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CASE REPORT

# Molecular dynamics simulation of *KIT* mutation in a patient with piebaldism, congenital cataract, and aphakic glaucoma

Fahrettin Duymus[1]*, Banu Bozkurt[2], Ali Sahin[3], Huseyin Babayev[3], Sibel Ersoy Evans[4], Betul Saylik[2], Tulin Cora[5]

## ABSTRACT

**Background:** Piebaldism (OMIM #172800) is a rare autosomal dominant genodermatosis characterized by congenital poliosis and stable patches of leucoderma. Piebaldism is caused by mutations in the *KIT* and *SNAI2* genes. The most common mutations are detected in the *KIT* gene.

**Case Presentation:** A 5-year-old boy, who was followed up for aphakic glaucoma after congenital cataract surgery, was consulted to the medical genetics and dermatology departments due to premature graying of the hair, white forelock in the frontal region of the scalp, whitening in the inner part of the eyebrows and eyelashes and patchy leukoderma with hyperpigmented islands inside on the extremities and trunk. DNA sequencing revealed a heterozygous missense c.1861G>A mutation in the *KIT* gene. Mutation was evaluated using *in silico* 3-D-structure analysis and bioinformatics tools.

**Conclusion:** The *KIT* gene has a critical role in melanoblast migration, proliferation, differentiation, and survival and molecular dynamics simulation and modeling proved that this variant inhibits the migration of melanoblasts and melanocytes by reducing the enzymatic activity of the *KIT* protein.

**Keywords:** Piebaldism, *KIT* gene, congenital cataract, aphakic glaucoma, molecular dynamics.

## Background

Piebaldism (OMIM #172800) is an autosomal dominant genodermatosis characterized by congenital poliosis and stable patches of leucoderma (1,2). In about 75% of the cases, mutations are detected in the *KIT* gene, which is mapped on chromosome 4q12 (NM_000222) and approximately 93 *KIT* mutations causing piebaldism were reported (2-5). Piebaldism may be confused with other inherited disorders of melanocyte development or melanin biosynthesis, such as Waardenburg syndrome, vitiligo, and a number of inherited or acquired diseases involving skin or hair hypopigmentation (1). Therefore, molecular and genetic analyses are very important in the differential diagnosis of disorders with pigmentation, which are caused by changes in the different stages of development of neural crest cells (6).

Herein, we discussed the clinical and genetical findings of a 5-year-old boy with piebaldism who was followed up for aphakic glaucoma after congenital cataract surgery and his family members in the light of current literature. The mutation was further evaluated by *in silico* 3-D-structure analysis and bioinformatics.

## Case Report

The patient was the third child of the family and had an uncomplicated vaginal delivery at term. He was operated on for a bilateral congenital cataract at the age of 4 months and admitted to us with the complaint of enlargement of the right eyeball. He had no heterochromia. Both eyes were aphakic and the intraocular pressures (IOP) were 38 mmHg oculus dexter (OD) and 13 mmHg oculus sinister (OS). The cup-to-disc was 0.9 in the right eye and 0.3 in the left eye. Topical prostaglandin-beta blocker combination was initiated in the right eye. As the patient had premature graying of the hair, white forelock

**Correspondence to:** Fahrettin Duymus
*Department of Medical Genetics, Konya City Hospital, Konya, Turkey.
**Email:** fahrettinduymus@gmail.com
*Full list of author information is available at the end of the article.*
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(poliosis) in the frontal region of the scalp, and whitening in the inner part of the eyebrows and eyelashes (Figure 1I a and b), he was consulted to the Department of Medical Genetics and Dermatology with the preliminary diagnosis of Waardenburg syndrome. On physical examination, in addition to the facial findings, there was multiple patchy leukoderma on the trunk, flexor surfaces of the forearms, face, and legs, with discrete skin-colored and hyperpigmented macules inside (Figure 1Ic and d). His audiometric examination was normal, and he had no obvious neurological defects. Similar skin and hair findings were present in the patient's mother, two siblings, grandmother, and grandmother's brother (Figure 1IIa-f). None had hearing loss in the audiometric examinations. They were diagnosed with piebaldism. Figure 1III shows the family tree.

