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Predictors of Development of Juvenile Multiple Sclerosis in Kazakh Population According to the DR-Genes of Major Histocompatibility Complex

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Abstract

The genetic factors associated with multiple sclerosis in Kazakhstan are not well understood. The aim of the study was to determine a major histocompatibility complex (MHC) genotype pattern and its clinical correlations in pediatric demyelinating diseases of multiple sclerosis (MS) spectrum in Kazakh population. For that a complex clinical, neuroimaging and immunogenetic examination was performed in 19 children (11 girls, 8 boys) with central nervous system (CNS) demyelination aged from 4 to 18 years. Clinically, 4 out of 19 children had leukoencephalitis (21%), and in 15 of 19 patients had MS (79%) including 6 presented with a clinically isolated syndrome. We found that presence of gene allele DRB1*15(02) and the associated alleles, combined in the haplotype DRB1*15(02)~DQA1*01:02~DQB1*06:02 point out a significantly higher risk of MS development, i.e. 7.7 fold in children and adolescents of the Kazakh ethnicity and a 3.12 fold risk for children of Russian ethnicity. We observed that presence of both DRB1*15 alleles (homozygotes) and of one DRB1*15 allele in combination with DRB1*03 in female teenagers presented with more aggressive "classical" MS; this aspect requires more profound clinical observations in a bigger cohort.

Keywords

Introduction

Demyelinating diseases of CNS in children are rare inflammatory autoimmune diseases. These diseases are also called neuroimmune diseases of CNS and include post-infectious acute disseminated encephalitis, MS, as well as Rasmussen encephalitis and Schilder's leukoencephalitis recognized as variants of MS. Among all acquired demyelination syndrome, which are usually monophasic, about 20% develop MS at later time point [1]. Thereby pediatric MS is a part of a big group of disorders related to acquire demyelination syndrome but it shares environmental and genetic risk factors associated with adult MS [1-3].

The main candidate genes which regulate the interaction between a macrophage and CD4+ T cells are MHC genes. This interaction starts the autoimmune reaction to myelin in MS and related conditions [1-3]. Also the important factor for this disturbance of immune tolerance to myelin and some other brain antigens is the phenomena of molecular mimicry between some viral (or other infection) antigens and myelin antigens, which in some immune circumstances resulted in violation of nature immune tolerance for own brain antigens [1, 3].

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Studies based on animal model showed that auto-immunity to myelin-associated/oligodendrocyte basic proteins is determined by DQB1*06:02 HLA class II genes [4]. Many authors recognize the differences in haplotypes of HLA class II, depending on the ethnicity of patients [1]. In various populations MS is associated with DRB1*15:01~DQA1*01:02~DQB1*06:02 as a linkage complex. In the other ethnicities such type of linkage is not observed, for instance, in Asians MS is associated with DRB1*15:01 only, and in African-Brazilian population-with DQB1*06 locus [2-4].

Demyelinating diseases are not systematically studied in Kazakhstan, where it has a population of 179942 million people, and 25% of them are children [5]. Kazakh population is multiethnic with 60% prevalence of Kazakhs (Asian ethnicity). 10-15 years ago Kazakh health professionals considered MS a "European disease". Since that we first started a search for these diseases, we collected mainly atypical or difficult cases from all over the country aiming to do a systematic registration. Investigation of HLA class II MHC genes linkage with MS is needed to find the genetic predictors of pediatric MS in multiethnic cohort. This is useful for development of selective targeted MS immune treatment.

The aim of the study was to investigate genes of the main complex of human histocompatibility (HC) associated with diseases of MS spectrum diseases in Kazakh population.

Materials and Methods

Complex clinical, neuroimaging and immunogenetic studies were performed in 2015-2017 in the Aksai Clinic of Kazakh National Medical University (KazNMU). Overall 19 children (11 girls, 8 boys) with demyelination of the central nervous system aged 4 to 18 years were enrolled. There were 8 children (5 girls and 3 boys) of Caucasian (Russian) origin, and 11(6 girls and 5 girls) were Kazakhs (Asians).

Investigation was performed with the agreement from Ethical Committee of Kazakh National Medical University (No 208 29/04/2015). Parents of all patients signed special informed consent form approving samples collection from their children.

