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# Melanocortin 2 Receptor Mutations and Clinical Significance in Case of Cushing Syndrome and Subclinical Cushing Syndrome and Primary Aldosteronism

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**KEYWORDS** Adrenocorticotropic Hormone Receptor. Adrenal Disease. Melonocortin-2-Receptor Mutation. Non-functional Adrenal Incidentalomas. SNP

**ABSTRACT** The researchers investigated whether single nucleotide polymorphisms (SNPs) of *M2CR* were associated with the development of adrenocortical diseases including cushing syndrome (CS), subclinical cushing syndrome (SCS) and primary aldosteronism (PA) in Turkish population. Two promoter SNPs [rs1893219 (853A/G) and rs1893220 (759 G/T)] were genotyped in 43 patients with adrenal adenomas. All patients were examined hormonally with dynamic tests. While 22 of the patients had non-functional (NF) adrenal adenomas; 21 of the patients (CS=9, SCS=10, PA=2) had functional adrenal adenomas. In rs1893219 the frequencies of the CC (AA) were found to be 2, 3 and 1 patients in CS, SCS and PA, respectively. In rs1893220 the frequencies of the AA (GG) genotype were found to be 2, 1 and 1 patients in CS, SCS and PA. Also, the C allele frequency of rs1893219 was found increased in the patients with functional adenomas. The results have shown that the *M2CR* gene may contribute to the development of adrenocortical diseases.

## INTRODUCTION

Clinically unapparent adrenal masses of the adrenal glands called as adrenal incidentalomas may be hormonally functional/non-functional or malignant/benign, thus the evaluation of the hormonal status in adrenal masses have become an important problem for clinicians (Nieman 2010). Cushing syndrome (CS) is a clinical disorder according to excessive secretion of cortisol and this clinical issue is a major diagnostic problem in clinical endocrinology (Yorke et al. 2017). Chronic exposure of over cortisol value causes too much clinical comorbidity as cardiovascular and metabolic disease thus resulting in increased mortality (Guignat and Bertherat 2010). Autonomous cortisol secretion by adrenal incidentalomas is defined by the change of the pituitary-adrenal axis due to the adrenal autonomy in the absence of the phenotype of hypercortisolism; known as subclinical cushing syndrome (SCS) (Terzolo et al. 2012). As a result of widespread use of imaging modalities (CT, MRG) SCS has been diagnosed increasingly. Chronically, slight cortisol secretion can have a clinical negative effect on cardiovascular system, bone metabolism or fasting blood glucose levels. The diagnosis of SCS is completely dependent on biochemical and hormonal evaluation. Although etiological diversity of hypercortisolism caused by CS or SCS is described by many authors, their researches are not clearly comprehended. Hypercortisolism depended on CS or SCS; genetic and molecular mechanisms play a substantial role in these phenomes (Albani et al. 2018). Although some recent mutations related to CS such as phosphodiesterase mutation (*PDE8B* and *PDE11A* ), protein kinase A (PKA) or *DAX1* mutations have been demonstrated in CS, these mutations have been linked primarily to pigmented adrenocortical disease (PPNAD) or ACTH independent macronoduler adrenal hyperplasia

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(AIMAH) (Lacroix et al. 2004; Stratakis et al. 2001).

Primary aldosteronism (PA) is the most common form of secondary hypertension and is generally derived from unilateral aldosterone producing adenoma or hyperplasia. Increased levels of aldosterone cause severe hypertension, hypokalemia and cardiovasculer diseases. KCNJ5, ATP1A1, ATP2B3, CACNA1D and CTNNB1 mutations have been recently determined in patients with aldosterone producing adenomas (APAs)

Melanocortin receptor family plays diverse physiological roles and melanocortin 2 (MC2R) receptor is one of the five members of melanocortin receptor family. This receptor family is related to G-protein coupled receptor and all of them are activated pro-opiomelanocortin (POMC) (Dores 2013). The *MC2R* gene is located in chromosome 18p11.2 and has two exons. The coding region for the 297-amino acid protein is located in the second exon (El Ghorayeb et al. 2018). *MC2R* is highly sensitive and specific receptor for ACTH activation and differ from other melanocortin receptor subtypes in terms of two molecular features. First, without longer ACTH stimulating, it could not be stimulated by α-, β-, or γ-MSH peptide. Second, it was only functional in adrenal or melanoma cells. After *MC2R* binding ACTH receptor, adenylyl cyclase is activated then glucocorticoid is synthesized and secreted in adrenal cortex (Fridmanis et al. 2014).

