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Expression of aldosterone synthase CYP11B2 was inversely correlated with longevity

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Abstract

Immunohistochemistry of human aldosterone synthase (CYP11B2) has revealed that most of aldosterone is autonomously produced in aldosterone-producing cell clusters (APCCs) beneath the capsule of adult adrenals rather than physiologically in the zona glomerulosa (ZG). APCCs have been occasionally found to harbor a somatic mutation of ion channel/pump genes, and number and size of APCCs increase with age until 50 years old. Herein, the objective of the study was to examine APCC development in 106 autopsied adrenals from 85 elderly individuals who died at ages from 50 to 103 years. We obtained the following results: (1) physiological CYP11B2 expression in ZG were attenuated in more elderly persons; (2) number and size of APCCs

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Author contributions

Writing the manuscript: TH, ZZ, TS, HA, CEGS, KM, KN; pathological section preparation: AS; immunohistochemistry: MY; scanning of slides: ZZ; APCC tracing and measuring: KM, KN, GAE; adrenal section tracing and area measuring: ZZ; IRB application: TH, KN, OM; preparation of the HSD3B2 antibody: CEGM.

Disclosure summary

Nothing to disclose.

decreased with age; (3) detachment of APCC from the capsule appeared to occur occasionally over the wide range of the ages; and (4) incidental micro aldosterone-producing adenomas (APAs) and possible APCC-to-APA transitional lesions (pAATLs) were found primarily in samples from persons aged 50–60 years but not in samples from more elderly persons; pAATL was a putative designation based on our previous results indicating that it consisted of subcapsular APCC-like portion and inner APA-like portions. Thus, the formation of the CYP11B2-expressing lesions as well as thickening of the ZG in the adrenals were inversely correlated with age of death in the individuals aged over 50 years. Considering that autopsy samples were used in this study, inactive production of aldosterone regardless of autonomous or physiological manners may have survival advantages in individuals aged over 50 years.

Keywords

Aldosterone-producing cell cluster (APCC); Aldosterone-producing adenoma (APA); Adrenal gland; Aldosterone synthase (CYP11B2)

1. Introduction

1.1. Subtypes of primary aldosteronism

Primary aldosteronism (PA) is the most common cause of secondary hypertension [1,2]. In adults, PA is primarily caused by aldosterone-producing adenoma (APA) or idiopathic hyperaldosteronism (IHA). Somatic mutations in ion channel/pump genes including *KCNJ5* (potassium channel, inwardly rectifying subfamily J, member 5), *ATP1A1* (ATPase Na^+/K^+ transporting subunit α1), *ATP2B3* (ATPase plasma membrane Ca^{2+} transporting 3), *CACNA1D* (calcium voltage-gated channel subunit α1D), *CACNA1H* (calcium voltage-gated channel subunit α1H), and *CTNNB1* (catenin β1) have been identified in adult-onset APAs [3–6]. Juvenile PA is very rare and is mainly caused by four types of familial hyperaldosteronism (types 1–4) [1,2,7]. Among these subtypes of familial PA, familial hyperaldosteronism type 3 is caused by germ-line mutations in ion channel/pump genes including *KCNJ5* [3]. In addition to these PA subtypes, our group has reported an interesting juvenile case of non-familial juvenile PA, which was suggested to be genetic mosaicism of a *KCNJ5* mutation that presumably occurred at mesodermal development [8]. Thus, the causes of PA are currently subclassified into six types (i.e. APA, IHA, and familial hyperaldosteronism types 1–4), whereas the pathology or aldosterone-producing lesions of PA, particularly for IHA, has yet to be elucidated.

1.2. APA, APCC, and pAATL

We previously established immunohistochemistry for aldosterone synthase (CYP11B2) and cortisol-synthesizing enzyme (11β-hydroxylase, CYP11B1) and achieved a pathological confirmatory diagnosis of APA [9]. APAs contain CYP11B2-expressing cells, CYP11B1-expressing cells, and double negative cells, whereas cortisol-producing adenomas possess CYP11B1-expressing cells and double negative cells [9].

We also reported that sites of CYP11B2 expression in normal adult adrenal glands were mainly aldosterone-producing cell clusters (APCCs) that attached to the capsule rather than

the zona glomerulosa (ZG) [9]. ZG is occasionally discontinuous, and aldosterone synthesis in ZG is mostly uninduced presumably due to autonomous production of aldosterone in APCCs. APCCs morphologically consist of subcapsular ZG-like cells and inner zona fasciculata (ZF)-like cells; the latter cells are histologically indistinguishable from the surrounding ZF cells expressing CYP11B1, thereby making APCCs undetectable with hematoxylin and eosin staining (H&E). They strongly express steroidogenic enzymes necessary for aldosterone synthesis including CYP11B2, but not CYP11B1, suggesting autonomous production of aldosterone [10]. Because they frequently harbor a somatic mutation in one of the ion channel/pump genes, *CACNA1D*, *ATP1A1*, and *ATP2B3*, which were already found in APAs (APA-associated mutations), we suspect that APCCs are precursors of APAs [3–6].

Furthermore, we have proposed possible APCC-to-APA transitional lesion (pAATL) as a putative transitional lesion developing from APCC to APA on the basis of accumulating indirect evidence: Morphological and steroidogenic characteristics of pAATLs indicate that they consist of a subcapsular APCC-like portion and an inner APA-like portion; distributions of somatic mutations of the ion channel/pump genes in the two different portions of pAATLs suggest that APCC is an origin that develops to APA as discussed later [11,12].

The pathologies, namely aldosterone-producing lesions, causing PA (except for familial hyperaldosteronism) have been classified into two categories; one, APAs that unilaterally produce excessive amounts of aldosterone together with different levels of cortisol; and the other, unilateral or bilateral adrenal hyperplasia with micro- and macro-nodules [1,2]. The functional evaluation using immunohistochemistry of CYP11B2 as well as other steroidogenic enzymes allows to revise the conventional classification especially adrenocortical hyperplasia with micro- or macronodules.

1.3. APA-associated mutations

The most frequent APA-associated mutations are those of *KCNJ5* [3]. Mutation of *CACNA1D*, *ATP1A1*, or *ATP2B3* have often been found in the rest of the cohort [4–6]. APCCs of normal individuals were found to have mutations of *the latter three genes* frequently, but not those of *KCNJ5* [10]. Larger pAATLs harbored *KCNJ5* mutations [12]. APAs are generally solitary, whereas multiple APCCs and/or pAATLs often exist together. APAs harboring *KCNJ5*-mutated cells are slightly larger than those of wild-type cells or those with other types of mutations, suggesting that *KCNJ5* renders a proliferative advantage *in vivo* [6,14,15]. On the other hand, Oki et al demonstrated that transduction of HAC15 cells with a lentivirus encoding a *KCNJ5* mutant (p.T158 A) exerted anti-proliferative effects [16]. The mechanisms responsible for the discrepancy of growth effects between *in vitro* and *in vivo* have not yet been elucidated.

