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Aberrant G protein-receptor expression is associated with DNA methylation in aldosterone-producing adenoma

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ABSTRACT

This study aimed to evaluate the methylation levels of G protein-coupled receptor (GPCR) related genes and the effects of methylation on mRNA expression levels in aldosterone-producing adenoma (APA). DNA methylation array and transcriptome analysis were applied in non-functioning adrenocortical adenoma (NFA) and APA. We investigated 192 GPCR-related genes and found hypo-methylation in the promoter region of 66 of these genes in APA. An integration study between microarray and methylation analysis revealed that *HTR4*, *MC2R*, *TACR1*, *GRM3*, and *PTGER1* showed hypo-methylation and up-regulation of mRNA in APA. qPCR analysis showed that *HTR4* and *PTGER1* expression was 9.3-fold and 6.6-fold higher in APAs than in NFAs, respectively, whereas expression of the other genes was not different between the groups. Methylation of *HTR4* and *PTGER1* at positions −229 and −666 from the transcription start site, respectively, showed a significant inverse correlation with their mRNA levels. Methylation levels were not associated with *KCNJ5* or *ATP1A1* mutations in human adrenal samples. We demonstrated an increased incidence of CpG island demethylation of GPCR-related gene in APA. The expression of two receptors, *HTR4* and *PTGER1*, showed a strong association with DNA methylation.

1. Introduction

Primary aldosteronism (PA) is the most frequent cause of secondary hypertension associated with autonomous excess aldosterone production and secretion (Funder et al., 2016). Patients with PA have an increased risk of cardiovascular disease compared with essential hypertension with similar blood pressure and risk profiles (Milliez et al., 2005, Mulatero et al., 2013). Therefore, the elucidation of autonomous excess aldosterone production machinery leads to detect therapeutic target for PA, and it is important to improve health and extend life span.

PA is mainly classified with aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism. APA is a form of secondary hypertension potentially curable by adrenalectomy (Funder et al., 2016). Recent research shows that 50–80% of APAs contain somatic mutations of *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D*, or *CTNNB1*, resulting in over-production of aldosterone (Akerstrom et al., 2016, Azizan et al., 2013, Beuschlein et al., 2013, Choi et al., 2011). On the other hand, it is well known that ectopic or aberrant membrane receptors such as G protein-coupled receptors (GPCR) are expressed in APAs and are associated with acceleration or suppression of aldosterone production by agonists or antagonists, respectively (Duparc et al., 2015, Perraudin et al., 2006, Ye et al., 2007, Zwermann et al., 2009). The regulation of the membrane receptor may be a therapeutic target for aldosterone production in APA. However, the mechanism of ectopic or aberrant membrane receptor expression in APA has not been elucidated.

Berger et al. defined an epigenetic trait as a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence (Berger et al., 2009). DNA methylation is a well-studied epigenetic modification that occurs mostly on the 5-carbon of cytosine residues in CpG dinucleotides. Hypomethylation of CpG in the promoter region potentiates gene transcription by facilitating the binding of transcription factors (Sen et al., 2016). Alteration of DNA methylation can influence the development or progression of some disorders including cancer, adenoma, and life style-related diseases (Maqbool et al., 2016). In fact, we and others have demonstrated that CYP11B2, the rate-limiting enzyme for aldosterone production, is demethylated in APA (Howard et al., 2014, Yoshii et al., 2016). In adrenal tissues, DNA methylation is likely to be involved in fetal adrenal development

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and adrenocortical cancer progression via its regulation of transcription (Legendre et al., 2016, Slieker et al., 2015).

Taken together, the transcription of GPCR or GPCR-related genes might be regulated by their DNA methylation in adrenal cells, however DNA methylation levels of GPCR or GPCR-related genes has not been studied. Therefore, we hypothesized that the expression of GPCRs might be regulated by DNA methylation in adrenal cells. The aims of our study were to clarify the DNA methylation levels of GPCR or GPCR-related genes in APA and the effects of DNA methylation on mRNA expression levels in APA.

