Spectrum of Disease Severity in Nonsyndromic Patients With Mutations in the *CEP290* Gene: A Multicentric Longitudinal Study

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Purpose. The purpose of this study was to perform a detailed longitudinal phenotyping and genetic characterization of 32 Italian patients with a nonsyndromic retinal dystrophy and mutations in the *CEP290* gene.

Methods. We reviewed the clinical history and examinations of 32 patients with a nonsyndromic retinal dystrophy due to mutations in the *CEP290* gene, followed up (mean follow-up: 5.9 years) at 3 Italian centers. The clinical examinations included: best corrected visual acuity (BCVA), optical coherence tomography (OCT), and full-field electroretinogram (ERG).

Results. Patients (mean age = 19.0 ± 3.4 years) had a mean BCVA of 1.73 ± 0.20 logMAR. Longitudinal analysis of BCVA showed a nonsignificant decline. Central retinal thickness (CRT) declined significantly with age at an exponential rate of 1.0%/year ($P = 0.001$). At disease onset, most patients (19/32; 49.4%) had nystagmus. The absence of nystagmus was significantly associated with better BCVA and more preserved CRT ($P < 0.05$). ERG showed undetectable responses in most patients (64.0%), whereas reduced scotopic and photopic responses were observed in four patients (16.0%) who had no nystagmus. We identified 35 different variants, among which 12 were novel. Our genotype-phenotype correlation analysis shows a significantly worse BCVA in patients harboring a loss-of-function mutation and the deep-intronic variant c.2991+1655A>G.

Conclusions. Our study highlights a mild phenotype of the disease, characterized by absence of nystagmus, good visual acuity, considerably preserved retinal morphology, and recordable ERG, confirming the wide spectrum of *CEP290*-related retinal dystrophies. Finally, in our cohort, the deep intronic variant c.2991+1655A>G was associated with a more severe phenotype.

Keywords: *CEP290* gene, early onset severe retinal dystrophy, Leber congenital amaurosis, retinitis pigmentosa

The *CEP290* gene, localized to chromosome 12q21.32, encodes a 290 kDa centrosomal protein called nephrocystin-6 (NPHP6), which is important for ciliary assembly and function.[1,2] Due to the diverse functions of cilia in humans,[3] biallelic mutations in *CEP290* (OMIM entry: 610142) lead to a phenotypic spectrum that ranges from isolated retinopathies,[4] such as Leber congenital amaurosis (LCA), to systemic disorders (e.g. Joubert syndrome, Senior-Loken syndrome, Bardet-Biedl syndrome, and Meckel syndrome).[5,6]

LCA is the most severe form among inherited retinal dystrophies, affecting children from birth and resulting in

severe visual impairment or blindness in early adolescence.[7] LCA has an estimated prevalence of 1 in 50,000 in Europe and North America.[8,9] In almost 80% of cases, the causative mutation can be found in one of the 23 genes currently known to be associated with this condition (http://sph.uth.edu/retnet).[7,10]

CEP290 is the most frequently mutated gene accounting for nearly 30% of LCA cases.[11–13] Causative mutations in *CEP290* have also been reported in patients with early onset severe retinal dystrophy (EOSRD) who showed a milder retinal phenotype with detectable photopic and scotopic electroretinogram (ERG) responses.[10] Robust genotype-phenotype correlations have proven difficult to establish due to the pleiotropic nature of *CEP290*-associated diseases and the variability of clinical presentations associated with the same variants.[5] For example, affected subjects sharing the same genotype, even siblings, display different disease phenotypes, both concerning the severity of the retinal disease and the presence of extraretinal features.[14] To further add to this complexity, the impact of specific *CEP290* mutations on the protein level and function cannot be accurately predicted as the *CEP290* transcript undergoes extensive nonsense-associated altered splicing and basal exon skipping.[15–17]

Approved treatments for *CEP290*-associated retinal disease are not currently available. However, a gene therapy approach based on the administration of an antisense oligonucleotide is being tested in clinical trials for patients carrying the deep-intronic c.2991+1655A>G variant.[18] This deep-intronic variant is the most recurrent *CEP290* mutation identified in more than half of LCA cases.[5,14,18,19] Additional therapeutic approaches based on lentiviral vector gene replacement and CRISPR/Cas9 are also being explored as a treatment for patients with mutations in the *CEP290* gene.[20,21] Understanding the natural disease course is crucial for the development and evaluation of experimental therapies as it allows to critically assess patients' eligibility and determine the optimal timeframe for therapeutic interventions. To date, only a few studies described the natural disease course in patients with mutations in the *CEP290* gene, either reporting longitudinal recordings in small cohorts of patients,[6,22,23] or providing only visual acuity data,[24] or focusing only on subjects with the deep-intronic c.2991+1655A>G variant.[21,25] Furthermore, a recent study[26] investigated the genotype - phenotype correlation in a large German cohort, reporting some findings which are in contrast with the results obtained in cohorts from other countries, thus suggesting that national studies are important to better characterize the *CEP290*-related phenotypes.

