CASE STUDY

Genetic Defect Underlying Progressive Blindness Uncovered by Strand's Clinical Exome Test

Patient Profile

Swati Koparkar*, a 33-year-old owner of a handicrafts boutique had been experiencing problems with her vision. She was adept at creating useful objects from eco-friendly materials like cane, grass, handmade paper and natural fabrics. Embellishing them with fine designs in paint and embroidery were also her prime skills. However, she had begun to make mistakes in the edges of designs that she was creating. She began to bump into furniture and objects to her right and left side, despite knowing the layout of her boutique and home. Driving at night was also becoming increasingly difficult. She had narrowly missed getting hit by a car, that came at her scooter, from the left side of an intersection.

Gender : Female

Age : 33 years

Location : Pune, Maharashtra

Diagnosis : Retinitis Pigmentosa

Strand Test : Clinical Exome

Conclusion :

- Hereditary cause of loss of vision established. Supplementary visual aids prescribed.

Medical Investigations

Swati consulted an ophthalmologist at a renowned hospital in Pune, in order to understand why she had suddenly begun to develop problems with her vision.

Routine visual tests indicated that Swati had developed 'Tunnel vision' wherein the peripheral vision was compromised. Partial degradation of the retinas of both eyes was evident in Swati's case, in a retinal examination. Since the ophthalmologist suspected the incidence of retinitis pigmentosa (RP), she suggested a genetic diagnostic test to Swati. The test would look for pathogenic variants of genes that cause familial RP. Swati's father was also diagnosed with retinitis pigmentosa. Hence, it was essential to understand if she also had inherited pathogenic genes that cause RP, or not.

Retinitis Pigmentosa

Retinitis pigmentosa is a group of inherited disorders that cause progressive loss of vision. Deterioration of the light-sensitive rod photoreceptor cells is the main reason for reduced visual acuity in this malaise. In the US and Europe, 1:3500 to 1:4000 people suffer from RP (Genetics Home Reference).

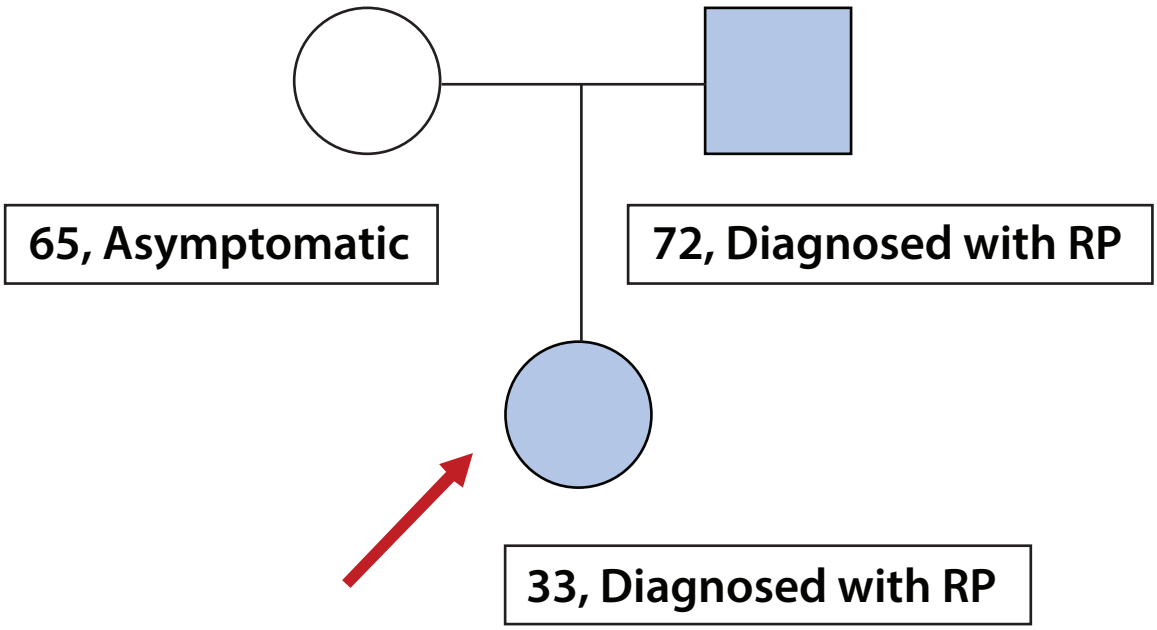
In India, a high incidence of 1:372 (rural) and 1:930 (urban) has been noted in a study (1). In another study, an incidence rate of 1:750 has been reported from Central India (2).

