

Cover letter

Dear Dr. Vladimir N. Uversky
Editor-in-Chief, Biomolecules

We greatly appreciate the efficient, professional and rapid processing of our paper by your team. Please find enclosed our revised manuscript entitled “*The role of MECP2 and CCR5 polymorphisms on the development and course of systemic lupus erythematosus.*” It has been revised according to the comments of the journal reviewers (see below). We hope that you will find our study interesting and appropriate for your readers.

Reviewers' comments and action taken:

Response to Reviewer 1

This paper measures MECP2 and CCR5 polymorphisms in whole blood samples from patients with lupus. The paper is limited by the following factors:

Comment 1. No mention is made as to whether the patients met the standard requirements for the classification of lupus

Thanks’ the Reviewer for comments. In Materials and Methods 2.1. Study group section we have information “All patients with SLE included in the study met the classification criteria of American College of Rheumatology (ACR) for this disease”, however, we also add this information: “SLE patients included in the study have the presence of at least four criteria”. Please refer to the revised version of the manuscript.

Comment 2. Table 2 lists the P value of every gene analyzed as well as the statistical significance, but only 11 meet the standard criteria of $p < 0.05$.

Thank you very much for the Reviewer's kind comments. All p-value which were not significant we corrected as NS. In table 2 we leave only significant p-value and p-value with a tendency. Please refer to the revised version of the manuscript.

Comment 3. There is no analysis as to whether the polymorphism affects the level of gene expression or affects the function of the associated protein.

We deeply appreciate the Reviewer's professional remarks. Unfortunately, at this moment, we don't have information on examined gene expression in our study group. Moreover, in this moment we are unable to do these analysis because we do not have a reagents. But we agree with the reviewer that these analysis may be important and we would like continue our research in this subject. We also did the bioinformatic analysis to check if examined SNPs affect the function of the associated protein. Computational functional prediction analysis was performed using MutationTaster and PROVEAN programs. The analysis showed that not MECP2 SNPs, but deletion of 32 bp in rs333 CCR5 might result in altered function of the associated protein. The amino acid sequence is changed, what consequences in frameshift. It leads to changes in splice site and also truncate the protein (might cause nonsense-mediated mRNA decay (NMD)).

We added information about software used to perform biostatistical analysis in Statistical analysis subsection as well as information about outcomes of biostatistical analysis in MECP2 and CCR5 polymorphisms and SLE susceptibility subsection. We added the two references in the Reference section. Please refer to the revised version of the manuscript.

Neither the entire work nor any part of its content has been published or accepted elsewhere. The authors declare no conflict of interest. All authors read and accepted revised version of the paper.

Your sincerely,

Agnieszka Paradowska-Gorycka, PhD

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National Institute of Geriatrics, Rheumatology and Rehabilitation
Poland, Warsaw

CCR5 rs333



mutation t@sting

[documentation](#)

Prediction	disease causing - long InDel	Model: <i>complex_aae</i> , prob: 1 (explain)
Summary	• amino acid sequence changed • frameshift • protein features (might be) affected • splice site changes • truncated protein (might cause NMD)	hyperlink

analysed issue		analysis result
name of alteration	no title	
alteration (phys. location)	chr3:46414947_46414978delGTCAGTATCAATTCTGGAAGAATTTCCAGACA	
HGNC symbol	CCR5	
Ensembl transcript ID	ENST00000343801	
Genbank transcript ID	NM_000579	
UniProt peptide	P51681	
alteration type	deletion	
alteration region	CDS	
DNA changes	c.554_585delGTCAGTATCAATTCTGGAAGAATTTCCAGACA cDNA.911_942delGTCAGTATCAATTCTGGAAGAATTTCCAGACA g.3315_3346delGTCAGTATCAATTCTGGAAGAATTTCCAGACA	

