Screening of pharmacogenetic variants associated with drug sensitivity in patients with papillary thyroid carcinoma using next generation sequencing

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ABSTRACT
Thyroid cancer is the most common malignant tumour of the endocrine system. One of the most frequent types of thyroid malignancy is papillary carcinoma. In our study, we performed next generation sequencing (NGS) using a cancer panel (Illumina; Illumina, San Diego, USA) to screen for pharmacogenetic susceptibility variants in blood samples of 10 patients with papillary thyroid cancer (PTC). We report variants rs1042522 (TP53), rs2228001 (XPC), rs2227983 (EGFR), rs13181 (ERCC2), rs17655 (ERCC5) and rs1799939 (RET), which were detected in the analyzed patients either in homozygous and/or heterozygous state previously known to be connected with pharmacogenetic sensitivity to certain drugs in oncology. The results showed the TruSight Cancer Panel to be a useful clinical tool for determination of oncotherapy-associated pharmacogenetic variants in the blood of patients.

KEYWORDS
Papillary thyroid cancer (PTC); drug sensitivity; next generation sequencing (NGS); pharmacogenetic variants

Introduction
Thyroid cancer is the most common malignancy of endocrine organs. The most frequent types of thyroid malignancy are papillary thyroid cancer (PTC) and follicular thyroid cancer [1]. Different methods have been applied for the identification of the most common mutations associated with thyroid carcinoma, including Sanger sequencing, pyrosequencing, real-time polymerase chain reaction (PCR) amplification, allele-specific PCR [2]. Most patients with PTC (70%) carry a mutation in some of the genes belonging to the MAPK-signaling pathway. The high frequency of these mutations in thyroid cancer leads to a high degree of dependency on this pathway and its downstream effectors MEK1 and MEK2 [3]. Several targeted agents have been developed against cancers with the BRAF V600E mutation but acquired resistance to these agents has emerged as a new obstacle for this treatment [4,5]. Several mechanisms of resistance to RAF inhibitors have been discussed [6,7]. However, the incidence of acquired drug resistance in patients with PTC still remains to be elucidated. It is important to identify and screen for pharmacogenetic variants connected with the therapeutic response in patients subjected to chemotherapy and/or targeted therapy. Next generation sequencing (NGS) is a powerful up-to-date method for the identification of such variants.

In this study, we applied a complex approach using NGS data for screening of variants known to be associated with drug sensitivity in order to prevent adverse drug effects in PTC patients.

Materials and methods
DNA extraction and NGS sequencing
Genomic DNA was extracted from blood samples of 10 individuals belonging to four families after obtaining a signed written informed consent form. DNA concentrations were measured by the Qubit 2.0 Fluorometric Quantitation system using the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

Sequencing was performed using the Illumina platform and the TruSight™ Cancer Sequencing Panel (Illumina, San Diego, CA, USA). The TruSight™ Cancer Sequencing Panel targets 94 genes, including genes associated with both common and rare cancers. The input DNA (50 ng) from each sample was randomly fragmented, tagged with unique adapter sequences, purified...
and amplified by a 12-cycle PCR using primers complementary to the adapter sequences flanking the fragments. The TruSight Cancer Content Set contains a set of 80-mer capture probes designed to target the exons of the 94 genes (Table 1), as well as 284 single-nucleotide polymorphisms (SNPs). The number of targeted exons is over 1700 and the cumulative size of the target region is 255 kb of the human genome. Sequencing was performed on an Illumina MiSeq System (San Diego, CA, USA).

### Data analysis

After sequencing was completed, analysis of the sequencing data was performed using the Softgenetics NextGene Software (version 2.3.3). The software automatically processes the data, performing alignment of the sequences to the human reference genome (GRCh37), filtering and variant calling. The variants found were further analyzed by mutation prediction software SIFT (version 5.2.2) and PolyPhen-2 (version 2.2.2) to determine what their possible effect on protein functionality might be. Variants were subsequently checked and analyzed using the PharmGKB database (https://www.pharmgkb.org/) for pharmacogenetic SNP variants associated with sensitivity to certain drugs.