Retinitis pigmentosa is also one of the manifestations of other complex inherited syndromes like Usher Syndrome, Bardet-Biedl Syndrome, Refsum disease, and Neuropathy, Ataxia & Retinitis pigmentosa (NARP) syndrome. If only visual symptoms are present, RP is termed as 'non-syndromic' RP. If other physiological symptoms are seen, then RP is termed as 'syndromic' RP. A distinction between these two types of RP can be made if the pathogenic genes involved are identified, using specifically designed tests.

Non-syndromic RP can be inherited in autosomal dominant, autosomal recessive or in an X-linked manner. Given that Swati's father was also suffering from RP, the inheritance pattern in this family seemed to be autosomal dominant.

** Name changed to protect patient privacy*

Family Tree Prior To Genetic Testing



Results of Genetic Testing

The Strand Clincal Exome Test for diagnosis of inherited disorders, specifically RP, was prescribed to Swati. This is a laboratory-developed test designed to identify mutations in 62 genes that are involved in the development of retinitis pigmentosa.

RESULT Positive for two heterozygous **'pathogenic'** variants in exon 35 and exon 38 of the *ABCA4* gene

Results

Positive for two heterozygous **'pathogenic'** variants, which were detected in exon 35 and exon 38 of the *ABCA4* gene.

Key Findings

Gene	Variation	Zygosity	Clinical significance
ABCA4	chr1:94486943C>T c.4871G>A p.Trp1624Ter	Heterozygous	Pathogenic
ABCA4	chr1:94480187_94480188insC c.5371dupG p.Ala1791GlyfsTer9	Heterozygous	Pathogenic

Genetic analysis showed that Swati had inherited two pathogenic mutations in the *ABCA4* gene. Light falling upon photoreceptor cells leads to conversion of this signal into an electrical signal that is transmitted to the brain, resulting in a perceived image. Toxic substances like N-retinylidene-PE, are produced in the photoreceptor cells as a result of this process.

The normal *ABCA4* gene codes for a protein that helps to remove N-retinylidene-PE from photoreceptor cells (Genetics Home Reference),(3). Toxic accumulation of this molecule results in loss of vision when the *ABCA4* gene is mutated, leading to compromised protein function (4,5).

Retinitis pigmentosa resulting from loss of function of *ABCA4* is termed as RP19 and is an autosomal recessive disorder (6). However, in Swati's case, although she is heterozygous for both variants, she started manifesting symptoms of RP, suggesting inheritance of one mutation per each copy of the *ABCA4* gene. This compound heterozygosity is possible only if her parents are carriers of one mutation and not the other.

Mutation-Specific Genetic Testing* of Swati's Parents

In order to ascertain Swati's compound heterozygous status, her parents' DNA samples were tested, specifically for the presence of the two identified mutations in the *ABCA4* gene. This is known as mutation-specific testing.

Mutation-specific testing (MST) is extended to a proband's family members as a confirmatory test for diagnosis of rare inherited disorders. Strand also offers MSTs to confirm the risk for inheritance of cancer and inborn errors of metabolism, to proband's relatives .

MST-Swati's Mother

Swati's mother is heterozygous for the c.4871G>A(p.Trp1624Ter) mutation in the *ABCA4* gene.

Results

- **Heterozygous** for the tested variant, c.4871G> (p.Trp 1624Ter), in the *ABCA4* gene.
- **Negative** for the tested variant, c.5371 dupG (p.Ala 1791GlyfsTer9), in the *ABCA4* gene.