2. Methods

2.1. Patients and samples

The inclusion criteria for patients and tissue samples have been described in a previous report (Yoshii et al., 2016). Briefly, the diagnosis of PA was based on the guidelines of the Japan Endocrine Society (Nishikawa et al., 2011). PA was diagnosed if the aldosterone renin ratio (aldosterone, ng/dL; plasma renin activity, ng/mL/hr) was greater than 20. The captopril challenge test, furosemide-upright test, and saline infusion test were performed to confirm the diagnosis. Adrenal venous sampling was performed for subtype diagnosis in all patients. Non-functioning adenoma (NFA) was diagnosed by radiological findings and endocrinological results showing cortisol or aldosterone excess as previously reported (Oki et al., 2012). Twelve NFA and 35 APA samples were obtained by surgery and stored at −80 °C until used for DNA methylation and quantitative polymerase chain reaction (qPCR) analysis. The clinical characteristics of the patients were shown in our previous report (Yoshii et al., 2016). The genotypes of the APAs included 5 *ATP1A1* mutations, 21 *KCNJ5* mutations, and 9 wild types. Five of 12 NFA and 19 of 35 APA samples were used for microarray analysis. This study was approved by the ethics committee of Hiroshima University, and written informed consent was obtained from all patients.

2.2. DNA genotyping and methylation analysis

Genomic DNA was extracted from NFA or APA samples by DNeasy blood and tissue kit (Qiagen, Hilden, Germany). Genotyping of the adenomas for *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D*, and *CTNNB1* was performed as previously described (Kishimoto et al., 2016). DNA methylation levels were determined using the Infinium HumanMethylation450 BeadChip kit (Illumina, San Diego, CA, USA), as previously described (Yoshii et al., 2016). Ninty-nine percent of the RefSeq genes and 96% of the CpG islands were covered. Methylation levels were shown as β values, which were then used to estimate the methylated signal intensity (Dedeurwaerder et al., 2011). The average β values were expressed as 0 to 1, representing completely non-methylated to completely methylated values, respectively.

2.3. RNA extraction, qPCR assay, and microarray analysis

Total RNA extraction and cDNA synthesis were performed as previously described (Kishimoto et al., 2016). PCR primers for *HTR4* (5-hydroxytryptamine receptor 4), *MC2R* (melanocortin 2 receptor), *TACR1* (tachykinin receptor 1), *GRM3* (glutamate metabotropic receptor 3), *PTGER1* (prostaglandin E receptor 1) and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) are shown in Table S1. Expression levels were assessed using SYBR-green-based gene expression assays (Takara Bio SYBR Premix EX Taq; Takara Bio Inc., Shiga, Japan). Gene expression levels were determined as arbitrary units normalized against *GAPDH* expression. Microarray analysis was performed using the SurePrint G3 Human Gene Expression 8 × 60K v2 array (Agilent Technologies Inc., Santa Clara, CA, USA).

2.4. Statistical analysis

Results were expressed as mean ± S.D. The differences were considered significant at $P < 0.05$. Analyses were performed using SPSS for Windows (release 24.0; SPSS Inc., Chicago, IL, USA). First, *t*-test was applied for the differences of DNA methylation or mRNA expression levels by DNA methylation array and transcriptome analysis, respectively. Significantly higher and lower DNA methylation levels of the genes in APA were denoted as “hyper-methylation” and “hypo-methylation”, respectively. Significantly higher and lower mRNA expression levels of the genes in APA were expressed as “up-regulation” and “down-regulation”, respectively. Second, the expression levels of *HTR4*, *MC2R*, *TACR1*, *GRM3*, and *PTGER1* mRNA detected by qPCR were analyzed by the *t*-test after logarithmic transformation, because they did not fit a normal distribution. The relationships between DNA methylation and mRNA expression levels or plasma aldosterone levels were analyzed by Spearman's test. Finally, differences of DNA methylation levels among APA genotypes were analyzed by one-way ANOVA followed by Bonferroni comparisons.

3. Results

3.1. Methylation levels of GPCR or GPCR-related genes in APA

We identified 192 GPCR or GPCR-related genes from the Gene Set Enrichment Analysis website (http://www.broadinstitute.org/gsea/index.jsp). From this list of genes, 185 had as DNA methylation lesion in the promoter region up to −1500 bases from transcription start site (TSS). Methylation at these sites was compared between NFAs (n = 12) and APAs (n = 35). APAs showed hypomethylation in 66 genes and hypermethylation 14 genes (Table 1 and Table S2). Six genes showed a mixture of hypo- and hypermethylation in APAs (Table 1 and Table S2). Ninety-nine genes showed no differences in DNA methylation between NFAs and APAs.

