

There is a possible molecular basis for the difference in the extent of thyroid cancer at the time of presentation and the difference in outcomes observed in adolescents and young adults as compared with older adults

Vriens MR, Moses W, Weng J, Peng M, Griffin A, Bleyer A, Pollock BH, Indelicato DJ, Hwang J, Kebebew E. Clinical and molecular features of papillary thyroid cancer in adolescents and young adults. *Cancer* 2010. 10.1002/cncr.25369 [doi]

SUMMARY

BACKGROUND

Thyroid cancer in adolescents and young adults (AYAs) is underreported, and similarly to thyroid cancer in adults, the incidence has doubled over the past 30 years. In this study, the molecular and clinical features of papillary thyroid cancer (PTC) in AYAs are compared with the same features among older patients. The disparity in the incidence and outcome in AYAs typically appears as more advanced disease with a higher prevalence of lymph-node and distant metastases as compared with adults, but nonetheless usually has a favorable outcome with therapy. In 2006, the National Cancer Institute (NCI) initiated a Progress Review Group on AYA to investigate the biologic basis of age-related differences in the outcome for AYAs with thyroid cancer.

MATERIALS AND METHODS

The data for the subjects of this study were derived from medical records of 1011 patients who had their initial therapy for conventional and follicular variant PTC that was initially treated at the University of California at San Francisco (UCSF) from January 1983 through December 2003 and had follow-up at UCSF for ≥ 5 years. The study variables were age, sex, tumor–node–metastasis (TNM) classification, type of surgery, radioiodine ^{131}I treatment, and clinical disease-free and overall survival. The patients were subdivided into two age groups: 15 to 39 years (AYA group) and ≥ 40 years using data in the Surveillance, Epidemiology, and End Results (SEER) program from 1973 through 2006 (n = 46,680). The National Cancer Institute SEER Program at the time of this study represented approximately 26% of all cancer cases in the U.S. population.

Genotyping for Somatic Mutations

Primary PTC tumor samples from 245 patients (AYA group, n = 109; ≥ 40 years group, n = 145) were genotyped for common activating mutations that occur in PTC, including the v-ras murine sarcoma viral oncogene homolog B1 (*BRAF*) valine to glutamine substitution at amino acid 600 (V600E) point mutation; the ret proto-oncogene/papillary thyroid cancer 1 (*RET/PTC1*), *RET/PTC3*, and neurotrophic tyrosine kinase receptor type 1 (*NTRK1*) rearrangements; and hotspot point mutations in v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*). Point mutations in *BRAF* V600E, *KRAS*, and *NRAS* were detected by polymerase chain reaction (PCR) and direct sequencing. *RET/PTC1*, *RET/PTC3*, and *NTRK1* were detected by using nested PCR. The number and type of mutation in the AYA group was compared with those in the ≥ 40 years group.

Analysis of Pathway-Specific Array Genes

Previous studies by the authors identified 39 genes in pathway-specific array analyses, which are genes involved in the cell cycle, angiogenesis, extracellular matrix, and cell adhesion, that had significantly different expression in thyroid cancer. The expression of these genes were compared between the AYA group (n = 16) and the ≥ 40 years group (n = 22) in the available samples. Also compared were the expression levels of genes that the authors associated previously with the extent of disease in thyroid cancer (epidermal growth factor receptor [*EGFR*], extracellular matrix protein 1 [*ECM1*], ephrin-B2 [*EFNB2*], and minichromosome maintenance complex component 7 [*MCM7*]) between the AYA group (n = 106) and the ≥ 40 years group (n = 151).

Genomewide Gene Expression Analysis

The authors used the Affymetrix Gene Chip (U133 Plus 2.0) array probe set, including 38,500 well-characterized human genes (Affymetrix), to identify differences in gene expression between the two age groups in 96 available PTC samples (AYA group, n = 46; ≥ 40 years group, n = 50). RNA samples were hybridized using the Human Genome U133 Plus 2.0 Chip (Affymetrix). The criteria used for differential gene expression between the two age groups were: (1) twofold higher or lower gene expression

Characteristics and Outcomes of Patients Who Had Surgery for Papillary Thyroid Cancer by Age Group

