

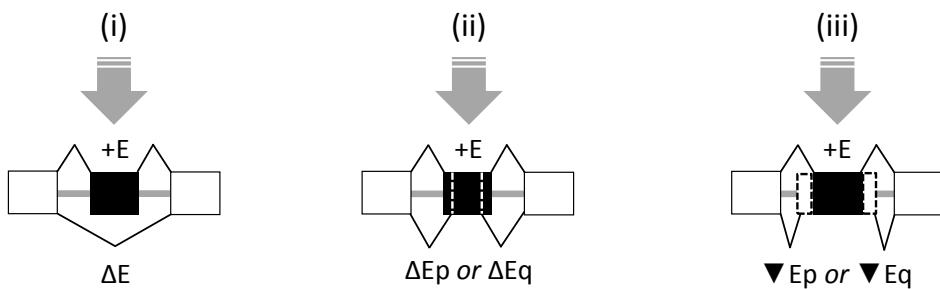
**18 MSH2 variants including:**

- 14 intronic variants at consensus splice site sequences (IVS $\pm$ 1,2)
- 3 intronic variants outside IVS $\pm$ 1,2
- 1 missense variant



**Impact on RNA splicing**  
*In cellulo splicing minigene-based assay*

**3 biotypes of variant-induced in-frame splicing anomalies**



mRNA

Exon skipping

 $\Delta E_3, \Delta E_4, \Delta E_5, \Delta E_{12}$ 

Segmental exonic deletion

 $\Delta E7q48, \Delta E15p36$ 

Segmental intronic retention

 $\nabla E4p24, \nabla E7p9, \nabla E12q30, \nabla E14p9$ 

Protein

Large internal deletion  
(del 49 to 93 aa)

p.Ala123\_Gln215del  
p.Ile216\_Gln264del  
p.Val265\_Gln314del  
p.Tyr588\_Gly669del

Small internal deletion  
(del 12 or 16 aa)

p.Ile411\_Gly426del  
p.Gly820\_AlA831del

Small internal insertion  
(ins 3 to 10 aa)

p.Glu215\_Ile216ins8  
p.Arg358\_Ser359ins3  
p.Gly669\_Pro670ins10  
p.Arg737\_Ser738ins3

**Impact on MSH2 function***Methylation tolerance-based functional assay***Loss of Function**

**Figure S2. Overview of the functional characterization of *MSH2* variants resulting into in-frame splicing alterations**