AA changes	S185Ifs*32		
position(s) of altered AA if AA alteration in CDS	185 (frameshift or PTC - further changes downstream)		
frameshift	yes		
known variant	Variant was neither found in ExAC nor 1000G. Search ExAC.		
regulatory features	H3K36me3, Histone, Histone 3 Lysine 36 Tri-Methylation H3K27me3, Histone, Histone 3 Lysine 27 Tri-Methylation		
phyloP / phastCons		PhyloP	PhastCons
	(flanking)	0.19	0
		-2.152	0
		-1.357	0
		3.918	0.535
		1.445	0.487
		0.241	0.052
		-0.07	0.002
		-0.449	0
		0.158	0
		0.448	0
		-0.049	0
		-0.81	0
		-0.112	0.012
		1.428	0.815
		1.646	0.918
		2.296	1
		6.052	1
		3.057	1
		3.488	1
		0.646	0.903

	1.344	0.82
	0.444	0.363
	0.621	0.169
	-1.983	0.001
	1.682	0.138
	2.341	0.144
	-0.033	0.013
	1.295	0.014
	0.66	0.007
	-0.942	0
	0.178	0
	1.218	0.001
	-3.056	0
	(flanking)	-1.704 0
	explain score(s) and/or inspect your position(s) in in UCSC Genome Browser	

	effect	gDNA position	score	wt detection sequence	exon-intron border
splice sites	Acc gained	3347	0.71	mu: CAGCTCTCATTTTCCATACATTAAAGATAGTCATCTTGGGG	gcc
	Acc gained	3345	0.98	mu: TGCAGCTCTCATTTTCCATACATTAAAGATAGTCATCTTGG	ctgc C
	Acc gained	3352	0.36	mu: CTCATTTTCCATACATTAAAGATAGTCATCTTGGGGCTGGT	
	Acc gained	3310	0.39	mu: ACCTGCAGCTCTCATTTTCCATACATTAAAGATAGTCATCT	tcca TACA
	Acc gained	3349	0.65	mu: GCTCTCATTTTCCATACATTAAAGATAGTCATCTTGGGGCT	c
	Donor gained	3351	0.70	mu: ATTAAAGATAGTCAT	
	Donor gained	3350	0.95	mu: CATTAAAGATAGTCA	
	Donor gained	3355	0.47	mu: AAGATAGTCATCTTG	
	Donor gained	3346	0.96	mu: CATACATTAAAGATA	TGCC

distance from splice site	565
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Kozak consensus sequence altered?	N/A
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conservation protein level for non-synonymous changes	species	match	gene	a	a	alignment	
	Human			1	8	LFYTCSSFFP YSCYQFW KNFQTLKI VILGLVLP LLVMVICYSGILKTLIRCRNEKRRH	RAVRLIFTIMIVYFLFWAPYNIVLLLNTFQEFFGLNNCSS SNRLDQAMQVTETLGMTHCCINPIIYAFVGEKFRNYLL

	no mutated	align	5							VFF	
		ment	n/a								
Ptroglo dytes	all identi cal	ENSPTRG0 0000014848	1 8 5			FFP	YSCYCFW	KNEQTILKI	VILGIVLFLIVMVICYS	GILKTIIRCRNEKKRE	RAVRLIFTIMIVYFLFWAPYNIVLLNTFQEFFGLNN CSSNRLDQAMQVTETLGMTHCCINPIYAFV
Mmulat ta	no homo logue										
Fcatus	partly conse rved	ENSFCAG0 0000003003	1 8 5			FFP	SNQYEFW	KNEQTILKMTI	LGIVLFLIVMIVCYS	AILRTILFRCRNEKKRE	RAVKLIFVIMIGYFLFWAPNNIVLLLSTFPESFGLNNO SSSNRLDQAMQVTETLGMTHCCINPIYAFV
Mmusculus	no homo logue										
Ggallus	partly conse rved	ENSGALG0 0000011732	1 9 2			TRYTCSAFYP	CEQRDEW	KCFELAKMNI	LGIVFMIMICS	YTIKTIICRNEKK- NKAVRLIFHIMIVYFFFWAPYN	
Trubripes	partly conse rved	ENSTRUG0 0000010083	1 6 7			PPA SNPECFPEYTS D	NANSEFW	SVESLIKMNI	LGIFLPIAIMLFCYS	QIIVRL- -CHSESSKKQAIRLILVVVAVFLFCWVPYNI	
Drerio	partly conse rved	ENSARG0 0000086616	2 4 6			NCTICASYPT	TEQWSYD S	KTACIFKMNVIC	ILFLSVICFCYS	MILIKLITVRS- -SRR T	QAMRLVVVMVVFCCWAPYNIAAFFKALELKKVL
Dmelanogaster	no homo logue										
Celegans	no homo logue										
Xtropicalis	no homo logue										