3.2. Integration analysis between methylation level and mRNA expression

Results of the integration study between microarray and methylation analysis are shown in Table 1. We focused on genes with an inverse association between methylation and gene expression, because this indicates that DNA methylation directly regulates the expression of these genes. *HTR4*, *MC2R*, *TACR1*, *GRM3*, and *PTGER1* showed hypomethylation and upregulation of mRNA in APAs (Table 1 and Fig. S1). Nine genes showed higher mRNA expression in APAs than NFAs (Table S3), but no differences in DNA methylation between APA and NFA. Thus, some GPCR and GPCR-related genes

Table 1
Relationship between DNA methylation and mRNA expression among GPCR in APA.

mRNA expression in APA compared with NFA	DNA methylation in APA compared with NFA		
	Hypo-methylation	Mixed	Hyper-methylation
up-regulation	5	0	2
not significant	61	6	12
down-regulation	0	0	0

A *t*-test was applied for the differences of DNA methylation or mRNA expression levels by DNA methylation array and transcriptome analysis. Significantly higher and lower DNA methylation levels of the genes in APA were denoted as “hyper-methylationis” and “hypo-methylation”, respectively. Significantly higher and lower mRNA expression levels of the genes in APA were expressed as “up-regulation” and “down-regulation”, respectively. The specific gene symbols were shown in Table S2. NFA, non-functioning adrenocortical adenoma; APA, aldosterone-producing adenoma; GPCR, G protein-coupled receptor.

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were highly expressed in APAs compared to NFAs, in agreement with previous reports (Duparc et al., 2015, Perraudin et al., 2006, Ye et al., 2007, Zwermann et al., 2009).

3.3. Relationship between GPCR gene methylation and mRNA levels

We performed qPCR analysis of *HTR4*, *MC2R*, *TACR1*, *GRM3*, and *PTGER1* gene expression in NFAs (n = 12) and APAs (n = 35). *HTR4* expression was 9.3-fold higher ($P < 0.001$, Fig. 1), and *PTGER1* expression was in 6.6-fold higher in APAs than in NFAs ($P = 0.003$, Fig. 1). There were no differences in *MC2R*, *TACR1*, or *GRM3* expression between APAs and NFAs (Fig. 1).

The presumed methylation sites of *HTR4* and *PTGER1*, which had inverse correlations between DNA methylation and mRNA expression, are depicted in Fig. 2. *HTR4* and *PTGER1* had 3 and 4 methylation sites, respectively, in their promoter regions. First, the methylation levels of *HTR4* and *PTGER1* were compared between NFAs and APAs. Two methylation sites −229 bases from the *HTR4* gene TSS and −666 bases from the *PTGER1* gene TSS were significantly hypomethylated in APAs (Table 2). Second, the relationship between DNA methylation at these sites and mRNA expression levels were analyzed by Spearman's test. Methylation levels at the above two sites had a significant inverse correlation with mRNA levels (Table 3). Finally, we analyzed the relationship between DNA methylation levels and plasma aldosterone levels. However, there were no correlations between them as shown in Table S4.

Table 2
Relationship between *HTR4* or *PTGER1* methylation levels and their mRNA expression levels.

DNA methylation site	Methylation Rate (β value)		Fold change (APA/NFA)	*P* value
	NFA average	APA average		
HTR4				
a	0.58 ± 0.20	0.57 ± 0.14	0.97	0.754
b	0.53 ± 0.19	0.34 ± 0.14	0.65	0.001
c	0.84 ± 0.06	0.83 ± 0.05	0.99	0.628
PTGER1				
d	0.72 ± 0.18	0.71 ± 0.13	0.99	0.920
e	0.26 ± 0.04	0.22 ± 0.04	0.82	0.001
f	0.06 ± 0.04	0.07 ± 0.05	1.15	0.561
g	0.06 ± 0.05	0.06 ± 0.08	1.00	0.957

The relationship between HTR4 or PTGER1 methylation levels and their mRNA expression levels were analyzed by Spearman's test. The methylation sites from "a" to "g" are shown in Fig. 2. NFA, non-functioning adrenocortical adenoma; APA, aldosterone producing adenoma.