### Objectives

The hypothesis of this study is to describe that *MC2R* mutations may lead to over or mild cortisol and even aldosterone secretion by ACTH receptor stimulation with the absence of detectable value of ACTH in adrenal cortex. And researchers also aim to demonstrate that *MC2R* mutations may have existed in cortisol and aldosterone secretion adrenal adenomas without hyperplasia. Besides, the researchers aim to demonstrate the *MC2R* mutations in non-functional adrenal incidentalomas. So the researchers' have different viewpoints about mutations in adrenal masses by comparing this mutation in two groups. Promoter single nucleotide polymorphism (SNP) of *MC2R* gene [rs1893219 (853A/G) and rs1893220 (759 G/T)] had been associated with CS, SCS or PA in Turkish population.

## METHODOLOGY

The Local Ethics Committee of the University of Cukurova approved this study. Written information consent was obtained from all subjects according to the Declaration of Helsinki. The researchers screened 43 patients with adrenal adenoma in their cohort. Considered patients are taken from southern province of Turkey (Adana) in Cukurova district. Patients were included when: 1) they had documented having adrenal masses; and 2) diagnosis was at 18 years old or older. At first; if there were only imaging results (CT, MRG) of the patients who were examined for any reason and detected to have adrenal mass, the existence of adrenal mass was confirmed through MRG examination in the researchers' center. The diagnosis of CS, SCS or PA in this study was based on hormonal evaluation with dynamic tests. All patients underwent an overnight 1 mg dexamethasone [DST] test after having performed basal cortisol and ACTH values. The suppression test was considered appropriate when morning cortisol value was below1.8 mcg/dL. If morning cortisol levels were higher than 1.8 mcg/dL, twice more low-dose DST suppression test was performed [2 mg, four times a day for two days]. The diagnosis of SCS (Morelli et al. 2010) was based on the presence of at least one of the following in addition to cortisol levels greater than 1.8 mcg/dL after 1-mg DST [R]: (1) urinary free cortisol (UFC) levels of >300 mcg/day in two of the three consecutive collections per 24-hour period, (2) ACTH levels of <10 pg/mL (<2.2 pmol/L), and (3) low serum DHEAS levels. Urinary metanephrine and normetanephrine excretion values were in normal limits for all patients. All patients with adrenal mass plasma-renin activity (PRA) and plasma aldosterone (PA) values were recorded. In patients with a Plasma Aldosterone/Renin ratio (ARR) >25, primary hyperaldosteronism (PHA) diagnosis was made via saline infusion confirmatory test.

All patients with CS and PHA were operated and their pathological diagnosis was shown in Table 1. Otherwise none of the SCS was operated because of not having clinical or metabolic indication.

**Table 1: Clinical characteristics of 43 patients with adenoma for whom measured hormonal profile**

| | *Age* | *Sex* | *Cortisol (µg/dL)* | *ACTH (pg/ml)* | *1mg DST (µg/dL)* | *4x0.5 mg DST (µg/dL)* | *Urine cortisol (µg/24h)* | *PAC (pg/mL)* | *Mass size (cm)* |
|---|---|---|---|---|---|---|---|---|---|
| SCSn=10 | 62.5±3.9 | F=8 M=2 | 16.5± 9.1 | 9±4.1 | 20 | 18 | 60.46 | 25± 10.1 | 2.5±1.4 |
| CSn=9 | 45±7.07 | F=8 M=1 | 24.3±15.0 | 4.4±3.1 | 24 | 20 | 95.98 | 18± 10.2 | 3.4±3.1 |
| PAn=2 | 46.5±6.3 | F=2 | 10.2± 7.0 | 15±0.1 | 18 | 20.1 | 20.8 | 240±118 | 1.5 |
| NFAIn=22 | 54.3±6.2 | F=15 M=7 | 12.1± 8.3 | 22±8.2 | 22 | 18 | 25.5 | 15± 5.1 | 2.2±1.3 |

