

Is *FKBP5* a genetic marker of depression? A case-control study and analysis of disease related traits.

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Abstract

Background

A dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been proposed as an important pathogenic factor in depression. Genetic variants of *FKBP5*, a protein of the HPA system modulating the glucocorticoid receptor, have been reported to be genetically associated with improved response to medical treatment and an increase of depressive episodes.

Methods

We examined three single nucleotide polymorphisms (SNPs) in *FKBP5*, rs4713916 in the proposed promoter region, rs1360780 in the second intron and rs3800373 in the 3'-untranslated region (3'-UTR), in a case-control study of Caucasian origin (affective psychosis: n= 248; controls: n= 188) for genetic association and association with disease related traits.

Results

Allele and genotype frequencies of rs4713916, rs1360780 and rs3800373 were not significantly different between cases and controls. Two haplotypes, G-C-T and A-T-G, accounted for 86.2% in controls. Odds ratios were not increased between cases and controls, except the rare haplotype G-C-G (OR 6.81), representing 2.1% of cases and 0.3% of controls. The frequency of rs4713916AG in patients deviated from expected Hardy-Weinberg equilibrium, the genotype AA at rs4713916 in monopolar depression (P= 0.011), and the two-locus haplotype rs1360780T - rs3800373T in the total sample (overall P= 0.045) were associated with short duration of disease.

Conclusion

Evidence for an association of *FKBP5* polymorphisms to affective psychosis is weak and challenges *FKBP5* as a genetic marker for depression. In summary, our data do not support a significant genetic contribution of *FKBP5* to affective psychosis in the analysed markers, and the findings are inconclusive regarding putative risk haplotypes or association with disease-related traits.

Background

Collaterally to a complex dysregulation of neurotransmission, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis has been proposed as an essential pathogenic factor in depressive disorders [1]. As a predictor of clinical improvement normalisation of the HPA-system is one of the basic findings [2] as well as a partial cortisol resistance among depressive patients resulting in an impaired negative feedback and elevated cortisol levels [1]. In search for the genetic and functional mechanisms underlying the HPA-dysfunction in depression, recent research focused on genes involved in HPA-axis regulation, such as the genes for corticotropin-releasing hormone or the glucocorticoid receptor (GR) with several cochaperones. Among the latest *FKBP5* gained growing interest.

FKBP5, synonymous to *FKBP51* and *FKBP54*, is located on chromosome 6p21, a chromosomal region associated with bipolar disorder and psychosis [3] and consists of 10 exons spanning ~156kb (UCSC Genome Browser, release August 2004). *FKBP5* is mainly expressed in brain, and in a wide range of human cell tissue, including muscle, liver, and thymus. Functionally, cortisol induces the *FKBP5* expression by glucocorticoid-response-elements in human lymphocytes [4]. The protein of 457 amino acids is composed of three domains, two immunosuppressants binding sites and a tetratricopeptide-repeat domain [5]. *FKBP5* reduces the receptor affinity for cortisol by a complex interaction with the mature hetero-oligomeric glucocorticoid receptor complex and is thought to diminish the dynein binding and nuclear translocation of the glucocorticoid-receptor complex [6]. In addition, *FKBP5* has been associated with glucocorticoid-resistance in new world monkeys which results from both expression of GRs that are less responsive and overexpression of *FKBP5* that further reduces GR responsiveness [7].

In a mutation scan aiming to detect genetic equivalents of HPA-dysregulation among the cochaperon gene cluster in depression, thirty single-nucleotide-polymorphisms (SNPs) were detected at the *FKBP5* locus, but no overall genetic association with disease was traceable in a panel of 317 patients with depression [8]. Three SNPs, namely rs4713916 in the putative promoter region, rs1360780 in the second intron, and rs3800373 in the 3'-UTR region, however, were found associated with improved response to antidepressant treatment. Patients with the rs1360780TT genotype had more than twice as many depressive episodes in the past. In lymphocyte cell-culture studies FKBP5 levels were increased for the risk TT-genotype, and the correlation of plasma cortisol and FKBP5 mRNA level was interpreted as a firm control of the HPA-axis by FKBP5 [8].