3.4. Association of APA genotype and DNA methylation levels of GPCR genes

DNA methylation levels were compared among APAs with

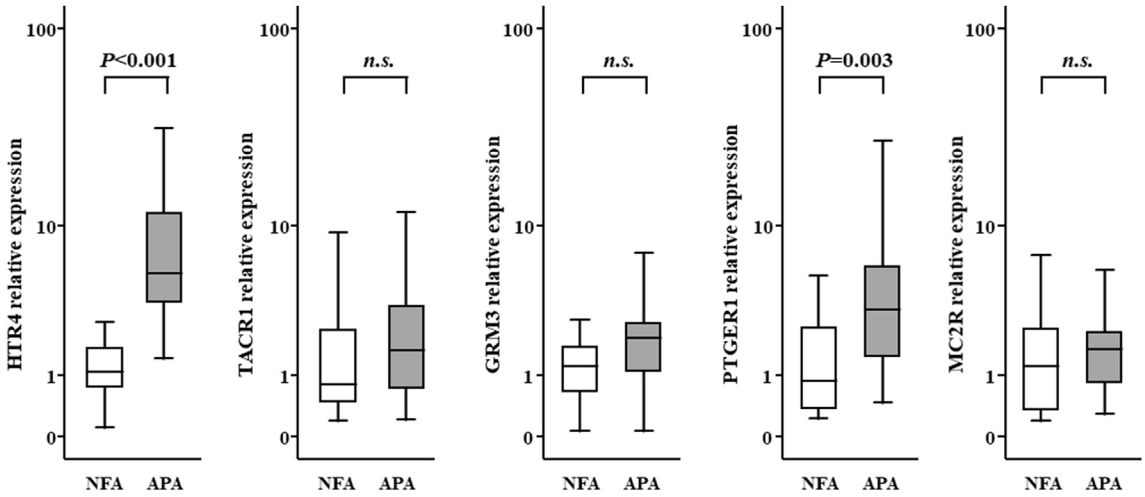

Fig. 1. mRNA expression levels of the GPCRs, *HTR4*, *MC2R*, *TACR1*, *GRM3*, and *PTGER1*, in aldosterone-producing adenoma (APA, n = 35) and non-functioning adrenocortical adenoma (NFA, n = 12). n.s., not significant.

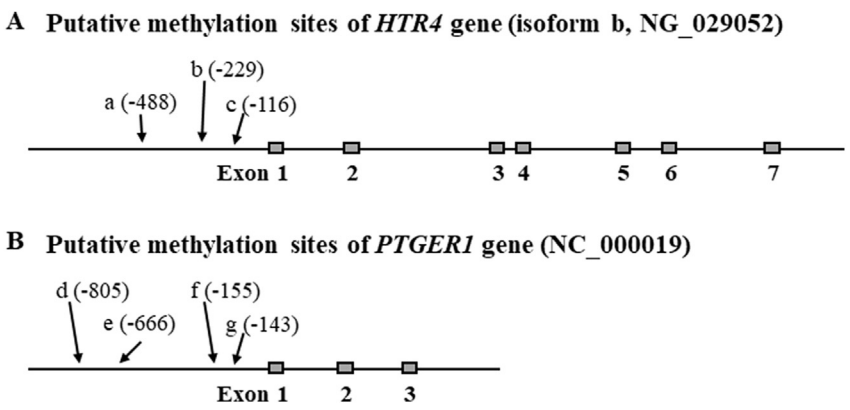

Fig. 2. Conceivable methylation sites of *HTR4* (A) and *PTGER1* (B) promoters. Gene accession numbers are represented in the parentheses of each heading. The locations from the transcription start site are indicated as numbers in parentheses.

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Table 3
Relationship between *HTR4* or *PTGER1* methylation levels and their mRNA expression levels.

