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Simple Summary: Despite the progress in elucidating the molecular mechanisms underlying differentiated thyroid cancer (DTC) thanks to genome sequencing, germline mutations responsible for genetic susceptibility to DTC are still poorly recognized. Since the genetic background predisposing to DTC may differ among populations, the study aimed to assess the prevalence of genetic germline mutations predisposing to DTC in a cohort of Polish individuals, using whole-genome sequencing (WGS). To date, this is the first analysis harnessing WGS data for the Slavic population - which accounts for over 4.5% of the world's inhabitants. The frequency of variants discovered in the Polish cohort (our study) – genetic reference for the Slavic population - was compared to the variant frequency estimated for the non-Finnish European population obtained from the gnomAD database. The results show statistically significant variants in *APC*, *MYH9*, *PALB2*, *PLCB1*, *PTEN*, *RET*, *STK11*, *BRCA1* and *TERT* genes, with different variant frequencies among the populations.

Abstract: The genome sequencing technologies reveal the molecular mechanisms of differentiated thyroid cancer (DTC). Unlike somatic mutation analysis from thyroidectomy samples, germline mutations showing genetic susceptibility to DTC are less understood. The study aimed to assess the prevalence of germline mutations predisposing to DTC in a cohort of Polish individuals based on their whole genome sequencing (WGS) data. We analyzed sequencing data from 1076 unrelated individuals, released openly for academic and clinical research as The Thousand Polish Genomes database (https://1000polishgenomes.com). The list of genes chosen for further analysis was based on the review of previous studies. The cohort contained 104 variants located within the coding and noncoding DNA sequences of 90 genes selected by ClinVar classification as pathogenic and potentially pathogenic. The frequency of variants in the Polish cohort (our study) was compared to the frequency estimated for the non-Finnish European population, obtained from the gnomAD database (gnomad.broadinstitute.org). Statistically significant variants included 23 genes. Even though the Polish population is genetically similar to other European populations, there are significant differences in variant frequencies contributing to the disease development and progression, such as *RET, CHECK2, BRCA1, SLC26A4* or *TERT*. Further studies are needed to identify genomic variants associated directly with DTC.

Keywords: thyroid cancer; whole-genome sequencing; non-medullary thyroid cancer; germline; genetics

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1. Introduction

Thyroid cancer is the most common malignant endocrine tumor [1]. It accounts for 1-2% of all neoplasms [2]. Its prevalence is steadily and the most rapidly rising among all cancers [3]. This increasing trend may be partly linked to an improved detection of smaller (< 2 cm) tumors thanks to more frequent and better ultrasound detection, fine-needle aspiration biopsies and the increased pathological reporting of incidental microcarcinomas [4,5]. In 2020, the World Health Organization (Global Cancer Statistics 2020: GLOBOCAN) reported 586202 new cases of thyroid cancer, of which 43646 resulted in death [6].

Thyroid cancer types are classified according to their histological characteristics (Figure 1) [7]. Differentiated thyroid cancer (DTC) is also known as non-medullary thyroid cancer (NMTC) and represents approximately 90% of all thyroid cancers [8]. Over 90% of thyroid cancer is sporadic, due to somatic genetic alterations [9]. Only 3-9% of all thyroid cancers are familial non-medullary thyroid cancer (FNMTC) cases, defined by the presence of thyroid cancer in 2 or more first-degree relatives, in the absence of predisposing environmental factors [10]. FNMTC can be divided into syndromic or non-syndromic FNMTC. It depends on whether the thyroid cancer is a part of one of many constellations of tumors (syndromic) or is the primary cancer (non-syndromic FNMTC) [11]. Out of all FNMTCs, only 5% in the syndromic form have well-defined driver germline mutations. On the contrary, 95% of FNMTC is non-syndromic with less well-defined genetic susceptibility [11].

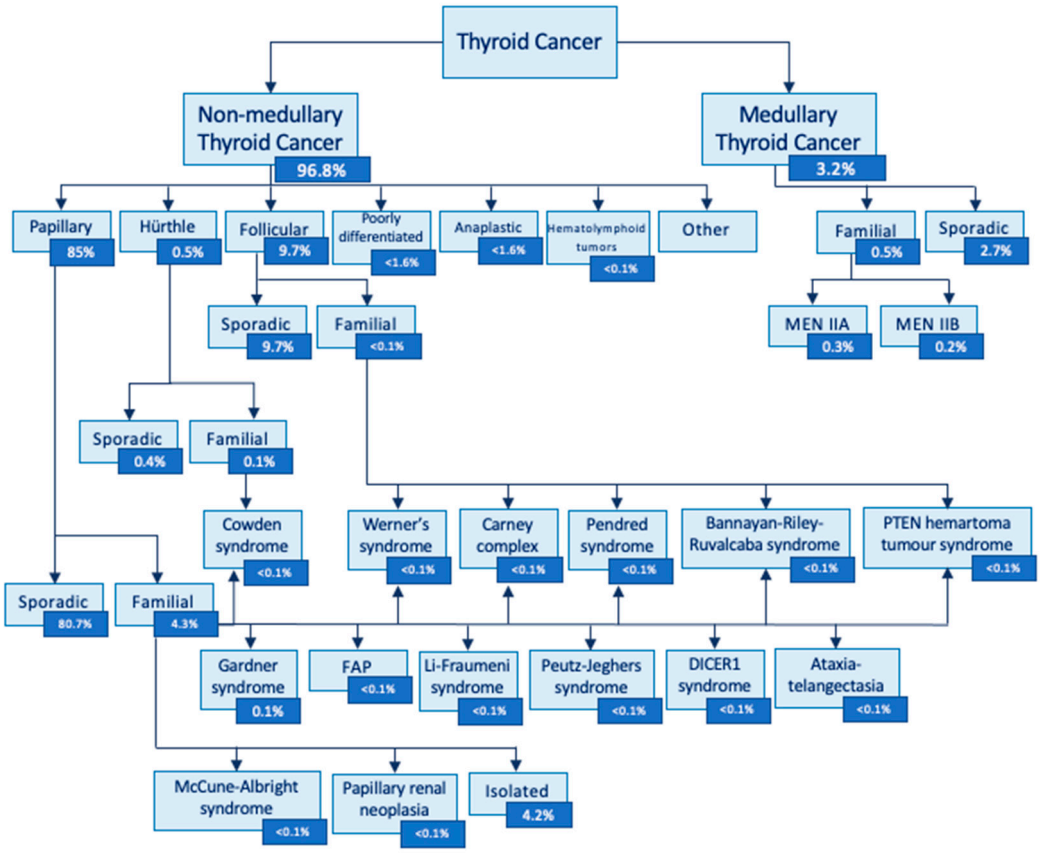

Figure 1. Thyroid cancer classification (modified from Vriens et al. 2009 [7], Hińcza et al. 2019 [8] and Kamani et al. 2022 [12].