Methods

Patients

We identified a total of 32 patients of Italian origin with a clinical diagnosis of LCA, EOSRD, or retinitis pigmentosa (RP) harboring two candidate pathogenic variants in the *CEP290* gene. Patients with neurologic or other systemic disorders were not included in this study. The patients included in the study were ascertained from three expertise centers for the care of patients with retinal dystrophy (i.e. 12 from the Referral Centre for Inherited Retinal Diseases of the University of Campania "Luigi Vanvitelli"; 9 from the Centre of Child Neuro-ophthalmology of the IRCCS C. Mondino Foundation; and 11 from the Department of Ophthalmology at the University of Florence). Medical records of all patients from 2008 to 2020 were reviewed. Data collection included date of birth, sex, self-reported age of onset of ocular symptoms (determined by the age at which patients or their parents first noticed nystagmus or visual abnormalities), age at diagnosis, presence of nystagmus, medical and ophthalmic history, extraocular symptoms, consanguinity, genetic testing results, and pedigree information.

As previously done,[27] clinical diagnosis was performed relying on the diagnostic criteria of LCA/EOSRD, according to Chacon-Camacho and Zenteno[28] and the diagnostic criteria of RP, according to Hamel.[29] Moreover, the medical records were reviewed to extract the findings of the following ophthalmological examinations: best corrected visual acuity (BCVA) measurements, slit lamp anterior segment examination, fundus imaging and examination, spectral domain optical coherence tomography (SD-OCT), and ERG.

BCVA measurements were performed using either a Snellen projection chart or Teller Acuity Cards.

Full-field ERG was recorded using corneal contact lens electrodes with a Ganzfeld stimulator (LACE Elettronica Electrophysiology System, Pisa, Italy) according to the standards of the International Society for Clinical Electrophysiology of Vision.[30]

SD-OCT data were collected from scans acquired using the Cirrus HD-OCT (Carl Zeiss, Dublin, CA, USA). In particular, SD-OCT images with a satisfactory signal quality (i.e. strength higher than 9)[31] were evaluated to determine the length of ellipsoid band (EZ) and to measure the central retinal thickness (CRT).

Patient data were reviewed and anonymized by the referring physician. All procedures of this retrospective observational study adhered to the tenets of the Declaration of Helsinki and were approved by the ethics board of each center.

Genetic Analysis

Total genomic DNA was extracted from peripheral blood using standard protocols or the DNeasy Blood and Tissue Kit (QIAGEN). Older analyses were performed by an Arrayed Primer Extension (APEX)-based genotyping microarray (www.asperbio.com; Asper Biotech, Ltd.) on different chip versions that comprised known mutations associated with LCA and EOSRD (LCA chip versions 2004–2009[32]). Whenever single heterozygous variants were identified in *CEP290*, Sanger sequencing of all exons was performed. More recently, samples were screened by targeted next generation sequencing (NGS) using panels of known retinopathy related genes with increasing complexity (in terms of number of targeted genes; ranging from 14 to 285 genes) that also comprise the deep intronic variant c.2991+1655A>G.[18] Finally, samples underwent either clinical exome sequencing (ClearSeq Inherited Disease Panel or Constitutional Panel enriched with the SureSelectQXT Target Enrichment system; Agilent Technologies) or whole-exome sequencing. The samples analyzed by clinical exome or whole-exome sequencing were also screened for the presence of the deep intronic variant c.2991+1655A>G by Sanger sequencing. When filtering the variants identified by NGS-based methods, only variants with a minor allele frequency (MAF) < 0.05 in the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org) were considered. Selected variants were validated by Sanger sequencing. Parents' samples for segregation analysis were

