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INVESTIGATION OF THE ASSOCIATION BETWEEN THE POLYMORPHISM *SLC2A9* RS2280205 AND GOUT IN VIETNAMESE POPULATION

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ABSTRACT

Gout, a predominant form of inflammatory arthritis, is characterized by the deposition of uric acid crystals in joints and other tissues. Genome-wide association studies (GWAS) have established the association between gout and genetic variants of solute carrier family 2 member 9 (*SLC2A9*). In this study, we assessed the association of the single nucleotide polymorphism *SLC2A9* rs2280205 with gout susceptibility in the Vietnamese population. Genomic DNA was extracted from the peripheral blood of 477 samples (160 gout patients and 317 healthy controls). The polymorphism rs2280205 of the *SLC2A9* gene was genotyped using the PCR-RFLP method. Results indicated that the allele distribution of this variant was in accordance with Hardy-Weinberg Equilibrium, and the genotype frequencies of rs2280205 GG/GA/AA were 0.65, 0.33, and 0.02, respectively. However, no relation was identified between *SLC2A9* rs2280205 and gout in this studied population. This research contributed a preliminary insight into the genetic factors in the development of gout in the Vietnamese population.

Keywords: gout, PCR-RFLP, rs2280205, *SLC2A9*, Vietnamese.

INTRODUCTION

Gout is a common form of inflammatory arthritis characterized by recurrent attacks of severe pain, redness, swelling, and tenderness in the joints, which severely reduce life quality and pose serious health threats. Gout has become more common worldwide, as its prevalence increased from < 1% to 6.8% (2009 - 2013), with an incidence of 0.58 to 2.89 cases per 1,000 person-years (Dehlin *et al.*, 2020). According to the Community Oriented Program for the Control of Rheumatic Disease (COPCORD), the prevalence of gout in Hanoi is approximately 0.14% (Minh Hoa *et al.*, 2003). This inflammatory condition is caused by the deposition of urate crystals in the affected joints and surrounding tissues, which occurs when the

uric acid level in the blood surpasses 0.41 mmol/l (6.8 mg/dl) (Dalbeth *et al.*, 2021). Gout patients have high risks for diabetes, obesity, chronic kidney disease, and cardiovascular disease. Among the main factors leading to an imbalance in uric acid levels, genetic factors also play an important role in the development of gout (Zhang *et al.*, 2016). The relationship between gout susceptibility and the variants in the *SLC2A9* gene has been studied in different ethnic groups, such as Germans and Americans (Hamajima *et al.*, 2011; Vitart *et al.*, 2008).

Located on chromosome 4p16, *SLC2A9* contains 13 exons and spans over a length of 214 kb (Vitart *et al.*, 2008). It is mainly expressed in the kidneys and liver (Liu *et al.*, 2011). The gene encodes a membrane protein GLUT9, which is responsible for the transport and reabsorption of uric acid across cell membranes, allowing the body to regulate serum urate levels in the body (Wang *et al.*, 2019). GWAS of German populations demonstrated the relationship between polymorphism *SLC2A9* rs2280205 and gout disease (Döring *et al.*, 2008). To investigate whether a similar linkage could be established in the Vietnamese population, we carried out a case-control association study of the variation rs2280205 in the *SLC2A9* gene.

MATERIALS AND METHODS

Study subjects

The study was approved by the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology. All subjects gave informed consent before blood collection. Peripheral blood samples from 477 participants, including 160 gout patients and 317 controls, were collected at the Nguyen Trai Hospital, Ho Chi Minh City, Vietnam. Participants in the control group were randomly recruited with no family history of diabetes or gout. Gout patients were determined following the criteria of the American College of Rheumatology (Neogi *et al.*, 2015).

