Differential central regulatory mineralocorticoidreceptor systems for anxiety and depression – could KCNJ5 be an interesting target for further investigations in major depression?

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Running title: MR systems in anxiety and depression

Word count: Manuscript: 2817 (without abstract, acknowledgment, references, tables and figures)

Figures: 4 **Tables:** 4 **Supplementary Tables:** 0 **Supplementary Figures:** 0

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Disclosure statement: The authors have nothing to disclose.

Keywords: primary aldosteronism, depression, anxiety, KCNJ5

Contributions: All authors contributed sufficiently to the manuscript in conceptualisation, data sampling, data analysis, writing, correction.

Conflict of interest: There is no conflict of interest.

Data availability: Data can be provided, if required

Funding: This work was supported by the Else Kröner-Fresenius Stiftung in support of the German Conn's Registry-Else-Kröner Hyperaldosteronism Registry (2013_A182, 2015_A171 and 2019_A104 to MR), the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 694913 to MR), by the Deutsche Forschungsgemeinschaft (DFG) (within the CRC/Transregio 205/1 "The Adrenal: Central Relay in Health and Disease" to CA, DH, HS and MR).

Abstract

The mineralocorticoid receptor (MR) is suggested to play a role in the pathophysiology of depression and anxiety. Main support comes from studies in patients with primary aldosteronism (PA) which suggested different central pathways for depression and anxiety mediated via the MR and gender differences. We investigated 118 patients with PA over 3 years using self-rating questionnaires for anxiety (GAD-7) and depression (PHQD) at baseline and once a year under specific treatment with adrenalectomy (ADX; n=48) or a MR antagonist (MRA; n= 70). Genotyping for KCNJ5 mutation was performed in resected tumors.

At baseline, patients treated by ADX or MRA had comparable scores for anxiety and depression. Females showed a better metabolic profile but higher scores of depression and anxiety, compared to males. Initiation of specific treatment for PA resulted in a better response in depressive symptoms after ADX and of anxiety under MRA treatment. However, GAD-7 and PHQD remained high in women over the three-year follow-up.KCNJ5 mutation, linked to co-secretion of hybrid steroids as 18-oxocortisol and 18-hydroxycortisol, was detected in 10 female and 2 male patients. They tended to have higher GAD and PHQD scores at baseline compared to patients without KNCJ5 mutation, but showed a significant better reduction in symptoms of anxiety during the 3-year follow up compared to patients without this mutation (all $p< 0.05$).

These data support a differentiated regulation of depression and anxiety by the MR. Moreover, genetic mutations such as KCNJ5 could affect the pathophysiology of these disorders by impacting in adrenal steroidogenesis.

Introduction

The renin-angiotensin-aldosterone-system (RAAS) participates in the pathophysiology of depression and anxiety. In patients with depression elevated aldosterone levels were observed (Emanuele, Geroldi, Minoretti, Coen, & Politi, 2005; Murck et al., 2003; Nowacki et al., 2020). Additionally, Segeda et al. 2017 (Segeda, Izakova, Hlavacova, Bednarova, & Jezova, 2017) suggested in their study salivary aldosterone to be associated with markers of chronicity, severity, duration and outcome of major depression. Aldosterone binds with high affinity to the mineralocorticoid receptor (MR) in the presence of the enzyme 11beta hydroxysteroid dehydrogenase type 2 (11betaHSD2). One key brain area co-expressing MR and 11betaHSD2 is the nucleus of the solitary tract (NTS) (Geerling & Loewy, 2006; 2009). This area is connected closely to brain areas that are known to be associated to the pathophysiology of depression as the nucleus accumbens, a regulator of motivation, (Shekhtman, Geerling, & Loewy, 2007; Shin, Geerling, & Loewy, 2008), the insula, related to interception (Shin et al., 2008) and prefrontal areas, that is involved in mood regulation (Shin et al., 2008) and physiological symptoms as higher salt preference and longer slow wave sleep (Buttner et al., 2015). Genotype studies suggested MR haplotype 1 to be related to a CRH hypoactivity and symptoms of atypical depression, whereas haplotype 2 may be protective against depression – especially in females. (Klok et al., 2011; Kumsta, Kliegel, Linden, DeRijk, & de Kloet, 2019). Patients with primary aldosteronism (PA) are a natural model for the interaction between the RAAS and symptoms of depression and anxiety. In preceding studies, we and others could show that patients with PA show more symptoms of depression and anxiety than the general population. Initiation of specific treatment for PA was followed by a significant reduction of depressive symptoms in patients with unilateral disease undergoing ADX, whereas in patients treated by MRA anxiety scores were significantly decreased, at least in a one-year short term follow-up, suggesting different regulatory pathways

for anxiety and depression mediated by MR blockade and cure of aldosterone excess (Murck et al., 2021). Gender differences in regulation were also reported, as women were found to be more affected by symptoms of depression and anxiety. The exact mechanisms behind that are still unclear (Apostolopoulou et al., 2014; Murck et al., 2020).

Patients with unilateral PA often feature somatic mutations (Fernandes-Rosa et al., 2014; Holler et al., 2019) . The most commonly mutated gene is KCNJ5, which is predominantly found in females (Fernandes-Rosa et al., 2014). Those patients have a specific hormonal pattern. They show a clinically pronounced expression of psychopathological symptoms than patients without this mutation. This is accompanied by the release of the hormones 18-Oxocortisol and 18-Hydroxycortisol (Rege, Turcu, & Rainey, 2020). The receptor affinity of these hormones is still unclear, but they may affect the clinical expression. Therefore, we hypothesized that this mutation might affect psychopathological symptoms in patients with PA.

Thus, the current study aimed to investigate the impact of KCNJ5-mutation on the severity and the course of depression and anxiety in PA and to provide data on the long-term course of depression and anxiety in patients with PA undergoing specific treatment.

Methods

Patients and methods

We screened data of 208 patients with PA, who were prospectively included in the Munich center of the German Conn's Registry between 2008 and 2017 and attended baseline visit and follow-up for at least three years after initiation of specific treatment for PA. Although 90 patients had to be excluded (e.g., missing questionnaires), 118 patients had a sufficient data set and could be included in the final analysis. All patients gave written informed consent, and the protocol of the German Conn's Registry was approved by the Ethics Committee of the University of Munich.

