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LETTER TO THE EDITOR

The Italian reappraisal of the most frequent genetic defects in hereditary optic neuropathies and the global top 10

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We read with great interest the recent publication by Rocatcher and colleagues[1] entitled 'The top 10 most frequently involved genes in hereditary optic neuropathies in 2186 probands', reporting on the experience of a national diagnostic centre for hereditary optic neuropathies (HON) in France. Similarly, our scientific institute is a national referral and diagnostic centre for HON in Italy, and in this correspondence, we present our neurogenetic assessments of 1097 HON patients. We offer a comparison between the two studies and comment on the global results obtained by merging the data, thus commenting on the largest cohort of HON cases presented to date.

In the Program of Neurogenetics at the IRCCS Institute of Neurological Sciences of Bologna, we run neurogenetic diagnostics and, since 2006, have been undertaking an assessment of the three common LHON pathogenic mutations (m.11778G>A/*MT-ND4*, m.3460G>A/*MT-ND1* and m.14484T>C/*MT-ND6*), complete mtDNA sequencing and direct Sanger sequencing of *OPA1*, the major contributor to dominant optic atrophy (DOA).

As next generation sequencing (NGS) became available, we implemented diagnostics of nuclear genes with two versions of targeted custom panels, composed of 36 and 50 genes, respectively (2014–18). If this analysis was negative, over the next few years (2018–21), we reanalysed these samples using whole exome sequencing (WES). In 2022, WES started to be used routinely in our centre, taking advantage of the NovaSeq6000 platform (Illumina). This is currently the standard algorithm followed by *in silico* panel analysis, prioritizing variants in known HON genes, to eventually discover new disease genes.

By including all patients referred for HON diagnostics over the period 2006–22, we here report our cohort of 1097 probands,

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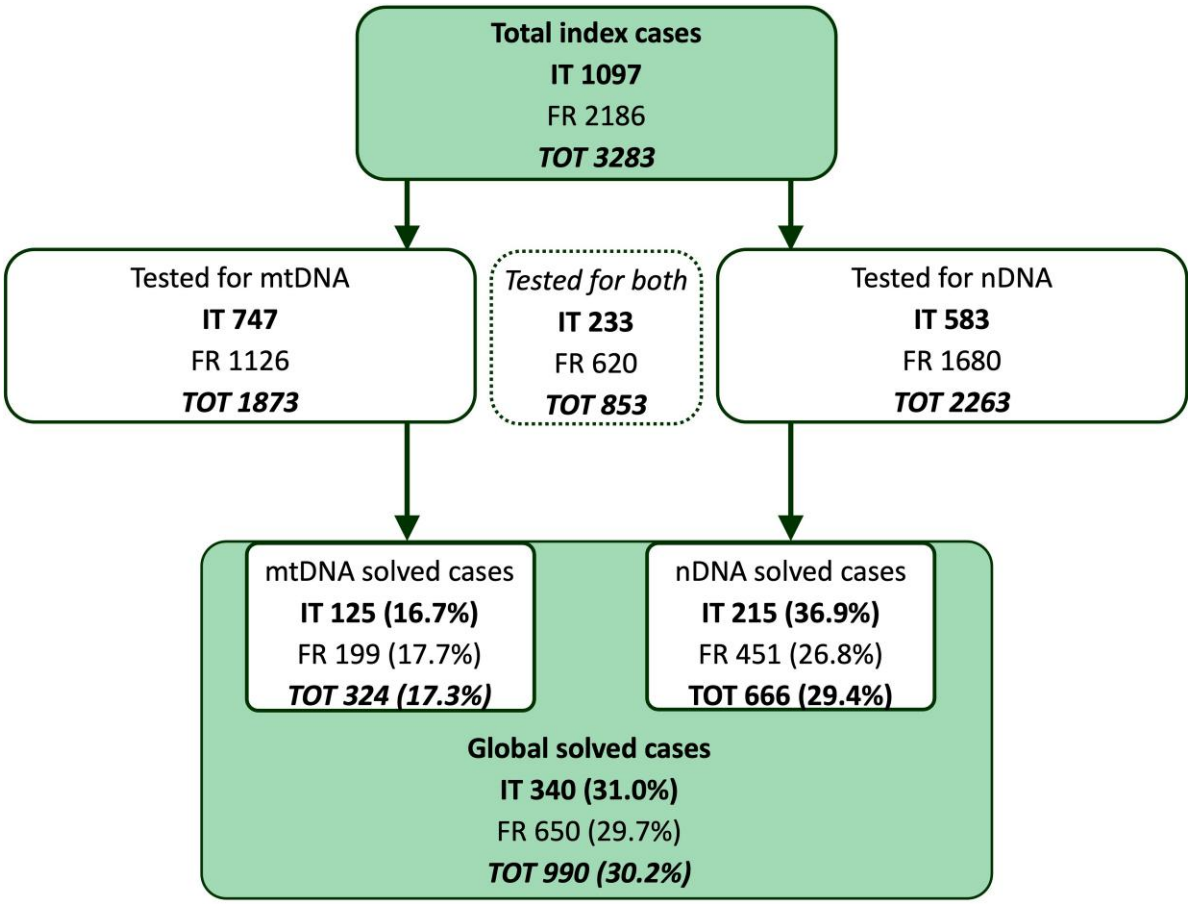

