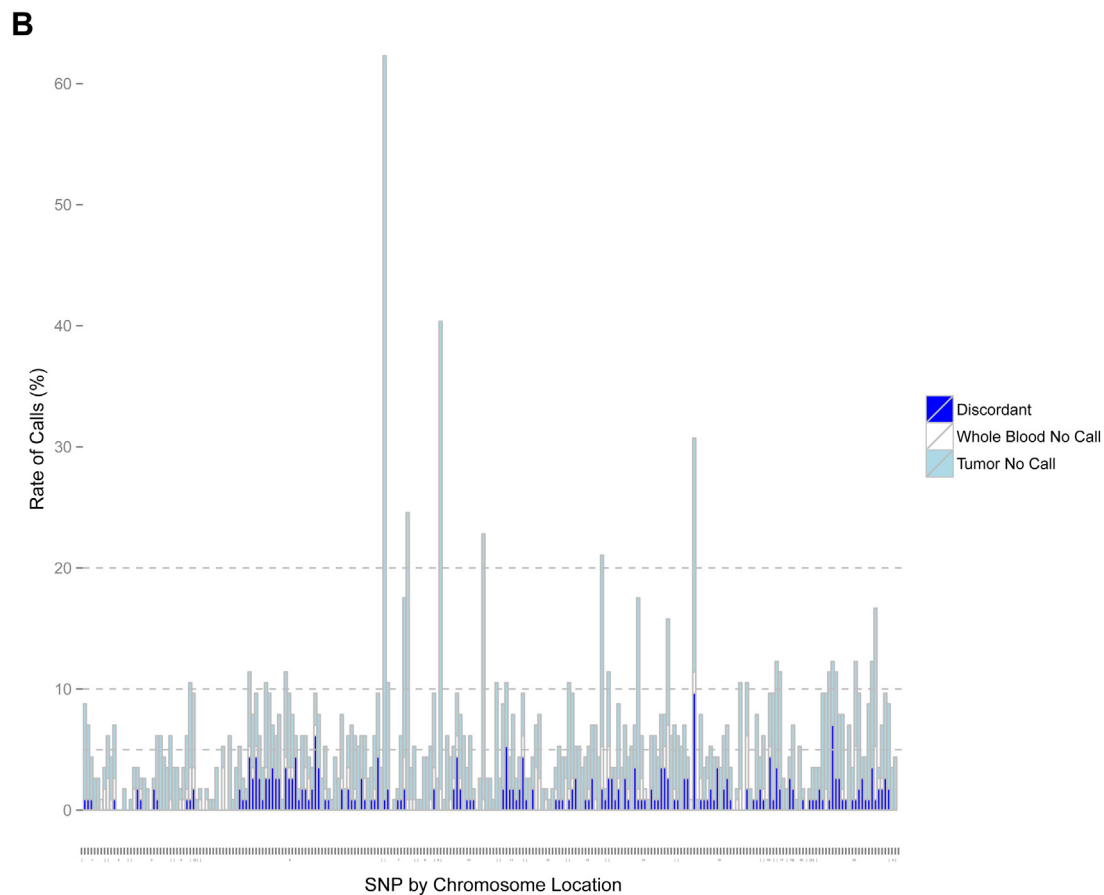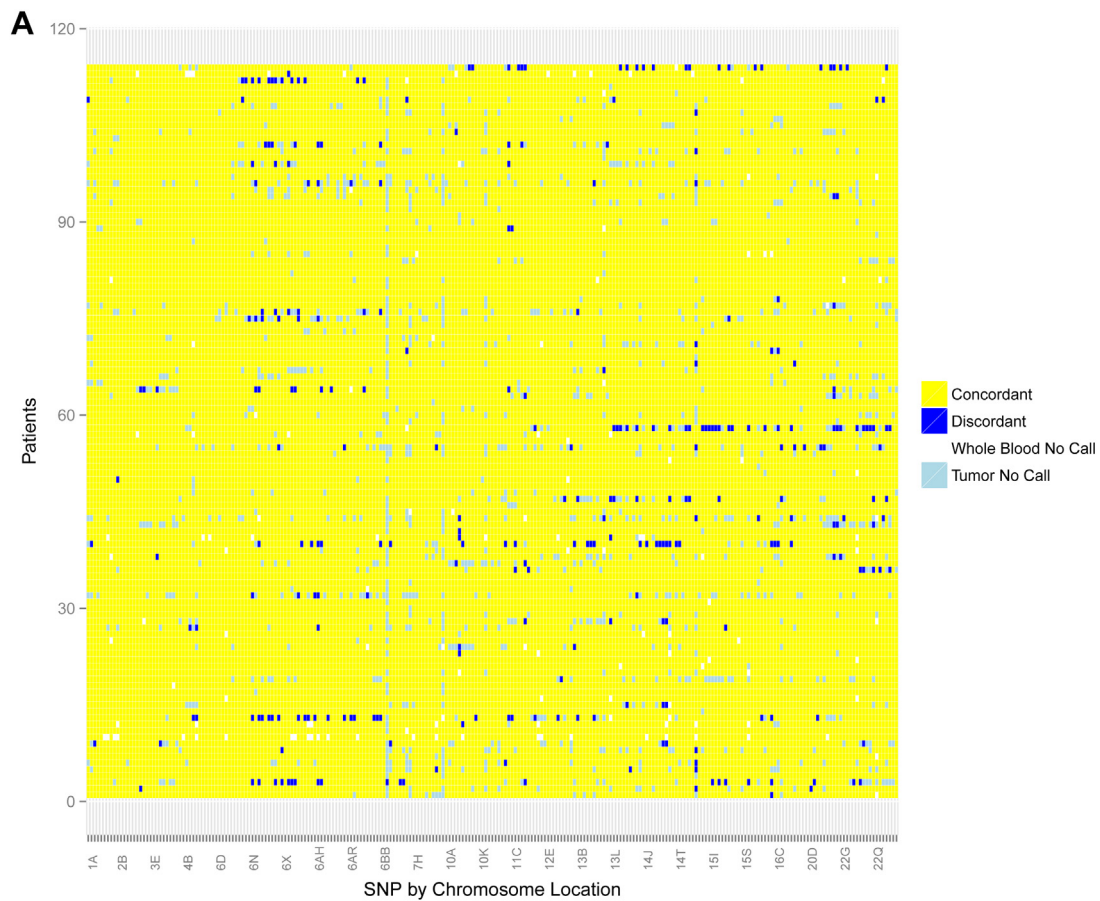
Figure 2 — Genotyping for FFPE-T. Concordance between FFPE-T and blood for all patients (n = 114) and SNPs (n = 247) that passed quality control. (A) Discordant calls (blue) and no-calls in either the tumor (light blue) or blood (white) are represented by colors. (B) The number of possible discordant calls (strict discordant calls plus no-calls in tumor or blood) for all SNPs with stacked bars colored similarly to Figure 1A. The horizontal lines indicate cutoffs of 5%, 10%, and 20%. Overall, the tumor genotyping was highly concordant with few SNPs that failed FFPE-T genotyping.