SNP Genotyping

The total DNA from the frozen blood samples was extracted using the Exgene Whole Blood DNA Extraction Kit (GeneAll, Korea) following the manufacturer's protocol. The purity and concentration of the DNA were checked using a NanoDrop™ One Spectrometer (Thermo Fisher). Then, all the DNA samples were diluted to 10 ng/µl with TE and preserved at -20°C. The DNA fragment containing the polymorphism *SLC2A9* rs2280205 was amplified by PCR using specific primers. The primers were designed using the Primer-BLAST tool (NCBI). The quality of the primers was evaluated using the OligoAnalyzer tool to prevent dimerization (Table 1).

The components used in the PCR reaction (7 µl) included 4.065 µl of nuclease-free water (H_2O); 0.7 µl DreamTaq buffer (10X); 0.42 µl dNTPs (2.5µM); 0.035 µl Taq DNA polymerase (5U/µL); 0.14 µl primer F/R (10 pM) and 1.5 µl DNA template (10 n/mL). The thermal cycle of the reaction was 95°C in 5 minutes; 35 cycles of 95°C in 35 seconds, 60°C in 40 seconds, and 72°C in 30 seconds; and finally, at 72°C in 8 minutes. Then, the PCR products were digested using the restriction enzyme (RE) *Hpa*II. The products were analyzed by electrophoresis on 2.5% agarose gel. The genotypes of *SLC2A9* rs2280205 were determined based on the number and size of DNA bands in the agarose gel (Table 1).

Table 1. General information on *SLC2A9* rs2280205 polymorphism in the study population.

Polymorphism	Primers (5' - 3')	PCR Size (bp)	Genotype	Fragment (bp)
SLC2A9 (rs2280205)	F: 5'GTAACCCCCTGTGGCATTCT3' R: 5'CATCCACGCCTCTCCACCTT3'	285	GG	177; 108
			GA	285; 177; 108
			AA	285; 108

Statistical analysis

The obtained result from the PCR-RFLP method was analyzed using R programming software version 4.3.1. and Microsoft Excel (Microsoft Corp., Washington, DC, USA). The Chi-squared test was used to calculate the Hardy-Weinberg equilibrium (HWE), and the relationship between the genotype of the polymorphism and gout susceptibility was assessed using the odds ratio and its 95% confidence interval, as well as the Chi-squared (χ^2) and Fisher's exact test. All statistical tests were two-sided, and the results were considered statistically significant if $p < 0.05$.

RESULTS

Genetic analysis of *SLC2A9* rs2280205

The region containing *SLC2A9* rs2280205 was amplified using the designed primer pair (Table 1) and verified on a 1% agarose gel. The electrophoresis result showed a bright, intense band with a specific size of 285 bp (Figure 1). The PCR products were then digested by the restriction enzyme (RE) *Hpa*II (Thermo Fisher) and visualized on a 2.5% agarose gel. The genotypes of the variant were determined by the number and size of DNA bands of *SLC2A9* rs2280205 on the agarose gel (Figure 2).

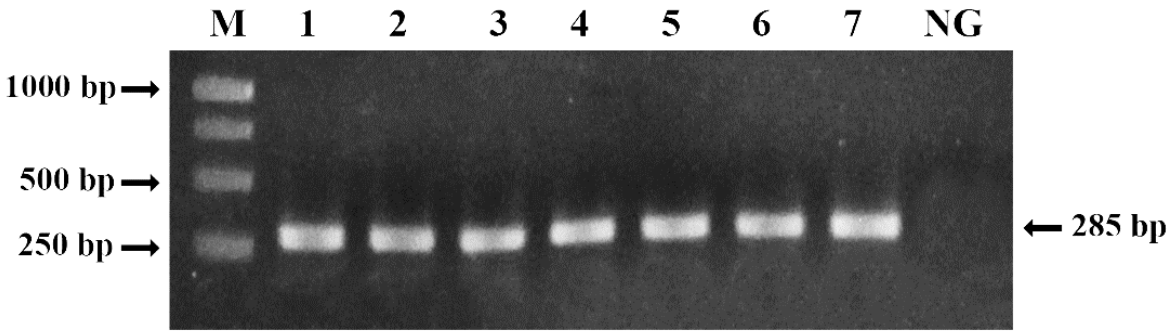

Figure 1. Analysis of PCR products of the DNA region containing *SLC2A9* rs2280205 by electrophoresis on a 1% agarose gel. M: 1 kb marker (Thermo Fisher), 1-7: PCR products of 7 representative samples, NC: Negative control.