### Results and discussion

Thyroid cancer is commonly treated by one or a combination of treatments, including surgery, hormone and radioactive iodine treatment, targeted therapy, external-beam radiation therapy and chemotherapy [8]. Recent data suggest that specific molecular profiles, such as the presence of *BRAF* in a combination with other oncogenic mutations (in *PIK3CA*, *AKT1*, *TERT* promoter, or *TP53* mutations) may serve as specific markers of less favorable outcome of PTC [9–11]. In order to predict the effect to specific drug therapy, we used the NGS data of our PTC patients (Cancer Panel, 94 genes) and screened for variants connected to drug sensitivity using a web available pharmacogenetic database (https://www.pharmgkb.org/). Out of 2858 SNPs in 94 cancer-related genes, we selected six variants most frequently present in our PTC patients known to be associated with drug sensitivity. They are presented in Table 2.

All variants in the six genes were classified as tolerated/benign according to SIFT and PolyPhen prediction parameters; 5 out of 6 were shown to be present in at least half of the patients (5 out of 10) (rs13181, rs1042522, rs2227983, rs2228001, rs1799939). One variant was detected in three patients (rs17655). Among them, *TP53* rs1042522 and *ERCC2* rs13181 have been reported in previous studies of thyroid cancer [12,13].

*TP53* rs1042522 was found in the majority of the analyzed patients (8/10 patients); three samples were heterozygous (GC), whereas five ones were homozygous (CC). The rs1042522 polymorphism has been widely studied as a predisposing variant to thyroid cancerogenesis [12] but little is known about its role in drug-induced toxicity. Recent data shows that the Pro allele of *TP53* is associated with a higher risk of osteoradionecrosis in patients with head and neck cancer on radiation therapy [14]. The GC genotype, which determines an increased risk of toxicity when treating patients with standard chemotherapeutic drugs (fluorouracil and cyclophosphamide), was present in three of our 10 patients (Table 2). This is important when considering the therapeutic regime of PTC patients and has to be taken into consideration, possibly resulting in lowering the dosage of the drugs.

The rs13181 polymorphism in *ERCC2* has long been known as a factor that influences the chemotherapeutic outcome in patients. In breast cancer patients, carriers of the GG and GT genotype have decreased risk of neutropenia when treated with docetaxel as compared to the other genotypes (GT) [16]. In thyroid cancer, *ERCC2* rs13181 has not been profoundly studied. One of our patients, with the GG genotype, was associated with decreased survival after application of platinum compounds.

*RET* is well known to be associated with PTC. The rate of *RAS* point mutations and *RET/PTC1* and *RET/PTC3* mutations are associated with increased survival of PTC patients compared to other mutations [17]. In conclusion, our study demonstrates the utility of NGS technologies in the clinical management of patients with PTC, highlighting the potential impact of pharmacogenomic testing on clinical decision-making.

### Table 1. An alphabetical list of the genes targeted by the capture probes of the TruSight Cancer Content Set.

| Gene    | Symbol  |
|---------|---------|
| AIP     | BUB1B   |
| ALK     | CDX73   |
| APC     | CDH1    |
| ATM     | CDK4    |
| BAP1    | CDKN1C  |
| BLM     | CDKN2A  |
| BMPR1A  | CEBPA   |
| BRCA1   | CEP57   |
| BRCA2   | CHEK2   |
| BRIP1   | CYLD    |

**Table 1.** An alphabetical list of the genes targeted by the capture probes of the TruSight Cancer Content Set.
rearrangements has been broadly studied in different populations and age groups [17–19]. The RET rs1799939 variant was present in seven analyzed samples in a heterozygous form (GA). It has been reported that the AA and GA genotypes are not associated with the overall survival of patients with carcinoma treated with sunitinib as compared to patients with the GG genotype [20]. None of our patients carried the risk-associated genotype GG, which is associated with drug toxicity of sunitinib.

Meta-analysis shows that there is no significant association between the rs2227983 polymorphism in EGFR and risk of cancer but the relation between the rs2227983 polymorphism and the chemosensitivity of anticancer drugs remains to be elucidated [21]. In our study, we found the AA genotype in only one patient. There is no evidence for chemosensitivity in thyroid cancer patients who are carriers of the A allele, although patients with NSCLC, colorectal and pancreatic neoplasms with the AA and AG genotype may have a decreased risk of rash when treated with epidermal growth factor receptor (EGFR) inhibitors, such as erlotinib, as compared to patients with the GG genotype [22,23].

