Correlating Genetic Aberrations with World Health Organization-defined Histology and Stage across the Spectrum of Thymomas

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ABSTRACT

Thymomas are thymic epithelial tumors. Because most of them are rich in neoplastic T-cells, recurrent genetic aberrations have been reported only in the rare, lymphocyte-poor WHO types A, B3, and C. We have now investigated virtually the whole spectrum of thymomas, including the commoner types AB and B2, microdissecting or culturing neoplastic cells from these lymphocyte-rich thymomas and applying 41 microsatellite markers covering 17 loci on 10 chromosomes. In 28 cases, comparative genomic hybridization data were available. Apart from type A, there was striking heterogeneity between thymomas. Allelic imbalances were seen in 87.3% of the 55 cases, and MSI in 9.9%. Losses of heterozygosity (LOHs) were much the commonest aberration. Overall, they were most prevalent at four regions on chromosome 6. Aberrations elsewhere, affecting mainly 8p11.21 and 7p15.3, suggested a cortical footprint because they recurred only in the thymopoietically active type AB and B thymomas. LOHs were also seen at the adenomatous polyposis coli (APC) locus (5q21-22) in subsets of these thymomas, whereas combined LOHs at the APC, retinoblastoma (13q14.3), and p53 (17p13.1) loci were confined to a subset of B3 thymomas that had possibly evolved from APC-hemizygous B2 thymomas by tumor progression; indeed, thymomas combining B2 plus B3 features are common. Notably, some AB and B thymomas shared LOHs despite their nonoverlapping morphology and different clinical behavior. Finally, allelic imbalances at 8p11.21 and 16q22.1 (CDH1) were significantly more frequent in stage IV metastatic thymomas. We conclude that the WHO-defined histological thymoma types generally segregate with characteristic genetic features, type A thymomas being the most homogeneous. Many findings support the view that B2 and B3 thymomas form a continuum, with evidence of tumor progression. However, our findings imply that types A and AB are biologically distinct from the others, any potential invasiveness being severely restricted by a medullary commitment in the precursor cell undergoing neoplastic transformation.

INTRODUCTION

Thymomas are the commonest human mediastinal tumors and frequently associated with MG (1–3). According to the recently published WHO classification (4), they are divided into types A (also called medullary), AB (mixed), B, and C thymomas. Type B thymomas are additionally subdivided into type AB and B thymomas. LOHs were seen in 87.3% of the 55 cases, and MSI in 9.9%. Losses of heterozygosity (LOHs) were much the commonest aberration. Overall, they were most prevalent at four regions on chromosome 6. Aberrations elsewhere, affecting mainly 8p11.21 and 7p15.3, suggested a cortical footprint because they recurred only in the thymopoietically active type AB and B thymomas. LOHs were also seen at the adenomatous polyposis coli (APC) locus (5q21-22) in subsets of these thymomas, whereas combined LOHs at the APC, retinoblastoma (13q14.3), and p53 (17p13.1) loci were confined to a subset of B3 thymomas that had possibly evolved from APC-hemizygous B2 thymomas by tumor progression; indeed, thymomas combining B2 plus B3 features are common. Notably, some AB and B thymomas shared LOHs despite their nonoverlapping morphology and different clinical behavior. Finally, allelic imbalances at 8p11.21 and 16q22.1 (CDH1) were significantly more frequent in stage IV metastatic thymomas. We conclude that the WHO-defined histological thymoma types generally segregate with characteristic genetic features, type A thymomas being the most homogeneous. Many findings support the view that B2 and B3 thymomas form a continuum, with evidence of tumor progression. However, our findings imply that types A and AB are biologically distinct from the others, any potential invasiveness being severely restricted by a medullary commitment in the precursor cell undergoing neoplastic transformation.

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High throughput sequencing analyses showed that the majority of thymomas displayed LOH at the APC locus (5q21-22) because it contributed to invasion in gastric and breast cancers (26, 27), and also the AIRE (at 21q22.3) locus because of its role in tolerance induction in the thymus (28) and the frequent occurrence of autoimmune in thymoma patients. The results suggest a spectrum of genetic alterations with diversity increasing from the relatively homogeneous type A through AB to B2 and possibly B2 to B3 progression.