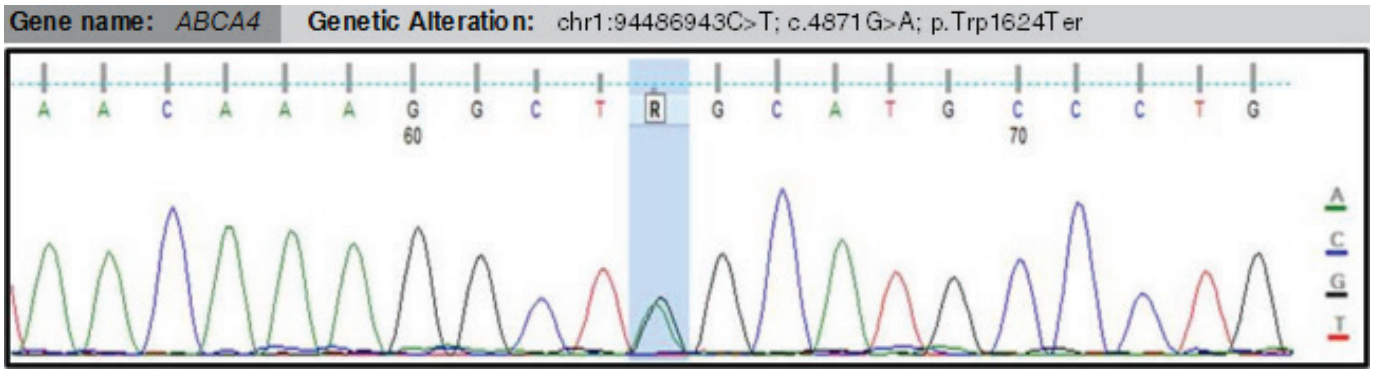


Figure 1. Sanger sequencing data (electrophoregram) from the individual showing a heterozygous nucleotide change 'G>A' at position c.4871 in the ABCA4 gene (RefSeq id: NM_000350). This variation was confirmed by sequencing with both forward and reverse primers.

The parameter marked with an * are not accredited by NABL and CAP.

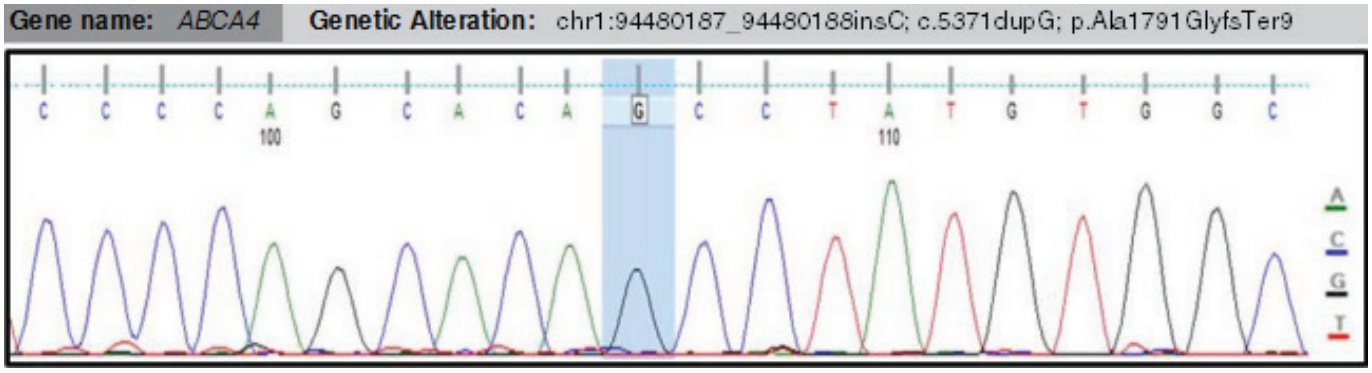


Figure 2: Sanger sequencing data (electrophoregram) from the individual showing the reference nucleotide 'G' at position c.5371 in the ABCA4 gene (RefSeq id: NM_000350). This finding was confirmed by sequencing with both forward and reverse primers.

MST-Swati's Father

Swati's father was also affected with retinitis pigmentosa and was undergoing treatment for the same. A DNA sample from Swati's father was also analysed for the presence of the two *ABCA4* gene variants. Her father is homozygous for the second mutation, c.5371dupG (p.Ala1791GlyfsTer9), in the *ABCA4* gene.

Results

- **Negative** for the tested variant, c.4871G>A (p.Trp 1624Ter), in the *ABCA4* gene.
- **Heterozygous** for the tested variant, c.5371 dupG (p.Ala 1791GlyfsTer9), in the *ABCA4* gene.