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In the last 30 years, the availability of the genome sequence has enabled progress in elucidating the molecular mechanisms underlying thyroid cancer. However, most studies involved somatic mutations analysis from fresh frozen paraffin embedded samples from thyroidectomy [13]. Driver mutations that promote cancer development were identified in over 90% of TC [14], but germline mutations showing genetic susceptibility to differentiated thyroid cancer are less studied. For the latter, genetic studies are vital. Despite the completion of the Human Genome Project [15], the information on the full spectrum of the human genetic variation remains incomplete [16]. Open, population-scale databases of human genetic variation are important for clinical genetics, biomedical research, prioritizing and tailoring genetic screening programs or improving guidelines for genetic counselling [17].

The high-quality of the sequencing data enabled the building of a unique repository of genetic variation in the Polish population, released publicly as the Thousand Polish Genomes database [17]. This database includes small and structural variants, runs of homozygosity, mitochondrial haplogroups, and novel variants identified in genomes of 1076 Poles.

The genetic background predisposing to differentiated thyroid cancer may differ among populations. To our best knowledge, there is no analysis including the Slavic population - accounting for over 4.5% of the world's inhabitants. The Polish population, which is homogenous and sedentary in its nature but influenced by many migrations of the past, is not unique and very similar to many other European populations, therefore can serve as a genetic reference for the Slavic populations as long as there are no broader studies in other countries [17].

The risk of thyroid cancer inheritance may be 8- to 12-fold higher for first-degree relatives compared to the general population [18,19]. It makes thyroid cancer one of the most heritable cancers, displaying Mendelian inheritance [12]. The comparison of the aggressiveness of the disease in FNMTC patients compared to sporadic cases brings ambiguous results [12]. It may result in a more aggressive disease course at a younger age, with larger tumors and more lymph node involvement [20–22]. FNMTC may also express clinical anticipation with presentation at a younger age, with more severe symptoms at the second generation (genetic anticipation) [23]. Early diagnosis based on screening enables identification of thyroid cancer tumors of smaller size with less lymph node metastases and thus requiring less extensive treatments, potentially improving the treatment outcome [24]. The penetrant mutations in susceptibility genes to FMNTC could be vital for identifying at-risk individuals, thereby making early diagnosis, and selecting appropriate treatment possible [12]. If there exists genetic heterogeneity in the risk assigned to particular susceptibility genes or/and significant differences in risk allele frequencies, genetic screening should be matched to the relevant population. Therefore, the aim of the study was to assess the prevalence of germline mutations predisposing to the differentiated thyroid cancer (FMNTC) in a cohort of Polish individuals and compare prevalence of these mutations to the non-Finnish European population.

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2. Materials and Methods

2.1. Gene Search Strategy

The first step was to define the list of genes, which germline mutations have been already linked to increased risk of developing differentiated thyroid cancer. The search strategy included Medical Subject Headings terms and keywords: "familial" OR "hereditary" AND "non-medullary thyroid cancer". Reference lists of all the selected articles, previous meta-analyses, and reviews were hand-searched for any additional articles. We included studies, regardless of their sample size, with the investigation of the association between germline mutations and differentiated thyroid cancer occurrence. We carried out the systematic review following the guidelines formulated in the Cochrane Handbook for Systematic Reviews of Interventions [25] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26]. We searched PubMed, MEDLINE, Academic Search Complete, CINAHL Complete, CINAHL, Scopus, Cochrane, Health Source: Nursing/Academic Edition, Web of Knowledge, MasterFILE Premier, Health Source-Consumer Edition, Agricola, Dentistry and Oral Science Source databases from January 2006 up to January 2023 to find all relevant, full-text journal articles written in English.

2.2. Data Extraction and methodology assessment

Two authors (MBo and MBr) independently selected publications, which fulfilled the inclusion criteria specified above and extracted data for the outcomes using a standardized data extraction form. The risk of bias in the included studies was independently assessed based on the Cochrane risk of bias tool [27]. All included studies were assessed using the Newcastle-Ottawa Scale [28]. Only studies rated with at least seven stars were included for further consideration. Abstracts/papers focused on somatic mutations were excluded. The final list of NMTC susceptibility genes was identified and divided into genes related to syndromic and nonsyndromic FNMTC.

2.3. Genetic study

The cohort analyzed in this study consisted of 1076 unrelated individuals of Polish origin. The median age of participants was 45.4 years, with predominance of males (697 vs. 525). The analysis of clinical data showed that the most common chronic diseases reported by the participants were hypertension (13.0%), cancer (4.6%), diabetes (4.0%), and hypothyroidism or Hashimoto's disease (3.0%). No health problems (excluding COVID-19 infection) were reported by 86% of participants. The sequence data encompassed over 1018 billion read pairs, yielding an average 35.26× read depth per genome. In every sample, over 91% of the reference genome was covered with at least 10 reads. The allelic frequencies of small and structural variants identified in this cohort were released openly for academic and clinical research as the Thousand Polish Genomes database (POL) [17].

The list of genes selected for further analysis (Figure 2 and Table 1) was based on the review of previous studies and included 90 genes involved in the development of DTC. The variants were annotated using the following resources: Ensemble Variant Effect Predictor v.108 [29], including references to databases of genomic variants from ClinVar v. 20221224 [30], and dbSNP build 154 [31], variant population frequencies from the 1000 Genomes Project, and gnomAD v2.0.1 and v3.0, as well as pathogenicity scores, such as Polyphen-2, SIFT and CADD [32]. All gene coordinates were padded with variants in the 10 kb range at both ends of the genes. The analyzed set contained 165736 variants in 90 genes. Following the ClinVar classification, we filtered pathogenic and potentially pathogenic variants for further analysis. The frequency of variants in the Polish cohort (our study) was compared to the frequency estimated for the non-Finnish European (NFE) population, obtained from the gnomAD database (accessed on 2023-01-19). Per gene cumulative allele frequencies were calculated and defined as the relative frequency of an allele (variant of a gene) at a particular locus in a particular gene in a population, expressed as a fraction or percentage [33].