3–6). Type C thymomas comprise a heterogeneous group of thymic carcinomas (4).

Although the immunological features of thymomas have been intensively studied (7–15), their genetic characterization has been hampered by the abundant nonneoplastic lymphocytes in ~70% of cases. Therefore, genetic characterization has concentrated on the rare WHO types A, B3, and C that harbor few lymphocytes (16, 17). For the majority of lymphocyte-rich type AB, B1, and B2 thymomas, there are only a few case reports of karyotypes revealed by classical cytogenetics (18–20). Using CGH and more sensitive/more finely localized microsatellite analyses, we previously defined some recurrent genetic abnormalities in WHO types A, B3, and C thymoma (16, 17). LOHs frequently occurred on chromosomes 3p, 6, 13q, and 16q (16), especially on the long arm of chromosome 6 (17). Other consistent LOHs were detected at 5q21-22, including the APC locus, and at 17p13.1, including the p53 locus (17). Comparing the allele types of types A, B3, and C thymoma, we inferred two mutually exclusive pathogenetic pathways characterized by either the 6q23.3-25.3 or 5q21-22 LOHs.

However, we could not address certain controversial issues because of the exclusion of the common types AB and B2: (a) whether the new WHO-defined thymoma types are just convenient descriptive labels or reflect true biologically distinct entities with specific genetic alterations, clinical features, and prognoses, analogous to most WHO-defined lymphoma types (21); (b) whether the various histological thymoma types represent a continuum ranging from the usually benign type A to the frankly malignant types B3 and C (22) or whether types A and AB show qualitatively distinct differentiation pathways (3, 6, 23); and (c) the supposedly close relationships between type A and AB thymomas and between types B2 and B3 remain hypotheses (3, 6, 24) based on clinical or morphological observations and require substantiation by genetic data.

To address these questions, we have now extended allelotyping studies to all WHO-defined thymoma types, except for the very rare WHO type B1 thymoma, of which we have not received a single fresh sample during the last 2.5 years. By contrast, the lymphocyte-rich and clinically most relevant types AB and B2 were studied here for the first time with a broad panel of microsatellite markers using laser-assisted microdissection or short-term primary thymoma epithelial cell cultures to isolate tumor DNA. To detect allelic imbalance or MSI at putative tumor suppressor gene loci, we applied microsatellite markers that proved useful in the previous study (17). Although we recently found several LOH hot spots on chromosome 6 using microsatellite analysis (25), the other chromosomes have not been studied across the spectrum of thymomas. Moreover, we added additional markers for CDH1 (at 16q22.1) because it contributes to invasion in gastric and breast cancers (26, 27), and also the AIRE (at 21q22.3) locus because of its role in tolerance induction in the thymus (28) and the frequent occurrence of autoimmunity in thymoma patients. The results suggest a spectrum of genetic alterations with diversity increasing from the relatively homogeneous type A through AB to B2 and possibly B2 to B3 progression.
**MATERIALS AND METHODS**

**Patients.** Fifty-seven patients (ages 29–84 years; mean, 59 years; 29 females, 28 males) with thymoma were selected for this study from our files. Among these, 55 thymomas were evaluated using microsatellite analysis and 28 were analyzed by CGH. Thymomas were classified according to the WHO system (4): type A (n = 11); AB (n = 18); B2 (n = 6); B3 (n = 18); and C (n = 4). Our type C thymomas included only epidermoid thymic carcinomas. Tumor stage was defined according to Masaoka’s staging system as modified by Shimosato and Mukai (2, 29): 12 cases were stage I; 25 cases were stage II (infiltration through the tumor capsule into the mediastinal fat); 11 cases were stage III; and 9 cases were stage IV. Among the 57 cases, type AB (n = 18) and B2 (n = 6) lymphocyte-rich thymomas were tested here for the first time with a broad panel of microsatellite markers. MG diagnosis was confirmed by detection of anti-<i>Cd8</i> antibodies in 32 cases. Among these MG patients, two had been pretreated with corticosteroids (both type A tumors).