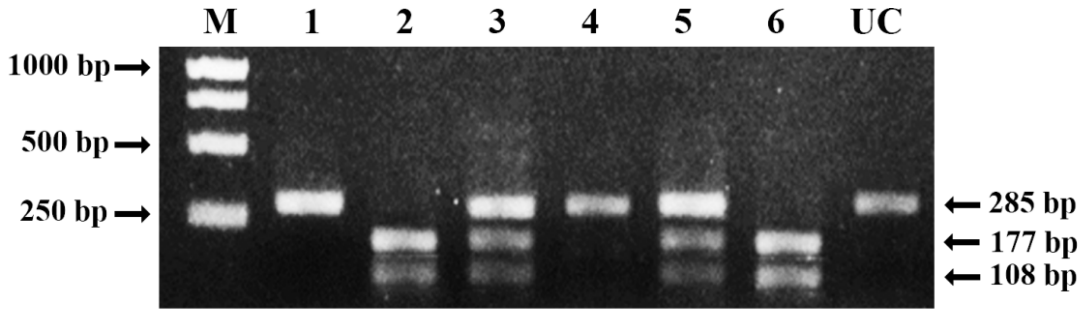

Figure 2. *Hpa*II-digested PCR products of some representative samples on agarose gel 2.5%. M: 1 kb marker (Thermo Fisher); 1-6: Digested products; UC: Undigested PCR product; 1, 4: Homozygous AA (1 band with 285 bp); 2, 6: Wild type GG (2 bands with 177 bp, 108 bp); 3, 5: Heterozygous GA (3 bands with 285 bp, 177 bp, 108 bp).

The obtained data showed that the distribution of *SLC2A9* rs2280205 polymorphism followed Hardy-Weinberg Equilibrium with $p > 0.05$ in the case, control, and whole population (Table 2). The distribution of the A minor allele was less common than the G major allele in the case group, with frequencies of 0.178 and 0.822, respectively. A similar pattern was observed in the control group, with the frequencies of the A allele and G allele being 0.194 and 0.806, respectively (Table 3). The GG/GA/AA genotype frequencies of *SLC2A9* rs2280205 were 0.65 (308/477), 0.33 (158/477) and 0.02 (11/477), respectively. The relationship between this variant and gout disease was evaluated by Chi-squared and Fisher's Exact test on three models (additive, dominant, and recessive), indicating that rs2280205 was not associated with gout disease in our sample ($p > 0.05$) (Table 4).

Table 2. Polymorphism *SLC2A9* rs2280205 in the study population of 477 individuals.

SNP_ID	Gene	Position	Variation type	Reference/ Alternate allele	MAF in control group	MAF in gout group
rs2280205	*SLC2A9*	4:9908299	Missense	G/A	0.194	0.178

Position refers to the GRCh38.p14 assembly; MAF: Minor allele frequency.

Table 3. *SLC2A9* rs2280205 alleles and genotypes of 477 individuals.

	Genotypes			Allele frequencies		HWE *p*-value
	GG	GA	AA	G	A	
Cases (n=160)	106	51	3	0.822	0.178	0.208
Controls (n=317)	202	107	8	0.806	0.194	0.416
Total (n=477)	308	158	11	0.811	0.189	0.098

Note: n: Number of samples; HWE: Hardy-Weinberg equilibrium; *p*-values were calculated using Chi-squared test.

Table 4. Association between *SLC2A9* rs2280205 and gout trait.