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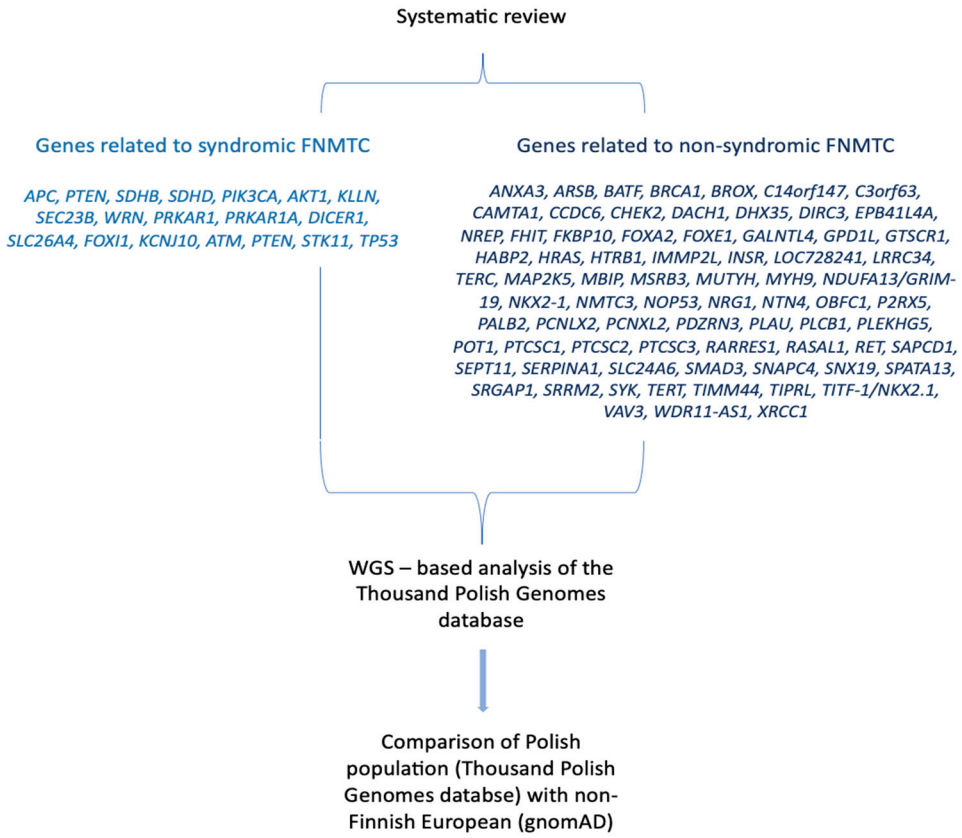

Figure 2. Workflow for assessing the prevalence of germline mutation predisposing to the differentiated thyroid cancer in a cohort of Polish individuals using a genome-wide association study.

Table 1. Genetic syndromes and genes related to syndromic familial non-medullary thyroid cancer; adapted from Kamani et al. [12].

Name	Gene	Mode of Inheritance	Thyroid cancer histological subtype*	Phenotypes other than thyroid cancer
FAP and Gardner's syndrome	*APC*	Autosomal dominant	PTC	Colorectal carcinoma, ampullary carcinoma, hepatoblastoma, medulloblastoma
Cowden syndrome	*PTEN, SDHB-D, PIK3CA, AKT1, KLLN, SEC23B*	Autosomal dominant	cPTC, fvPTC, FTC	Multiple hamartomas, follicular thyroid carcinoma, benign thyroid nodules, breast cancer, endometrial cancer
Werner syndrome	*WRN*	Autosomal recessive	PTC, FTC, ATC	Premature ageing, scleroderma-like skin changes, cataracts, subcutaneous calcifications, muscular atrophy, diabetes
Carney complex	*PRKAR1*	Autosomal dominant	PTC, FTC	Spotty skin pigmentation, cardiac myxomas, endocrine tumors
DICER1 syndrome	*DICER1*	Autosomal dominant	PTC, DTC	Endocrine tumors (thyroid, parathyroid, pituitary, pineal gland, endocrine pancreas, paragangliomas, medullary, adrenocortical, ovarian, and testicular tumors
Pendred syndrome	*SLC26A4, FOXI1, KCNJ10*	Autosomal recessive	PTC, FTC, ATC	Sensorineural deafness/hearing impairment, goiter, and an abnormal organification of iodide with or without hypothyroidism
Ataxia-telangiectasia	*ATM*	Autosomal recessive	PTC	Cerebellar degeneration, telangiectasia, immunodeficiency, recurrent sinopulmonary infections, radiation sensitivity,

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				premature aging, lymphoid cancer, poor growth, gonadal atrophy, insulin resistant diabetes
Bannayan-Riley-Ruvalcaba syndrome	*PTEN*	Autosomal dominant	PTC, FTC	Macrocephaly, hamartomatous tissue overgrowth, lipomas, pigmented macules on the penis, developmental delay, large birth weight, joint hyperextensibility, endometrial cancer, renal cell carcinoma, Lhermitte–Duclos disease
Peutz-Jeghers syndrome	*STK11*	Autosomal dominant	PTC, DTC	Gastrointestinal polyposis, mucocutaneous pigmented macules, breast cancer, uterine cancer, cervical cancer, lung cancer, ovarian cancer, testicular cancers
PTEN hamartoma tumor syndrome	*PTEN*	Autosomal dominant	FTC, PTC, fvPTC	Breast cancer, endometrial cancer, gastrointestinal hamartomas, Lhermitte-Duclos disease, macrocephaly, macular pigmentation of the glans penis, multiple mucocutaneous lesions, autism spectrum disorder, colon cancer, esophageal glycogenic acanthosis, lipomas, mental retardation, renal cell carcinoma, testicular lipomatosis, thyroid adenoma, multinodular goiter
Li-Fraumeni syndrome	*TP53*	Autosomal dominant	cPTC, fvPTC	Adrenocortical carcinomas, breast cancer, central nervous system tumors, osteosarcomas, soft-tissue sarcomas, leukemia, lymphoma, gastrointestinal cancers, cancers of head and neck, kidney, larynx, lung, skin, ovary, pancreas, prostate, and testis

*PTC (papillary thyroid cancer), FTC (follicular thyroid cancer), ATC (anaplastic thyroid cancer), cPTC – classical variant of PTC, fvPTC (follicular variant of PTC), DTC – differentiated thyroid cancer.

