Penetrance of the *ABCA4* p.Asn1868Ile Allele in Stargardt Disease

We read with interest the manuscript by Runhart et al.,[1] which further confirmed our recent discovery of the c.5603A>T (p.Asn1868Ile) variant in the *ABCA4* gene as the causal mutation in Stargardt (STGD1) and related diseases.[2] We had demonstrated that the variant, which is highly frequent in all populations, especially in the general population of non-Finnish Europeans (minor allele frequency [MAF] ~7%) and therefore considered benign, is actually causal when in *trans* with a loss-of-function allele.[2] It is reassuring that Runhart and colleagues validated all conclusions of our study, that the variant is frequent in STGD1, segregates with the disease phenotype, and results in a predominantly late-onset disease phenotype that at times can be misdiagnosed as age-related macular degeneration (AMD). The only new conclusion, which came to our attention, is that the penetrance of the p.Asn1868Ile allele, when in *trans* with a "severe" *ABCA4* mutation, is estimated at less than 5%. This conclusion was based on calculations of allele frequencies from the gnomAD database and the observation of two sibling pairs where the genotype did not properly segregate with the disease.

We find this conclusion of extremely low penetrance of the variant incompatible with the authors' own data showing its segregation with the disease in families, which the authors correctly use to support pathogenicity. As a basic principle of genetics, no segregation would be observed even at 50% penetrance (10 times higher than proposed) in the case of a recessive disease. The phenotypic variability associated with this variant adds further uncertainty to the assessment of "non-segregation" as the authors concede that even those individuals with no clinical signs of STGD1 can develop the disease at a later time since they do document disease onset in some cases at a very advanced age, over 70 years.[1] Therefore, it is unclear how the study corroborates the conclusion of <5% penetrance with their own data. The authors may also want to re-examine the penetrance calculations with respect to the following considerations:

First, using MAFs from the general population data in gnomAD, instead of those specific to their Dutch patient population, is incorrect. Significant differences in *ABCA4* allele frequencies have been described not only between racial groups[3,4] but also between ethnicities/nationalities.[5,6] It is clear from the manuscript that many frequent deleterious mutations are very rare or absent in the Dutch population and even the frequency of the p.Asn1868Ile has not been established in The Netherlands.

Second, the prevalence of ABCA4 disease is not known. The 1:10,000 estimate comes from the textbook chapter by Blacharski in 1988,[7] which is frequently cited but likely not read, since it states "We have seen this condition much more commonly than retinoblastoma, which has been estimated at 1 in 15,000 live births. Fundus flavimaculatus is not as common as retinitis pigmentosa, which has a prevalence of no more than 1 in 5000. We have roughly estimated the incidence to be between 1 in 8000 and 1 in 10,000." We suggest that this is not an appropriate way to determine a disease prevalence, especially as a variable for statistical calculations. The actual prevalence of ABCA4 disease is very difficult to estimate due to enormous clinical heterogeneity, variable age of onset, and (still) incomplete genetic data.

Third, we stated[2] that the p.Asn1868Ile is penetrant in cases where the other allele is "deleterious" or "loss-of-function,"[2,8] not "severe."[1] Differentiating between these two categories is very difficult, but it is clear that the functional outcome of an allele cannot be determined by its appearance; that is, even many nonsense mutations may not result in a complete loss-of-function while certain missense alleles do.[9,10] In fact, we suggested, based on the empirical data, that cases with p.Asn1868Ile are a "litmus test" for severity of the allele in *trans*, including all classes of mutations.[2]

Fourth, the authors suggest that both genetic and environmental modifiers are the cause of the very low penetrance. Modifiers certainly play a role in expression of ABCA4 disease, but these have to be specifically identified to make such a statement. For example, although the c.2588G>C ([p.Gly863Ala, Gly863del]) variant had been previously considered a "mutation,"[11] we presented new data showing that it is in fact a modifier for the p.Asn1868Ile variant. It does not cause the disease alone, but the complex allele, together with the p.Asn1868Ile mutation, becomes a fully penetrant, disease-causing mutation.[2]

In summary, there is a glaring inconsistency between the almost perfect segregation data and the authors' conclusion of very low penetrance of the p.Asn1868Ile variant. The latter stems from using questionable assumptions in their estimation of the disease prevalence, MAF of variants in their population, and the functional consequence of the variants. The published data[1,2] suggest the exact opposite, at least 95% penetrance.

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