A c.4253+43G>A

| | _ | NM_ | 000350.2(A | BCA4):c.4253+43G | i>A - [c.4197 (Ex | on 28) - c.4253+14 | 43 (Intron 28) |] Alam | ut Vi | sual v.2.7 rev. 1 |
|-----------------------|---------|------------------------|------------|------------------|-------------------|--------------------|----------------|----------------|-------|--------------------------|
| SpliceSiteFinder-like | [0-100] | | | | | | | | | 5000 1.2.7 101.1 |
| MaxEntScan | [0-12] | | | | | | | | | |
| NNSPLICE 5' | [0-1] | | | | | | | | | |
| GeneSplicer 🥌 | [0-15] | -1. | 6 | | | | | | | |
| Human Splicing Finder | [0-100] | | | | | | | | | |
| | | 425 | | 4253+10 | 4253 | | 253+30 | 4253+4 | - | 4253+50 |
| Reference Sequence | TCT | T C A G <mark>G</mark> | TGCGC | GGACTCGG | GGTCACCA | ATTCTCCTC | CTGTGGG | ST T T IGGG | CAC | CTGGGTCAC |
| SpliceSiteFinder-like | [0-100] | | | | | | | | | |
| MaxEntScan | [0-16] | | | | | | | | | |
| NNSPLICE | [0-1] | | | | | | | | | |
| GeneSplicer 🥌 | [0-15] | | | | | | | | | |
| Human Splicing Finder | [0-100] | | | | | | | | | |
| Branch Points | [0-100] | | | | | | | | | L L |
| SpliceSiteFinder-like | [0-100] | | | | | | | | | |
| MaxEntScan | [0-12] | | | | | | | | | |
| NNSPLICE 5' | [0-1] | | | | | | | | | |
| GeneSplicer 🐱 | [0-15] | -1. | 2 | | | | | | | |
| Human Splicing Finder | [0-100] | | | | | | | | | |
| | | 425 | | 4253+10 | 4253 | | 253+30 | 4253+4 | | 4253+50 |
| Mutated Sequence | ГТСТ | T C A G G | TGCGC | GGACTCGG | GGTCACCA | ATTCTCCTC | CTGTGGG | ST T T G G G A | CAC | CTGGGTCAC |
| SpliceSiteFinder-like | [0-100] | | | | | | | | | |
| MaxEntScan | [0-16] | | | | | | | | | |
| NNSPLICE | [0-1] | | | | | | | | | |
| GeneSplicer 🥌 | [0-15] | | | | | | | | | |
| Human Splicing Finder | [0-100] | | | | | | | | | interactive |
| Branch Points | [0-100] | L | | U | | | | | U | l biosoftwa r e [|



Changes: SC35 motif 3.75>3.39, and new SF2/ASF motif 2.09

| Predicted signal | Prediction algorithm | cDNA Position | Interpretation | | |
|------------------|----------------------------|----------------------------|--|--|--|
| ESS Site broken | 1 - Sironi et al Motif 2 | ggtttgggåcåcc | Alteration of an intronic ESS site. | | |
| | 2 - Fas-ESS hexamers | | Probably no impact on splicing. | | |
| New ESE Site | 1 - HSF Matrices - 9G8 | gtttggggesectgggte | | | |
| | 2 - ElEs from Zhang et al. | 2 | Creation of an intronic ESE site. Probably no impact on splicing. | | |
| | 3 - ESE-Finder - SF2/ASF | 36 38 40 42 44 46 48 50 52 | | | |

B c.6006-609T>A

| | NM | _000350 |).2(ABCA4):c. | 6006-609 | Г>А - [с.6006 | -708 (Intron 43) | - c.6006-5 | 09 (Intron 43)] | Jamut V | /isual v.2. | 7 rev |
|-----------------------|---------|---------|---------------|------------|------------------------|------------------|------------|-----------------|---------|-------------|----------|
| SpliceSiteFinder-like | [0-100] | | | | | | | | | 15000 1120 | ., |
| MaxEntScan | [0-12] | | | | | | | | | | |
| NNSPLICE 5' | [0-1] | | | | | | | | | | |
| Genespricer | [0-15] | | | | | | | | | | |
| Human Splicing Finder | [0-100] | | | | | | | | | | |
| | -630 | | 006-620 | | 06-610 | 6006-60 | | 6006-590 | | 06-580 | 600 |
| Reference Sequence | TTCA | TAGA | СССТАА | GCTA | T A A G A A | GAAAAGG | ATGAG | ATTAGAC | TAAATG | TCTAT | GT A T A |
| SpliceSiteFinder-like | [0-100] | | | | | | | | | | |
| MaxEntScan | [0-16] | | | | | | | | | | |
| | [0-1] | 1.0 | | | | | | | | | |
| GeneSplicer 🥌 | [0-15] | 5.8- | | | | | | | | | |
| Human Splicing Finder | [0-100] | | | | 71.6 68 | | | | | | |
| Branch Points | [0-100] | | | | | | | | • [O | | |
| SpliceSiteFinder-like | [0-100] | | | | | | | | | | |
| MaxEntScan | [0-12] | | | | | | | | | | |
| NNSPLICE 5' | [0-1] | | | | | | | | | | |
| GeneSplicer 🐱 | [0-15] | | | | | | | | | | |
| Human Splicing Finder | [0-100] | | | | | | | | | | |
| | -630 | | 006-620 | | 06-610 | 6006-60 | | 6006-590 | | 06-580 | 600 |
| Mutated Sequence | TTCA | TAGA | СССТАА | GCTA | A A A G A A | GAAAAGG | ATGAG | ATTAGAC | TAAATG | TCTAT | GT A T A |
| SpliceSiteFinder-like | [0-100] | | | | | | | | | | |
| MaxEntScan | [0-16] | | | | | | | | | | |
| NNSPLICE | [0-1] | 1.0 | | | | | | | | | |
| GeneSplicer 🥌 | [0-15] | 6.2- | | | | | | | | | |
| Human Splicing Finder | [0-100] | | | | 71.7 <mark>5</mark> 66 | | | | | intera | |
| Branch Points | [0-100] | | | <u>ا</u> ۲ |] 0 0 0 0 0 | | | | - [] - | biosoft | ware L |



