

1 **Title page**

2 **DC-SIGN Polymorphisms Associated with Risk of Hepatitis C Virus**

3 **Infection Among Men who Have Sex with Men but not Among Injecting**

4 **Drug Users**

5 **Running Title: DC-SIGN SNPs and HCV susceptibility**

6

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10 **observational Study of Acute infection with Hepatitis C) study group and the ACS**

11 **(Amsterdam Cohort Studies)**

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48 **Abstract**

49 We aimed to identify whether genetic polymorphisms within L-SIGN or DC-SIGN correlate with
50 HCV susceptibility. An MSM and an IDU cohort of HCV cases and multiple-exposed uninfected
51 controls were genotyped for numerous L-SIGN and DC-SIGN polymorphisms. DC-SIGN SNPs -
52 139, -871 and -939 correlate with HCV acquisition in the MSM cohort only. When the same
53 SNPs were introduced into a transcription activity assay they demonstrated a reduction in
54 expression with predicted alteration in binding of transcription factors. DC-SIGN promoter
55 SNPs correlate with risk of HCV acquisition via sexual but not IDU exposure, likely through
56 modulation of mRNA expression levels.

57

58 **Keywords:** HCV; HIV-1; Lectins, DC-SIGN; polymorphism, single nucleotide; MSM; sexual
59 transmission

60

61 **Introduction**

62 Hepatitis C virus (HCV) represents a major global health burden, with 350.000 people dying
63 annually from HCV-related liver disease.[1] Intravenous drug use is now the major
64 transmission route. Nevertheless, since 2000, sexual transmission has been reported
65 frequently among HIV-infected men who have sex with men (MSM) and is associated with
66 high-risk sexual behavior. Interestingly, some individuals remain uninfected despite practicing
67 high-risk behavior(s). Studies have shown that ultimately 10-20% of injecting drug users (IDU)
68 do not seroconvert, suggesting a biological reason why some individuals are less prone to
69 contract HCV. [2]

70 DC-SIGN (dendritic cell specific ICAM-grabbing non-integrin, CD209) and L-SIGN (DC-
71 SIGN related, CD209L) are c-type lectins, which have been implicated to play a role in HCV
72 transmission and infection.[3] DC-SIGN is a calcium-dependent cell surface lectin on the
73 surface of dendritic cells (DCs).[4] DCs are localized in skin and mucosal tissues and may serve
74 as a replication reservoir for HCV.[4,5] L-SIGN is mainly expressed on liver and lymph node
75 sinusoidal endothelial cells. It shares 77% amino acid identity with DC-SIGN and it has been
76 shown to capture several viruses including HCV.[3] Whereas the DC-SIGN neck region on exon
77 4 is highly conserved (7 repeats in the majority of individuals) the L-SIGN neck region is very
78 variable.[6] This repeat region has been suggested to affect disease susceptibility and outcome
79 for HIV-1 infection. [7–10]

80 The objective of this study was to analyze the frequency of previously reported genetic
81 variations in DC/L-SIGN genes in individuals from two well-defined cohorts at risk of HCV
82 infection who either seroconverted or remained uninfected. We identified three DC-SIGN SNPs
83 that were associated with HCV susceptibility through high risk sexual exposure but not with
84 IDU. Furthermore, we assessed whether these SNPs in the DC-SIGN promoter affect its activity.

85

86 **Patients and Methods**

87 *Study populations*

88 *1. MSM cohort (MOSAIC)*

89 Sixty-two HIV-1 infected, Western European MSM participating in the MSM Observational
90 Study of Acute Infection with Hepatitis C (MOSAIC) cohort were included. Risk behavior data
91 was available from behavioral questionnaires collected at 6 month intervals. Participants were
92 categorized as multiple exposed uninfected (MEU, n = 30) or multiple exposed infected (MEI,
93 n=32) based on reported behavioral risk factors at inclusion or any of the follow up visits,
94 which have been shown to be associated with increased risk of acquiring HCV sexually in the
95 MOSAIC cohort. Distribution of risk factors (i.e. no or inconsistent condom use, anal
96 intercourse with an HCV-infected sex partner, fisting, use of sex toys, rectal bleeding during or
97 after sex, and group sex) is summarized in supplemental Table 1. The MOSAIC study was
98 approved by the Institutional Review Board of the Academic Medical Center under assigned
99 study numbers NL26485.018.09 and NL48572.018.14.