Statistical significance of odds ratios for each variant was estimated with Fisher's exact test and corrected for false discovery rate (FDR), where only variants with q-value < 0.05 were considered significant.

2.4. Rechecking data in genetic resources

Variants with significant differences in allele frequency among the Polish population (Thousand Polish Genomes database) and the non-Finnish European population (gnomAD) were clinically annotated using resources such as: ClinGen, NCCN guidelines, OMIM, Genetics Home Reference, GeneCards, ClinVar, and Gene-NCBI to describe their role and possible impact on DTC occurrence.

3. Results

Among the 90 genes considered, 19 were related to syndromic FNMTC (Table 1) and 71 were related to non-syndromic FNMTC. Following the ClinVar classification, out of the 165736 variants located in those genes, we selected pathogenic and potentially pathogenic ones for further analysis. The alleles frequencies of variants in the Polish cohort (our study) were compared to the frequencies estimated for the non-Finnish European population obtained from the gnomAD database. Among them, 23 had significantly different frequencies in the Polish population than in non-Finnish European: *APC, ARSB, ATM, BRCA1, CHEK2, DICER1, GPD1L, INSR, KCNJ10, MYH9, PALB2, PLCB1, PLEKHG5, PTEN, RET, SEC23B, SERPINA1, SLC26A4, SMAD3, STK11, TERT, TOE1,* and *WRN*. Most of those genetic variants are more frequent among Polish population, except from *DICER1* and *KCNJ10*.

Among genes related to syndromic FNMTC (Table 1) those significantly characteristic for Polish population and with the highest OR were (Table 2, Table S1) *APC* (rs201375478; OR = 22.99), *ATM* (rs3092859; OR = 13.55), *DICER1* (rs117358479; OR = 4.93), *KCNJ10* (rs145947380; OR = 11.76), *SEC23B* (rs138198461; OR = 4.77), *SLC26A4* (rs17154362; OR = 15.55), *STK11* (rs587782259; OR = 24.31), *WRN* (rs4987238; OR = 5.65). Among genes related to nonsyndromic FNMTC (Table 1) the ones significantly characteristic for the Polish population and with the highest OR were *ARSB* (rs200040980; OR = 8.62), *BRCA1* (rs80357087; OR = 17.02), *CHEK2* (rs121908698; OR = 47,42), *MYH9* (rs762239398; OR = 126,00), *PALB2* (rs377085677; OR = 21.08), *PTEN* (rs180953647; OR = 14.47), *RET* (rs377767388) (OR = 50.59), *TERT* (rs377216965) (OR = 42.15). The genetic variants with an impact as modifiers according to ClinVar were: *ARSB* (rs72764913), *ATM* (rs879796523), *DICER1* (rs1555368535), *KCNJ10* (rs116418256, rs192835895, rs56656397), *PALB2* (rs138200248), *PCLB1* (rs532302075), *PTEN* (rs180953647),

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SERPINA1 (rs11558258), *SLC26A4* (rs17154362), *SMAD3* (rs958007552), *STK11* (rs587782259), *TOE1* (rs3219466).

Table 2. Genes with significant (q-value <0.05) differences in pathogenic variant burden among Polish population (Thousand Polish Genomes database) compared with non-Finnish European (gnomAD); as indicated in the Table 1 in supplement, most of the genetic variants analyzed are more frequent among Polish population, however two exceptions are indicated in red below.

Gene	Type of thyroid cancer*	Hereditary syndromes	Other cancers	Reference
APC	PTC with cribriform pattern	FAP and Gardner's syndrome	Colorectal cancer, ampullary carcinoma, hepatoblastoma, medulloblastoma	Kamani et al. 2022 [34]; Cetta et al. 2015 [34]2023-09-07 8:41:00 AM
ARSB	DTC	N/A	N/A	Figlioli et al. 2014 [35]
ATM	PTC	Ataxia-telangiectasia	Cerebellar degeneration, telangiectasia, immunodeficiency, recurrent sinopulmonary infections, radiation sensitivity, premature ageing, lymphoid cancer, poor growth, gonadal atrophy, insulin resistant diabetes	Kamani et al. 2022 [12]
	PTC, DTC	N/A	N/A	Dombernowsky et al. 2008 [36]; Akulevich et al. 2009 [37]; Gu et al. 2014 [38]; Wójcicka 2014 [39]
BRCA1	PTC	N/A	N/A	Wójcicka et al. 2014 [39]
CHEK2	Non-syndromic DTC	N/A	Breast cancer, prostate cancer	Wang et al. 2019 [40]
	PTC	N/A	N/A	Wójcicka et al. 2014 [41]; Siołek et al. 2015 [42]; Zhou et al. 2021[41]
DICER1	PTC, DTC	DICER1 syndrome	Endocrine tumors (parathyroid, pituitary, pineal gland, endocrine pancreas, paragangliomas, medullary, adrenocortical, ovarian, and testicular tumors	Rutter et al. 2016 [43]
			Nephroblastoma, NMTC pleuropulmonary blastoma, cystic nephroma, multinodular goiter, thyroid adenoma, sex cord tumor	Zhou et al.2021 [41]

