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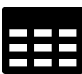

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Risk assessment of *FLT3* and *PAX5* variants in B-acute lymphoblastic leukemia: a case - control study in a Pakistani cohort

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AIMS: B-cell acute lymphoblastic leukemia (B-ALL) is amongst most prevalent cancers of children in Pakistan. Genetic variations in *FLT3* are associated with auto-phosphorylation of kinase domain that leads to increased proliferation of blast cells. Paired box family of transcription factor (*PAX5*) plays a critical role in commitment and differentiation of B-cells. Variations in *PAX5* are associated with the risk of B-ALL. We aimed to analyze the association of *FLT3* and *PAX5* polymorphisms with B cell leukemia in Pakistani cohort. **METHODS:** We collected 155 B-ALL subject and 155 control blood samples. For analysis, genotyping was done by tetra ARMS-PCR. SPSS was used to check the association of demographic factors of SNPs present in the population with the risk of B-ALL. **RESULTS:** Risk allele frequency 'A' of *FLT3* non-synonomous polymorphism rs35958982 was conspicuous and showed positive association (OR= 2.3, CI=1.2-4.5, P=0.00526) but genotype frequency (OR=3.67, CI=0.75-18.10, P=0.088) failed to show any association with the disease. *PAX5* polymorphism rs3780135 risk allele 'A' frequency was more in B-ALL subjects than ancestral allele frequency 'G'(OR=2.17,CI=1.37-3.43, P=0.0002). Genotype frequency analysis of *PAX5* polymorphism exhibited the protective effect (OR=0.55,CI=0.72-1.83, P=0.029). *FLT3* intronic polymorphism rs12430881, minor allele frequency 'G' (OR=1.15,CI=1.37-3.43, P=0.0426) and genotype frequency 'GA' (OR=2.52 P=0.0061) showed positive association with the B-ALL. Family history of cancer (OR=15.42, P=0.000134) and consanguinity (OR=1.87, P=0.05) were found to be associated with B-ALL whereas, detailed analysis of these factors showed no association with risk genotypes of variants. **CONCLUSION:** In present study, a strong risk of B-cell acute lymphoblastic leukemia was associated with *FLT3* polymorphism rs12430881. However, *PAX5* polymorphism rs3780135 showed the protective effect. Additionally, other demographic factors like family history, smoking and consanguinity were also found to be important in risk assessment. We anticipate that the information from genetic variations in

this study can aid in therapeutic approach in the future.

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2 # leukeima: a case-control study in a Pakistani cohort

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13 ## Abstract

14 **AIMS:** B-cell acute lymphoblastic leukemia (B-ALL) is amongst most prevalent cancers of
15 children in Pakistan. Genetic variations in *FLT3* are associated with auto-phosphorylation of
16 kinase domain that leads to increased proliferation of blast cells. Paired box family of
17 transcription factor (*PAX5*) plays a critical role in commitment and differentiation of B-cells.
18 Variations in *PAX5* are associated with the risk of B-ALL. We aimed to analyze the association
19 of *FLT3* and *PAX5* polymorphisms with B cell leukemia in Pakistani cohort.
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21 genotyping was done by tetra ARMS-PCR. SPSS was used to check the association of
22 demographic factors of SNPs present in the population with the risk of B-ALL.
23 **RESULTS:** Risk allele frequency 'A' of *FLT3* non-synonomous polymorphism rs35958982 was
24 conspicuous and showed positive association (OR= 2.3, CI=1.2-4.5, P=0.00526) but genotype
25 frequency (OR=3.67, CI=0.75-18.10, P=0.088) failed to show any association with the disease.
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27 ancestral allele frequency 'G'(OR=2.17,CI=1.37-3.43, P=0.0002). Genotype frequency analysis
28 of *PAX5* polymorphism exhibited the protective effect (OR=0.55,CI=0.72-1.83, P=0.029). *FLT3*
29 intronic polymorphism rs12430881, minor allele frequency 'G' (OR=1.15,CI=1.37-3.43,
30 P=0.0426) and genotype frequency 'GA' (OR=2.52 P=0.0061) showed positive association with

31 the B-ALL. Family history of cancer (OR=15.42, P=0.000134) and consanguinity (OR=1.87,
32 P=0.05) were found to be associated with B-ALL whereas, detailed analysis of these factors
33 showed no association with risk genotypes of variants.
34 **CONCLUSION:** In present study, a strong risk of B-cell acute lymphoblastic leukemia was
35 associated with *FLT3* polymorphism rs12430881. However, *PAX5* polymorphism rs3780135
36 showed the protective effect. Additionally, other demographic factors like family history,
37 smoking and consanguinity were also found to be important in risk assessment. We anticipate
38 that the information from genetic variations in this study can aid in therapeutic approach in the
39 future.