Table 1. Clinical Findings in the Patients With Mutations in the *CEP290* Gene

Parameters	Study Cohort ($n = 32$)*	
Age (y)	19.0 ± 3.4	
Age at diagnosis (y)	4.2 ± 1.6	
Hyperopia ($n = 23$)	12 (52.1%)	
Myopia ($n = 23$)	7 (30.4%)	
Parameters	**Right Eye**	**Left Eye**
BCVA (logMAR)	1.73 ± 0.20	1.65 ± 0.20
CRT (μm) ($n = 12$)	199.7 ± 16.7	203.3 ± 17.4
EZ-band width (μm) ($n = 10$)	1915.4 ± 569.5	1974.3 ± 557.7
ERG Findings	**Subgroup ($n = 25$)**	
Undetectable scotopic and photopic ERG	16 (64.0%)	
Undetectable scotopic with detectable photopic ERG	5 (20.6)	
Detectable scotopic and photopic ERG	4 (16.0%)	

* Data are expressed as mean ± standard error of mean or counts (frequency).

available for 21 patients. In all analyzed cases (patient IDs: 2, 3, 5–7, 12, 13, 17, 20, 22, 23, and 25–34), segregation confirmed that the identified variants were present in trans in the proband. Variant positions are reported with reference to the NM_025114 transcript.

Statistical Analysis

Continuous variables are reported as mean ± standard error of the mean (SEM) and categorical variables are reported as counts (frequency).

The natural history of disease was analyzed using previously applied methods.[33,34] Briefly, linear regression, estimated by a generalized estimating equation (GEE), was fitted on the data of the baseline visit to estimate the mean rate of change per year of age for each outcome measure. Moreover, GEE was fitted to estimate the decline over the follow-up period (i.e. using baseline values as offset). Asymmetry in BCVA between the 2 eyes was defined as a difference of 0.3 logMAR, which is the threshold for clinically significant changes.[35] GEEs were applied because this method could accommodate the inter-eye correlation and the longitudinal correlation by adopting an appropriate covariance structure, as previously described.[36] BCVAs were converted to logMAR using the values of 2.7 for hand motion, 2.8 for light perception, and 2.9 for no light perception. All the other measures were also log-transformed to estimate the exponential rate of progression.

Differences in age at study entrance and age at diagnosis of the patients stratified according to their genotype were evaluated with analysis of variance with Bonferroni correction for multiple comparisons and *t*-tests. Fisher exact tests and Pearson χ^2 tests were performed to explore differences for dichotomous and categorical variables, respectively.

P values lower than 0.05 were considered statistically significant.

Results

Clinical Characterization of the Cohort of Patients With *CEP290*

A total of 32 patients (mean age = 19.0 ± 3.4 years) from 29 families with a clinical diagnosis of LCA/EOSRD (20/32; 62.5%) or RP (12/32; 37.5%) harboring disease-causing mutations in the *CEP290* gene were recruited for this study.

The main clinical findings of the cohort are summarized in Table 1. The self-reported age at onset of the first symptom was 4.2 ± 1.6 years (median = 0.4 years). At disease onset, more than half of the patients presented nystagmus (12/32; 37.5%) or roving eye movements (7/32; 21.9%), whereas six patients (18.8%) showed a decreased visual acuity. Night blindness (2/32; 6.3%) and visual field constriction (3/32; 9.4%) were less frequently reported at disease onset.

Fundus examination showed 18 patients (56.3%) with RPE dystrophy (salt-and-pepper retinal dystrophy; i.e. fine pigment clumping and dispersion), and 9 patients (28.1%) with a typical RP fundus. RP with macular involvement (2/32; 6.4%) and pericentral RP (1/32; 3.1%) were less frequently observed. Finally, a normal fundus appearance was found in two patients (6.3%). We observed that patients with a typical RP fundus (aged 35.2 ± 5.7 years) were significantly older ($P < 0.001$) than patients with RPE dystrophy (aged 7.5 ± 2.4 years). We did not detect significant changes in fundus oculi over the follow-up period (mean = 5.9 years; 24 patients), particularly in patients with RPE dystrophy (mean follow-up = 5.8 years; 13 patients). Moreover, we observed a significantly delayed disease onset in patients with an RP fundus compared to those with RPE dystrophy (9.5 ± 3.8 years vs. 1.1 ± 2.7 years; $P = 0.009$).

Refraction data were available for 23 patients and the most recently measured mean spherical equivalent, averaged between eyes, was 1.6 ± 1.0 D (range = −7.75 D to +10 D). More than half (12/23; 52.1%) were hyperopic, with seven patients (30.4%) having high hyperopia (i.e. >5 D). Seven patients (30.4%) were myopic, one of whom (4.3%) with high myopia (i.e. <−6 D). The remaining four patients (17.4%) did not have significant refractive errors.