Diagnosis and subtyping of PA was performed in accordance with Endocrine Society Practice Guidelines (Funder et al., 2016). In short, screening was performed using the aldosterone-to-renin-ratio (ARR). Prior to screening and testing antihypertensive medication with impact on ARR was stopped or whenever possible changed to medication with limited impact on ARR (e.g., verapamil, doxazosin). If ARR was abnormal, patients underwent confirmatory testing using saline infusion and/or captopril challenge test. PA subtyping was performed using adrenal vein sampling (Betz et al., 2011). In case of unilateral disease of PA all patients were offered unilateral adrenalectomy. Patients with unilateral disease, who had contraindications for adrenalectomy or refused surgery and all other patients were medically treated with MRA. At time of diagnosis and at each visit patients underwent standard procedures including collection of anthropometric data, laboratory testing and clinical characteristics such as current medication.

DNA sequenzing

Genomic DNA was extracted from fresh frozen adrenal tissues, and DNA fragments were amplified as described before (Yang et al., 2019).

Questionnaires

To assess symptoms of depression and anxiety we used the brief patient's health questionnaire (PHQ-9) and the 7-item generalized anxiety disorder scale (GAD-7) respectively. Both questionnaires are well-established and validated. Details are reported elsewhere (Gendreitzig et al., 2021; Gilbody, Richards, Brealey, & Hewitt, 2007; Murck et al., 2020; Ruiz et al., 2011).

Statistical analysis

All values are expressed as mean ± standard deviation, if not mentioned otherwise. Data between groups were compared using Mann-Whitney U test, or chi-square test for numerical or categorical variable, respectively. Within-group changes from baseline to follow-up were calculated by Wilcoxon signed-rank test and McNemar's test for numerical or categorical variable, respectively. Spearman's Rank correlation coefficient was used to perform bivariate correlation analysis. Stepwise multiple regression analysis was used for multivariate analysis.

Two-tailed probability values of <5% were considered to be statistically significant. Statistical analysis was performed using standard statistical software (IBM SPSS Statistics for Windows, Version 26. Armonk, NY: IBM Corp.).

Results

Characteristics of the total cohort

In the current study the data of 118 patients with PA were analyzed. The cohort was predominately male and overweight with a long-lasting hypertension of more than 10 years (Tables 1+2). As expected, at baseline patients had high blood pressure and plasma aldosterone levels, whereas serum potassium was at the lower limit of the normal. GAD-7 and PHQD were both increased to a pathological level with a mean score of 5.0 and 6.9 respectively. Women had significantly lower systolic (140 vs 156 mmHg, $p< 0.001$) and diastolic blood pressure levels (91 vs 96 mmHg, $p= 0.020$) as well as lower BMI (24.6 vs 29.5, $p< 0.001$) compared to men. Moreover, the lipid and glucose profile of women including HDL-cholesterol ($p< 0.001$), triglycerides ($p= 0.004$), LDL-cholesterol ($p< 0.001$) and HbA1c ($p=0.012$) was significantly lower than in men. GAD-7 (5.6 vs 4.7 $p= 0.348$) and PHQD score (8.4 vs 6.1, $p= 0.295$) were numerically higher in women but did not reach significance.

In the total cohort, PHQD significantly decreased at one-year follow-up and tended to be even lower at three-year follow-up. Interestingly, mean PHQD remained pathologic over the three-year follow-up in women. GAD-7 decreased slightly at each follow-up visit and became significant not before the three-year follow-up (5.0 vs 4.0, $p< 0.001$).

Baseline characteristics of patients treated with either MRA or ADX

While 70 patients received MRA treatment 48 patients underwent ADX. Both cohorts were comparable for age, BMI, duration of hypertension, markers of glucose and lipid metabolism and blood pressure levels, but patients assigned to ADX required higher defined doses of antihypertensives (3.0 vs 2.0, $p= 0.009$; Tables 1+2). As reported before, patients assigned to ADX featured higher plasma aldosterone (268 vs 181, $p< 0.001$) and pro-BNP levels (180 vs 107, $p= 0.039$), whereas potassium levels were significantly lower (3.3 vs 3.7, $p< 0.001$). GAD-7 and PHQD did not differ between the subgroups.

Follow-up characteristics of patients treated with MRA

One year after initiation of treatment with MRA, aldosterone and renin levels significantly increased and potassium levels as well as blood pressure normalized (Table 1). GAD-7 and PHQD significantly decreased at one-year follow-up. While the decrease in GAD-7 was stable over the years and remained highly significant compared to baseline, the level of significance for the decrease in PHQD was at a borderline level at the two- and three-year follow-up. Moreover, PHQD score at three-year follow-up was still pathologically increased whilst GAD-7 was normalized (Figures 1+2).

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178 ***Follow-up characteristics of patients treated with ADX***

179 One year after initiation of treatment with ADX, aldosterone, renin and potassium levels as
180 well as blood pressure widely normalized (Table 2). While GAD-7 was unaltered, PHQD
181 significantly decreased at one-year follow-up (7.9 vs 6.4, p= 0.039). At two- and three-year follow-
182 up patients showed stable blood pressure and aldosterone levels. However, GAD-7 and PHQD
183 decreased step-by-step at each visit. Consequently, at three-year follow up PHQD but also GAD-7
184 were significantly decreased compared to the baseline visit (Figures 1+2).

185

186 ***Impact of treatment modality on GAD-7 and PHQD***

187 Although there was a strong correlation between the reduction in GAD-7 and PHQD at all
188 follow-up visits (r= 0.477, r=0.499, r=0.540 all p< 0.001), in multivariate analysis we could detect
189 a significant better reduction for PHQD in patients treated by ADX at three-year follow-up
190 (p=0.023)., whilst improvement of GAD-7 was significant with MRA treatment at one-year follow-
191 up but not at two- and three-year follow-up. While the reduction of GAD-7 was significant in
192 patients under MRA treatment at 1-year follow-up (p= 0.031), we could no longer observe a
193 significant association with treatment modality at two and three-year follow-up.

194

195 ***Genotyping in patients after ADX***

196 In 27 patients, who underwent ADX genotyping for somatic mutations in resected tumors
197 was performed. The most frequent mutation was the KCNJ5 mutation, which could be detected in
198 44% (n=12) of patients. Those patients (KCNJ5-group) were predominately female (n=10) and
199 showed a strong phenotype for depression (8.8 vs 5.1 in patients without KCNJ5 mutation) and
200 anxiety (5.8 vs. 4.0 in patients without KCNJ5 mutation, Tables 3a+b). This was also illustrated by

the fact that 55% of patients in the KCNJ5-group but only 27% without KCNJ5 mutation had abnormal GAD-7 score. In line with these findings patients with KCNJ5 mutation showed significantly more pronounced decrease at one year (p= 0.032), two-year (p= 0.036) and three-year follow-up (p=0.043) in GAD-7 compared with patients without detection of KCNJ5 mutation (Figures 3+4). The association between the decrease in GAD-7 and the presence of KCNJ5 mutation was independent of differences in sex distribution according to linear regression analysis (p= 0.023).