40 Introduction

41 According to the Punjab cancer registry report, acute lymphoblastic leukemia (ALL) is a
42 predominant malignancy of children and it makes up most prevalent cancer in Punjab, Pakistan,
43 The worldwide incidence rate is 1-4.75 per 100,000 people. In Pakistan ALL contributes to
44 17.9% among all cancers. It is characterized by mutation in blast cells in hematopoietic stem
45 cells, spleen, neurons, gonads , lymph nodes, and hepatic cells (Portell et al., 2013). Although,
46 B-ALL is very common in children but it may also occur in adult populace (Forero et al., 2013).
47 Several demographic parameters like gender, age, family history and biological factors also play
48 an important role in the prevalence of disease. Other factors like exposure to UV, radiations,
49 lifestyle may also increase risk of disease (Levine *et al.*, 2016; Acharya *et al.*, 2018). Mutation in
50 certain genes involved in different processes like apoptosis, proliferation, and differentiation of
51 B-cells may also cause B-ALL. These genetic alterations largely effect the prediction and
52 therapeutic approach used for medication and therapy of ALL (Tasian and Hunger, 2017).
53 FMS-like tyrosine kinase (*FLT3*) belongs to class III receptor tyrosine kinase (RTK) family.
54 Structurally, FLT3 consist of an extracellular domain at the amino terminus. This domain
55 comprise of immunoglobulin-like transmembrane region and intracellular juxta-membrane
56 domain (JMD). At carboxyl terminus, there are two kinase domains, separated by a kinase insert
57 region (Gilliland and Griffin, 2002). FLT3 is expressed in normal human bone marrow
58 especially in CD34+ hematopoietic stem, brain (Çakmak-Görür *et al.*, 2019) and gonads
59 (Matthews et al., 1991; Small et al., 1994) and encodes 1000 amino acid protein in humans. In
60 the hematopoietic tissues, Binding of FL with its receptor causes auto-phosphorylation of
61 tyrosine residues present in the kinase domain and stimulate growth of progenitor cells in the

62 marrow and blood (Marhäll *et al.*, 2018).This results into downstream activation of signaling
63 pathways that are involved in regulation of cell cycle or apoptotic including (PI3K), caspase-9
64 and Ras/Raf pathways and causes multiplied proliferation of cells, reduced cell apoptosis, and
65 inhibition of cell differentiation of B-cells (Zhang and Broxmeyer, 2000).
66 In hematologic malignancy, 70% to 100% increased expression of FLT3 in acute myeloid
67 leukemia (AML) and acute lymphoblastic leukemia (ALL) are reported previously (Brown *et al.*,
68 2005; Griffith *et al.*, 2016). Rosnet and colleagues reported 3 of 5 ALL subjects with increased
69 expression of FLT3 on leukemia blasts (Rosnet *et al.*, 1996).Carow and coworkers found higher
70 RNA expression in bone marrow in 33 of 33 subjects of ALL. These studies signify the critical
71 role of *FLT3* expression in proliferation of blast cells (Carow *et al.*, 1996).
72 B-cell-specific activator protein (*PAX5*) encodes transcription factors that are the member of
73 paired box domain. *PAX5* plays imperative role in the commitment of B-cell lineage from blast
74 cells as it controls the differentiation of pro B cell to pre-B cells (Fuxa and Skok, 2007; Lang *et*
75 *al.*, 2007). In pre-pro-B cells the immunoglobin gene rearrangement starts and matures into pro–
76 B cells. From pro-B stage expression of *PAX5* initiate to pre-B cells. In late B-lymphoposis,
77 PAX5 maintains the function of mature B cells (Shahjahani *et al.*, 2015).
78 In B-cell malignancies, *PAX5* act as an oncogene. Down-regulation of PAX5 halts B-cells
79 reverts B-cell precursors (BCPs) to progenitors (pro B-cell stage) (Schebesta *et al.*, 2007; Carotta
80 and Nutt, 2008). Conversely, uncontrolled proliferation of B-cells leads to the abnormal
81 expression of PAX5 in precursor cells and inhibit T cell proliferation (Souabni *et al.*, 2007). It is
82 reported that in childhood acute lymphoblastic leukemia (ALL), translocations and mutation in
83 *PAX5* are more prevalent (Bousquet *et al.*, 2007; Nebral *et al.*, 2009; Santoro *et al.*, 2009;
84 Iacobucci and Mullighan, 2017). Alternative splicing of *PAX5* in exon 7 to exon 9 results into
85 five isoforms. These isoforms are more expressed in primary B-cell lymphoma tissues and
86 cancerous cell lines (Zwollo *et al.*, 1997; Arseneau *et al.*, 2009).

