Table S1: Exons covered at ≥5 depth in the *ABCC6* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon2** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon3** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon4** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon5** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon6** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon7** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon8** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon9** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon10** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon11** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon12** | x | | x | x | x | | x | x | x | x | x | x | x | x | x | x |
| **Exon13** | x | | x | x | x | x | x | x | | x | x | x | x | x | x | x |
| **Exon14** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon15** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon16** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon17** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon18** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon19** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon20** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon21** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon22** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon23** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon24** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon25** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon26** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon27** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon28** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon29** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon30** | | | x | x | x | | x | x | x | x | x | x | x | x | x | x | |
| **Exon31** | | | | | | | | | | | | | | | | | |
| **% total exons** | 84 | 77 | 97 | 97 | 97 | 94 | 97 | 97 | 94 | 97 | 97 | 97 | 97 | 97 | 97 | 97 | |

Table S2: Exons covered ≥5 depth in the *GGCX* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | | | | | | | | | | | | | | | | |
| **Exon2** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon3** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon4** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon5** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon6** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon7** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon8** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon9** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon10** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon11** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon12** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon13** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon14** | x | | x | x | x | x | | x | x | x | x | x | x | x | x | x |
| **Exon15** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon16** | | | x | | | x | | | | x | x | | | x | x | |
| **Exon17** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon18** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon19** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon20** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon21** | x | x | x | x | x | x | | x | x | x | x | x | x | x | x | x |
| **Exon22** | | | | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon23** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon24** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon25** | | | | | | | | | | | | | | | | |
| **% total Exons** | 68 | 52 | 88 | 88 | 88 | 92 | 80 | 88 | 88 | 92 | 92 | 88 | 88 | 92 | 92 | 88 |

Table S3: Exons covered ≥5 depth in the *ENPP1* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | | | x | x | | | x | | x | x | | | x | x | | x |
| **Exon2** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon3** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon4** | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon5** | | | x | x | x | | | x | x | x | | x | x | x | x | x |
| **Exon6** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon7** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon8** | | x | x | x | x | x | x | x | x | x | x | x | | x | x | x |
| **Exon9** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon10** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon11** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon12** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon13** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon14** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon15** | | | | | | | | | | | | | | | | |
| **% total exons** | 60 | 73 | 93 | 93 | 87 | 80 | 87 | 87 | 93 | 93 | 80 | 87 | 87 | 93 | 87 | 93 |

Table S4: Exons covered ≥5 depth in the *VKORC1* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | | | | | | | | | | | | | | | | |
| **Exon2** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon3** | | | | | | | | | | | | | | | | |
| **% total exons** | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |

Table S5: Exons covered ≥ 20 reads in the *ABCC6* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon2** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon3** | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon4** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon5** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon6** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon7** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon8** | | | x | x | x | | x | x | x | x | x | x | x | x | x | x |
| **Exon9** | | | x | x | x | | | x | x | x | x | x | x | x | x | x |
| **Exon10** | | | x | x | | | | x | | | | x | x | x | | x |
| **Exon11** | | | | | | | | | | | | | x | x | x | |
| **Exon12** | | | | x | | | | | | | | | x | x | x | |
| **Exon13** | | | | | | | | | | | | | | | | |
| **Exon14** | | | x | x | | | | x | | | x | x | x | x | x | x |
| **Exon15** | | | x | | x | | | x | x | x | | x | x | x | x | x |
| **Exon16** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon17** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon18** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon19** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon20** | | | x | x | | | x | x | x | x | x | x | x | x | x | x |
| **Exon21** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon22** | | | x | x | | x | x | x | | | | | | x | x | |
| **Exon23** | | | | | | | | | | | | | x | | | |
| **Exon24** | | | | x | x | | x | | | | x | | | x | | x |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon25** | | | x | | | | | x | | | | | x | | | |
| **Exon26** | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon27** | | | | | | | | | | | | | | | | |
| **Exon28** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon29** | | | x | x | | | | x | | | x | x | x | x | | x |
| **Exon30** | | | | | | | | | | | | | | | | |
| **Exon31** | | | | | | | | | | | | | | | | |
| **% of total Exon** | 3 | 13 | 74 | 74 | 58 | 48 | 58 | 74 | 58 | 58 | 65 | 68 | 81 | 81 | 71 | 71 |