100

101 *2. IDU cohort (ACS)*

102 Sixty-two Western European participants from the Amsterdam Cohort Studies (ACS) among
103 IDU were selected, who started injecting drugs intravenously before 1990, which was a period
104 with high incidence of HCV among drug users (up to 27.5/100 person years in the 1980s in this
105 cohort).[11] The ACS among IDU was an open prospective cohort study recruiting drug users
106 between 1985 and 2016 investigating the epidemiology, the natural history and pathogenesis
107 of HIV-1 infection and other blood-borne and/or sexually transmitted diseases. Participants
108 who injected more than 2 years and remained HCV seronegative during follow-up (n = 40)
109 were classified as MEU where as 22 MEI seroconverted for HCV during follow up. Total
110 duration of injecting drugs and follow up was similar for MEU and MEI (supplemental Table 1).
111 The ACS study was approved by the Institutional Review Board of the Academic Medical
112 Center under assigned study numbers MEC 07/182 and MEC 09/040.

113

114 *DNA isolation and genotyping*

115 DNA was isolated from 200 μ l participant serum utilising the QIAamp DNA blood mini kit
116 according to the manufacturer's protocol (Qiagen). The number of repeat domains within the
117 L-SIGN repeat region was determined for each subject by PCR. PCR reactions contained 5 μ l of
118 template DNA, 400nM forward primer, 400nM reverse primer, 2.5 mM MgCl₂, 0.2 mM dNTPs,
119 0.1 mg/mL Bovine Serum Albumin (BSA), 1.25 units FastStart Taq DNA polymerase in a total
120 volume of 25 μ L 1x Faststart PCR buffer.

121 L-SIGN SNP rs2277998 was assessed using the Ready-to-use hot start reaction mix for
122 High Resolution Melting (HRM) curve analysis using the LightCycler[®] 480 (Roche). The reaction
123 contained 2.0 μ l DNA template, 2.5 mM MgCl₂, 8 ng α -casein, 450 nM Fwd primer (Biolegio)
124 and 450 nM Rev primer (Biolegio) in a total volume of 20 μ L 1x HRM master mix.

125 To assess reported DC-SIGN SNPs in the promoter region at positions -939 (rs735240),
126 -871 (rs735239), -336 (rs4804803) and -139 (rs2287886), a DNA fragment covering
127 approximately 1000 bp upstream of the ATG translation start site was amplified with two
128 primer sets. The amplicons were sequenced in both directions with the same primers using Big
129 dye terminator according to manufacturer's instructions (Applied Biosystems, Inc., Norwalk
130 CT). Primers and amplification conditions are summarized in supplemental Table 2.

131

132 *Cell culture*

133 HEK 293T/17 cells (ATCC number: CRL-11268) were cultured in DMEM (Invitrogen)
134 supplemented with 10% FCS, 1x MEM Non-Essential Amino Acids (Gibco), 100 U/ml penicillin
135 and 100 U/ml streptomycin. Cells were incubated at 37°C in 5% CO₂ and passaged twice a
136 week upon 90% confluence.

137

138 *Construction DC-SIGN promoter expression construct*

139 The DC-SIGN promoter variants were constructed by amplifying the DC-SIGN promoter region
140 from DNA from one study participant with the -139A, -871A and -939G variants using primers
141 tailed with *XhoI* and *HindIII* restriction sites. The amplicons were cloned into the pGL10.4
142 vector[luc2] (Promega) at the *XhoI* and *HindIII* sites. Promoter variants (see supplemental
143 Figure 1) were established by site directed mutagenesis. Mutations were made with the
144 QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies) with specific mutagenic
145 primers (see Supplemental Table 2).

146

147 *Transfection of 293T cells with promoter constructs and analysis of luciferase expression*

148 293T/17 cells were transfected with the various DC-SIGN promoter constructs and a Renilla
149 luciferase expression plasmid (pRL-CMV) (Promega) for normalization in a 50:1 ratio using
150 Xtremegene (Invitrogen) according to manufacturer's protocol. Cells were incubated 24 hours
151 and lysed with Passive lysis buffer (Promega). 5 μ l of the lysate was used to measure Firefly
152 and Renilla luciferase activity with Dual-Glo luciferase assay system (Promega) according to
153 manufacturer's protocol.