**Microdissection.** Frozen sections (7-μm thick) were stained with anti-CD3 antibody (Immunotest, Marseille, France) using the Dako ChemMate DAB kit K5001 (Dako, Glostrup, Denmark), followed by hematoxylin. By laser-assisted microdissection, we collected cells from neoplastic epithelium-rich areas that were clearly separate from lymphocyte-rich areas in 15 type AB thymomas. Tumor cells were laser-microdissected on the IX 70 microscope with a broad panel of microsatellite markers. MG diagnosis was confirmed by detection of anti-<i>Cd8</i> antibodies in 32 cases. Among these MG patients, two had been pretreated with corticosteroids (both type A tumors).

**Short-Term Primary Culture of Neoplastic Thymic Epithelial Cells.** Epithelial cells from short-term primary cell cultures were used as a source of tumor DNA in six type B2 thymomas, which, by definition, contain abundant nonneoplastic lymphocytes throughout the tumor, as also in two of the 17 type AB thymomas. Fresh surgical specimens of thymoma were minced and cultured for 5 days in the presence of 1.35 mM 2-deoxyguanosine (Sigma, Steinheim, Germany) in MEM-v-Val (Life Technologies, Inc., Ltd., Paisley, United Kingdom) supplemented with 10% FCS. 2-Deoxyguanosine-treated tissue fragments were incubated in 0.25% trypsin-0.02% EDTA for 30 min at 37°C and vortexed vigorously. Dispersed cells were cultured for 2 days and only adherent tumor cells were then collected; their purity was >95% as confirmed by labeling with a mixture of anticytokeratin 8 and 18 antibodies (CAM 5.2; Becton-Dickinson, San Jose, CA, USA). Only tumor cells harvested from the first passage of primary cultures within 1 week after surgical resection were used. These cultured cells were clearly an appropriate source of tumor DNA: (a) primary cultures from lymphocyte-poor type A (n = 1) and B3 (n = 3) thymomas showed genotypes identical to those of cells from the same patient’s tumor block (data not shown); and (b) genotypes of thymic epithelial cells from a primary culture of a hyperplastic thymus showed no sign of <i>in vitro</i> acquisition of genetic aberrations (data not shown).

**DNA Extraction and Microsatellite Analysis.** DNA extraction was performed according to conventional protocols (30). Microdissected or whole tumor tissue (types A, AB, B3, and C thymomas) or pellets of cultured tumor cells (types AB and B2) were digested using proteinase K. DNA was extracted using phenol-chloroform, followed by ethanol precipitation. Control DNA was obtained from peripheral blood lymphocytes or adjacent remnant thymus tissue not involved by the tumor.