SCS: Subclinical Cushing Syndrome, CS: Cushing Syndrome, PA: Primary Aldosteronism, NFAI: Non-functional Adrenal Incidentaloma, DST: Dexamethasone-supression Test, PAC: Plasma Aldosterone Concentrations

DHEAS, ACTH and cortisol values were analyzed using polymerase chain reaction [PCR] method, enzymatic-labeled chemiluminescent immunometric assay method, and chemiluminescence [Beckman DXI 800 auto analyzer], respectively. PRA and PA levels were measured through radioimmunoassay method. High performance liquid chromatography [HPLC] method was used to analyze urine cortisol and metanephrine values.

### Genetic Analyzes

Genomic DNA was prepared from peripheral blood leukoyctes using DNA isolation kit. Selected SNP of MC2R (rs1893219 (853 A/G) and rs1893220 (759 G/T) genotyping was conducted by direct sequencing using the following primers for each SNP: sense, 5 -GGCAATGCCTTGCTTTTCTCTG3; antisense, 5 -AGGGAAGGGCT ACTGTTGGT-3 , 387 bp) and rs1893220 (sense, 5 -GGCAATGCCTTGCTTTTC TCTG-3 ; antisense, 5 -AGGGAAGGGCTACTGTTGGT-3 , 323 bp). The entire coding sequence of the MC2R gene was amplified by PCR (Kyratec Termocycle, Australia). PCR products were purified using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) from on ABI 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA). BLAST analysis was performed using GenBank database for each sample with the obtained sequence analysis results and the type was determined. SNP genotyping analysis was performed by aligning with the sequence analysis results using the online MultAlin program (http://multalin.toulouse.inra.fr/multalin/).

### Statistical Analysis

The differences in genotype of rs1893219 (853 A/G) and rs1893220 (759 G/T) were analyzed statistically using Fischer's exact test and chi-square test. The associations between allele frequencies in all groups were evaluated by computing the odds ratios (OR) and their confidence intervals using Hardy-Weinberg equilibrium. $P<0.05$ was considered statistically significant.

## RESULTS

Majority of the patients with adrenal masses (33, 76.7%, n=43) were female. Twenty-two (22) of the patients had non-functional adrenal adenomas, 9 patients were diagnosed as an over cushing syndrome and the other 10 patients were diagnosed as a SCS. Two (2) of the patients were evaluated as PA. Majority of the patients (18, 85.7%, n=21) with functional adrenal mass were female. The mean age of the patients with CS, SCS, PA were 45±7.07, 62.50±3.99, 46.50±6.36 respectively. In rs1893219 the frequencies of the CC (AA) (25% and 5.1%) were found increased in patients with functional adrenal mass; especially SCS and PA compared to the patients with non-functional adenomas. In rs1893219 the frequencies of the TT (GG), TC (GA) were 16.66 percent (n=4) and 58.33 percent (n=14) respectively. In rs1893220 the frequencies of the AA (GG) genotype were found increased in the patients with functional adenomas (16.6%) compared to patients with non-functional adenomas (5.26%). In the allele frequency analysis, rs1893219 was also associated with functionality of the adrenal masses, and the frequency of the C allele was found increased in the patients with functional adenomas (n=26, 54.16%) compared to the patients with non-functional adenomas (n=15, 39.47%). In rs1893220 the frequency of the A allele was also found increased in the patients with functional adenomas (41.66%, n=20) compared to the patients with nonfunc-

tional adenomas (36.8%, n=14). But, no significant differences were observed in allele frequency of MC2R gene between functional and non-functional adenomas patients ($p<0.05$).

The genotype and allele frequencies of two examined SNPs are demonstrated in Tables 2a-2d and Figures 1 and 2. Both SNPs were related with SCS, CS and PA. The meta-analysis indicated that patients who carried *MC2R* rs1893219 CC genotype showed significantly having diagnosed as PA or CS. Otherwise patients with *MC2R* rs1893220 are also related with CS and PA. The CC, AC and AA MC2R genotypes were identified in 5 (55.55%), 2 (22.22%) and 2 (22.22%) of CS patients, respectively. In SCS patients, CC, AC and AA genotypes of MC2R were found in 2 (20%), 7 (70%) and 1 (10%), respectively. The CC and AC M2CR genotypes were identified in 1 (50%) and 1 (50%), respectively in two SNPs. Interestingly, one of the patient with PA had homozygous mutation while the other patient had heterozygous mutation. Otherwise patients with non-functional adrenal incidentaloma had mostly heterozygous mutations by genotyped with rs1893219 and rs1893220.

