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Genetic Association Studies of the Chromosome 15 GABA-A Receptor Cluster in Migraine With Aura

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Recently, a novel susceptibility locus for migraine with aura (MA) on chromosome 15q containing three GABA-A receptor subunits has been identified by linkage analysis in several large pedigrees. To further study the role of this locus in MA etiology we genotyped 56 SNPs capturing the known common haplotype variations of these three candidate genes in a sample comprising 270 MA patients and 273 matched controls. In a single marker analysis, four SNPs displayed nominally significant ($P < 0.05$) association with MA. However, after permutation-based correction for the number of tests performed, the *P*-values of these SNPs were non-significant. Furthermore, a replication study of two of these SNPs in a second independent sample of 379 MA patients and 379 controls did not result in a significant finding. We also compared haplotype estimates based on case–control genotypes. Again we could not demonstrate a significant association with the phenotype after correction for multiple testing. In summary, we found no convincing evidence for an involvement of common SNPs at the GABA-A receptor cluster on 15q11-q12 in the pathophysiology of MA. **© 2007 Wiley-Liss, Inc.**

KEY WORDS: GABA-A receptor; migraine; association study

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INTRODUCTION

Migraine with aura (MA) is a multifactorial disease with a strong and largely unknown genetic component contributing to its etiology. In some families MA displays a seemingly autosomal dominant mode of inheritance, with multiple individuals from consecutive generations being affected and presenting with homogeneous clinical features. Recently, Russo et al. [2005] performed a linkage analysis in a series of such families from Italy and reported a new susceptibility locus for MA in the genomic region 15q11-q13 with a maximum LOD score of 5.56 obtained with a parametric two-point linkage analysis. This region contains three genes encoding different subunits of γ-aminobutyric acid (GABA)-A receptors (*GABRA5*, *GABRB3*, and *GABRG3*).

GABA is a major inhibitory neurotransmitter of the mammalian central nervous system. The pathophysiological concept of migraine as a result of CNS hyperexcitability as well as the use of GABAergic anticonvulsant medications for migraine prophylaxis [Wenzel et al., 2006] adds some functional plausibility to the positional evidence for an involvement of GABA-A receptors in the etiology of MA. However, no disease-causing mutation in the five families with evidence for linkage to the GABA-A receptor subunit cluster has been identified [Russo et al., 2005].

A reasonable approach to further investigate a contribution of *GABRA5*, *GABRB3*, and *GABRG3* to the pathogenesis of MA is a systematic single nucleotide polymorphism (SNP)-based association study in a case–control approach. Here we present the results from genotyping 56 SNPs which capture the predicted common haplotype variations of the three candidate genes in a sample of German origin.

MATERIAL AND METHODS

Patients

Altogether 649 German MA patients were included in the study. All patients gave their written informed consent and were diagnosed as described before [Todt et al., 2005] according to the revised criteria of the Headache Classification Subcommittee of the International Headache Society [HCSIHS,

TABLE I. Case–Control Samples

Sampling unit	Samples		
	Basic (= sample 1)	Replication (= sample 2)	Full (= sample 1+2)
MA cases (male and female)	270	379	649
Male MA cases	77	74	151
Female MA cases	193	305	498
Controls (male and female)	273	379	652
Male controls	82	78	160
Female controls	191	301	492

Case–control samples 1 and 2. All patients were interviewed by experienced physicians, were of German descent, and attended a highly specialized pain clinic. The diagnosis was made according to the revised International Headache Society criteria. The control sample was also of Caucasian descent and was gender matched. The controls completed a questionnaire enabling us to identify migraine-suspicious individuals.

2004] by experienced physicians (see Table I for the number and gender of patients belonging to the two sub-samples used in different parts of this study). A detailed description of clinical features of the patient sample is given in Table II. The study was approved by the local university ethics committees. The control sample consisted of 652 German individuals, who were interviewed by a psychiatrist with a 1-year training in neurology. The migraine prevalence (MA and migraine without aura) in the control sample was approximately 15% and hence within the published prevalence rates for migraine [Palotie et al., 2002].

