Supplemental data

Case#1:

Case#1 was diagnosed with unilateral primary aldosteronism (PA) based on the Clinical Practice Guideline of PA from the Endocrine Society of the United States (1) and treated as follows:

A 54-year-old male patient was referred to Keio University Hospital for the further evaluation of his hypertension, high aldosterone-renin ratio (ARR = plasma aldosterone concentration [PAC: 190 pg/mL] / plasma renin activity [PRA: 0.2 ng/mL/hr] = 950 [cut-off value: <200 (1)]), and hypokalemia (2.9 [normal range: 3.5-4.8] mEq/L). On his first visit, he had a normal appearance with obesity (height: 174.2 cm, weight: 94.0 kg, body mass index [BMI]: 31.0 kg/m²) and high blood pressure (162/101 mmHg) under 3 antihypertensive agent treatments (candesartan [12 mg/day], amlodipine [10 mg/day], and carvedilol [10 mg/day]). His blood test was normal, except for hypokalemia (3.4 mEq/L). Computed tomography revealed a slightly enlarged left adrenal gland with no apparent adrenocortical tumor.

In order to confirm PA, several endocrinological tests were performed while controlling his hypertension and hypokalemia using doxazosin (2 mg/day), long-acting diltiazem (100 mg/day), and a slow-release potassium chloride supplement (1,800 kg/day) after stopping the 3 antihypertensive agents described above. The amount of urinary aldosterone excreted was 25.2 μg/day, even under increased sodium intake to >200 mmol/day for 3 days (oral sodium loading test [cut-off values: >12 μg/day at the Mayo Clinic and >14 μg/day at the Cleveland Clinic (1)]). The 50-mg captopril suppression (challenging) test did not suppress PAC (before administration, 217 pg/mL vs. 90 min after, 242 pg/mL). Adrenal venous sampling (AVS) was performed after the infusion of 250 mg cosyntropin (synthetic adrenocorticotropin hormone). The PAC to plasma cortisol concentration ratio (PAC [47,500 pg/mL] / PCC [821.1 μg/dL] = 57.8) of the left adrenal central vein was markedly higher than that of the right adrenal central vein (9,920/1,510.5=6.6) (lateralized ratio = 8.8 [cut off value: >4]), suggesting that the left adrenal gland produced more aldosterone than the right. The PAC/PCC ratio of the right adrenal central vein was
markedly lower than that of the inferior vena cava (PAC [222 pg/mL] / PCC [21.7 μg/mL] = 17.9), suggesting that aldosterone production in the right adrenal was suppressed due to the low level of circulating renin (contralateralized ratio: 0.37 [cut-off value: <1]). Thus, the patient was diagnosed with PA due to possible left adrenal hyperplasia.

Laparoscopic adrenalectomy was performed. The extracted fresh adrenal was cut into several pieces. Small tissues were taken from cut surface of a piece for fresh frozen preparation (Control#1 and sAPL#1, see ‘Fresh frozen tissue preparation’ in the following Supplemental Method). The remaining tissue pieces were processed to prepare formalin-fixed paraffin-embedded (FFPE) specimens for pathological and immunohistochemical analyses. H&E stained sections showed many nodular formations without apparent capsule, where nodules consisted of eosinophilic or clear cells with round nucleus (Figure 1A, C, and G). No apparent histological abnormality was identified outside of the nodules in H&E stained sections, and it was diagnosed as nodular hyperplasia (Figure 1A). After adrenalectomy, his PA was cured and his hypertension was improved. Six years after adrenalectomy, his blood pressure is well-controlled with amlodipine (5 mg/day) and olmesartan (20 mg/day), and his PAC (73 pg/mL) and active renin concentration (10.6 unit [normal range: 3.6-36.2]) are still within normal ranges.

