A frequent variant in the Japanese population determines quasi-Mendelian inheritance of rare retinal ciliopathy

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ABSTRACT

Hereditary retinal degenerations (HRDs) are Mendelian diseases caused by ultra-rare mutations and leading to progressive blindness. Following the genomic screening of 331 unrelated Japanese patients, we identified a disruptive *Alu* insertion and a nonsense variant (p.Arg1933*) in the ciliary gene *RP1*. Surprisingly, none of these changes were rare alleles in Japan. p.Arg1933* was almost polymorphic (frequency = 0.6%, amongst 12,000 individuals), did not cause disease in homozygosis or heterozygosis, and yet was considerably enriched in patients vs. controls (frequency = 2.1%, i.e. a 3.5-fold enrichment; *p*-value = 1.29×10^{-6}). Familial co-segregation and exome-wide association analyses showed that p.Arg1933*could act as a Mendelian mutation, *in trans* with the *Alu* insertion, but also caused disease in association with at least one allele elsewhere in the genome, according to a non-Mendelian pattern of heredity. Our results suggest that rare conditions such as HRDs can be paradoxically determined by relatively common variants, following a quasi-Mendelian model linking monogenic and complex inheritance.

MAIN TEXT

Together with intellectual disabilities, hereditary retinal degenerations (HRDs, comprising retinitis pigmentosa and allied diseases) represent a group of conditions for which both genetic and allelic heterogeneity is the highest in humans[1,2]. To date, almost 300 genes and thousands of mutations have been identified as causative of HRD, and the detection of novel disease genes and variants continues at a steady pace[3]. Considering that the overall prevalence of HRDs does not exceed 1 in 2,000 individuals, the average contribution of any given HRD gene to the disease is incredibly small. Similarly, apart from two DNA variants that appear to be relatively frequent in the general population and determine a specific form of the disease (p.Asn1868Ile and p.Gly863Ala in *ABCA4*)[4,5], the largest majority of mutations are so rare that are seldom detected in more than one pedigree, worldwide. In addition, although HRDs affect people from the five continents, their specific allelic assortment seems to be population-specific[6,7]. For instance, similar to other islanders or groups of people that have experienced relative historical isolation, Japanese carry certain alleles, including pathogenic ones, which are not found elsewhere in the world[8]. Furthermore, lack of significant reduction in fitness before the reproductive age, associated with such an elevated heterogeneity with respect to other Mendelian conditions, have led to the consequence that the number of recessive mutations that are detected heterozygously in the general, unaffected population is remarkably high and may affect up to one person in two[9].

Despite such an extraordinary variability and abundance of mutations, HRD is almost invariantly inherited as a monogenic, Mendelian trait, for which the presence of only one (dominant) or two (recessive) mutations in the same gene, genome-wide, is at the same

time a necessary and sufficient condition for pathogenicity[10]. At the other end of the spectrum of ocular conditions having a genetic component lies age-related macular degeneration (AMD), another retinal disease affecting people aged 50 and over. AMD is a *bona fide* complex disease with a relatively high prevalence (1 in 13 individuals), favored by the presence of polymorphic SNPs, highly-penetrant rare variants, and environmental factors[11]. Between these two pillars of inheritance, there is an intermediate zone, consisting in a few examples for which extremely rare mutations in more than one gene are associated with Bardet-Biedl syndrome, a retinal ciliopathy displaying sometimes digenic triallelic inheritance[12-14].

RP1 is one of the several HRD genes identified to date, and one of the few causing disease by more than one Mendelian pattern of inheritance. Originally described as linked to autosomal dominant retinitis pigmentosa (adRP)[15-17], a subtype of HRD, it was later shown to be associated with a recessive form of the same disease (arRP)[18]. To date, at least 60 mutations have been reported in *RP1*, most of which cluster within its last exon (exon 4), cumulatively accounting approximately for 5.5% and up to 4.5% of all adRP and arRP cases, respectively[19,20]. However, some DNA variants in the far 3' end of the gene, including nonsense variants, appear not to cause disease, at least not according to a dominant or recessive pattern of inheritance[21,22]. *RP1* encodes a multi-modular protein of 2156 amino acids, which is a member of the doublecortin family and is present in the ciliary axoneme of both rods and cones, the light-sensing neurons of the retina[23,24]. Mutations in *RP1* thus determine visual loss as a consequence of a ciliopathic phenotype affecting these specialized cell types.