Genetic Analysis

Initial SNP-genotyping on genomic DNA of sample 1 was performed on an Illumina™ platform according to the manufacturer's protocol. Genotyping of SNPs rs4533233 and rs1432133 in the replication sample was carried out on a TaqMan™ platform with assays designed by Applied Biosystems, Foster city, CA (assay IDs C_428305_10 and C_2078454_10).

SNP Selection

The haplotype-tagging (ht-)SNPs (Fig. 1) were selected from the HapMap database (http://www.hapmap.org; version September 2004) to discriminate between all predicted common haplotypes (with an estimated frequency >5%) within haplotype blocks in the Central European sample. Haplotype blocks were defined as regions in which >85% of total haplotype diversity is covered by common haplotypes, using the program hapblock [Zhang et al., 2004].

Statistics

Allele and genotype distributions were compared between cases and controls by a chi-square test with the appropriate degrees of freedom. Tests of HWE were performed using a χ^2 goodness-of-fit test. For the comparison of haplotype frequencies between cases and controls we used the program famhap [Becker and Knapp, 2004]. This program estimates maximum-likelihood haplotype frequencies using an expectation-maximization algorithm and compares haplotype frequencies by a permutation-based strategy.

Power Calculation

The power analysis was performed with the Genetic Power Calculator [Purcell et al., 2003]. We estimate that under the assumption of complete linkage disequilibrium (LD) between the marker tested and the disease-causing variant, we had 84% power to detect a true difference in allele frequency between the 270 MA cases and 273 controls with a single-marker association analysis (α of 0.05), further assuming a frequency of the disease-associated allele A of 0.18, a relative risk of 1.5 for genotype Aa and of 2.25 for genotype AA, and a prevalence of MA in the general population of 8%. Using the same parameters, the estimated power increases to 99.6% in a sample of 649 cases and 652 controls.

RESULTS

Single Marker Analysis

Among the 56 SNPs in the GABA-A receptor cluster (Fig. 1) genotyped in the initial sample (270 MA cases and 273 control individuals), four SNPs displayed nominally significant ($P < 0.05$) allelic association with MA (Table III). The smallest observed allelic and genotypic P-values in this initial sample were 0.015 and 0.009, respectively, for rs8031708 in *GABRG3* (data for genotypic P-values are not shown). However, after permutation-based correction for the number of tests per-

TABLE II. Clinical Characteristics of Patients With Migraine With Aura (MA)

Characteristic	Basic sample (= sample 1)	Replication sample (= sample 2)	Full sample (= sample 1+2)
Subjects (no.)	270	379	649
Men/women (no.)	77/193	74/305	151/498
Age (years)	48 ± 13.5	46 ± 12.5	47 ± 13.0
Mean age at onset (years)	19.5 ± 11.1	19.1 ± 10.4	19.3 ± 10.7
Mean duration of one attack (hr)	44.9 ± 23.7	52.3 ± 22.2	49.2 ± 23.1
Mean number of attacks / month	2.6 ± 1.4	2.7 ± 1.3	2.7 ± 1.4
Visual aura symptoms (%)	96.6	94.1	95.1
Sensory aura symptoms (%)	40.4	39.7	40.0
Dysarthria/aphasia (%)	32.8	31.6	32.1
Nausea (%)	87.4	92.9	90.6
Vomiting (%)	66.0	68.6	67.5
Photophobia (%)	91.6	91.8	91.7
Phonophobia (%)	88.9	91.5	90.5
Unilateral location (%)	84.6	85.2	84.9
Pulsating pain (%)	80.8	83.8	82.6