## DISCUSSION

Herein this report the results of screening for known genes associated with CS, SCS or PA in a cohort of 43 patients with adrenal. This study confirmed that *MC2R* is a gene which its related mutations can contribute to PA, SCS and CS. The primary association occurs with single nucleotide polymorphism rs1893219 and rs1893220. Particularly, the frequencies of the major alleles of both rs1893219 and rs1893220 (the C allele and the A allele, respectively) were increased in the patients with functional adenomas. Especially; both the patients with PA had also homozygous mutations in the two SNPs. All the patients with NFAI had heterozygous mutations. CC and AA haplotypes consisting of rs1893219 and rs1893220 were related to hypercortisolism and hyperaldosteronism.

ACTH stimulation is the primary initial factor for secreting cortisol in adrenal cortex and according to this process; abnormal ACTH between its receptor interactions can lead to over secretion of glucocorticoid or mineralocorticoid (CS and PA) and changing adrenocortical tissue (Arnsten 2009). ACTH carries out its actions

**Table 2a: Genotype frequencies of the SNP in MC2R gene in functional and non-functional patients**

| | *rs1893219* | | | *rs1893220* | | |
|---|---|---|---|---|---|---|
| | *TT (GG)* | *TC (GA)* | *CC (AA)* | *CC (TT)* | *AC (GT)* | *AA (GG)* |
| Functional | 4 (16.66%) | 14 (58.33%) | 6 (25.01%) | 8 (33.33%) | 12 (50.01%) | 4 (16.66%) |
| NF | 5 (26.31%) | 13 (68.42%) | 1 (5.2 6%) | 6 (31.57%) | 12 (63.15% | 1 (5.26%) |
| | | P: 0.203 | | | | P: 0.466 |

**Table 2b: Alleles frequencies of the SNP in peripheral blood MC2R gene in functional and non-functional patients**

| | | *rs1893219* | | *rs1893220* | |
|---|---|---|---|---|---|
| | *Total* | *T (G)* | *C (A)* | *C (T)* | *A (G)* |
| Functional | 48 | 22 (45.83%) | 26 (54.16%) | 28 (58.33%) | 20 (41.66%) |
| NF | 38 | 23 (60.552%) | 15 (39.47%) | 24 (63.15%) | 14 (36.84%) |
| | | OR: 1.812 | P: 0.177 | OR: 1.224 | P: 0.649 |

**Table 2c: Genotype frequencies of the SNP in MC2R gene in CS, SCS and PA patients**

| | *rs1893219* | | | *rs1893220* | | |
|---|---|---|---|---|---|---|
| | *TT (GG)* | *TC (GA)* | *CC (AA)* | *CC (TT)* | *AC (GT)* | *AA (GG)* |
| CS | 3 (33.33%) | 4 (44.44%) | 2 (22.22%) | 5 (55.55%) | 2 (22.22%) | 2 (22.22%) |
| SCS | 0 | 7 (70%) | 3 (30%) | 2 (20%) | 70 (70%) | 1 (10%) |
| PA | 0 | 1 (50%) | 1 (50%) | 0 | 1 (50%) | 1 (50%) |