Clinical diagnosing of the MS and related conditions were performed using McDonald criteria (2010, 2017, https://www.mstrust.org.uk/a-z/mcdonald-criteria). Brain MRI in a 1.5 Tesla in FLAIR regime was performed in 19 children.

Genotyping of HLA DRB1, DQA1, DQB1 (LOCUS 6P21) genes

Genotyping of HLA DRB1, DQA1, DQB1 (LOCUS 6P21) genes was done at the Institute of Immunology of the Russian Academy of Medical Sciences, Moscow by Professor Boldyreva M.N. and Atchabarov Research Institute in KazNMU (test kits DNA-technology, Russia). The data were normalized to a healthy population of Kazakh (Asians) [6], and Russian (Europeans) [7], (Institute of Immunology, Russian Academy of Medical Sciences). Genomic DNA was isolated

from peripheral blood by salting-out procedure according to a standard protocol [8]. The HLA-DRB1, HLA-DQA1 and HLA-DQB1 genes were detected by a real-time PCR using the DNA-Technology kits (Russia) and detecting amplifiers with four channels and 96 holes, DT-96, (https://www.dna-technology.ru/files/images/broshura/HLA.pdf). Genotyping at loci DRB1, DQA1, DQB1 was performed at the level of allele groups (low resolution). The amplification mode: 94.0 °C-30 sec, 64.0 °C-15 sec (5 cycles); 94.0 °C-10 sec, 64.0 °C-15 sec (45 cycles); 25.0 °C-15 sec-50 cycles, "Melting curve", $\Delta t = 1$ °C; Tcon = 75 °C. The following genes were tested: HLA-DRB1: DRB1* 01, 03, 04, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16; DQA1*01:01, 01:02, 01:03, 02:01, 03:01, 04:01, 05:01, 06:01; DQB1*02, 03:01, 03:02, 03:04, 03:05, 04:01/04:02, 05:01, 05:02/05:04, 05:03, 06:01, 06:02-8.

Statistical analysis

The data was statistically processed by the SPSS version 16. All genetic data are presented in absolute numbers and their percentages. Clinical data are shown as the average values of the clinical symptom with a standard deviation (M ± m), the significance was assessed by the Student's criteria ($p < 0.05$ was considered a significant difference between samples). The genetic data (group of alleles) of our patients and the population standard were calculated by direct counting and compared using the $\chi2$ criterion in the Fisher test for small samples and relative risk was calculated (RR), $p < 0.01$ was considered significant.

Results

Clinical characteristics of patients

Out of 19 children enrolled in this study 11 were ethnical Kazakhs (Asians) and 8 children were Russians (Europeans). Clinically 15 out of 19 (78.9%) had multiple sclerosis including 6 children (40%) with clinically isolated syndrome (4-opticoneuritis and 2-ataxia). 4 out of 19 children (21.1%), had leukoencephalitis with symptoms of encephalopathy and local neurological signs (Table 1). Infectious-toxic syndrome including hyperthermia of 37.6-38.2 °C, drowsiness, general malaise, weakness, loss of appetite in the onset of the disease was noted in all 4 children with leukoencephalitis. Patients with MS were elder than children with leukoencephalitis (14.4 ± 2.5 years versus 5.25 ± 0.75, $p < 0.01$). The average duration of the disease in MS patients was 21.77 ± 17.28 months, in leukoencephalitis patients - 20.75 ± 9.25 months. Furthermore, clinical picture of MS was presented with exacerbations and remissions, overall in 5 of 19 (26%) had 1-2 relapses, 9 of 19 (50%) children had 3 relapses, and 4 of 19 (21%) patients had 4 relapses.

In most of the patients symptoms of ataxia were observed: trunk ataxia was diagnosed in 12 (63%) and limb ataxia - in 11 of 19 children (58%). At the first presentation 4 children (21%) had general symptoms of encephalopathy, including headache and vomiting, thus, they were diagnosed with leukoencephalitis, 2 of them had generalized convulsions. Local neurological signs as paresis and paralysis were observed

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in 10 of 19 (52%); chorea-like hyperkinesis were detected in 3 of 19 (16%). Symptoms of optic neuritis were seen in 7 children (37%), strabismus was seen in 3 children (15%).