Table 1 – No-call and discordant call rates in each sample type across all SNPs (n = 247) and by genotype.

			Blood	FFPE-T	Comparison p-value[i]	FFPE-LN	Comparison p-value[i]	FFPE-T vs FFPE-LN p-value
No calls	Overall[a]		0.5% (135/28158)	4.3% (1221/28158)	<0.001	3.0% (843/28158)	<0.001	<0.001
	By genotype[b]	Homozygous	NA	1.8% (346/18900)	<0.001	2.5% (468/18900)	<0.001	NA
		Heterozygous	NA	9.5% (864/9123)		4.0% (363/9123)		
Strict discordant calls[c]	Overall[d]		NA	1.1% (306/26813)	NA	0.3% (84/27192)	NA	<0.001
	By genotype[e]	Homozygous	NA	0.2% (33/18554)	<0.001	0.2% (32/18432)	<0.001	NA
		Heterozygous	NA	3.3% (273/8259)		0.6% (52/8760)		
	Direction of discordance[f]	Hom → Het	NA	10.8% (33/306)	NA	28.6% (24/84)	NA	NA
		Het → Hom	NA	89.2% (273/306)		61.9% (52/84)		
		Hom → Hom	NA	0% (0/306)		9.5% (8/84)		
Possible discordant calls[g]	Overall[h]		NA	5.9% (1651/28158)	NA	3.7% (1050/28158)	NA	<0.001