protein features	start (aa)	end (aa)	feature	details
	167	198	TOPO_DOM	Extracellular (Potential).

199	218	TRANSMEM	Helical; Name=5; (Potential).
219	235	TOPO_DOM	Cytoplasmic (Potential).
236	260	TRANSMEM	Helical; Name=6; (Potential).
261	277	TOPO_DOM	Extracellular (Potential).
278	301	TRANSMEM	Helical; Name=7; (Potential).
302	352	TOPO_DOM	Cytoplasmic (Potential).
321	321	LIPID	S-palmitoyl cysteine.
321	321	MUTAGEN	C->A: Small reduction in palmitoylation. Cell surface expression reduced by 50%. Greatly reduced palmitoylation. Cell surface expression greatly reduced; when associated with A-323 or A-324. No palmitoylation. Cell surface expression greatly reduced. HIV entry reduced by 50%; when associated with A-323 and A- 324.
323	323	LIPID	S-palmitoyl cysteine.
323	323	MUTAGEN	C->A: Small reduction in palmitoylation. Cell surface expression reduced by 50%. Greatly reduced palmitoylation. Cell surface expression greatly reduced; when associated with A-321 or A-324. No palmitoylation. Cell surface expression greatly reduced. HIV entry reduced by 50%; when associated with A-321 and A- 324.
324	324	MUTAGEN	C->A: Small reduction in palmitoylation. Cell surface expression reduced by 50%. Greatly reduced palmitoylation. Cell surface expression greatly reduced; when associated with A-321 or A-323. No palmitoylation. Cell surface expression greatly reduced. HIV entry reduced by 50%; when associated with A-321 and A- 323.
324	324	LIPID	S-palmitoyl cysteine.
336	336	MUTAGEN	S->A: APO-RANTES-stimulated phosphorylation reduced by 15%; APO- RANTES-stimulated phosphorylation reduced by 30-50%; when associated with A-337 or A-342 or A-349; APO-RANTES-stimulated phosphorylation reduced by 80%; when associated with A-337 and A-342 or A-349; No APO-RANTES-stimulated phosphorylation; when associated with A-337; A-342 and A349; abolishes interaction with ARRB2; when associated with S-337; S-342 and S- 349.
336	336	MOD_RES	Phosphoserine; by BARK1.
337	337	MOD_RES	Phosphoserine; by BARK1.
337	337	MUTAGEN	S->A: APO-RANTES-stimulated phosphorylation reduced by 18%; APO- RANTES-stimulated phosphorylation reduced by 30-50% on APO-RANTES stimulation; when associated with A-336 or A-342 or A-349; APO-RANTES-stimulated phosphorylation reduced by 80%; when associated with A- 336 and A-342 or A-349; No APO-RANTES-stimulated phosphorylation; when associated with A-336; A-342 and A349; abolishes interaction with ARRB2; when associated with S-336; S-342 and S-349.
342	342	MOD_RES	Phosphoserine; by BARK1.
342	342	MUTAGEN	S->A: APO-RANTES-stimulated phosphorylation reduced by 42%. Phosphorylation reduced by 50% on APO- RANTES stimulation; when associated with A-336 or A-337 or A-349; APO-RANTES- stimulated phosphorylation reduced by 80% when associated with A-336 and A-337 or A-349; No APO-RANTES-stimulated phosphorylation; when associated with A- 336; A-337 and A349; abolishes interaction with ARRB2; when associated with S-336; S-337 and S-349.
349	349	MUTAGEN	S->A: APO-RANTES-stimulated phosphorylation reduced by 43%; APO- RANTES-stimulated phosphorylation reduced by 30-50%; when associated with A-336 or A-337 or A-342; APO-RANTES-stimulated phosphorylation reduced by 80%; when associated with A-336 and A-337 or A-342; No APO-RANTES-stimulated phosphorylation stimulation; when associated with A-336; A-337 and A347; abolishes interaction with ARRB2; when associated with S-336; S-337 and S-342.
349	349	MOD_RES	Phosphoserine; by BARK1.