Most patients (26/32; 81.3%) had clear lens in both eyes. Only four patients (12.5%) developed subcapsular posterior cataract in both eyes, one (3.1%) was pseudophakic, and one (3.1%) had a nuclear opacity in one eye and a subcapsular posterior cataract in the other.

In the study cohort, mean BCVA was 1.73 ± 0.20 logMAR in the right eyes and 1.65 ± 0.20 logMAR in the left eyes. The analysis of inter-eye asymmetry values revealed a very low asymmetry in terms of BCVA (mean = 0.04 ± 0.02; median = 0.00 logMAR), as shown in Figure 1a. Measurements of BCVA in the best-seeing eye revealed no statistically ($P = 0.400$) significant association with age (Fig. 1b). Furthermore, a significantly worse BCVA correlated with

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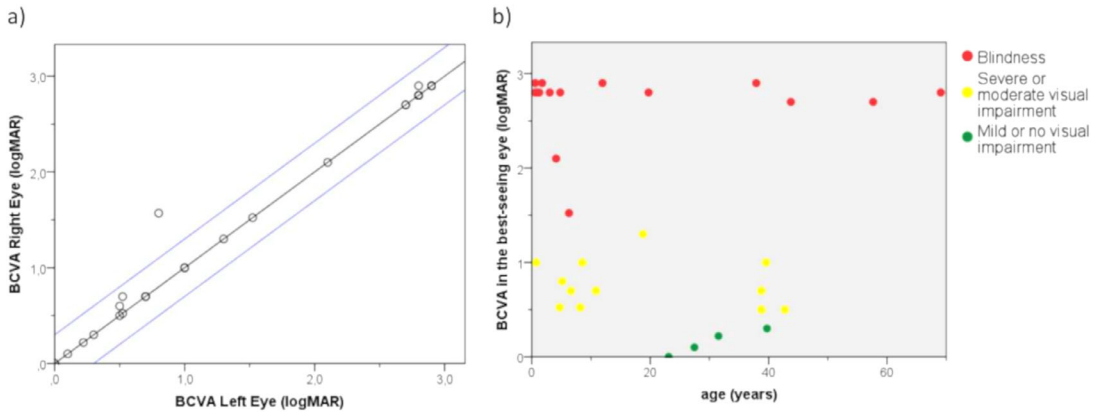

Figure 1. Comparison of BCVA measurement between both eyes measured at the baseline visits (**a**). The blue lines represent the limit of the test–retest variability of 3 lines (0.3 logMAR), which corresponds to 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters. BCVA measurements of the best-seeing eye in dependence of age (**b**). The values are colored according to the World Health Organization (WHO) criteria for blindness (BCVA < 20/400; *red*), severe or moderate visual impairment (20/60 < BCVA ≤ 20/400; *yellow*), mild or no visual impairment (BCVA ≥ 20/60; *green*).

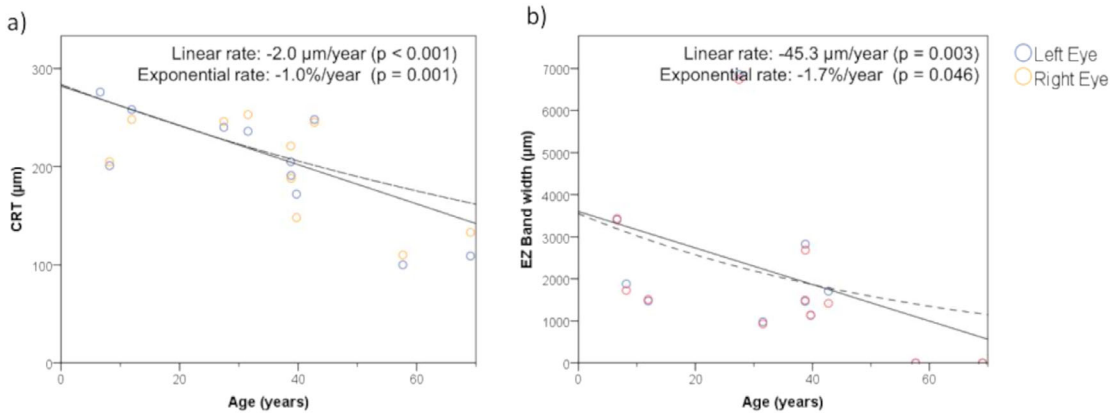

Figure 2. OCT measurements in dependence of age: CRT (**a**) and EZ band width (**b**). Linear and exponential models of progression with age are represented by *continuous line* and *dotted line*, respectively.

the presence of nystagmus or roving eye movements (β = 1.28 logMAR; SE = 0.39; P = 0.001).