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GPD1L	DTC	N/A	N/A	Figloli et al. 2014 [35]
INSR	PTC	N/A	N/A	Son et al. 2017 [44]
	FTC	N/A	N/A	Lai et al. 2017 [45]
KCNJ10	ATC	Pendred	Sensorineural deafness/hearing impairment, goiter, and an abnormal organification of iodide with or without hypothyroidism	Liu et al. 2016 [46]; Yang et al. 2023 2023-09-07 8:41:00 AM
MYH9	PTC, FTC	N/A	N/A	Wang et al. 2017 [47]
PALB2	PTC	N/A	N/A	Kamihara et al. 2022 [48]
PLCB1	PTC	N/A	N/A	Bakhsh et al. 2018 [49]
PLEKHG5	PTC	N/A	N/A	Sarquis et al. 2020 [50]
PTEN	cPTC, fvPTC, FTC	Cowden syndrome	Multiple hamartomas, follicular thyroid carcinoma, benign thyroid nodules, breast cancer, endometrial cancer	Bevan et al. 2001 [51]; Pilarski et al. 2013 [52]; Hendricks et al. 2021 [53]; Bubien et al. 2013 [54]; Jonker et al. 2020 [55]; Ngeow et al. 2020 2023-09-07 8:41:00 AM
	PTC, FTC	Bannayan-Riley-Ruvalcaba syndrome	Macrocephaly, hamartomatous tissue overgrowth, lipomas, pigmented macules on the penis, developmental delay, large birth weight, joint hyperextensibility, endometrial cancer, renal cell carcinoma, Lhermitte–Duclos disease	
	FTC, PTC, fvPTC	PTEN hamartoma tumor syndrome	Breast cancer, Endometrial cancer, FTC, Gastrointestinal hamartomas, Lhermitte-Duclos disease, Macrocephaly, Macu- lar pigmentation of the glans penis, Multiple mucocutaneous lesions, Autism spectrum disorder, Colon cancer, Esophageal glycogenic acanthosis, Lipomas, Mental retardation, Renal cell carcinoma, Testicular lipomatosis, PTC, fvPTC, thyroid adenoma, MNG	
RET	PTC	FAP	Colorectal carcinoma, ampullary carcinoma, hepatoblastoma, medulloblastoma	Cetta et al. 2015 [34]

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SEC23B	cPTC, fvPTC, FTC	Cowden syndrome	Multiple hamartomas, follicular thyroid carcinoma, benign thyroid nodules, breast cancer, endometrial cancer	Yehia et al. 2015 [56]
SERPINA1	PTC	N/A	N/A	Vierlinger et al. 2011 [57]
SLC26A4	FTC, DTC	Pendred syndrome	Sensorineural deafness/hearing impairment, goiter, and an abnormal organification of iodide with or without hypothyroidism	Makhlouf et al. 2016 [58]
SMAD3	DTC	N/A	N/A	Gudmundsson et al. 2017 [59]
	PTC	N/A	N/A	Zhang et al. 2014 [60];
STK11	PTC, DTC	Peutz-Jeghers syndrome	Gastrointestinal (GI) polyposis, mucocutaneous pigmented macules, breast cancer, uterine cancer, cervical cancer, lung cancer, ovarian cancer, testicular cancers	Buryk et al. 2015 [61]; Wei et al. 2016 [62]
TERT	DTC	N/A	N/A	Gudmundsson et al. 2017 [59]
	PTC	N/A	N/A	Kim et al. 2022 [63]; Alzahrani et al. 2022 2023-09-07 8:41:00 AM
	ATC	N/A	N/A	Abe et al. 2021 [64]
TOE1	PTC	FAP	Colorectal carcinoma, ampullary carcinoma, hepatoblastoma, medulloblastoma	ClinVar [30]
WRN	PTC, FTC, ATC	Werner syndrome	Premature aging, scleroderma-like skin changes, cataracts, subcutaneous calcifications, muscular atrophy, diabetes	Lauper et al. 2013 [65]

* PTC (papillary thyroid cancer), FTC (follicular thyroid cancer), ATC (anaplastic thyroid cancer), cPTC – classical variant of PTC, fvPTC (follicular variant of PTC), DTC – differentiated thyroid cancer.

Figure 3 shows allele frequencies of variants in 25 genes with significantly different allele frequency between POL and gnomAD NFE. Table 2 describes genes with significant differences in allele frequencies between POL and gnomAD NFE.

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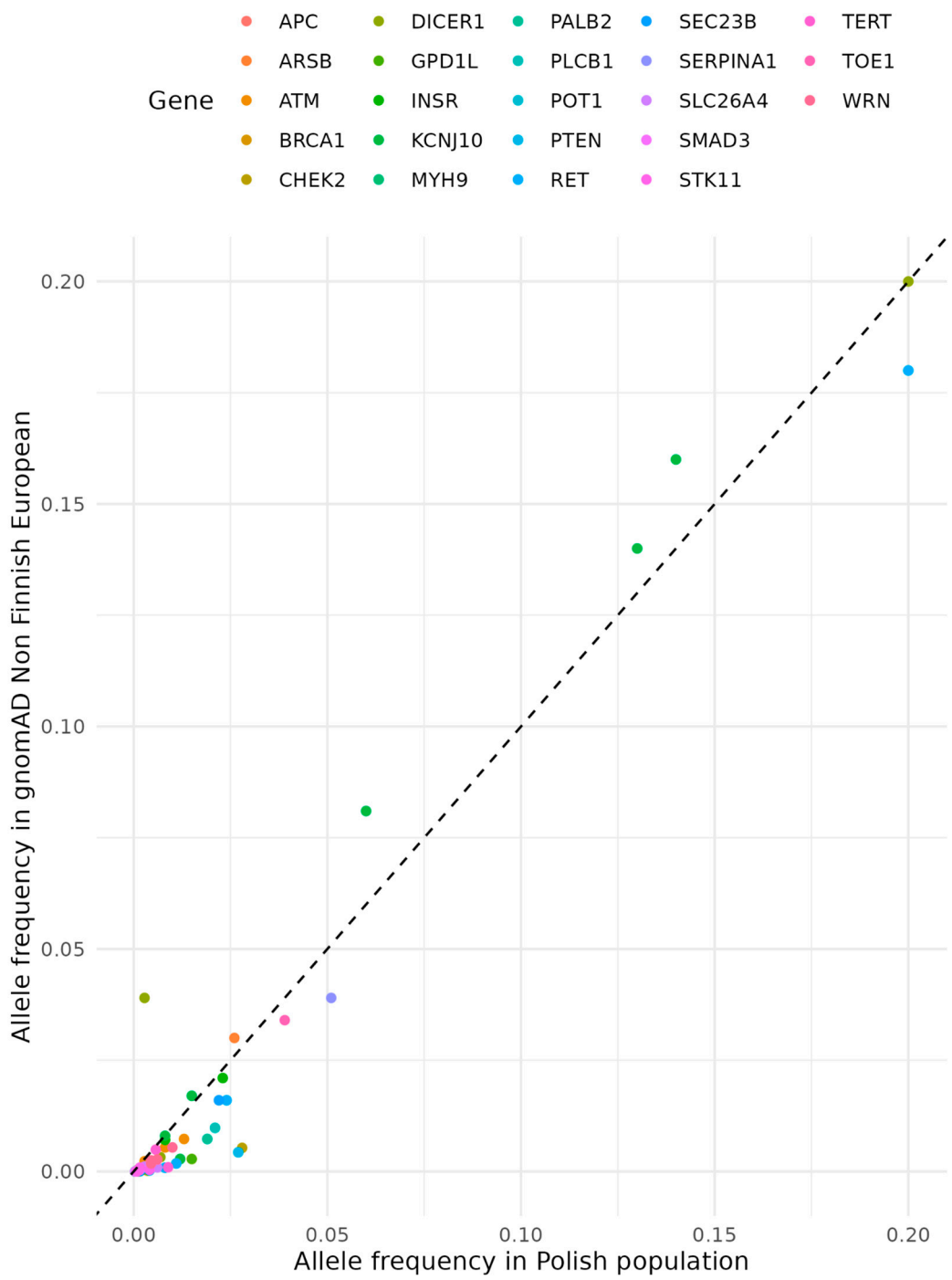