Discussion

This is to our knowledge the first study that investigates a long-term follow-up for depressive symptoms and anxiety in patients with PA in response to treatment. We could confirm former results of short-time studies showing patients with PA presenting more symptoms of depression and anxiety, and one year of starting a specific treatment, patients after ADX showing a significant reduction of depressive symptoms, whereas MRA treatment led to a significant decrease in anxiety scores (Murck et al., 2021).

In the present study we found similar results in a smaller sample over a longer period. Patients after ADX continuously improved in their depression scores over the three years period. Scores for anxiety tended to be higher in the first year but could significantly improve after three years. MRA treated patients improved over time in anxiety scores, but still showed high scores for depressive symptoms above the cut-off after three years of follow up. This supports our former data that MR-antagonists are able to reduce anxiety independently from aldosterone concentration. We confirmed that normalization of aldosterone levels could mainly have a positive influence on symptoms of depression. This points out to different regulatory pathways for depression and anxiety.

Of special interest is a subgroup of patients carrying the KCNJ5 mutation, mainly females. They were compared to a group of patients, in whom genotyping of adrenal tumors was performed but KCNJ5-mutation could not be detected. This group was comparable for baseline parameters such as age, BMI as well as plasma aldosterone and renin levels but consisted only of males (Tables 3+4). Both groups are rather small, but they may offer interesting insights. Patients carrying KCNJ5 mutation had the highest scores for depression and anxiety but responded favourably for both symptoms after ADX, an effect that was even significantly more pronounced than in patients without KCNJ5 mutation. This data might suggest that the presence of KCNJ5 mutation t be a factor for the peculiarity of psychopathological symptoms. This could be in line with somatic findings, reporting that the KCNJ5 mutation is responsible for a pronounced hypertensive symptomatology in patients with PA (Fernandes-Rosa et al., 2014; Holler et al., 2019). A recent study found these patients to have less abdominal fat and metabolic disorders (Chen et al., 2021). Interestingly patients with KCNJ5 are known to show a specific steroid pattern, with higher levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol. Earlier studies suggest 18-oxocortisol secretion to be under control of ACTH and the renin-angiotensin system (Gomez-Sanchez, Zager, Foecking, Holland, & Ganguly, 1989; Yamakita et al., 1993). In Cushing´s disease, which has a big impact on psychiatric symptoms, these steroids were elevated, too (Ueshiba, Shimojo, & Miyachi, 1997). As our patients with KCNJ5 mutation are more affected by anxiety and depression at baseline we encourage the idea that these steroids might have a central effect. 11betaHSD2 might play a role in this hypothesis as in the absence of 11betaHSD2, which inactivates cortisol to cortisone, cortisol is the main ligand of the MR. Interestingly, Hellal-Levy et al. 1999 (Hellal-Levy et al., 1999) showed in their study an almost equal affinity of 18-oxocortisol at the MR and GR in contrast to 18-hydroxycortisol, which can be transformed to 18-oxocortisol by 11betaHSD2. The

work of Williams et al. 2016 (Williams et al., 2016) about the steroid profile of patients with hyperaldosteronism featuring KCNJ5 mutation suggested a stimulation of the MR by 18-oxocortisol, although the potency of stimulating the MR is lower than the potency of aldosterone. The activation of the MR by aldosterone and 18-oxocortisol seems to add to the effect of aldosterone to induce symptoms of anxiety and depression in patients featuring KCNJ5 mutation. 18-oxocortisol might not be affected by the 11betaHSD2, that is responsible for the deactivation of cortisol in certain brain areas as the NTS, a nucleus that is related to a number of physiological changes, which links this system to the pathophysiology of depression (Buttner et al., 2015; Murck, Buttner, Kircher, & Konrad, 2014; Murck, Ploch, & Montgomery, 2018) This, however, assumes a co-activation of the MR by 18-oxocortisol and aldosterone. As 18-hydroxycortisol can be transformed to 18-oxocortisol by the 11betaHSD2, that could additionally intensify the effect.

Of interest in this context might be a subgroup of patients with additional cortisol co-secretion (Arlt et al., 2017). These patients were not found to differ significantly concerning symptoms of anxiety and depression from patients without cortisol co-secretion, but they responded more favorable to specific treatment (Gendreitzig et al., 2021). Again, females with cortisol co-secretion were found to have significantly higher scores of anxiety and depression compared to females without cortisol co-secretion (Gendreitzig et al., 2021). Unfortunately, we do not have the information about cortisol co-secretion in all patients with mutations, but patients in the KCNJ5 group had numerically higher mean values of cortisol in the DST (3.5 vs. 2.0, p=0.229; n=7) and they show a similar psychopathological pattern as described by Gendreitzig et al 2021 (Gendreitzig et al., 2021).

Somatic parameters normalized as expected in both treatment groups. Patients were sufficiently controlled for blood pressure and electrolytes and parameters were normalized after one year of specific treatment. As expected, markers of lipid metabolism were slightly increased

after treatment, which is in accordance with our previous findings probably due to changes in renal function or change of medication (Adolf, Berends, Connelly, Reincke, & Dullaart, 2020) (Adolf et al., 2016) . Interestingly, lipid and glucose profile in both treatment groups remained significantly improved in women compared to men. Of special interest in the subgroup of patients with mutations is that males show – despite a comparable BMI – a worse lipid and glucose pattern with significantly higher LDL-cholesterol ($p= 0.019$) and plasma glucose ($p< 0.001$) and significantly lower HDL-cholesterol levels ($p=0.035$) and additionally do not respond as favourably as women, who are initially more severely affected by symptoms of depression and anxiety. But they show a better response in psychopathology and, as expected for the mutation, had a better metabolic profile (Chen et al., 2021).