**Case#2:**

Case#2 was diagnosed with unilateral PA based on the Clinical Practice Guideline of PA from the Japan Endocrine Society (2) and treated as follows:

A 41-year-old male patient was referred to Yokohama Rosai Hospital for the further evaluation of his hypertension, which was diagnosed when he was 31 years old (148/96 mmHg). In his first visit to Yokohama Rosai Hospital, he had a normal appearance without obesity (height: 184.4cm, weight: 76.5 kg, BMI: 22.5 kg/m²) and high blood pressure (143/104mmHg) under 2 antihypertensive agent treatments (amlodipine [5 mg/day] and atenolol [50 mg/day]). His blood test was normal including serum potassium (4.4 mEq/L), except for high ARR (PAC [182 pg/mL] / PRA [0.5 ng/mL/hour] = 364). Computed tomography revealed no evidence of adrenocortical abnormalities in his adrenal glands.
In order to confirm PA, several endocrinological tests were performed while controlling his hypertension with amlodipine only (10 mg/day). The saline-loading test showed high PAC after loading (70 pg/mL [cut-off value: >60 pg/mL in the Clinical Practice Guideline of PA from the Japan Endocrine Society (2)]). The captopril suppression test resulted in high ARR (PAC [394 pg/mL] / PRA [0.7 ng/mL/hour] = 562.9 [cut-off value: >200 in the Japanese guideline]) 90 min after its administration. Thus, he was diagnosed with PA.

AVS was performed under cosyntropin stimulation as previously reported (Panels A and B in Supplemental Figure 1) (3). The PAC/PCC ratio in the right adrenal central vein (PAC [20,200 pg/mL] / PCC [297 μg/mL] = 68.0) was higher than that in the left adrenal central vein (3,840/153=25.1) (lateralized ratio = 2.7 [cut-off value: >2.6 in the Japanese guideline]). The PAC/PCC ratio in the femoral vein (222/21.7=10.2) was lower than that in the left adrenal vein (contralateralized ratio: 2.5 [cut-off value: > 1.0 in the Japanese guideline]). This result suggested that, although the bilateral adrenals may be producing aldosterone autonomously, the contribution of the right adrenal was greater than that of the left adrenal.

In addition to standard AVS, we simultaneously performed super-selective AVS (ssAVS, Supplemental Figure 1B-C, E-R), in which we collected blood samples from small adrenal tributary veins (smaller upstream branches of the adrenal veins) using the specialized microcatheter, GOLD CREST Micro Catheter (catalog#: KCV29S1S-OM, Koshin Medical Inc., Tokyo, Japan; details in reference (4)). ssAVS enabled us to remove the requirement of PAC/PCC normalization and evaluate PAC alone since the tributary samples did not contain non-adrenal venous blood contamination. PAC values of more than 14,000 pg/mL in any of the venous tributary samples indicated autonomous aldosterone production in the corresponding area (5). In Case#2, we inserted the microcatheter into the inferior (green arrow in Supplemental Figure 1B) and lateral (pink arrow in Supplemental Figure 1C) venous tributaries of the right adrenal gland and determined that catheterization was successful because PCC values were more than 10-fold higher (312 and 242 μg/mL, respectively) than that of the femoral vein (21.7 μg/mL). PAC values from these tributaries were higher than the cut-off value of 14,000 pg/mL (14,500 and 23,600 pg/mL).
pg/mL, respectively), suggesting that multiple areas of the right adrenal cortex produced aldosterone autonomously. We also succeeded in catheterization of the superior and lateral tributaries of the left adrenal gland (PCCs were 221 and 371μg/mL, respectively), and the corresponding PAC from these tributaries were only 8,380 and 2,790 pg/mL (lateral and superior tributaries, respectively), suggesting that the left adrenal was not producing aldosterone autonomously (Supplemental Fig1. E-F). In summary, ssAVS indicated that the right adrenal was the responsible lesion in Case#2-PA and the lesions producing aldosterone in the right adrenal were not focal.

We performed laparoscopic right adrenalectomy to treat Case#2-PA. The extracted adrenal gland was cut into several pieces and processed to prepare FFPE specimen for pathological and immunohistochemical analyses. The adrenal H&E histology showed normal layered structure throughout blocks#1-#7 except for several noduels, and was diagnosed as nodular hyperplasia (Figure 1K). One week after surgery, his blood pressure was controlled with only 5mg/day of amlodipine. His ARR values had normalized in the 9 days after surgery (PAC [101 pg/mL] / PRA [0.8 ng/mL/hr] = 126.3) and have remained normal for 3 years (108.0/1.4=77.1). In conclusion, ssAVS in the right adrenal gland showed a consistent result with immunohistochemistry for CYP11B2 (Figure 1), and the patient was successfully treated by adrenalectomy.

Supplemental method

Ethical reviews and approvals

This study was approved by the Medical Ethics Committee of the School of Medicine, Keio University (approval #20090018 from the Department of Urology and #20030061 from the Department of Internal Medicine) and Yokohama Rosai Hospital (approval #24-10). Written informed consent for immunohistochemical and molecular analyses using adrenal tissues was obtained from Cases#1 and #2.