Brain MRI on a 1.5 Tesla in FLAIR regime was performed in all 19 children. No gadolinium positive lesions were observed in children with leukoencephalitis. In contrast, 6 out of 15 children (40%) from the group of patients with multiple sclerosis had gadolinium positive (Gd+) lesions. The presence of T2-active foci was detected in 11 of 15 patients (73%) with MS (including in 2 cases in spinal cord), where the mean number of foci was 5.42 ± 2.83. In all 15 MS patients were active demyelinating lesions (T2-active and/or Gd+). In children with leukoencephalitis T2-active foci were diagnosed in all children (100%) (Table 1). Reduction of brain volume was not evident, only 3 children with leukoencephalitis had shown decrease in brain volume 6-8 months after the disease debut.

Table 1: Main clinical and neurovisualization characteristics of the children with MS spectrum diseases.

| Characteristic	Patients with MS (M ± m)	Children with Leukoencephalitis (M ± m)
Number of patients	15	4
Age (years)	14.4 ± 2.5	5.25 ± 0.75
Disease duration at the time of sampling (months)	21.8 ± 17.28	20.75 ± 9.25
Number of relapses	3.7 ± 1.7	3 ± 0.5
Number of T-2 lesions on brain MRI	6 ± 3.73	3 ± 1
Number of patients with Gadolinium+ lesions	6 (40%)	0

Hereby, clinical stratification determined 2 main variants of chronic demyelination. First variant is children with MS (also including clinically isolated syndrome) and chronic demyelination process. Second variant is leukoencephalitis of Schilder type, which is also considered as variant of MS by some authors but steadily progressing without not remissions for long time, progressing loss of brain volume (more similar to primary progressive MS) [1].

Genotyping of DRB1, DQA1, QB1 gens allels of MHC of patients with MS spectrum diseases

In table 2, we present results of genotyping of the genes of HLA DRB1, DQA1, DQB1 (locus 6p21) of 19 children (11 girls, 8 boys) with demyelinating diseases of MS spectrum. In table 3 these data are shown in comparison with the same gene genotyping results of healthy population of Kazakhs (Asians) and Russians (Europeans).

As shown in table 2 and 3, a haplotype DRB1*15, DQA1*01:02, DQB1*06:02 was the most prominent in our patients. Theses alleles were detected in 34.2% from all DR-alleles (number of all alleles doubles the number of patients).

In comparison, in Kazakh Normal it was seen in 6.3% of DR-alleles (χ2 = 31.2, p = 0.000004, relative risk = 7.7), in Russian normal – in 14% (χ2 = 9.8, p = 0.0025, relative risk = 3.17). Comparison of the results is shown in figure 1, where the Y-axis is the DRB1 gene allele; X-axis represents percentage of the population carrying a certain genotype. Together figure 1, table 2 and 3 give a hint on linkage disequilibrium of DRB1*15 allele and associated genes, as they were most prevalent in the group of patients with MS-associated diseases. So haplotype DRB1*15~DQA1*01:02~DQB1*06:02 predict the 7.7 fold increase of relative risk (RR) of MS development in the Kazakh population, and 3.17 fold in Russians.
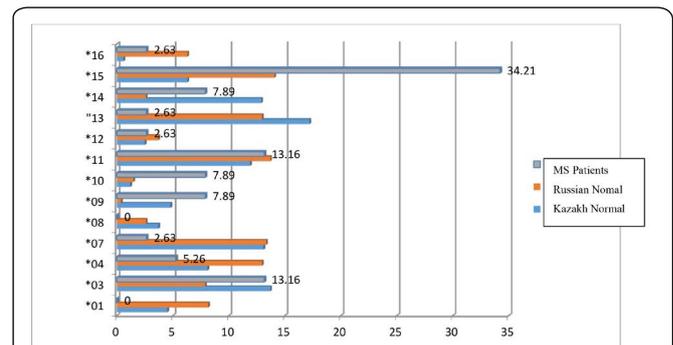
Figure 1: Genotyping results of DRB-1 genes in comparison with the norm of Kazakh and Russian populations: the Y-axis of the DRB1 gene allele, X-axis is % of the population.