To our knowledge this is the first study describing a 3-years follow up on anxiety and depression in patients with PA. Although the sample size is small, all patients are well characterized. Both treatments could show their effectiveness in PA and its related symptoms with different key psychiatric aspects. We confirm the hypothesis of different regulatory pathways for depressive symptoms and anxiety mediated by the MR. Also, we suggest a pathophysiological role for 18-hydroxycortisol and 18-oxocortisol, which might influence psychopathological symptoms by modulation of the MR. Therefore, patients with KCNJ5 mutation, mainly females, should be initially monitored more intensively for symptoms of anxiety and depression as this is related to their quality of life. Males should be monitored in the long term for metabolic symptoms and their psychopathological status.

Acknowledgements

This work was supported by the Else Kröner-Fresenius Stiftung in support of the German Conn's Registry-Else-Kröner Hyperaldosteronism Registry (2013_A182, 2015_A171 and 2019_A104 to MR), the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 694913 to MR), by the Deutsche Forschungsgemeinschaft (DFG) (within the CRC/Transregio 205/1 "The Adrenal: Central Relay in Health and Disease" to CA, DH, HS and MR).

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Table Legends

All Tables

GAD: cut-off = 5

PHQD: cut-off = 5

Table 1: Baseline and follow-up characteristics of patients with primary aldosteronism undergoing MRA treatment.

Data are given as mean ± standard deviation.

†: Due to incomplete data GAD-7 (n=68), PHQD (n=67), plasma glucose (n=67) and Cortisol after DST (n= 39) were performed with a reduced number of patients as listed in brackets.

Abbreviations: DBP: diastolic blood pressure; DDD: defined daily doses; DST: dexamethasone suppression test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Table 2: Baseline and follow-up characteristics of patients with primary aldosteronism undergoing ADX

Data are given as mean ± standard deviation.

†: Due to incomplete data GAD-7 (n=45), plasma glucose (n=47), HDL-Cholesterol and LDL-Cholesterol (n=46), triglycerides (n=46) and cortisol after DST (n= 18) were performed with a reduced number of patients as listed in brackets.

Abbreviations: ADX: Adrenalectomy; DBP: diastolic blood pressure; DDD: defined daily doses; DST: dexamethasone suppression test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Table 3a: Baseline and follow-up characteristics of patients with primary aldosteronism undergoing ADX without KNJC5 mutation.

Data are given as mean ± standard deviation.

†: Due to incomplete data plasma glucose (n=14) HDL-Cholesterol and LDL-Cholesterol (n=13) were performed with a reduced number of patients as listed in brackets.

Abbreviations: DBP: diastolic blood pressure; DDD: defined daily doses; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Table 3b: Baseline and follow-up characteristics of patients with primary aldosteronism undergoing ADX with KNJC5 mutation.

Data are given as mean ± standard deviation.

†: Due to incomplete data GAD-7 (n=11) was performed with a reduced number of patients as listed in brackets.

Abbreviations: DBP: diastolic blood pressure; DDD: defined daily doses; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Figure Legends

Figure 1: **Course of GAD-7 score in patients treated either by MRA or ADX.**

Mean and 95 per cent confidence interval of GAD-7 are shown.

Abbreviations: ADX: adrenalectomy; MRA: mineralocorticoid receptor antagonist treatment.

Figure 2: **Course of PHQD score in patients treated either by MRA or ADX.**

Mean and 95 per cent confidence interval of PHQD are shown.

Abbreviations: ADX: adrenalectomy; MRA: mineralocorticoid receptor antagonist treatment.

Figure 3: **Course of PHQD score in patients treated by ADX according to presence of KCNJ5 mutation.**

Mean and 95 per cent confidence interval of PHQD are shown.

Figure 4: **Course of GAD-7 score in patients treated by ADX according to presence of KCNJ5 mutation.**

Mean and 95 per cent confidence interval of GAD-7 are shown.

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Table 1:

Patient characteristics	**MRA cohort** (n=70)						
	Before treatment initiation	**Follow-up after one year**	**Follow-up after two years**	**Follow-up after three years**	**p (1 year)**	**p (2 years)**	**p (3 years)**
Age [years]	52 ± 11	--	--	--	--	--	--
Sex [f/m]	25/45	--	--	--	--	--	--
Duration of hypertension [months]	135 ± 111	--	--	--	--	--	--
BMI [kg/m²]	27.1 ± 4.7	27.2 ± 4.7	27.2 ± 4.7	27.3 ± 4.9	0.724	0.488	0.279
Cortisol after DST [µg/dl] †	1.9 ± 1.7	--	--	--	--	--	--
Late night salivatory cortisol [ng/ml] †	1.2 ± 0.7	--	--	--	--	--	--
Plasma aldosterone [ng/l]	181 ± 146	280 ± 211	299 ± 248	303 ± 190	<0.001	<0.001	<0.001
Plasma renin concentration [mU/l]	6.3 ± 5.4	30 ± 61	38 ± 75	60 ± 164	<0.001	<0.001	<0.001
Antihypertensive agents [DDD]	2.0 ± 1.8	2.8 ± 2.7	2.7 ± 3.0	2.8 ± 2.6	0.003	0.101	0.003
SBP [mmHg]	149 ± 20	131 ± 14	131 ± 20	129 ± 19	<0.001	<0.001	<0.001
DBP [mmHg]	93 ± 11	86 ± 9	86 ± 12	86 ± 12	<0.001	<0.001	<0.001
Serum sodium [mmol/l]	141 ± 3	139 ±2	139 ± 3	140 ± 2	0.003	0.002	0.038
Serum potassium [mmol/l]	3.7 ± 0.4	4.2 ± 0.3	4.3 ± 0.4	4.4 ± 0.4	<0.001	<0.001	<0.001
Plasma glucose [mg/dl] †	100 ± 15	104 ± 26	105 ± 24	105 ± 21	0.151	0.087	0.115
HDL-Cholesterol [mg/dl]	61 ± 17	57 ± 17	57 ± 15	58 ± 16	0.001	0.001	0.020
LDL-Cholesterol [mg/dl]	117 ± 30	119 ± 31	116 ± 31	115 ± 32	0.143	0.564	0.453
Triglycerides [mg/dl]	105 ± 46	124 ± 56	123 ±79	132 ± 77	0.001	0.112	0.002
GAD-7 †	5.1 ± 4.2	4.1 ± 4.1	4.0 ± 3.3	4.2 ± 3.6	0.001	0.028	0.013
PHQD †	6.3 ± 5.5	4.8 ± 4.5	5.4 ± 5.2	5.2 ± 4.6	0.002	0.197	0.042

Data are given as mean ± standard deviation.

