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On variants and disease-causing mutations: Case studies of a *SEMA4A* variant identified in inherited blindness

Laura Bryant, **Olga Lozynska**, **Grace Han**, **Jessica I.W. Morgan**, **Xiaowu Gai**, **Albert M. Maguire**, **Tomas Aleman**, and **Jean Bennett***

Center for Advanced Retinal and Ocular Therapeutics (CAROT) and F.M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, University of Pennsylvania, 310 Stellar-Chance Labs, 422 Curie Blvd., Philadelphia, PA, 19104, USA; Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania Perelman School of Medicine, 51 N. 39th Street, Philadelphia, PA 19104, USA

Three different missense variants in *SEMA4A* have been identified in retinitis pigmentosa F(RP) patients and presumed to be pathogenic: p.R354H, p.F350C and p.R713Q[1]. p.R354H and p.F330C were reported to be recessive mutations while p.R713Q was reported to be a dominant mutation. The variants were classified as pathogenic since: 1) they segregated with the disease in the respective families, and 2) they were not found in 100 ethnically matched normal-sighted control individuals[1]. Here we review laboratory results relating to *SEMA4A* variants and present data that contradict previous conclusions that particular *SEMA4A* variants are pathogenic.

Nogima et al created knock-in mouse lines for *SemA4a* missense variant[2]. Of the three variants, only the p.F350C variant resulted in retinal degeneration in mice[2]. As the authors mention, it is possible that the difference in *SEMA4A* sequence between human and mouse can account for the differences in the effects of the variants between these two species. However, it is also possible that the variant is not pathogenic and is merely a benign polymorphism or a risk factor for blindness that is not sufficient to cause disease on its own. Additional studies in the ARPE19 human retinal pigmented epithelium (RPE) cell line demonstrated that the p.D345H and p.F350C variants do not properly localize to the cell membrane and also cause deficits in phagocytosis or ER stress response to oxidative stress[3]. Conversely, the p.R713Q variant did not affect phagocytosis, ER stress response or protein localization[3].

After obtaining consent and collecting blood samples for DNA (UPenn IRB #808828), we analyzed whole exome sequencing data from patients with genetic forms of retinal degeneration seen at the Scheie Eye Institute Department of Ophthalmology. We discovered three unrelated subjects who were heterozygous or homozygous for the p.R713Q variant of *SEMA4A*. Further analyses showed that the variant did not segregate with the disease in any of the families. The details are as follows:

*Correspondence to: Jean Bennett, Center for Advanced Retinal and Ocular Therapeutics (CAROT), 309C Stellar-Chance Labs, 422 Curie Blvd, Philadelphia, PA 19104; voice: 215-898-0915; Fax: 215-573-7155; jebennet@mail.med.upenn.edu.

Family A: This family has autosomal dominant retinal degeneration which is manifest as retinitis pigmentosa in some individuals (II-2) and macular dystrophy in others (II-4, III-1, II-1). The p.R46X mutation in *PRPH2*, known to be pathogenic, segregates with the disease. The proband is a 74 year old woman with macular dystrophy (II-4, Figure 1A) who has been followed for the past 39 years. While most of the relatives tested had retinal degeneration and were heterozygous for the p.R713Q variant in *SEMA4A*, the brother of the proband, II-7, was heterozygous for the *SEMA4A* variant (but not the *PRPH2* mutation) and had no symptoms or signs of retinal/macular degeneration.

Family B: A 43yo female (proband II-2, Figure 1B) presented with unilateral pigmentary retinal degeneration. She had been symptomatic since age 19. Examination was notable for marked asymmetry, with bone spicules and peripheral to central retinal degeneration in the right eye only and asymmetric ERGs and visual fields. Over the next 14 years, lattice degenerative changes commenced in the left eye. Neither parent had a history of retinal disease. The proband II-2 was diagnosed with simplex RP. Neither the 30yo son nor the 32yo daughter of the proband shows signs of retinal disease. The proband is homozygous for the p.R713Q variant of *SEMA4A*. The son (III-1) and the daughter (III-2) are heterozygous for the p.R713Q variant of *SEMA4A*. Based on the pedigree of this family, it would be possible for the p.R713Q variant of *SEMA4A* to cause AR disease, but it is not consistent with a dominant mutation. We were unable to positively identify the pathogenic mutation(s) in this proband after exploring numerous potential candidate genes (including common variants involved in AR and ADRP as well as *PER3, HOXD1, DLEC1, ALS2CL, COL4A1, MRPS31,* and *STARD8*). We suspect that either a novel gene is responsible or potentially a *de novo* mutation in a modifier gene arose in the more severely affected retina.

Family C: An otherwise healthy 67yo male (proband II-3, Figure 1C) presented with light perception only vision. An ophthalmic exam revealed widespread pigmentary changes, retinal thinning and vessel attenuation. He had been diagnosed with simplex RP in his 20's. His sisters and brothers and two sons (each in their 30's) had normal vision. Genetic testing revealed that the proband is heterozygous for the p.R713Q variant in *SEMA4A*. Three of his unaffected siblings are also heterozygous for the variant. Additionally, his unaffected 43yo son is homozygous for the p.R713Q variant of *SEMA4A*. The fact that an unaffected family member is homozygous for the mutation indicates that this mutation is insufficient to cause disease. The pathogenic mutations in this family is likely to be in *USH2A* as the proband has compound heterozygous mutations in *USH2A* (p.R4192H and p.R1653*) and no other family member has mutations in both alleles.