1.4. Cell fate in normal adrenal cortex

Previous studies on cell fate of the adrenals of mice indicated that precursor cells located in the subcapsular region differentiated to cells initially expressing CYP11B2, then moved inwards losing the ability to express CYP11B2 but gaining the ability to express CYP11B1 [17,18]. They further migrate centripetally to the cortico-medullary junction, at which they

undergo apoptosis. Human adrenal cortices are assumed to undergo similar phenotypic changes and cell migration, and the cells are possibly removed by apoptosis in the cortico-medullary junction.

Although the fate of APCCs is currently unknown, we previously reported that numbers and sizes of APCCs steadily increase in individuals at least up to 50 years old [19]. Nanba et al. also observed similar changes in APCCs with aging [20]. However, it currently still remains unclear whether APCCs continue to increase in individuals older than 50 years. Therefore, we herein examined expression of CYP11B2 using autopsied adrenals from elderly individuals older than 50 years.

2. Material and methods

2.1. Ethics

The use of human samples in the present study was approved by the Institutional Review Board of the Saitama Medical University International Medical Center (approval #17–197). Animal experiments for immunization and generation of hybridoma cells were performed in the Cell Engineering Corporation (Osaka, Japan) and approved by the Animal Care and Use Committee of the institution as S-251.

2.2. Sample collection

Specimens were from a bank of autopsy samples of the Saitama Medical University Hospital. Cases that had not been diagnosed as PA were retrospectively and randomly selected for this study based on age as follows (Table 1): Two cases each (the newest and second newest) in every age from 50 to 98 years were included, except for 1 case each at ages of 59, 65, 70, 77, 83, 85, 95, and 98 years as well as no case at ages of 82, 93, and 96 years due to the absence of adrenal samples. Additionally, 1 case aged 103 years was included in order to expand the age range. Eighty-five cases comprising 33 females and 52 males were analyzed in the present study. These cases were registered in the database at the Japanese Society of Pathology with specific identification numbers (Byori Boken Syuho, http://pathology.or.jp/kankoubutu/JSP-hyou.html) starting with ‘A’ (Table 1 and Supplemental Table 1). Causes of death listed in Table 1 were taken from the database, but not from each patient’s clinical record. One adrenal sample was available from each of 65 cases, while two and three samples were from 19 and 1 cases, respectively (* in Table 1, samples from the left and right adrenals). Serial sections from the 106 adrenal samples were used for histochemical (hematoxylin and eosin) and immunohistochemical analyses (CYP11B2 and HSD3B2), as previously reported [11,21,22]. Images of stained sections were obtained by scanning with the Aperio ScanScope® XT slide scanner (Leica Biosystems, Nussloch, Germany). All scanned images can be downloaded from National Bioscience Database Center in Japan Science and Technology Agency (https://humandbs.biosciencedbc.jp/en/hum0181-v1).

2.3. CYP11B2 immunohisotchemistry

A mouse monoclonal anti-human CYP11B2 antibody was prepared, validated, and used as described in Supplemental Materials and Methods.

2.4. Sample selection

Sections stained for HSD3B2 were used to exclude non-adrenal samples, low-quality adrenal samples, and less steroid-producing cases. HSD3B2 signal intensities were measured using the Aperio Positive Pixel Count algorithm set in Aperio ImageScope software v.12.2.1.5005 (Leica Biosystems). Briefly, the edge of an HSD3B2-stained adrenal section was traced (green line in Supplemental Fig. 2A), and HSD3B2 signal intensities inside the traced line were converted into pseudo color images (Supplemental Fig. 2A and B images were converted into Supplemental Fig. 2C and D). HSD3B2 positivity was assessed using the Aperio Positive Pixel Count Algorithm, in which areas of weakly positive (yellow in Supplemental Fig. 2D), positive (orange), and strongly positive (red) cells were calculated as follows: positivity = ([area of weakly positive] + [area of positive] + [area of strongly positive]) / ([area of weakly positive] + [area of positive] + [area of strongly positive] + [area of negative]) ('HSD3B2 positivity' in Supplemental Table 1 and Table 1). Thirty-one samples with positivity less than 20% were excluded (*italicized* in Table 1); therefore, 75 samples from 64 cases (24 females and 40 males) were used in analyses (bold in Table 1).

2.5. Measurement of APCC

Three examiners (KN, KM, and GAE) independently recognized and then traced for measurements of sizes and numbers of APCCs on CYP11B2-stained sections using Aperio ImageScope software, as shown in SupplementalFig. 2E ('APCC area' in Supplemental Table 1). Adrenal areas, percentages of the sum of APCC areas over the whole adrenal area (AA/WAA, %), and numbers of APCCs (NOA/mm^2) were calculated and used in statistical analyses as previously reported (Table 1 and Supplemental Table 1). Variations among examiners were primarily due to disagreements in recognition of APCCs; some examiners judged a positive region as APCC and others judged it as a small population of positive cells in ZG. Statistically, NOA (APCCs/mm^2) and AA/WAA values from the 3 examiners strongly correlated with each other (r: 0.766 – 0.915, $p < 0.0001$ in NOA; r: 0.729 – 0.839, $p < 0.0001$ in AA/WAA; Supplemental Fig. 3), and the average numbers of the three examiners were used for statistical analyses.

2.6. Measurement of CYP11B2-positive ZG thickness

In each sample, three examiners (TS, KM, and GAE) independently selected representative areas of normal ZG, in which no APCC, pAATL, or APA existed, and assessed the representative thickness of CYP11B2-positive ZG for the sample using the following scale: ZG (−) (no positive ZG cells), ZG (+) (0–1 cell layer was positive for CYP11B2), ZG (++) (2–5 cell layers were positive), and ZG (+++) (more than 5 cell layers were positive). Based on the three examiners' measurements, the 'average' ZG thickness for each sample was evaluated by taking the most common assignment, i.e. if two examiners assigned ZG (++) and one examiner assigned ZG (+), the 'average' ZG thickness was ZG (++). Although the three examiners' measurements agreed in many samples (25 samples, 39%), they assigned three different thicknesses in 6 samples (9%), and, thus, a fourth examiner (KN) independently measured these samples in order to select the most common thickness assignment. Retrospectively, the samples that the three examiners did not agree on mostly had CYP11B2-negative ZG areas with only small areas with positive signals in ZG. Such

samples might lead some examiners to focus their attention incorrectly on the positive areas. All of these samples were assigned ZG (−) when the measurements of the fourth examiner were considered.