Immunohistochemistry

Archival FFPE surgical specimens of Cases#1 and #2 were used for double
immunohistochemical staining of aldosterone synthase (CYP11B2 and steroid 11β-hydroxylase (CYP11B1) (6). Furthermore, single staining for 3βHSD and CYP17 was performed as previously reported (6), which essentially showed identical results in that 3βHSD co-localized with CYP11B2 and CYP11B1, whereas CYP17 co-localized with CYP11B1 only (data not shown).

**Fresh frozen tissue preparation**

Immediately after adrenalectomy in Case#1, a suspected aldosterone-producing lesion (sAPL#1 in Table 1) and adjacent normal adrenal tissue (Control#1) were macroscopically collected from a nodular portion and a brown adrenocortical portion of the extracted and cut adrenal gland. These samples were flesh-frozen in liquid nitrogen, and were kept at -80 ºC until DNA/RNA extraction.

**DNA and RNA isolation from fresh frozen tissues, cDNA generation from RNA, and quantitative real-time polymerase chain reaction (qPCR) analysis using cDNA**

Using the AllPrep DNA/RNA Mini Kit (catalog#: 80204, Qiagen, Valencia, CA), DNA/RNA #21 and #22 (Table 1) were prepared from sAPL#1 and Control#1, respectively. cDNA samples were generated from RNA#21 and #22 using the High-Capacity cDNA Reverse Transcription Kit (catalog#: 4368814, Thermo Fisher Scientific, Waltham, MA). These cDNAs were used for qPCR analyses of CYP11B2 as previously reported (6). The 18S ribosomal RNA was used for the normalization of sample loading (TaqMan ribosomal RNA control reagents, catalog#: 4308329, Thermo Fisher Scientific). The delta delta Ct method was used to calculate fold differences (7).

**Direct Sanger sequencing for APL#1**

To analyze if DNA#21 have KCNJ5 mutation, it was amplified by a polymerase chain reaction (PCR, 30 cycles) using the following forward and reverse primers: 5’-CTGTTCTTCCGGCTCTTCAATTTGG-3’ (primer name: ‘KCNJ5-1st-F’) and 5’-AGGGTCTTCGCTCTTTCTT-3’ (‘KCNJ5-1st-R’), respectively (product size: 296 bp). The PCR product was subsequently diluted 10,000-fold with nuclease free water and 1µl of the diluent was again amplified by PCR (30 cycles) using the following primers, which were
designed inside the ‘KCNJ5-1st-F’ and ‘KCNJ5-1st-R’: 5’-TCATTTGGTGCTTGCTT-3’ (‘KCNJ5-nested-F’) and 5’-TGACGATGGAGCCCAGGA-3’ (‘KCNJ5-nested-R’, PCR product size: 219 bp). This nested PCR reaction was performed using the PrimeSTAR HS kit (catalog#: R010A, TAKARA, Tokyo, Japan). The final PCR product was purified using ExoSAP-IT for PCR Product Cleanup (#78200, Affymetrix, Santa Clara, CA), followed by Sanger sequencing using the ‘KCNJ5-nested-F’ primer.

**DNA and RNA isolation from FFPE tissues, cDNA generation from RNA, and qPCR analysis using cDNA**

FFPE tissues were manually macrodissected for the isolation of DNA and RNA as previously reported (8). Briefly, a set of 3 – 6 serially sectioned slides were identified, followed by H&E staining of the first slide and CY11B2/CYP11B1 double immunostaining of the last slide. Macrodissection was accomplished using a disposable scalpel on the remaining unstained sections by localizing pAA TLs or double-negative nodules using H&E- and CYP11B2/CYP11B1-stained slides as a reference (Supplemental Figure4, 5, 6, 8 and 9). In each case, an area of the normal adrenal cortex (zona fasciculata and medulla) was similarly dissected as controls (DNA#1 and #8 from Cases#1 and 2, respectively). DNA and RNA were isolated using the Qiagen Allprep FFPE DNA/RNA kit (catalog#: 80234, Qiagen), according to the manufacturer’s instructions. The isolation protocol was modified by extending the xylene incubation to five minutes, centrifugation during deparaffinization to five minutes, and by eluting in a volume of 20 µl. cDNA samples were generated from RNA using the High-Capacity cDNA Reverse Transcription Kit (catalog#: 4368814, Thermo Fisher Scientific). These cDNAs were preamplified using the TaqMan PreAmp Master Mix Kit (catalog#: 4384267, Thermo Fisher Scientific) with the primer/TaqMan probe mix for CYP11B2 (6) and the 18S ribosomal RNA gene, according to the manufacturer’s instructions. Preamplified cDNAs were used in the qPCR analysis of CYP11B2 and the 18S ribosomal RNA gene using the same primer/TaqMan probe mix for preamplification.
Next Generation Sequencing (NGS) of adrenal DNA