DNA methylation site	r	*P* value
HTR4		
a	0.057	0.702
b	−0.336	0.021
c	0.030	0.842
PTGER1		
d	−0.112	0.455
e	−0.353	0.015
f	−0.208	0.161
g	−0.091	0.545

The relationship between HTR4 or PTGER1 methylation levels and their mRNA expression levels were analyzed by Spearman's test. The methylation sites from a to g are shown in Fig. 2.

ATP1A1 mutation, *KCNJ5* mutation, and wild type genotypes. The methylation levels of site "b" in *HTR4*, where significant correlation between methylation and mRNA levels were found, did not show methylation differences among APAs with different genotypes (Fig. 3A). The methylation levels of site "e" in *PTGER1* showed no difference among APAs with different genotypes (Fig. 3B).

4. Discussion

We demonstrated that 33.8% (65 of 192) of GPCR or GPCR-related genes were hypo-methylated in the promoter region in APAs. The integration study of DNA methylation and gene expression analysis identified five genes whose expression might be directly regulated by DNA methylation, and two genes showed inverse correlation between DNA methylation and mRNA expression in adrenal tumors. Methylation levels were not associated with *KCNJ5* or *ATP1A1* mutation in human adrenal samples.

Our study demonstrated that the promoters of GPCR or GPCR-related genes are associated with a much higher incidence of CpG island demethylation in APAs. ACTH, glucagon, somatostatin, parathyroid hormone and glutamate metabotropic receptors genes, which were hypomethylation in APAs, have been were associated with aldosterone production (Ye et al., 2007, Zwermann et al., 2009). However, there were no differences in expression of these receptors between NFAs and APAs in our microarray analysis (Fig. S1). These genes are unlikely to be regulated only by DNA methylation, because there was no inverse correlation between DNA methylation and mRNA expression levels in adrenal samples. They may also be regulated by transcription factors and/or other epigenetic mechanisms. Importantly, these genes are in a state that facilitates gene transcription in APAs, and their ectopic or aberrant expression could lead to further aldosterone production.

We found that the methylation levels of *HTR4* and *PTGER1* in APAs are lower than those in NFAs, and these methylation levels are associated with their mRNA levels. Since hypo-methylation of CpG in the promoter region potentiates gene transcription by facilitating the binding of transcription factors (Sen et al., 2016), the expression levels of transcription factors that bind to these regions would not be different between NFAs and APAs. Therefore, methylation level is considered a rate-limiting factor for receptor expression. Otherwise, no or low levels of transcription factors would be present to bind to these regions in adrenal adenomas. Remarkably, our study found that two GPCR genes, *HTR4* and *PTGER1*, may be regulated by DNA methylation state.

HTR4 is serotonin receptor subtype known to be expressed in the adrenal gland according to human tissue specific expression analysis using RNA-seq and proteomics (Fagerberg et al., 2014). Importantly, HTR4 is up-regulated in APAs and serotonin increases aldosterone secretion *in vivo* and *in vitro* (Duparc et al., 2017, Ye et al., 2007, Zwermann et al., 2009). Duparc et al. reported that serotonin secreted from mast cells stimulates aldosterone production through paracrine regulation in APAs (Duparc et al., 2015). Our data indicate that the expression of HTR4 and subsequent stimulation of aldosterone production in APAs may be regulated by methylation of the *HTR4* gene.

PTGER1 encodes prostaglandin E receptor 1, one of the four receptors identified for prostaglandin E2. This is the first study to show up-regulation of *PTGER1* in APAs. In adrenal glomerulosa cells, prostaglandin E2 increased aldosterone production by stimulating the conversion of cholesterol to pregnenolone (Campbell et al., 1986). A selective agonist for prostaglandin E receptor 1 inhibits the up-regulation of aromatase expression following epidermal growth factor treatment in human adrenocortical carcinoma (H295R) cells (Watanabe et al., 2006). Aldosterone induces vascular inflammation via cyclooxygenase-2 expression and activation which sequentially produce prostaglandin E2 (Yoshimoto and Hirata, 2007), and thus *PTGER1* up-regulation may lead to further aldosterone production in APAs. Taken together, APAs have high expression of *PTGER1* which might be regulated by DNA methylation, and the expression might induce aldosterone overproduction and tumor progression in APAs.