2.7. Statistical analysis

Comparisons of AA/WAA and NOA values among the 3 examiners were analyzed by the Kruskal-Wallis one-way analysis of variance on ranks. The relationships between AA/WAA and NOA and between age and AA/WAA or NOA were analyzed by Pearson's product moment correlation. The relationships between NOA or AA/WAA and categorized ZG thickness were analyzed by Spearman's rank order correlation. Comparison of average ages among categorized ZG thickness was analyzed by a one-way analysis of variance. Comparisons of average ages between patients with and without detached APCCs and between patients with and without pAATLs/APA were analyzed using an unpaired Student's *t*-test. P-values less than 0.05 were considered to be significant.

3. Results

3.1. Decrease in numbers and sizes of APCC with age

Adrenal samples from autopsy cases were retrospectively selected for analyses; histological and immunohistochemical staining was performed as previously reported (see images of all samples in Supplemental Fig. 1) [9,10,23]; low-quality samples were excluded (see 'Sample selection' in the Materials and Methods and Supplemental Fig. 2A–D). Percentages of the sum of APCC areas over the whole adrenal area (AA/WAA, %) and numbers of APCCs (NOA, /mm^2) were calculated as detailed in the Materials and Methods. Seventy-five samples from 64 cases (24 females and 40 males) with ages ranging from 50 to 103 years were used. Supplemental Fig. 2E shows a representative CYP11B2-stained section used for measurement (APCCs are outlined). Table 1 summarizes patient characteristics and measurement outcomes, and Supplemental Table 1 includes the details of the measurements.

As expected, NOA and AA/WAA strongly correlated (r = 0.804, p = 1.201E-015, Pearson's product moment correlation analysis), although the r value of males (r = 0.886, p = 3.044E-014) was greater than that of females (r = 0.617, p = 0.00131; Fig. 1A). NOA significantly decreased with age (r = −0.356, p = 0.00390, Pearson's product moment correlation analysis, Fig. 1B). Similarly, AA/WAA significantly decreased with age (r = −0.316, p = 0.0111, Pearson's product moment correlation analysis, Fig. 1C). When males and females were analyzed separately, the decrease in NOA was significant both in males (r = −0.391, p = 0.0126) and females (r = −0.356, p = 0.0039) as well as those in AA/WAA was significant both in males (r = −0.356, p = 0.0243) and females (r = −0.316, p = 0.0111). Similar results were observed even including samples which HSD3B2-positivity was larger than 0% (Supplemental Fig. 4A–C) and 10% (Supplemental Fig. 4D–F). These results indicated that the numbers and sizes of APCC decreased with age of death after 50 years old.

3.2. Depression of ZG expression of CYP11B2 with age

In addition to APCCs, the adrenal samples differed in thickness of the CYP11B2-positive ZG layer (Table 1, Supplemental Fig. 1), depending on the physiological upregulation of aldosterone synthesis and responsiveness of ZG, similar to rat adrenals under different conditions of sodium intake [24]. In order to observe age-associated changes, the thickness of representative CYP11B2-positive ZG areas (in which no APCC, pAATL, or APA existed) in each sample was measured as follows: ZG (−) (no positive ZG cells, n = 16, 25%), ZG (+) (0–1 cell layer was positive for CYP11B2, n = 15, 23%), ZG (++) (2–5 cell layers were positive, n = 25, 39%), and ZG (+++) (more than 5 cell layers were positive, n = 8, 13%). Bilateral samples (* in Table 1) generally showed a similar ZG thickness on both sides. Median NOA in ZG (−), ZG (+), ZG (++), and ZG (+++) samples were 0.0101 (0.00303–0.0408) /mm^2, 0.0248 (0.00944–0.0512) /mm^2, 0.0332 (0.0199–0.0478) /mm^2, and 0.0623 (0.0271–0.121) /mm^2, respectively (Fig. 2A). Accordingly, NOA correlated with ZG thickness ($r = 0.389$, $p = 0.0015$; Spearman's rank order correlation). Similarly, median AA/WAA in ZG (−), ZG (+), ZG (++), and ZG (+++) samples were 0.269% (0.0631 – 0.540%), 0.266% (0.127 – 0.783%), 0.371% (0.150 – 0.728%), and 1.09% (0.750–2.45%), respectively (Fig. 2B), and correlated with ZG thickness ($r = 0.424$, $p = 0.000478$). These results suggested that the number and size of APCCs were associated with the thickness of CYP11B2-postive ZG. The mean ages of CYP11B2-positive ZG thickness were not significantly different between ZG (−) and ZG (+) (76.7 ± 3.4 [SEM] and 82.3 ± 3.8 years old, respectively, $p = 0.228$), whereas those of ZG (++) and ZG (+++) were significantly younger than that of ZG (+) (70.2 ± 2.6 and 56.1 ± 1.8 years old, respectively; one-way analysis of variance followed by pairwise multiple comparison procedures with the Holm-Sidak method).

3.3. Detachment of APCC from capsule

APCCs that seemed to be detached from the capsule (pink dotted line in Fig. 3A and B) were detected in 14 cases (21.9%, # in Table 1). The ages of patients with detached APCCs distributed from 51 to 103 years, and the average age of patients with and that without detached APCCs were not significantly different from each other (72.5 [56.5–84.8] and 74.0 [60.0–86.3] years old, respectively, $p = 0.826$; the Mann-Whitney rank sum test). In addition, an APCC expressing less CYP11B2 (blue-outlined area in Fig. 3C) than other APCCs in the same section (Fig. 3D) was also observed. APCC detachment appeared to occur in a wide age range without age-associated changes. These were the first observations obtained with samples from the elderly.

3.4. Regression of incidental APA with age

We noted that some samples contained non-functional tumors (5 cases, 7.8%) and pAATLs/APAs (6 cases, 9.4%) (Table 1). Multiple pAATLs existed in one case (Fig. 4A, pink arrowheads) and consisted of subcapsular APCC-like portions and inner APA-like portions as previously reported (* and ** in Fig. 4B, respectively) [11,21]. In another case, multiple APCCs (black arrowheads) and pAATLs (pink arrowheads) existed in one adrenal (Fig. 4C). An APA in A-5690 (areas outlined with blue dots in Fig. 4D–F) was found just below a pAATL (the area outlined with red dots in Fig. 4F). This APA might have grown out from

the pAATL because there was no apparent border between these lesions (APCC-like and APA-like lesions are denoted as * and **, respectively, in Fig. 4F). The APA expressed CYP11B2 weakly and heterogeneously, and the positivities of three arbitrarily selected areas were 0, 3, and 17% (boxes in Fig. 4F). The APAs of A-5754 (Fig. 4G and H) and A-5976 (Fig. 4I and J) showed a similar CYP11B2 expression pattern: weak and heterogeneous. An APA of A-5993 showed a stronger expression of CYP11B2, which could have caused PA (Fig. 4K–N). The average age of patients with pAATLs/APAs was significantly younger than those without them (59.8 ± 4.5 and 74.2 ± 4.5 years old, respectively, $p = 0.0227$, unpaired Student's *t*-test), suggesting that APAs are not common in older individuals.