Longitudinal analysis of BCVA data collected in 24 patients over a mean follow-up period of 5.9 years (SEM = 0.9; range = 0.4 to 13.1 years; median = 5.2 years; average number of visits = 3.7) showed a nonsignificant decline of 0.004 logMAR/year (SE = 0.004; P = 0.375). Bilateral BCVA progression was observed only in four patients (16.7%), whereas one patient (4.2%) showed a worsening of BCVA in one eye, which developed subcapsular posterior cataract.

Measurements of CRT by SD-OCT imaging (available in 12 patients) showed a mean CRT of 199.7 ± 16.7 µm in the right eyes and 203.3 ± 17.4 µm in the left eyes. We observed a significant decrease of CRT with age at a linear rate of -2.0 µm/year (95% confidence interval [CI] = −3.2 to −1.2 µm/year; P < 0.001) and at an exponential rate of −1.0%/year (95% CI = −1.6 to −0.4 %/year; P = 0.001), as shown in Figure 2a. SD-OCT scans of acceptable quality were also available for six patients with nystagmus (out of 19; 31.6%). By applying age-adjusted models, we found that nystagmus was associated with a significant CRT decrease (β = −55.2 µm; SE = 24.8; P = 0.026). We did not observe significant differences in CRT between patients with RP and patients with RPE dystrophy (P = 0.161).

The EZ band was detectable in 10 patients. The mean width of the EZ band was 1915.4 ± 569.5 µm in the right eyes and 1974.3 ± 557.7 µm in the left eyes. The EZ band width significantly decreased with age at a linear rate of −45.3 µm/year (95% CI = −75.7 to −14.9 µm/year; P = 0.003) and at an exponential rate of −1.7%/year (95% CI = −3.4 to 0%/year; P = 0.046), as shown in Figure 2b. Moreover, patients with nystagmus showed a significantly reduced EZ band width (β = −2610.8 µm; SE = 1158.4; P = 0.024).

ERG examinations, performed in 25 patients, showed undetectable scotopic and photopic responses in 16 patients (64.0%), undetectable scotopic with markedly reduced photopic in 5 patients (20.0%), and reduced scotopic and photopic responses in 4 patients (16.0%). Patients with undetectable photopic and scotopic ERG had a significantly worse BCVA compared to those with reduced scotopic and photopic responses (β = +1.80 logMAR; SE = 0.27; P < 0.001). Moreover, patients with undetectable photopic and scotopic ERG showed a significantly smaller EZ band width both compared to those with undetectable scotopic and markedly reduced photopic (P < 0.001) or

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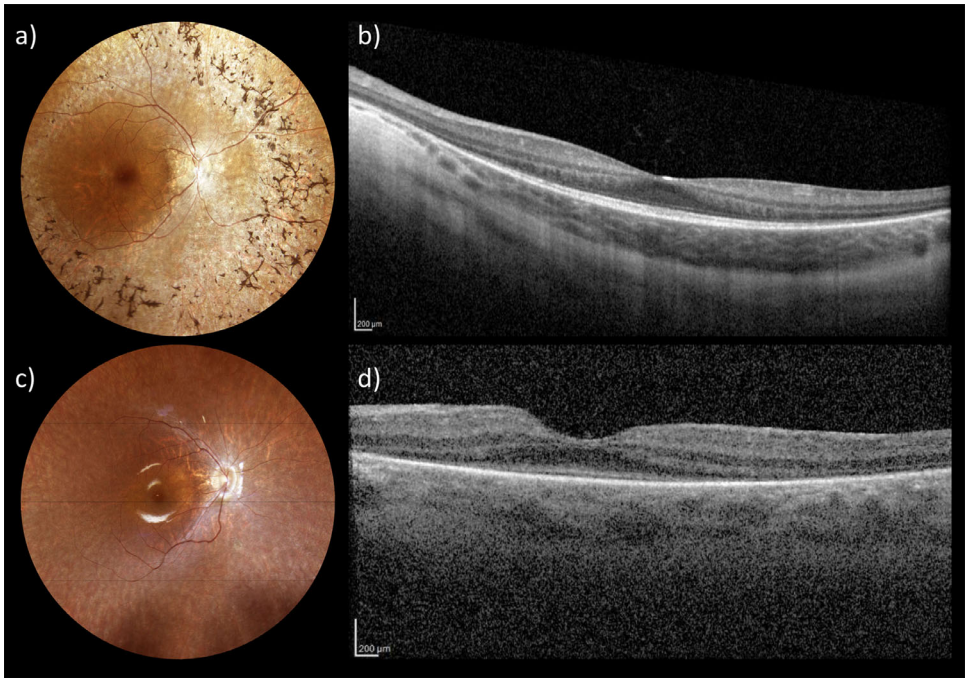