Table 2: Genotyping of DRB1, DQA1, DQB1 gens alleles of MHC of patients with MS spectrum diseases.

No samples	Male/ female	Age of onset, years	DRB1		DQA1		DQB1	
1	f	16	*03*	15	05:01	**01:02**	02	**06:02-8**
2	f	12	*03*	10	050:1	01:01	02	05:01
3	m	7	09	11	03:01	05:01	03:03	03:02
4	m	12	10	**15**	01:01	**01:02**	05:01	**06:02-8**
5	m	10	09	14	03:01	01:01	03:03	05:03
6	m	14	13	**15**	**01:02**	**01:02**	**06:02-8**	**06:02-8**
7	f	18	*03*	11	05:01	05:01	02	03:01
8	f	14	09	**15**	03:01	**01:02**	03:03	**06:02-8**
9	f	15	04	**15**	03:01	01:03	03:02	06:01
10	f	16	10	**15**	01:01	**01:02**	05:01	**06:02-8**
11	m	16	*03*	11	05:01	05:01	02	03:01
12	f	11	11	**15**	05:01	**01:02**	03:01	**06:02-8**
13	f	14	**15**	15	**01:02**	**01:02**	**06:02-8**	**06:02-8**
14	f	17	12	**15**	05:01	**01:02**	03:01	**06:02-8**
15	m	9	04	**15**	03:01	**01:02**	03:02	**06:02-8**
16	m	4	11	**15**	05:01	**01:02**	03:01	**06:02-8**
17	f	5	**15**	16	**01:02**	**01:02**	**06:02-8**	05:02/4
18	f	18	14	14	01:01	01:01	05:03	05:02/4
19	m	12	03	07	05:01	02:01	02	02

As it is seen in table 2 there were 12 patients with typical "MShaplotype"-DRB1*15(02)~DQA1*01:02~DQB1*06:02.

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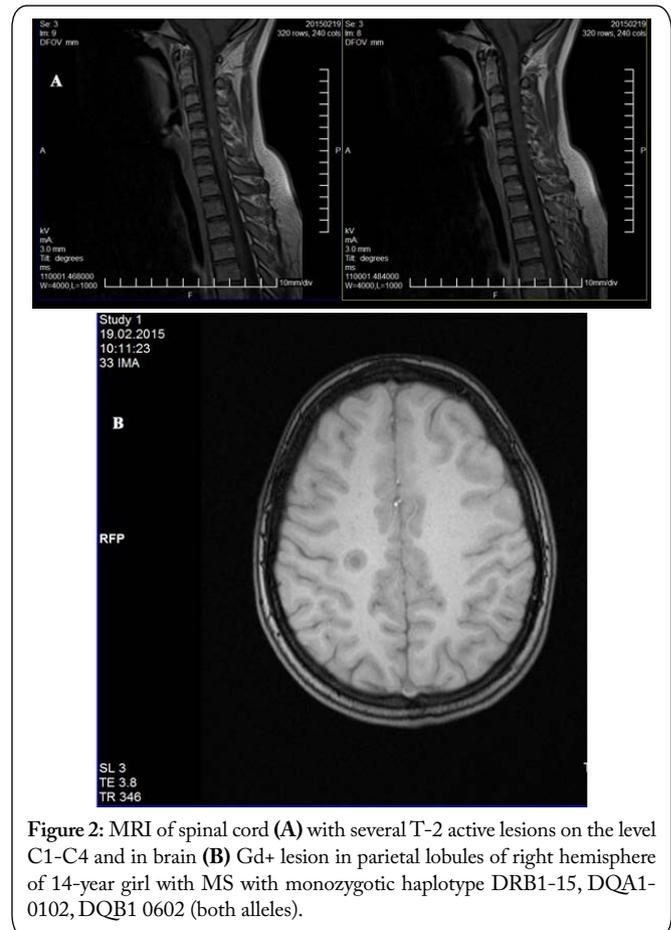
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Clinically 8 of 12 were girls (6 with classical MS, 2 with leukoencephalitis), 4 of 12 with were boys (3 with MS, 1 with leukoencephalitis).