4. Discussion

In the present study, autopsy adrenal samples from patients older than fifty years were employed for examination of CYP11B2 expression with immunohistochemistry. We observed that (1) physiological CYP11B2-positive ZG thickening was less common in the more elderly individuals; (2) numbers and sizes of APCCs decreased with age, particularly in males; (3) detachment and regression of APCC were noted in the adrenals over the wide age range; and (4) incidental pAATLs/APAs were found primarily in samples from patients aged 50–60 years, but not in older individuals. These observations suggest that autonomous aldosterone production as well as physiological production in individuals who live to old ages, such as eighty and older, is less active than those who died earlier.

Direct interpretation of the results may be a notion that the aldosterone-producing lesions or cells regress in life-span of each person with aging after fifty years old. However, since this study used autopsy cases, it is inevitable that the sample collection may be inhomogeneous. Thus, individuals who died later may have fewer or less active aldosterone-producing lesions or cells in the first place than those who died in the fifties. Autonomous aldosterone production may lead to comorbidities and mortalities. In fact, patients with hyperaldosteronism have higher mortality rates [25]. The direct interpretation described above could reflect survival advantages of low levels of aldosterone production.

We previously reported that the NOA and AA/WAA of APCCs steadily increased up to 50 years old using autopsied adrenals [18]. Following our study, Nanba et al. obtained similar findings using normal adrenals from renal transplant donors, who were presumably in good health, thus different from our cohort, aged 9 months to 68 years [20]. Since the two studies carried out with different manners of sample collection gave similar results, it is very likely that numbers and sizes in APCC increased with aging up to fifty years old.

Physiological synthesis of aldosterone in ZG is upregulated by RAS. The current result that the physiological thickening of CYP11B2-positive ZG was diminished in the elderly is consistent with the previous results from human studies showing that RAS is suppressed in elderly individuals [26–29]. Nanba et al. also reported that the adrenals from young renal transplant donors exhibited a layered pattern of CYP11B2 expression in ZG, whereas adrenal glands from older individuals displayed progressively less normal CYP11B2 expression [20]. In addition, they showed that older individuals had less ability to enhance

aldosterone production upon sodium restriction [20], supporting our current results described above.

Immunohistochemisty of CYP11B2 has been changing the conventional concepts of aldosterone production in humans. Subsequently to the discovery of APCC, we further proposed pAATLs as a new form of a culprit lesion [11,12,30]. As described, pAATL was a description by this research group uniquely based on our own data derived from a few cases. Since formation of pAATL seems a transient event, it would be so quite infrequent that researchers seldom encountered with it. One of reasons why we found pAATL would be that we could examine a large number of human adrenals, including so-called adrenal hyperplasia, using in-house generated antibodies to CYP11B2.

In addition to the morphological and steroidogenic characteristics of pAATLs as mentioned earlier, the mutation analyses of the ion channel/pump genes using separately micro-dissected APCC-like portions and APA-like portions gave the following results [31]: (i) Small pAATLs harbor mutations in the genes such as *CACNA1D* except for *KCNJ5*, whereas large pAATLs harbor *KCNJ5* mutations; (ii) mAPA-like portions from two of the three large pAATLs harbored mutations (*KCNJ5* [p.G151R] and *ATP1A1* [p.L337 M]), whereas their corresponding APCC-like portions did not, suggesting their role in the formation of mAPA; (iii) another lesion carried novel mutations in *ATP1A1* (p.Ile322_Ile325del and p.Ile327Ser) in both the mAPA-like and APCC-like portions, thereby supporting these portions having a clonal origin. These findings led us to propose that pAATL could be a candidate for a transitional lesion from APCC to APA. As described, the proposal is supported by the indirect evidence while it has not yet been proven by solid evidence. Thus, it deserves to be a hypothesis for studies on development of culprit lesions causing excessive aldosterone.

Recently, a classical terminology “adrenocortical micronodule” was used for aldosterone-producing lesions [13]. This is a common term of histopathology and lacks functional evaluation for aldosterone production. Since the “micronodules” in the report seemed to consist of APCC-like and micro APA-like portions, they could be called pAATLs according to our definition. Furthermore, in our experiences, so-called adrenal hyperplasia causing PA had morphologically-recognized micronodules negative for CYP11B2 [12] in addition to those positive for it. Thus, the term micronodule may cause confusion in description of pathological PA subtypes. Therefore, the lesions in adrenal hyperplasia can be categorized as APCC, pAATL or small APA based on functional evaluation for expression and distribution of steroidogenic enzymes including CYP11B2. According to the criteria, the pathological classification for PA may be (i) APAs, (ii) multiple APCCs, and (iii) pAATLs.

Lineage tracing studies in mice have shown that the adrenal cells originate in the subcapsular area of the adrenals in which precursor cells differentiate into ZG cells, migrate centripetally with transdifferentiation into ZF cells, and then reach the corticomedullary junction at which they undergo apoptosis [17,18]. These schemes are consistent with the fact that APCCs of humans develop beneath the capsule. On the other hand, a question arises why APCCs do not extend to the bottom of the cortex. Also, the present work raised another question what mechanisms detach APCCs from the capsule, though there was a possibility

that the way to section irregularly-folded adrenals made APCCs look detached. The current detection of the detached APCCs and APCCs with weak-CYP11B2-staining leads us to speculate that they are examples of APCC migrating inward and undergoing de-differentiation or transition to ZF phenotype. Further observational and molecular studies are needed in order to solve the questions, confirm the outcomes of the present study, and prove our hypothesis.