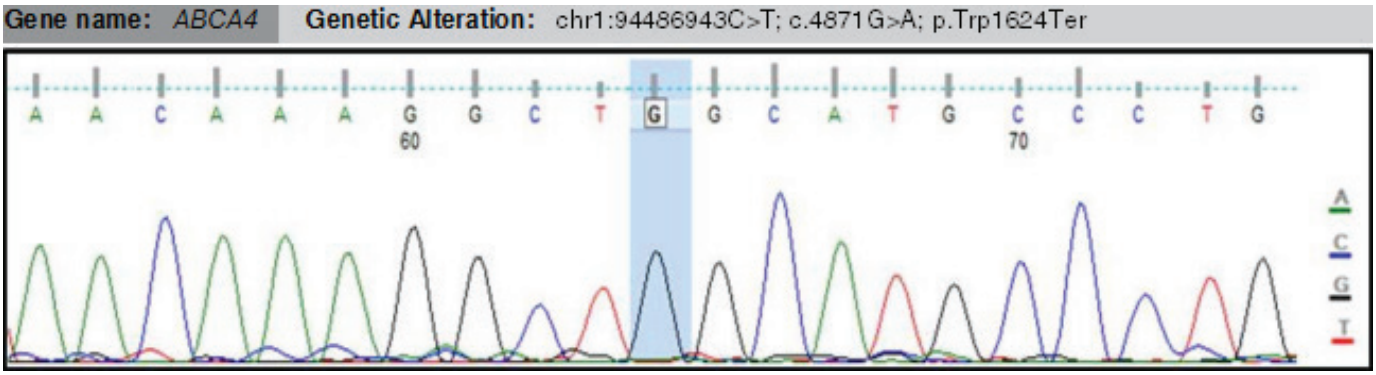


Figure 3: Sanger sequencing data (electrophoregram) from the individual showing the reference nucleotide 'G' at position c.4871 in the ABCA4 gene (RefSeq id: NM_000350). This finding was confirmed by sequencing with both forward and reverse primers.

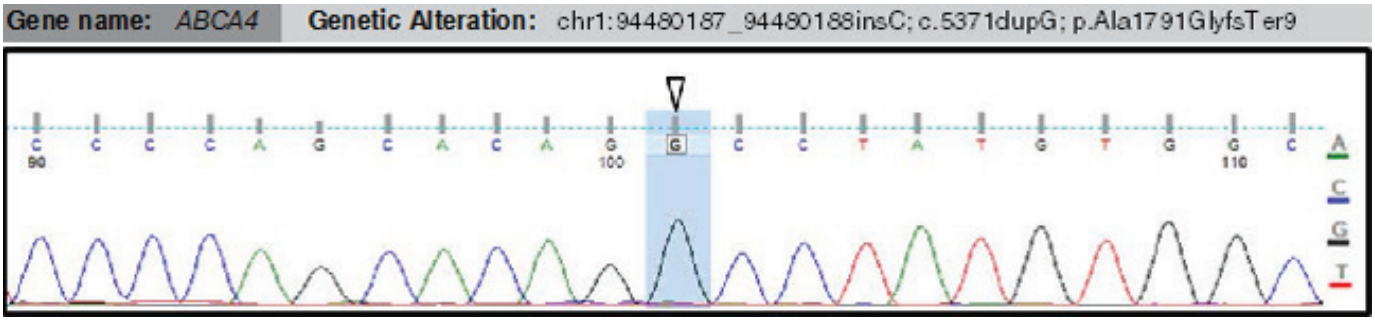
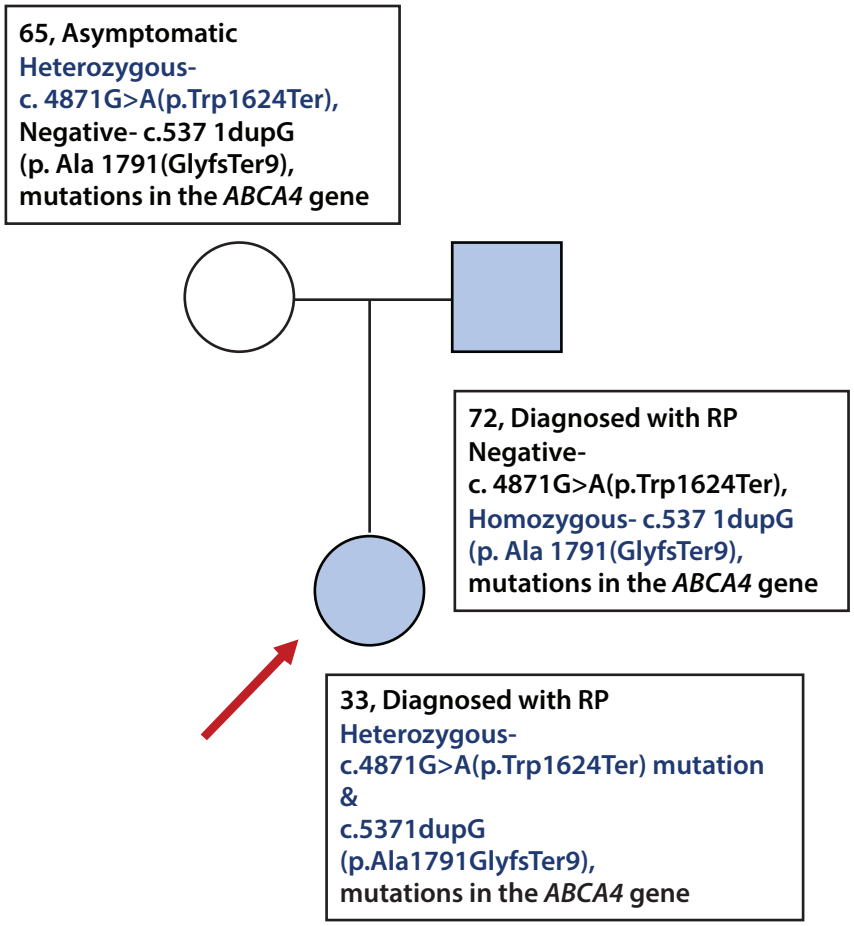