Changes: SRp40 motif 2.71>3.53

| Predicted signal | Prediction algorithm | cDNA Position | Interpretation | | |
|------------------|-------------------------------------|---------------------|-----------------------------------|--|--|
| | 1 - EIEs from Zhang et al. | ctaagctataagaagaaaa | | | |
| New EQE Obs | 2 - HSF Matrices - Tra2-β | 1 | Creation of an intronic ESE site. | | |
| New ESE Site | 3 - RESCUE ESE Hexamers | | Probably no impact on splicing. | | |
| | 4 - ESR Sequences from Goren et al. | -815 -810 -805 -800 | | | |

C c.5196+1056A>G

| | NM_000350.2(ABCA4):c.5196+1056A>G - [c.5196+956 (Intron 36) - c.5196+1156 (Intro | on 36)] |
|-----------------------|--|----------------------|
| SpliceSiteFinder-like | [0-100] | |
| MaxEntScan | [0-12] 4.4 | |
| NNSPLICE | [0-1] 0.8 | |
| GeneSplicer 💙 | [0-15] | |
| Human Splicing Finder | [0-100] 80.5 | |
| | | +1060 5196+1070 51 |
| | GGAATTCCTGAAGATATGATTTCTATCTTACTCAGCTTAGTAAGC | AGCTATCACTTAACAATA |
| SpliceSiteFinder-like | [0-100] | |
| MaxEntScan | [0-16] | |
| NNSPLICE | [0-1] | |
| GeneSplicer 🐸 | [0-15] | |
| Human Splicing Finder | | |
| Branch Points | | |
| SpliceSiteFinder-like | [0-100] 81.6 | |
| MaxEntScan | [0-12] 8.7 | |
| NNSPLICE | [0-1] 1.0 | |
| GeneSplicer 🚽 | [0-15] | |
| Human Splicing Finder | [0-100] 91.1 | |
| | | +1060 5196+1070 51 |
| Mutated Sequence | ggaattcctgaagatatgatttctatcttactcagcttggtaagc | AGCTATCACTTAACAATA |
| SpliceSiteFinder-like | [0-100] | • |
| MaxEntScan | [0-16] | V |
| NNSPLICE | [0-1] | 0 |
| GeneSplicer 🥣 | [0-15] | 11 |
| Human Splicing Finder | | |
| Branch Points | | |

Cryptic donor strongly activated

| | | | · · · | | | · · | · · · | Alamut | VISUAL V. Z. / |
|-----------------------|---------|-----------|----------|-----------|--------|------------|-----------|--------|----------------|
| SpliceSiteFinder-like | [0-100] | | | | | | | | |
| MaxEntScan | [0-12] | - | | | | | | | |
| NNSPLICE 5' | [0-1] | | | | | | | | |
| GeneSplicer 🥌 | [0-15] | | | | | | | | |
| Human Splicing Finder | [0-100] | | | | | | | | |
| | þ | 5196+1280 | 5196+12 | 90 5196 | 6+1300 | 5196+1310 | 5196+1320 | 519 | 6+1330 |
| Reference Sequence | Α C T T | AGAAAAG | TGCGGAGG | СТТТТААТ | TTTAC | ττττττάααα | GATAAGAAA | TAGAA | TTTACAC |
| SpliceSiteFinder-like | [0-100] | | | | | 8 | 1.3 | | |
| MaxEntScan | [0-16] | | | | | | 5.3= | | |
| NNSPLICE 2' | [0-1] | | | | | | 0.5 | | |
| GeneSplicer 🤳 | [0-15] | | | | | | 6.3- | | |
| Human Splicing Finder | [0-100] | | | | | 8 | 1.7 | | intera |
| Branch Points | [0-100] | | | 65.2 78.1 | 1 71.9 | 000 | | - 00 | biosof |

Probable nearest downstream cryptic splice acceptor at c.5196+1318

Splicing from these cryptic sites would result in a 262 bp pseudoexon and frameshift.

| Predicted signal | Prediction algorithm | cDNA Position | Interpretation | | |
|------------------|----------------------|---|---|--|--|
| N. D. O'le | 1 - HSF Matrices | agcttggtaagcagc | Activation of an intronic cryptic donor site. | | |
| New Donor Site | 2 - MaxEnt | 1,052 1,054 1,056 1,058 1,060 1,062 1,064 | Potential alteration of splicing. | | |



Supplemental Figure 1. Predicted effects of the three non-coding *ABCA4* variants on splicing and exonic splicing enhancers and silencers (ESE and ESS). Assessed using 5 different algorithms (SpliceSiteFinder, MaxEntScan, NNSPLICE, GeneSplicer, Human Splicing Finder) via Alamut software (<u>http://www.interactive-biosoftware.com</u>).