SNP (Gene)	Model	Case (n = 160)	Control (n = 317)	OR	95% CI	*p*-value
rs2280205 (*SLC2A9*)	**Additive**					0.8277[(2)]
	GG	106 (66.25%)	202 (63.72%)	1		
	GA	51 (31.88%)	107 (33.75%)	0.909	0.601-1.364	0.644[(1)]
	AA	3 (1.87%)	8 (2.53%)	0.738	0.151-2.672	0.754[(2)]
	Dominant					
	GG	106 (66.25%)	202 (63.72%)	1		
	GA+AA	54 (33.75%)	115 (36.28%)	0.896	0.598-1.334	0.586[(1)]
	Recessive					
	GG+GA	157 (98.12%)	309 (97.48%)	1		
	AA	3 (1.88%)	8 (2.52%)	0.762	0.157-2.739	0.758[(2)]
	Alleles					
	G	263 (82.2%)	511 (80.6%)	1		
	A	57 (17.8%)	123 (19.4%)	0.902	0.633-1.272	0.554[(1)]

Note: n: Number of samples; n (%): Number of individuals (percentage); OR: Odds ratio; 95% CI: Confident intervals; *p*-values were calculated by either Chi-squared test [(1)] or Fisher's exact test [(2)].

DISCUSSION

Gout is one of the most prevalent metabolic disorders in humans caused by inflammatory responses to the deposition of monosodium urate crystals (MSU), which occurs in the presence of exceeded uric acid levels in the blood. The pathogenesis of gout is influenced not only by environmental factors but also by the genetic variation of

individuals. Previous research has shown the effects of hereditary factors on serum UA concentration and gout disease. Among these factors, *SLC2A9* is considered to be a causative factor of gout disease (Dehghan *et al.*, 2008; Hollis-Moffatt *et al.*, 2009; Dalbeth *et al.*, 2021; Dehlin *et al.*, 2020; Klopp *et al.*, 2008).

As a member of the solute carrier family 2 (SLC2), *SLC2A9* is expressed mainly in the kidneys, which are responsible for maintaining the homeostasis of uric acid in the blood (Vitart *et al.*, 2008; Liu *et al.*, 2011). Several studies have successfully investigated the associations between polymorphisms in the *SLC2A9* gene and gout disease in different populations: Caucasians, Māori, and Solomon (Dehghan *et al.*, 2008; Hollis-Moffatt *et al.*, 2009; Tu *et al.*, 2010).

The "A" minor allele of *SLC2A9* rs2280205 (chr4:9908299, G>A) is a substitution mutation, modifying the translated amino acid from proline to leucine at position 350 of the peptide sequence (p.Pro350Leu), which might affect the function of the protein (Xing *et al.*, 2015). Moreover, the functional effect of missense variant rs2280205 of the *SLC2A9* gene was predicted *in silico* using the PhD-SNPg, software used to predict the pathogenicity of single nucleotide polymorphisms in the human genome (Capriotti, Fariselli, 2017). These results suggested that *SLC2A9* rs2280205 was a promising candidate for an association study. In addition, the amino acid proline at position 350 is highly conservative and remains unchanged in several primate species, such as chimp, orangutan, gibbon, rhesus, and baboon (https://genome.ucsc.edu), suggesting the importance of this protein among this species. Moreover, a GWAS study on 4,162 participants in Germany figured that the polymorphism *SLC2A9* rs2280205 connected with gout disease with $p = 1.83 \times 10^{-7}$ (Döring *et al.*, 2008). However, recent studies indicated that there was no association between *SLC2A9* rs2280205 and gout in the Czech (250 samples) and Cameroonian (60 samples) populations (Hurba *et al.*, 2014; Nkeck *et al.*, 2018). The findings of this study suggested that this polymorphism has not been associated with gout disease. The differences in genetics, lifestyle, or environmental factors could be explained for the conflict results. Our research provided evidence for further research on the impact of the *SLC2A9* rs2280205 polymorphism on gout among the Vietnamese population.