Table S6: Exons covered ≥ 20 reads in the *ENPP1* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | | | | | | | | | | | | | | | | |
| **Exon2** | | | | | | | | | x | | | | x | | x | x |
| **Exon3** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon4** | | | x | x | | x | | x | x | x | x | x | x | x | x | x |
| **Exon5** | | | | | | | | | | | | | | | | |
| **Exon6** | | | | | | | | | x | | | | | | | |
| **Exon7** | | | | | | | | | x | | | x | | | | |
| **Exon8** | | | | | | | | | x | x | | | | | x | |
| **Exon9** | | | | x | x | x | | x | x | x | x | x | x | x | x | x |
| **Exon10** | | | | | x | x | | x | x | x | | x | x | x | x | |
| **Exon11** | | | | | | | | | x | x | | | | | x | |
| **Exon12** | | | x | x | x | x | x | x | x | x | | x | x | x | x | |
| **Exon13** | | | | | | | | | x | | | x | | | | |
| **Exon14** | | | | | | | | | | x | x | | | | | |
| **Exon15** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon16** | | | | | | | | | | | | | | | | |
| **Exon17** | | | x | | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon18** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon19** | | | x | | | | | | | | | | | | | |
| **Exon20** | | | | | x | x | | x | x | x | | x | x | x | x | x |
| **Exon21** | | | | | | | | | x | | | x | | | x | |
| **Exon22** | | | | | | | | | | | | | | x | | |
| **Exon23** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon24** | | | x | | x | x | | | x | x | x | x | x | | x | x |
| **Exon25** | | | | | | | | | | | | | | | | |
| **% of total** | 0 | 0 | 36 | 28 | 40 | 44 | 24 | 40 | 72 | 56 | 36 | 56 | 48 | 44 | 60 | 40 |
| **Exon** | | | | | | | | | | | | | | | | |

Table S7: Exon covered ≥ 20 reads in the *GGCX* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | | | | | | | | | | | | | | | | |
| **Exon2** | | | x | x | x | | x | x | x | x | x | | x | x | x | x |
| **Exon3** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon4** | | | | x | | | | | x | x | | | x | x | | x |
| **Exon5** | | | | | | | | | | | | | | | | |
| **Exon6** | | | | x | | | | | x | | | x | | x | x | x |
| **Exon7** | | | | x | x | | x | x | x | | | | x | x | x | x |
| **Exon8** | | | | | | | x | | | | | | | | | |
| **Exon9** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon10** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon11** | | | x | | x | | x | | x | | x | | x | x | | x |
| **Exon12** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon13** | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Exon14** | | | x | x | | x | | | x | x | x | x | x | x | x | x |
| **Exon15** | | | | | | | | | | | | | | | | |
| **% of total Exon** | 0 | 0 | 53 | 67 | 53 | 40 | 60 | 47 | 73 | 53 | 53 | 47 | 67 | 73 | 60 | 73 |

Table S8: Exon covered ≥ 20 reads in the *VKORC1* gene

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exon1** | | | | | | | | | | | | | | | | |
| **Exon2** | | | | | x | x | | | x | | x | x | x | x | x | x |
| **Exon3** | | | | | | | | | | | | | | | | |
| **% of total Exon** | 0 | 0 | 0 | 0 | 33 | 33 | 0 | 0 | 33 | 0 | 33 | 33 | 33 | 33 | 33 | 33 |

Table S9: List of polymorphisms found in *ABCC6, ENPP1, GGCX* and *VKORC1* in the16 patients.

| Gene | Patient | Polymorphism | | |
|---|---|---|---|---|
| | | **Exonic** | **Intronic** | **UTR3** |
| *ABCC6* | P1 | NM_001171:c.G3803A:p.(R1268Q)<br>NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.G1245A:p.(V415V)<br>NM_001171:c.T1233C:p.(N411N) | c.1338+20C>G<br>c.1338+7C>A | |
| | P2 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.T1233C:p.(N411N) | c.3633+55C>T<br>c.2787+62T>C<br>c.1635+48C>T | |
| | P3 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.C1896A:p.(H632Q)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.C1890G:p.(T630T)<br>NM_001171:c.G1245A:p.(V415V)<br>NM_001171:c.T1233C:p.(N411N) | c.2787+62T>C<br>c.1338+20C>G<br>c.1338+7C>A | c.*17G>A |
| | P4 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.C1896A:p.(H632Q)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.A793G:p.(R265G)<br>NM_001171:c.C2490T:p.(A830A)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.C1890G:p.(T630T) | c.2787+62T>C | |
| | P5 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.C1896A:p.(H632Q)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.C2490T:p.(A830A)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.C1890G:p.(T630T) | c.2787+62T>C<br>c.1635+48C>T<br>c.1338+20C>G | |