†: Due to incomplete data GAD-7 (n=68), PHQD (n=67), plasma glucose (n=67), late night salivatory cortisol (n= 50) and cortisol after DST (n= 39) were performed with a reduced number of patients as listed in brackets.

Abbreviations: DBP: diastolic blood pressure; DDD: defined daily doses; DST: dexamethasone suppression test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Table 2:

Patient characteristics	ADX cohort (n=48)						
	Before treatment initiation	**Follow-up after one year**	**Follow-up after two years**	**Follow-up after three years**	**p (1 year)**	**p (2 years)**	**p (3 years)**
Age [years]	51 ± 11	--	--	--	--	--	--
Sex [f/m]	20/28	--	--	--	--	--	--
Duration of hypertension [months]	136 ± 134	--	--	--	--	--	--
BMI [kg/m²]	28.4 ± 5.2	28.2 ± 4.6	28.7 ± 5.1	28..5 ± 4.8	0.814	0.150	0.165
Cortisol after DST [µg/dl] †	2.0 ± 1.4	--	--	--	--	--	--
Late night salivatory cortisol [ng/ml] †	1.5 ± 1.0	--	--	--	--	--	--
Plasma aldosterone [ng/l]	268 ± 152	72 ± 54	89 ± 55	100 ± 62	<0.001	<0.001	<0.001
Plasma renin concentration [mU/l]	6.5 ± 7.1	38 ± 73	43 ± 81	37 ± 60	<0.001	<0.001	<0.001
Antihypertensive agents [DDD]	3.0 ± 2.4	2.0 ± 2.5	1.8 ± 1.9	1.8 ± 2.1	0.009	0.005	0.001
SBP [mmHg]	150 ± 21	132 ± 15	131 ±15	131 ± 17	<0.001	<0.001	<0.001
DBP [mmHg]	94 ± 12	87 ± 11	87 ± 9	87 ±10	<0.001	<0.001	<0.001
Serum sodium [mmol/l]	141 ± 2.6	139 ±3	139 ± 2	140 ± 2	<0.001	<0.001	0.127
Serum potassium [mmol/l]	3.3 ± 0.4	4.2 ±0.4	4.3 ± 0.4	4.3 ± 0.4	<0.001	<0.001	<0.001
Plasma glucose [mg/dl] †	104 ± 20	99 ± 14	101 ± 17	98 ± 27	0.045	0.167	0.635
HDL-Cholesterol [mg/dl] †	61 ± 14	56 ± 15	57 ± 15	56 ± 15	<0.001	0.004	0.001
LDL-Cholesterol [mg/dl] †	122 ± 37	118 ± 38	116 ± 36	114 ± 44	0.926	0.611	0.220
Triglycerides [mg/dl] †	91 ± 38	118 ± 53	120 ± 58	120 ± 55	<0.001	<0.001	<0.001
GAD-7 †	4.9 ± 3.8	5.4 ± 4.6	4.9 ± 4.1	3.8 ±3.3	0.362	0.896	0.006
PHQD †	7.9 ± 6.2	6.4 ± 5.8	5.6 ± 5.1	4.7 ±3.9	0.039	0.005	<0.001

Data are given as mean ± standard deviation.

†: Due to incomplete data GAD-7 (n=45), plasma glucose (n=47), HDL-cholesterol and LDL-cholesterol (n=46), triglycerides (n=46), late night salivatory cortisol (n=22) and cortisol after DST (n= 18) were performed with a reduced number of patients as listed in brackets.

Abbreviations: ADX: Adrenalectomy; DBP: diastolic blood pressure; DDD: defined daily doses; DST: dexamethasone suppression test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Table 3a:

Patient characteristics	**ADX cohort without KCNJ5 mutation** (n=15)						
	Before treatment initiation	**Follow-up after one year**	**Follow-up after two years**	**Follow-up after three years**	**p (1 year)**	**p (2 years)**	**p (3 years)**
Age [years]	54 ± 7	--	--	--	--	--	--
Sex [f/m]	0/15	--	--	--	--	--	--
Duration of hypertension [months]	140 ± 173	--	--	--	--	--	--
BMI [kg/m²]	29.0 ± 2.9	29.2. ± 2.9	29.4± 3.1	29.0 ± 2.9	0.530	0.117	0.532
Cortisol after DST [µg/dl] †	2.0 ± 1.5	--	--	--	--	--	--
Late night salivatory cortisol [ng/ml] †	2.6 ± 1.4	--	--	--	--	--	--
Plasma aldosterone [ng/l]	283 ± 170	55 ± 23	80 ± 25	82 ±45	0.001	0.001	0.001
Plasma renin concentration [mU/l]	7.6 ±7.7	63.1 ±110.3	81.6 ± 83.5	57.5 ± 71.0	0.002	0.001	0.001
Antihypertensive agents [DDD]	3.5 ±2.5	2.9 ± 2.9	2.5 ± 2.6	2.7 ±2.4	0.426	0.140	0.163
SBP [mmHg]	162 ± 26	137 ± 10	132 ± 13	134 ± 19	0.003	0.002	0.003
DBP [mmHg]	96 ±13	89 ±7	88 ± 6	87 ± 10	0.030	0.024	0.010
Serum potassium [mmol/l]	3.3 ± 0.2	4.3 ± 0.5	4.2 ± 0.3	4.2 ±0.4	0.001	0.001	0.001
Plasma glucose [mg/dl] †	113 ±21	102 ± 16	110 ± 17	94 ± 43	0.022	0.441	0.208
HDL-Cholesterol [mg/dl]	53 ±10	45 ± 12	46 ± 14	46 ± 11	0.013	0.019	0.004
LDL-Cholesterol [mg/dl]	135 ±28	137 ± 33	128 ± 36	122 ±51	0.701	0.432	0.182
Triglycerides [mg/dl]	108 ±39	150 ± 58	160 ± 64	157 ± 77	0.028	0.014	0.033
GAD-7 †	4.0 ±3.0	5.3 ± 3.2	4.5 ±2.5	3.9 ± 2.0	0.195	0.240	>0.999
PHQD †	5.1. ± 2.5	5.1 ±3.2	5.3 ± 3.5	3.7 ± 2.4	0.821	0.873	0.040

Data are given as mean ± standard deviation.

†: Due to incomplete data plasma glucose (n=14) HDL-cholesterol and LDL-cholesterol (n=13), late night salivatory cortisol (n=4) and cortisol after DST (n=4) were performed with a reduced number of patients as listed in brackets.