For microsatellite analyses, we used the 41 highly selected repeats that revealed frequent allelic imbalances in our previous study and in exactly the same way (17). D3S1293 and D3S1283 at 3p24-26 (FHTI), D5S882 and D5S346 at 5q21-22 (APC), D6S105, D6S1666, and D6S1560 at 6p21.3 (MHIC), D6S1596, D6S284, and D6S460 at 6q14-15, D6S1592 and D6S447 at 6q21, D6S441, D6S290, D6S473, D6S442, D6S1708, and D6S1612 at 6q25.2-25.3, D7S493 and D7S673 at 7p15.3, D8S1374 and D8S1820 at 8p12.1-21.1, D8S532 and D8S258 at 8p11.21, D13S153 and D13S194 at 13q14.3 (RB), D16S663 and D16S405 at 16p13, D16S3031 and D16S3095 at 16q22.1 (CDH1), D16S516 and D16S460 at 16q23-24, TP53CA and p53 at 17p13.1 (P53), D18S535, D18S1127, and D18S1129 at 18q21 (BCL2 and DCC), and D21S49 and D21S171 at 21q22.3 (AIRE). The primer sequences for the amplification of microsatellite repeats were derived from the Genome Data Base.4 Chromosomal locations of markers were derived from the gene map on the National Center for Biotechnology Information web site and Ensembl Human Genome server.5,6 PCR primers were synthesized at MWG Biotech (Munich, Germany) or Applied Biosystems (Weiterstadt, Germany), and one oligonucleotide of each primer pair was labeled with fluorescent dye phosphoramidites FAM, HEX, NED, or TAMRA. Paired normal and tumor DNA samples from each thymoma patient were amplified with the AmpliTaq Gold enzyme (Applied Biosystems, Foster City, CA) using 50 ng of genomic DNA as template under conditions specified by Genome Data Base. Thirty PCR cycles were carried out in an MWG Primus Gold thermal cycler (MWG Biotech) in a total volume of 20 μl. The annealing temperatures ranged from 55°C to 61°C. Aliquots of the PCR products were mixed with Genescan 350-ROX (Applied Biosystems, Warrington, United Kingdom) as a size standard and formamide-denatured and electrophoresed on a 4.5% polyacrylamide gel using a 377 DNA Sequencer (Applied Biosystems). The automatically collected data were analyzed using Genescan and Genotyper software. Because homozygosity and MSI render any locus uninformative for allelic imbalances, we studied only heterozygous cases, determining ratios of the alleles in both the normal and the tumor samples. Any ratios differing by >20% were additionally evaluated for possible allelic imbalances. For determination of LOH or amplification in the cases without CGH data, the unchanged allele was first identified by comparison with other microsatellites showing no change in the same multiplex PCR and then the ratios between the alleles at each locus were calculated. An increase of at least 40% in the tumor:control ratio was designated amplification; a decrease by at least 40% is an LOH (25). MSI was detected as the appearance of new bands not seen in the genomic DNA (25). Any genetic aberrations were checked at least twice more.

**CGH.** When sufficient DNA was available, CGH analysis was performed as described previously (16). Briefly, tumor DNA was labeled with biotin-16-DUTP, whereas normal DNA extracted from placenta was labeled with digoxigenin-11-DUTP by nick translation (Roche Diagnostics, Mannheim, Germany). Equal amounts of test and reference DNA (1 μg each) were cohybridized on commercially available metaphase slides (Vysis, Downers Grove, IL). Detection of biotin- and digoxigenin-labeled probes was accomplished with FITC avidin (Vector Laboratories, Burlingame, CA) or Cy3-conjugated antidigoxigenin (Dianova, Hamburg, Germany), respectively. 4,6-Diamidino-2-phenylindole counterstain was used for chromosome identification after antibody detection. Signals were visualized under a Zeiss Axioshot fluorescence microscope and analyzed with the ISIS digital image analysis system (MetaSystems, Altlussheim, Germany). At least 15 metaphases/case were analyzed. For identification of chromosomal imbalances, ratios of 1.25 and 0.8 were used as upper and lower thresholds, respectively.

**Immunohistochemistry for hMLH1 and hMSH2.** In cases showing increased levels of MSI, we performed immunohistochemical staining for the mismatch repair gene products hMLH1 and hMSH2 (anti-MLH1 antibody was from hybridoma clone G168-15 from PharMingen, San Diego, CA; anti-hMSH2 antibody was from hybridoma clone FE11 from Calbiochem, San Diego, CA) on formalin-fixed, paraffin-embedded tissue sections using standard immunoperoxidase techniques. Normal lymphoid cells on the same slide served as a control.

**Statistical Analysis.** Statistical differences were analyzed by χ² test or Fisher’s exact test using the commercially available Stat View statistical program (Abacus Concepts, Inc., Berkeley, CA).

**RESULTS**

**Overall Frequencies of Genetic Aberrations in Thymoma.** We found genetic aberrations at one or more of the 17 chromosomal regions studied in 87.3% of cases. There were allelic imbalances in 48 of 55 cases (87.3%). Most of these were LOHs, whereas only 7 cases (12.7%) showed amplifications at certain loci (Fig. 1). MSI was found in 5 of 55 cases (9.9%; Figs. 2 and 3).