In the framework of a Whole-Genome Sequencing screening effort of Japanese patients, we identified a novel, unusual mutation consisting in the insertion of a mobile *Alu*

element in exon 4 of the *RP1* gene (m1, or NM_006269.1:c.4052_4053ins328/p.Tyr1352Alafs*9) in a female individual from a recessive HRD family. This insertion caused the disruption of the reading frame by introducing 328 additional nucleotides, including a premature termination codon in the canonical *RP1* coding sequence. The mother of the proband was heterozygous for this variant and the proband's affected brother was also a homozygote, in support of the notion that this was indeed a recessive HRD mutation (Fig. 1a). Subsequently, targeted screening for this *Alu* insertion in an additional 220 and 330 European and Japanese patients (all forms of HRDs, isolate or recessive cases, not genetically pre-screened), respectively, as well as in 524 Japanese controls, identified 15 other affected Japanese individuals (Supplementary Table 1) and one heterozygous Japanese control carrying this insertion. In total, six patients were homozygous for the mutation (12 alleles), which co-segregated with the disease as a classical Mendelian, recessive allele (not shown), while 10 carried it heterozygously. Altogether, these findings indicate that this *Alu* insertion is not only clearly pathogenic [*p*-value = 1.66×10^{-8}, by Chi-square (1,047:1 vs. 640:22; wt alleles in controls:mutations in controls vs. wt alleles in patients:mutations in patients, all Japanese)], but it is also a rather prevalent cause of retinal degeneration within the Japanese islands, possibly second only to the most frequent mutation so far identified in this country, i.e. NM_001142800.1:c.4957dup in *EYS*[25-27].

Remarkably, 6 of the 10 individuals who carried the *Alu* insertion heterozygously were in fact compound heterozygotes for either of two other changes in *RP1*: a novel frameshift mutation (c.4196del/p.Cys1399Leufs*5, m2, two unrelated individuals) and a nonsense variant c.5797C>T/p.Arg1933* (m3, four unrelated individuals) that was previously identified in the general population and is present in dbSNP as entry #

rs118031911. Again, both variants, detected by direct Sanger sequencing, co-segregated with the disease within their respective families, according to an autosomal recessive pattern of inheritance (Fig. 1bcd and data not shown).

Frameshift c.4196del/p.Cys1399Leufs*5 was absent from 3,480 Japanese control chromosomes and was reported in the gnomAD database[28] to have an allele frequency of $5.44x10^{-5}$ in East Asia, indicating that this DNA variant is a very rare allele, as it is the case of most HRD mutations.

In contrast, the rs118031911/T allele, despite being virtually absent in many world populations, was found to be relatively frequent in East Asians (Supplementary Figure 1). In particular, our direct screening of 12,379 Japanese individuals with no retinal degeneration showed the presence of rs118031911/T in 145 subjects, 142 heterozygotes and 3 homozygotes (148 alleles), validating the notion that this DNA variant is in fact almost polymorphic in Japan (allele frequency = 0.6%). All these subjects were examined by fundoscopy and, in addition, we evaluated clinically one of the three homozygotes (the only one who could be re-assessed, in agreement with our Institutional Review Boards protocol) by a very thorough ophthalmological examination. At age 28 y.o., she had no visual symptoms and displayed no ocular abnormalities: she had normal visual acuity (20/20 in both eyes), intact visual field (Goldmann perimetry), and no evidence of retinal degeneration through slit lamp examination and fundoscopy. Furthermore, optical coherence tomography imaging used to assess detailed retinal structures showed no sign of retinal thinning and electroretinogram, a test allowing objective detection of minimal retinal dysfunction even in the absence of subjective symptoms, showed normal responses. Finally, absence of late-onset HRD, who could have escaped detection in a 28 y.o. individual, was confirmed by the assessment of the fundi of the other two rs118031911/T homozygotes,

who displayed no signs of retinal degeneration at ages of 78 and 79 years, respectively. Altogether, both population based-data and direct clinical assessments confirm that rs118031911/T does not cause *per se* HRD, in heterozygosis or in homozygosis.