Table 3: Genotyping results of DRB1 gene in comparison with the norms of Kazakh and Russian populations.
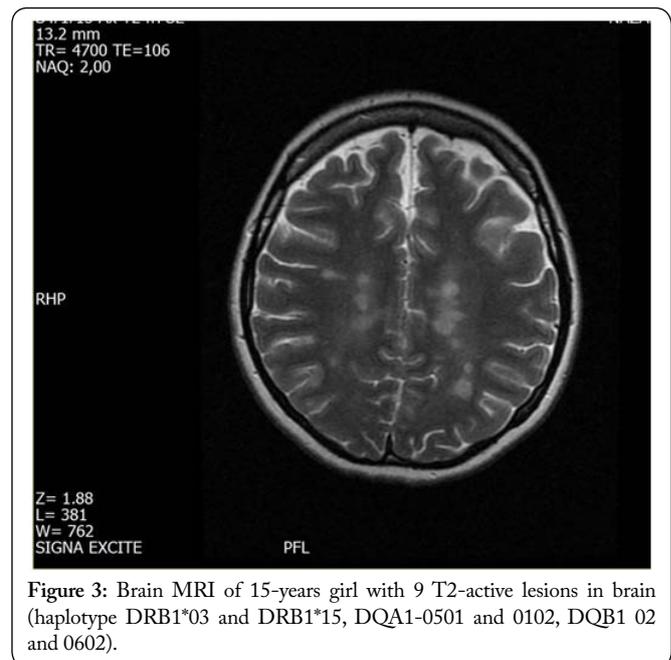
Alleles	Kazakh normal		Russian normal		MS patients	
DRB1	%	abs	%	abs	%	abs
*01	4.5	14	8.15	22	0	0
*03	13.7	43	7.88	21	13.16	5
*04	8.1	25	12.96	35	5.26	2
*07	13.1	41	13.33	36	2.63	1
*08	3.7	11	2.59	7	0	0
*09	4.8	15	0.37	1	7.89	3
*10	1.2	4	1.48	4	7.89	3
*11	11.9	37	13.7	37	13.16	5
*12	2.5	8	3.7	10	2.63	1
"13	17.2	54	12.96	35	2.63	1
*14	12.9	40	2.59	7	7.89	3
*15	6.3	20	14.07	38	34.21	13
*16	0.6	2	6.3	17	2.63	1
Number of alleles		314		270		38
RR of DRB1*115	7.7		3.17			
P-value	0.000004		0.0025			
χ2	31.2		9.8			

As a striking example, we describe the clinical case of 14 years old girl who was diagnosed as monozygotic on haplotype DRB1*15(02)~DQA1*01:02~DQB1*06:02, compared to the other MS patients she had the highest number of relapses, 3 during the first year of the disease with full clinical remissions, but within that year she developed gadolinium positive (Gd+) lesion in the brain and several T-2 lesions in spinal cord (Figure 2A and 2B). Nevertheless, clinically during the remission of the disease she had mild disability according to EDSS 1.0 score. So she developed more aggressive relapsing-remitting course of MS with three relapses fully reversing on high dose intravenous methylprednisolone (her spinal cord and brain MRI pictures presented on figure 2A and 2B).

Quite interesting results was obtained for patients harboring DRB1*03 allele, which was detected in 5 patients (3 Kazakh and 2 Russians). This allele was previously described to have a recessive association with MS, whereas the DRB1*15 associated in a dominant mode [2]. DRB1*03 allele showed no association with increased risk of MS development in Kazakh (13.16% vs 13.7%, RR = 0,95, p = 0.2, χ2 = 0.0083) and Russian populations (13.16% vs 7.88%, RR = 1.8, p = 0.1, χ2 = 1.247). However, one16-year old Russian female with

Figure 2: MRI of spinal cord **(A)** with several T-2 active lesions on the level C1-C4 and in brain **(B)** Gd+ lesion in parietal lobules of right hemisphere of 14-year girl with MS with monozygotic haplotype DRB1-15, DQA1-0102, DQB1 0602 (both alleles).

DRB1*03 carrying either a "classical" DRB1*15 developed a relapsing-remitting MS with very high activity on MRI (9 T2- active periventricular lesions, with one big Gadolinium positive lesions). This female teenager developed MS 6 months after first episode of optic neuritis (Figure 3).