There were no correlations between DNA methylation levels of GPCR or GPCR-related genes and plasma aldosterone levels. The methylation levels would determine their expression levels to collaborate with transcription factors (Sen et al., 2016). In addition, aldosterone levels were determined by the receptor expression levels and the plasma ligand levels. Therefore, in analyzing together with transcription levels and ligand levels, we could predict the

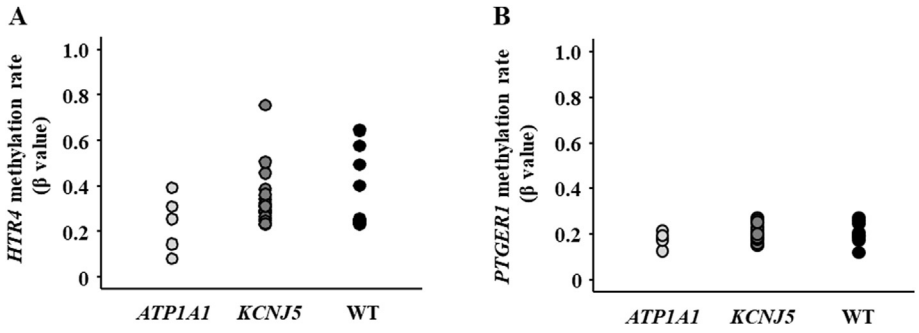

Fig. 3. Difference in *HTR4* and *PTGER1* methylation levels among genotypes of aldosterone-producing adenomas (APA). (A) The methylation levels of site "b" in *HTR4* among different APA genotype are shown. Methylation values for *ATP1A1* (n = 5), *KCNJ5* (n = 21), and wild type (WT, n = 9) were 0.25 ± 0.12, 0.35 ± 0.12, and 0.37 ± 0.16, respectively. (B) The methylation levels of site "e" in *PTGER1* among APA genotype are shown. Methylation values for *ATP1A1*, *KCNJ5*, and WT were 0.19 ± 0.03, 0.22 ± 0.04, and 0.22 ± 0.04, respectively.

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plasma aldosterone levels related with DNA methylation levels of GPCR or GPCR-related genes. As mentioned above, these genes are in a state that facilitates gene transcription to produce more aldosterone in APAs.

Some GPCR or GPCR-related genes may be regulated by intracellular signaling, but not by DNA methylation. *GNRHR* and *LHCGR* are well known to be highly expressed in APAs (Ye et al., 2007, Zwermann et al., 2009), but their methylation levels were not different between NFAs and APAs (data not shown). We have previously reported that *GNRHR* and *LHCGR* are regulated by a *KCNJ5* mutation that induces the activation of intracellular calcium signaling (Kishimoto et al., 2016). Therefore, it is thought that the regulation of some ectopic or aberrant receptors is independent of DNA methylation state in APAs.

This study reports a comprehensive integration analysis of DNA methylation and mRNA expression related to GPCR in APAs. We demonstrated that GPCR or GPCR-related genes had a much higher incidence of CpG island demethylation in APAs, and due to this demethylation, some receptors were in a state that would facilitate gene transcription. The expression of two receptors, HTR4 and PTGER1, had a strong association with DNA methylation; furthermore, increased HTR4 expression may stimulate aldosterone production in APAs. This study provides important information regarding the molecular mechanisms of ectopic or aberrant receptor expression in APAs and identifies therapeutic or diagnostic targets that could be studied further.

Disclosure statements

The authors have nothing to disclose.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.mce.2017.08.019.

References

Akerstrom, T., Maharjan, R., Sven Willenberg, H., Cupisti, K., Ip, J., Moser, A., Stalberg, P., Robinson, B., Alexander Iwen, K., Dralle, H., Walz, M.K., Lehnert, H., Sidhu, S., Gomez-Sanchez, C., Hellman, P., Bjorklund, P., 2016. Activating mutations in CTNNB1 in aldosterone producing adenomas. Sci. Rep. 6, 19546.