Abbreviations: DBP: diastolic blood pressure; DDD: defined daily doses; DST: dexamethasone suppression test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Table 3b:

Patient characteristics	ADX cohort with KCNJ5 mutation (n=12)						
	Before treatment initiation	**Follow-up after one year**	**Follow-up after two years**	**Follow-up after three years**	**p (1 year)**	**p (2 years)**	**p (3 years)**
Age [years]	49 ± 10	--	--	--	--	--	--
Sex [f/m]	10/2	--	--	--	--	--	--
Duration of hypertension [months]	163 ±144	--	--	--	--	--	--
BMI [kg/m²]	27.4 ±5.0	27.5 ± 4.9	28.2 ±4.9	28.2 ± 4.8	0.508	0.239	0.071
Cortisol after DST [µg/dl] †	3.5 ± 2.1	--	--	--	--	--	--
Late night salivatory cortisol [ng/ml] †	1.8 ± 1.2	--	--	--	--	--	--
Plasma aldosterone [ng/l]	281 ±162	67 ± 47	83 ± 70	115 ± 60	0.003	0.012	0.012
Plasma renin concentration [mU/l]	7.5 ± 8.5	31.5 ± 45.7	31.3 ± 62.4	42.5 ± 81.2	0.015	0.084	0.041
Antihypertensive agents [DDD]	2.7 ± 3.2	0.6 ± 1.2	1.9 ± 1.7	0.5 ± 1.0	0.012	0.789	0.012
SBP [mmHg]	139 ± 16	121 ±18	126 ±17	127 ± 22	0.011	0.034	0.023
DBP [mmHg]	90 ±10	81 ± 11	87 ±13	85 ± 13	0.031	0.346	0.146
Serum potassium [mmol/l]	3.2 ± 0.3	4.1 ±0.3	4.3 ± 0.4	4.4 ± 0.3	0.002	0.002	0.002
Plasma glucose [mg/dl]	91 ± 7	97 ±9	93 ± 9	97 ± 9	0.168	0.755	0.015
HDL-Cholesterol [mg/dl]	67 ± 18	64 ± 15	64 ± 14	65 ± 16	0.529	0.272	0.455
LDL-Cholesterol [mg/dl]	100 ± 39	111 ± 30	119 ± 38	118 ±46	0.346	0.041	0.209
Triglycerides [mg/dl]	81 ± 30	102 ±44	111 ±50	119 ± 42	0.055	0.055	0.006
GAD-7 †	5.8 ± 5.3	4.0 ± 3.3	3.8 ±2.7	3.9 ±4.8	0.079	0.123	0.046
PHQD	8.8 ± 6.1	5.3 ±4.7	4.7 ± 4.3	4.5 ±2.8	0.097	0.029	0.041

Data are given as mean ± standard deviation.

†: Due to incomplete data GAD-7 (n=11), late night salivatory cortisol (n=3) and cortisol after DST (n=3) were performed with a reduced number of patients as listed in brackets.

Abbreviations: DBP: diastolic blood pressure; DDD: defined daily doses; DST: dexamethasone suppression test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MRA: mineralocorticoid receptor antagonist; SBP: systolic blood pressure.

Figure 1

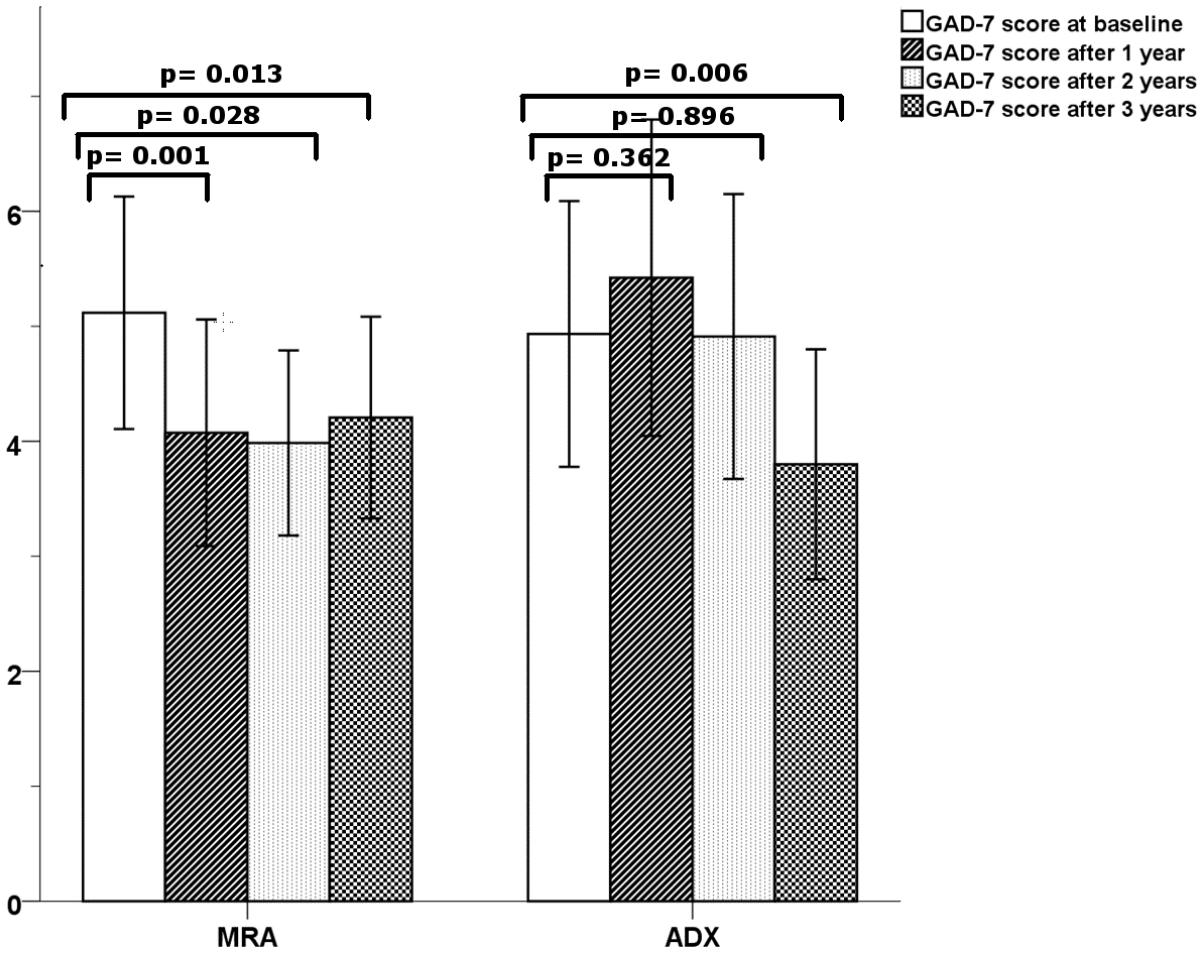

GAD-7 score at baseline
GAD-7 score after 1 year
GAD-7 score after 2 years
GAD-7 score after 3 years
p= 0.013
p= 0.028
p= 0.001
p= 0.006
p= 0.896
p= 0.362
6
4
2
0
MRA
ADX