There are reports that the variants XPC rs2228001 and ERCC5 rs17655 show a chemosensitivity effect [24–26]. Four of our patients carried the risk-associated genotype GT in XPC. These patients could be expected to potentially develop toxicity when treated with cisplatin. According to Caronia et al. [24,25] and Sakano et al. [24,25], in patients with osteosarcoma and urinary bladder neoplasms, the GT genotype carriers have an increased risk of toxicity with cisplatin treatment, including hearing loss and neutropenia. Patients with the TT genotype may have a decreased, but not absent, risk of toxicity with cisplatin treatment as compared to patients with the GG or GT genotype [24,25]. Similarly, the rs17655 variant in the ERCC5 gene is considered a survival-modifying factor in ovarian cancer [26]. The authors suggest that patients with the CG genotype and early

| Gene | Chr. position | SIFT/ PolyPhen | SNP | Genotype | Number of patients with the SNP | Drug sensitivity | Type of polymorphism |
|------|--------------|----------------|-----|----------|--------------------------------|-----------------|---------------------|
| TP53 | 17           | Tolerated/ Benign | rs1042522 | Heterozygote GC | 3 | Increased risk of toxicity with cyclophosphamide and fluorouracil treatment | Missense |
|      |              |                |     | Homozygote GG           | 0 |                                |                |
|      |              |                |     | Homozygote CC           | 5 | Decreased risk of toxicity with cyclophosphamide and fluorouracil treatment | Missense |
| ERCC2 | 19       | Tolerated/ Benign | rs13181 | Heterozygote TG | 6 | Higher survival when treated with platinum compounds | Missense |
|      |            |                |      | Homozygote TT           | 1 |                                |                |
|      |            |                |      | Homozygote GG           | 0 | Decreased survival when treated with platinum compounds | Missense |
| RET  | 10         | Tolerated/ Benign | rs1799939 | Heterozygote GA | 7 | Not associated with drug toxicity when treated with sunitinib | Missense |
|      |            |                |      | Homozygote AA           | 0 |                                |                |
|      |            |                |      | Homozygote GG           | 0 | Associated with drug toxicity when treated with sunitinib | Missense |
| EGFR | 7           | Tolerated/ Benign | rs2227983 | Heterozygote GA | 5 | Decreased risk of rash when treated with EGFR inhibitors | Missense |
|      |            |                |      | Homozygote AA           | 1 |                                |                |
|      |            |                |      | Homozygote GG           | 0 | Increased risk of rash when treated with EGFR inhibitors | Missense |
| XPC  | 3           | Tolerated/ Benign | rs2228001 | Heterozygote GT | 4 | Increased risk of toxicity with cisplatin treatment | Missense |
|      |            |                |      | Homozygote GG           | 0 |                                |                |
|      |            |                |      | Homozygote TT           | 3 | Decreased risk of toxicity with cisplatin treatment | Missense |
| ERCC5 | 13     | Likely benign  | rs17655 | Heterozygote GC | 3 | Increased progression-free survival when treated with platinum-based chemotherapy | Missense |
|      |            |                |      | Homozygote CC           | 0 |                                |                |
|      |            |                |      | Homozygote GG           | 0 | Decreased progression-free survival when treated with platinum-based chemotherapy | Missense |

Note: The table shows the gene; chromosome localization; prediction parameters (SIFT and PolyPhen); SNP name; genotypes; number of individuals with this genotype; drug sensitivity associated with the rs (https://www.pharmgkb.org/); mutation type.
stage ovarian cancer may have increased overall survival, whereas patients with the CC genotype and late stage ovarian cancer may have decreased overall survival when treated with platinum-based chemotherapy, as compared to patients with the GG genotype [26]. According to our NGS data, only three of our patients were revealed to be heterozygote carriers (GC), which, on the other hand, defines increased progression-free survival after platinum treatment.

Conclusions

Many genetic markers in oncology have been recently revealed to confer sensitivity to drug therapy. In this study, we report six variants (rs13181, rs2227983, rs1042522, rs2228001, rs17655, rs1799939) in cancer-related genes known to be associated with drug sensitivity. These variants are important for the future prognosis of the treatment outcome in thyroid cancer. Thus, the NGS sequencing method could be beneficial as a screening approach to detect pharmacogenetic variants of known importance for the therapeutic response in cancer patients.

Disclosure statement

None declared.

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