Given these genetic and clinical findings, we tried to replicate the association of the FKBP5 gene locus with depression and disease-related variables in a case-control study of probands with similar ethnic background.

Methods

The sample encompassed 248 cases (154 males) with recurrent depression and bipolar disorder according to ICD10 [9]. These patients also fulfilled the more restricted criteria of monopolar depression (n = 57), and manic depression (n = 191; Table1) in differentiated psychopathology [10]. Diagnosis in differentiated psychopathology was made by repeated personal examinations of a single investigator (GS). The probands were recruited from 1996 to 2005 at the Department of Psychiatry and Psychotherapy at of the University of Würzburg. The 188 volunteer control subjects (105 males) were recruited from the blood donor centre at the University of Würzburg. All subjects were unrelated and of German Caucasian descent. The Ethics Committee of the University of Würzburg had approved the study, and informed consent was obtained from all subjects.

PCR for allelic discrimination was performed in a final reaction volume of 20 μ l containing 20ng genomic DNA and 10 μ l of 2 \times TaqMan[®] Universal PCR Master Mix (Applied Biosystems) and 1 μ l of 20 \times TaqMan[™] SNP genotyping assay including fluorescent tags specific for the wild type allele and the variant allele. Marker amplification was performed in microtiter plates on Biometra machines (Whatman). PCR amplification conditions were according to the manufacturer's recommendation [10 min at 95°C followed by 15 sec at 92°C and 60 sec at 60°C for 40 cycles]. Allelic discrimination with endpoint detection of fluorescence was performed at 60°C on an ABI prism 7000 sequence detection system followed by analysis with an appropriate software package (Applied Biosystems).

Fisher's exact test and Armitage's trend test were used to compare allelic and genotypic distributions between cases and controls. Haplotype frequencies and global association were calculated with the program FAMHAP [11], which does not specify a confidence interval for Odds ratios. The exact test proposed by Weir [12] was applied for Hardy-Weinberg equilibrium (HWE). For quantitative traits, we performed the Kruskal-Wallis test and analysis of variance (ANOVA) for single marker analyses, and for haplotypes by using haplotype trend regression (HTR) [13]. With the endpoint in 2005, one of us assessed the clinical data by chart analysis blind to genotyping (Table 1). Age at onset was defined as age at first contact with psychiatric service, duration of disease was measured by the period of time between first hospitalisation and age at recruitment. The number and period of in-patient treatments was assessed for all hospitalisations for which medical charts were available. The preponderance of males is reflecting the recruitment procedure on wards for acutely admitted males, and does not necessarily point towards gender differences in affective psychoses.

Results

Allele and genotype frequencies of rs4713916, rs1360780 and rs3800373 were not significantly different between cases and controls (Table 2). We observed neither gender differences nor differences in the clinical subgroups of monopolar affective psychosis and manic-depression (data not shown). For rs4713916, the genotype distribution in cases deviated from HWE ($P=0.048$) due to an excess of heterozygous individuals. The standardized linkage disequilibrium (LD) among controls was $D' = 0.751$ for rs4713916 and rs1360780, $D' = 0.750$ for rs4713916 and rs3800373, and $D' = 0.971$ for rs1360780 and rs3800373. A case-control study with 248 cases and 188 controls had a power of 80% to detect (at $\alpha = 0.05$) an association with a susceptibility allele, under the assumption that the susceptibility allele has a population frequency of 0.3 and the effect of this allele is recessive with a relative risk of 2.8.

In the studied population we found two haplotypes, G-C-T and A-T-G, with a cumulated frequency of 86.2% in controls (Table 3). Odds ratios were not increased between cases and controls, except the rare haplotype G-C-G (OR 6.81) representing 2.1% of cases, and 0.3% of controls.