87 Previous studies showed that presence of single nucleotide polymorphisms (SNPs) in genome
88 maybe risk causing or protective for the disease and it may also alters pharmacokinetic and
89 pharmacodynamics properties of drugs (Kumanayake, 2013; Pui, 2015; Tasian and Hunger,
90 2017). We selected two non-synonomous SNPs one *FLT3* SNP rs35958982, 557 (Val > Ile) at
91 position Chr13:28034336 (GRCh38.p12) and second *PAX5* SNP rs3780135, 293(Thr > Ile)
92 position Chr9:36840626 (GRCh38.p12). An intronic *FLT3* SNP rs12430881 (A>G) position

Chr13:28020665 (GRCh38.p12) was also selected. The change in amino acid sequence due to non-synonomous SNP alters the protein structure implicating its expression and function. Current study is designed to evaluate role of *FLT3* and *PAX5* genes in B cell lymphoblastic leukemia. For this purpose, a case control analysis was conducted to evaluate SNP rs35958982, rs3780135 and rs12430881 association with B cell acute lymphoblastic leukemia (B-ALL) incidence.

Materials & Methods

Study subjects

The present study was conducted at the University of Punjab, Pakistan and Ethical approval was also granted to carry out the study within its facilities (Ethical Application Ref: sbs/222/18). Blood samples were collected during the period of January 2017 to February 2017 from Children's Hospital, Lahore, Pakistan. Study population comprised of 155 cases and 155 controls less than 15 years of age. The diagnostic criteria for B-ALL cases include B cell positive marker (CD19, CD10, CD22, and CD20) confirmed by flow cytometry analysis. Cases with relapsed and newly diagnosed B-ALL were also included. All 310 subjects recruited ~~were~~ consented to participate in this study after filling the questionnaire. The subjects with any other type of leukemia, blood infectious disease, and B-ALL subjects greater than 15 years of age were excluded from the study. Family history with cancer, parental consanguinity (first and second degree relatives) and smoking status (>100 cigarettes in lifetime) were gathered by questionnaire interviewed.

Genotyping:

Venous blood samples of cases and controls were collected in EDTA vials. DNA extraction was done using Sam brook 2001 organic protocol. Genes and SNPs associated with B-ALL were screened using DisGeNET platform (Queralt-Rosinach *et al.*, 2016) and were verified by dbSNP database (Sherry *et al.*, 2001). Presence of the selected SNPs in Pakistani population was confirmed by ENSEMBLE (Frankish *et al.*, 2017). In order to identify the SNPs, tetra arm primers were designed using Primer1 software (Ye *et al.*, 2001). Tetra arm PCR was done using protocol 26 using advanced primus 96 (PeqLab) thermal cycler.

rs35958982: Forward outer primer: TGTGACAAATTAGCAGGGTTAACAC; Reverse outer primer: CACAGAAGAGATCACAGAAGGAGTCT; Forward inner primer:

123 GAAACTCCCATTTGAGATCATATTCA; Reverse inner primer:
124 AGACAGAGACAAGCAGACATTCG
125 **rs3780135:** Forward outer primer: CTCTTCCAGGCTCCCCCGAC**;** Reverse outer primer:
126 GGGCGGCAGCGCTATAAGAA**;** Forward inner primer:
127 ACCCCAGCTCTAGATGGCGAAG**;** Reverse inner primer:
128 ATAGGTGCCATCAGTGTTTGGTGC
129 **rs12430881:** Forward outer primer: GTTTGTCTCCTCTTCATTGGCA**;** Reverse outer primer:
130 GCCTCAGTGTCATCTTCGAATT**;** Forward inner primer:
131 CCTTTTATCTTCACATCAGGCCT**;** Reverse inner primer:
132 CTTAGTAGAGATGGGGTTTTGCC
133 For SNP rs35958982, PCR programme was optimized as follow:
134 An initial denaturation at 94 °C for 4 min, followed by 30 cycle; denaturation at 94 °C for 1 min,
135 annealing at 58.4 °C for 1.5 min, extension at 72 °C for 1 min and a final extension at 72 °C for
136 10 min.
137 For SNP **rs3780135**, PCR programme propensity was optimized as follow:
138 An initial denaturation at 94 °C for 4 min, followed by 30 cycle; denaturation at 94 °C for 1 min,
139 annealing at 56.6°C for 1.5 min, extension at 72 °C for 1 min and a final extension at 72 °C for
140 10 min.
141 For SNP **rs12430881**, PCR programme was optimized as follow:
142 An initial denaturation at 94 °C for 4 min, followed by 30 cycle; denaturation at 94 °C for 1 min,
143 annealing at 58.8°C for 1.5 min, extension at 72 °C for 1 min and a final extension at 72 °C for
144 10 min.
145 PCR products were further analyzed by gel electrophoresis (fig: 1, 2, 3).