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4 Internet address: http://gdbwww.gdb.org/.
6 Internet address: http://www.ensembl.org/.
Overall, the aberrations at four regions on chromosome 6 and 13 regions on nine other chromosomes give a strong impression of heterogeneity. Types B2, B3, and C thymomas usually showed genetic aberrations on multiple chromosomes, although such multiple aberrations were rare in type A and intermediate in type AB thymoma (Fig. 4). Types B and C showed more allelic imbalances than types A or AB (Fig. 1A). In all types, they were most frequent on chromosome 6, especially at 6q25.2-25.3 and 6p21.3, as shown in Fig. 1B. In addition, we saw amplifications at three regions on chromosome 16 (including CDH1), one region on chromosome 5 (including APC), and one region on chromosome 18 (including BCL-2 and DCC) in a total of seven thymomas (Fig. 1B).

Although we found MSIs in only five thymomas (1 AB, 1 B2, and 3 B3), they frequently involved multiple sites; in case no. 36, they occurred at 44% of informative loci (MSI-high) and at 10 and 20% (MSI-low) in case nos. 39 and 40 (all type B3). Nevertheless, we found no sign of defective expression of the mismatch repair proteins hMLH1 and hMSH2; nuclear staining was strongly positive in these thymomas (data not shown).

**Frequent and Multiple LOHs on Chromosome 6 in all Thymoma Types.** The two regions most frequently affected were on chromosome 6-6p21.3 (MHC) and 6q25.2-25.3 (Fig. 2); interestingly, these showed LOHs in all thymoma types, whereas those at 6q14-15 and 6q21 were less common in types A and AB (Fig. 2) such as those at the other chromosomal regions (Fig. 3). We found deletions of one entire chromosome 6 (monosomy 6) in one type B2, three B3, and one C thymomas using both microsatellite analysis and CGH. One type B3 (case no. 36) with numerous MSIs and one type C (case no. 53), exhibited an apparently complete loss of chromosome 6 by CGH, whereas microsatellite analysis detected small regions of chromosome 6 with retention of heterozygosity in both samples. Monosomy 6 detected by CGH occurred significantly more frequently in types B–C than types A–AB (P = 0.0045). No amplifications were seen at any of the four regions on chromosome 6.
Frequent Allelic Imbalances at 13q14.3 (RB), 16q22.1 (CDH1), 8p11.21, and 7p15.3 in Non-A Type Thymomas. Results for chromosomes other than 6 are shown in Fig. 3; recurring aberrations are summarized in Table 1. Only 3 of 11 type A thymomas showed LOHs there, affecting multiple loci only in one (case no. 11). By contrast, genetic aberrations were more frequent in some AB thymomas, especially at 13q14.3 (RB) and 7p15.3, strikingly, also in 22 of 28 with types B2, B3, or C and involving 8p11.21, 16q22.1 (CDH1), and 17p13.1 (p53) in addition. Thus, in these cases, LOHs at multiple regions were also commoner (Fig. 4). We noted amplifications (square) more often on chromosome 16 in the B2 and B3 samples and MSIs (circle) at multiple sites in the B3s especially (Fig. 3).

Whereas type A thymomas showed recurrent alterations only on chromosome 6, the aberrations at other loci become increasingly diverse as one scans from types AB through B2 to B3 in Table 1 and Fig. 3. Their prevalence approached 100% only in type C, where we also saw unusual LOHs at 16q23-24.1. Interestingly, in two type B2 thymomas, there were amplifications at 16q22.1 (CDH1; Fig. 3), whereas recurrent LOHs in the B2s were at 5q21-22, 7p15.3, and 8p11.21 (Fig. 3); in addition, three advanced stage III or IV type B3s shared a unique combination of LOHs at 5q21-22 (APC), 13q14.3 (RB), and 17p13.1 (p53; case nos. 44, 45, and 46 marked * in Table 1 and Fig. 3). Surprisingly, this co-occurred with LOH at 6q25.2-25.3 in one B3 (case no. 46), although we had previously concluded that these were mutually exclusive (16, 17). Finally, recurring LOHs at 5q21-22, 7p15, and 8p11 in both type B2 and B3 thymomas, plus the generally higher frequencies of genetic aberrations in the B3s (including shared MSIs at 7p15.3), are consistent with B2 to B3 progression in some cases.