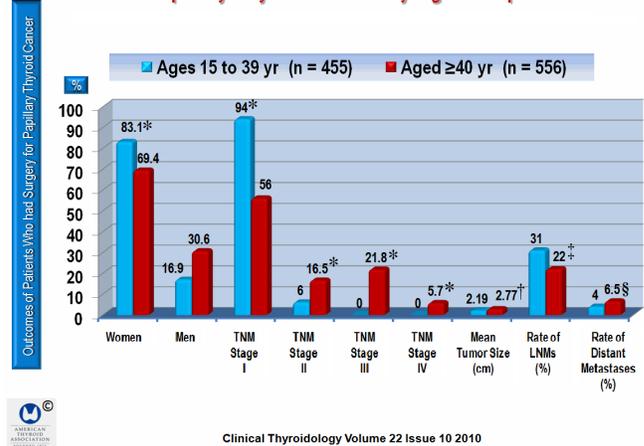


Figure 1. This figure shows the characteristics and outcomes of patients who had surgery for papillary thyroid cancer by age group. *P<0.001. †P = 0.75. ‡P<0.08. §P = 0.08. All comparisons are between the AYA group and the ≥ 40 years group. LNM = lymph-node metastases; TNM = tumor–node–metastasis. The data for this figure and Figures 2 and 3 are derived from Table 2 of Vriens et al.

levels, and (2) P value <0.5, determined by t-test. The study also used gene set enrichment analysis to determine any differences in biologic pathways between the two age groups.

RESULTS

Study Subjects (Figure 1)

DEMOGRAPHICS OF STUDY SUBJECTS (FIGURE 1)

Of the 1011 patients who had been treated for PTC over 20 years at UCSF, 455 (45%) were in the AYA group (15 to 39 years) and 556 (55%) were in the older group (≥40 years) (Figure 1). There were significantly more women in the AYA group (83.1%) as compared with the ≥40 years group (69.4%; (P<0.001) (Figure 1). Also, a greater proportion of patients in the AYA group had TNM stage I disease as compared with the ≥40 years group (94% vs. 56%), respectively (P<0.001), which turned out this way because age is included in the TNM staging system (P<0.01) (Figure 1).

Mean tumor size was smaller in the AYA group as compared with the ≥40 years group,(2.19 cm vs. 2.77 cm, P = 0.075) (Figure 1). The incidence of lymph-node metastases was significantly greater in the AYA group versus the ≥40 years group (31% vs. 22%, P = 0.008) (Figure 1). In addition, the rate of distant metastases was lower in the AYA group as compared with the ≥40 years group (4% vs. 6.5%; P = 0.08) (Figure 1).

Initial Surgery (Figure 2)

Surgery was lobectomy in 6.8% of AYAs and 16.7% of adults ≥40 years of age (P< 0.001), and subtotal or near-total thyroidectomy was performed in 16.5% and 16.4%, respectively (P not significant). Total thyroidectomy was performed in 74.3% and 63.3%, respectively (P<0.001) (Figure 2).

Duration of Disease-free and Overall Survival (Figures 2 and 3)

A total of 64.5% of the AYA group and 57.1% of the ≥40 years group had no recurrences or were free of disease (0.017%); 7.1% of the AYA group had a recurrence, as compared with 6.1% of the ≥40 years group (Figure 2). Death occurred in 2.4% of the AYA

group and 21.8% of the ≥40 years group (P<0.001) (Figure 2).

Mean (±SD) disease-free survival rates were 597.68±15.2 weeks in the AYA group and 531.11±15 weeks in the ≥40 years group (P = 0.126) (Figure 3).

Median disease-free survival for the AYA group was 568 (95% confidence interval [CI], 493.1 to 623), and 497.57±15 for the ≥40 years group (Figure 3).

Mean overall survival rates were 615.30±14.1 weeks for the AYA group and 532.08±13.1 weeks for the ≥40 years group (P = 0.0013) (Figure 3).

Median overall survival rates were significantly higher in the AYA group (580.29; 95% CI, 525.3 to 641.0; P = 0.013), as compared with the ≥40 years group (476.29; 95% CI, 436.3 to 522.4; P = 0.0013).