Figure 2

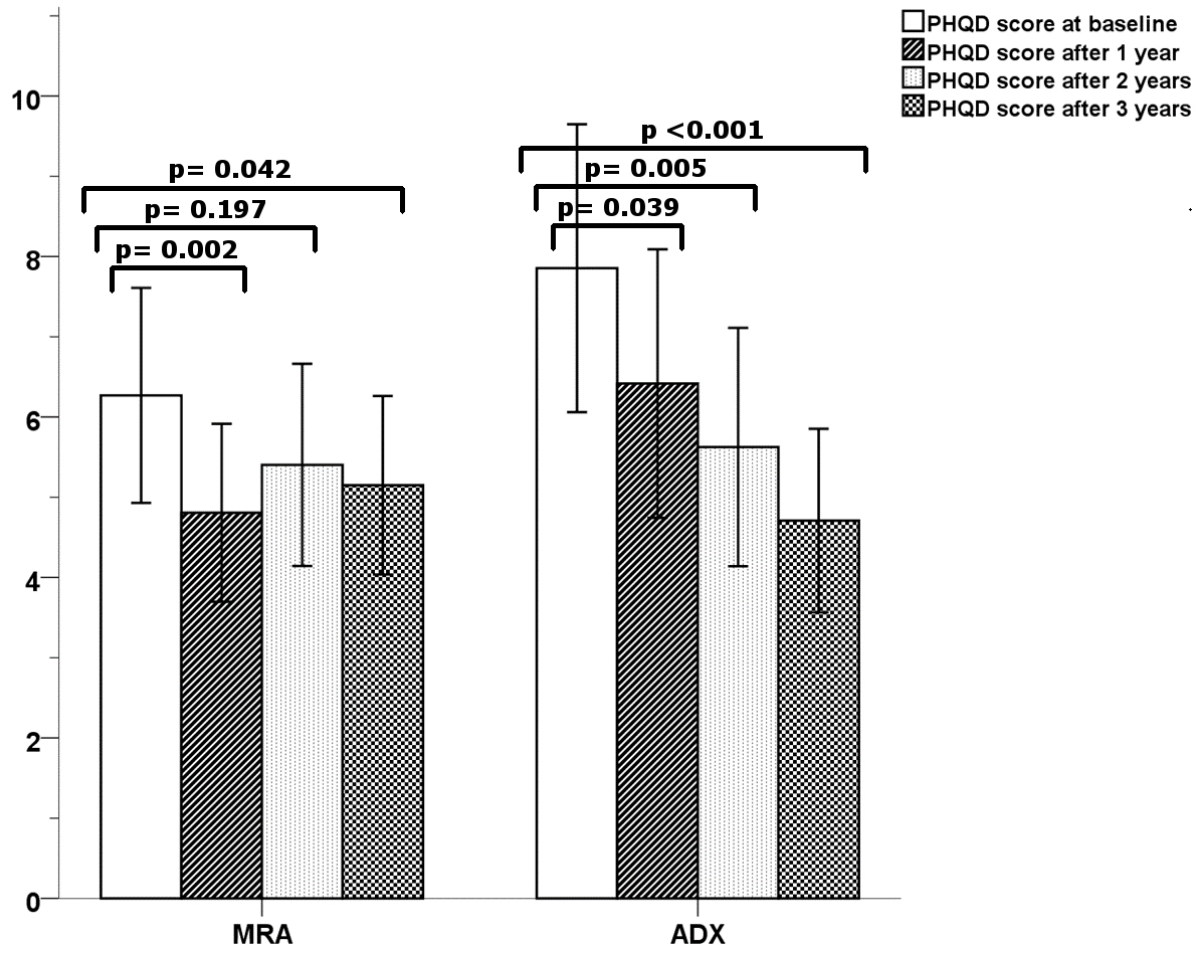

PHQD score at baseline
PHQD score after 1 year
PHQD score after 2 years
PHQD score after 3 years
10
8
6
4
2
0
p= 0.042
p= 0.197
p= 0.002
p <0.001
p= 0.005
p= 0.039
MRA
ADX