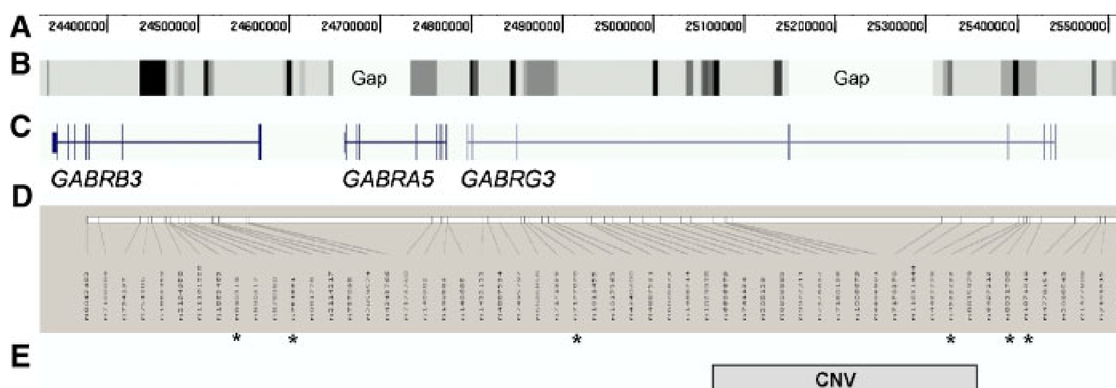

Fig. 1. Schematic representation of the chromosome 15 GABA-A receptor cluster. (**A**) Chromosome 15 position in bases, based upon NCBI *Build* 35 of the human genome. (**B**) Recombination rates within this genomic region as estimated by the International HapMap project. Darker regions have higher and lighter regions have lower recombination rates. Regions with higher recombination rates have a greater haplotype diversity. No genomic sequence data are available for the two gaps, possibly due to segmental structural variation within the GABA-A receptor cluster. (**C**) Genomic structure of the three positional candidate genes. Small vertical bars represent 5′-untranslated exons, large vertical bars coding exons. (**D**) Relative position and rs-IDs of the 56 SNPs genotyped in this study. Note that SNP density is highest in genomic regions with high recombination rates and hence greater haplotype diversity. SNPs with a deviation from Hardy–Weinberg equilibrium are marked by an asterisk. (**E**) Location of a copy number variant (CNV) identified in 1 out of 270 individuals [Redon et al., 2006] which overlaps with one of the sequence gaps shown in (B).

formed, the P-values of this SNP increased to 0.64 and 0.82, respectively. Of note, rs8031708 deviated from Hardy–Weinberg equilibrium (HWE) in the control sample. In total, 4 of the 56 SNPs displayed significant Hardy–Weinberg disequilibrium (HWD) in the control sample (Fig. 1 and Table III), all resulting from a deficit of heterozygotes, and two SNPs displayed significant HWD in the case sample, one resulting from a deficit and one resulting from an excess of heterozygotes. Three of these six SNPs in HWD (rs4533233, rs8031708, and rs1871019) cluster within 25 kb of genomic sequence and are located in proximity to a reference sequence gap in the current NCBI genome assembly, potentially caused by a structural polymorphism [Zody et al., 2006]. It is possible that this HWD results from causes that are specific to the GABA-receptor locus, because the ascertained genotypes were part of a larger study without any atypically strong HWD at other loci (unpublished work). As however, in a very recent systematic study of copy number variations (CNV) in HapMap individuals [Redon et al., 2006] just 1 out of 270 individuals showed a gain/loss at this locus (Fig. 1), a common CNV as cause of the HWD seems questionable and it could, at least partially, also constitute a type I error.

To avoid discounting a true positive case–control difference in the SNP data that results from weak genetic effects on disease susceptibility (resulting in only moderately significant P-values), we genotyped the *GABRG3* SNPs rs1432133 and rs4533233 in our second sample (379 MA cases and 379 controls). Allele call rates on the ABI-Taqman platform used for these experiments were 99.9% for rs1432133 and 95.8% for rs4533233. The initially significant results could not be replicated here ($P > 0.05$; data not shown). The same holds true for the combined analysis of sample 1 and sample 2 (649 MA cases and 652 control individuals) for rs1432133 ($P = 0.31$) and rs4533233; however the latter still showed a non-significant trend (uncorrected $P = 0.07$).

Haplotype Analysis

Since our SNPs were selected to achieve maximal power by discriminating common haplotypes, we also performed a haplotype-based comparison of case–control genotypes. To account for the variable degree of LD between the genotyped SNPs, we partitioned the three GABA-A receptor genes into blocks of discernible LD ($D' > 0.1$ for more than 90% of SNP pairs) and evaluated all possible SNP combinations within all LD blocks. The rationale behind this strategy is that the haplotype analysis can provide meaningful information only in the presence of LD between SNPs. Among a total of 714 evaluated SNP-combinations, we found 288 for which the global haplotype distribution significantly deviated from the random expectation with $P < 0.05$, 80 combinations with $P < 0.01$, and 4 combinations with $P < 0.001$ (Supplementary Table 1). Most of these SNP-combinations cluster in a 110 kb sized LD-block spanning from rs8035979 to rs2169637 that contains the SNP found to be most significant in the single marker analysis. After permutation-based correction for multiple testing of different marker combinations within this LD block, the P-value of the most significant marker combination increased to 0.027. When further applying a Bonferroni correction for the number of 16 largely independent LD blocks, this P-value falls above the formal significance level.

