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Inglehearn, CF [orcid.org/0000-0002-5143-2562](https://orcid.org/0000-0002-5143-2562), Yahya, S [orcid.org/0000-0002-3710-1011](https://orcid.org/0000-0002-3710-1011), Smith, CEL [orcid.org/0000-0001-8320-5105](https://orcid.org/0000-0001-8320-5105) et al. (7 more authors) (2023) *Reply*. *Ophthalmology*, 130 (3). e9-e10. ISSN 0161-6420

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**Re Yahya et al: Late onset autosomal dominant macular degeneration caused by deletion of the CRX gene. Reply to Mustafi and Chao.**

We thank Mustafi and Chao for their considered response to our paper. We described a case series of eight patients with macular degeneration (MD), all of whom carry the same 126 kb deletion encompassing the entire gene encoding the transcription factor CRX. In our paper<sup>1</sup> we suggest this constitutes strong evidence for a haploinsufficiency (lack of normal protein) disease mechanism, despite the published observation of apparently asymptomatic cases with heterozygous putative null *CRX* variants. We go on to suggest these cases were young and may later develop MD, and we accept that, given the variation in age at onset (20-78 years), there may also be cases of non-penetrance.

Mustafi and Chao propose an alternative explanation; that in the patients in our case series, the deleted allele is paired with a trans-acting hypomorphic allele, making this in effect a recessive disease in which a mild allele is paired with the more severe deleted allele. In proposing this, and citing selected examples from the literature, they highlight a problem in human genetics terminology. The terms hypomorphic, modifier or variable penetrance allele are often used more or less interchangeably in a variety of contexts to describe the action of alleles with widely varying frequencies and effect sizes.

If, by a hypomorphic allele, they mean a rare allele with a near-Mendelian effect size, such as those they cite in the *RPGR* and *RDH12* genes, we accept that such a hypothesis is theoretically possible but suggest that our proposed mechanism, haploinsufficiency, is far more likely. In our paper, we stated that no second potential disease causing alleles were identified in seven cases, the eighth having been screened only by breakpoint PCR. To be certain that such variants had not been excluded through filtering, we re-examined exomes from four *CRX* deletion carriers for whom data were readily accessible within the time available to respond, using a conservative filtering strategy. No single variant was found in multiple cases, as had been described for the *RDH12* allele cited by Mustafi and Chao, and no two cases shared the same haplotype in the trans allele. Furthermore, no variant with a minor allele frequency below 1% and a CADD score >10 was observed. Yet the hypothesis proposed by Mustafi and Chao requires that such alleles are present in every case. Indeed one would have to infer that a hypomorphic allele or alleles had come into the family from partners in both generations for the mother and daughter pair described in our paper. Given that, for all but one of our cases, we have only exome or targeted sequence data, we cannot exclude the possibility of such a variant outside the coding sequence, but based on frequency alone, the presence of such an allele seems unlikely.

Alternatively, Mustafi and Chao may envisage a relatively common allele, such as the ABCA4 p.Asn1868Ile variant highlighted in their third cited example, which is present in around 7% of the population. This may indeed be the case, but it would then be impossible to prove an effect in such a small cohort of patients. That task would be made even harder if, as they suggest, the variant(s) in question lay outside the coding sequences of the gene. One class of non-coding variants that have been shown to alter penetrance in rare diseases<sup>2</sup> are expression/splicing QTLs (eQTLs and sQTLs), but no significant eQTLs/sQTLs have been characterised for *CRX* to date<sup>3</sup>.

Mustafi and Chao suggest that long-read sequencing would more readily detect such variants. The variants most commonly missed by short read sequencing are low complexity regions and complex rearrangements, but the hypomorphic variant examples cited by Mustafi and Chao are SNPs, which are easily detected by conventional short-read sequencing. We suggest that the challenge in characterising such variants is not in detecting them, but in proving they have an effect.

In conclusion, we feel that the approach we have used is a valid and appropriate one, and that, while we accept their proposed alternative explanation is possible, haploinsufficiency remains the most likely cause of disease in these patients.

**Chris F Inglehearn**

**Samar Yahya**

**Claire E L Smith**

**James A Poulter**

**Manir Ali**

**Carmel Toomes**

*Leeds Institute of Medical Research, University of Leeds, St James's University Hospital, Leeds, UK.*

**Jamie Ellingford**

**Graeme C Black**

*Manchester Centre for Genomic Medicine, St. Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, UK.*

**Gavin Arno**

**Andrew R Webster**

*NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust & UCL Institute of Ophthalmology, London, UK.*

## References

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