CONCLUSION

The genotype distribution of the rs2280205 polymorphism in the *SLC2A9* gene was examined using the PCR-RFLP method in the Vietnamese population, revealing the frequency of GG/GA/AA as 0.65, 0.33, and 0.02, respectively. Statistical analysis indicated that *SLC2A9* rs2280205 was not associated with gout. Further extensive research on various single nucleotide polymorphisms of the *SLC2A9* gene is needed to enhance our understanding of associations between different genetic variants and gout in the Vietnamese population.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPLEMENTARY

Genotype results of all samples.

No.	Lab code	Genotype
1	HG_C_024	GG
2	HG_C_025	GA
3	HG_C_026	GA
4	HG_C_027	GG
5	HG_C_028	GG
6	HG_C_029	GG
7	HG_C_030	GA
8	HG_C_031	GG
9	HG_C_032	GA
10	HG_C_033	GA
11	HG_C_034	GG
12	HG_C_035	GG
13	HG_C_036	GA
14	HG_C_037	GA

No.	Lab code	Genotype
15	HG_C_038	GG
16	HG_C_039	GG
17	HG_C_040	GA
18	HG_C_042	GG
19	HG_C_043	GG
20	HG_C_044	GA
21	HG_C_045	GG
22	HG_C_046	GA
23	HG_C_047	GG
24	HG_C_048	GG
25	HG_C_049	GA
26	HG_C_050	GA
27	HG_C_051	GA
28	HG_C_052	GG
29	HG_C_053	GG
30	HG_C_054	GG
31	HG_C_055	GA
32	HG_C_056	GG
33	HG_C_057	GA
34	HG_C_058	GA
35	HG_C_059	GG
36	HG_C_060	GG
37	HG_C_061	GG
38	HG_C_062	GA
39	HG_C_063	GG
40	HG_C_064	GA
41	HG_C_065	GA
42	HG_C_066	GA
43	HG_C_067	GG
44	HG_C_068	GG
45	HG_C_069	GG

No.	Lab code	Genotype
46	HG_C_070	GA
47	HG_C_071	GA
48	HG_C_072	GG
49	HG_C_073	GA
50	HG_C_074	GG
51	HG_C_075	GG
52	HG_C_076	GA
53	HG_C_077	GG
54	HG_C_078	GA
55	HG_C_079	AA
56	HG_C_080	GA
57	HG_C_081	GG
58	HG_C_082	GA
59	HG_C_083	GG
60	HG_C_084	GA
61	HG_C_085	GG
62	HG_C_086	GG
63	HG_C_087	GG
64	HG_C_088	GG
65	HG_C_089	GG
66	HG_C_090	GA
67	HG_C_091	GG
68	HG_C_092	GA
69	HG_C_093	GG
70	HG_C_094	GG
71	HG_C_095	AA
72	HG_C_096	GA
73	HG_C_097	GG
74	HG_C_098	GA
75	HG_C_099	GA
76	HG_C_100	GG

No.	Lab code	Genotype
77	HG_C_101	GA
78	HG_C_102	GA
79	HG_C_103	GG
80	HG_C_104	GG
81	HG_C_105	GG
82	HG_C_106	GG
83	HG_C_107	GG
84	HG_C_108	GA
85	HG_C_109	GG
86	HG_C_110	GA
87	HG_C_111	GG
88	HG_C_112	GG
89	HG_C_113	GG
90	HG_C_114	GG
91	HG_C_115	GG
92	HG_C_116	AA
93	HG_C_117	GG
94	HG_C_118	GG
95	HG_C_119	GG
96	HG_C_120	GA
97	HG_C_121	GA
98	HG_C_122	GA
99	HG_C_123	GG
100	HG_C_124	GA
101	HG_C_125	GA
102	HG_C_126	GG
103	HG_C_127	GG
104	HG_C_128	AA
105	HG_C_129	GG
106	HG_C_130	GG
107	HG_C_131	GA