| | | | | |
|---|---|---|---|---|
| | P6 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.C2835T:p.(P945P)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.G1245A:p.(V415V)<br>NM_001171:c.T1233C:p.(N411N) | c.2787+62T>C<br>c.1338+20C>G<br>c.1338+7C>A | |
| | P7 | NM_001171:c.G3803A:p.(R1268Q)<br>NM_001171:c.C3190T:p.(R1064W)<br>NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.A2400G:p.(G800G) | c.2787+62T>C<br>c.1635+48C>T<br>c.1338+20C>G<br>c.1338+7C>A | |
| | P8 | NM_001171:c.G3803A:p.(R1268Q)<br>NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.C1896A:p.(H632Q)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.A793G:p.(R265G)<br>NM_001171:c.C2490T:p.(A830A)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.C1890G:p.(T630T) | c.2787+62T>C | |
| | P9 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.A2400G:p.(G800G) | c.2787+62T>C | c.*17G>A |
| | P10 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.C1896A:p.(H632Q)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.C2490T:p.(A830A)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.C1890G:p.(T630T) | c.2787+62T>C<br>c.1338+20C>G<br>c.1338+7C>A | |
| | P11 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.A2400G:p.(G800G) | c.4042-30C>T<br>c.3633+55C>T | |
| | P12 | NM_001171:c.A2542G:p.(M848V)<br>*NM_001171:c.C1353T:p.(S451S)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.G1245A:p.(V415V)<br>NM_001171:c.T1233C:p.(N411N) | *c.4042-35G>A<br>c.3507-16T>C<br>c.2787+62T>C<br>c.1338+20C>G<br>c.1338+7C>A | |

| | | | | |
|---|---|---|---|---|
| | P13 | *NM_001171:c.C1353T:p.(S451S)<br>NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.G1245A:p.(V415V)<br>NM_001171:c.T1233C:p.(N411N) | *c.4042-35G>A<br>c.3507-16T>C<br>c.2787+62T>C<br>c.1338+20C>G<br>c.1338+7C>A | |
| | P14 | *NM_001171:c.C1353T:p.(S451S)<br>NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.G1245A:p.(V415V)<br>NM_001171:c.T1233C:p.(N411N) | *c.4042-35G>A<br>c.3507-16T>C<br>c.2787+62T>C<br>c.1338+20C>G<br>c.1338+7C>A | |
| | P15 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.C2835T:p.(P945P)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.G1245A:p.(V415V)<br>NM_001171:c.T1233C:p.(N411N) | c.2787+62T>C<br>c.1338+20C>G<br>c.1338+7C>A | |
| | P16 | NM_001171:c.A2542G:p.(M848V)<br>NM_001171:c.C1896A:p.(H632Q)<br>NM_001171:c.T1841C:p.(V614A)<br>NM_001171:c.C2490T:p.(A830A)<br>NM_001171:c.A2400G:p.(G800G)<br>NM_001171:c.C1890G:p.(T630T) | c.2787+62T>C<br>c.1635+48C>T<br>c.1338+7C>A | |
| *GGCX* | P1 | NM_001142269:c.A666C:p.(G222G) | c.44-32G>A | |
| | P2 | NM_001142269:c.A666C:p.(G222G)<br>NM_000821:c.C189T:p.(S63S) | c.2084+45G>C<br>c.44-32G>A | |
| | P3 | NM_001142269:c.C1071T:p.(T357T)<br>NM_001142269:c.C1047T:p.(R349R)<br>NM_001142269:c.G803A:p.(R268Q)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P4 | NM_001142269:c.C1047T:p.(R349R)<br>NM_001142269:c.G803A:p.(R268Q)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P5 | NM_001142269:c.C1071T:p.(T357T)<br>NM_001142269:c.A666C:p.(G222G) | | |