Figure 3

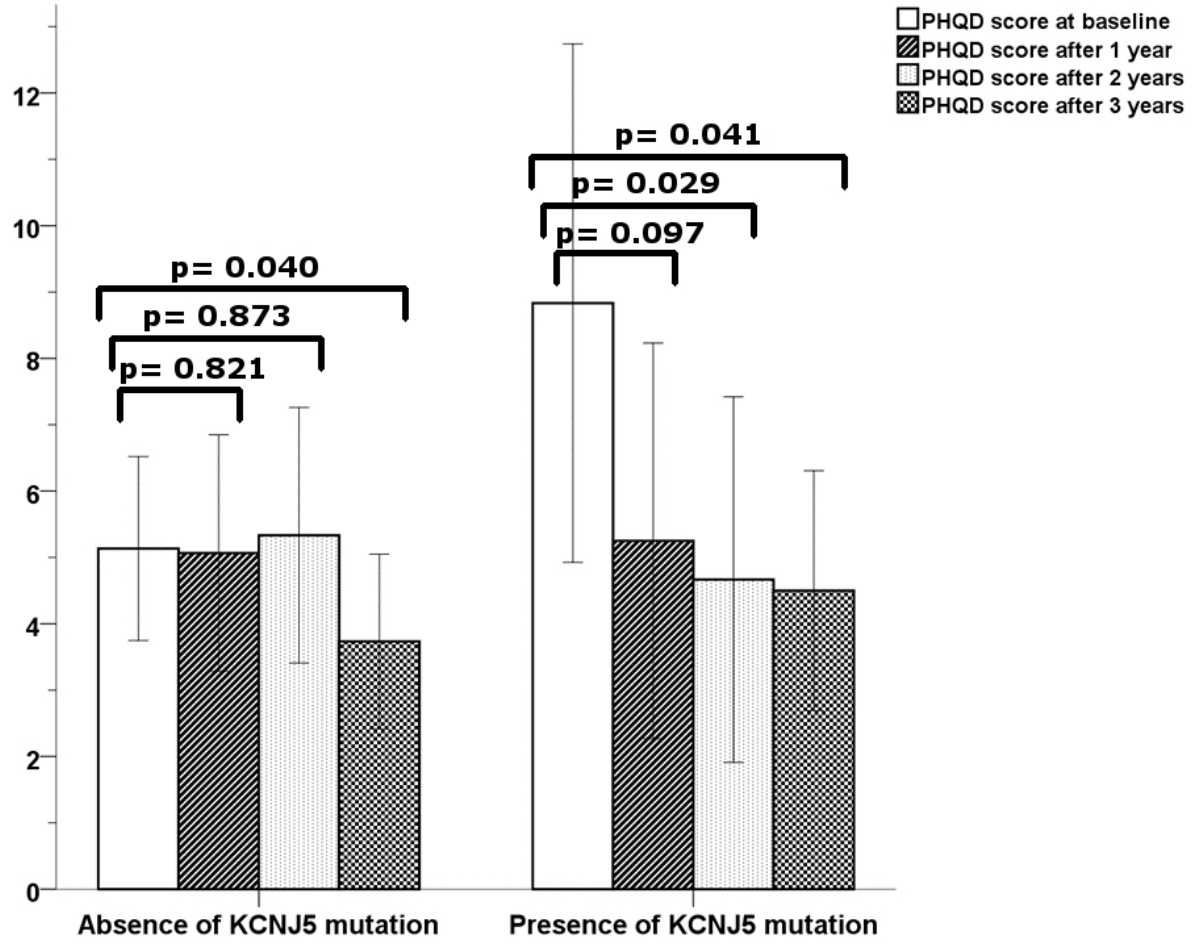
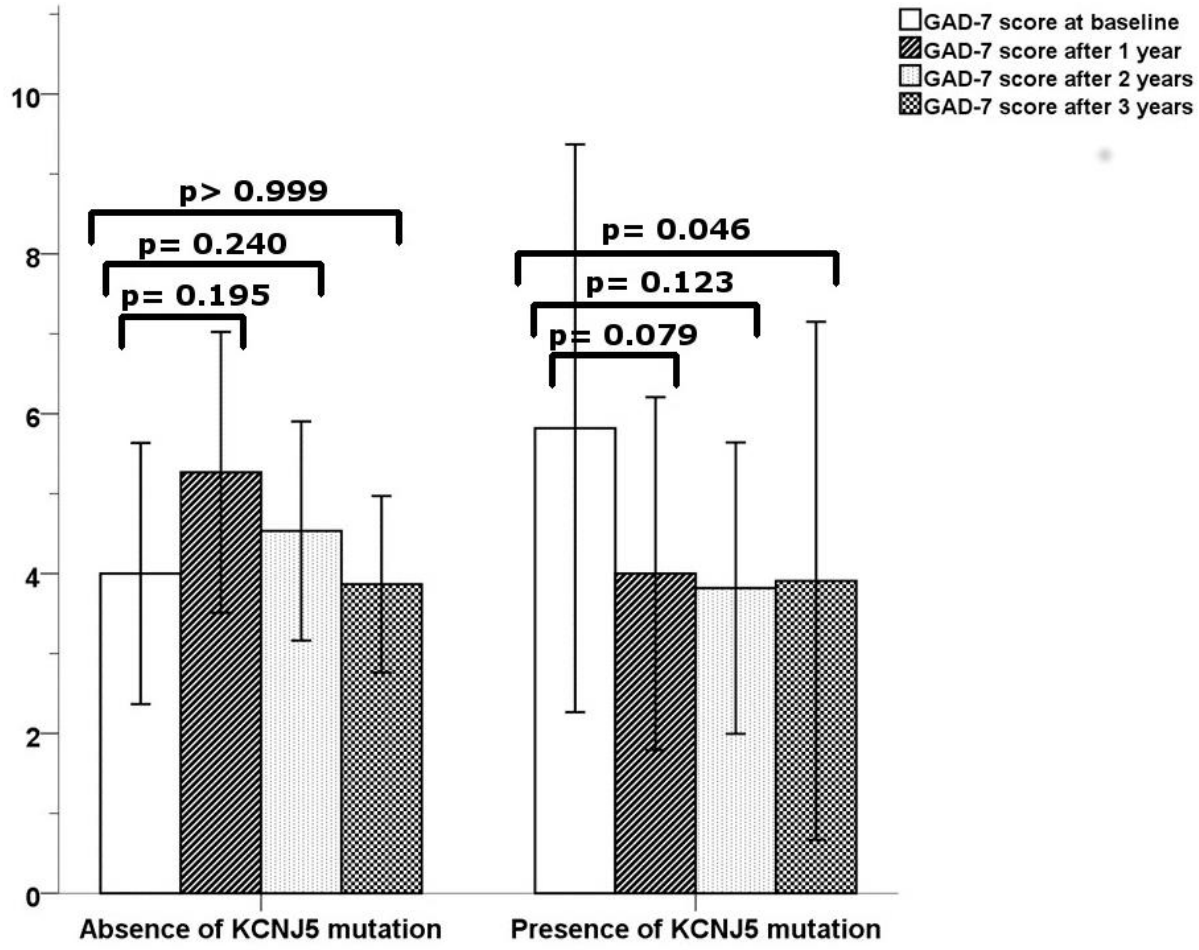

PHQD score at baseline
PHQD score after 1 year
PHQD score after 2 years
PHQD score after 3 years
12
10
8
6
4
2
0
p= 0.040
p= 0.873
p= 0.821
p= 0.041
p= 0.029
p= 0.097
Absence of KCNJ5 mutation
Presence of KCNJ5 mutation

Figure 4


GAD-7 score at baseline
GAD-7 score after 1 year
GAD-7 score after 2 years
GAD-7 score after 3 years
10
8
6
4
2
0
p> 0.999
p= 0.240
p= 0.195
p= 0.046
p= 0.123
p= 0.079
Absence of KCNJ5 mutation
Presence of KCNJ5 mutation