Analysis of FKBP5 genotypes and disease related variables produced only one positive association: in monopolar depression ($n= 57$) rs4713916AA was related to a short duration of disease ($P= 0.011$; without Bonferroni's correction). Although rs1360780 has been previously described as most significant marker [8], we observed no association of rs1360780 with disease variables (Figure 1), such as age at first hospitalisation (ANOVA $P= 0.98$; Kruskal-Wallis $P= 0.94$), number of in-patient treatments (ANOVA $P= 0.72$; Kruskal-Wallis $P= 0.53$) or total period

in hospital (ANOVA $P= 0.67$; Kruskal-Wallis $P= 0.49$). We found no association of three-locus haplotypes with clinical variables, neither in the sub-samples nor the combined group (data not shown). In the total sample the two-locus haplotype combination rs1360780T - rs3800373T was associated with duration of disease at overall p-value of 0.045.

Discussion

At the *FKBP5* locus allele and genotype frequencies of rs4713916, rs1360780 and rs3800373 were not significantly different between patients with affective psychosis and controls. The putative risk haplotype G-C-G occurred at a frequency of 2.1% among cases with Odds ratio of 6.4. Further findings were deviation of HWE among patients at rs4713916, an association of rs4713916 (genotype analysis) and rs1360780 - rs3800373 (two-locus haplotype analysis) with duration of disease. However, we failed to identify a clear-cut association of genetic markers with the clinical course of affective psychosis in the total sample, measured by age at onset, age of first hospitalisation, frequency and period of in-patient treatment.

We confirm the notion of Binder and colleagues [8], that none of the SNPs has a major effect on affective disorder, neither with monopolar depression nor with manic depression. Our sample size of 248 patients and the ethnic background was comparable to their study [8] with 317 cases of unipolar or bipolar depression with assessment for treatment response. Regarding the power to detect an association, we had a probability of 80% under the assumption that the susceptibility allele has a population frequency of 0.3 and the effect of this allele is recessive with a relative risk of 2.8 (at $\alpha = 0.05$). The main clinical findings by Binder and colleagues [8] were related to the rare homozygous genotype rs1360780TT, located in intron 2 of *FKBP5*. The frequency of 9.7% in the present sample was similar to the previous data of 9.1%. Despite this congruence, we found rs1360780TT not associated with an increased frequency of depressive episodes, although our sample had a mean follow-up history of >10 years and a mean number of five episodes requiring in-patient treatment. In this longitudinal approach we did unfortunately not evaluate treatment response of single depressive/manic episodes. However, frequency and duration of in-

patient treatment in relation to duration of the disease seem reliable and valuable items to assess the severity and the naturalistic course of affective psychosis.

Our findings at the *FKBP5* locus were complex and partially contradictorily. Unlike Binder and colleagues [8] we found weak evidence for involvement of rs4713916, located at the presumed promoter region in the vicinity of a putative glucocorticoid response element [4]. Genotype distribution for rs4713916 in patients deviated from HWE and a short duration of disease was associated with rs4713916AA in monopolar depression ($P= 0.011$). In contrast, we observed a risk haplotype rs4713916G - rs1360780C - rs3800373G not exceeding 2.1% of the sample and the two-locus haplotype rs1360780T - rs3800373T associated with a short duration of disease (overall $P= 0.045$). Thus, our results may be the effect of chance findings due to sample stratification, although haplotype and haplotype-related analyses were based on permutation-based tests. Given the differences of LD between the promoter region and distal areas, different genetic variants may be relevant at *FKBP5* in depression. Variants at the promoter region may influence the transcription of *FKBP5*, whereas other functional variants in downstream regions may act as independent risk factors in other cases [14]. Reflecting the complex phenomenology of depression, *FKBP5* may play a decisive role only in some cases of depression, and other genes involved in the glucocorticoid system are responsible for the disturbances of the HPA-axis during depressive episodes [15, 16]. *FKBP5* could be linked to several basic mechanisms of stress related phenomena, as polymorphisms in *FKBP5* were associated with peritraumatic dissociation in medically injured children [17].