**Table 2d: Alleles frequencies of the SNP in peripheral blood MC2R gene in CS, SCS and PA patients**

| | | *rs1893219* | | *rs1893220* | |
|---|---|---|---|---|---|
| | *Total* | *T (G)* | *C (A)* | *C (T)* | *A (G)* |
| CS | 18 | 10 (55.55%) | 8 (44.44%) | 12 (66.66%) | 6 (33.33%) |
| SCS | 23 | 10 (43.47%) OR: 1.621 | 13 (56.52%) P: 0.445 | 11 (47.82%) OR: 1.636 | 9 (39.13%) P: 0.463 |
| PA | 4 | 1 (25%) OR: 2.301 | 3 (75%) P: 0.490 | 1 (25%) OR: 3.666 | 3 (75%) P: 0.294 |

through activating of human ACTH receptor genes (*MC2R). MC2R* gene includes two exons and is intronless within the coding sequence. *MC2R* activation is the first step in the stimulation of the ACTH receptor pathway (Cooray and Clark 2011). So far several studies have described inactivating mutations of the *MC2R* gene associated with familial glucocorticoid deficiency (FGD) (Chung et al. 2010). In contrast, one of the studies reported that the activating mutations of the *MC2R* gene and one of these patients were diagnosed with PMAH (Fragoso et al. 2013). This reason has encouraged the researchers for searching on the *MC2R* gene in the range of adrenal hyperplastic and neoplastic pathologies.

Latronico et al. (1995) amplified the polymerase chain reaction and genotyped the entire exon of the *MC2R* gene in 25 adrenocortical tumors (17 adenomas and 8 carcinomas), but they did not find missense or silent mutations. However, Beuschlein et al. (2001) researched about constitutive activating of G protein couplet receptor including ACTH-R gene family; they observed MC2R LOH in two of four informative adrenocortical carcinomas which suggested a role for MC2R that could contribute to development of adrenal tumorigenesis by the way of cellular dedifferentiation in adenomas and carcinomas. The possibility of an ACTH receptor activating mutation as the cause of hypercortisolism was also reported in a patient with hypercortisolemia with the absence of detectable value of ACTH with *MC2R* mutation. Aloi et al. (1995) demonstrated the patient with hypercor-