length of protein	strongly truncated protein, might cause NMD (-137 AA / more than 10% missing)
AA sequence altered	yes
position of stopcodon in wt / mu CDS	1059 / 648
position (AA) of stopcodon in wt / mu AA sequence	353 / 216
position of stopcodon in wt / mu cDNA	1416 / 1005
poly(A) signal	N/A

conservation nucleotide level for all changes - no scoring up to now	N/A
position of start ATG in wt / mu cDNA	358 / 358
chromosome	3
strand	1
last intron/exon boundary	347
theoretical NMD boundary in CDS	cannot be calculated, too little distance between start ATG and last intron/exon boundary
length of CDS	1059
coding sequence (CDS) position	553 / 586
cDNA position (for ins/del: last normal base / first normal base)	910 / 943
gDNA position (for ins/del: last normal base / first normal base)	3314 / 3347
chromosomal position (for ins/del: last normal base / first normal base)	46414946 / 46414979
original gDNA sequence snippet	CAGCTCTCATTTTCCATACAG TCAGTATCAATTCTGGAAGAATTTCCAGAC ATTAAAGATAGTCATCTTGGG
altered gDNA sequence snippet	CAGCTCTCATTTTCCATACATTAAAGATAGTCATCTTGGG
original cDNA sequence snippet	CAGCTCTCATTTTCCATACAG TCAGTATCAATTCTGGAAGAATTTCCAGAC ATTAAAGATAGTCATCTTGGG
altered cDNA sequence snippet	CAGCTCTCATTTTCCATACATTAAAGATAGTCATCTTGGG
wildtype AA sequence	MDYQVSSPIY DINYYTSEPC QKINVKQIAA RLLPPLYSLV FIFGFVGNML VILILINCKR LKSMTDIYLL NLAISDLFFL LTVPFWAHYA AAQWDFGNTM CQLLTGLYFI GFFSGIFFII LLTIDRYLAV VHAVFALKAR TVTFGVVTSV ITWVVAVFAS LPGIIFTRSQ KEGLHYTCSS HFPYSQYQFW KNFQTLKIVI LGLVLP LLVM VICYSGILKT LLRCRNEKKR HRAVRLIFTI MIVYFLFWAP YNIVLLNTF QEFFGLNNCS SSNRLDQAMQ VTETLGMTHC CINPIIYAFV GEKFRNYLLV FFQKHIAKRF CKCCSIFQQE APERASSVYT RSTGEQEISV GL*
mutated AA sequence	MDYQVSSPIY DINYYTSEPC QKINVKQIAA RLLPPLYSLV FIFGFVGNML VILILINCKR LKSMTDIYLL NLAISDLFFL LTVPFWAHYA AAQWDFGNTM CQLLTGLYFI GFFSGIFFII LLTIDRYLAV VHAVFALKAR TVTFGVVTSV ITWVVAVFAS LPGIIFTRSQ KEGLHYTCSS HFPYIKDShL GAGPAAACHG HLLLGNPkNS ASVSK*
speed	0.13 s

All positions are in basepairs (bp) if not explicitly stated differently.