Azizan, E.A., Poulsen, H., Tuluc, P., Zhou, J., Clausen, M.V., Lieb, A., Maniero, C., Garg, S., Bochukova, E.G., Zhao, W., Shaikh, L.H., Brighton, C.A., Teo, A.E., Davenport, A.P., Dekkers, T., Tops, B., Kusters, B., Ceral, J., Yeo, G.S., Neogi, S.G., McFarlane, I., Rosenfeld, N., Marass, F., Hadfield, J., Margas, W., Chaggar, K., Solar, M., Deinum, J., Dolphin, A.C., Farooqi, I.S., Striessnig, J., Nissen, P., Brown, M.J., 2013. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. Nat. Genet. 45, 1055–1060.

Berger, S.L., Kouzarides, T., Shiekhattar, R., Shilatifard, A., 2009. An operational definition of epigenetics. Genes Dev. 23, 781–783.

Beuschlein, F., Boulkroun, S., Osswald, A., Wieland, T., Nielsen, H.N., Lichtenauer, U.D., Penton, D., Schack, V.R., Amar, L., Fischer, E., Walther, A., Tauber, P., Schwarzmayr, T., Diener, S., Graf, E., Allolio, B., Samson-Couterie, B., Benecke, A., Quinkler, M., Fallo, F., Plouin, P.F., Mantero, F., Meitinger, T., Mulatero, P., Jeunemaitre, X., Warth, R., Vilsen, B., Zennaro, M.C., Strom, T.M., Reincke, M., 2013. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. Nat. Genet. 45, 440–444, 444e441-442.

Campbell, W.B., Brady, M.T., Gomez-Sanchez, C.E., 1986. Effects of angiotensin, prostaglandin E2 and indomethacin on the early and late steps of aldosterone biosynthesis in isolated adrenal cells. J. Steroid Biochem. 24, 865–870.

Choi, M., Scholl, U.I., Yue, P., Björklund, P., Zhao, B., Nelson-Williams, C., Ji, W., Cho, Y., Patel, A., Men, C.J., Lolis, E., Wisgerhof, M.V., Geller, D.S., Mane, S., Hellman, P., Westin, G., Åkerström, G., Wang, W., Carling, T., Lifton, R.P., 2011. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Science 331, 768–772.

Dedeurwaerder, S., Defrance, M., Calonne, E., Denis, H., Sotiriou, C., Fuks, F., 2011. Evaluation of the Infinium methylation 450K technology. Epigenomics 3, 771–784.

Duparc, C., Andre, C., Menard, J., Godouet-Getti, B., Wils, J., Cailleux, A.F., Moreau-Grange, L., Louiset, E., Lefebvre, H., 2017. l-Lysine acts as a serotonin type 4 receptor antagonist to counteract in vitro and in vivo the stimulatory effect of serotonergic agents on aldosterone secretion in man. Horm. Metab. Res. 49, 269–275.

Duparc, C., Moreau, L., Dzib, J.F., Boyer, H.G., Tetsi Nomigni, M., Boutelet, I., Boulkroun, S., Mukai, K., Benecke, A.G., Amar, L., Gobet, F., Meatchi, T., Plouin, P.F., Zennaro, M.C., Louiset, E., Lefebvre, H., 2015. Mast cell hyperplasia is associated with aldosterone hypersecretion in a subset of aldosterone-producing adenomas. J. Clin. Endocrinol. Metab. 100, E550–E560.

Fagerberg, L., Hallstrom, B.M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., Habuka, M., Tahmasebpoor, S., Danielsson, A., Edlund, K., Asplund, A., Sjostedt, E., Lundberg, E., Szigyarto, C.A., Skogs, M., Takanen, J.O., Berling, H., Tegel, H., Mulder, J., Nilsson, P., Schwenk, J.M., Lindskog, C., Danielsson, F., Mardinoglu, A., Sivertsson, A., von Feilitzen, K., Forsberg, M., Zwahlen, M., Olsson, I., Navani, S., Huss, M., Nielsen, J., Ponten, F., Uhlen, M., 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol. Cell Proteomics 13, 397–406.

Funder, J.W., Carey, R.M., Mantero, F., Murad, M.H., Reincke, M., Shibata, H., Stowasser, M., Young Jr., W.F., 2016. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. J. Clin. Endocrinol. Metab. 101, 1889–1916.