Gross Genetic Aberrations Detected by CGH Analysis. CGH was performed in the 28 cases in which sufficient DNA was available. The results of 18 cases are already published (16); we now add one

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**Table 1: Allelotyping of WHO-Defined Thymoma Subtypes**

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Fig. 3. Genetic aberrations on other chromosomes than 6 in thymoma. Status of each locus is indicated: [●], LOH; [□], amplification; [□], MSI; [☑], retention of heterozygosity; and [□], homozygosity. In the CGH column: (−), no gain or loss; NA, not analyzed by CGH.
type A, four conventional AB (including case no. 56), one AB with unusual histological heterogeneity (case no. 57; Fig. 5), and four B2 thymomas. Monosomy 6 was detected in one B2, four B3, and two C thymomas (Fig. 2). Seven cases showed losses at 13q (including the RB locus): one AB; five B3; and one C. Thus, when both types of analysis were performed in a given case, the CGH findings confirmed the results of the microsatellite analysis (Figs. 2 and 3).

In two cases (nos. 56 and 57), only CGH was performed because nonneoplastic tissue was not available. Loss at 5q21-q31 (including the APC locus) was the only aberration detected in case no. 56 (conventional type AB by morphology). By contrast, the carcinomalike area in an unusual AB thymoma with heterogeneous histology showed a complex pattern of genetic imbalances (case no. 57 Fig. 5, a–c). Chromosomal gains were found at 1q, 5q15-qter, 17q, and Xq22-qter. Chromosomal losses occurred at 3p14-pter, 10p, 11q21-a, and Xq22-qter. The focal high-grade change in invasiveness despite very similar LOHs (Table 3). For example, although one type of AB thymoma (case no. 19) had three LOHs, it was fully encapsulated (stage I), whereas one B3 (case no. 48) that shared two of them (at 7p15.3 and 8p11.21) exhibited pleural dissemination (stage IVA). Other similar, if less extreme, contrasts are highlighted in Table 3. Taken together, these pairs suggest that the correlation between genetic and clinicopathological thymoma features is not simple. That is supported by the apparent intratumoral progression revealed by CGH in case no. 57. The focal high-grade change in this otherwise conventional AB thymoma was accompanied by complex aberrations, including gains at 1q and LOHs at 16q and 17p. Although these changes are characteristic of advanced stage B3 thymomas and thymic epidermoid carcinomas (Table 2; Refs. 16, 17), no such morphological patterns were seen in the high-grade focus of this

**Discussion**

This study reports five major new findings about the genetics of thymomas in addition to their obvious heterogeneity: (a) the various WHO-defined histological thymoma types, including the lymphocyte-rich AB and B2 systematically studied here for the first time, exhibit different profiles of genetic aberrations; (b) some of the lymphocyte-poor type B3 thymomas are genetically closely related to the lymphocyte-rich B2 thymomas and may arise from them by gain of genetic aberrations; (c) only WHO type A thymomas exhibit a generally homogeneous profile of genetic abnormalities mainly involving chromosome 6, in contrast with the heterogeneity in the other thymoma types; (d) metastatic behavior shows a significant correlation with allelic imbalances at 8p11.21 and 16q22.1; and (e) some type A and AB thymomas share apparently similar profiles of genetic aberrations with type B or C thymomas but maintain their distinctive morphology and benign clinical behavior.

**Frequent LOHs on Chromosome 6.** The frequent LOHs on chromosome 6 might play several important roles in thymoma evolution. Interestingly, the hitherto elusive lymphocyte-rich AB and B2 types closely resembled the previously studied lymphocyte-poor types A, B3, and C thymomas in their frequent LOHs at 6q25 (Figs. 1B and 2; Refs. 16, 17). This suggests nearby tumor suppressor genes in this region that is lost early in thymomagenesis. Its rarity in other epithelial neoplasms (31, 32) and non-Hodgkin’s lymphoma (33) implies a specific role in normal thymic epithelial homeostasis. In addition, because localized chromosome 6 LOHs were common in all thymoma subtypes, whereas complete deletions (i.e., monosomy 6) were found only in clinically malignant types B2, B3, and C of at least stage III (Fig. 2), loci elsewhere on this chromosome may influence histological phenotype and prognosis.