DISCUSSION

We genotyped 56 SNPs in a large MA sample by a systematic haplotype-based case–control association study. The study design (with respect to sample size, ethnic, and gender constitution) aimed at minimizing the probability of false findings due to, for example, population stratification. If disregarding multiple testing, we found 4 out of 56 SNPs at the GABA-A receptor locus nominally associated with MA in our sample of 270 cases and 273 control individuals. These differences could not be replicated in a larger sample (sample 2). We therefore conclude that the initial differences are likely to be false positives, resulting from the large number of tests performed. Furthermore, we found several nominally significant differences in the estimated haplotype distribution between cases and controls in the genomic region spanning the most significant SNPs. These differences also did not withstand the correction for multiple testing and may therefore be attributed to random fluctuations in the data. Nevertheless, we cannot exclude a potential role of these markers, and future studies might find it interesting to evaluate them in other samples of patients with migraine or related phenotypes with a possible involvement of the GABAergic system. The conclusion that these differences in haplotype distribution are false positives is further strengthened by the report submitted in parallel by Oswell et al. to this journal. Due to a completely independent

TABLE III. Genotyping Results and Statistical Analysis

SNP rs-ID	Gene	Distance (bp)	Allele1/2 (strand)	Deviation from HWE (*P*-value) in		Minor allele frequency in		Allelic *P*-value	OR (95% CI)
				Controls	Cases	Controls	Cases		
rs8042482	*GABRB3*	—	A/G (−)	0.410	0.990	44.3 (A)	44.03 (A)	0.940	1.01 (0.79–1.28)
rs7166689	*GABRB3*	13,657	A/C (+)	0.250	0.170	30.33 (C)	34.51 (C)	0.131	1.22 (0.94–1.57)
rs754197	*GABRB3*	45,582	A/G (+)	0.920	0.810	48.35 (A)	45.71 (A)	0.390	1.11 (0.87–1.41)
rs754186	*GABRB3*	8,320	A/G (+)	0.180	0.500	31.62 (A)	32.65 (A)	0.781	1.04 (0.8–1.34)
rs1863459	*GABRB3*	4,862	A/G (−)	0.850	0.750	16.54 (G)	17.54 (G)	0.646	1.08 (0.78–1.48)
rs2194958	*GABRB3*	14,733	A/G (−)	0.410	0.500	40.81 (G)	44.59 (G)	0.204	1.17 (0.92–1.49)
rs11161328	*GABRB3*	3,735	A/G (+)	0.860	0.250	28.23 (G)	24.81 (G)	0.180	1.2 (0.92–1.58)
rs1863463	*GABRB3*	1,376	C/G (−)	0.690	0.760	13.24 (C)	14.18 (C)	0.564	1.11 (0.78–1.56)
rs890319	*GABRB3*	1,114	A/G (−)	0.0003	0.980	32.54 (A)	35.21 (A)	0.367	1.12 (0.87–1.44)
rs890317	*GABRB3*	8,567	A/C (+)	0.270	1.000	27.39 (A)	24.44 (A)	0.269	1.17 (0.89–1.53)
rs878960	*GABRB3*	6,735	A/G (−)	0.210	0.750	35.06 (G)	34.59 (G)	0.903	1.02 (0.79–1.3)
rs754661	*GABRB3*	5,341	A/G (+)	0.011	0.220	43.57 (G)	42.35 (G)	0.746	1.04 (0.82–1.32)
rs981778	*GABRB3*	22,963	A/G (+)	0.360	0.810	38.7 (A)	39.55 (A)	0.709	1.05 (0.82–1.34)
rs2114217	*GABRB3*	2,789	A/T (−)	0.600	0.100	16.91 (A)	15.67 (A)	0.545	1.1 (0.8–1.53)
rs737098	*GABRB3*	7,141	A/G (+)	0.790	0.920	27.21 (G)	26.87 (G)	0.896	1.02 (0.78–1.33)
rs2059574	*GABRB3*	29,873	A/T (+)	0.270	0.760	48.35 (A)	46.83 (A)	0.666	1.05 (0.83–1.34)
rs4243766	*GABRB3*	3,582	A/T (+)	0.620	0.420	21.32 (T)	25.56 (T)	0.100	1.27 (0.96–1.68)
rs7173260	*GABRA5*	202,972	A/G (+)	0.940	0.310	46.69 (A)	43.66 (A)	0.387	1.11 (0.87–1.41)