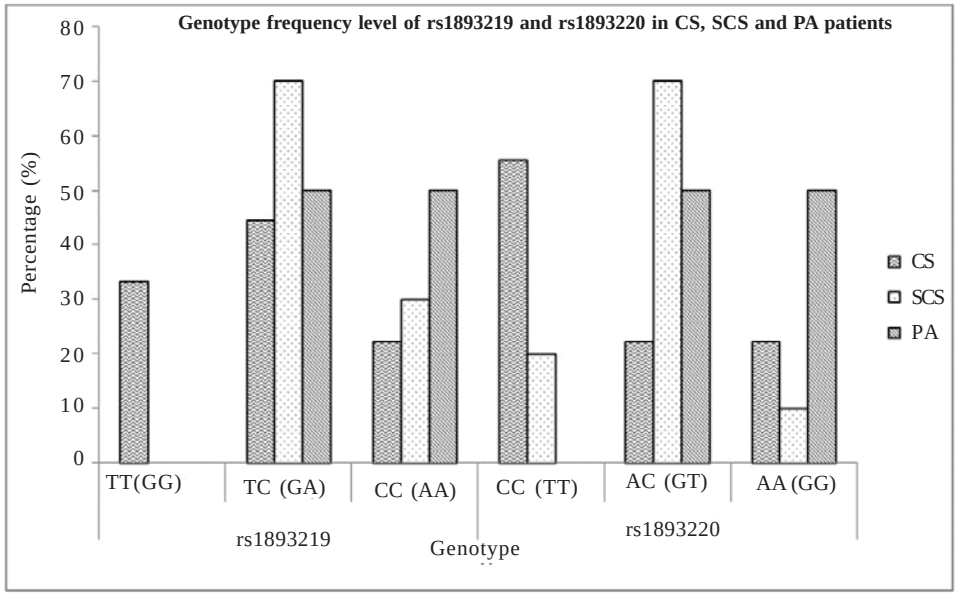


**Fig. 1. Genotype frequencies (%) of the SNP in MC2R gene in CS, SCS and PA**

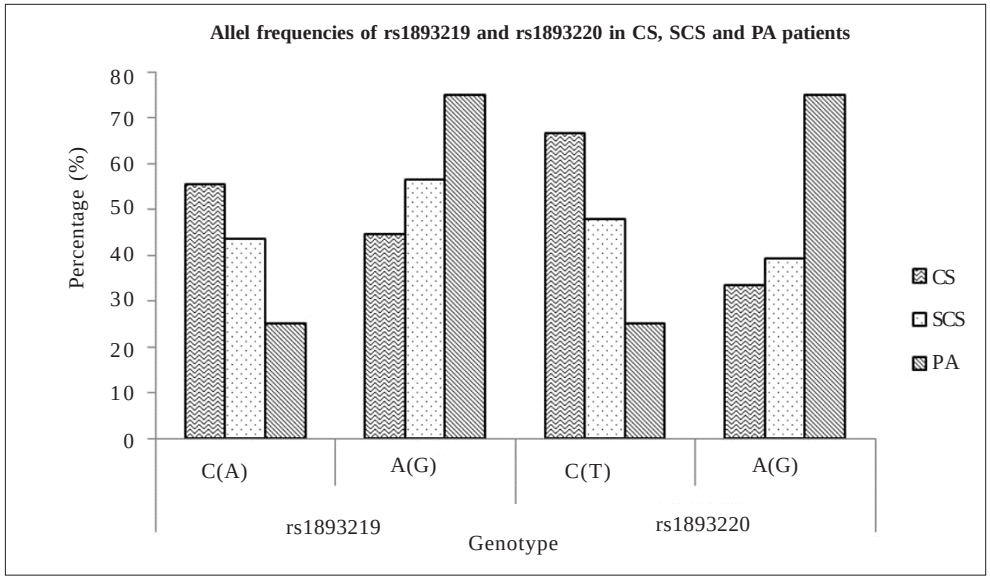


**Fig. 2. Allele frequencies (%) of the SNP in MC2R gene in CS, SCS and PA**

tisolemia had germline missense mutation of the *MC2R* that resulted in the substitution of Phe 278 by Cys (F278C) in the C terminal tail.

Another hypothesized hypercortisolism related with *MC2R* mutations has been indicated in constitutive activation of the human ACTH-R associated with clinical hypersensitivity (Swords et al. 2002; Swords et al. 2004). Swords et al. (2002) reported that the wild type (WT) *MC2R* showed rapid early desensitization to recurrent stimulation with ACTH and WT *MC2R* gene and could increase basal activation and impaired desensitization. Some studies (Swords et al. 2004) reported that constitutive activity of the human ACTH receptor related with missense mutation in *MC2R* gene and this mutation could be the cause of elevation activity of the ACTH responsiveness despite the normal dose of ACTH value. In the researchers' study, 4 patients of CS and 7 of the SCS patients with adrenal adenoma for rs1893219 and 2 patients of CS and 7 of the SCS patients for rs1893220 heterozygous mutations of *MC2R* gene plasma ACTH value of the patients' were also completely suppressed. The researchers speculated that constitutive activation of the ACTH receptor related with homozygous or heterozygous mutations may trigger in the presence of the hypercortisolemia. Therefore, the researchers suggested that CS and SCS have the heterozygous etiology and related to cause by activating *MC2R* gene.

Although mutations in *MC2R* gene seem to be related with solely hypercortisolemia, recent studies indicate that *MC2R* has a major role in the autocrine/paracrine processes occurring in the adrenal gland in both physiological and pathological conditions (Lefebvre et al. 2016). Arnaldi et al. (1998) researched that ACTH-R was expressed in all patients' tissue including non-secreting adenomas, aldosterone producing adenomas and androgen secreting adenomas. They highlighted that the overexpression of the ACTH-R was more abundant than other hormone secreting tissues. Despite the variety of the diurnal rhythm, renin-angiotensin system or fluid electrolyte balance primarily influence aldosterone synthesis; this mutation can cause tumorigenesis by supporting the role of ACTH on aldosterone secretion in these tumors. In this study; patients with primary aldosteronism the frequency of major alleles (C and A, respectively) in two SNPs was seventy-five percent. One of the patients with PA had homozygous mutation while the other patient had heterozygous mutation.

In addition, previous studies demonstrated that blocking MC2 receptor can also be used to treat medically for cushing syndrome or disease

(Bouw et al. 2014; Igaz et al. 2008; Newfield 2010; Rauschecker and Stratakis 2012). Furthermore, studies suggested that antagonism of this receptor could provide a useful therapeutic aid and can lead to innovative pharmacological therapies as an alternative to other surgical treatments in CS, ectopic ACTH syndrome or congenital adrenal hyperplasia (Clark et al. 2016; Mircescu et al. 2000).