In NGS, 5 µl of each isolated DNA from FFPE samples (DNA#1-3, #5, #7-12, #25-28), 10 ng of each isolated DNA from frozen tissues (DNA#21 and #22), and 40 pg of control male DNA (catalog#: 360486, lot#: 1404103, Thermo Fisher Scientific, shown as DNA#52) were utilized. These DNA samples were PCR-amplified using the novel primer mix APA_v13, which was designed to cover twelve reported APA-associated mutation regions in \textit{ATP1A1} (9,10), \textit{ATB2B3} (10), \textit{KCNJ5}(9,11,12), and \textit{CACNA1D} (9,13) using Ion AmpliSeq Designer software version 3.4 (https://www.ampliseq.com, primer information is in Supplemental Table 1, all nucleotide numbers are based on the Genome Reference Consortium GRCh37/hg19 assembly). Amplified DNA was ligated with barcode and adaptor sequences to generate a library for NGS. These library generation steps were performed using Ion AmpliSeq library kit 2.0 (catalog#: 4475345, Thermo Fisher Scientific) and the Ion Express Barcode Adapters 1-16 kit (catalog#: 4471250, Thermo Fisher Scientific) according to manufacturer’s instructions, except for the additional amplification cycles used in some of the DNA samples due to small starting quantities (see the exact cycle #s in Supplemental Table 3). Amplified templates were prepared from the library using the Ion PGM Template OT2 200 Kit (catalog#: 4480974, Thermo Fisher Scientific) on Ion One Touch 2 equipment (Thermo Fisher Scientific) according to the manufacturer’s instructions. NGS of amplified templates was performed by Ion 314 Chip Kit v2 (catalog#: 4482261, Thermo Fisher Scientific) using Ion PGM Sequencing 200 Kit v2 (catalog#: 4482006, Thermo Fisher Scientific) following the manufacturer’s instructions.

Data analysis was performed in Torrent Suite 4.0.2 software as described (14), with alignment by the Torrent Mapping Alignment Program (TMAP) using default parameters, and variant calling by the Torrent Variant Caller plugin using default low-stringency somatic variant settings. Sequencing variants were annotated using Ion Reporter 4.2 software (Thermo Fisher Scientific). The called variants were filtered to identify potential driving somatic mutations by removing synonymous or non-coding variants; those with frequencies > 0.01 in normal populations from ESP6500 or 1000 genomes; and those with
flow-corrected read depths (FDP) < 50, flow variant allele-containing reads (FAO) < 10, variant allele fractions (FAO/FDP) < 0.10, or the flow variant allele calling forward to reverse read ratio (FSAF/FSAR) <0.2 or >5. These filtering criteria were more stringent than previously validated criteria for calling single nucleotide/indel variants from AmpliSeq data (8). All somatic variants were visualized by Integrative Genomics Viewer (IGV) 2.3 (Supplemental Figure 1). The variant frequency in matched controls was determined using IVG since low frequency variants were not called by the Torrent Variant Caller plugin.

We carried out Sanger DNA sequencing to confirm the mutations in KCNJ5, ATP1A1, and ATP2B3 genes using the remaining isolated DNA from FFPE samples (DNA#1-3, #5, #7-12, #25-28), however, the attempts were not successful even with nested PCR amplification protocol, most likely due to the limited amounts of remaining DNA, which is highly fragmented due to FFPE treatment. Nonetheless, we believe that the NGS results are reliable since the mutations were identified in a number of sequence reads and passed our stringent filtering criteria described above.

Statistics

In the qPCR analysis, P values were calculated by the unpaired Student’s t-test using delta Ct values and P values <0.05 were considered to be significant.

Supplemental Figure 1 Adrenal venography for regular AVS and super-selective AVS.