Howard, B., Wang, Y., Xekouki, P., Faucz, F.R., Jain, M., Zhang, L., Meltzer, P.G., Stratakis, C.A., Kebebew, E., 2014. Integrated analysis of genome-wide methylation and gene expression shows epigenetic regulation of CYP11B2 in aldosteronomas. J. Clin. Endocrinol. Metab. 99, E536–E543.

Kishimoto, R., Oki, K., Yoneda, M., Gomez-Sanchez, C.E., Ohno, H., Kobuke, K., Itcho, K., Kohno, N., 2016. Gonadotropin-releasing hormone stimulate aldosterone production in a subset of aldosterone-producing adenoma. Med. Baltim. 95, e3659.

Legendre, C.R., Demeure, M.J., Whitsett, T.G., Gooden, G.C., Bussey, K.J., Jung, S., Waibhav, T., Kim, S., Salhia, B., 2016. Pathway implications of aberrant global methylation in adrenocortical cancer. PLoS One. 11, e0150629.

Maqbool, F., Mostafalou, S., Bahadar, H., Abdollahi, M., 2016. Review of endocrine disorders associated with environmental toxicants and possible involved mechanisms. Life Sci. 145, 265–273.

Milliez, P., Girerd, X., Plouin, P.F., Blacher, J., Safar, M.E., Mourad, J.J., 2005. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. J. Am. Coll. Cardiol. 45, 1243–1248.

Mulatero, P., Monticone, S., Bertello, C., Viola, A., Tizzani, D., Iannaccone, A., Crudo, V., Burrello, J., Milan, A., Rabbia, F., Veglio, F., 2013. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. J. Clin. Endocrinol. Metab. 98, 4826–4833.

Nishikawa, T., Omura, M., Satoh, F., Shibata, H., Takahashi, K., Tamura, N., Tanabe, A., Task Force Committee on Primary Aldosteronism, The Japan Endocrine Society, 2011. Guidelines for the diagnosis and treatment of primary aldosteronism–the Japan Endocrine Society 2009. Endocr. J. 58, 711–721.

Oki, K., Yamane, K., Nakanishi, S., Shiwa, T., Kohno, N., 2012. Influence of adrenal subclinical hypercortisolism on hypertension in patients with adrenal incidentaloma. Exp. Clin. Endocrinol. Diabetes 120, 244–247.

Perraudin, V., Delarue, C., Lefebvre, H., Do Rego, J.L., Vaudry, H., Kuhn, J.M., 2006. Evidence for a role of vasopressin in the control of aldosterone secretion in primary aldosteronism: in vitro and in vivo studies. J. Clin. Endocrinol. Metab. 91, 1566–1572.

Sen, P., Shah, P.P., Nativio, R., Berger, S.L., 2016. Epigenetic mechanisms of longevity and aging. Cell 166, 822–839.

Slieker, R.C., Roost, M.S., van Iperen, L., Suchiman, H.E., Tobi, E.W., Carlotti, F., de Koning, E.J., Slagboom, P.E., Heijmans, B.T., Chuva de Sousa Lopes, S.M., 2015. DNA methylation landscapes of human fetal development. PLoS Genet. 11, e1005583.

Watanabe, M., Noda, M., Nakajin, S., 2006. Effect of epidermal growth factor and prostaglandin on the expression of aromatase (CYP19) in human adrenocortical carcinoma cell line NCI-H295R cells. J. Endocrinol. 188, 59–68.

Ye, P., Mariniello, B., Mantero, F., Shibata, H., Rainey, W.E., 2007. G-protein-coupled receptors in aldosterone-producing adenomas: a potential cause of hyperaldosteronism. J. Endocrinol. 195, 39–48.

Yoshii, Y., Oki, K., Gomez-Sanchez, C.E., Ohno, H., Itcho, K., Kobuke, K., Yoneda, M., 2016. Hypomethylation of CYP11B2 in aldosterone-producing adenoma. Hypertension 68, 1432–1437.

Yoshimoto, T., Hirata, Y., 2007. Aldosterone as a cardiovascular risk hormone. Endocr. J. 54, 359–370.

Zwermann, O., Suttmann, Y., Bidlingmaier, M., Beuschlein, F., Reincke, M., 2009. Screening for membrane hormone receptor expression in primary aldosteronism. Eur. J. Endocrinol. 160, 443–451.

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