Figure 3. Allele frequencies of ClinVar variants in 23 DTC associated genes. All displayed variants are statistically significant (q-value < 0.05) in difference in frequency between POL and gnomAD NFE. Most of those genetic variants are more frequent among Polish population, except from *DICER1* and *KCNJ10*.

4. Discussion

Between 5% and 15% of NMTC cases occur due to germline mutations [20]. Despite the mounting evidence for the heritability of thyroid cancer, at least partially, to date only a handful of genetic variants have been convincingly associated with a higher risk of this cancer [12]. As the studies have shown, the high heritability of thyroid cancer may occur likely due to the contributions of many rare but high-penetrance genetic variants in some cases, or common, low-penetrance variants in the other [66]. The first attempt to identify germline mutations with the risk for cancer

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was already done about ten years ago using GWAS technique, finding five single nucleotide polymorphisms (SNPs; rs965513, rs944289, rs966423, rs2439302, and rs116909374) to be associated with papillary thyroid carcinoma (PTC) in the cohort of Polish individuals [67]. Each of the variants showed highly significant but moderate to low disease risk, therefore the cumulative risk has been assessed and concluded that it is less significant than previously suggested, thus, the clinical use may not be feasible [67].

Among genes analyzed in our study, 19 were related to syndromic FNMTC and 71 were genes related to non-syndromic FNMTC. Within this group statistically significant variants included *APC, ARSB, ATM, BRCA1, CHEK2, DICER1, GPD1L, INSR, KCNJ10, MYH9, PALB2, PLCB1, PLEKHG5, PTEN, RET, SEC23B, SERPINA1, SLC26A4, SMAD3, STK11, TERT, TOE1, WRN* and indeed many of them have been already described as important in the initiation or progression and development of thyroid cancer. However, not many of them have ever been described in the context of population frequency. Most of those genetic variants are more frequent among Polish population (Thousand Polish Genomes database) than in non-Finnish European (gnomAD), except from *DICER1* and *KCNJ10*. Below, we briefly discuss each of the genes with the perspective of thyroid cancer contribution and known prevalence in other populations.

APC gene variants are well known for their contribution to the familial adenomatous polyposis (FAP), Gardner syndrome, however papillary thyroid carcinoma (PTC) associated with FAP is very rare [68]. Even though in most cases papillary thyroid carcinoma associated with FAP occurs in females in their 30s and rarely in the elderly, there are several cases described where the disease occurred among patients in other age groups, as well as among members of the same family. As a rare extracolonic manifestation of the FAP, thyroid cancer, not solely PTC, is diagnosed in about 2.6% of all FAP patients globally, however the molecular background of this malignancy remains unknown [69,70]. The most significant variants occurring more frequently among Polish population (see Table S1) are missense variants of conflicting interpretation of pathogenicity. All of them have been described before and known to be cancer related.

The expression level of *ARSB* in the thyroid cancer tissue is low [71], however its significance in thyroid cancer development and the prevalence of its variants has been already reported among the Italian population [35,72,73]. In our cohort three variants reached statistical significance, however their prevalence is not very different from other analyzed populations in gnomAD. This gene has not been described profoundly in the context of thyroid cancer and may be an interesting novel candidate for further studies. The protein encoded by *ARSB* gene is located in the lysosome and it is known to participate in the regulation of cell adhesion, cell migration and invasion for example in colonic epithelium [74]. In the central nervous system, is a regulator of neurite outgrowth, as well as neuronal plasticity, acting through the control of sulphate glycosaminoglycans and neuronal levels (ARSB_HUMAN, P15848).

ATM gene is crucial when it comes to the response to ionizing radiation-induced DNA damage, and therefore it has been long suspected it might be involved in thyroid cancer [75]. The cBioPortal lists somatic *ATM* mutations in 2.4% of thyroid cancers [76]. Mutations of the *ATM* gene are responsible for ataxia telangiectasia, a rare inherited disorder characterized by progressive ataxia, but also radiosensitivity, many cell-cycle checkpoint defects, genome instability, and a predisposition to cancer of all types [75]. The expression of the gene in the thyroid is high, especially in glandular cells [77]. It has been reported that the individual susceptibility to DTC may be attributable to the genetic variants of the *ATM* gene [75]. However, little is known about the population prevalence of the *ATM* mutations among thyroid cancer patients, even if there are several interesting case reports describing this association [78]. In our cohort several variants of the *ATM* gene have been discovered as significantly more frequent, many of them with clearly pathogenic status (see Table S1).

BRCA1, similarly to the *CHEK2* and *PALB2* genes, are engaged in the DNA repair mechanisms, therefore absolutely crucial for cancer protection. In our cohort only 3 variants of this gene have been significantly more frequent than in gnomAD cohort, two of them are of pathogenic status and one missense variant of conflicting interpretation status. However, among *CHEK2* gene variants all significant mutations are of conflicting interpretation of pathogenicity status and all of them appear

to be more frequent among analyzed cohort. It has been suggested that CHEK2 mutations generally predispose to thyroid cancer, together with familial aggregations of breast and thyroid cancer and even to double primary cancers of the breast and thyroid, with several cases already described among the Polish population [42]. It plays a role in maintaining genomic stability, and it also acts as a tumor suppressor [79]. However, in the recent work of Cieszyńska et al. of all women with breast cancer analyzed in the study, only 0.49% developed a second case of primary thyroid cancer [80]. Interestingly, the ten-year risk of thyroid cancer development was higher in women who also carried a *CHEK2* mutation (1.5%) than among women who carried no other mutations (0.9%) [80]. Among Polish females, according to the study, following a diagnosis of breast cancer, the age-specific annual incidence rates of thyroid cancer increased at least 4 times [80]. Some genes have already had extensive research done in the thyroid cancer perspective within the Polish population, just like aforementioned *CHEK2*. Pathogenic variants in this gene were reported in both PTC and FTC, although not with the same prevalence: about 15.6% of patients with PTC had mutations in *CHEK2* gene [42,81]. Interestingly, *CHEK2* mutation carriers reported a family history of breast cancer 2.2 times more frequently than non-carriers [42].