| | | | | |
|---|---|---|---|---|
| | P6 | NM_001142269:c.C1047T:p.(R349R)<br>NM_001142269:c.G803A:p.(R268Q)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P7 | NM_001142269:c.C1071T:p.(T357T)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P8 | NM_001142269:c.C1071T:p.(T357T)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P9 | NM_001142269:c.C1047T:p.(R349R)<br>NM_001142269:c.G803A:p.(R268Q)<br>NM_001142269:c.A666C:p.(G222G) | c.2085-21C>T | |
| | P10 | NM_001142269:c.C1071T:p.(T357T)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P11 | NM_001142269:c.C1047T:p.(R349R)<br>NM_001142269:c.G803A:p.(R268Q)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P12 | NM_001142269:c.C1047T:p.(R349R)<br>NM_001142269:c.G803A:p.(R268Q)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P13 | NM_001142269:c.C1047T:p.(R349R)<br>NM_001142269:c.G803A:p.(R268Q)<br>NM_001142269:c.A666C:p.(G222G) | | |
| | P14 | NM_001142269:c.A666C:p.(G222G) | | |
| | P16 | NM_001142269:c.C1071T:p.(T357T)<br>NM_001142269:c.A666C:p.(G222G) | | |
| *ENPP1* | P1 | NM_006208:c.A517C:p.(K173Q) | | |
| | P6 | NM_006208:c.A517C:p.(K173Q) | | |
| | P8 | NM_006208:c.C2320T:p.(R774C) | | |
| | P10 | NM_006208:c.C2320T:p.(R774C) | | |
| | P11 | NM_006208:c.A2661G:p.(A887A) | | |

| | | | | |
|---|---|---|---|---|
| | P12 | NM_006208:c.A517C:p.(K173Q)<br>NM_006208:c.T1056G:p.(A352A)<br>NM_006208:c.T1329C:p.(D443D)<br>NM_006208:c.A2661G:p.(A887A) | | |
| | P13 | NM_006208:c.A517C:p.(K173Q)<br>NM_006208:c.T1056G:p.(A352A)<br>NM_006208:c.T1329C:p.(D443D)<br>NM_006208:c.A2661G:p.(A887A) | | |
| | P14 | NM_006208:c.A517C:p.(K173Q)<br>NM_006208:c.T1056G:p.(A352A)<br>NM_006208:c.T1329C:p.(D443D) | | |

Table S10: Minor Allele Frequency (MAF) of polymorphisms in *ABCC6*, *GGCX*, and *ENPP1*

| Gene | Polymorphisms | | | | | Patient ID |
|---|---|---|---|---|---|---|
| | **Amino acid change** | **Location in gene** | **Hom/het** | **ESP5400_ALL** | **1000g2012feb_ALL** | |
| ***ABCC6*** | c.4042-35G>A | intronic | het | 0.0015% | 0.0005% | P12, P13 |
| | NM_001171:c.C1353T:p.(S451S) | exonic | het | 0.0008% | 0.0005% | P12, P13 |
| ***GGCX*** | NM_000821:c.C189T:p.(S63S) | exonic | het | 0.0028% | 0.0014% | P2 |
| ***ENPP1*** | NM_006208:c.T1329C:p.(D443D) | exonic | het | 0.0185% | 0.01% | P12, P13, P15 |

Hom: homozygous; Het: heterozygous

Table S11: List of possible homozygous mutations in two PXE-patients with one *ABCC6* mutation, after filtering based on population frequency of ≤1% and an *in silico* functional prediction of 'deleterious' (SIFT) or 'probably damaging' (polyphen)

| Patient | Rs number | Population frequency | Gene | Nucleotide change | Change in protein | Type of mutation | Variant location | Function of the gene |
|---|---|---|---|---|---|---|---|---|
| F5 | NA | NA | *PTPRH* | c.2512C>A | p.(P838T) | missense | Exon | - Receptor-type tyrosine-protein phosphatase H<br>- Regulates a variety of cellular processes including cell growth, differentiation, mitotic cycle and oncogenic transformation |
| F5 | NA | NA | *PTPRH* | c.3046C>A | p.(P1016T) | missense | Exon | |
| F16 | NA | NA | FUK | c.380T>C | p.(L127P) | missense | Exon | - Fucokinase<br>- Involved in reutilization of fucose from the degradation of oligosaccharides |