In his 1-month follow-up examination, right eye IOP was 30 mmHg and 360° diode cyclodestruction was done. As IOP did not decrease after cyclodestruction, Ahmed glaucoma tube implantation surgery was performed and IOP decreased to 12 mmHg. In his final examination, the right iris color was slightly darker than the left iris due to topical prostaglandin analog use, the right pupil was temporally slanted, and the tube was in place (Figure 1IVa and b). The eye examinations of the family members were normal, with no refractive errors, no iris heterochromia, and no pigmentary changes in the retina or choroid (Figure 1Va-d).

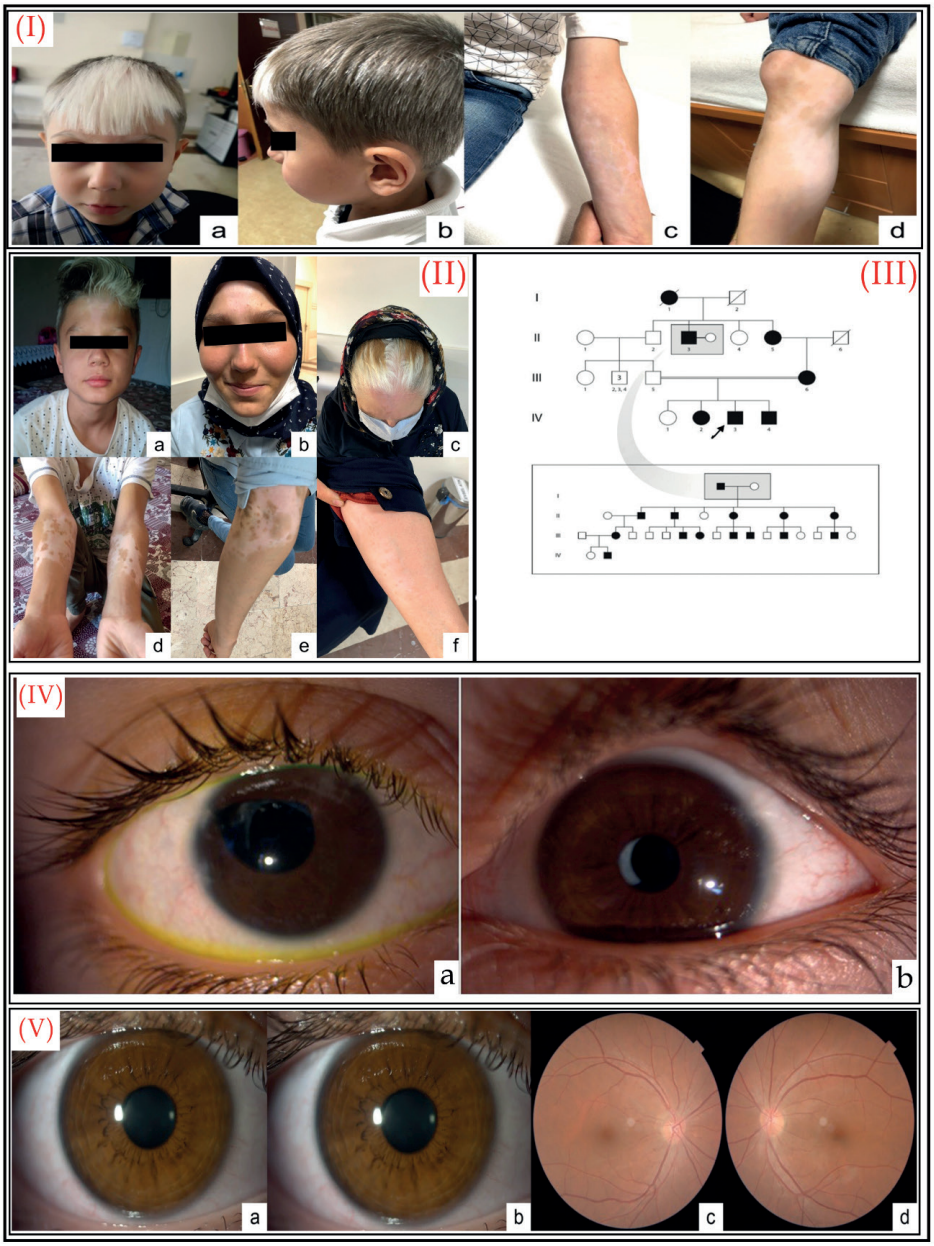


***Figure 1.*** *(I a-d) Phenotypes of the patient: note premature graying of the hair, white forelock (poliosis) in the frontal region of the scalp, multiple patchy leukoderma on the trunk, flexor surfaces of the forearms, face, and legs. (IIa-f) Skin and hair findings of the patient's affected family members. (III) Pedigree of the patient. (IV and Va-d) Eye examination findings of the patient.*

### *Genetic analysis*

Informed consent was taken from the mother and the family members. Genomic DNA was obtained from the patient's blood sample and DNA sequencing revealed a heterozygous missense c.1861G>A mutation (p.Ala 621Thr) in exon 12 of the *KIT* gene mapped on chromosome 4q12 (NM_000222) (Figure 2II). This variant in the tyrosine kinase 1 domain of the *KIT* gene was classified as possibly pathogenic according to the interpretation guidelines of the American College of Medical Genetics.