DICER1 gene mutations are crucial for DICER1 syndrome associated with familial pleuropulmonary blastoma), cystic nephroma and ovarian Sertoli-Leydig cell tumors [22]. Co-occurrence of DTC with Sertoli-Leydig cell tumor is highly suggestive of DICER1 syndrome [22]. Most patients with DICER1 syndrome diagnosed with DTC (PTC and FTC) had prior exposure to radiation and chemotherapy for the treatment of associated malignancy [22]. However, recently PTC in a patient without past medical history of oncological treatment was reported [43] and the risk of DTC was 16- to 24-times increased over a DICER1 patient's lifetime [43]. In our study the frequency of *DICER1* variants were like the comparator, except for one variant with slightly higher prevalence among Polish population, but not clearly pathogenic (see Table S1).

GPD1L mutations in patients with DTC were described in the Italian, Polish and Spanish populations [35], whereas *INSR* only in the Korean population [44]. In our cohort only one variant of the *GPD1L* gene occurs statistically more frequently than in the gnomAD control group, possessing the status of conflicting interpretation of pathogenicity. Similarly, *INSR* variants discovered in our study are more frequent among Polish cohort than in the gnomAD control group, all of which also possess the status of conflicting interpretation of pathogenicity (see Table S1).

KCNJ10 mutations were described in patients with Pendred syndrome, which is an autosomal recessive syndrome linked to sensorineural deafness/hearing impairment, goiter, and an abnormal organification of iodide with or without hypothyroidism, as well as to DTC [12]. Little is known about the frequency of its variants among other population, however in our cohort at least eight variants seem to be important (see Table S1). Interestingly, 5 of them are in the 3'UTR region of the gene.

PLEKHG5 germline variants in familial non-syndromic PTC (classical and follicular variant) were identified in a Brazilian population [50]. In our study one missense variant was found to be significantly more frequent among Polish population. Its protein product regulates autophagy of synaptic vesicles in axon terminal of motoneurons (by similarity). Involved in the control of neuronal cell differentiation [82]. Also plays a role in angiogenesis through regulation of endothelial cells chemotaxis [83].

MYH9 encodes myosin-9 and potentially impacts the risk of PTC by interacting with a long noncoding RNA (encoded by the *PTCS2* gene) and *FOXE1* gene [47]. Only one missense variant has been found to be relevantly more frequent among Polish cohort, even though it has the status of conflicting interpretation of pathogenicity. It may also be an interesting candidate gene for further studies on DTC. This gene is a novel cancer stem cell marker and claimed as a prognostic indicator in esophageal cancer that promotes oncogenesis through the PI3K/AKT/mTOR axis [84].

InDel intronic variant within *PLCB1* was the first mutation identified in familial multiple papilloid adenomata-type DTC patients and in a subset of patients with sporadic DTC [85]. In patients who were carriers of this mutation, multinodular goiter progressed to PTC (follicular variant) through overexpression of phospholipase C beta 1 (PLCB1) [86]. Among Polish population

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three variants gained statistical significance with higher prevalence but having conflicting interpretation of pathogenicity.

PTEN hamartoma tumor syndrome (PHTS) embodies a group of diseases caused by germline mutations in the phosphatase and tensin homolog (*PTEN*) gene located at 10q23.31. They include Cowden syndrome, PTEN-related Proteus syndrome, Proteus-like syndrome, Bannayan-Riley-Ruvalcaba syndrome (BRRS), and adult Lhermitte-Duclos disease (LDD) [12]. Approximately 6 to 38% of PHTS patients develop thyroid cancer with a median age of diagnosis between 31 and 37 years [12]. A risk of DTC is 26 to 39 times higher than for individuals without a *PTEN* mutation [87]. In our study three variants located in the noncoding regions have been found as appearing more frequently among Polish population, however little is known about them in the context of DTC cancer.

Although *RET* gene mutations are crucial for medullary thyroid cancer, a germline *RET* oncogene mutation may be also linked to NMTC, especially as a part of FAP syndrome [34]. In our study several genetic variants of this gene have been found as being significantly more frequent among Poles, most of them are missense variants or located in the 3′UTR or 5′UTR regions.

SEC23B mutation was identified in patients with FTC as a component of the Cowden syndrome in a WGS study [56]. In a *SEC23B* pathogenic variant carriers, Yehia et al. found a significantly increased age-adjusted standardized incidence rate of DTC when compared with the general population [56]. Apart from DTC, the Cowden syndrome may cause hamartomas, breast, kidney, colon, endometrium, and brain tumors, mucocutaneous lesions and macrocephaly [12]. Again, in our population discovered variants are more frequent, one of them possessing status of being pathogenic (see Table S1). *SEC23B* encodes Sec23 Homolog B, a component of coat protein complex II (COPII). It is involved in vesicle trafficking, responsible for transporting proteins from the endoplasmic reticulum to the Golgi apparatus [56].

SERPINA1 (α1-AntiTrypsin) located at 14q32.13, is highly expressed in colorectal cancer, cutaneous squamous cell carcinoma, and papillary thyroid carcinoma [50]. The role of this gene in thyroid tumorigenesis is not fully understood. However, in colorectal cancer cell invasion and migration have been associated with *SERPINA1* upregulation of fibronectin. Mutations in this gene may also present a mitogenic action, stimulating malignant cell proliferation [50]. One variant of this gene located in the 5′UTR region has been found as statistically more prevalent among Polish population.

Among the Korean population, it has been reported that *SLC24A6* gene, which encodes a mitochondrial sodium and calcium ion exchanger, is important for the risk of developing thyroid cancer [44,88]. However, its relationship to DTC pathogenesis is not established yet, even though an interesting finding was the stronger genetic variant association with FTC rather than PTC in that Korean study [44,88]. This striking finding suggested that at least some genetic markers of susceptibility to DTC are different between thyroid cancer types. In our study many variants seem to be more frequent among analyzed population, most of which are missense variants of conflicting interpretation of pathogenicity, whereas one discovered variant is likely pathogenic as a frameshift mutation.