No.	Lab code	Genotype
108	HG_C_132	GG
109	HG_C_133	GG
110	HG_C_134	GG
111	HG_C_135	GG
112	HG_C_136	GG
113	HG_C_137	GG
114	HG_C_138	GG
115	HG_C_139	GG
116	HG_C_140	GG
117	HG_C_141	GA
118	HG_C_142	GA
119	HG_C_143	GG
120	HG_C_144	GG
121	HG_C_145	GA
122	HG_C_146	GG
123	HG_C_147	GA
124	HG_C_148	GG
125	HG_C_149	GG
126	HG_C_150	GG
127	HG_C_151	GA
128	HG_C_152	GG
129	HG_C_153	GG
130	HG_C_154	GG
131	HG_C_155	GG
132	HG_C_156	GG
133	HG_C_157	GA
134	HG_C_158	GA
135	HG_C_159	GA
136	HG_C_160	GG
137	HG_C_161	GG
138	HG_C_162	GG

No.	Lab code	Genotype
139	HG_C_163	GA
140	HG_C_164	GG
141	HG_C_165	GG
142	HG_C_166	GA
143	HG_C_167	GG
144	HG_C_168	GG
145	HG_C_169	GG
146	HG_C_170	GG
147	HG_C_171	GG
148	HG_C_172	GG
149	HG_C_173	GA
150	HG_C_174	GG
151	HG_C_175	GG
152	HG_C_176	GG
153	HG_C_177	GG
154	HG_C_178	GA
155	HG_C_179	GA
156	HG_C_180	GG
157	HG_C_181	GG
158	HG_C_183	GG
159	HG_C_184	GG
160	HG_C_185	GG
161	HG_C_186	GG
162	HG_C_187	GG
163	HG_C_188	GG
164	HG_C_189	GA
165	HG_C_190	GG
166	HG_C_191	GA
167	HG_C_192	GG
168	HG_C_193	GA
169	HG_C_195	GG

No.	Lab code	Genotype
170	HG_C_196	GG
171	HG_C_198	GG
172	HG_C_199	GA
173	HG_C_200	GA
174	HG_C_201	GG
175	HG_C_202	GG
176	HG_C_203	AA
177	HG_C_204	GA
178	HG_C_205	GA
179	HG_C_206	GG
180	HG_C_207	GA
181	HG_C_208	GA
182	HG_C_209	GA
183	HG_C_210	GG
184	HG_C_211	GA
185	HG_C_212	GG
186	HG_C_213	GG
187	HG_C_214	GG
188	HG_C_215	GG
189	HG_C_216	GG
190	HG_C_217	GA
191	HG_C_218	GG
192	HG_C_219	GG
193	HG_C_220	GG
194	HG_C_221	GA
195	HG_C_222	GG
196	HG_C_223	GA
197	HG_C_224	GG
198	HG_C_225	GG
199	HG_C_226	GA
200	HG_C_227	GG

No.	Lab code	Genotype
201	HG_C_228	GA
202	HG_C_229	GG
203	HG_C_230	GA
204	HG_C_231	GG
205	HG_C_232	GG
206	HG_C_233	GA
207	HG_C_234	GG
208	HG_C_235	GG
209	HG_C_236	GA
210	HG_C_237	GG
211	HG_C_239	GA
212	HG_C_240	GG
213	HG_C_241	GG
214	HG_C_242	GG
215	HG_C_243	GA
216	HG_C_244	GA
217	HG_C_245	GG
218	HG_C_246	GG
219	HG_C_247	GG
220	HG_C_248	GA
221	HG_C_249	GG
222	HG_C_250	GG
223	HG_C_251	GA
224	HG_C_252	GG
225	HG_C_253	GG
226	HG_C_254	GG
227	HG_C_255	GA
228	HG_C_256	GG
229	HG_C_257	GA
230	HG_C_258	GG
231	HG_C_259	GG