A: Right adrenal central venography. Green and pink arrows mark the corresponding positions of microcatheter markers in panels B and C, respectively. B: Selective venography of the right adrenal lateral tributary vein. A green arrow indicates a marker of the microcatheter. C: Selective venography of the right adrenal inferior tributary vein. A pink arrow indicates a marker of the microcatheter. D: Left adrenal central venography. Blue and yellow arrows mark the corresponding positions of microcatheter markers in panels E and F. E: Selective venography of the left adrenal lateral vein. A blue arrow indicates a marker of
the microcatheter. F: Selective venography of the right adrenal superior tributary vein. A yellow arrow indicates a marker of the microcatheter. Scale bars indicate 1 cm. Note that the actual front edge of the microcatheter was 0.8 mm from the markers. PAC: plasma aldosterone concentration. PCC: plasma cortisol concentration.

**Supplemental Figure 2 Sanger Sequence results**

A known heterozygous somatic mutation, p.Gly151Arg (12), was found in the genomic DNA sample from sAPL#1 (DNA#21). This mutation was also detected by NGS at a variant allele frequency of 32.5% (Table 1).

**Supplemental Figure 3**

**Macrodissected portions for DNA#1 (control, A-F) and DNA#2 (pAATL#2, G-L) (Table 1).** A and G, Hematoxylin and eosin staining. B and H, immunohistochemistry for aldosterone synthase (CYP11B2, blue) and steroid 11β-hydroxylase (CYP11B1, brown), respectively. C-F and I-L, Formalin-fixed paraffin-embedded section images after macrodissection. Scraped areas were used to prepare DNA and RNA. The areas surrounded by black dots indicate a pAATL lesion (pAATL#2). pAATL: A possible APCC-to-APA transitional lesion. Enriched populations of tissues were macrodissected from zF (Control#2, C-F) and pAATL#2 (I-L). All panels are in the same magnification, and scale bars indicate 1 mm.

**Supplemental Figure 4**

**Macrodissected portions for DNA#11-12 (pAATL, A-F) and DNA#25-27 (G-L) (Table 1).** A and G, Hematoxylin and eosin staining. B and H, immunohistochemistry for aldosterone synthase (CYP11B2, blue) and steroid 11β-hydroxylase (CYP11B1, brown), respectively. C-F and I-K, Formalin-fixed paraffin-embedded section images after macrodissection. Scraped areas were used to prepare DNA and RNA. The areas surrounded by black dots indicate pAATL lesions (pAATL#3 and #4). pAATL: A possible APCC-to-APA transitional lesion. Enriched population of tissues were macrodissected from APCC-like
and APA-like portions of pAATL#2 (#11 and #12 in C-F, respectively) as well as from APCC-like portion (#26 in I-J), APA-like portion (#27 in J-K), and their ‘border’ these portions (#25 in K) of pAATL#4 (Table 1). All panels are in the same magnification, and scale bars indicate 1 mm.

Supplemental Figure 5

Macrodissected portions for DNA#3 (A-F) and DNA#28 (DNN#2, G-L) (Table 1). A and G, Hematoxylin and eosin staining. B and H, immunohistochemistry for aldosterone synthase (CYP11B2, blue) and steroid 11β-hydroxylase (CYP11B1, brown), respectively. C-F and I-L, Formalin-fixed paraffin-embedded section images after macrodissection. Scraped areas were used to prepare DNA and RNA. The areas surrounded by black dots indicate a double-negative nodule (DNN, negative both for CYP11B2 and CYP11B1). Enriched population of tissues were macrodissected from DNN#1 (C-F) and DNN#2 (I-L). All panels are in the same magnification, and scale bars indicate 1 mm. M: medulla.