In summary, we describe three families with retinal degeneration and in which the *SEMA4A* p.R713Q variant was observed in both affected and unaffected individuals. Our findings are inconsistent with the dominant pattern of inheritance currently ascribed to the *SEM4A* p.R713Q variant[1]. Not only is there a lack of segregation of the mutation with disease, but also one of the unaffected family members in family C is homozygous for the variant, thus eliminating the possibility that the variant leads to a recessive disease. These results are consistent with the results from the mouse model generated by Nogima et al that was homozygous for the p.R713Q missense *Sema4a* variant. This mouse did not show any signs of retinal degeneration.[2,3] It is possible that the p.R713Q missense *Sema4a* change could

lead to disease when combined with a mutation in another gene, but it is not sufficient to cause disease in isolation.

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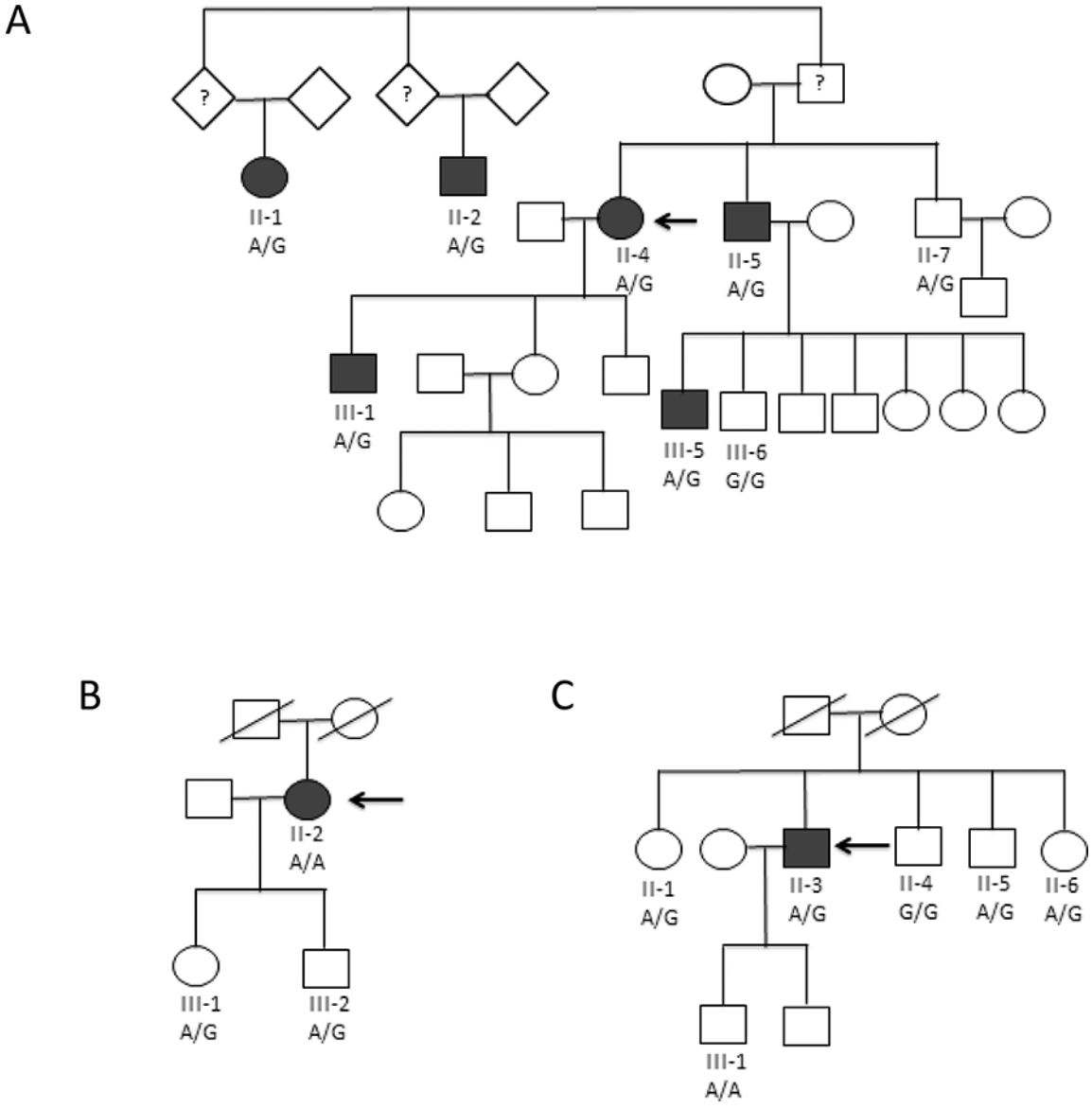

Figure1:

Family Pedigrees and clinical findings. Three families carry the p.R713Q missense variant in *SEMA4A* (c.2138G>A). A) Family A has a dominant inheritance pattern with multiple affected individuals. B) Family B has one affected member with autosomal recessive retinitis pigmentosa. C) Family C has one affected member with autosomal recessive retinitis pigmentosa.