Figure 1 Workflow of the Italian and French studies and the global results.

presenting the results in parallel with Rocatcher *et al.*,[1] as well as providing the global results obtained by merging the two cohorts (Fig. 1). In considering the Italian probands suspected of having LHON ($n = 747$), we found 125 (16.7%) to be LHON positive. These cases were stratified for the three common pathogenic variants as follows: 74 carried the m.11778G>A/*MT-ND4* pathogenic variant (59.2%); 20 the m.3460G>A/*MT-ND1* pathogenic variant (16.0%); 18 the m.14484T>C/*MT-ND6* pathogenic variant (14.4%); and one case harboured a double mutation, m.3460G>A /*MT-ND1*+ m.14484T>C/*MT-ND6*. In all, these variants represented 89.6% of the LHON-positive cases (Supplementary Table 1). We found nine cases with rare LHON pathogenic variants and three probands with combinations of rare variants (Supplementary Table 1) as reported previously.[2] Among the rare LHON variants, only m.10254G>A/*MT-ND3* and m.14487T>C/*MT-ND6* were found twice in independent maternal lineages. Considering the nuclear genes underlying DOA or recessive optic atrophy (ROA), or the rare X-linked optic atrophy (XOA), we had a total of 583 probands, for which diagnosis was reached in 215 cases (36.9%). The nine most frequently represented genes were found in at least in three independent families (*OPA1* = 125, *WFS1* = 20, *ACO2* = 19, *AFG3L2* = 8, *RTN4IP1* = 5, *SPG7* = 4, *SDHA* = 4, *DNAJC30* = 3 and *SSBP1* = 3), and three genes were found equally in two independent families (*C19orf12*, *SLC25A46* and *UCHL1*). The other 18 genes were found to harbour pathogenic variants in single cases. All of these results are summarized in Fig. 1 and Table 1. The overall diagnostic yield of the 1097 Italian HON patients reached 31.0% (340/1097) (Fig. 1).

Reassuringly, the results for patients suspected of having LHON were very similar to those of the French cohort with regard to the mtDNA analysis (Fig. 1). One emerging difference was the occurrence of specific combinations of rare mtDNA variants in our cohort, for which the pathogenicity has been validated experimentally in cybrids and further certified by strict maternal inheritance.[2] We note that some of the variants listed remain in a grey area for stringent genotype–phenotype association and are also reported to occur in the general population. For example, m.3395A>G/*MT-ND1* has been associated with multiple phenotypes, including deafness, diabetes, cerebellar syndrome and LHON (www.mitomap.org). Similar considerations may apply to m.9957T>C/*MT-CO3*, associated with MELAS, progressive encephalopathy, hypertrophic cardiomyopathy and chronic progressive external ophthalmoplegia (www.mitomap.org). Complete mtDNA sequencing remains thus the gold standard for LHON cases that are negative for the three common mutations, particularly if maternal inheritance is obvious.