In summary, our data do not support a significant genetic contribution of *FKBP5* to affective psychosis in the analysed markers, and the findings are inconclusive regarding putative risk

haplotypes or association with disease-related traits. For further scrutiny, the putative promoter region seems as promising as coding and functional intronic sequences of *FKBP5*.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MG carried out the molecular genetic studies and drafting of the manuscript, ME performed laboratory assays, SJ participated in the coordination of the study and MJ performed the chart analysis. MM and MK contributed the data-analysis, interpretation of the data and drafting of the manuscript, GS participated in the design and coordination of the study, interpretation of the data, and drafting of the manuscript. All authors read and approved the final manuscript.

Table 1: Demographic and disease related traits in the sample of probands with affective psychosis

	Monopolar (n=57)				Bipolar (n=191)				Total sample (n=248)					
	Male (n=32)		Female (n=25)		Male (n=122)		Female (n=69)		Male (n=154)		Female (n=94)		All cases (n= 248)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age at onset (years)	42.1	16.5	41.6	15.9	33	12.1	32.3	12.9	34.3	14.3	35.1	15.1	34.6	14.5
Age at first hospitalisation (years)	47.9	17.9	45.1	13.3	36.2	13.6	38.8	14.7	38.6	15.3	40.5	14.5	39.3	15
Age at assessment (years)	53.8	16.8	55.9	11.8	44.6	15.4	48.7	15.1	46.6	16.1	50.7	14.6	48.1	15.6
Period of time in hospital (weeks)	33.2	24.8	47.8	53.2	38.5	56.6	46.9	59	37.4	51.6	47.1	57.2	41.1	53.9
Duration of disease at assessment (years)	12.3	14.2	14.6	12	12.9	11.2	16.5	13.4	12.8	11.9	16	13	14	12.4
Suicide attempts (number)	0.4	0.6	0.48	1	0.8	1.7	0.8	1.6	0.7	1.5	0.7	1.5	0.7	1.5
In-patient treatments (number)	3.4	2.8	5.8	4.9	4.8	6.9	5.1	4.8	4.5	6.3	5.3	4.8	4.8	5.8

SD: Standard deviation

Table 2: Allele and genotype distribution of polymorphisms spanning the *FKBP5* locus

	Controls						Cases							
	Allele		Genotype				Allele		P-value ⁺	Genotype				
	1 (%)	2 (%)	11	12	22	HWE	1 (%)	2 (%)		11	12	22	HWE	P-value*
rs4713916 A/G	110 (29.3)	266 (70.7)	18	74	96	0.501	160 (32.3)	336 (67.7)	0.375	19	122	107	0.048	0.115
rs1360780 C/T	262 (69.7)	114 (30.3)	94	74	20	0.348	338 (68.2)	158 (31.8)	0.658	114	110	24	0.733	0.857
rs3800373 T/G	275 (73.1)	101 (26.9)	102	71	15	0.594	344 (69.4)	152 (30.6)	0.229	115	114	19	0.200	0.865