AA/aa: amino acid; CDS: coding sequence; mu: mutated; NMD: nonsense-mediated mRNA decay; nt: nucleotide; wt: wildtype; TGP: 1000 Genomes Project

[rs1734787](#)

X:153325446

A/C



mutation
t@sting

[document](#)
[ation](#)

Prediction

polymorphism

Model: *without_aae*, prob:

0.999999245737463 ([explain](#))

Summary

[hyperlink](#)

analysed issue

analysis result

name of alteration	no title
alteration (phys. location)	chr23:153402581T>G show variant in all transcripts IGV
HGNC symbol	MECP2
Ensembl transcript ID	ENST00000303391
Genbank transcript ID	NM_004992
UniProt peptide	N/A
alteration type	single base exchange
alteration region	3'UTR
DNA changes	cDNA.15332794A>C g.0A>C
AA changes	N/A

position(s) of altered AA if AA alteration in CDS	N/A
frameshift	N/A
known variant	Variant was neither found in ExAC nor 1000G. Search ExAC.
regulatory features	H2BK20ac, Histone, Histone 2B Lysine 20 Acetylation H2BK5ac, Histone, Histone 2B Lysine 5 Acetylation H2BK120ac, Histone, Histone 2B Lysine 120 Acetylation DNase1, Open Chromatin, DNase1 Hypersensitive Site E2F6, Transcription Factor, E2F6 Transcription Factor Binding H3K4me3, Histone, Histone 3 Lysine 4 Tri-Methylation H2AZ, Histone, Histone 2A variant Z Promoter Associated, Regulatory Feature, Promoter like regulatory feature CTCF, Transcription Factor, CCCTC-binding factor
phyloP / phastCons	PhyloP PhastCons (flanking) -0.169 0.001 explain score(s) and/or inspect your position(s) in in UCSC Genome Browser
splice sites	no abrogation of potential splice sites
distance from splice site	39369
Kozak consensus sequence altered?	N/A
conservation protein level for non-synonymous changes	N/A
protein features	N/A
length of protein	N/A
AA sequence altered	N/A
position of stopcodon in wt / mu CDS	N/A

position (AA) of stopcodon in wt /
mu AA sequence

N/A

position of stopcodon in wt / mu
cDNA

N/A

poly(A) signal

signal is predicted to be ok

conservation

nucleotide level for all changes - no
scoring up to now

N/A

position of start ATG in wt / mu
cDNA

251 / 251

chromosome

23

strand

-1

last intron/exon boundary

628

theoretical NMD boundary in
CDS

327

length of CDS

1461

coding sequence (CDS) position

N/A

cDNA position

(for ins/del: last normal base / first
normal base)

15332794

gDNA position

(for ins/del: last normal base / first
normal base)

0

chromosomal position

(for ins/del: last normal base / first
normal base)

153402581



mutation t@sting

[documentation](#)

Prediction

polymorphism

Model: *without_aae*, prob:

0.999999245737463 ([explain](#))

Summary

[hyperlink](#)

analysed issue

analysis result

name of alteration	no title
alteration (phys. location)	chr23:153402581T>G show variant in all transcripts IGV
HGNC symbol	MECP2
Ensembl transcript ID	ENST00000453960
Genbank transcript ID	NM_001110792
UniProt peptide	N/A
alteration type	single base exchange
alteration region	3'UTR
DNA changes	cDNA.15332599A>C g.0A>C
AA changes	N/A
position(s) of altered AA if AA alteration in CDS	N/A
frameshift	N/A
known variant	Variant was neither found in ExAC nor 1000G. Search ExAC.