We reached a slightly higher efficiency in diagnosing nuclear genes (36.9% compared with 26.8% in the French cohort) (Fig. 1), which might be attributed partially to the recent use of WES as the default technique, followed by *in silico* panel screening. This flexible approach enlarges the number of genes screened, compared with the fixed number of 87 genes included in the custom targeted gene panel reported by Rocatcher *et al.*[1] Therefore, we found pathogenic variants in 30 genes, of which 18 were in single families (8.4%), whereas in the French cohort, only 21 genes were found to carry pathogenic variants, with seven singleton cases (1.5%). Remarkably, significant agreement

Table 1 Ranking of nuclear genes associated to HON in Italian, French and global cohorts

Nuclear genes									
	Italian cohort ($n = 215$)			France cohort ($n = 451$)			Total ($n = 666$)		
Gene	*n*	%	Ranking	*n*	%	Ranking	*n*	%	Ranking
OPA1	125	58.1%	1 (=)	166	36.8%	1 (=)	291	43.7%	1
WFS1	20	9.3%	2 (=)	131	29.0%	2 (=)	151	22.7%	2
ACO2	19	8.8%	3 (=)	46	10.2%	3 (=)	65	9.8%	3
SPG7	4	1.9%	6 (↓)	27	6.0%	4 (=)	31	4.7%	4
AFG3L2	8	3.7%	4 (↑)	14	3.1%	6 (↓)	22	3.3%	5
RTN4IP1	5	2.3%	5 (↑)	11	2.4%	7 (↓)	16	2.4%	6
MFN2	0	0.0%	–	15	3.3%	5 (↑)	15	2.3%	7
TMEM126A	1	0.5%	13 (↓)	10	2.2%	8 (=)	11	1.7%	8
NR2F1	0	0.0%	–	9	2.0%	9 (=)	9	1.4%	9
FDXR	1	0.5%	13 (↓)	5	1.1%	10 (=)	6	0.9%	10
SLC25A46	2	0.9%	10 (↑)	3	0.7%	11 (=)	5	0.8%	11
SSBP1	3	1.4%	8 (↑)	2	0.4%	13 (↓)	5	0.8%	11
DNM1L	1	0.5%	13 (=)	3	0.7%	11 (↑)	4	0.6%	13
SDHA	4	1.9%	6 (↑)	0	0.0%	–	4	0.6%	13
BTD	1	0.5%	13 (↑)	2	0.4%	13 (↑)	3	0.5%	15
DNAJC30	3	1.4%	8 (↑)	0	0.0%	–	3	0.5%	15
C19orf12	2	0.9%	10 (↑)	0	0.0%	–	2	0.3%	17
MECR	1	0.5%	13 (↑)	1	0.2%	15 (↑)	2	0.3%	17
OPA3	1	0.5%	13 (↑)	1	0.2%	15 (↑)	2	0.3%	17
UCHL1	2	0.9%	10 (↑)	0	0.0%	–	2	0.3%	17
C12ORF65	0	0.0%	–	1	0.2%	15 (↑)	1	0.2%	21
CACNA1F	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
CISD2	0	0.0%	–	1	0.2%	15 (↑)	1	0.2%	21
DNMT1	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
IBA57	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
LYST	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
MMP19	0	0.0%	–	1	0.2%	15 (↑)	1	0.2%	21
MTFMT	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
NDUFA10	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
NDUFAF2	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
NDUFAF4	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
NDUFB11	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
NDUFS2	0	0.0%	–	1	0.2%	15 (↑)	1	0.2%	21
NDUFV2	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
PDSS1	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
SLC52A2	1	0.5%	13 (↑)	0	0.0%	–	1	0.2%	21
TIMM8	0	0.0%	–	1	0.2%	15 (↑)	1	0.2%	21