146 **Statistical analyses:**

147 Statistical studies were performed using IBM SPSS 23. Chi-square test was conducted to
148 compare categorical data. Allele and genotype association between SNPs and B-ALL were
149 calculated by computing odds ratio (OR) and 95% confidence interval (CI). A logistic regression
150 model was used to analyze interaction between SNPs and covariates. The probability level
151 accepted for significance was $P < 0.05$.

152 ## Results

153 Family history of cancer and parental consanguinity showed significant association with B-ALL
154 while, there was no association with smoker parents. Subjects with family history of any type of
155 cancer showed high risk of having B-ALL (OR=15.42, P=0.000134). Smoker parents were also
156 considered to increase the risk of cancer but our results are contradictory to this study as no
157 significant association was between smokers in B-ALL subjects was found (OR=0.85, P=0.58).
158 In the present study, more B-ALL subjects were product of parental consanguinity and showed
159 highly significant association with the risk of B-ALL (OR=1.87, P=0.05) (Table:1). Education
160 level of the parents was also considered (at least matriculation degree). Our result showed that
161 mothers (39.4%) and fathers (16.3%) were educated.
162 Our data showed that none of the subjects and their parents was exposed to radiations.
163 Furthermore, 18 patients had liver hepatomegaly, having size 11.7 ± 3.3, nine cases had
164 nephropathy, having size of right kidney 9.47 ± 2.9 and left kidney 10.01 ± 2.3. Symptoms like
165 night sweating, dizziness, abdominal pain, vomiting , bruises, pallor, enlarged lymph nodes,
166 cough with blood, loose stools, jaundice, pedal edema, pain, dehydration, hepatosplenomegaly,
167 atypical blast cells mild abdominal ascites, low leukocytes, and thrombocytopenia were also
168 recorded.
169 In our cohort, rs35958982 encoding isloleucine form of codon frequency in subjects was 13.4%
170 and 5.6% in controls. Moreover, statistical analysis showed positive association of allele
171 frequency (OR= 2.3, CI=1.2-4.5, P=0.00526) and no association of genotype frequency
172 (OR=3.67,CI=0.75-18.10, P=0.088) with the disease. Another polymorphism rs3780135, a minor
173 allele frequency in subjects was 47.1% and 32.58% in controls showing positive association with
174 B-ALL (OR=2.17, CI=1.37-3.43, P=0.0002). The genotype frequency showed protective effect
175 with B-ALL (OR=0.55, CI=0.72-1.83, P=0.029). The SNP rs12430881 allele frequency
176 (OR=1.15, CI=1.37-3.43, P=0.0426) and genotype frequency (OR=2.52, CI=1.28 -4.95,
177 P=0.0061) showed strong association with the disease is shown in table: 2.
178 Multivariate regressions analyses was performed after adjusting baseline for conventional B-
179 ALL risk factors such as family history, smoking and parental consanguinity. Outcome of the
180 disease in rs3780135 and rs12430881was more among subjects with parental smoking status
181 having genotypes GG (OR=1.08, CI=0.46-2.5) and GG (OR=1.05, CI=0.45-2.42) respectively.
182 The outcome of GA genotype was more in positive consanguinity (OR=1.152, CI=0.5-2.65) but
183 none of risk factors were associated with the disease (table: 3).

184 ## Discussion

185 According to previous studies, association of First and second-degree family history of cancer
186 signifies genetic and environmental risk factor for causing acute lymphoblastic leukemia. Our
187 study also showed positive association of family history with B-ALL (OR=15.42,
188 P=0.000134).Previously, parental smoking has also been associated with the prevalence of ALL
189 but our study showed contrary results (OR=0.85, P=0.58) (Belson *et al.*, 2007). Parental
190 consanguinity is still practiced in Pakistan, which results into minor allele pool and contributes
191 with occurrence of diseases. Our results are in accordance with (Steinberg and Steinfeld, 1960;
192 Urtishak *et al.*, 2016) which states that familial occurrence of leukemia exist (OR=1.87, P=0.05).
193 Education of parents was taken into account as more education leads to awareness of the disease
194 and child health. Some studies found a correlation between parental exposure to radiation before
195 conception, that may be due to their work (Gardner, 1991) or the X-rays (Shu *et al.*, 2002). In
196 our analysis, none of the patient or their parent was ever exposed to radiations. Hepatomegaly
197 and nephropathy are often seen in B-ALL subjects having chemotherapy. Malfunctioned
198 leucocytes in liver and kidney leads to enlargement of these organs (Rasool *et al.*, 2015).
199 Another study states that hepatomegaly and nephropathy can also results due to toxicity of
200 chemotherapy given to the subjects (Giamanco *et al.*, 2016).
201 It is well established that cancer risk is affected by numerous variants having any effect of risk or
202 protection. The degree of penetrance of a certain genotype in the population and environmental
203 factors is a major cause of cancer (Fletcher and Houlston, 2010). The information given by
204 allelic and genotypic data of single nucleotide polymorphism in a population propose the
205 possible genetic markers for cancer risk and predict possible targeted therapies(Griffith *et al.*,
206 2016; Wu and Li, 2018).
207 In this study, *FLT3* SNP rs35958982 is a germline polymorphism present in transmembrane
208 region in exon 13. It is a nonsynonymous variant as V (Val) > I (Ile). This change in amnio acid
209 alters the structure of the protein. Previously, the high throughput DNA sequence analysis done
210 to check frequency of rs35958982 with leukemiogenesis in drivers and passengers showed no
211 association with AML (Fröhling *et al.*, 2007). In contrast, present study display the association of
212 SNP rs35958982 with the disease (OR= 2.3, CI=1.2-4.5, P=0.00526). Detailed analysis of
213 genotype frequency in the population showed no association with B-ALL (OR=3.67,CI=0.75-
214 18.10, P=0.088). This might be due to the fact that *FLT3* SNP rs35958982 is rare in acute