154

155 *Prediction of transcription factor (TF) binding sites*

156 TF binding sites were predicted using the PROMO database (<http://alggen.lsi.upc.es/>) which
157 uses TRANSFAC for prediction. [16]

158

159 *Statistical analysis*

160 DC/L-SIGN SNP genotype frequencies between MEU and MEI were compared using logistic
161 regression. Initially, an additive/dominance deviation joint 2 degree of freedom test (with two
162 genotype-dependent variables in the regression, one with 0/1/2 coding and the second with
163 0/1/0 coding) was carried out. Subsequently, in case of dominance deviation ($p < 0.1$), a
164 dominant or recessive genetic model was assumed, otherwise an additive genetic model was

165 assumed in the logistic regression model used to estimate the odds ratio (OR) and
166 corresponding 95% confidence interval. A p value <0.05 was considered statistically significant
167 and all analyses were carried out using SPSS software (IBM, version 20).

168

169 **Results**

170 ***DC-SIGN -139GG, -871GG and -939AA are associated with reduced HCV susceptibility in MSM***

171 Patient characteristics are summarized in supplemental Table 1. In the MSM cohort, three DC-
172 SIGN SNPs were significantly associated with HCV infection (Table 1). The -139GG was found
173 more frequently in MEU (63.3% in MEU compared to 37.5% in MEI). Additionally, -871GG
174 (36.7% in MEU compared to 12.5% in MEI) and the -939AA (53.3% in MEU compared to 21.9%
175 in MEI) were found more often in MEU, indicating that -139GG, -871GG and -939AA genotypes
176 protect against HCV acquisition (OR: 0.35 $p=0.045$, OR: 0.23 $p=0.027$ and OR: 0.23 $p=0.009$
177 respectively). The -336 SNP was not significantly associated with HCV susceptibility. In the ACS
178 IDU cohort, no significant associations were found between SNPs and HCV susceptibility.

179

180 ***No associations between L-SIGN polymorphisms and HCV susceptibility***

181 No association with HCV susceptibility was found for L-SIGN SNP rs2277998. In addition, the L-
182 SIGN repeat distribution between MEI and MEU was similar for both cohorts (supplementary
183 Table 3). No significant difference in zygosity for the L-SIGN repeat was found between MEI
184 and MEU (OR: 0.982 $p=0.961$) (supplementary Table 4).

185

186 ***DC-SIGN promoter SNPs affect promoter activity***

187 We tested the effect of the promoter variants within the DC-SIGN promoter on transcription
188 activity by using luciferase promoter constructs (Figure 1). The -139G caused a 2.6 fold
189 reduction ($p<0.001$), the -871G a 3.3 fold reduction ($p<0.001$) and the -939A a 1.4 fold

190 reduction ($p=0.086$). This data suggests that the DC-SIGN promoter variants affect
191 transcription levels and thereby protein and cell surface expression patterns.
192 Next, we investigated whether the observed decrease in DC-SIGN promoter activity for specific
193 SNPs could be due to alterations in TF binding sites, by a *in silico* comparison of predicted TF
194 binding sites of promoter variants (Figure 1B). The variants at the -139, -871 and -939 sites do
195 affect multiple predicted TF binding sites, with some putative sites lost (GR, C/EBP, Pr-B, Pr-A,
196 HOXD9, HOXD10) and some TF binding sites gained (GR-Alpha, AP-2Alpha). This would indicate
197 that the SNPs identified within the DC-SIGN promoter region can modulate activity through
198 differential binding of transcription factors.

199

200 **Discussion**

201 Here we investigated whether polymorphisms in DC-SIGN and L-SIGN correlated susceptibility
202 to HCV infection in two well-defined cohorts consisting of individuals at high risk of HCV
203 infection through sexual or intravenous exposure. We selected polymorphisms based on what
204 has been reported within the literature for HCV as well as other infectious agents. In the MSM
205 cohort we identified an association of HCV susceptibility with three DC-SIGN SNPs. These SNPs
206 were not associated with HCV susceptibility in the IDU cohort. No effects were found for the
207 DC-SIGN -336 SNP, the L-SIGN SNP rs2277998 and repeat polymorphism in either cohort.