Figure 3. Example of a patient with reduced scotopic and photopic ERG, associated with diagnosis of RP, no nystagmus, pigment deposits visible at the color fundus image (**a**), a more preserved EZ band detectable by SD-OCT (**b**); and of a patient with undetectable photopic and scotopic ERG, associated with a diagnosis of LCA, nystagmus, RPE dystrophy shown by the color fundus image (**c**), associated with a less preserved EZ band as evaluable by SD-OCT (**d**).

to patients with reduced scotopic and photopic responses ($P < 0.001$). Undetectable ERG responses were associated with nystagmus/roving eye movements. In particular, almost all patients (14/16; 87.5%) with undetectable photopic and scotopic ERG presented nystagmus or roving eye movements, whereas all patients with detectable photopic and scotopic ERG responses (4/4; 100%) had no nystagmus or roving eye movements. Moreover, an undetectable photopic and scotopic ERG was observed in almost all patients with RPE dystrophy (12/15; 80%) and in only four of the seven patients with RP (57.1%). Figure 3 shows the comparison of fundus imaging and SD-OCT between a patient with detectable photopic and scotopic ERG responses and one with undetectable ERG.

Molecular Characterization of the *CEP290* Cohort

The 32 patients of Italian origin included in this study were from unrelated families, with the exception of 2 pairs of siblings (IDs 6 and 7, and 14 and 15) and an aunt-nephew pair (IDs 22 and 23; Table 2). In the study cohort, we identified 35 different variants in *CEP290*. They comprised 15 frameshifts, 10 nonsense mutations, 5 splice site variants, 6 missense variants, and one in-frame deletion of a single amino acid. Twelve of the identified variants were novel (see Table 2) and included four missense variants whose pathogenicity was hypothesized based on in silico predictions and allele frequency criteria. Interestingly, almost all patients (97%) harbored at least one truncating mutation, whereas only one patient (ID 8) carried two missense variants (see Table 2). In particular, 25 patients (78%) were found to harbor 2 truncating variants, whereas 5 patients (IDs 2–4, 6, and 7; 16%) were compound heterozygous for a truncating and a missense variant. The most frequent mutation was the deep intronic variant c.2991+1655A>G,[18] detected in 14 patients (44%). The second most recurrent variation, found in five patients (16%), was a single base pair deletion c.(1666del) causing a frameshift p.(Ile556Phefs*17) predicted to introduce a premature stop codon.

Genotype - Phenotype Correlation Analysis

To assess whether patients' genotypes associated with disease severity, we stratified the cohort according to the number of *CEP290* loss-of-function (LoF) alleles identified in each subject. To this aim, we adopted a conservative approach and considered as bona fide LoF mutations only truncating mutations (namely, nonsense variants and frameshifts) predicted to produce no full-length product. We did not consider splice-site variants as bona fide LoF mutations because the impact of splice defects on the transcript and protein product cannot be reliably predicted in silico. Due to its relevance (i.e. high frequency and therapeutic opportunities), we also sought to assess the impact of the deep-intronic variant c.2991+1655A>G. This variant generates a strong splice donor site, leading to aberrant splicing, with the subsequent insertion of a cryptic exon and a premature termination codon at amino acid position 998 p.(Cys998*).[18] A small amount of properly spliced mRNA is still produced in patients homozygous for c.2991+1655A>G, suggesting a hypomorphic *CEP290* mutation.[18] Based on this evidence, we classified the intronic variant c.2991+1655A>G as a separate category from the bona fide LoF mutations.