regulatory features	H2BK20ac, Histone, Histone 2B Lysine 20 Acetylation H2BK5ac, Histone, Histone 2B Lysine 5 Acetylation H2BK120ac, Histone, Histone 2B Lysine 120 Acetylation DNase1, Open Chromatin, DNase1 Hypersensitive Site E2F6, Transcription Factor, E2F6 Transcription Factor Binding H3K4me3, Histone, Histone 3 Lysine 4 Tri-Methylation H2AZ, Histone, Histone 2A variant Z Promoter Associated, Regulatory Feature, Promoter like regulatory feature CTCF, Transcription Factor, CCCTC-binding factor
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phyloP / phastCons	PhyloP PhastCons (flanking) -0.169 0.001 explain score(s) and/or inspect your position(s) in in UCSC Genome Browser
splice sites	no abrogation of potential splice sites
distance from splice site	39404
Kozak consensus sequence altered?	N/A
conservation protein level for non-synonymous changes	N/A
protein features	N/A
length of protein	N/A
AA sequence altered	N/A
position of stopcodon in wt / mu CDS	N/A
position (AA) of stopcodon in wt / mu AA sequence	N/A
position of stopcodon in wt / mu cDNA	N/A
poly(A) signal	signal is predicted to be ok

conservation nucleotide level for all changes - no scoring up to now	N/A
position of start ATG in wt / mu cDNA	56 / 56
chromosome	23
strand	-1
last intron/exon boundary	469
theoretical NMD boundary in CDS	363
length of CDS	1497
coding sequence (CDS) position	N/A
cDNA position (for ins/del: last normal base / first normal base)	15332599
gDNA position (for ins/del: last normal base / first normal base)	0
chromosomal position (for ins/del: last normal base / first normal base)	153402581

[rs17435](#)

X:153311980

Prediction

polymorphism

Model: *without_aae*, prob: 0.999999245737463 ([explain](#))

Summary

[hyperlink](#)

analysed issue

analysis result

name of alteration	no title
alteration (phys.	chr23:153402581T>A show variant in all transcripts IGV

location)

HGNC symbol	MECP2
Ensembl transcript ID	ENST00000303391
Genbank transcript ID	NM_004992
UniProt peptide	N/A
alteration type	single base exchange
alteration region	3'UTR
DNA changes	cDNA.15331448A>T g.0A>T
AA changes	N/A
position(s) of altered AA	N/A
if AA alteration in CDS	
frameshift	N/A
known variant	Variant was neither found in ExAC nor 1000G. Search ExAC.
regulatory features	H2BK20ac, Histone, Histone 2B Lysine 20 Acetylation H2BK5ac, Histone, Histone 2B Lysine 5 Acetylation H2BK120ac, Histone, Histone 2B Lysine 120 Acetylation DNase1, Open Chromatin, DNase1 Hypersensitive Site E2F6, Transcription Factor, E2F6 Transcription Factor Binding H3K4me3, Histone, Histone 3 Lysine 4 Tri-Methylation H2AZ, Histone, Histone 2A variant Z Promoter Associated, Regulatory Feature, Promoter like regulatory feature CTCF, Transcription Factor, CCCTC-binding factor
phyloP / phastCons	PhyloP PhastCons (flanking) -0.169 0.001 explain score(s) and/or inspect your position(s) in in UCSC Genome Browser

splice sites	no abrogation of potential splice sites
distance from splice site	39369
Kozak consensus sequence altered?	N/A
conservation protein level for non-synonymous changes	N/A
protein features	N/A
length of protein	N/A
AA sequence altered	N/A
position of stopcodon in wt / mu CDS	N/A
position (AA) of stopcodon in wt / mu AA sequence	N/A
position of stopcodon in wt / mu cDNA	N/A
poly(A) signal	signal is predicted to be ok
conservation nucleotide level for all changes - no scoring up to now	N/A
position of start ATG in wt / mu cDNA	251 / 251
chromosome	23
strand	-1
last intron/exon boundary	628
theoretical NMD boundary in CDS	327

length of CDS	1461
coding sequence (CDS) position	N/A
cDNA position (for ins/del: last normal base / first normal base)	15331448
gDNA position (for ins/del: last normal base / first normal base)	0
chromosomal position (for ins/del: last normal base / first normal base)	153402581