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| **Table S1: Primers used for sequencing *AHI1* exons** | | |
|  |  |  |
| **Primer name** | **Sequence** | **Length PCR product (bp)** |
| AHI1\_ex4FW | ACATCTCCTTTGTTTCGATAGG | 167 |
| AHI1\_ex4RV | CAAGAATCCCTTAAAATAAACCAAAC |
| AHI1\_ex5FW | aaaggtcagagggatacaggtg | 542 |
| AHI1\_ex5RV\_new | ttcctgtaggacagcactcaag |
| AHI1\_ex6FW | AAAATGAATCCAAAGTGTTAATCC | 596 |
| AHI1\_ex6RV\_new | TGAAGGAAAGCCACCAAATC |
| AHI1\_ex7\_1FW\_new | TAATCCTCCCAATAATTCCACA | 490 |
| AHI1\_ex7\_1RV\_new | GCTTGCATCAATTCTTCATCC |
| AHI1\_ex7\_2FW\_new | AGGCGTTGATCATCAGAAAAG | 513 |
| AHI1\_ex7\_2RV\_new | TCCTCATCAGCAAGATAGGAAG |
| AHI1\_ex8FW\_new | ACCACCTATGATACCTATTTGACAC | 581 |
| AHI1\_ex8RV\_new | TCAGAGAAGACTCAAGTGATACAAAC |
| AHI1\_ex9FW\_new | ATGGTTTGCTGTTGTCTGGC | 455 |
| AHI1\_ex9RV | CTGACTTTCAACTACCAATGGC |
| AHI1\_ex10FW | TTATTTCTGGTTGCCTCACC | 413 |
| AHI1\_ex10RV | TCCTAGAGATTAAATTCTCACACAAC |
| AHI1\_ex11FW | AAATGGACCCTCCCTAACTG | 438 |
| AHI1\_ex11RV\_new | gaatgaGCATAACCTGAGCTTG |
| AHI1\_ex12FW\_new | CATTTGGGCTACCTTTTGTC | 652 |
| AHI1\_ex12RV\_new | caactcctgCTTTAAATCAACC |
| AHI1\_ex13FW | ACAGGACTGTAGTTTTAAGCAGC | 533 |
| AHI1\_ex13RV | tgcttatatacacatgctaggcac |
| AHI1\_ex14FW\_new | ATGGTTTTTCACCATTCTGC | 595 |
| AHI1\_ex14RV\_new | TCCCCATGAGATTTATTCATCC |
| AHI1\_ex15FW | TGTGCTGCAAATGTCTTTGG | 380 |
| AHI1\_ex15RV | TTATGACAGTCCCTTCTTGGG |
| AHI1\_ex16FW | TGCTTGCTTAAGGTTCATTGG | 432 |
| AHI1\_ex16RV | GCGCAATCATCAGTACATAACC |
| AHI1\_ex17FW | TTCTTTGACTGTTTTACTGGGG | 380 |
| AHI1\_ex17RV\_new | ATCAGTCAGCCATCAGGAGG |
| AHI1\_ex18FW | GCATCCTATACAGTGGAATTGG | 341 |
| AHI1\_ex18RV | gagtgtggggacactgcttag |
| AHI1\_ex19FW\_new | tgggactagctctcctttattctg | 564 |
| AHI1\_ex19RV\_new | AGGCCATGAAAGAACAAACC |
| AHI1\_ex20FW\_new | AGAAGGAGGAGGGTCAGTGG | 477 |
| AHI1\_ex20RV\_new | TGATTCCAACATAAGGGCAC |
| AHI1\_ex21FW\_new | TCCCAGTTTACATGGCCTTC | 622 |
| AHI1\_ex21RV\_new | CCTAAATTCAGGTTGTCTGTTGC |
| AHI1\_ex22FW\_new | TCACAGTGCCATTTGTTTGG | 378 |
| AHI1\_ex22RV\_new | TGAGCTCCAATAATGAAAGTACAC |
| AHI1\_ex23FW | TCATGTGTCCTGGTTTGTATAATAAG | 402 |
| AHI1\_ex23RV\_new | AACTTACTTTTGAAATAACCTTGGTG |
| AHI1\_ex24FW\_new | GGCTTAGCTAACCTTGAGTCAG | 390 |
| AHI1\_ex24RV\_new | TGCAGGGATATAACTTTTGGC |
| AHI1\_ex25FW\_new | TGCTTCTCCTGCTGTGTTCC | 448 |
| AHI1\_ex25RV | CATATGCAAAGGTTACAAAGACAG |
| AHI1\_ex26FW\_new | TTGCAAATTGCCGTAACAAG | 498 |
| AHI1\_ex26RV\_new | TCCCATCACTTAGGCTGTGAC |
| AHI1\_ex27FW | TTCTCTCCCCATTCAGGAAG | 306 |
| AHI1\_ex27RV | CAAGCTAAACTCCTTTAAAATCAAC |
| AHI1\_ex28FW\_new | CTCATTAGTTCTGCCGGATG | 341 |
| AHI1\_ex28RV | tggtatttgtccttgctgacc |
| AHI1\_ex29FW | TGCCTGAGCACACTAAGATTTG | 286 |
| AHI1\_ex29RV\_new | AACTGAACTCAAAGGCCACG |

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| **Table S2: Homozygous and possible compound heterozygous variants after filtering WES data of patient A-II-1, B-II:2 and C-II:1** | | | | | | | | | |
|  |  |  |  |  |  |  |  |  |  |
| **Patient** | **Chr** | **Genomic position** | **Reference** | **Variant** | **Gene** | **Transcript** | **Nucleotide change** | **Protein change** | **Interpretation of phenotypic effect of the gene** |
| A-II:1 | 6 | 135754173 | T | A | *AHI1* | ENSG00000135541 | c.2258A>T | p.Asp753Val | Gene is associated with a syndromic retinal dystrophy |