rs140682	*GABRA5*	10,413	A/G (−)	0.470	0.760	45.4 (A)	43.28 (A)	0.454	1.1 (0.86–1.39)
rs140683	*GABRA5*	5,978	A/T (+)	0.390	0.290	42.65 (A)	43.28 (A)	0.772	1.04 (0.81–1.32)
rs140685	*GABRA5*	124	A/G (−)	0.460	0.400	48.53 (A)	45.9 (A)	0.358	1.12 (0.88–1.42)
rs1432133	*GABRG3*	39,887	A/G (+)	0.290	0.920	41.91 (G)	47.95 (G)	0.045	1.28 (1.01–1.62)
rs4887534	*GABRG3*	5,827	C/G (+)	0.050	0.250	39.89 (G)	43.47 (G)	0.226	1.16 (0.91–1.48)
rs7495797	*GABRG3*	12,289	A/G (+)	0.840	0.840	17.65 (G)	19.78 (G)	0.360	1.15 (0.85–1.57)
rs6606858	*GABRG3*	23,963	A/T (+)	0.090	0.100	36.58 (T)	36.01 (T)	0.823	1.03 (0.8–1.32)
rs7171856	*GABRG3*	4,105	A/T (−)	0.660	0.060	22.06 (T)	21.83 (T)	0.934	1.01 (0.76–1.35)
rs7177870	*GABRG3*	4,914	A/G (−)	0.430	0.003	49.45 (A)	48.51 (A)	0.760	1.04 (0.82–1.32)
rs1011455	*GABRG3*	13,919	A/G (+)	0.260	0.490	40.44 (G)	41.98 (G)	0.511	1.08 (0.85–1.38)
rs1017363	*GABRG3*	7,010	A/G (−)	0.900	0.460	27.39 (G)	24.25 (G)	0.239	1.18 (0.9–1.55)
rs4340300	*GABRG3*	7,585	A/G (−)	0.400	0.220	8.82 (A)	6.9 (A)	0.227	1.32 (0.84–2.06)
rs4887531	*GABRG3*	35,917	A/T (+)	0.490	0.190	15.44 (A)	16.04 (A)	0.762	1.05 (0.76–1.46)
rs6606873	*GABRG3*	4,944	A/G (−)	0.590	0.190	44.49 (A)	46.64 (A)	0.471	1.09 (0.86–1.39)
rs1468714	*GABRG3*	13,445	A/G (+)	0.500	0.180	37.68 (G)	33.96 (G)	0.215	1.17 (0.91–1.5)
rs1029938	*GABRG3*	9,807	A/G (−)	1.000	0.180	25 (G)	27.61 (G)	0.332	1.14 (0.87–1.5)
rs6606879	*GABRG3*	19,653	A/G (−)	0.200	0.980	45.76 (A)	46.08 (A)	0.859	1.02 (0.8–1.3)
rs741124	*GABRG3*	13,033	C/G (−)	0.540	0.950	47.23 (C)	49.06 (C)	0.587	1.07 (0.84–1.36)
rs208129	*GABRG3*	20,150	A/T (−)	0.700	0.740	42.65 (A)	39.93 (A)	0.344	1.12 (0.88–1.43)
rs6606885	*GABRG3*	22,937	A/G (+)	0.370	0.760	38.6 (G)	41.98 (G)	0.275	1.14 (0.9–1.46)
rs9972311	*GABRG3*	11,390	A/G (+)	0.880	0.810	34.01 (A)	30.04 (A)	0.178	1.19 (0.92–1.54)
rs2376832	*GABRG3*	24,235	A/G (+)	0.490	0.250	15.44 (G)	18.1 (G)	0.266	1.2 (0.87–1.65)
rs7180136	*GABRG3*	12,660	C/G (−)	0.880	0.720	42.28 (G)	45.9 (G)	0.227	1.16 (0.91–1.47)
rs1006679	*GABRG3*	2,084	A/G (+)	0.610	0.820	45.4 (A)	45.34 (A)	0.991	1.0 (0.79–1.27)
rs6606891	*GABRG3*	6,923	A/G (−)	0.890	0.210	29.41 (G)	29.48 (G)	0.991	1.0 (0.77–1.3)
rs7170270	*GABRG3*	232,092	A/G (+)	0.990	0.620	48.9 (A)	45.71 (G)	0.077	1.24 (0.98–1.57)
rs11631444	*GABRG3*	21,845	A/G (+)	0.930	0.290	46.32 (G)	47.57 (G)	0.589	1.07 (0.84–1.36)
rs4432228	*GABRG3*	33,666	A/G (−)	0.320	0.080	38.79 (A)	36.38 (A)	0.397	1.11 (0.87–1.42)
rs4533233	*GABRG3*	29,894	C/G (+)	0.760	0.046	32.72 (C)	38.99 (C)	0.034	1.31 (1.02–1.68)
rs8035979	*GABRG3*	4,798	A/T (−)	0.090	0.720	45.77 (A)	48.69 (T)	0.060	1.26 (0.99–1.6)
rs6497213	*GABRG3*	3,425	A/C (−)	0.650	0.220	37.5 (C)	31.53 (C)	0.044	1.29 (1.01–1.66)
rs8031708	*GABRG3*	4,843	A/G (+)	0.006	0.980	46.51 (G)	46.08 (A)	0.015	1.34 (1.06–1.71)
rs1871019	*GABRG3*	11,110	A/G (+)	0.013	0.770	48.52 (G)	44.4 (G)	0.199	1.17 (0.92–1.49)
rs4778154		38,548	A/G (+)	0.090	0.110	43.75 (A)	44.22 (A)	0.914	1.01 (0.8–1.29)
rs3098543		24,650	A/G (+)	0.430	0.230	28.86 (G)	27.99 (G)	0.743	1.05 (0.8–1.36)
rs1477808		3,712	A/G (−)	0.260	0.270	47.98 (A)	49.07 (A)	0.718	1.04 (0.82–1.33)
rs3101615		4,333	A/G (−)	0.460	0.810	18.38 (A)	16.79 (A)	0.508	1.11 (0.81–1.52)
rs2169637		16,978	A/C (+)	0.160	0.130	24.08 (A)	21.08 (A)	0.214	1.2 (0.9–1.59)