No.	Lab code	Genotype
232	HG_C_260	GG
233	HG_C_261	GG
234	HG_C_263	GA
235	HG_C_264	GG
236	HG_C_265	GG
237	HG_C_266	GG
238	HG_C_267	GA
239	HG_C_268	GG
240	HG_C_269	GG
241	HG_C_270	AA
242	HG_C_271	AA
243	HG_C_272	GG
244	HG_C_273	GG
245	HG_C_274	GA
246	HG_C_275	GG
247	HG_C_276	GG
248	HG_C_277	GG
249	HG_C_278	GA
250	HG_C_280	GG
251	HG_C_281	GG
252	HG_C_282	GA
253	HG_C_283	GA
254	HG_C_284	GG
255	HG_C_285	GG
256	HG_C_286	GG
257	HG_C_287	GG
258	HG_C_288	GG
259	HG_C_289	GG
260	HG_C_290	GG
261	HG_C_291	GA
262	HG_C_292	GG

No.	Lab code	Genotype
263	HG_C_293	GG
264	HG_C_294	GG
265	HG_C_295	GG
266	HG_C_296	GG
267	HG_C_297	GA
268	HG_C_298	GG
269	HG_C_299	GG
270	HG_C_300	GG
271	HG_C_301	GG
272	HG_C_302	GA
273	HG_C_303	GG
274	HG_C_304	GG
275	HG_C_305	GG
276	HG_C_306	GG
277	HG_C_307	GG
278	HG_C_308	GG
279	HG_C_309	GA
280	HG_C_310	GG
281	HG_C_311	GG
282	HG_C_312	GA
283	HG_C_313	GG
284	HG_C_314	GA
285	HG_C_315	AA
286	HG_C_317	GG
287	HG_C_318	GG
288	HG_C_319	GA
289	HG_C_320	GG
290	HG_C_321	GA
291	HG_C_322	GA
292	HG_C_323	GG
293	HG_C_324	GA

No.	Lab code	Genotype
294	HG_C_325	GA
295	HG_C_326	GG
296	HG_C_327	GG
297	HG_C_328	GG
298	HG_C_329	GA
299	HG_C_331	GA
300	HG_C_332	GG
301	HG_C_333	GG
302	HG_C_334	GG
303	HG_C_335	GA
304	HG_C_336	GG
305	HG_C_337	GG
306	HG_C_338	GA
307	HG_C_340	GG
308	HG_C_341	GG
309	HG_C_342	GG
310	HG_C_343	GA
311	HG_C_344	GG
312	HG_C_345	GA
313	HG_C_346	GA
314	HG_C_347	GG
315	HG_C_349	GA
316	HG_C_350	GG
317	HG_C_351	GG
318	HG_P_005	GG
319	HG_P_006	GG
320	HG_P_007	GG
321	HG_P_008	GG
322	HG_P_009	GA
323	HG_P_010	GG
324	HG_P_011	GG

No.	Lab code	Genotype
325	HG_P_012	GG
326	HG_P_013	GG
327	HG_P_014	GA
328	HG_P_015	GG
329	HG_P_016	GG
330	HG_P_017	GG
331	HG_P_018	AA
332	HG_P_019	GG
333	HG_P_020	GG
334	HG_P_021	GA
335	HG_P_022	GG
336	HG_P_023	GA
337	HG_P_024	GG
338	HG_P_025	GG
339	HG_P_026	GA
340	HG_P_027	GG
341	HG_P_028	GA
342	HG_P_029	GG
343	HG_P_030	GG
344	HG_P_031	GG
345	HG_P_032	GA
346	HG_P_033	GA
347	HG_P_034	GG
348	HG_P_037	AA
349	HG_P_038	GG
350	HG_P_039	GG
351	HG_P_040	GA
352	HG_P_041	GG
353	HG_P_042	GG
354	HG_P_043	GG
355	HG_P_044	GA