HWE: P-value of Weir's test for HWE

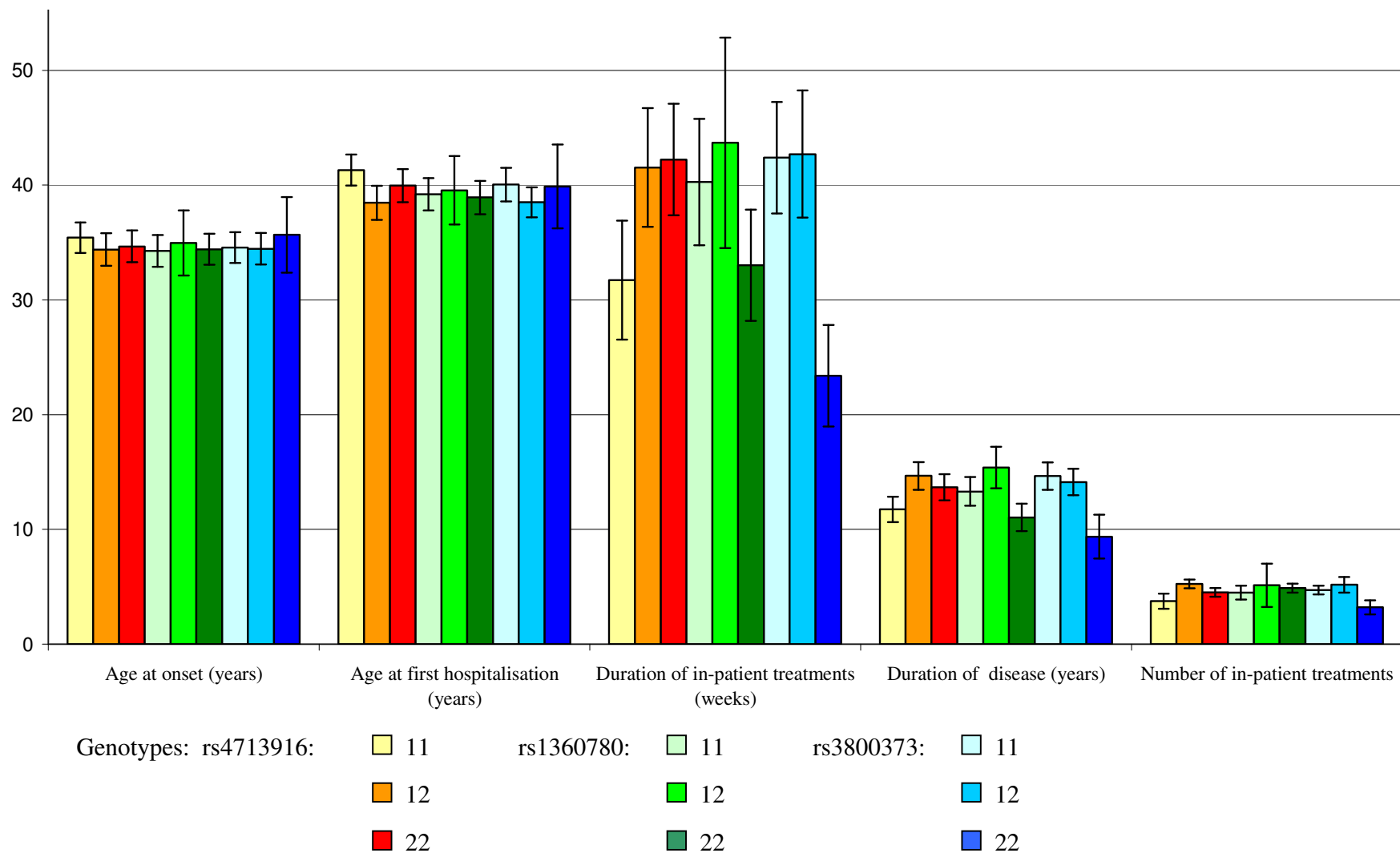
+ : P-value of Fisher's exact test for comparison of allele frequencies between cases and controls

* : P-value of Armitage's trend test for comparison of genotype frequencies between cases and controls

Table 3: Haplotype frequencies of markers at *FKBP5* calculated by FAMHAP [11]

rs4713916	rs1360780	rs3800373	Controls (%)	Cases (%)	Odds ratio
G	C	T	64.3	60.7	0.86
A	T	G	21.9	24.8	1.18
A	C	T	4.8	5.2	1.10
G	T	G	4.4	3.6	0.80
A	T	T	2.4	2.1	0.88
G	T	T	1.7	1.4	0.82
G	C	G	0.3	2.1	6.81
A	C	G	0.3	0.1	0.51

Figure 1: Genotype-specific mean values of clinical variables in affective psychosis for three SNPs in *FKBP5* (n=248).