NA: not applicable

Table S12: List of possible second gene mutations in two PXE-patients with one *ABCC6* mutation, after filtering based on population frequency of ≤1% and an *in silico* functional prediction of 'deleterious' (SIFT) or 'probably damaging' (polyphen)

| Patient | Rs number | Population frequency | Gene | Nucleotide change | Aminoacid change | Type of mutation | Variant location | Function of the gene |
|---|---|---|---|---|---|---|---|---|
| P5, 16 | rs138052077 | 0.0005 | *ZC3H12C* | c.658C>T | p.(A220T) | Missense | Exon | - Zinc finger CCCH-type containing 12C<br>- Acts as RNase and regulates the levels of target RNA species |
| P5, 16 | rs141683209 | 0.000617 | *TULP4* | c.4396G>A | p.(V1466M) | Missense | Exon | - Tubby like protein 4<br>- Mediates the ubiquitination and subsequent proteasomal degradation of target proteins. |
| P5 | rs146857860 | 0.000396 | *PCDHGA2* | c.529C>A | p.(H177A) | Missense | Exon | - Protocadherin gamma subfamily A, 2<br>- Involved in neuronal connections in the brain. |
| P5 | rs146857860 | 0.000396 | *PCDHGA2* | c.529C>A | p.(H177A) | Missense | Exon | |
| P5, 16 | rs138308587 | 0.000566 | *ACAA1* | c.1166C>T | p.(T389M) | Missense | Exon | - Acetyl-CoA acyltransferase 1<br>- Involved in the beta-oxidation system of the peroxisomes |
| P5, 16 | rs138308587 | 0.000566 | *ACAA1* | c.887C>T | p.(T296M) | Missense | Exon | |
| P16 | rs139927121 | 0.000242 | *SPANXC* | c.103C>T | p.(P35S) | Missense | Exon | - Sperm protein associated with the nucleus on the X chromosome, family member C<br>- Maturation of spermatozoa |
| P16 | rs201057336 | 0.001 | *SUPV3L1* | c.1565A>G | p.(T522C) | Missense | Exon | - Suppressor of Var1, 3-Like 1<br>- Plays a role in mitochondrial RNA metabolism |
| P16 | rs146579435 | 0.00022 | *FLNB* | c.5974C>T | p.(A1992C) | Missense | Exon | - Filamin B, beta<br>- Regulates intracellular communication |
| P16 | rs146579435 | 0.00022 | *FLNB* | c.6007C>T | p.(A2003C) | Missense | Exon | |
| P16 | rs146579435 | 0.00022 | *FLNB* | c.6100C>T | p.(A2034C) | Missense | Exon | |
| P16 | rs138073376 | 0.0005 | *PPEF2* | c.2026G>A | p.(G676L) | Missense | Exon | - Protein phosphatase, EF-hand calcium binding domain 2<br>- Acts as calcium sensing regulator of ionic currents, energy production or synaptic transmission. |
| P16 | rs144374710 | 0.000662 | *PRR15L* | c.50A>T | p.(L17I) | Missense | Exon | - Proline rich 15L<br>- Unknown function |
| P16 | rs150626138 | 0.00022 | *CEACAM1* | c.442T>C | p.(S148P) | Missense | Exon | - Carcinoembryonic antigen-related cell adhesion molecule 1<br>- Plays a role in differentiation and arrangement of tissue structure, angiogenesis, apoptosis, tumor suppression, metastasis, and the modulation of innate and adaptive immune responses |
| P16 | rs150711066 | 0.000742 | *PALLD* | c.1040C>T | p.(T347M) | Missense | Exon | - Palladin<br>- Organizing the structure of cytoskeleton |
| P16 | rs139560362 | 0.000909 | *QTRT1* | c.349C>A | p.(L117M) | Missense | Exon | - Queuine tRNA-ribosyltransferase 1<br>- Involved in tRNA modification |
| P16 | rs142863092 | 0.000396 | *PVRL1* | c.629G>A | p.(A210H) | Missense | Exon | - Poliovirus receptor-related 1<br>- Involved in organization of adherens junctions and tight junctions in epithelial and endothelial cells |