208 We studied four SNPs in the DC-SIGN promoter region, of which three (-139, -871 and -
209 939) were found to correlate with HCV susceptibility in MSM, with -139G showing the
210 strongest effect. Although the same SNPs have previously been associated with other
211 infectious diseases, this is the first time SNPs have been reported to be associated with
212 susceptibility to sexual transmission of HCV. Interestingly, the -139G SNP has also been
213 reported to protect against sexual transmission of HIV-1.[14]

214 It has been published previously that the combination of -139G and -939A in the DC-
215 SIGN promoter region significantly reduces DC-SIGN expression on immature DCs compared to

216 -139A and -939G.[15] We now show that the -139G and -871G SNP independently cause a
217 reduction in promoter activity, while the -939A variant failed to reach statistical significance (p
218 = 0.085). The DC-SIGN promoter encodes multiple TF binding sites which are *in silico* predicted
219 to be affected by the -139, -871 and -939 variants. This strongly suggests that the decreased
220 promoter activity observed *in vitro* is (at least partly) caused by a reduction in TF binding,
221 which will require further testing.

222 As our study was small, our observations clearly need to be confirmed in larger
223 cohorts. However, the functional data supports the associations of the SNPs with protection
224 against HCV acquisition. Collectively, our data suggest that DC-SIGN plays a role in HCV
225 acquisition via sexual and not intravenous exposure. This effect appears to be mediated by
226 reduced DC-SIGN expression, which suggest that DC-SIGN on DCs plays a role in sexual
227 transmission of HCV, similar to its role in HIV infection. [4]. We hypothesize that DCs transfer
228 HCV to the liver through DC-SIGN; individuals with the protective genotypes will have lower
229 DC-SIGN expression, resulting in a reduced susceptibility to sexual acquisition of HCV.
230 Alternatively, DC-SIGN expression on DCs at mucosal surfaces may influence HCV antigen
231 capture and induction of localized immune responses and modulate mucosal protection
232 against HCV acquisition, which does not play a role in intravenous exposure. Further studies
233 into the exact mechanism behind DC-SIGN affecting HCV infection susceptibility are warranted
234 to better understand how DC-SIGN expression levels might influence immune responses, as
235 well as mechanisms of transmission.

236

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245 Service of Amsterdam, the Academic Medical Center of the University of Amsterdam, Sanquin
246 Blood Supply Foundation, the University Medical Center Utrecht, and the Dutch HIV
247 Monitoring Foundation.

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249

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289 their relevance for human cytomegalovirus reactivation and disease after allogeneic stem-cell
290 transplantation. *Clin Microbiol Infect* 2008; **14**:228–234.

291

292

293 **Table and Figure Legends**

294 **Table 1** Distribution of DC/L-SIGN SNPs in MEI and MEI individuals

295 rs2287886 GG, rs735240 AA and rs735239 GG genotypes are significantly associated with protection against HCV

296 acquisition in the MOSAIC (MSM) cohort. No significant associations within the ACS (IDU) cohort.

297

298 **Figure 1** Effect SNPs on DC-SIGN promoter activity *A*, The -139 SNP causes a reduction of 2.6 fold ($p=0.0005$), the -871

299 SNP of 3.3 fold ($p=0.0009$) and the -939 SNP a 1.4 fold (not significant). *B*, Protective SNPs affect TF binding sites in the

300 DC-SIGN promoter. Putative binding of TFs to DC-SIGN promoter sequences with and without SNPs. Some TFs do not

301 bind anymore to the sequence containing protective SNPs (orange), some bind both sequences (blue) and some bind

302 exclusively to the SNP containing the protective variant (green).