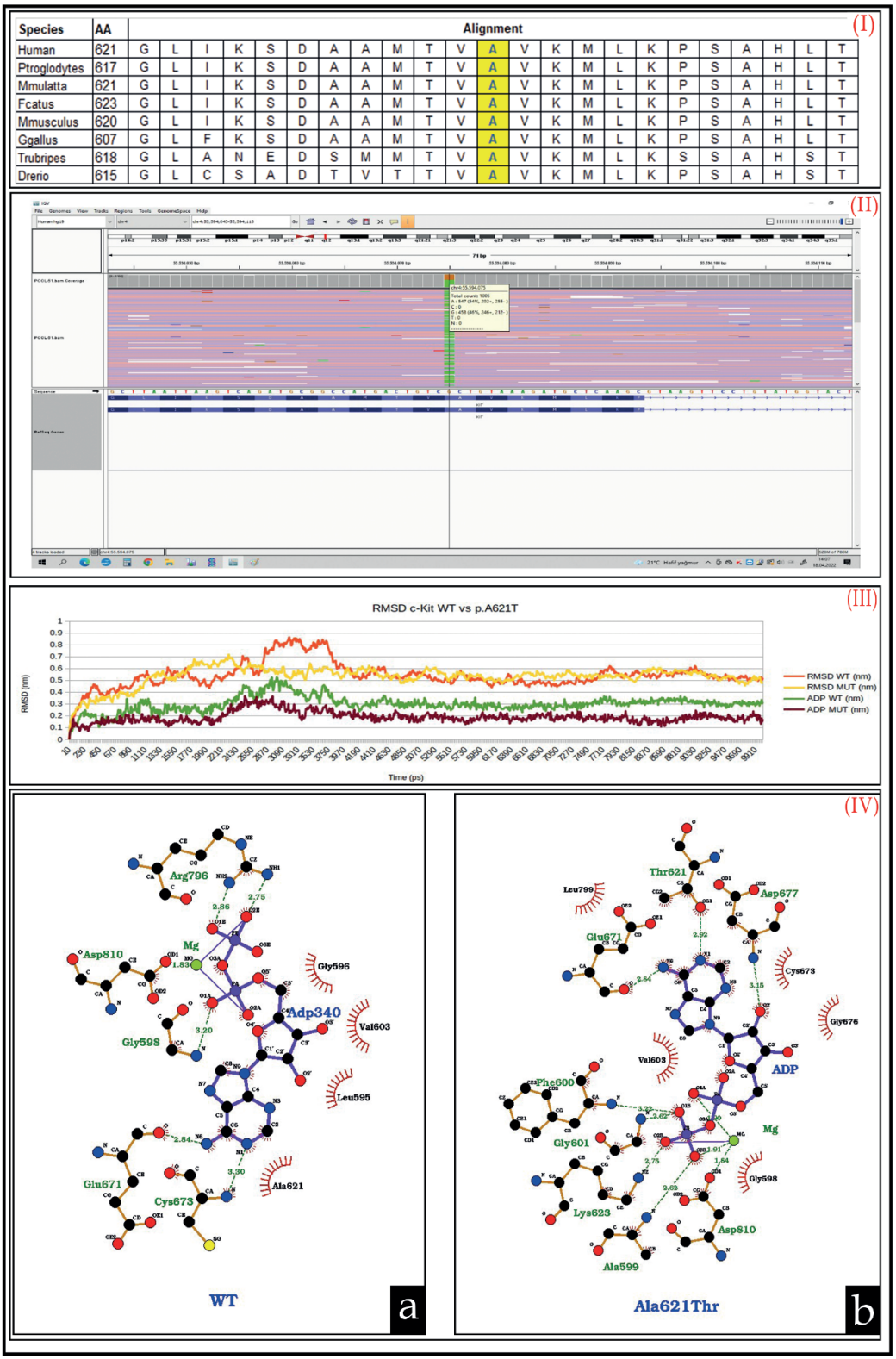


***Figure 2.*** *(I) Conservation protein level for non-synonymous changes. (II) Mutation in the KIT gene (c.1861 G > A). (III) The RMSD plots for both mutant and wild-type. (IVa and b) The interaction scheme of the ADP ligand with the protein.*

Alanine 621 is an interspecies conserved amino acid (Figure 2I) and *in silico* 3-D-structure analysis and bioinformatics tools showed that this variant can lead to abnormal conformation which may be pathogenic.

### *Molecular dynamics and modeling*

The structure (1T45) of the wild-type kinase domain of ADP-bound *KIT* protein was retrieved from the Protein Data Bank (7). Missing amino acids in the structure were added using SWISS-MODEL (8). The mutant construct (Ala621Thr) was generated using the UCSF Chimera (9). Two systems were simulated using the TIP3P water model and the CHARMM36 force field (10). Each complex was dissolved with water and neutralized with 0.15 M counter $Na^+$ and $Cl^-$ ions in a dodecahedron box. After electro-neutralization, each system was subjected to energy minimization using the steepest descent algorithm until a maximum force of 1,000 kJ $mol^{-1}$ $nm^{-1}$ was achieved. Canonical ensemble (NVT) group was used at 310 K with the Nose-Hoover method to maintain the temperature of each system, while the pressure was balanced using an Isothermal–isobaric ensemble (NPT) group at 1 bar with the Parrinello-Rahman algorithm. Finally, a 10 ns generation run was performed for each system, starting from different random initial speeds. Orbital data were recorded at 10 ps time intervals. Molecular dynamic stability parameters were analyzed using the GROMACS and VMD version 1.9.1 toolkits (11). Except for the first 4 ns, the root mean square deviation (RMSD) plots for both mutant and wild-type have a similar shape throughout the simulation, as shown in Figure 2III. The PRODIGY-LIGAND web server was used to predict ADP binding to WT and mutant protein (12). It was observed that the binding affinity of ADP to mutant protein ($\Delta G$ = -6.5 kcal/mol) was higher than the wild type ($\Delta G$ = -6.2 kcal/mol). The interaction scheme of the ADP ligand with the protein was analyzed using LigPlot+ (13) (Figure 2IVa and b). It was observed that threonine added to the construct as a result of mutation interacted with the number one nitrogen in the ADP structure.