No.	Lab code	Genotype
356	HG_P_045	GG
357	HG_P_046	GA
358	HG_P_047	GA
359	HG_P_048	GA
360	HG_P_049	GG
361	HG_P_050	GG
362	HG_P_051	GG
363	HG_P_052	GG
364	HG_P_053	GA
365	HG_P_054	GG
366	HG_P_055	GA
367	HG_P_056	GA
368	HG_P_057	GA
369	HG_P_058	GG
370	HG_P_059	GG
371	HG_P_060	GG
372	HG_P_061	GG
373	HG_P_062	GA
374	HG_P_063	GG
375	HG_P_064	GG
376	HG_P_066	GG
377	HG_P_067	GG
378	HG_P_068	GG
379	HG_P_069	GA
380	HG_P_070	GG
381	HG_P_071	GG
382	HG_P_072	GA
383	HG_P_073	GG
384	HG_P_074	GA
385	HG_P_075	GG
386	HG_P_076	GA

No.	Lab code	Genotype
387	HG_P_077	GG
388	HG_P_078	GA
389	HG_P_079	GA
390	HG_P_080	GA
391	HG_P_081	GA
392	HG_P_082	GA
393	HG_P_084	GA
394	HG_P_085	GG
395	HG_P_086	GA
396	HG_P_087	GG
397	HG_P_088	GG
398	HG_P_089	GG
399	HG_P_090	GG
400	HG_P_091	GG
401	HG_P_092	GG
402	HG_P_093	GG
403	HG_P_094	GA
404	HG_P_095	GA
405	HG_P_096	GG
406	HG_P_097	GG
407	HG_P_098	GG
408	HG_P_099	GG
409	HG_P_100	GG
410	HG_P_101	GG
411	HG_P_102	GG
412	HG_P_103	GG
413	HG_P_104	GG
414	HG_P_105	GG
415	HG_P_106	GA
416	HG_P_107	GG
417	HG_P_108	GA

No.	Lab code	Genotype
418	HG_P_109	GA
419	HG_P_110	GA
420	HG_P_111	GG
421	HG_P_112	AA
422	HG_P_113	GG
423	HG_P_114	GG
424	HG_P_115	GG
425	HG_P_116	GG
426	HG_P_117	GA
427	HG_P_118	GG
428	HG_P_119	GA
429	HG_P_120	GA
430	HG_P_121	GA
431	HG_P_122	GA
432	HG_P_123	GG
433	HG_P_124	GG
434	HG_P_125	GA
435	HG_P_126	GG
436	HG_P_127	GG
437	HG_P_128	GG
438	HG_P_129	GG
439	HG_P_130	GA
440	HG_P_132	GG
441	HG_P_133	GA
442	HG_P_134	GG
443	HG_P_135	GG
444	HG_P_136	GA
445	HG_P_137	GG
446	HG_P_138	GG
447	HG_P_139	GG
448	HG_P_140	GG

No.	Lab code	Genotype
449	HG_P_141	GA
450	HG_P_142	GG
451	HG_P_143	GG
452	HG_P_144	GG
453	HG_P_145	GG
454	HG_P_146	GG
455	HG_P_147	GG
456	HG_P_148	GG
457	HG_P_149	GG
458	HG_P_150	GG
459	HG_P_151	GA
460	HG_P_152	GG
461	HG_P_154	GG
462	HG_P_155	GG
463	HG_P_156	GA
464	HG_P_157	GG
465	HG_P_158	GG
466	HG_P_159	GG
467	HG_P_160	GG
468	HG_P_161	GA
469	HG_P_162	GA
470	HG_P_163	GA
471	HG_P_164	GG
472	HG_P_165	GG
473	HG_P_166	GG
474	HG_P_167	GG
475	HG_P_168	GG
476	HG_P_169	GG
477	HG_P_170	GA