However, specific screening for the rs118031911/T allele in the same cohort of 331 Japanese HRD patients mentioned above led to the identification of 10 additional heterozygotes (14 alleles in total) showing that its frequency in HRD patients was 2.1% (14 alleles out of 662) (Supplementary Figure 1). The 3.5-fold enrichment of rs118031911/T in patients vs. controls (148 alleles out of 24,758 = 0.6%) was highly significant [*p*-value = 1.29×10^{-6}, by Chi-square (24,610:148 vs. 648:14)], indicating that this relatively common variant has in fact an effect on retinal health. WES analysis of these 10 patients detected no mutations in HRD genes that could explain their phenotype, according to a Mendelian fashion of inheritance. Considering that rs118031911/T introduces a nonsense codon in the *RP1* open reading frame and was found *in trans* with respect to the *Alu* insertion in some patients, it is not unlikely that it could represent a hypomorphic variant contributing to the mutational load of genes involved in retinal homeostasis. In other words, despite being benign when considered as a Mendelian allele (monoallelically or biallelically), rs118031911/T could exert a pathogenic function in conjunction with DNA changes in other genes, according to an oligogenic pattern of inheritance that was previously modeled for hereditary ciliopathies[29-31].

We tested this hypothesis by performing an Exome-wide association study (ExWAS) on low frequency variants (minor allelic frequency between 1% and 5%, further details in Supplementary Methods) from the 10 patients mentioned above carrying rs118031911/T heterozygously and no other recognized mutation in *RP1* vs. 3,554 Japanese controls from the 3.5KJPN database[32]. This analysis did not identify any variant within the *RP1* sequence,

in support of the hypothesis that these 10 patients are not compound heterozygotes for an additional, undetected *RP1* mutation *in trans* with respect to rs118031911/T, such as a deletion or a non-coding pathogenic DNA change. Furthermore, the presence of false positive signals due to possible geographical proximity or genetic relatedness of the 10 carriers of rs118031911/T was excluded by performing principal component analysis of WES calls from these patients and from 137 unrelated HRD Japanese patients not carrying rs118031911/T, sequenced by the same procedures (Supplementary Figure 2) and by computing PLINK's PI_HAT for all their possible pairwise combinations (Supplementary Table 2). The association test identified one variant that was significantly enriched in the 10 patients vs. controls in the gene *KIF13B* (NM_015254.3:c.834-35T>C, rs117338543, Table 1, Fig. 2, Supplementary Figure 3). We validated these changes by targeted sequencing of 21 additional patients (Supplementary Table 1), again carrying rs118031911/T heterozygously, no HRD mutations and no other *RP1* pathogenic allele *in trans*, identified by panel sequencing in an independent cohort of 1,394 Japanese HRD patients vs. 125 control Japanese from the JGA-NGS dataset[33]. Once more, cryptic relatedness or geographical proximity was excluded by PLINK's PI_HAT (Supplementary Table 2) and by manually inspecting the haplotype surrounding rs118031911/T in 11 randomly sampled individuals (Supplementary Table 3). The variant in *KIF13B* was indeed replicated and showed to be independently significant in these additional cohorts (Table 1), providing a combined allele frequency of 0.19 in the 31 (10+21) rs118031911/T heterozygotes, vs. a 0.0322 frequency in controls (Table 1, Fig. 2). Altogether, these results indicate that the rs118031911/T nonsense likely acts in concert with at least another DNA change (and possibly with more than one) to determine a pathological phenotype in a non-Mendelian fashion.

The extreme genetic heterogeneity of retinal degenerations, together with the elevated number of pathogenic and hypomorphic changes in HRD genes that are detected in the unaffected population, have evoked the theoretical possibility that non-Mendelian, oligogenic inheritance could be responsible for these conditions[10]. Digenic heredity has been clearly demonstrated for specific combinations of mutations[34-36] in particular pedigrees or in individual cases, including digenic triallelic transmission of Bardet-Biedl syndrome [12,37]. For these patients, the presence of two (diallelic) or three (triallelic) mutations at two different loci (digenism) causes disease, presumably by compromising the overall function of gene products that belong to the same complex or are part of the same biochemical pathway. This model seems to be particularly true for genes encoding for proteins that form or play a role within the cell primary cilium, according to the paradigm of "mutational load" put forward by N. Katsanis and coworkers[38]. In these instances, accumulation of rare variants (which individually may have a little effect) in multiple ciliary genes can produce a pathological phenotype that is connected to ciliary function and result in a "ciliopathy", including retinal ciliopathies[39-41]. Indeed, KIF13B is a ciliary protein belonging to the kinesins superfamily of microtubule motors and involved in physiological processes that relate to organelle and vesicle transport, possibly having a synergic function with respect to RP1[42]. Recently, KIF13B has been shown to localize in the centrosomes and primary cilia of mammalian cells[43], and its depletion in retinal pigment epithelium cells affects in fact the homeostasis of their cilia[44]. Notably, other KIF proteins have been implicated in HRDs, such as for instance KIF7 and KIF11[45,46].