|  | 6 | 135754257 | C | T | *AHI1* | ENSG00000135541 | c.2174G>A | p.Trp725\* |
| B-II:2 | 2 | 219506931 | C | G | *ZNF142* | ENSG00000115568 | c.G4308C | p.Q1436H | Targets of protein are unknown |
|  | 2 | 219508090 | C | T | *ZNF142* | ENSG00000115568 | c.G3149A | p.R1050K |
|  | 6 | 135754341 | G | A | *AHI1* | ENSG00000135541 | c.C152T | p.P51L | Gene is associated with a syndromic retinal dystrophy |
|  | 6 | 135787041 | G | - | *AHI1* | ENSG00000135541 | c.606delC | p.P202fs |
|  | 7 | 21882211 | C | T | *DNAH11* | ENSG00000105877 | c.C10762T | p.L3588F | Gene is associated with disease symptoms that are not present in patient |
|  | 7 | 21901467 | C | T | *DNAH11* | ENSG00000105877 | c.11224-4C>T | r.spl? |
|  | 8 | 131070318 | T | C | *ASAP1* | ENSG00000153317 | c.A3197G | p.K1066R | Protein function seems not be related to the retina |
|  | 8 | 131104231 | C | T | *ASAP1* | ENSG00000153317 | c.G2560A | p.A854T |
|  | 9 | 35819211 | T | C | *FAM221B* | ENSG00000204930 | c.A1034G | p.H345R | Protein function is unknown |
|  | 9 | 35819909 | C | A | *FAM221B* | ENSG00000204930 | c.G1022T | p.X341L |
|  | 16 | 88496148 | T | G | *ZNF469* | ENSG00000225614 | c.T2270G | p.L757R | Gene is associated with disease symptoms that are not present in patient |
|  | 16 | 88501809 | G | A | *ZNF469* | ENSG00000225614 | c.G7931A | p.R2644Q |
| C-II:1 | 1 | 54605319 | - | GTT | *CDCP2* | ENSG00000157211 | c.1224\_1225insAAC | p.P408delinsPT | Protein function seems not be related to the retina |
|  | 1 | 54605336 | C | G | *CDCP2* | ENSG00000157211 | c.G1207C | p.V403L |
|  | 1 | 160133978 | G | A | *ATP1A4* | ENSG00000132681 | c.G811A | p.D271N | Mouse model shows no retinal phenotype |
|  | 1 | 160134047 | G | T | *ATP1A4* | ENSG00000132681 | c.G880T | p.A294S |
|  | 1 | 228412195 | C | T | *OBSCN* | ENSG00000154358 | c.C2689T | p.R897C | Gene is predominantly expressed in muscle tissues |
|  | 1 | 228562402 | G | A | *OBSCN* | ENSG00000154358 | c.G22612A | p.V7538M |
|  | 2 | 71607348 | G | A | *ZNF638* | ENSG00000075292 | c.2266-4G>A | r.spl? | Protein regulates genes which proteins have no function in the retina |
|  | 2 | 71650080 | C | T | *ZNF638* | ENSG00000075292 | c.C256T | p.P86S |
|  | 2 | 71650482 | A | G | *ZNF638* | ENSG00000075292 | c.A658G | p.I220V |
|  | 2 | 179577968 | C | T | *TTN* | ENSG00000155657 | c.G23161A | p.E7721K | Gene is associated with disease symptoms that are not present in patient |
|  | 2 | 179606001 | T | C | *TTN* | ENSG00000155657 | c.A10870G | p.I3624V |
|  | 6 | 33131600 | A | C | *COL11A2* | ENSG00000204248 | c.4813-5T>G | r.spl? | Gene is associated with disease symptoms that are not present in patient |
|  | 6 | 33157099 | G | T | *COL11A2* | ENSG00000204248 | c.C230A | p.P77Q |
|  | 6 | 135751083 | G | A | *AHI1* | ENSG00000135541 | c.C491T | p.P164L | Gene is associated with a syndromic retinal dystrophy |
|  | 6 | 135754344 | T | C | *AHI1* | ENSG00000135541 | c.A149G | p.H50R |
|  | 9 | 34485183 | G | T | *DNAI1* | ENSG00000122735 | c.G92T | p.W31L | Gene is associated with disease symptoms that are not present in patient |
|  | 9 | 34501134 | A | G | *DNAI1* | ENSG00000122735 | c.1020-2A>G | r.spl? |
|  | 11 | 2424138 | G | A | *TSSC4* | ENSG00000184281 | c.G275A | p.R92H | Gene is associated with disease symptoms that are not present in patient |