Abbreviations: Het = Heterozygous genotype call, Hom = Homozygous genotype call.
a Overall no-call rate calculated by: # no-calls/28158 genotype calls (247 SNPs in 114 patients).
b No-call rate by genotype calculated by: # no-calls/# calls of that genotype. Note this excludes 135 SNPs with no-calls in blood.
c Strict discordance defined as a genotype call was made in both blood and FFPE-T (or FFPE-LN) and the calls were different.
d Overall strict discordant call rate calculated by: # genotype calls in FFPE-T (or FFPE-LN) that are different from genotype call in blood/# of genotype calls in FFPE-T (or FFPE-LN) and blood. Note that any sample with a no-call in FFPE-T (or FFPE-LN) or blood excluded from calculation.
e Strict discordance rate by genotype calculated by: # genotype calls in FFPE-T (or FFPE-LN) that are different from genotype call in blood/# of genotype calls in FFPE-T (or FFPE-LN) and blood of that genotype. Note that any sample with a no-call in FFPE-T (or FFPE-LN) or blood excluded form calculation.
f Type of discordance with the genotype call in blood first and the genotype call in the tissue of interest second. Percentage calculated as the # of discordant calls in that direction/the number of total discordant calls.
g Possible discordance defined as either a strict discordant call was made or a genotype call was not made in blood or FFPE-T (or FFPE-LN).
h Overall possible discordance calculated by: # of genotype calls in FFPE-T (or FFPE-LN) that are different from genotype calls in blood plus number of no-calls in FFPE-T (or FFPE-LN) or blood/28158 genotype calls (247 SNPs in 114 patients).
i P-values compare FFPE-T or FFPE-LN vs. blood, FFPE-T vs. FFPE-LN, or homozygous vs. heterozygous within sample type, as appropriate.

discordance rate >10%. SNPs that passed both metrics were considered analytically validated.

2.6. Statistical methods

Comparisons of call rates (no-calls, strict discordant calls, possible discordant calls) between tissue types (blood, FFPE-T, FFPE-LN) were analyzed using mixed effects logistic regression with random effects for subjects and SNPs and primary covariate of interest being tissue type. Similar models were used for differences by genotype with the covariate of interest being the interaction between genotype and tissue type. Spearman Correlation was used to measure the association between no-call or discordant call rate and DNA concentration. All statistical analyses were performed using SAS v9.3.

3. Results

3.1. Sample quality control

Flow diagrams that describe the filtering of patients and SNPs are displayed in Figure 1. After exclusion of four patients' samples due to unacceptable genotype call rates in blood (<95%, n = 1) or FFPE-T (<80%, n = 3), 114 patients with matched samples were included in the analysis. There was a statistically

significant correlation between FFPE-T DNA concentration and call rate (r = 0.48, p < 0.0001, Supplementary Figure 1). Quality control was then performed on the 304 individual SNPs, with 247 passing all quality control metrics. These 247 SNPs were ordered by chromosomal location and assigned labels that indicate the chromosome (#) and their position (letter) (Supplementary Table 2). The eliminated SNPs (n = 57, See Supplementary Table 3) failed due to low call rate (<95%, n = 24), low minor allele frequency (MAF) (<2%, n = 31), or significant deviation from HWE (p < 0.0002, n = 2) in blood sample genotyping.