## CONCLUSION

In this study, the researchers sequenced the rs1893219 (1032T/C) and rs1893220 (936C/A) coding area of MC2R gene. They found that two promoter SNPs of this gene were not associated with CS, SCS and PA patients. Although there was not statistical significance; CC genotype was more common in patients with hypercortisolemia (CS, SCS). So *MC2R* mutations may linked with overt cortisol secretion.

## RECOMMENDATIONS

There is a need for further and more comprehensive study including more patients to understand the role *MC2R* mutations play in occurrence of overt hormone secretion.

## REFERENCES


Albani A, Theodoropoulo M, Reincke M 2018. Genetics of cushing's disease. *Clin Endocrin,* 88: 3-12.

Aloi JA, Carey RM, Thorner MO, Malchoff CD, Orth DN, Walsh B, Macgillivray D, Marchoff D 1995. Episodic, ACTH-Independent, Cushing's Syndrome Associated with a Point Mutation of the ACTH Receptor. *Paper Presented in Program of the 77th Annual Meeting of the Endocrine Society,* June 1995, Washington DC.

Arnaldi G, Mancini V, Constantini C, Giovagnetti M, Petrelli M, Masini A, Bertagna X, Mantero F 1998. ACTH receptor mRNA in human adrenocortical tumors: Overexpression in aldosteronomas. *Endocr Res,* 24(3-4): 845-849.

Arnsten AFT 2009. Stress signaling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci,* 10(6): 410-422.

Beuschlein F, Fassnacht M, Klink A, Allolio B, Reincke M 2001. ACTH-receptor expression, regulation and role in adrenocortical tumor formation. *Eur J Endocrinol,* 144: 199-206.

Bouw E, Huisman M, Neggers SJ, Themmen AP, van der Lely AJ, Delhanty PJ 2014. Development of potent selective competitive-antagonists of the melanocortin type 2 receptor. *Mol Cell Endocrinol,* 394: 99-104.

Chung TTL, Chan LF, Metherell LA, Clark AJL 2010. Phenotypic characteristics of familial glucocorticoid deficiency (FGD) type 1 and 2. *Clin Endocrinol,* 72: 589-594.

Clark AJ, Forfar R, Hussain M, Jerman J, Mclver E, Taylor D, Chan L 2016. ACTH antagonists. *Front Endocrinol,* 7: 101

Cooray SN, Clark AJ 2011. Melanocortin receptors and their accessory proteins. *Mol Cell Endocrinol,* 331(2): 215-221.

Dores RM 2013. Observations on the evolution of the melanocortin receptor gene family: Distinctive features of the melanocortin-2 receptor. *Front Neurosci,* 7: 28.

El Ghorayeb N, Bourdeau I, Lacroix A 2018. Pathophysiology and genetic landscape of adrenocortical tumors and hyperplasias. In: A Levine (Eds): *Adrenal Disorders*. Cham: Humana Press, pp. 147-171.

Fragoso MC, Alencar GA, Lerario AM, Bourdeau I, Almeida MQ, Lacroix A 2015. Genetics of primary macronodular adrenal hyperplasia. *J Endocrinol,* 224(1): 31-43.

Fridmanis D, Petrovska R, Pjanova D, Sclöth HB, Klovins J 2014. Replacement of short segments within transmembrane domains of MC2R disrupts retention signal. *J Mol Endocrinol,* 53(2): 201-215.

Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF 2016. The management of primary aldosteronism: Case detection, diagnosis, and treatment: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab,* 101:1889-1916.

Guignat L, Bertherat J 2010. The diagnosis of cushing's syndrome: An endocrine society clinical practice guideline: Commentary from a European perspective. *Eur J Endocrinol,* 163(1): 9-13.

Igaz P, Tömböl Z, Szabó, PM, Likó I, Rácz K 2008. Steroid biosynthesis inhibitors in the therapy of hypercortisolism: Theory and practice. *Curr Med Chem,* 15(26): 2734-2747.

Lacroix A, Baldacchino V, Bourdeau I, Hamet P, Tremblay J 2004. Cushing's syndrome variants secondary to aberrant hormone receptors. *Trends Endocrinol Metab,* 15(8): 375-382.