When SEER data were subdivided into the two age groups (15 to 39 years, n = 16,983; ≥40 years, n = 29,697), there was a significant difference in the sex distribution between the AYA and the ≥40 years group (P<0.001); and as in the California study group, more AYA patients were women (80%, vs. 71% in the ≥40 years group (Figure 3).

SEER Database

In the SEER data the mean tumor size was comparable to that in the California study group (2.05 cm for the AYA group vs. 2.16 cm for the ≥40 years group, P not significant). The number of lymph-node metastases was comparable for both groups (33% for AYAs vs. 34% for those ≥40 years), as was the number of distant metastases (6% for both groups). Similar to the California group, the SEER data showed a median overall survival in the AYA group that was significantly higher than that in the group ≥40 years of age (588 weeks vs. 296 weeks); however, disease-free survival data were not available in the SEER database.

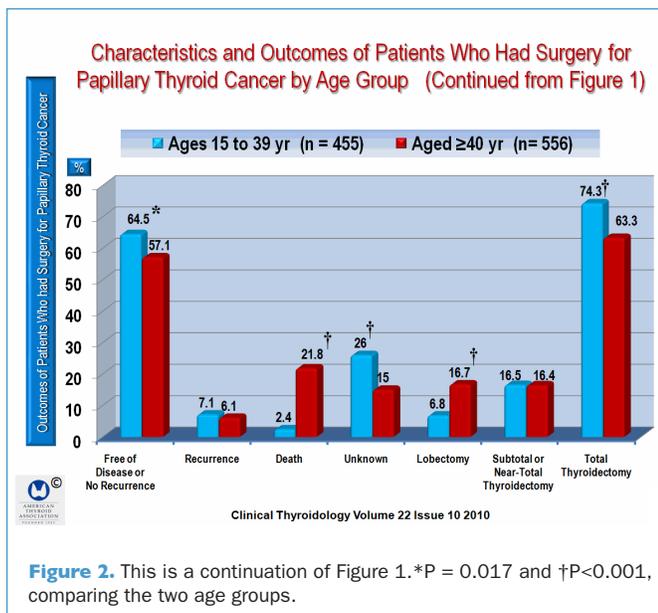


Figure 2. This is a continuation of Figure 1. *P = 0.017 and †P<0.001, comparing the two age groups.

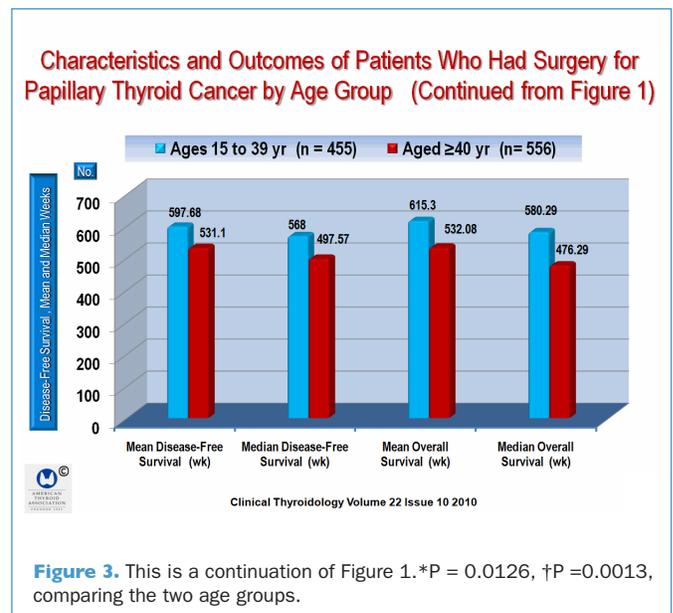


Figure 3. This is a continuation of Figure 1. *P = 0.0126, †P = 0.0013, comparing the two age groups.

Genotype in Adolescent and Young Adult Papillary Thyroid Cancer

The authors underscore that previous studies of age with differentiated thyroid cancer mainly addressed the finding that older age (>45 years) was associated with a worse prognosis, increasing the recurrence and mortality rates of the disease. The present study takes a significantly different approach to this disease.