The 6p21.3 LOH, spanning the MHC locus, was common in types A, AB, B3, and C but was seen in only one of six B2 thymomas, a possible difference that requires confirmation. In theory, loss of a MHC haplotype might affect intratumorous T-cell selection (7); however, the LOH at 6p21.3 did not associate significantly with MG in this study (P > 0.05) and nor did the LOH of the putative autoimmune-related AIRE locus on chromosome 21 (28). Alternatively,
**Signs of Tumor Progression.** Whereas type A thymomas were the most homogeneous, with recurrent LOHs restricted to chromosome 6, the other types typically showed multiple recurrent allelic imbalances at other chromosomal loci, sometimes without aberrations on chromosome 6 (Figs. 2 and 3). Surprisingly, AB thymoma was more akin to type B in this respect, despite sharing a singularly benign clinical behavior with type A. Some type B2 and B3 thymomas shared recurrent genetic features as well, notably LOHs at 5q21-22 (APC), 7p15, and 8p11, suggesting B2 to B3 progression. However, the combined loss of regions 5q21-22 (APC), 13q14.3 (RB), and 17p13.1 (p53) was unique to a subset of more invasive type B3 thymomas (in Table 1). The APC gene is involved both in intercellular adhesion through β-catenin and E-cadherin and in the control of proliferation via the WNT pathway (35, 36). Inherited or acquired APC mutations initiate a variety of tumors (35–40) indicating the APC-dependent adenoma-colon carcinoma sequence (also involving the APC, RB, and p53 loci) that predisposes to both sporadic and familial colorectal cancer (37, 38), a sequence strikingly echoed by the combined LOHs in this subset of B3 thymomas (in Table 1).

The shared aberrations at 5q21-22, 7p15 and 8p11 and the combined LOHs at 3p14.2, 5q21-22, 13q14.3, and 17p13.1 only in some type B3 thymomas suggests that a subset of B3s may arise from B2s; that is also supported by the frequent occurrence of both B2- and B3-like areas in combined thymomas (3, 4, 6, 24). In line with this progression, B2 and especially B3 thymomas have proved aggressive in most clinicopathological studies (6, 41, 42). On the other hand, we saw LOHs at 5q21-22 (APC) not only in type B but also (by CGH) in one AB thymoma (case no. 56), which therefore seems closer to type B than to A. Furthermore, these recurrent LOHs in type AB, B2, and B3, but not A, thymomas correlate with the generation of abundant thymocytes, as in normal thymic cortex, and may imply a cortical footprint.

**Correlates of Invasiveness/Metastasis.** The LOH at 8p11.21 was seen in approximately one-third of both type B2 and B3 thymomas and appears to be independent of the LOHs at 5q21-22 or 6p25.2-25.3. LOHs at 8p have been observed in many cancers, including gastrointestinal (43, 44), hepatic (45), oral and laryngeal (46), pulmonary (47), prostate (48), breast (49), and ovarian carcinomas (50). Although there is a candidate LZTS1 tumor suppressor near 8p22 (51), imbalances at 8p21.2-21.1 proved to be ~2-fold less frequent there than at 8p11.21, perhaps implying additional more centromeric candidates.

A third recurrent feature in occasional type B thymomas (16.7%) was the MSI that affected multiple loci both on chromosome 6 and elsewhere. The isolated MSIs at 7p15.3 in one of six B2 samples (case no. 31) and at 16q23-24.1 in 1 of 17 ABs (case no. 22) may be unusual events, for example, in this rare monophasic variant of the AB type that is lymphocyte-rich throughout the tumor (case no. 22). It would be interesting to compare the genetic profiles of thymoma epithelial cells cultured from such cortex-like areas and lymphocyte-poor (medulla-like) areas that occur side-by-side in the commoner composite variant of AB thymomas. In any event, the findings suggest that, in some cases, MSI may represent a third route to thymoma
development in addition to the previously suggested pathways via 6q25.2-25.3 or 5q21-22.