The way by which rs117338543 in *KIF13B* could exert a negative role on ciliary functions is currently unclear. Its presence within an intron may suggest possible interference with respect to the splicing process, as it has been observed for similar variants

in other HRD genes, including ciliary ones[47-51]. However, *in silico* predictors do not recognize a significant effect for rs117338543, and therefore additional functional experiments will have to be performed to test this hypothesis, possibly in a context that recreates *in vitro* or *ex vivo* the splicing conditions of a photoreceptor cell, such as for instance patient-derived differentiated iPS cells. Further investigation of additional variants present in *KIF13B*, within rs118031911/T carriers from the discovery cohort, allowed recognizing 7 rare variants that could have a frequency compatible with that of hypomorphic changes (not shown). However, again, none of them could be immediately recognized as being clearly deleterious by *in silico* tools. A classical digenic (specifically, biallelic or triallelic) mode of action for rs118031911/T on HRD with respect to known disease genes was also tested, by comparing the frequency of this variant in patients for whom the molecular causes of retinal degenerations were identified (i.e. "solved" cases) vs. "unsolved" HRD cases vs. controls. As expected, a comparison of unsolved vs. controls showed significance, as reported above, whereas solved vs. controls did not show any significant enrichment for rs118031911/T (6 rs118031911 variants over 722 alleles for solved vs. 148 over 24,758 for controls, *p-value* = 0.46, OR = 1.4, CI = 0.50-3.14). Comparison of solved vs. unsolved HRD cases showed borderline non-significant enrichment for rs118031911/T in unsolved cases (*p-value* = 0.07, OR = 2.30, CI = 0.94-6.76), possibly indicating that either well-defined diallelism or triallelism does not take place for this variant or, simply, that we did not have enough power to detect it.

In this work we show that two specific *RP1* alleles are responsible for a relatively large number of Mendelian HRD cases in Japan. Interestingly, none of these two changes is a rare allele at all, compared to the average frequencies of classical HRD mutations. The first, the c.4052_4053ins328/p.Tyr1352Alafs*9 *Alu* element insertion in *RP1*, seems to be the

second most common HRD recessive mutation described so far in Japan, and its frequency may even be underestimated, since insertional events of mobile elements are difficult to detect by conventional screening techniques. The second variant, c.5797C>T/p.Arg1933* or rs118031911/T, is even more frequent, and by far more interesting. Despite introducing a premature stop codon in the *RP1* open reading frame, this DNA change is almost polymorphic in East Asia and does not cause disease either in heterozygous or homozygous carriers. However, this same change may act as pathogenic allele in a Mendelian fashion (with another *RP1* mutation *in trans*), or in association with rare variants in at least another gene, according to a non-Mendelian, possibly oligogenic pattern of inheritance. Although we currently ignore the molecular mechanisms leading to this unusual model of pathogenicity, it is probably the consequence of an increased global mutational load with threshold effect, determined by the accumulation of variants with different pathogenic potential. The presence of one or of two rs118031911/T alleles likely produces a load that is below this pathological threshold, while the co-occurrence of extra variants could result in the crossing of such a limit for normal retinal homeostasis. This hypothesis is supported by the evidence that rs118031911/T may be pathogenic in conjunction with very severe mutations, such as the insertion of the *Alu* element in *RP1* mentioned above, which completely ablates the open reading frame of the gene. We term this model of inheritance “quasi-Mendelian”, to define the differential behavior (Mendelian or non-Mendelian) that specific alleles may have with respect to different genotypes at the same locus or elsewhere in the genome.

In conclusion, it seems that, at least for *RP1*-associated HRD, disorders displaying a Mendelian pattern of inheritance may also genetically behave like multigenic conditions, for which both polymorphic (having a low effect) and rare (having a rather high effect) variants can determine pathogenesis (Supplementary Figure 4). The low prevalence of HRD and the

even lower percentage of HRD patients carrying rs118031911/T prevents us at the moment to increase the power of our analysis and to propose more defined models of pathogenicity, since the identification of the 31 heterozygotes used in this study corresponds roughly to the screening of 10 million Japanese individuals. However, this work provides a clear proof of concept that a non-negligible proportion of HRDs can be caused by inheritance mechanisms that transcend the Mendelian model, to be investigated in detail by future, very large-scale and population-specific sequencing endeavors, such as for instance the 100,000 genomes project[52]. Furthermore, our findings suggest that oligogenic heredity of human diseases (and perhaps of other characters) may not be limited to a low number of cases with hyper-rare conditions, as shown up to now[53-55], but could extend to more frequent phenotypes and represent a bridge between monogenic and complex inheritance.

