

A Sadeghi-Tari¹, M Jamshidian-Tehrani¹, A Nabavi¹,
S Sharif-Kashani², E Elami¹, N Hassanpour¹ and
KA Zamani¹

¹Eye Research Center, Farabi Eye Hospital, Tehran
University of Medical Sciences, Tehran, Iran

²Department of Radiology, Tehran University of
Medical Sciences, Imam Khomeini Hospital, Tehran,
Iran

E-mail: mansooreh_2468@yahoo.com

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Sir, Identification of a novel *NRL* mutation in a Chinese family with retinitis pigmentosa by whole-exome sequencing

Retinitis pigmentosa (RP) is a group of inherited retinal
degenerative disorders affecting ~1 in 4000 individuals
worldwide.¹ Because of the great genetic heterogeneity
of RP, technologies based on the next-generation

sequencing are increasingly used to screen mutations or
identify novel causative genes in RP patients for
investigative and diagnostic purposes.² Here, we report a
Chinese family suffering from autosomal dominant RP
(adRP). The genetic cause of the family was further
investigated.

The proband (II:2 in Figure 1a, 43 years old) complained
of night blindness since childhood, followed by visual
field loss and reduction of visual acuity. Fundus
examination revealed bone-spicules pigmentation in the
mid-peripheral retina (Figure 1b). Her 14-year-old
daughter (III:1) also reported night blindness before age
10 years. No apparent pigmentation could be seen at the
time of the last ophthalmologic examination (Figure 1b).
To identify the possible causing mutation(s), we performed
whole-exome sequencing using the proband's genomic
DNA. Through a multistep bioinformatics pipeline
(Supplementary Information), candidate variants were
selected and validated by Sanger sequencing and
segregation analysis. A novel c.147_149del (p.Ser50del)
variation in *NRL* was identified as the most likely cause of
the family (Figure 1c). The variation was absent in 250
normal controls.

The neural retina leucine zipper (*NRL*) gene encodes a
237-aa basic motif-leucine zipper transcription factor of

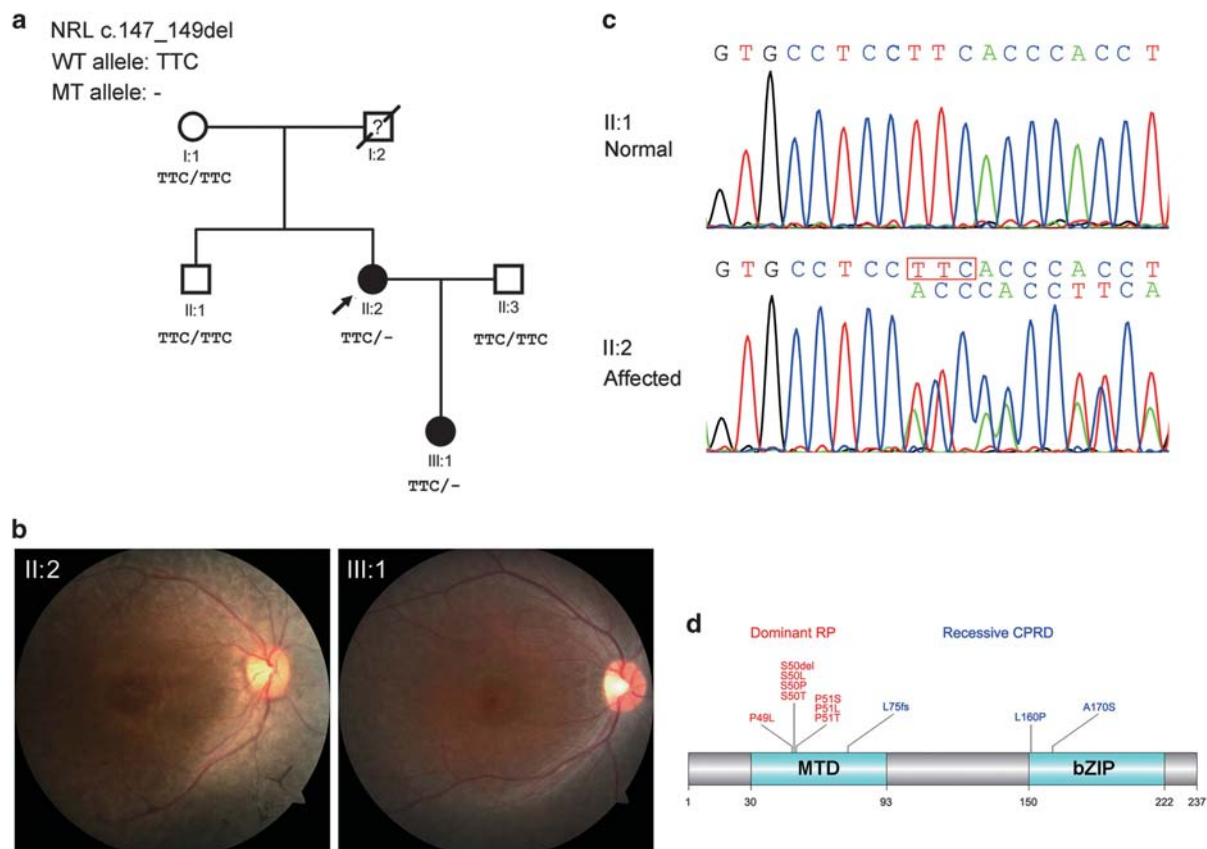


Figure 1 Identification of a novel *NRL* mutation in a Chinese adRP family. (a) Pedigree of the family. Circles, females; squares, males; filled symbols, affected individuals; empty symbols, unaffected individuals; arrow, proband; question mark, unexamined individual. (b) Fundus photographs of the two patients. II:2, the proband, 43 years old; III:1, 14 years old. (c) Sanger sequencing of the c.147_149delTTC mutation. The deleted nucleotides were marked with a red box. (d) Schematic structure of the *NRL* protein. The red color- and blue color-labeled *NRL* mutations associated with dominant retinitis pigmentosa and recessive clumped pigmentation, respectively.