We divided our cohort into three genotypic categories. Group A comprised nine patients that harbored an LoF variant together with a presumed nontruncating change

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Table 2. *CEP290* Variants Identified in the Study Cohort

		Nucleotide Change		Protein Change		Reference	
Family	**Patient ID**	**Variant 1**	**Variant 2**	**Variant 1**	**Variant 2**	**Var. 1**	**Var. 2**
F1	8	c.1298A>G	c.2252G>A	p.(Asp433Gly)	p.(Arg751Gln)	37	†
F2	6, 7	c.1466T>C	c.1666del	p.(Leu489Pro)	p.(Ile556Phefs*17)	†	38
F3	2	c.1664A>T	c.7394_7395del	p.(Lys555Ile)	p.(Glu2465Valfs*2)	†	†
F4	3	c.6401T>C	c.1666del	p.(Ile2134Thr)	p.(Ile556Phefs*17)	37	38
F5	1	c.180+1G>T	c.7031_7034del	p.(?)	p.(Leu2344Hisfs*2)	18	†
F6	4	c.2723G>A	c.5493del	p.(Arg908Gln)	p.(Ala1832Profs*19)	†	38
F7	5	c.2991+1655A>G	c.1666del	p.Cys998*	p.(Ile556Phefs*17)	18	38
F8	11	c.2991+1655A>G	c.1666del	p.Cys998*	p.(Ile556Phefs*17)	18	38
F9	26	c.2991+1655A>G	c.5041_5046delAT	p.Cys998*	p.(Val1680fs*1683)	18	†
F10	29	c.2991+1655A>G	c.1860_1863delAAGA	p.Cys998*	p.(Arg621Ilefs*2)	18	14
F11	30	c.2991+1655A>G	c.3292G>T	p.Cys998*	p.(Glu1098*)	18	14
F12	33	c.2991+1655A>G	c.180+1G>A	p.Cys998*	p.(?)	18	†
F13	21	c.2991+1655A>G	c.2991+1655A>G	p.Cys998*	p.Cys998*	18	18
F14	24	c.2991+1655A>G	c.5813_5817del	p.Cys998*	p.(Thr1938Asnfs*15)	18	18
F15	27	c.2991+1655A>G	c.1219_1220del	p.Cys998*	p.(Met407Glufs*13)	18	14
F16	28	c.2991+1655A>G	c.566C>G	p.Cys998*	p.(Ser189*)	18	39
F17	17	c.2991+1665A>G	c.1593C>A	p.Cys998*	p.(Tyr531*)	18	14
F18	22, 23	c.2991+1665A>G	c.2991+1665A>G	p.Cys998*	p.Cys998*	18	18
F19	25	c.2991+1665A>G	c.934G>T	p.Cys998*	p.(Glu312*)	18	†
F20	13	c.3012delA	c.4732G>T	p.(Lys1004fs)	p.(Glu1578*)	27	40
F21	32	c.4705-1G>T	c.3811C>T	p.(?)	p.(Arg1271*)	41	38
F22	18	c.4962_4963del	c.6604del	p.(Glu1656Asnfs*3)	p.(Ile2202Leufs*24)	14	14
F23	12	c.5709+2T>G	c.384_385del	p.(?)	p.(Asp128fs)	†	13
F24	9	c.5813_5817del	c.6916A>T	p.(Thr1938Asnfs*15)	p.(Arg2306*)	18	27
F25	10	c.6604del	c.6836T>A	p.(Ile2202Leufs*24)	p.(Leu2279*)	14	†
F26	14, 15	c.6916A>T	c.1987A>T	p.(Arg2306*)	p.(Lys663*)	27	42
F27	31	c.7341_7344dupACTT	c.1189+1G>A	p.(Ser2449Thrfs*7)	p.(?)	†	42
F28	34	c.1187_1188del	c.7341_7344dupACTT	p.(Lys396Argfs*25)	p.(Ser2449Thrfs*7)	27	†
F29	20	c.1219_1220del	c.4661_4663del	p.(Met407Glufs*13)	p.(Glu1554del)	14	14

† Variant reported for the first time in this study.

Table 3. Clinical Features of Patients Stratified According to the *CEP290* Variants

Parameters	**Group A* (*n* = 9)**	**Group B* (*n* = 6)**	**Group C* (*n* = 10)**	***P* Value**
Age (y)	25.7 ± 6.2	16.7± 7.3	17.7 ± 7.5	0.629
Age at diagnosis (y)	14.3 ± 7.1	7.4 ± 3.2	2.1 ± 0.9	0.083
Undetect. scotopic and photopic ERG†	2/6 (33.3%)	3/ 4 (75.0%)	7/8 (87.5%)	
Undetect. scotopic with detect. photopic ERG†	2/6 (33.3%)	1/4 (25.0%)	1/8 (12.5%)	0.181
Detect. scotopic and photopic ERG†	2/6 (33.3%)	0/4 (0%)	0/8 (0%)	