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LEGENDS TO FIGURES

Figure 1 Pedigrees of representative families segregating different *RP1* mutations.

Figure 2 Manhattan plot for exome-wide association (ExWAS) between 10 patients with retinal degeneration carrying rs118031911/T heterozygously and 3,554 Japanese controls (discovery test, green and blue dots). The red dot indicates the value relative to KIF13B (rs117338543) following the combined analysis of the discovery and the validation tests (validation: 21 additional heterozygous rs118031911/T carriers vs. 125 controls). The genome-wide significance threshold is indicated by the dotted line.

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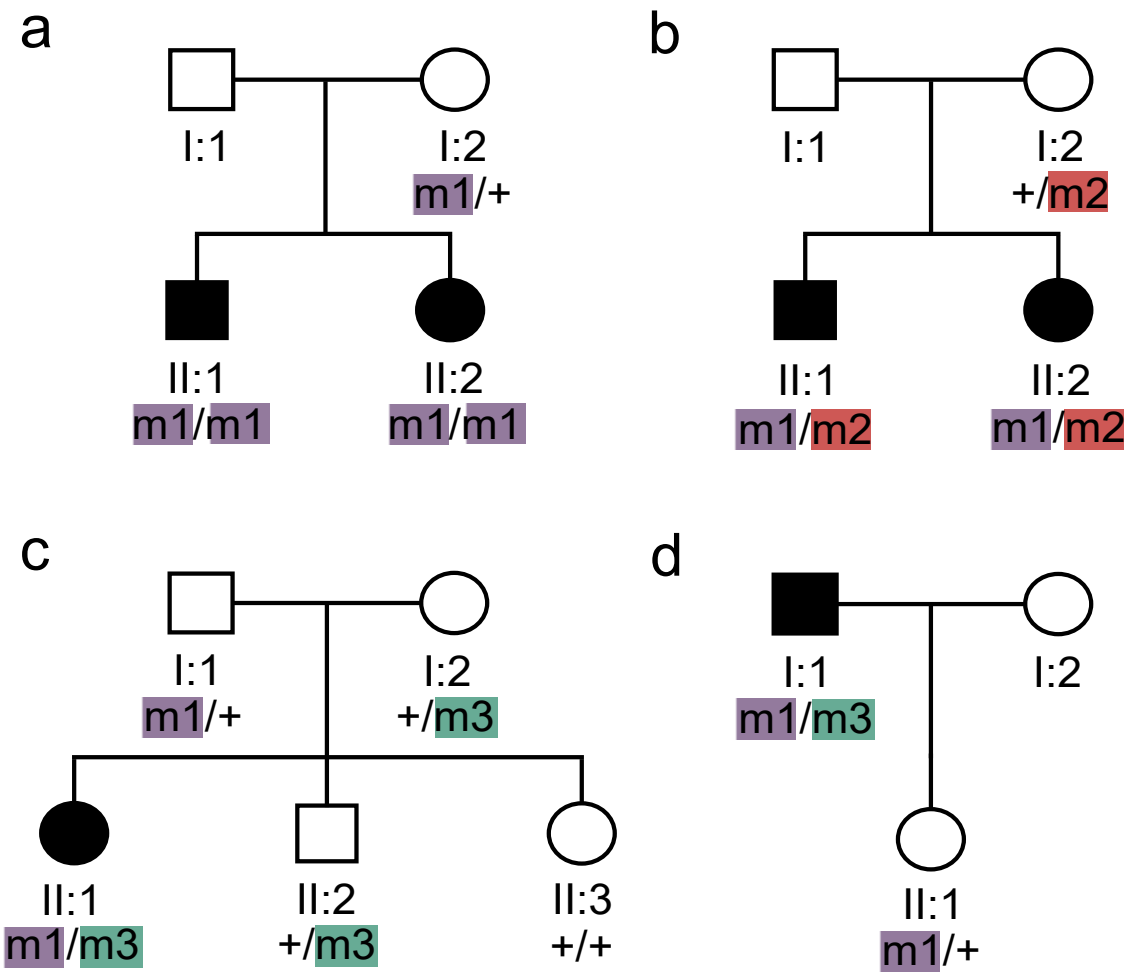

m1: c.4052_4053ins328/p.Tyr1352Alafs*9 (*Alu* insertion)
m2: c.4196del/p.Cys1399Leufs*5
m3: c.5797C>T/p.Arg1933*

Figure 1. Pedigrees of representative families segregating different *RP1* mutations.