Genotyping results and statistics for 56 SNPs analyzed in Sample 1. *P*-values <0.05 are shaded. HWE, Hardy–Weinberg equilibrium; OR, Odds ratio; CI, Confidence intervals.

study design their collection of SNPs only partially overlaps with our assortment (11 from 30 analyzed SNPs), however, 14 of the non-overlapping SNPs of their study are in strong LD ($D' > 0.8$) with one or more SNPs included in our study.

The genomic complexity of the GABA-A receptor cluster is demonstrated by the recent finishing of the chromosome 15 DNA sequence that was unable to close two gaps within this region [Zody et al., 2006]. The authors explain these gaps by the

possible existence of structural polymorphisms, which may include CNVs. Indeed, a very recent genome-wide screening for CNVs identified one such polymorphism spanning the sequence gap in *GABRG3* in 1 out of 270 individuals genotyped in the HapMap project [Redon et al., 2006]. Therefore, we cannot exclude that this CNV or other structural polymorphisms may slightly interfere with SNP-genotyping results and may be a cause of the observed HWD in a few markers, although rather common CNVs at this locus were not demonstrated. As the study of Redon et al. showed that CNVs constitute a significant part of the human genome, this possible obstacle of SNP-based association studies has to be addressed in general in the future. Thus, also future studies of the GABA-A receptor cluster may include different technical approaches currently not available as a standard methodology that might detect a putative contribution of CNVs within the GABA-A receptor cluster to the pathogenesis of MA or other diseases of interest. In summary, based on our comprehensive genotyping of SNPs, we currently cannot conclude that this locus plays a major role in the molecular etiology of MA.

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