5. Conclusions

The present results suggest that, considering that autopsy samples were used in this study, formation of autonomous lesions for aldosterone production occurred less frequently in individuals who lived to older ages. Inactive production of aldosterone regardless of autonomous or physiological manners may have survival advantages in individuals aged over 50 years.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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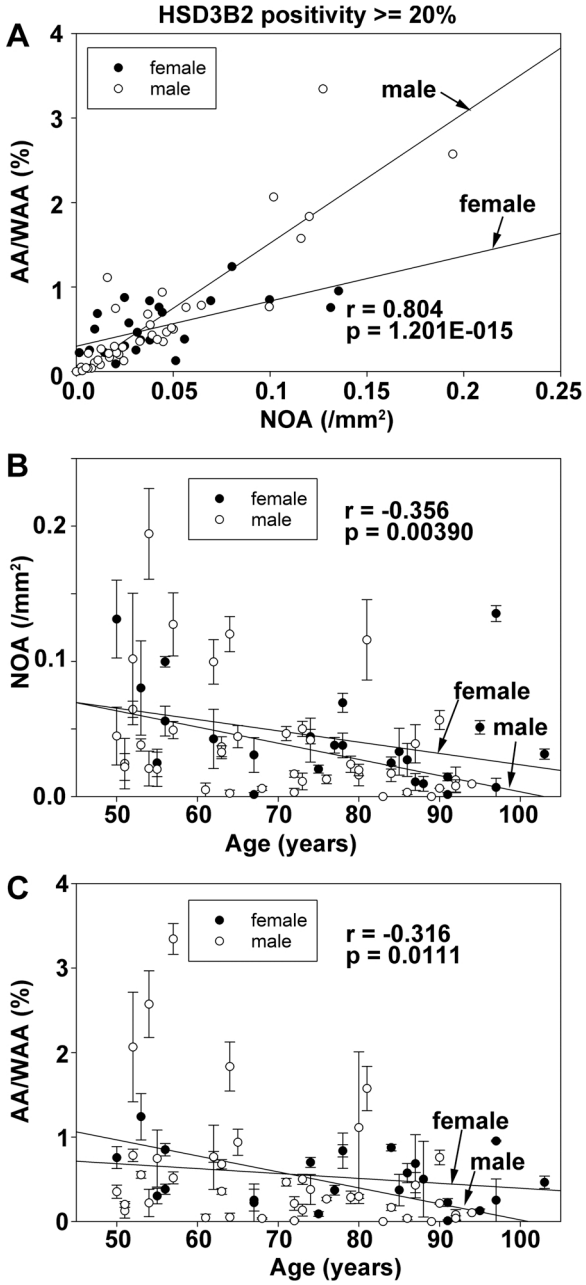

Fig. 1.

Relationship between adrenal senescence and APCCs.

A: Correlation between the average value of AA/WAA (%) and that of NOA (/mm^2). B: Correlation between age (years) and NOA (/mm^2) with the SEM of three examiners. C: Correlation between age (years) and AA/WAA (%) with the SEM of three examiners.

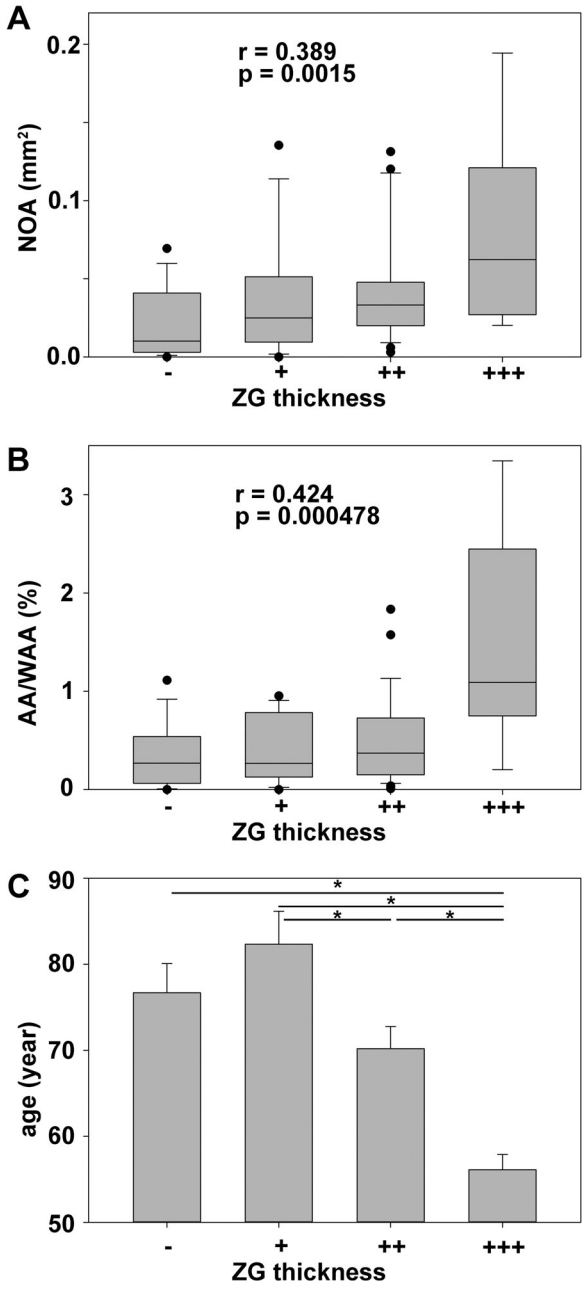

Fig. 2.

ZG thickness *vs* NOA, AA/WAA, and age.

A and B: Correlation between NOA and ZG thickness and between AA/WAA and ZG thickness, respectively. The boundary of the box indicates the 25th and 75th percentiles, and the line within the box marks the median. Whiskers below and above the box indicate the 10th and 90th percentiles, respectively. r and p values were calculated by Spearman's rank order correlation analysis. C: Correlation between age and ZG thickness. Bars and error bars indicate the average and SEM, respectively. These average values were compared by a one-way analysis of variance followed by pairwise multiple comparison procedures (Holm-Sidak method). "*" indicates a significant difference between the ZG thickness groups.