Figure 3: Brain MRI of 15-years girl with 9 T2-active lesions in brain (haplotype DRB1*03 and DRB1*15, DQA1-0501 and 0102, DQB1 02 and 0602).

Thus, the presence of gene allele DRB1-15 (02) and the associated alleles, DQA1*01:02 and DQB1*06:02, combined

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in the haplotype DRB1*15(02)~DQA1*01:02~DQB1*06:02 indicate significantly high association of 7.7 fold MS risk development in children and adolescents of the Kazakh ethnicity and a 3.12 fold risk for children of Russian ethnicity.

Discussion

In our study we summarized the genetic association of pediatric MS in multiethnic population. Thus, 7 from 12 children with DRB1*15 were Russian, only 5 Asian (Kazakh). All of them had classical MS: 10 of 12 patients had a teenage debut of the MS disease that within 11 years transformed into a "classical" variant of MS. The monozygotic for haplotype DRB1*15:01~DQA1*0102~DQB1*0602 Russian 14-year old girl showed the most fast developing of "classical" variant of MS with 3 relapses during the first year of the disease and very evident MRI and clinical picture (opticospinal lesions with so-called "dissemination in space and time", figure 2A and 2B). The others were heterozygotes for this haplotype and had 2 relapses within a year and predominantly brain involvement of the process. Therefore, our results correspond to the published findings concerning DR15-allel linkage with early onset of MS, mainly in females [1-3, 7, 9-11]. This predicts MS development at younger age, predominantly in female population, but does not influence the course, outcomes, clinical features and paraclinical indexes in the CSF or characteristic abnormalities on MRI imaging of the central nervous system [9, 10].

Numerous studies pointed that combined haplotype of DRB1*15:01~DQA1*01:02~DQB1*06:02 predisposes to MS. The strong association between DRB1*15:01 and DQA1*01:02 was particularly described, although DQB1 0602 is usually linked with DRB1*15:01 [1-3]. However, in African–Brazilian MS patients the strongest association was observed with DQB1*06:02 rather than DRB1*15:01 [11]. The most of authors underline that HLA association of MS with DR-genes is more haplotypic rather than allelic [2, 9-11]. Moreover, it is important to mention that HLA-DRB1*15 is not considered as a susceptibility allele for MS in whole population, and not predicting the clinical course and severity of the disease in general [10]. However, in children with the first episode of acquired demyelination syndrome, presence of one or both copies of DRB1*15 increase risk of follow-up diagnosing MS for 37%, while without such allele only for 17% ($p < 0.001$) [1].

In our study, we proved the high risk of MS in patients with classical combined haplotype DRB1*15~DQA1*01:02~DQB1*06:02. We observed that presence of both DRB1*15 alleles (homozygotes) and of one DRB1*15 allele in combination with DRB1*03 in female teenagers correlated with more aggressive "classical" MS. We hope to collect more data to further strengthen this observation, taking into account that the same phenomena was shown by Disanto et al. [1] on a bigger cohort of pediatric patients (37%, 34 from 93 MS children) but not well determined for adult MS population.

Allele DRB1*03 which was detected in 5 children (4 of them are boys) from our study did not increase risk of the disease development, but in one case of a teenage girl with DRB1*15, confirmed previously established data about its recessive effect in MS population in Europe, Africa and Asia [2, 11-13].

It is remarkable that DRB1*15:01 in Asian Mongolian-like population, as shown in Japanese studies, is predisposed to "classic" Caucasian MS [12]; the authors point that DRB1*0405-positive MS patients have younger age at disease onset, lower EDSS scores and a lower frequency of MS-like brain lesions [12-14].

Summarizing, in the Kazakh population the presence of the haplotype DRB1-15 (02) DRB1*15~DQA1*01:02~ DQB1*06:02 which is associated with the higher level of cellular and humoral immunity to myelin proteins even after the first episode of demyelination. Such patients require early application of a disease modifying treatment which current protocols indicate mostly for adults, thereby, a delay of selective therapy start worsens the disease prognosis.

Author Contribution

Acknowledgement

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