Advanced tumor spread (stage IV) correlated primarily with allelic imbalances, particularly LOHs at 8p11.21 and 16q22.1 (CDH1), but not with any others (Table 2). That echoes findings in other malignancies, including LOHs at 8p in advanced colorectal and breast cancers (44, 49), that seem not to involve LZZTS1 at 8p22. In addition, reduced expression of E-cadherin, encoded by the CDH1 gene at 16q22.1, may impair adhesion among tumor cells, therefore, favoring invasion or metastasis; CDH1 deletions or mutations have been observed in invasive gastric (26), breast (27), and prostate carcinomas (52). Taken together, our results implicate a variety of pathways in metastatic thymomas. However, tumor stage is subject to other influences such as early discovery by chance on routine screening. More systematically, associated autoimmune disorders (especially MG) may lead to earlier diagnosis of thymomas; because MG is most common in type B2 (2–6, 53), advanced stages may be underrepresented in this type.

Notably, several pairs of A/B, A/C, AB/B, or AB/C thymomas with very similar LOHs differed strikingly in their invasiveness (Table 3). Therefore, we suspect that additional loci must also be involved, although we have screened most of the candidates best known to confer aggressiveness to a wide histogenetic spectrum of other tumors (3). Therefore, we suspect that additional loci must also be involved, although we have screened most of the candidates best known to confer aggressiveness to a wide histogenetic spectrum of other tumors (3). To these arguments, we can now add: the frequent occurrence in case no. 46 (type B3, stage III) of LOHs at both 5q21-22 and 6q25.2-25.3, arguing that these pathways are not mutually exclusive as previously inferred (17).

In conclusion, type A thymomas appear distinct and relatively homogeneous, with genetic aberrations mainly on chromosome 6. By contrast, the diverse changes in other subtypes suggest that, genetically, type AB thymomas are true intermediates between type A and B thymomas and that some B3 thymomas may arise from B2s by tumor progression. However, the distinctively benign behavior of types A and AB strongly suggests some additional genetic silencer(s); these could explain the imperfect correlation between genotype and phenotype in Table 3. Considering also the accumulating evidence that the diverse cortical and medullary epithelial subtypes (55, 56) derive from a common precursor (56, 57), we therefore propose that the stage at which neoplastic transformation occurs (e.g., in an uncommitted precursor or one with a medullary or cortical commitment) influences the biology and phenotype of the resulting thymoma.

**ACKNOWLEDGMENTS**

We thank Evi Werder and Christel Kohaut for expert technical assistance and Elke Oswald for advice on cell culture methods.

**REFERENCES**


### Table 3

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<th>Shared LOHs</th>
<th>Different WHO types, modified Masaoka’s stage (no. case numbers)</th>
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<tr>
<td>5q15-31 (APC)</td>
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<tr>
<td>6p21.3 (MHC)</td>
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<td>7p15.3 and 8p11.21</td>
<td>A stage F (no. 3), AB stage I (no. 13), B3 stage II (no. 43), IVB (no. 50)</td>
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<td>16q22.1 (CDH1)</td>
<td>AB stage I (no. 19), B3 stage III (no. 41), B3 stage IV (no. 48)</td>
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<td>17p13.1 (p53)</td>
<td>AB stage I (no. 11), B3 stage IV (no. 51), C stage IVB (no. 54)</td>
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* LOHs were detected by microsatellite analysis.

* Capital letters A, AB, B, C represent WHO thymoma types.

* In AB case no. 56, only CGH was applied, because only tumor material but no nonepithelial tissue was available. Numbers in parenthesis represent the individual case numbers as applied in Figs. 2 and 3.

* The pairs with the most discrepant clinicopathological features are highlighted by bold letters.