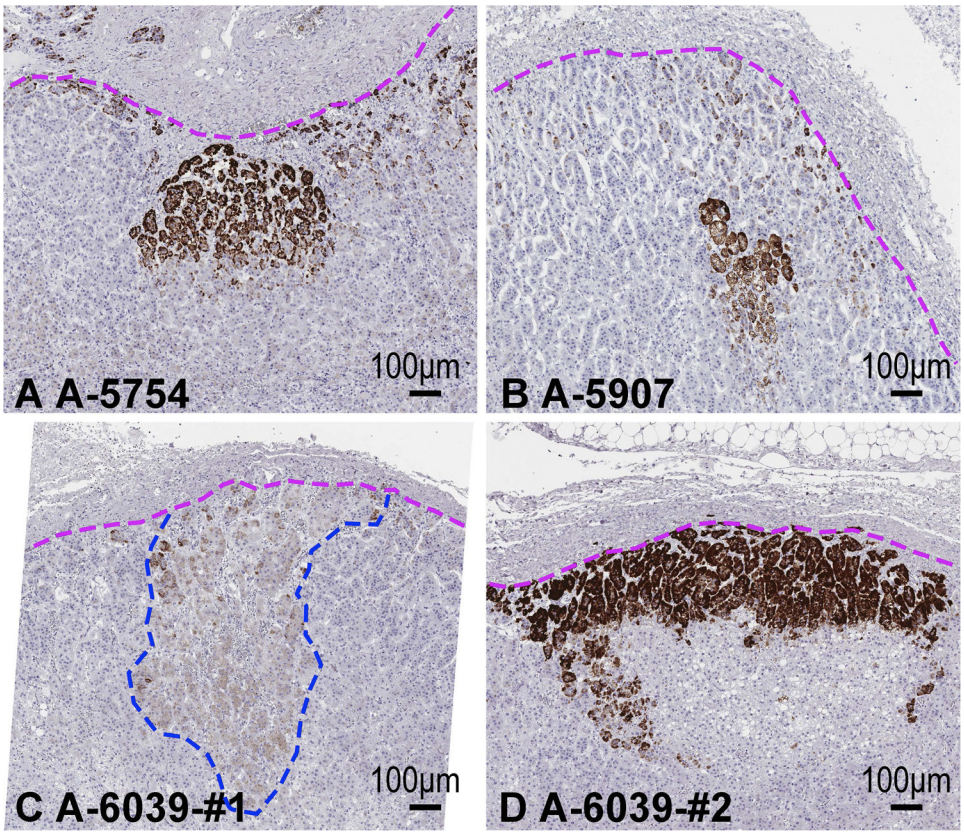

Fig. 3.

Detached and disappearing APCCs.

A and B: APCCs detached from the adrenal capsule (pink dotted line) in samples A-5754 (A) and A-5907 (B). C and D: APCC with weaker CYP11B2 expression (C) than another APCC (D) in the same A-6039 section. A large APCC in D is shown with cells that are migrating into the zona fasciculata.

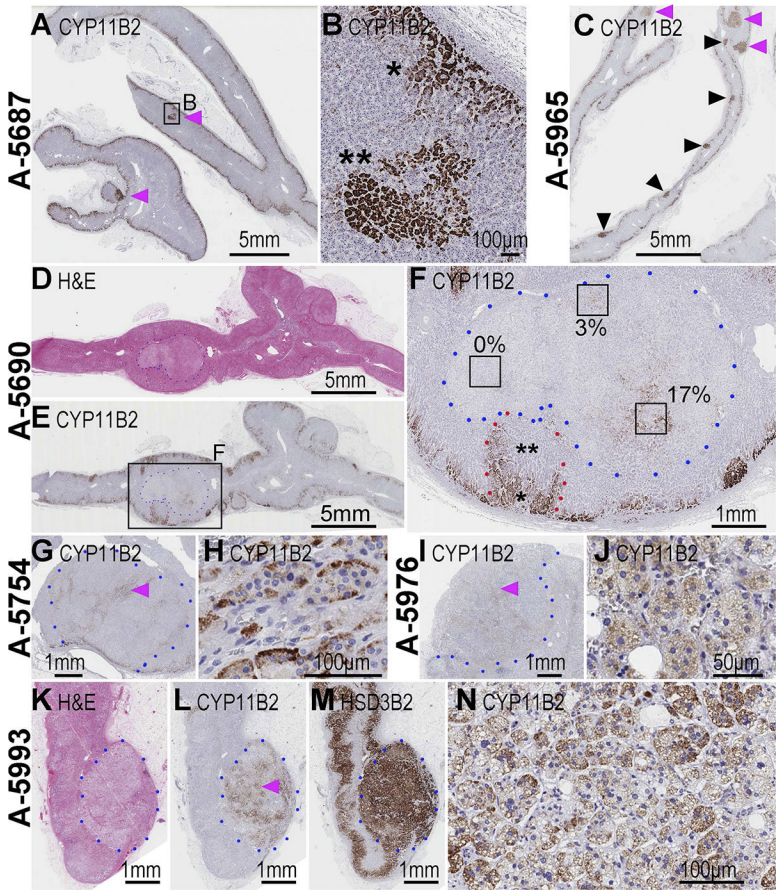

Fig. 4.

pAATLs and incidental APAs.

A: An example of a pAATL (purple arrowheads). A frame indicating the portion of the enlarged image in panel B. B: An enlarged pAATL, which consisted of a subcapsular APCC-like portion and inner APA-like portion. C: Another example of a pAATL (pink arrowheads). This sample had multiple APCCs. D and E: Hematoxylin and eosin staining (H&E) and CYP11B2 staining of an adrenal with pAATL (surrounded by red dots) and incidental APA (surrounded by blue dots), respectively. A frame indicating the enlarged image in panel F. F: Enlarged image of pAATL (surrounded by red dots) and incidental APA (surrounded by blue dots), which were histologically separated from each other. Frames with percentages indicate the positivity of CYP11B2. G and H: An incidental APA with a small CYP11B2-positive area and its enlarged image (pink arrowhead in G), respectively. I and J: Another incidental APA with a small CYP11B2-positive area and its enlarged image (pink arrowhead in I), respectively. K-M: H&E, CYP11B2 staining, and HSD3B2 staining of an incidental APA with a relatively large area positive for CYP11B2. The pink arrowhead in panel L indicates the enlarged image in panel N.

Table 1:

Case characteristics.

Case_ID		sex	age	cause of death	block number	other lesions	ZG thickrless					HSD3B2 positivity	NOA (APCC:s/mm^2, mean ± S.E.M.)			AA/WAA (%, mean ± S.E.M.)			Figure
							statistics	KN	KM	TS	GAE								
A-3944	*	**female**	**95**	**uterine cervical cancer**	**12**		+					**0.351**	0.0512	±	0.0049	0.1269	±	0.0213	
					13							**0.288**							
A-4165		**male**	**89**	**cholangiocarcinoma**			+					**0.28**	0	±	0	0	±	0	
A-4617	*	*male*	*89*	*brain infarction*	*32*							*0.065*	0.0088	±	0.0047	0.077	±	0.0487	
					33							*0.158*							
A-4769	*	*female*	*94*	*chronic heart failure*	*1*							*0.053*	0.0005	±	0.0005	0.0064	±	0.0064	
					2							*0.005*							
A-5092	*	**female**	**97**	**congestive heart failure, aortic stenosis,**	**22**		−					**0.214**	0.0068	±	0.0068	0.2515	±	0.2515	
					23							*0.14*							
A-5400	#	**male**	**92**	**colon cancer, liver metastasis**		**non func T**	++					**0.484**	0.0123	±	0.0094	0.0807	±	0.0602	
A-5407		*male*	*98*	*brain infarction*								*0.126*	0.0043	±	0.0043	0.0026	±	0.0026	
A-5454		**female**	**91**	**acute myocardial infarction**			+					**0.395**	0.0145	±	0.0029	0.2248	±	0.0455	
A-5550		**female**	**50**	**T-cell lymphoma**			++					**0.202**	0.1313	±	0.0288	0.7565	±	0.1301	
A-5576	*	**male**	**52**	**sudden death**	**29**		+					**0.384**	0.0645	±	0.006	0.7828	±	0.0736	
					51		−	−	+	−	++	**0.43**							
A-5624		**male**	**51**	**lung cancer**			++					**0.288**	0.0244	±	0.0186	0.1267	±	0.0861	
A-5687	#	**male**	**55**	**sepsis due to pneumonia**		**pAATL**	+++					**0.406**	0.0202	±	0.0127	0.7461	±	0.3369	**4A-B**
A-5690	*	**male**	**52**	**septic shock**	**4**	**APA**	+++					**0.437**	0.1018	±	0.0487	2.0654	±	0.6485	**4D-F**
					5							**0.359**							
A-5699		**male**	**94**	**senility**			−					**0.287**	0.0093	±	0	0.104	±	0.0031	
A-5737	*	**female**	**91**	**pancreatic cancer**	**18**		−					**0.452**	0.0015	±	0.0015	0.0091	±	0.0091	
					19							**0.431**							