Table S13: List of primers used in Sanger sequencing

| Gene | Exon | Forward primer 5'-3' | Reversed primer 5'-3' |
|---|---|---|---|
| ***ABCC6*** | exon 1 | GGTCCAAAGTGTTTAGGAAGTC | GCAGCCCGAGAGATCTGCAGC |
| | exon 2 | GATCCAAAAAGTTGCCTGGC | TGTCCCCTGCCTCCCCCGAA |
| | exon 3-4 | TCCCAGTTGGACATGGGGC**C** | TATAAGTGTGTGCATCGTG**T** |
| | exon 5 | CCTCCTCTGTCTCCATTCCTTAT | AGACTGAGACCTCAAAGTGG |
| | exon 6 | CACAGTTCGTCCTGTCTTCCTAC | TGGCCCTGGAGAAGCAGCTGT |
| | exon 7 | GTGACTTACCCAGGGTCACAC | ATGATGAGCTTTTCTGAAG**T** |
| | exon 8 | CCCCCAACTCCCATGATTG**C** | AAGGATGCCACTAAGAGACC |
| | exon 9 | AGGCACCTCCTCTCACCAGC | GGTGACAGAGCAAGACTCC**A** |
| | exon 10 | TGGGTGGAGAAGCCACCTGG | GGGGGACTCCGTTCAAAT |
| | exon 11 | TCTGAGAGCTGGGCTCCTCG | CAGGACCTGTGGCTTCCTCC |
| | exon 12 | GGTGGCTGTCAGGGTGCAGG | CCACCTACCTCACCCTGCCC |
| | exon 13 | AGGCTGCCCTATCCATGCTTGC | GGAAGCTGGAGCCAGGTGTAG |
| | exon 14 | AGACACCGACACCCAAAACC | TGCTGGCTTGCCATTATGGG |
| | exon 15 | CTCTCCGATGCAGAGGCC | CTACACCACCTCTCAGGTG |
| | exon 16 | TCACAGAGGCGGGCTGAACC | AGTGGGACTTTCAGGATGGG |
| | exon 17 | AACCTATCCCACCTCTGTCC | TCGGGGACCCAAATGACTCC |
| | exon 18 | CAGTTTCACCCTGTAGATGC | ATGCTAAGTGCTTCCTCTGC |
| | exon 19 | CATGTGTGTAACTTCTACCC | GCCAGTAGGACCCTTCGAGC |
| | exon 20 | GTGTCAAAGTGGGTATCTGG | TCAGCTACTTCAGCTTCAGC |
| | exon 21 | CCTGAGGGTTAGGCACATAC | GCTACATTTGGTGGGAGGAC |
| | exon 22 | TCGCCTGGCCTTGCCTCAACC | GTAAAGGAGTCCTGAGCACC |
| | exon 23 | CCGCTCCTGAGGGTCTCCAG | GCTGGGTGAAACCTCATATATGG |
| | exon 24 | CTACCTCTCTATGTCTGTGG | ATATGACCTCAGGTCTCACC |
| | exon 25 | CTTGTGCCCAGAGAAGCATC | AGGGGTCCGACAGTCTCTG |
| | exon 26 | CTGTTGCAAGCCCTCAAGTG | AGCAGATGTCAACAGGGACC |
| | exon 27 | GTGAAGTCTTAGAGGAAGGC | TTGTCCCTGGAGTCCTTTGG |
| | exon 28 | AATGCCCACAAACCCTCTGG | ACCATGCCTCCCATCTTTGC |
| | exon 29 | AAAGATGGGAGGCATGGTG | TCAGAGCTTGGAATTGCAGATA |
| | exon 30 | ACCCACACATCACCATGTGC | ACTGCAGGGCTGCTGTGAGG |
| | exon 31 | AGAGCCTCTCTGTCTCCCTCT | TCCAGCACTGCAGGCTGTGC |
| ***GGCX*** | exon 7 | AAGAGATCTGGAGCACTGG | TTTACACAGAGTCGGCGATG |

*ABCC6*-specific primers designed based on sequence differences between *ABCC6* and its two pseudo genes. Underlined nucleotides eliminate the amplification of both pseudogenes, underlined nucleotides eliminate *ABCC6-Ψ2*, nucleotides in bold eliminate *ABCC6-Ψ1*.