lymphoblastic leukemia (B-ALL) and have low penetrance. It has been studied that disruption of FLT3 gene leads to deficiency of B-lymphoid progenitors, suggesting critical role of FLT3 in survival and proliferation of blast cells (Zriwil et *al.*, 2018).

Bodian *et al.*, studied allele frequency of paired box domain polymorphism rs3780135 in different populations i.e. African 34%, African European 49%, Central Asain 85%, East Asian 94%, European 95% and Hispanic 88% (Bodian *et al.*, 2014). Pakistan lies in South East Asia and risk allele frequency found in this study is 47.1% less than the previous study. In another study, by Firtina *et al.*, found polymorphism rs3780135 in B-ALL subjects with increased mRNA expression of PAX5 suggesting role of SNP with increased polymorphism of blast cell (Firtina *et al.*, 2012). In Pakistani population, allele 'A' frequency (47.1%) was significantly more in B-ALL subjects than in controls (52.9%) (OR=2.17, CI=1.37-3.43, P=0.0002). Heterozygous allele GA (38.7 %) was more in population than homozygous risk allele AA (27.74 %) showing significant difference in frequency (CI=0.72-1.83, P=0.029) and having a protective effect (OR=0.55). It has been well established that PAX5 is involved in repression of T-cells, activation of B-cell proliferation from blast cell and presence of any variant in this gene affects the pathway leading to increased expression. This increased expression of *PAX5* is associated with B-ALL (Firtina *et al.*, 2012).

Present study analyze the intronic rs12430881 showing significant increase frequency of risk allele 'A' (29 %) in B-ALL subjects than controls (22%) (OR=1.15, CI=1.37-3.43, P=0.0426). Genotypic analysis showed the frequency of homozygous genotype 'AA' (20%) more than heterozygous genotype 'GA' (18.06%) (OR=2.52, CI=1.28 -4.95, P=0.0061) having positive association with the disease.

Conclusions

The findings of the present study significantly demonstrate that *FLT3* SNP rs12430881 correlates with the increase risk of B-ALL. In contrary, *PAX5* SNP rs3780135 has protective effect. None of the risk factor including family history, parental consanguinity and smoking was found to be associated with B-ALL risk causing variant. To eliminate limitation and validate the result of the present study, comprehensive studies with increased sample size including other demographic and environmental factors may be done. This study can lend a helping hand to the personalized drugs development approaches against B-ALL in the future.

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Table 1 (on next page)

Association of demographic factors with disease

Parameters with P<0.05 is significant studied are linked to B-ALL. (*) show significant.

1

Parameters	Patients (%)	Control (%)	OR	Chi-square	p-value
Age (mean)	7.3	11.7			
A positive family history	16.77	1.29	15.42	14.59	0.000134*
A negative family history	83.22	98.7			
Smoking by parent	38.06	41.94	0.85	0.31	0.58
No smoking parent	61.93	58.06			
Parental cousin marriage	33.55	21.29	1.87	3.78	0.05*
No cousin marriage	66.45	78.7			
Females	36.13	46.45	0.65	3.41	0.065
males	63.87	53.54			

2

3

Table 2(on next page)

Allele and genotype frequency

Allelie and genotype frequencies of patients and controls. * shows significant values.