Figure 4: Sanger sequencing data (electrophoregram) from the individual showing a homozygous duplication of nucleotide 'G' at position c.5371 in the ABCA4 gene (RefSeq id: NM_000350). This variation was confirmed by sequencing with both forward and reverse primers.

Family Tree Prior To Genetic Testing



Conclusions

- Swati, a 33-year-old woman from Pune was diagnosed with retinitis pigmentosa (RP) based on her progressive loss of vision.
- The diagnosis of RP was confirmed by a genetic test wherein two distinct mutations in the *ABCA4* gene were identified.
- Swati is likely to be compound heterozygous for both these mutations. Essentially, both copies of the *ABCA4* gene, in her genome, bear one mutation each.
- Mutation-specific testing was extended to her parents to confirm the compound heterozygosity.
- Swati's mother is a carrier of the c.4871G>A(p.Trp1624Ter) mutation but not the second mutation identified in this case.
- Conversely, Swati's father is homozygous for the c.5371dupG (p.Ala1791GlyfsTer9) mutation and is negative for the first mutation identified. His diagnosis of RP can be ascribed to the homozygosity of this mutation in his genome.
- The c.5371dupG (p.Ala1791GlyfsTer9) mutation was suspected to be autosomal dominant. However, the mutation analysis indicates that this mutation is autosomal recessive.
- The family was counselled about their genetic status. Swati was given advice about the use of visual aids and strategies to cope with loss of peripheral vision.

Strand Clinical Exome Test

The Strand® Clinical Exome test is a Laboratory Developed Test (LDT) that was developed and its performance characteristics determined by Strand Center for Genomics and Personalized Medicine at Strand Life Sciences.

Genes Evaluated

ABCA4, ABHD12, AIPL1, ARL6, BEST1, C2orf71, C8orf37, CA4, CDHR1, CERKL, CLRN1, CNGA1, CNGB1, CRB1, CRX, DHDDS, EYS, FAM161A, FLVCR1, FSCN2, GUCA1B, HGSNAT, IDH3B, IMPDH1, IMPG2, KLHL7, LRAT, MAK, MERTK, NR2E3, NRL, OFD1, PDE6A, PDE6B, PDE6G, PRCD, PRKCG, PROM1, PRPF3, PRPF31, PRPF6, PRPF8, PRPH2, RBP3, RDH12, RGR, RHO, RLBP1, ROM1, RP1, RP2, RP9, RPE65, SAG, SEMA4A, SNRNP200, SPATA7, TOPORS, TTC8, TULP1, USH2A, ZNF513

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