The SMAD family member 3 gene (*SMAD3*) show higher expression in the thyroid than in most other tissues. It supports a potential role for this factor in predisposition to thyroid cancer [8]. *SMAD3* is an important transcriptional mediator of transforming growth factor-b (TGF-b) signaling associated with PTC [12,59]. Wang et al. investigated the downstream mechanisms by which alterations of *SMAD3* contribute to thyroid cancer susceptibility [89]. Only two variants of conflicting interpretation of pathogenicity have been found in this study as being slightly more prevalent among Polish cohort.

Mutation in serine/threonine kinase 11 (*STK11*) gene is a causative agent for Peutz-Jeghers Syndrome (PJS). It is an autosomal dominant syndrome characterized by hamartomatous polyps and mucocutaneous hyperpigmentation with a 4-fold increase in cancer risk (regardless of a primary side) as compared to the general population [12]. The increased cancer risk has been connected to the P53 pathway [90]. PJS has been associated with multiple cases of thyroid cancer of PTC, FTC, tall cell variant PTC, and follicular variant PTC subtypes [12,62]. Only one intronic variant has been

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significantly more frequent among Polish cohort and it is in the intronic region of the gene, thus, may possess a regulatory function.

TOE1 gene product inhibits cell growth rate and cell cycle: it induces *CDKN1A* gene expression as well as TGF-beta expression. Moreover, it also mediates the inhibitory growth effect of *EGR1* [91]. *TOE1* mutations were associated with PTC occurring in patients with FAP. They also suffered from colorectal carcinoma, ampullary carcinoma, hepatoblastoma, medulloblastoma, PTC did not affect their survival [92]. In our study one intronic variant seems to be slightly more prevalent among Polish population, compared with gnomAD cohort, although it has a conflicting interpretation of pathogenicity at this moment.

TERT gene encodes a protein which is an essential component of the telomere length maintenance complex and is weakly expressed in normal thyroid tissue. However, it is known to be reactivated in many human cancers, including thyroid cancer through the transcriptional regulation [88,93]. It is correlated with a more severe form of the cancer disease, and mutations in the *TERT* promoter in thyroid cancer were suggested as having a prognostic potential if coexisting with the *BRAFV600E* mutation [88,94]. In our study two variants of *TERT* gene have been found as occurring more frequently among Polish population, both with conflicting interpretation of pathogenicity.

Mutations in the WRN gene are associated with Werner syndrome [65,73]. It is a rare autosomal recessive disorder classified as one of the progeria syndromes, thus associated with premature ageing and increased risk for several malignancies, especially melanoma, meningioma, soft tissue sarcoma, leukemia, osteosarcoma, but also differentiated thyroid cancer, usually follicular carcinoma [65,73,95]. It may increase the cancer risk irrespective of their location. Several missense variants have been found to occur more frequently among our cohort than in a control gnomAD group, however all of them with conflicting interpretation of pathogenicity.

It is important to notice the significance of the population studies, since in every cohort there might be several founder variants typical for the population, absent or very rare in other groups. Due to the historical influences in many populations some genetic variants may be more prevalent, and this information is very useful for clinical geneticists and other healthcare professionals, since they may provide us with additional hints at the point of diagnosis: facing financial constraints in many countries genetic testing is still not very popular and analysis of selected variants or genes, instead of huge panels or WGS, is simply cheaper and faster, thus, more available.

Despite our best efforts, there are several limitations to the study. First, we decided to focus only on the protein-coding genes, since the knowledge about other elements of the genome, especially being involved in the thyroid cancer risk, is still in its infancy. In our study we decided to focus on protein-coding genes, since the knowledge on non-coding elements of the genome, being involved in the thyroid cancer risk, is still in its infancy. Only two studies indicate association of PTC with mutations within miRNA genes in the Polish population [73,96]. Secondly, a reliable comparison of allele frequency between populations suffers from a low number of available data. Even the gnomAD cohort contains collected data for participants from many different populations, but usually of a small sample size that does not allow for an accurate allele frequency estimation. Especially when it comes to the WGS data, there are still only a few large, populational databases. Despite interesting findings, it is important to stress that only carefully selected genes have been analyzed in our research. Even if the entire gene sequence was taken into consideration, still there might be other elements of the genome with equally important significance, or even contributing more to the regulation of gene expression. Also, epigenetic factors are known to be crucial in many cancer stages of development, therefore further studies are crucial to fully understand the thyroid cancer intricacies.

5. Conclusions

Despite the progress in elucidating the molecular mechanisms underlying differentiated thyroid cancer (DTC) thanks to genome sequencing, germline mutations responsible for genetic susceptibility to DTC are still poorly recognized. The aim of this study was to assess the prevalence of genetic germline mutations predisposing to the differentiated thyroid cancer in a cohort of Polish individuals using whole genome analysis to make a basis for further tailored prophylaxis. Undoubtedly more

research is needed to provide a more extensive background on the penetrance, molecular function and functional consequences of the genetic variants presented here, which can further clarify the etiology of thyroid cancer and probably also support the identification of disease risk in family members of NMTC patients, not solely among Polish population, but serving as a hint for further populational studies. Finally, many studies describing the genetic predisposition to NMTC are case-control designs. What is needed are genome-wide association studies (GWAS), preferably based on next generation sequencing, and family-based exome sequencing. Genomic studies should be followed by transcriptome (e.g., Single-Cell RNA Sequencing) and/or molecular (droplet digital polymerase chain reaction (ddPCR) or Sanger sequencing) analyses, and linkage studies to identify and confirm the new susceptibility loci associated with NMTC [97].

Supplementary Materials: Table S1: Genes with significant (q-value <0.05) differences in pathogenic variant burden among Polish population (Thousand Polish Genomes database) compared with non-Finnish European (gnomAD)

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The idea for this study was raised in August 2022, bioinformatics analysis performed in autumn 2022 and the manuscript written in the first half of 2023. The datasets presented in this study can be found in an online repository: https://1000polishgenomes.com [access date: October 2022]. Full cohort description can be found in the following paper: https://doi.org/10.3390/ijms23094532

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