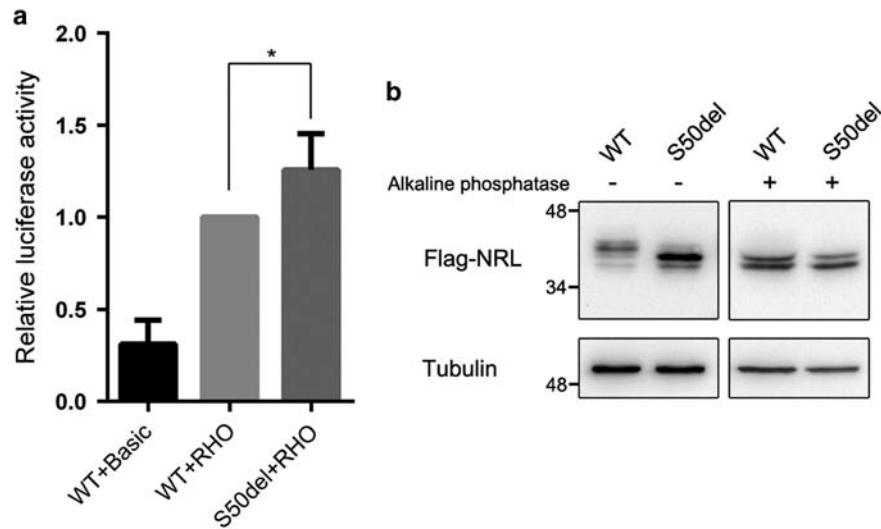


Figure 2 Effects of the S50del mutation on the transactivation ability and phosphorylation modification of NRL protein. (a) Dual-luciferase reporter assay of the WT and S50del mutant NRL proteins. Relative luciferase activities of the three groups, WT+basic, WT+RHO, and S50del +RHO, were shown as mean \pm SD. * $P < 0.05$. (b) Electrophoretic mobility shift assay of the Flag-tagged WT and S50del mutant NRL. Tubulin was used as a loading control. Samples treated with alkaline phosphatase were used as dephosphorylated controls.

the Maf subfamily, which has a critical role in the regulation of photoreceptor development and function. Currently, all known adRP-causing mutations in *NRL* are located in the Pro49, Ser50, and Pro51 codons (Figure 1d), including P49L,³ S50T, S50P, S50L, P51T, P51L, and P51S.⁴ These mutations in *NRL* have been proven to reduce the phosphorylation of NRL and increase its transactivation ability.⁴

To see whether the S50del mutation showed similar effects like other *NRL* mutations causing adRP, we first measured the transactivation abilities of WT and S50del mutant NRL on the human *RHO* (encoding rhodopsin) promoter⁵ using the dual-luciferase reporter assay. As expected, the transcriptional level of *RHO* promoter was higher when co-transfected with Ser50del mutant NRL as compared with WT NRL (Figure 2a). Furthermore, we examined the phosphorylated isoforms of WT and S50del mutant NRL protein by western blot. In the WT lane, multiple bands could be observed, which represented the phosphorylated isoforms of NRL according to previous studies. However, the S50del mutant NRL protein showed a smaller and concentrated band, indicating a remarkable reduction of phosphorylation modification (Figure 2b). After treated with alkaline phosphatase, which removes phosphate groups from proteins, both of the WT and S50del groups did not exhibit a multiple-band pattern. These results were in agree with previous studies, and further proved that the S50del mutation accelerates the transcriptional activation ability of NRL and decreases its phosphorylation level just like those known adRP-causing mutations in *NRL*.

In summary, through whole-exome sequencing, we identified a novel *NRL* mutation (c.147_149del, p.Ser50del) leading to adRP in a Chinese family, which alters the phosphorylation modification and transactivation ability of NRL. Our study widens the

mutation spectrum of *NRL* and provides insight on the functional studies of *NRL*.

Conflict of interest

The authors declare no conflict of interest.

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Y Qin^{1,4}, F Liu^{1,4}, S Yu¹, L Yang¹, M Gao¹, Z Tang¹,
AY Guo², M Zhang³, P Li³ and M Liu¹

¹Department of Genetics and Developmental Biology,
Key Laboratory of Molecular Biophysics of Ministry of
Education, College of Life Science and Technology,
Huazhong University of Science and Technology,
Wuhan, PR, China

²Department of Bioinformatics and Systems Biology,
Key Laboratory of Molecular Biophysics of Ministry of

Education, College of Life Science and Technology,
Huazhong University of Science and Technology,
Wuhan, PR, China

³Department of Ophthalmology, Union Hospital,
Tongji Medical College, Huazhong University of
Science and Technology, Wuhan, PR, China

Email: 44592236@qq.com

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⁴These authors contributed equally to this work.

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