3.2. Genotyping performance by tissue and sample

Concordant, discordant, and no-calls in FFPE-T for all patients and SNPs, and the summary rate of discordance per SNP, are displayed in Figure 2A and B, respectively. The summary no-call and discordant call rates in all three tissue types are reported in Table 1. Across the 28,158 genotype calls in the 247 SNPs among 114 patients the overall no-call rates were higher in the FFPE-T (4.3%) than FFPE-LN (3.0%), and higher in either compared to blood (0.5%) (p < 0.001 for all comparisons) (Supplementary Figure 2). The pattern was similar for strict discordant calls, with the strict discordance rate being greater in FFPE-T than FFPE-LN (1.1% vs. 0.3%, p < 0.001). Assuming the blood genotype is correct, heterozygous samples were

significantly more likely than homozygous samples to have no-calls or strict discordant calls in both FFPE-T and FFPE-LN (p < 0.001 for all comparisons). Of the strict discordant calls, the majority was heterozygous samples incorrectly called homozygous (FFPE-T: 89.2% of 306 discordant calls, FFPE-LN: 61.9% of 84 discordant calls).

When assessed by patient sample sets across 247 SNPs the discordance was not randomly distributed. The majority of FFPE-T samples had no strict discordant calls (64/114 = 56.1%), while 6 (4.2%) had strict discordance rates >5%. There was a weak, but statistically significant inverse correlation between FFPE-T DNA concentration and strict discordance (r = −0.20, p = 0.031, Supplementary Figure 3).

3.3.   *Analytical validation of SNPs*

Looking within each SNP across all 114 samples, 29 unique SNPs failed at least one performance metric in FFPE-T genotyping. Four SNPs (4/247 = 1.6%) had a strict discordance rate >5% (Table 2, Figure 3), 28 (11.3%) SNPs had a possible discordance rate >10% (Table 3), and 3 SNPs failed both metrics. Overall 88.3% (218/247) of the SNPs were analytically validated (Supplementary Figure 4).

4.   **Discussion**

In this study, we assessed the genotyping performance in DNA extracted from FFPE-T samples for 247 cancer pharmacogenetic SNPs genotyped on a custom Sequenom panel. Overall we found adequate call rates (95.7%) and very strong concordance (98.9%) to germline genotyping, consistent with previous publications (Goetz et al., 2005; Horn et al., 2010; Rae et al., 2003, 2013; van Huis-Tanja et al., 2013), reaffirming the analytic validity of genotyping stored tumor specimens for prospective−retrospective secondary analyses of clinical trials. The no-call rate in FFPE-T (4.3%) is below typical thresholds used for quality control in pharmacogenetic analyses (<5%) and the extremely low rate of strict discordant calls (1.1%) is well below reasonable thresholds of concern for correlative pharmacogenetic research. Analytic validity was demonstrated for genotyping 218 unique candidate SNPs on our custom platform from DNA extracted from FFPE-T.

There are several theoretical concerns with genotyping FFPE-T DNA. The first is the degradation of DNA from formalin fixation and long-term storage. Assessment of matched FFPE-LN enables us to isolate the contribution of FFPE processing and storage to the genotyping error seen in this study. The no-call rate in FFPE-LN was approximately 70% of that of FFPE-T, suggesting that most of the non-calling in FFPE-T is attributable to the FFPE process, consistent with what we reported previously for matched CYP2D6 genotyping (Rae et al., 2013). The extremely low rate of discordance in FFPE-LN (0.3%) is in line with discordance rates from comparisons of germline and FFPE non-tumor tissue (0%−2.6%) (Adank et al., 2006; Cannon-Albright et al., 2011; Hagleitner et al., 2011; Vos et al., 2015; Xie et al., 2006; Zhang et al., 2015) and similar to the 3% discordance reported in a comparison of matched fresh frozen and FFPE ovarian tumors genotyped on a genome-wide genotyping array (E. R. Thompson et al.,

Study label	Gene	rsID	Strict discordance (# FFPE-T discordant/ # genotype calls in blood and tumor)
6AI	ESR1	rs2347869	6% (7/110)
11B	PGR	rs500760	6% (6/108)
15E	CYP19A1	rs9806371	12% (11/90)
22E	COMT	rs4633	7% (8/108)

Table 2 — SNPs with strict discordance > 5% in FFPE-T.

2005). In a secondary analysis we identified a slight increase in FFPE-T strict discordance rate as the amount of time in FFPE storage increased (spearman correlation r = 0.22, p = 0.017), which is consistent with an increasing loss of DNA quality reported by others (Hedegaard et al., 2014). These findings clearly demonstrate that the FFPE processing and long-term storage have a small, but real, effect on genotyping performance.