1

Gene/SNP	Alleles/ Genotype	Controls (%)	Cases (%)	Odd Ratio	95% class interval	Chi-square	P value
rs35958982	A	5.6	13.4	2.3	1.2-4.5	7.79	0.00526*
	G	94.4	86.6				
	AA	1.85	6.5	3.67	0.75-18.10	2.9	0.088
	GA	7.6	13.8				
	GG	90.7	79.6				
rs3780135	A	32.58	47.1	2.17	1.37-3.43	13.63	0.0002*
	G	67.42	52.9				
	AA	17.42	27.74				
	GA	30.32	38.7	0.55	0.39-0.95	4.72	0.029*
	GG	52.26	33.55				
rs12430881	G	22	29	1.15	0.72-1.83	4.11	0.0426*
	A	78	71				
	GG	9.03	20	2.52	1.28 -4.95	7.51	0.0061*
	GA	25.8	18.06				
	AA	65.16	61.93				

2

Table 3 (on next page)

Logistic regression model

ORs were obtained from logistic regression model with adjustment for family history, smoking and consanguinity.

1

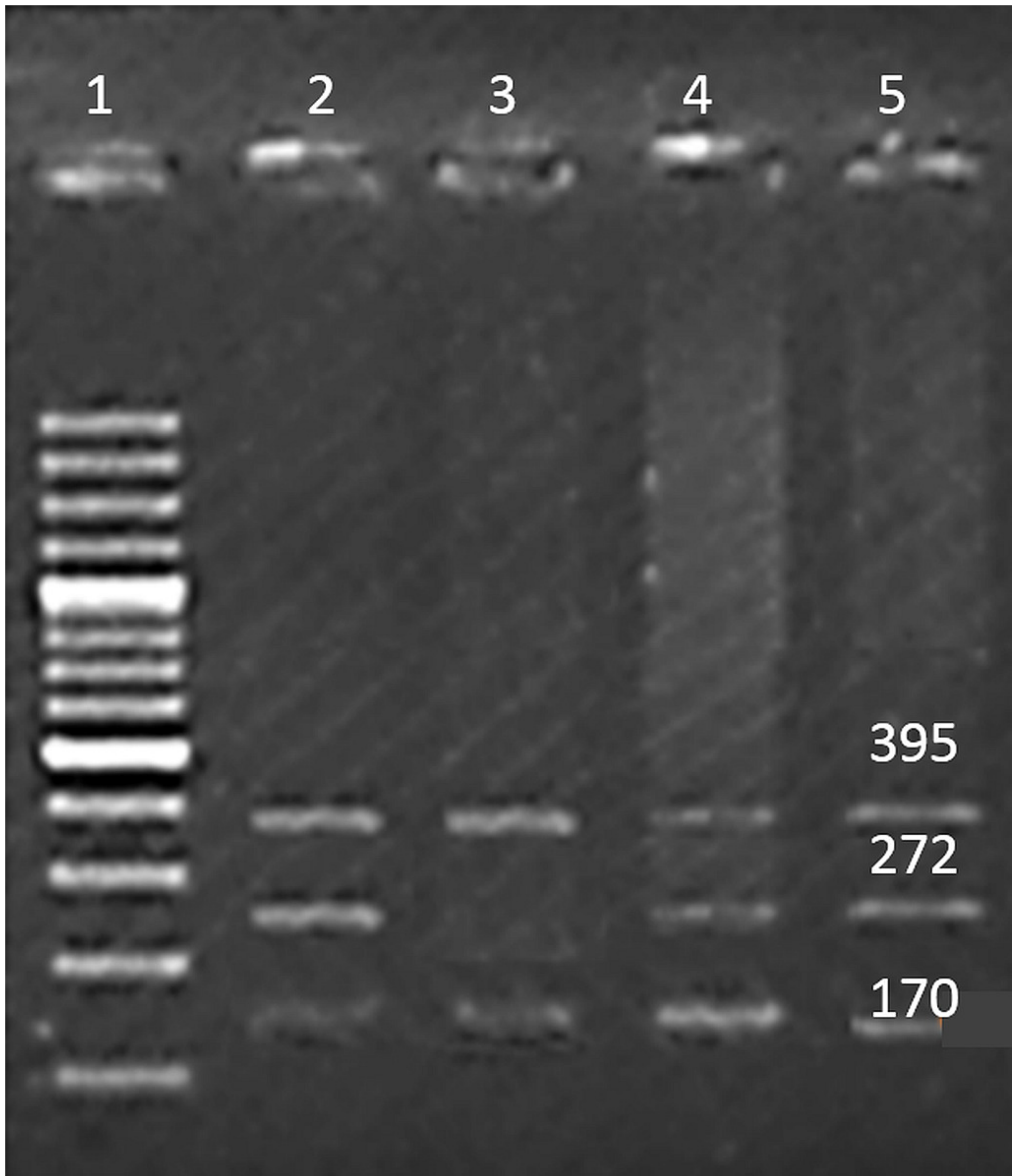
FLT3	OR (95% CI)			P$_{(interaction)}$-value
	GG	GA	AA	
Family history status				
Yes / No	1	0.917(.18-4.56)	0.5(.06-4.15)	0.729
Smoking status				
Yes / No	1	0.3(0.084-1.094)	0.46(0.1-2.12)	0.15
Consanguinity status				
Yes / No	1	0.86(0.24-3.1)	0.94(0.2-4.37)	0.965
rs3780135	**GG**	**GA**	**AA**	**P$_{(interaction)}$-value**
Family history status				
Yes / No	1	0.6(1.7-0.21)	0.44(0.15-1.3)	0.294
Smoking status				
Yes / No	1	1.08(0.46-2.5)	1.3(0.59-2.9)	0.754
Consanguinity status				
Yes / No	1	0.62(0.26-1.44)	0.6(.26-1.35)	0.4
rs12430881	**AA**	**AG**	**GG**	**P$_{(interaction)}$-value**
Family history status				
Yes / No	1	0.579(0.16-2.15)	1.8(.65-4.98)	0.286
Smoking status				
Yes / No	1	1.05(0.45-2.42)	1.08(0.45-2.6)	0.982
Consanguinity status				
Yes / No	1	1.152(0.5-2.65)	0.497(0.18-1.35)	0.317