Analysis of the most common somatic mutations revealed that the number and type of somatic mutations detected in PTCs among AYAs as compared with adults age ≥40 years were similar. A total of 39% of patients in the AYA group had no mutation, as compared with 44% of the patients in group ≥40 years of age. A cutoff of 40 years was chosen to divide patients into two groups, because it is the official definition of the upper end of the AYA age range according to the NCI Progress Review Group on AYA Oncology. In the current study, the mortality rate was significantly higher in the group ≥40 years of age, and disease-free survival was significantly lower than that in the AYA group. The difference in the survival rate in the current study was concordant with the SEER database, perhaps because of confounding factors such as different stage, race/ethnicity distribution, or differences in treatment and care.

A greater number of the AYA group had multiple somatic mutations (18% vs. 10%; P = 0.06). The prevalence of BRAF mutations alone was not significantly different between the two groups (42% in the AYA group vs. 35% in the ≥40 years group) (Figure 4). A matched analysis between the AYA group and the ≥40 years group found that according to the extent of disease at the time of diagnosis (tumor size, ±2 cm), lymph-node status, and distant metastasis status had no significant difference in the type and number of mutations that existed between the two groups (Figure 4).

Gene Expression Differences in Pathway-Specific Array Genes

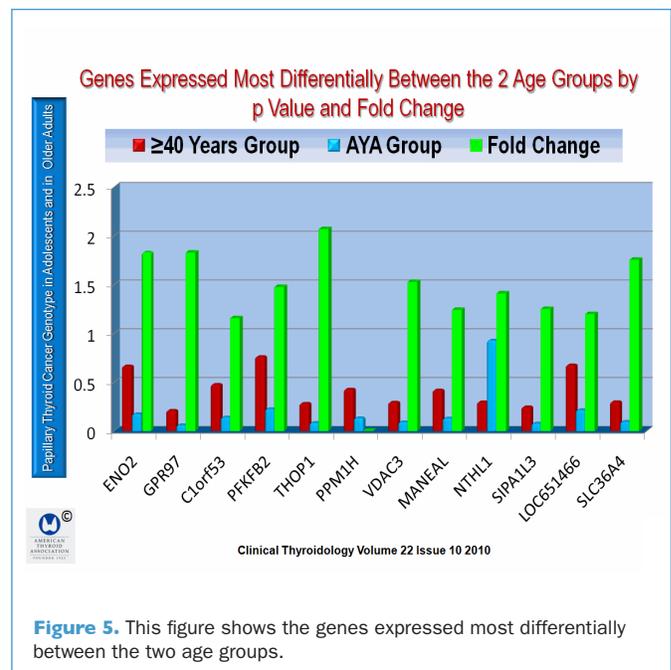
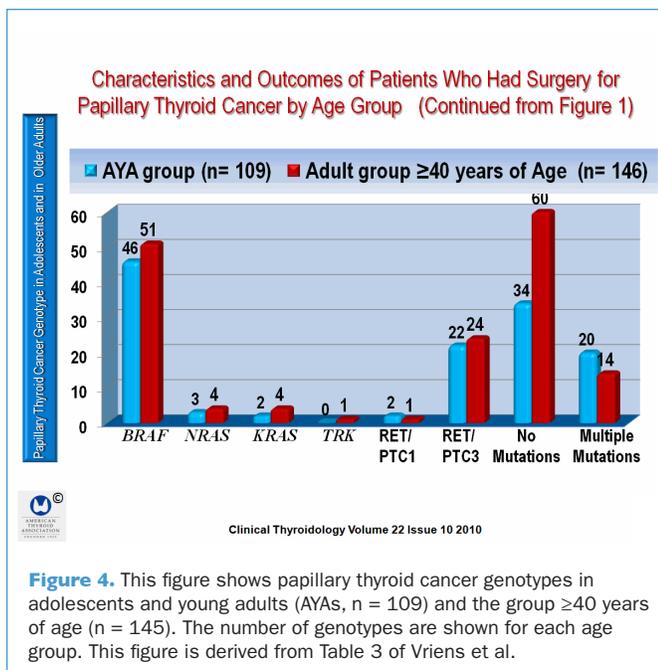
Of the 39 genes analyzed, 3 (ECM1, v-erb-2 erythroblastic leukemia viral oncogene homolog 2 [ERBB2], and urinary plasminogen activator [UPA]) had expression levels that differed significantly between the AYA group and the ≥40 years group. The mean delta-Ct expression of ECM1 was significantly lower for patients in the AYA group (5.47±3.41 vs. 6.69±3.09; P = 0.0031). The same was true for ERBB2 (71.9±60.2 vs. 95.83±39.2; P = 0.01), and for UPA (0 vs. 0.031±0.078; P = 0.025). A matched analysis between the AYA group and the group ≥40 years of age according to the extent of disease at presentation (tumor size, ±2 cm, lymph-node status, and distant metastasis status) found that only ECM1 had significant differential expression (P = 0.02) (Figure 5).