Somatic genetic aberrations, including LOH, are the second potential concern with FFPE-T genotyping. The strict discordance in FFPE-LN only accounted for 27.2% of that seen in FFPE-T, suggesting that the tumor genome may introduce some genotyping errors, as has been documented by some groups for individual SNPs (Goetz et al., 2014; Marisi et al., 2014). It is even possible that the discordance seen in a given patient could depend on the section of tumor from which the DNA was extracted due to intra-tumor heterogeneity. Alternatively, several other groups have reported high concordance (>98.9%) between comparisons of germline and frozen (non-FFPE) tumor (Marsh et al., 2005; A. Thompson et al., 2010) and matched FFPE normal and malignant tissue (Ahern et al., 2010; Schneider et al., 2006). This high concordance in direct comparisons of tumor versus normal that were similarly processed and stored confirms
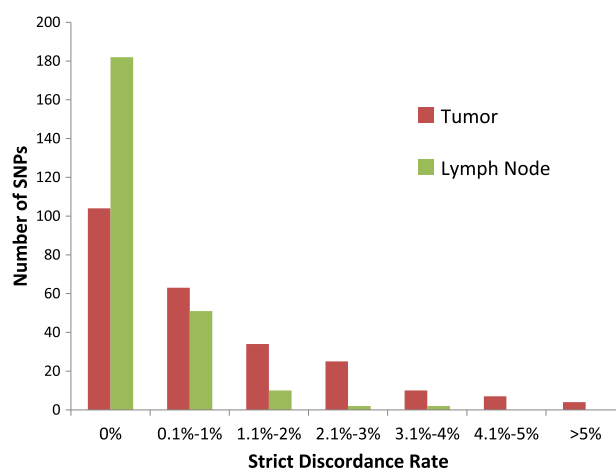
Figure 3 — Strict discordance rates for FFPE-T and FFPE-LN. Histogram of the strict discordance rates in FFPE-T and FFPE-LN, each compared with blood, for all SNPs included in the analysis. Overall the discordance rates were quite low, with very few SNPs having more than 1% discordant calls between either tissue of interest and blood.

that the tumor genome introduces very little genotyping error. The enrichment of discordance within heterozygous samples could be taken as evidence that tumor LOH interferes with genotyping accuracy a small amount, however, LOH is not the only, or even the most likely, explanation for this enrichment. The Sequenom MassARRAY calls genotypes by signal detection from each allele; therefore, accurate genotype calling of heterozygous samples can be more challenging, particularly for assays with differential binding efficiencies. This alternative hypothesis is supported by the coincident enrichment in heterozygous samples for no-calls, which would not be a consequence of LOH, and strict discordant calls in heterozygous FFPE-LN samples, which do not contain LOH.

The final concern with FFPE-T genotyping is the comparatively low yield of DNA extracted from these samples. We found that no-call and discordant call rates were higher in FFPE-T with lower DNA concentration. DNA extraction yield can vary greatly depending on the tumor sample type (i.e. core biopsy or section), size, and tumor content and genotyping platforms vary in their minimal DNA requirement and sensitivity to low DNA concentration. Thus, ensuring adequate sample availability, maximizing DNA extraction, and using genotyping platforms that are less sensitive to the amount of DNA template could further improve genotyping performance from FFPE-T.

Filtering samples with low genotype call rate is a standard aspect of genotyping quality control; three tumor samples with call rate <80% were eliminated from this analysis. Retaining these samples in the analysis would have increased the rate of tumor no-calls and discordant calls, potentially leading to classification of additional SNPs as FFPE-T failures. Conversely, setting a more stringent threshold, such as call rate <90%, would have eliminated additional samples and decreased the amount of discordance and the number of FFPE-T SNP failures. Similarly, adjusting the discordance thresholds above which SNPs were classified as FFPE-T failures has a direct consequence on the proportion of SNPs considered analytically validated, as depicted by the horizontal lines in Figure 2B.