2

Figure 1

SNP rs35958982

Well 1 indicates the DNA leader (100bp), an amplicon (395bp) is outer band. Amplicon 272bp: allele ‘A’ and amplicon of 170bp allele ‘G’.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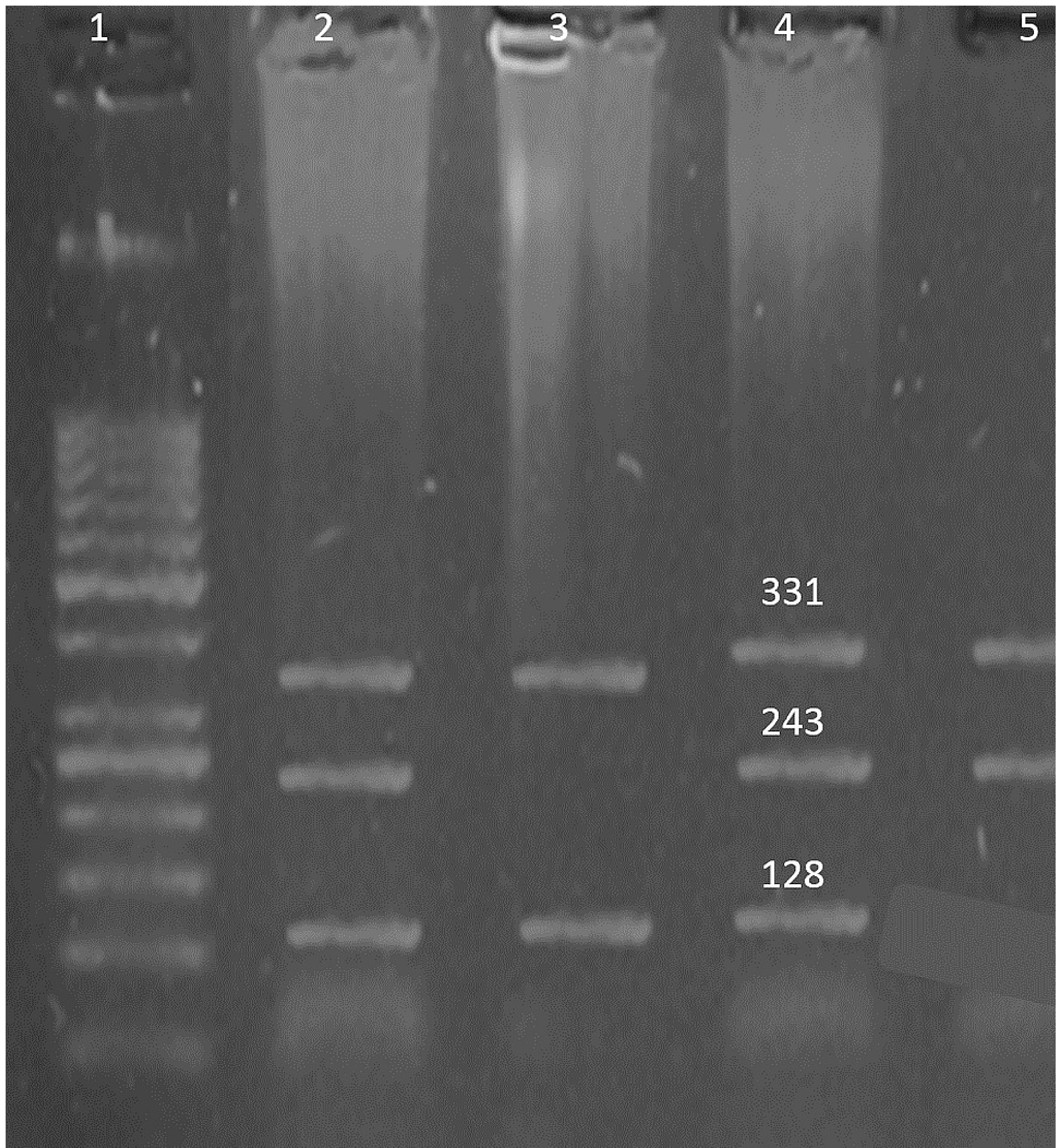
395
272
170