|  | 11 | 2424246 | C | T | *TSSC4* | ENSG00000184281 | c.C383T | p.P128L |
|  | 13 | 113819381 | G | A | *PROZ* | ENSG00000126231 | c.G520A | p.G174R | Gene is associated with disease symptoms that are not present in patient |
|  | 13 | 113824800 | C | T | *PROZ* | ENSG00000126231 | c.C647T | p.T216I |

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| **Table S3: *AHI1* variants detected in three non-syndromic visual impairment patients** | | | | | | | | | |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Patient** | **Nucleotide change** | **Protein change** | **PhyloP** | **Grantham** | **CADD\_PHRED** | **MutationTaster** | **PolyPhen-2 (HumVar score)** | **SIFT value** | **AF ExAC (%)** | **AF In-house exome database (%)** | **Effect on WD40 domain via protein modelling** | **Effect on core ciliairy parameters** | **Effect on basal body enrichment** |
| A-II:1 | c.2174G>A | p.Trp725\* | NA | NA | 43 | NA | NA | NA | 0.002 | ND | NA | NA | NA |
|  | c.2258A>T | p.Asp753Val | 4.7 | 152 | 32 | Disease causing | 1 | 0 | ND | ND | Mild | None | Less enrichment |
| B-II:2 | c.660delC | p.Ser221fs | NA | NA | 25.8 | NA | NA | NA | ND | ND | NA | NA | NA |
|  | c.2090C>T | p.Pro697Leu | 5.5 | 98 | 32 | Disease causing | 1 | 0 | ND | ND | Mild | ND | Less enrichment |
| C-II:1 | c.2087A>G | p.His696Arg | 4.6 | 29 | 26.1 | Disease causing | 0.999 | 0 | ND | ND | Mild | ND | ND |
|  | c.2429C>T | p.Pro810Leu | 5.4 | 98 | 31 | Disease causing | 0.999 | 0.01 | ND | ND | None | ND | ND |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| NA: Not applicable | |  |  |  |  |  |  |  |  |  |  |  |  |
| ND: Not determined | | |  |  |  |  |  |  |  |  |  |  |  |
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| **Table S4: Variants detected in *AHI1* exon 16 till 19 in 209 RP patients** | | | | | |
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| **Variant** | **Amino acid change** | **rs number** | **AF RP cohort (%)** | **AF in-house exome database (%)** | **AF ExAC European (Non-finnish) (%)** |
| c.2223T>C | p.Asp741Asp | rs227361 | 1.44 | 1.83 | 1.68 |
| c.2488C>T | p.Arg830Trp | rs13312995 | 1.91 | 2.43 | 5.86 |
| c.2505G>A | p.Arg835Arg | rs41288017 | 0.48 | 0.49 | 0.78 |
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| **Table S5: Clincal characteristics of three retintis pigmentosa patients with *AHI1* mutations** | | | | | | | | | | | | | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Patient number (gender)** | ***AHI1* mutations** | **Age of onset** | **Age at examination** | **Visual acuity** | | **Refraction (diopters)** | | **Color vision** | **Funduscopy** | **Autofluoresencse** | **OCT** | **Visual field** | **ERG** |
| **OD** | **OS** | **OD** | **OS** |
| Patient A-II:1 (F) | p.Trp725\* p.Asp753Val | 53 years | 55 | 1 | 0.8 | +1.25 | +0.75 | HRR: BE: mild RG defect, mild BY defect. Panel D-15: RE: mild errors: no specific axis; LE: saturated version: 1 error; desaturated version: tritan defect. | Mild pallor optic discs, attenuated vessels, yellowish discoloration of the macula with RPE changes, mid-perpiheral RPE atrophy with bone-spicule pigmentations | Spotty hypoautofluorescence in the macula encirceld by relative hyperautofluorescence, loss of autofluorescence along vascular arcades | Intact outer retina layers posterior pole | Goldmann: altitudinal defect (upper quadrants) with midperiheral absolute scotoma lower quadrants | Full field: Dark adapted: remnant isolated rod responses, significantly reduced mixed responses with prolonged implicit times a-wave, normal implicit times b-wave. Light adapted: 30 Hz flicker: (mildly) reduced with prolonged implicit times, single flash responses: (mildly) reduced amplitudes with prolonged implicit times. Generalised retinal dysfunction with rod-cone pattern. |