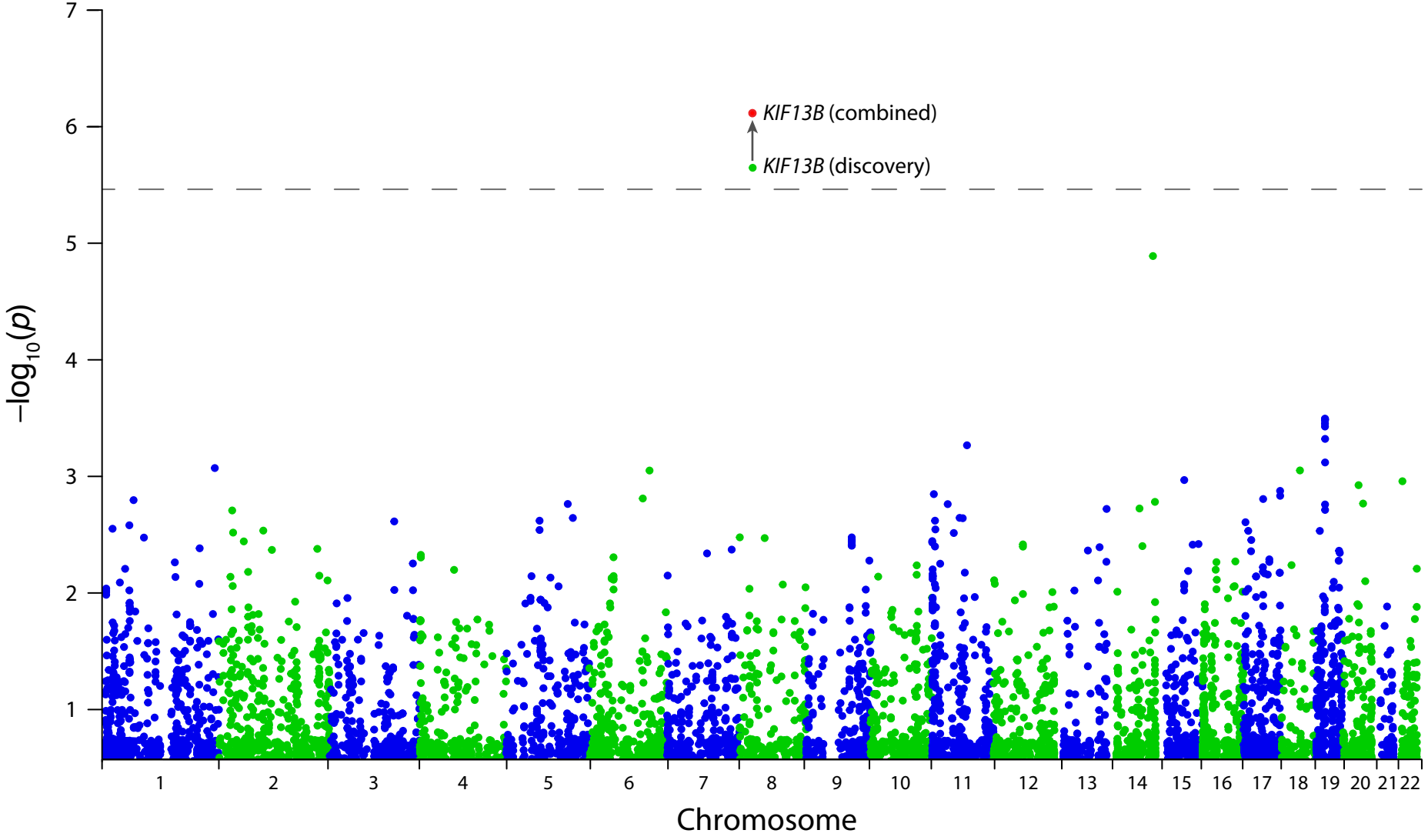

Figure 2. Manhattan plot for exome-wide association (ExWAS) between 10 patients with retinal degeneration carrying rs118031911/T heterozygously and 3,554 Japanese controls (discovery test, green and blue dots). The red dot indicates the value relative to *KIF13B* (rs117338543) following the combined analysis of the discovery and the validation tests (validation: 21 additional heterozygous rs118031911/T carriers vs. 125 controls). The genome-wide significance threshold is indicated by the dotted line.