The generalizability of these findings to other SNPs, tumor types, or genotyping protocols is limited. It is not possible to extrapolate our findings to SNPs that were not interrogated in our system and we could not identify any genes or genetic regions that were particularly difficult to genotype due to the small number of individual genes represented by multiple SNPs (data not shown). Analytic validity of our platform may not extend to FFPE-T samples from other cancer types given the differences in frequency of somatic genetic mutation, rearrangement, and LOH across cancers (Cancer Genome Atlas Network, 2012a; Cancer Genome Atlas Network, 2012b; Cancer Genome Atlas Research Network, 2012). The SNPs that were analytically validated in this study are likely specific

Table 3 – SNPs with possible discordance > 10% in FFPE-T.

Study label	Gene	rsID	# FFPE-T strict discordant calls	# FFPE-T no-calls	# blood no-calls	Possible discordance[a]
4E	VEGFC	rs1485766	1	8	3	10.5%
6O	ESR1	rs2046210	5	7	1	11.4%
6T	ESR1	rs3853250	3	8	1	10.5%
6Z	ESR1	rs1709181	4	8	1	11.4%
6BD	OPRM1	rs1799971	1	70	0	62.3%
7A	Intergenic	rs2097903	2	10	0	10.5%
7F	ABCB1	rs2032582	2	15	3	17.5%
7G	CYP3A	rs10273424	0	27	1	24.6%
9A	SLC28A3	rs7853758	0	44	2	40.4%
10M	CYP2C8	rs11188172	0	25	1	22.8%
10Q	CYP17A1	rs4919687	0	12	0	10.5%
11B[b]	PGR	rs500760	6	6	0	10.5%
12M	NCOR2	rs1812730	1	10	1	10.5%
13J	HTR2A	rs6311	2	18	4	21.1%
13L	SLC10A2	rs2301159	3	7	3	11.4%
14I	ESR2	rs1952585	1	18	1	17.5%
14R	AKT1	rs2494732	3	10	5	15.8%
15E[b]	CYP19A1	rs9806371	11	22	2	30.7%
15S	CYP19A1	rs730154	0	10	2	10.5%
15U	CYP19A1	rs4774585	2	5	5	10.5%
16D	SPG7	rs12960	4	10	0	12.3%
17A	PELP1	rs9436	2	11	0	11.4%
22D	COMT	rs6269	1	12	0	11.4%
22E[b]	COMT	rs4633	8	6	0	12.3%
22F	COMT	rs4818	3	10	0	11.4%
22L	EP300	rs20551	1	8	5	12.3%
22Q	NDUFA6	rs1801311	4	10	0	12.3%
22R	CYP2D6	rs28371738	1	13	5	16.7%

a Calculated as: # of strict discordant calls + FFPE-T no-calls + blood no-calls/number of patients (n = 114).
b Also failed due to strict discordance >5% in FFPE-T (Table 2).

to the genotyping platform (Nasri et al., 2010) and multiplex assays (Horn et al., 2010) utilized within this protocol. Therefore, others should perform assessments of analytic validity for genotyping their SNPs on their platform using their samples, which may have distinct pre-analytic features that affect genotyping performance (Bass et al., 2014).

5. Conclusions

In conclusion, genotyping from FFPE-T for breast cancer specimens has slightly higher rates of no-calls but is highly concordant to genotyping from germline DNA. The small loss of genotyping performance from FFPE-T is caused primarily by lower DNA yield, which is easily identified and overcome prior to analysis. The success rate could likely be improved by limiting multiplexing (Horn et al., 2010) or performing targeted amplification prior to genotyping (Baak-Pablo et al., 2010), if desired. While germline DNA remains the gold standard for pharmacogenetic analyses, FFPE-T DNA is a viable alternative for prospective–retrospective analyses of clinical trials. Analyses utilizing DNA isolated from FFPE should report whether the source tissue was normal, tumor, or a mixture of the two, and document analytic validity for genotyping each SNP using the appropriate tissue source and genotyping platform. We report here analytic validity for 218 cancer pharmacogenetics SNPs that can be accurately genotyped from breast FFPE-T on our custom Sequenom Multiplex array.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molonc.2015.07.002.

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