| Patient B-II:2 (F) | p.Pro221fs p.Pro697Leu | 27 years | 38 years | 1.8 | 1.8 | -7.75 DS (spherical equivalent) | -7.25 DS (spherical equivalent) | Not performed | Cataract surgery 2012 both eyes (posterior subcapsular cataract). Pale discs, attenuated vessels, widespread retinal atrophy, no increased pigmentary change both eyes, left macula demarcated circular chorioretinal atrophy lesion | Small island central autofluorescence remaining (2005 imaging), loss of autofluorescence corresponding to atrophy, loss of any residual normal autofluorescence (2014 imaging) | Extensive loss of outer retinal layers both eyes | Humphrey Field Analyser: 30-2 programm (2006) MD R -30.28DB, L -30.17DB, generalised field loss, central 10 degrees better preserved | Pattern ERG probably undetectable both eyes. Full field: rod specific ERG undetectable. Cone flicker reduced amplitude and profoundly delayed, photopic single flash reduced amplitude and markedly delayed. Moderately severe generalised retinal dysfunction involving both rod and cone systems. |
| Patient C-II:1 (F) | p.His696Arg p.Pro810Leu | 37 years | 39 years | 0.2 | 0.2 | Myope | Myope | HRR: mild RG defect | Posterior subcapsular cataract both eyes. Myopic changes (tilted discs, peripapillary atrophy) both eyes. Disc pallor and peripheral pigmentary retinopathy. | Ring of increased autofluorescence both maculae, reduced autofluorescence mid-peripheries | Centrally preserved inner segment ellipsoid band both eyes | Not performed | Pattern ERG markedly reduced bilaterally. Full field: rod ERGs are severely reduced bilaterally, bright flash ERG a waves are markedly reduced R>L, flicker ERGs are markedly delayed bilaterally and markedly reduced R>L, single flash ERGs are reduced bilaterally R>L. Consistent with moderately severe generalised retinal dysfunction in which rods are slightly more affected than cones and right eye is slightly more affected than left eye. |

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| **Table S6: Statistical summary of signal enrichment at the base of cilia for AHI1 wild type and AHI1 mutants** | | | | | |
|  | **AHI1 wildtype** | **AHI1 mutants** | | | |
| **P697L** | **D753V** | **D719G** | **W725R** |
| **N** | 38 | 36 | 40 | 32 | 34 |
| **Mean (Signal enrichment at the base of cilia)** | 2.4786 | 1.6067 | 1.6047 | 1.6804 | 1.5438 |
| **Std. Deviation** | 0.5272 | 0.2959 | 0.3509 | 0.3836 | 0.3566 |
| **Std. Error** | 0.0855 | 0.0493 | 0.0555 | 0.0678 | 0.0612 |
| **Difference of mean values between wildtype and mutants** |  | 0.8719 | 0.8739 | 0.7982 | 0.9348 |
| **Minimum** | 1.5674 | 0.9264 | 0.7307 | 1.1366 | 0.8380 |
| **25% Percentile** | 2.0583 | 1.4019 | 1.3298 | 1.4503 | 1.3379 |
| **Median** | 2.3855 | 1.5960 | 1.6162 | 1.6697 | 1.4622 |
| **75% Percentile** | 2.9573 | 1.7469 | 1.9045 | 1.8210 | 1.7252 |
| **Maximum** | 3.9068 | 2.3356 | 2.4174 | 2.8693 | 2.5260 |
|  |  |  |  |  |  |