The down arrow (↓) indicates the lower ranking in the national cohort as compared to the global cohort; the up arrow (↑) indicates the higher ranking in the national cohort as compared to the global cohort; the equal symbol (=) indicates the same ranking in the national cohort compared with the global cohort.

was, however, reached in terms of the top three most frequent genes, *OPA1*, *WFS1* and *ACO2* (76% in both cohorts). There was a higher frequency of *OPA1* in the Italian cases but a higher frequency of *WFS1* in the French cohort, whereas *ACO2* was similarly represented in both case series as the third most frequent gene.[3] The combination of *AFG3L2* and *SPG7*, which are assumed to follow a similar pathogenic mechanism, as the two proteins work in a dimeric complex, has also been found in a growing subset of DOA cases, reaching 5% and 9%, respectively, in the Italian and French cohorts.[4,5]

Another growing subgroup of HON cases seems to be represented by the constellation of genes directly or indirectly affecting complex I, which includes all nuclear-encoded NDUF complex I subunits, *DNAJC30*,[6] *TMEM126A*[7] and possibly *RTN4IP1*[8] genes, with the peculiarity that *DNAJC30* leads to a phenotype indistinguishable from maternally inherited LHON. We were somewhat surprised by the absence of the *SDHA* gene in the French panel of HON genes, as in our cohort, mutations in *SDHA* were relatively frequent, being found in four cases (about 2%) and thus entering in the top nine genes found in Italians.[9] Similarly, we have listed *SSBP1* in the top nine genes.[10]

On the downside of both studies, it must be considered that we have not distiguished paediatric onset forms of HON from those with onset in young-adulthood, possibly impacting the overall ranking of the top 10 genes. Moreover, a similar confounding effect may be derived from different clinical phenotypes being presented as a single entity, considering that even distinct pathogenic variants within the same gene (e.g. *OPA1*, *WFS1*) can cause both isolated and syndromic optic atrophies.

As mentioned earlier, when WES is performed as the default analysis, it may improve the diagnostic yield, especially with the use of human phenotype ontology (HPO) terms to better shape the filtering of genes and variants, ultimately contributing to diagnostic success. Overall, we think that switching to WES as the default method of analysis provides greater flexibility in the follow-up screening of negative cases and allows for the discovery

of new disease genes. Indeed, we and other groups are currently validating new HON genes that will further enrich the diagnostic landscape.

To conclude, by merging the results from the two cohorts of French and Italian probands, we provide the global top 10 genes in 3283 individuals, confirming *OPA1* ($n = 291$), *WFS1* ($n = 151$), *ACO2* ($n = 65$), *SPG7* ($n = 31$), *AFG3L2* ($n = 22$), *RTN4IP1* ($n = 16$), *MFN2* ($n = 15$), *TMEM126A* ($n = 11$), *NR2F1* ($n = 9$) and *FDXR* ($n = 6$), as well as a consistent similar diagnostic profile for mtDNA-related LHON (Fig. 1 and Table 1). Thus, the top 10 genes are consolidated by the contribution of the Italian cohort, with *OPA1*, *WFS1* and *ACO2* accounting for 76% of diagnosed HON, due to a nuclear gene defect. In addition, our cohort contributes to the better classification of the top 20 genes, with *SSBP1* ($n = 5$), *SLC25A46* ($n = 5$), *SDHA* ($n = 4$), *DNM1L* ($n = 4$), *DNAJC30* ($n = 3$) and *BTD* ($n = 3$) being represented by at least three cases. An enriched list of lower frequency genes is also provided according to the global cohort analysis (Table 1), consolidating a priority list for HON genetic diagnosis. The contribution of data from other large case series, especially from ethnically different populations, will further enhance our knowledge regarding the genetic causes of HON and their population-specific characteristics.

Data availability

Raw data were generated at IRCCS Istituto delle Scienze Neurologiche di Bologna, Italy. Derived data supporting the findings of this study are available from the corresponding author on request.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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