Case_ID			sex	age	cause of death	block number	other lesions	ZG thickrless					HSD3B2 positivity	NOA (APCC:s/mm^2, mean ± S.E.M.)						AA/WAA (%, mean ± S.E.M.)			Figure
								statistics	KN	KM	TS	GAE											
A-5746			**male**	**86**	**infectious endocarditis**		**non func T**	+					**0.407**	0.003	±	0.0015	0.0375	±	0.0192			
A-5754		*#*	**male**	**51**	**liver cirrhosis**		**APA**	+++					**0.464**	0.0219	±	0.0099	0.2022	±	0.0333	**3 A, 4 G-H**		
A-5790		*#*	**female**	**103**	**brain tumor**			+					**0.245**	0.0314	±	0.0038	0.4637	±	0.0735			
A-5797	*	*#*	**male**	**53**	**pyothorax**	**12**		−					**0.312**	0.0381	±	0.0046	0.5531	±	0.0403			
						13							**0.279**									
A-5814			**male**	**90**	**primary macroglobulinemia**			−					**0.264**	0.0061	±	0	0.2154	±	0.0106			
A-5843			**male**	**92**	**pneumonia, choronic heart failure**			+					**0.369**	0.0078	±	0.0045	0.0388	±	0.0275			
A-5845	*		*male*	*60*	*sepsis*	*16*							*0.056*	0.0047	±	0.0009	0.06	±	0.0147			
						17							*0.104*									
A-5846			*male*	*60*	*systemic lupus erythematosus*								*0.044*	0.0021	±	0.0021	0.0108	±	0.0108			
A-5847			**male**	**50**	**dermatomyositis, interstitial pneumonia**		**non func T**	++					**0.22**	0.0448	±	0.0213	0.3531	±	0.0786			
A-5850			**male**	**63**	**T-cell lymphoma**			++					**0.223**	0.0368	±	0.0074	0.678	±	0.0553			
A-5853		*#*	**male**	**62**	**rupture of esophagus varix, liver cirrhosis**			++					**0.463**	0.0995	±	0.0165	0.7657	±	0.0651			
A-5861			*male*	*58*	*Fournier's gangrene*								*0.157*	0	±	0	0	±	0			
A-5864			**male**	**87**	**tuberculous pleuritis**			+					**0.282**	0.039	±	0.0142	0.4301	±	0.1498			
A-5869			**male**	**63**	**multiple myeloma**			++					**0.285**	0.0327		0.0047	0.3572	±	0.0341			
A-5876			**female**	**86**	**sepsis**			++					**0.355**	0.0271	±	0.0122	0.5739	±	0.1134			
A-5885			*male*	*66*	*toxic epidermal necrolysis*								*0.107*	0.0058	±	0.0058	0.024	±	0.024			
A-5886	*		**male**	**54**	**mediastinal tumor**	**38**		+++					**0.357**	0.1943	±	0.0335	2.5731	±	0.3939			
						39							**0.309**									
A-5890			**male**	**90**	**chronic obstructive pulmonary disease**			++					**0.336**	0.0566	±	0.007	0.7581	±	0.0861			

Case_ID		sex	age	cause of death	block number	other lesions	ZG thickrless					HSD3B2 positivity	NOA (APCC:s/mm^2, mean ± S.E.M.)					AA/WAA (%, mean ± S.E.M.)		Figure
							statistics	KN	KM	TS	GAE									
A-5900	*	*male*	*71*	*origin unknown cancer*	*4*							*0.092*	0	±	0	0	±	0		
					5							*0.082*								
					6							*0.014*								
A-5907	#	**female**	**87**	**origin unknown cancer**			−					**0.381**	0.0108	±	0.0062	0.6849	±	0.3427		**3B**
A-5918		*female*	*75*	*angina pectoris*								*0.038*	0	±	0	0	±	0		
A-5933	*	**male**	**84**	**hepatocellular cancer**	**24**		++					**0.219**	0.0171	±	0.0059	0.1658		0.0348		
					25							**0.379**								
A-5940	*	**male**	**68**	**acute myeloid leukemia**	**48**		++					**0.305**	0.006	±	0.0015	0.0366	±	0.008		
					49							**0.2**								
A-5942		**male**	**54**	**diabetes mellitus, chronic renal failure**			++					**0.205**	0.0207	±	0.0129	0.2199	±	0.1623		
A-5947		*male*	*70*	*T-cell lymphoma*								*0.032*	0.0338	±	0.0135	0.4776	±	0.0516		
A-5950		**female**	**53**	**aplastic anemia**			+++					**0.458**	0.0803	±	0.0349	1.2402	±	0.2748		
A-5956	*	*male*	*58*	*Wegener's granulomatosis*	*37*							*0.183*	0.0337	±	0.0148	0.3076	±	0.1402		
					38							*0.143*								
A-5965		**male**	**57**	**autoimmune hemolytic anemia**		**pAATL/ APA**	+++					**0.209**	0.1273	±	0.0233	3.3451	±	0.1821		**4C**
A-5972		**female**	**55**	**sepsis**			++					**0.247**	0.0249	±	0.0101	0.302	±	0.0909		
A-5976		**male**	**64**	**multiple myeloma**		**APA**	−					**0.326**	0.0024	±	0.0024	0.0494		0.0494		**4I-J**
A-5980		**male**	**64**	**sepsis**			++					**0.272**	0.1202	±	0.013	1.8349	±	0.2901		
A-5987		**female**	**56**	**heart failure, hyperkalemia**			−	−	++	−	+	**0.315**	0.0558	±	0.0111	0.3821	±	0.0601		
A-5989		**female**	**74**	**heart failure, pneumonia**			++					**0.275**	0.0443	±	0.0053	0.7004	±	0.0582		
A-5992		**female**	**56**	**fulminant hepatitis**			+					**0.426**	0.0997	±	0.0038	0.8492	±	0.0739		
A-5993		**male**	**80**	**multiple organ failure**		**APA**	−					**0.438**	0.016	±	0.008	1.1119	±	0.8975		**4K-N**
A-5994		**male**	**81**	**aspiration pneumonitis**			++					**0.254**	0.1159	±	0.0297	1.5742	±	0.2628		