Latronico AC, Reincke M, Mendonça BB, Arai K, Mora P, Allolio B, Wajchenberg BL, Chrousos GP, Tsigos C 1995. No evidence for oncogenic mutations in the adrenocorticotropin receptor gene in human adrenocortical neoplasms. *JCEM,* 80(3): 875-877.

Lefebvre H, Thomas M, Duparc C, Bertherat J, Louiset E 2016. Role of ACTH in the interactive/paracrine regulation of adrenal steroid secretion in physiological and pathophysiological conditions. *Front Endocrinol,* 7: 98.

Mircescu H, Jilwan J, N'Diaye N, Bourdeau I, Tremblay J, Hamet P, Lacroix A 2000. Are ectopic or abnormal membrane hormone receptors frequently present in adrenal cushing's syndrome? *J Clin Endocrinol Metab,* 85(10): 3531-3536.

Morelli V, Masserini B, Salcuni AS, Eller-Vainicher C, Savoca C, Viti R, Coletti F, Guglielmi G, Battista C, Iorio L, Beck-Peccoz P, Ambrosi B, Arosio M, Scillitani A, Chiodini I 2010. Subclinical hypercortisolism: Correlation between biochemical diagnostic criteria and clinical aspects. *Clin Endocrinol,* 73(2): 161-166.

Newfield RS 2010. ACTH receptor blockade: A novel approach to treat congenital adrenal hyperplasia, or cushing's disease. *Med Hypotheses,* 74(4): 705-706.

Nieman LK 2010. Approach to the patient with an adrenal incidentaloma. *JCEM,* 95(9): 4106-4113.

Rauschecker M, Stratakis CA 2012. Molecular genetics of adrenocortical tumor formation and potential pharmacologic targets. *Minerva Endocrinol,* 37(2): 133-139.

Rossi GP 2011. A comprehensive review of the clinical aspects of primary aldosteronism. *Nat Rev Endocrinol*, 7: 485-495.

Scholl UI, Goh G, Stolting G, de Oliveira RC, Choi M, Overon JD, Fonseca AL, Korah R, Starker LF, Kunstman JW, Prasad ML, Hartung EA, Mauras N, Benson MR, Brady T, Shapiro JR, Loring E, Nelson-Williams C, Libutti SK, Mane S, Hellman P, Westin G, Akerström G, Björklund P, Carling T, Fahlke C, Hidalgo P, Lifton RP 2013. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet,* 45(9): 1050-1054.

Stratakis CA, Kirschner LS, Carney JA 2001. Clinical and molecular features of the carney complex: Diagnostic criteria and recommendations for patient evaluation. *J Clin Endocrinol Metab,* 86(9): 4041-4046.

Swords FM, Baig A, Malchoff DM, Thorner MO, King PJ, Hunyady L, Clark AJ 2002. Impaired desensitization of a mutant adrenocorticotropin receptor associated with apparent constitutive activity. *Mol Endocrinol*, 16(12): 2746-2753.

Swords FM, Noon LA, King PJ, Clark AJ 2004. Constitutive activation of the human ACTH receptor resulting from a synergistic interaction between two naturally occurring missense mutations in the MC2R gene. *Mol Cell Endocrinol,* 15(2): 149-154.

Tan GC, Negro G, Pinggera A, Tizen Laim NMS, Mohamed Rose I, Ceral J, Ryska A, Chin LK, Kamaruddin NA, Mohd Mokhtar N, Jamal AR, Sukor N, Solar M, Striessnig J, Brown MJ, Azizan EA 2017. Aldosterone-producing adenomas: histopathology-genotype correlation and identification of a novel CACNA1D mutation. *Hypertension,* 70(1): 129-136.

Terzolo M, Pia A, Reimondo G 2012. Subclinical cushing's syndrome: Definition and management. *Clin Endocrinol,* 76: 12-18.

Yorke E, Atiase Y, Akpalu J, Sarfo-Kantanka O 2017. Screening for cushing syndrome at the primary care level: What every general practitioner must know. *Int J Endocrinol*, Article ID #1547358, 6 pages.

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