Genomewide Gene Expression Analysis

A cluster analysis of the gene expression profile found no distinct clustering in 96 PTC samples according to patient age (AYA group, n = 46; ≥40 years group, n = 50). The 12 most differentially expressed genes between the two age groups found that none of the genes had significantly differential expression (Figure 5). There were no differentially expressed gene sets or pathways in a gene set enrichment analysis using a false discovery rate of ≥25%. For a nominal P value <1%, however, 10 gene sets were expressed differently in both groups (1 gene set was up-regulated in the AYA group, and 9 gene sets were down-regulated in the AYA group). Leading-edge analysis of these gene sets found that 1 gene, carbonic anhydrase II (CA2), was the most common in 3 of these 10 gene sets, and CA2 was down-regulated in the AYA group.

CONCLUSION

There is a possible molecular basis for the difference in the extent of disease at the time of presentation and the difference in outcomes observed in adolescents and young adults as compared with older adults



COMMENTARY

This study found that AYA patients with PTC have a different scope of disease at presentation and have better outcomes as compared with outcomes in older patients. No significant differences were found in the type or number of somatic mutations that occurred in PTC between the AYA group and the ≥40 years group. However, the study identified several genes that were expressed differentially between the AYA group and the ≥40 years group (*ECM1*, *ERBB2*, *UPA*, *PFKFB2*, and *MEIS2*). However, only *ECM1* had differential expression when a matched analysis was performed. These findings thus suggest a possible molecular basis for the difference in the extent of disease at the time of presentation and the difference in outcome observed in the AYA group. The authors note that the function of *ECM1*, an extracellular matrix protein, has not been characterized completely in carcinogenesis.

In a serial analysis of gene expression in both benign and malignant thyroid tumors, *ECM1* expression was higher in malignant tissue samples (1;2). Moreover, the level of *ECM1* expression was higher in older patients who had differentiated

thyroid cancer of follicular-cell origin. The authors suggest that the findings that aggressive tumors are capable of growth without interactions between the cell surface and the extracellular matrix for survival and cell-cycle progression suggests that the decreased expression of *ECM1* in more aggressive differentiated thyroid cancers might reflect cell anchorage-independent tumor growth (3;4).

This study thus found that AYA patients with PTC had a different extent of disease at the time of diagnosis and had better outcomes as compared with older patients. The analysis suggested several candidate genes (*ECM1*, *ERBB2*, *UPA*, *PFKFB2*, *MEIS2*, and *CA2*) might account for the differences in outcomes in younger patients as compared with older patients.

This is a superb study. The authors suggest that such studies may provide new screening, diagnostic, and therapeutic options for the evaluation and treatment of common cancers in the AYA population that has been under study. I have no doubt that the authors are correct in this prediction.

— Ernest L. Mazzaferri, MD, MACP

References

1. Pauws E, Sijmons GG, Yaka C et al. A novel homeobox gene overexpressed in thyroid carcinoma. *Thyroid* 2004;14:500-5.
2. Pauws E, Veenboer GJ, Smit JW et al. Genes differentially expressed in thyroid carcinoma identified by comparison of SAGE expression profiles. *FASEB J* 2004;18:560-1.
3. Kebebew E, Peng M, Reiff E et al. *ECM1* and *TMPRSS4* are diagnostic markers of malignant thyroid neoplasms and improve the accuracy of fine needle aspiration biopsy. *Ann Surg* 2005;242:353-61.
4. Kebebew E, Peng M, Reiff E et al. Diagnostic and extent of disease multigene assay for malignant thyroid neoplasms. *Cancer* 2006;106:2592-7.

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