Figure 2

SNP rs3780135

Well 1 indicates the DNA leader (50bp), an amplicon (331bp) is outer band. Amplicon 243bp: allele ‘A’ and amplicon of 128bp: allele ‘G’


1
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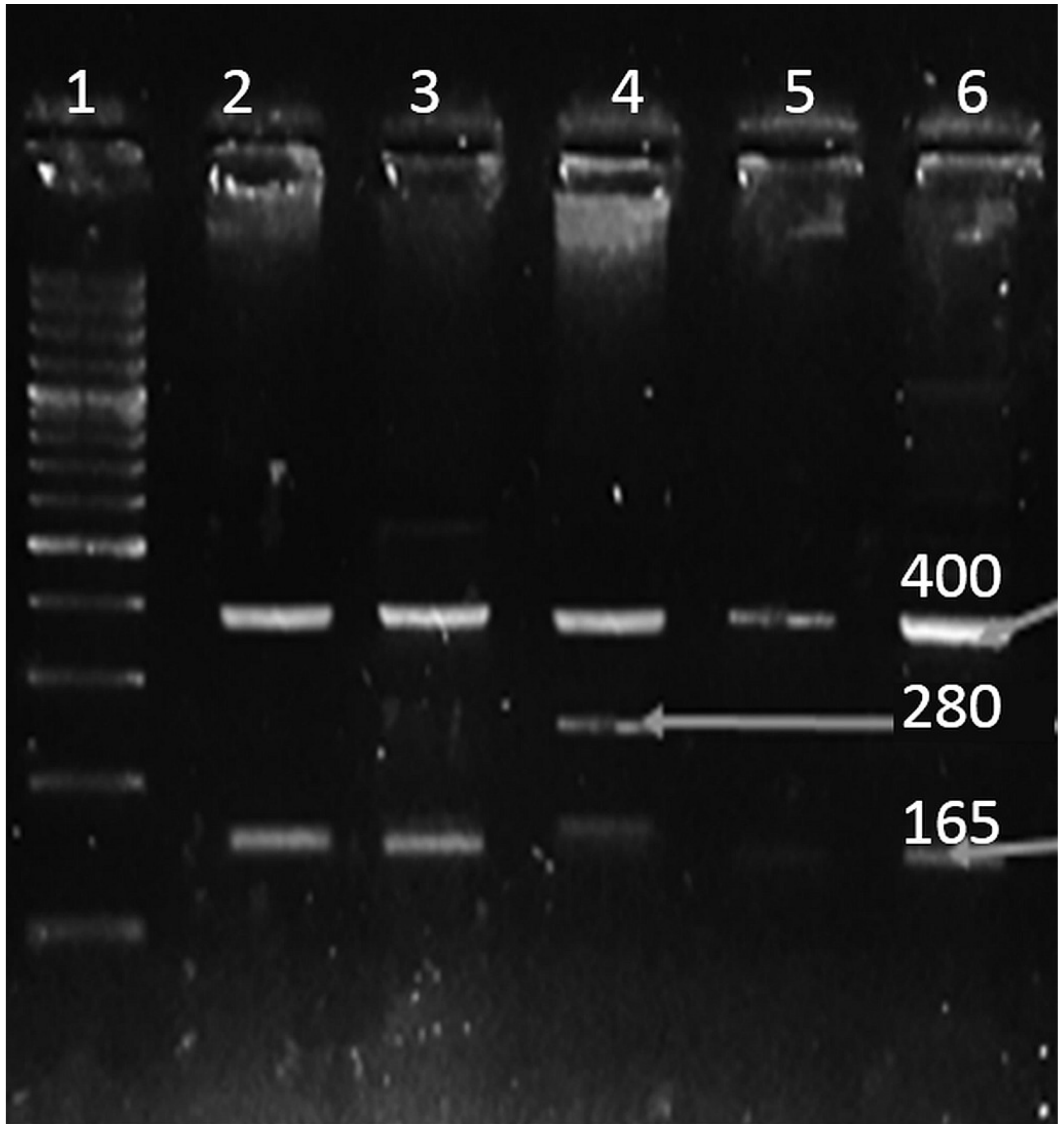
331
243
128

Figure 3

SNP rs12430881

Well 1 indicates the DNA leader (100bp), an amplicon (400bp) is outer band. Amplicon 165bp: allele G and amplicon of 280bp: allele A.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400
280
165