Case_ID			sex	age	cause of death	block number	other lesions	ZG thickrless					HSD3B2 positivity	NOA (APCC:s/mm^2, mean ± S.E.M.)				AA/WAA (%, mean ± S.E.M.)			Figure
								statistics	KN	KM	TS	GAE									
A-5996	*		*female*	*69*	*follicular lymphoma*	*23*							*0.199*	0.0299	±	0.0069	0.4409	±	0.0677		
						24							*0.186*								
A-6004			**male**	**76**	**post surgical hemorrhage, gastric**			+					**0.394**	0.0127	±	0.0032	0.2663	±	0.0198		
A-6007	*	#	**female**	**77**	**colon cancer, liver metastasis**	**22**	**non func T**	++					**0.47**	0.0379	±	0.0058	0.371	±	0.058		
						23	**non func T**						**0.42**								
A-6012	*		*female*	*68*	*rectal ulcer, hepatic abscess*	*21*							*0.158*	0	±	0	0		0		
						22							*0.108*								
A-6017			*female*	*88*	*toxic epidermal necrolysis*								*0.054*	0	±	0	0	±	0		
A-6020	*		**male**	**71**	**infectious aortic aneurism**	**21**		++					**0.309**	0.0465	±	0.0052	0.4653		0.0461		
						22							**0.37**								
A-6025			*female*	*79*	*pancreatic cancer, liver metastases*								*0.003*	0	±	0	0	±	0		
A-6026			*female*	*69*	*remitting seronegative symmetrical synovitis with pitting edema*								*0.119*	0.0781	±	0.0232	0.8481	±	0.2656		
A-6028			*male*	66	*hemorrhagic shock*								*0.178*	0.0259	+	0	0.7815	±	0.0942		
A-6030			*male*	63	*interstitial pneumonia*								*0.173*	0.0615	+	0.009	0.5256	±	0.0573		
A-6031			**female**	**88**	**hepatocellular cancer**			+					**0.274**	0.0094	±	0.0054	0.5015		0.4482		
A-6032			**female**	**97**	**gastric submucosal tumor**		**non func T**	+					**0.648**	0.1354	±	0.0059	0.9525	±	0.0093		
A-6033			**female**	**78**	**autoimmune hemolytic anemia**			++					**0.245**	0.0378	±	0.0091	0.8364	±	0.0723		
A-6037		#	**male**	**73**	**carbon dioxide narcosis**			−	−	+	−	++	**0.379**	0.05	±	0.0056	0.4996	±	0.0599		
A-6039		#	**female**	**78**	**multiple myeloma**			−	−	+	−	++	**0.386**	0.0693	±	0.007	0.8375	±	0.2084	3C-D	

Case_ID		sex	age	cause of death	block number	other lesions	ZG thickrless					HSD3B2 positivity	NOA (APCC:s/mm^2, mean ± S.E.M.)					AA/WAA (%, mean ± S.E.M.)		Figure
							statistics	KN	KM	TS	GAE									
A-6041		**male**	**79**	**lung tumor**		**non adrenal T**	−					**0.22**	0.0239	±	0.006	0.2863	±	0.0744		
A-6043	*#*	**male**	**83**	**acute leukemia**			−	−	+	−	+++	**0.42**	0	±	0	0		0		
A-6044	*#*	**male**	**72**	**pneumonia**			+					**0.349**	0.0167	±	0.0024	0.2135	±	0.0794		
A-6045	*#*	**male**	**57**	**pneumonia**			++					**0.324**	0.0491	±	0.0064	0.5166		0.0702		
A-6049		*female*	*81*	*multiple system atrophy*								*0.093*	0.0327	±	0.003	0.795	±	0.2027		
A-6050		*female*	*76*	*acute myocardial infarction, multiple organ failure*								*0.043*	0	±	0	0	±	0		
A-6054		**female**	**67**	**idiopathic bacterial peritonitis**			+					**0.551**	0.0307	±	0.0129	0.2537		0.1297		
A-6055		**male**	**74**	**rheumatoid arthritis, interstitial pneumonia**			−	−	+++	−	++	**0.364**	0.0417	±	0.0163	0.3794	±	0.1781		
A-6056		**female**	**75**	**myelodysplastic syndromes, sepsis**			++					**0.356**	0.0203	±	0.0029	0.0892	±	0.0282		
A-6058	*#*	**male**	**72**	**multiple hepatic metastases of origin unknown carcinoma**			++					**0.287**	0.003	±	0.003	0.0083	±	0.0083		
A-6060		**female**	**67**	**ovarian tumor**			−					**0.236**	0.0015	±	0.0015	0.2219	±	0.2219		
A-6061	*	**female**	**62**	**small-intestinal hemorrhage**	**17**		+++					**0.442**	0.0426	±	0.0219	0.7604	±	0.3806		
					18							**0.563**								
A-6062		**male**	**73**	**pulmonary flbrosis**			++					**0.427**	0.0111	±	0.0062	0.1348	±	0.0706		
A-6063	*#*	**female**	**84**	**systemic lupus erythematosus**			+					**0.305**	0.0248	±	0.0045	0.8752		0.039		
A-6066		**male**	**61**	**multiple myeloma**			−					**0.344**	0.005	±	0.005	0.044	±	0.044		
A-6068		**male**	**5**	**hepatocellular cancer**			+++					**0.37**	0.0443	±	0.0082	0.9379	±	0.1536		
A-6069		**female**	**85**	**diffuse large B-cell lymphoma**			++					**0.451**	0.0332	±	0.0173	0.3724	±	0.1863		
A-6071		**male**	**80**	**aspiration pneumonitis**			++					**0.312**	0.0196	±	0.0043	0.297	±	0.0248		

Case_ID		sex	age	cause of death	block number	other lesions	ZG thickrless					HSD3B2 positivity	NOA (APCC:s/mm^2, mean ± S.E.M.)			AA/WAA (%, mean ± S.E.M.)		Figure
							statistics	KN	KM	TS	GAE							
A-6072	*	*male*	*59*	*diffuse large B-cell lymphoma*	*35*							*0.026*	0	±	0	0	± 0	
					36							*0.177*						

NOA: number of APCCs/mm2, AA/WAA: APCC area per whole adrenal area (%),

*: cases who had 2–3 sample blocks,

#: samples with detached APCCs, n.a.: not available, bold characters: HSD3B2-positivity> =20%, italic characters: HSD3B2-positivity<20%.