Supplemental Figure 6 IGV validation

Visualized deletion/mutation site of ATP1A1 (Chr1:116,932,268 - Chr1:116,932,287) in DNA#26 (APCC-like area) and #27 (APA-like area) using Integrative Genome View software version 2.3.40. Reference (R) DNA and amino acid sequences (hg19) are shown at the bottom row. Each line above the reference sequences represents one sequence read. The reads from each DNA (#26 and 27) are grouped and indicated at the left. For example, the last nucleotide (C) in DNA#26 was read 7,080 times and that in DNA#27 was read 8,011 times. The gray shaded region in each read indicates the sequences that were matched to the Reference Sequence (hg19). The sequence fragments (F) only partially matched to the reference sequence had shorter gray shades and were indicated as F at the right edge. Black bars indicate deletion sites detected on each read. Red, green, blue, and brown bars indicate different variant sites on each read to the nucleotide bases T, A, C, and G, respectively. Purple dots indicate the insertion sites detected (details of the inserted nucleotides were not shown). Red, green, and brown arrowheads indicate single nucleotide variant sites on chr1:116932268 (synonymous), chr1:116932284 (synonymous), and chr1:116932286 (c.980T>G, p.Ile327Ser), respectively (Supplemental Table 2). The chr1:116932268
(red) variant was only observed in Library #26. The black bracket at the top indicates the deletion site of \( ATP1A1 \) (c.962_973del, p.Ile322_Ile325del) in Library #26. Most chr1:116932284 (green arrowhead) and chr1:116932286 (brown arrowhead) variants were identified in the same reads containing the p.Ile322_Ile325 deletion (black bracket) in DNA#26. These deletion and single nucleotide variants were also identified in DNA#27, suggesting that these deletion/mutation combinations were not amplification artifacts in pAATL#4. Moreover, the amino acid sequences of the deletion/mutation sites were highly conserved among humans, rhesus monkeys, mice, dogs, elephants, chickens, x. tropicalis, zebrafish, and lampreys according to the UCSC Genome Browser (http://genome.ucsc.edu), suggesting that these deletion/mutations may be pathological. Consequently, DNA#26 and #27 had c.962_973del ([flow corrected variant allele-containing reads / flow corrected read depth] = 752/1972 [38.1 %] and 441/1911 [23.1 %], respectively) and c.980T>G (795/1994 [39.9 %] and 468/1996 [23.5%], respectively) variants (see Supplemental Table 3).

Supplemental Figure 7

Macrodissected portions for DNA#5 (A-F) and DNA#9-10 (G-L) (Table 1). A and G, Hematoxylin and eosin staining. B and H, immunohistochemistry for aldosterone synthase (CYP11B2, blue) and steroid 11\( \beta \)-hydroxylase (CYP11B1, brown), respectively. C-F and I-K, Formalin-fixed paraffin-embedded section images after macrodissection. Scraped areas were used to prepare DNA and RNA. The areas surrounded by black dots indicate pAATL lesions. On DNA#5, it should be noted that enriched population of tissue from pAATL#5 was taken from sections in C and F, however macrodissected tissue from the sections in D and E was contaminated by tissue from the surrounding areas, which may have slightly affected the NGS results. On DNA#9 and #10, enriched population of tissues were macrodissected from APCC-like portion (DNA #9) and APA-like portion (DNA#10) of pAATL#7. All panels were in the same magnification, and scale bars indicate 1 mm. pAATL: A possible APCC-to-APA transitional lesion.

Supplemental Figure 8

Macrodissected portion for DNA#7 (A-F) and DNA#8 (G-L) (Table 1). A and G, Hematoxylin and
eosin staining. B and H, immunohistochemistry for aldosterone synthase (CYP11B2, blue) and steroid 11β-hydroxylase (CYP11B1, brown), respectively. C-F and I-K, Formalin-fixed paraffin-embedded sections after macrodissection. Scraped areas were used to prepare DNA and RNA. The areas surrounded by black dots indicate pAATL#6. Enriched population of tissues were macrodissected from pAATL#6 (C-F) and Control#3 (I-L). All panels were in the same magnification, and scale bars indicate 1 mm.

pAATL: A possible APCC-to-APA transitional lesion.

References


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**A** central

![Central image with markers and PAC, PCC values]

PAC: 20,200 pg/mL
PCC: 297 µg/mL

**B** lateral

![Lateral image with markers and PAC, PCC values]

PAC: 14,500 pg/mL
PCC: 312 µg/mL

**C** inferior

![Inferior image with markers and PAC, PCC values]

PAC: 23,600 pg/mL
PCC: 242 µg/mL

**D** central

![Central image with markers and PAC, PCC values]

PAC: 3,840 pg/mL
PCC: 153 µg/mL

**E** lateral

![Lateral image with markers and PAC, PCC values]

PAC: 8,380 pg/mL
PCC: 221 µg/mL

**F** superior

![Superior image with markers and PAC, PCC values]

PAC: 2,790 pg/mL
PCC: 371 µg/mL
DNA#1, zF (Control#2), block#6

DNA#2, the largest pAATL (pAATL#2), block#6