## Discussion

Piebaldism results from mutations of the *KIT* gene, which encodes a cell-surface type III receptor tyrosine kinase, whose ligand is the stem/mast cell growth factor (2-4). Mutations in the *KIT* gene cause a dominant-negative inhibition and decrease the enzymatic activity of tyrosine kinase (3). In our patient, alanine-threonine exchange (c.1861G>A) (p. Ala 621Thr) was in the tyrosine kinase domain, and molecular dynamics analyses confirmed that this change restricted the enzymatic activity of the *KIT* protein and inhibited the migration of melanoblasts and melanocytes. It was observed that threonine added to the construct interacted with number one nitrogen in the ADP structure and affected the conformation of the protein. We presumed that this interaction might stabilize the ADP structure and slow down the ADP/adenosine triphosphate (ATP) exchange required for activation.

In the literature, there is only one study reporting multifocal, tiny, whitish deposits in the posterior pole of the retina in a 20-year-old female with mild intellectual disability who had a heterozygous deletion in the 4q12 chromosome region (14). These lesions were described by multimodal imaging as mild hyper-autofluorescence in the late phase of fluorescein angiography and small elevations in the retinal pigment epithelium with staining. To the best of our knowledge, congenital cataract has not been reported in patients with piebaldism so far. In our patient, a congenital cataract was detected in both eyes, and after bilateral uneventful cataract surgeries at the age of 4 months, aphakic glaucoma developed in the right eye. As IOP could not be lowered with medical antiglaucomatous treatment, diode cyclophotocoagulation and afterward Ahmed tube implantation were performed. Although eye findings associated with piebaldism are not very common in the literature, there are many articles discussing WS eye findings. Iris heterochromia or dysplastic iris can be seen frequently in WS type 2 (15-17). In our patient, iris pigmentation was increased in the right eye due to prostaglandin analog use. In the fundus examination, there were no remarkable pigmentary changes in the retina. Fundus angiography or optical coherence tomography could not be performed due to nystagmus. There were no iris heterochromia or retinal findings in the family members. In the literature, the association between WS and congenital cataracts has been shown in two cases (18,19). Pigmentary and open-angle glaucoma had been reported before in some cases with WS (20-22).

In this case report, we detected a missense mutation in exon 12 of the *KIT* gene (c.1861G>A) (p.Ala 621Thr) in a child with piebaldism, congenital cataract, and glaucoma. For the first time in the literature, the mutation in the *KIT* gene was evaluated using *in silico* 3-D-structure analysis and bioinformatics tools and it was shown that threonine added to the construct interacted with the number one nitrogen in the ADP structure and affected the conformation of the protein.

## Conclusion

The *KIT* gene has a critical role in melanoblast migration, proliferation, differentiation, and survival, and molecular dynamics simulation and modeling proved that this variant inhibits the migration of melanoblasts and melanocytes by reducing the enzymatic activity of the *KIT* protein.

**List of Abbreviations**

| | |
|---|---|
| IOP | Intraocular pressures |
| OD | Oculus dexter |
| OS | Oculus sinister |
| ADP | Adenosine diphosphate |
| NVT | Canonical ensemble |
| NPT | Isothermal–isobaric ensemble |
| RMSD | Root mean square deviation |
| ATP | Adenosine triphosphate |

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None.

**Declaration of conflicting interests**
The authors declare that they have no conflict of interest regarding the publication of this case report.

**Consent for publication**

Written informed consent for publication of their details was obtained from the patient/study participant/parent/guardian/next of kin.

**Ethical approval**

Ethical approval is not required at our institution to publish an anonymous case report.

**Author details**

Fahrettin Duymus[1], Banu Bozkurt[2], Ali Sahin[3], Huseyin Babayev[3], Sibel Ersoy Evans[4], Betul Saylik[2], Tulin Cora[5]

1. Department of Medical Genetics, Konya City Hospital, Konya, Turkey
2. Department of Ophthalmology, Faculty of Medicine, Selcuk University, Konya, Turkey
3. Faculty of Medicine, Selcuk University, Konya, Turkey
4. Department of Dermatology, Faculty of Medicine, Hacettepe University, Ankara, Turkey
5. Department of Medical Genetics, Faculty of Medicine, Selcuk University, Konya, Turkey

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