303

304 **Supplemental Table legends**

305 **Supplemental Table 1** Patients characteristics from the MOSAIC and ACS cohorts

306 **Supplemental Table 2** Primers and PCR conditions used for analysis of the DC/L-SIGN polymorphisms

307 **Supplemental Table 3** Distribution L-SIGN repeat region among MEI and MEU individuals

308 **Supplemental Table 4** Zygosity L-SIGN repeat region compared between MEI and MEU individuals

309 No difference in L-SIGN zygosity between MEI and MEU individuals.

310

311 **Supplemental Figure legends**

312 **Supplemental Figure 1** Graphical representation of the DC-SIGN promoter and expression plasmid pGL4.10 construct

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322 Table 1

MOSAIC						MEI vs MEU			
	genotype	MEI (n)	MEI (%)	MEU (n)	MEU (%)	Dominance deviation ^a	OR	95% CI	p value
L-SIGN rs2277998	AA	1	3%	4	13%	0.09 ^b	0.83 (GG vs AG+AA)	0.29 to 2.37	0.73
	AG	11	34%	6	20%				
	GG	20	63%	20	67%				
DC-SIGN - 139	AA	3	9%	6	20%	0.01 ^b	0.35 (GG vs AG+AA)	0.12 to 0.97	0.04 ^c
	AG	17	53%	5	17%				
	GG	12	38%	19	63%				
DC-SIGN - 336	AA	22	69%	25	83%	0.99	2.32 (per G allele)	0.74 to 7.32	
	AG	9	28%	5	17%				
	GG	1	3%	0	0%				
DC-SIGN - 871	AA	16	50%	12	40%	0.08 ^b	0.23 (GG vs AG+AA)	0.06 to 0.85	0.03 ^c
	AG	12	38%	6	20%				
	GG	4	13%	11	37%				
DC-SIGN - 939	AA	7	22%	16	53%	<0.01 ^b	0.23 (AA vs AG+GG)	0.07 to 0.69	0.01 ^c
	AG	18	56%	5	17%				
	GG	7	22%	8	27%				
ACS						MEI vs MEU			
	genotype	MEI (n)	MEI (%)	MEU (n)	MEU (%)	Dominance deviation	OR	95% CI	p value
L-SIGN rs2277998	AA	1	4,50%	2	5%	0.85	1.06 (per A allele)	0.44 to 2.56	0.896
	AG	10	45,50%	17	43%				
	GG	11	50,00%	21	53%				
DC-SIGN - 139	AA	5	22,70%	10	25%	0.22	0.70 (per A allele)	0.36 to 1.38	0.3
	AG	5	22,70%	16	40%				
	GG	12	54,50%	14	35%				
DC-SIGN - 336	AA	15	68,20%	28	70%	0.50	1.20 (per G allele)	0.55 to 2.60	0.65
	AG	4	18,20%	9	23%				
	GG	3	13,60%	3	8%				
DC-SIGN - 871	AA	13	59,10%	18	45%	0.21	0.79 (per G allele)	0.36 to 1.74	0.56
	AG	6	27,30%	18	45%				
	GG	3	13,60%	4	10%				
DC-SIGN - 939	AA	4	18,20%	7	18%	0.56	0.87 (per A allele)	0.42 to 1.79	0.71
	AG	8	36,40%	18	45%				
	GG	10	45,50%	15	38%				

^a P-value of dominance deviation test^b Dominance deviation p-value < 0.1^c Statistically significant (<0.05)

325

326 **Supplementary Table 1**

Characteristics	MOSAIC		ACS	
	MEI	MEU	MEI	MEU
n (total=124)	32	30	22	40
Mean age ± SD	43.0 ±6.9	48.5 ±7.9	52.0 ±6.9	52.8 ±7.2
% Male gender	100%	100%	50%	72.5%
% Dutch Nationality	87.5%	96.7%	86.4%	92.5%
% HIV positive at entry	100%	100%	0%	0%
HIV seroconversion during follow-up	n.a	n.a	13.6%	0%
Median start date of Follow-up (IQR)	22/2/2011 (4/2/2010- 2/8/2011)	14/2/2011 (19/5/2010- 20/12/2011)	23/2/1988 (15/1/1987- 08/02/1992)	20/10/1992 (12/09/1988- 22/04/1998)
Median time of follow-up ± SD	4.01 ± 1.80	3.78 ± 1.30	14.96 ± 5.65	14.31 ±5.62
Mean duration IDU in years	4 IDU in last 6 months (no duration)	n.a	7.21 ±3.42	8.45±4.83
% Reported sharing of needles[§]	0%	0%	75%	55%
Having an HCV-infected sex partner*	7	1	n.a	n.a
Fisting[§]				
With steady partner	9	5	n/a	n/a
With casual partner(s)	10	8	n/a	n/a
Use of sex toys[§]				
With steady partner	13	12	n/a	n/a
With casual partner(s)	15	4	n/a	n/a
Rectal bleeding during or after sex[§]				
With steady partner	0	10	n/a	n/a
With casual partner(s)	15	8	n/a	n/a
Groupsex[§]	24	23	n/a	n/a
Rectal bleeding during or after sex*	17	5		
CD4 count last negative moment(cases)/last visit (controls)*	523±138	621±222	n.a	n.a
CD4 count nadir	277±160	269±179	n.a	n.a
Baseline Mosaic Risk score (medium)*[#]	2,9	1,1		