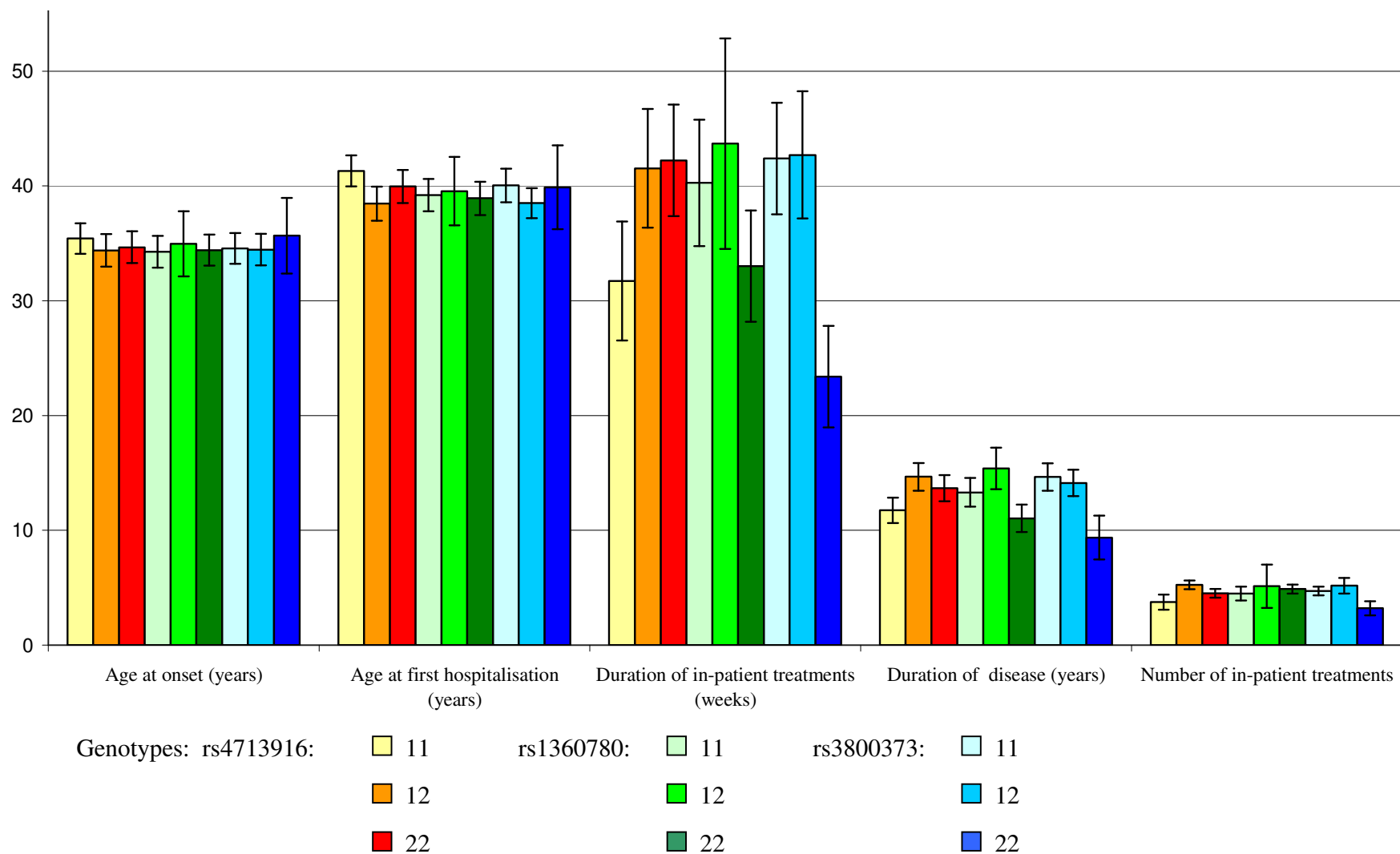
Using ANOVA and Kruskal-Wallis tests none of the markers reached statistical significance for association with disease-related variables of affective psychosis (I = Mean values including SD).

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Figure 1: Genotype-specific mean values of clinical variables in affective psychosis for three SNPs in *FKBP5* (n=248).



Using ANOVA and Kruskal-Wallis tests none of the markers reached statistical significance for association with disease-related variables of affective psychosis (I = Mean values including SD).