Influence of *CYP2C8*3* and *ABCG2* C421A genetic polymorphisms on trough concentration and molecular response of imatinib in Egyptian patients with chronic myeloid leukemia

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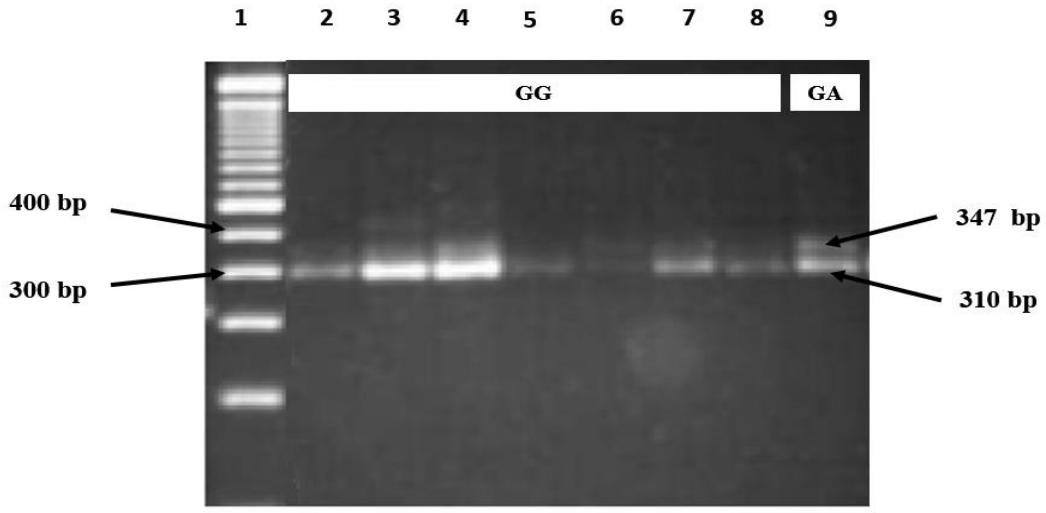
Details of the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of *CYP2C8*3* and *ABCG2* C421A polymorphisms:

a- **Polymerase chain reaction amplification of DNA samples:** A 50 µL reaction mixture was prepared in a 0.2 mL PCR tube following the manufacturer's protocol. The mixture included 25 µL of COSMO PCR Master Mix, 2 µL each of forward and reverse primers, 11 µL of nuclease-free water, and 10 µL of the DNA template.

b- **Restriction fragment length polymorphism analysis**: The amplified PCR products for each gene were digested with the appropriate restriction enzymes according to the manufacturer's protocol. For each sample, a 30 µL restriction reaction mixture was prepared, containing 17 µL of nuclease-free water, 2 µL of buffer, 10 µL of PCR product, and 1 µL of the restriction enzyme.

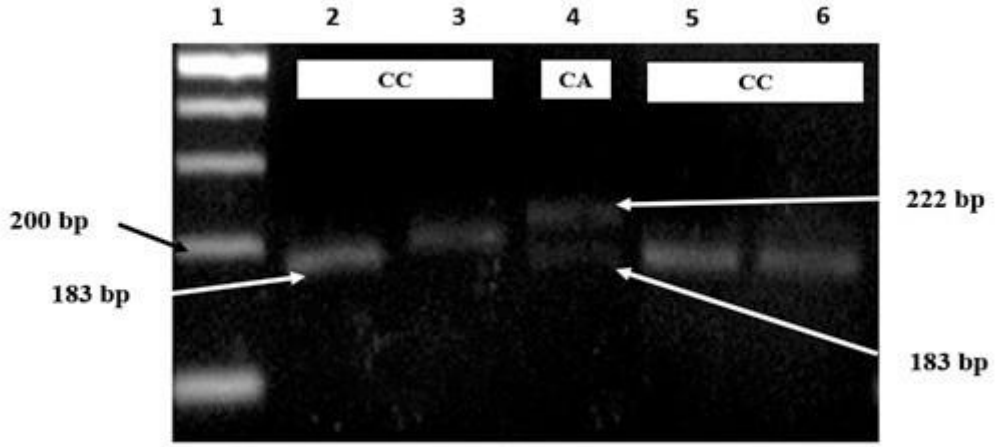
The primer sequences, PCR thermal conditions, restriction enzymes, and incubation temperatures for each SNP are presented in the following table:

	***CYP2C8*3* (G416A; rs11572080)**	***ABCG2* C421A (rs2231142)**
Primer sequence: - Forward	5' AGGCAATTCCCCAATATCTC-3'	5'-TGTTGTGATGGGCACTCTGATGTG-3'
- Reverse	5'-ACTCCTCCACAAGGCAGTGA-3'	5'-ATCAGAGTCATTTTATCCACAC -3'
PCR thermal conditions: - Initial denaturation	94 °C for 2 minutes.	94 °C for 10 minutes.
- Denaturation	94 °C for 30 seconds	
- Annealing	48 °C for 80 seconds.	53 °C for 30 seconds
- Extension	72 °C for 1 minute.	53 °C for 30 seconds
- Number of cycles	35 cycles	
- Final extension	72 °C for 7 minutes.	
PCR product [base pair (bp)]	347	222
Restriction enzyme	*Bse*RI	*Hpy*CH4III
Digestion temperature and duration	37 °C for 15 minutes	37°C for 1 hours
Fagments length after digestion (bp): - Homozygous wild type - Heterozygous type	**GG:** 310. **GA:** 347 and 310.	**CC:** 183. **CA:** 222 and 183



Gel electrophoresis of *CYP2C8*3* (G416A; rs11572080) polymorphism following PCR-RFLP analysis.

Lane 1 contains a 100 bp DNA ladder. Lanes 2–8 display the 310 bp band, indicating the wild-type homozygous genotype (GG; *CYP2C8*1/*1*). Lane 9 shows the 347 bp and 310 bp bands, representing the heterozygous genotype (GA; *CYP2C8*1/*3*).



Gel electrophoresis of *ABCG2* C421A (rs2231142) polymorphism following PCR-RFLP analysis.

Lane 1 contains a 100 bp DNA ladder. Lanes 2, 3, 5, and 6 display the 183 bp band, indicating the wild-type homozygous genotype (CC). Lane 4 shows both the 222 bp and 183 bp bands, representing the heterozygous genotype (CA).