327

328 n.a. = not applicable,

329 * = p < 0,05

330 [§] reported at least once331 [#] [10]

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344 **Supplementary table 2¹**

Name	Orientation	Primer sequence 5'→3'	fragment length
L-SIGN repeat	Fwd	CCTAAGTCAGGAACAATCCGA	284bp, 353bp, 422bp, 491bp, 560bp, 629bp, 698bp (3/4/5/6/7/8/9 repeats, respectively)
	Rev	GAACTCACAAATGCAGTCTTCAAATC	
L-SIGN SNP rs2277998	Fwd	GTCTAACTCCCAGCGGA	45bp
	Rev	TGGCAGGCGGTGACG	
DC-SIGN promotor PCR 1	Fwd	GCAGTCTTGGTTCCTTGGAG	630bp
	Rev	ACTTGCACTGCCTCCTCAGT	
DC-SIGN promotor PCR 2	Fwd	TGCTGCTGTCCTCATTTTTG	638bp
	Rev	AGCATACAGAAACCCCGTTG	
Mutagenesis primer -139	Fwd	TAGGGATCTGTCATCCAAAAGGCTAGTGGAAAGCATCAGAGCA	
	Rev	TGCTCTGATGCTTTCCACTAGCCTTTTGGATGACAGATCCCTA	
Mutagenesis primer -871	Fwd	AGTACTAGTACATTTAATAACGTAGATAAATCTCACAAAACAG	
	Rev	CTGTTTTGTGAGATTTATCTACGTTATTAAATGTACTAGTACT	
Mutagenesis primer -939	Fwd	CACACTGTAAGATTTGATTTTATGTGAATTTTGAGAACAGGCA	
	Rev	TGCCTGTTCTCAAATTCACATAAAATCAAATCTTACAGTGTG	

¹ Amplification conditions:

L-SIGN repeat: denaturation at 95°C for 5 min, followed by 45 cycles at 95°C for 30s, 60°C for 30s and 72°C for 1 min and a final extension step at 72°C for 10 min.

L-SIGN SNP rs2277998: 50°C for 2 min, denaturation at 95°C for 10 min, followed by 45 cycles at 95°C for 15s and 60°C for 15s, 72°C for 20s, followed by an HRM protocol of 95°C for 1 min, 40°C for 1 min and a fluorescence acquisition step at 60°C for 45s.

DC-SIGN SNPs: denaturation at 95°C for 5 min, followed by 5 cycles at 94°C for 30s, 61°C for 30s (-0.5°C every cycle) and 72°C for 45s followed by 32 cycles at 94°C for 30s, 60°C for 30s and 72°C for 45s and a final extension step at 72°C for 10min.

345 **Supplementary Table 3**

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MOSAIC		MEI vs MEU						
	genotype	MEI (n)	MEI (%)	MEU (n)	MEU (%)	OR	95% CI	p value
DC-SIGN-139	AA	3	11%	2	25%	0.25 (GG vs AG+AA)	0.105 - 1.30	0.09
	AG	16	59%	1	13%			
	GG	8	30%	5	63%			
DC-SIGN-871	AA	15	56%	4	50%	0.08 (GG vs AG+AA)	0.01 - 0.59	< 0.01
	AG	10	37%	0	0%			
	GG	2	7%	4	50%			
DC-SIGN-939	AA	5	19%	5	63%	0.14 (AA vs AG+GG)	0.02 - 0.77	0.02
	AG	16	59%	0	0%			
	GG	6	22%	3	50%			

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351 **Supplementary Table 4**

	genotype n(%)											
	4/5	4/6	4/7	5	5/6	5/7	5/9	6	6/7	6/9	7	7/9
MEI	0 (0.0)	0 (0.0)	5 (9.6)	2 (3.8)	2 (3.8)	9 (17.3)	1 (1.9)	1 (1.9)	5 (9.6)	1 (1.9)	22 (42.3)	4 (7.7)
MEU	1 (1.5)	1 (1.5)	1 (1.5)	6 (8.8)	5 (7.4)	13 (19.1)	1 (1.5)	3 (4.4)	13 (19.1)	0 (0.0)	24 (35.3)	0 (0.0)

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355 **Supplementary Table 5**

	homozygous	heterozygous	OR	p value
MEI	25 (48.1%)	27 (51.9%)	0.9820	0.9608
MEU	33 (48.5%)	35 (51.5%)		
Total	58 (48.3%)	62 (51.7%)		

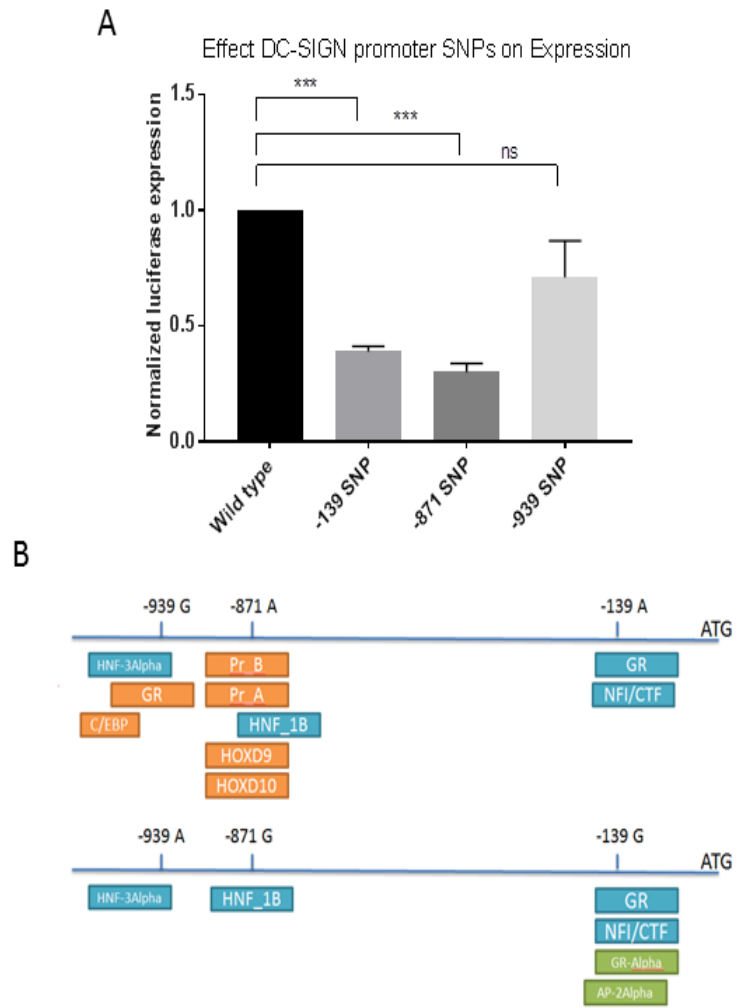
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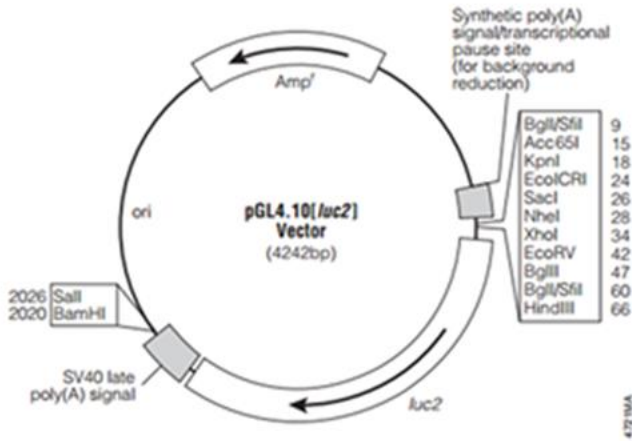


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362 Fig 1

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SNPs:

- 139 (rs2287886) **A** or **G**
- 871 (rs735239) **A** or **G**
- 939 (rs735240) **G** or **A**

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366 Fig S1