Parameters	**Right Eye**	**Left Eye**	**Right Eye**	**Left Eye**	**Right Eye**	**Left Eye**	**A vs. B**	**A vs. C**	**B vs. C**
BCVA (logMAR)	1.3 ± 0.3	1.1 ± 0.3	1.4 ± 0.5	1.4 ± 0.5	2.6 ± 0.2	2.6 ± 0.2	0.730	<0.001	0.012
CRT (µm)	202 ± 46	195 ± 48	206 ± 21	209 ± 18	161 ± 28	150 ± 41	0.837	0.337	0.076
EZ-band width (µm)	783 ± 416	894 ± 429	1466 ± 120	1487 ± 152	1341	1411	0.101	0.606	0.918

* Group A: an LoF and a nontruncating variant; group B: two LoF variants; Group C: an LoF and the deep intronic variant c.2991+1655A>G.
† Undetect.: undetectable; detect.: detectable.

(i.e. missense, in-frame changes, and splice-site variants). Group B consisted of six patients that carried biallelic LoF variants. Group C comprised 10 compound heterozygous patients for an LoF mutation and the deep-intronic variant c.2991+1655A>G. Groups of patients who were either homozygous for the variant c.2991+1655A>G (n = 2) or compound heterozygotes for missense variants (n = 1) were not analyzed further because of the low number of available cases. We then compared the clinical parameters of the patients in the three groups. Patients' age, age at diagnosis, and OCT measurements were not statistically different (Table 3). In terms of visual function, patients harboring an LoF mutation and the deep-intronic variant c.2991+1655A>G (group C) had a significantly worse BCVA

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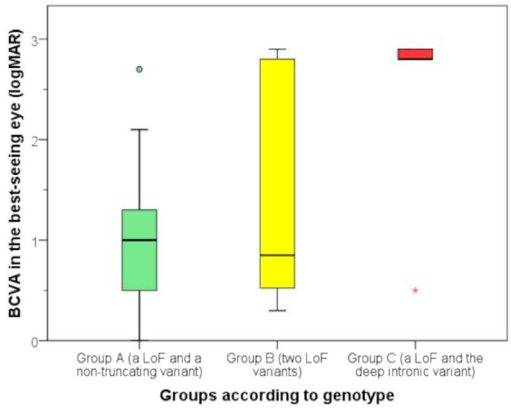

Figure 4. Comparison of BCVA measurements (best-seeing eye) in patients stratified according to the genotype. Patients harboring an LoF and the deep intronic variant c.2991+1655A>G (group C) had a significantly worse BCVA compared to the other groups.

compared to patients from groups A ($P < 0.001$) and B ($P = 0.012$; Fig. 4). Finally, detectable scotopic and photopic ERG responses were only present in group A (see Table 3).

Inter-Familiar and Intrafamiliar Phenotypic Variability

We observed a large inter-familiar variability in the two families (F13, F18; patients' IDs 21–23) that were homozygous for the deep-intronic variant c.2991+1655A>G. One patient (ID 21) had a BCVA of 0.5 logMAR up to the last assessment (at 16 years of age) whereas the other case (ID 23) had a worse BCVA (1.5 logMAR) at baseline (6 years of age), which increased to 2.1 logMAR at the last assessment (18 years of age). The affected aunt of patient 23 (ID 22) also showed a severe phenotype. However, she was enrolled in the current study at 44 years of age and previous medical records were not available. The clinical presentation of the other two unrelated cases (IDs 5 and 11) that were both compound heterozygous for c.2991+1655A>G and c.1666del could not be compared because of the difference in their age at the study baseline (39 and 79 years, respectively).

On the other hand, the comparison between the two pairs of siblings who had the same genotype and comparable ages did not reveal significant intra-familiar variability. For example, patients 14 and 15 who were compound heterozygous for 2 nonsense mutations (i.e. p.[Arg2306*], p.[Lys663*]) showed a similar EOSRD phenotype with RPE dystrophy, moderately impaired BCVA (logMAR 0.5 – 0.7), and undetectable photopic and scotopic ERG in the first decade of life. Likewise, patients 6 and 7, who were compound heterozygous for a missense p.(Leu489Pro) and a frameshift variant p.(Ile556Phefs*17), shared a similar phenotype of RP with typical fundus appearance, preserved BCVA (0.1–0.2 logMAR), and detectable photopic and scotopic ERG at least until the third decade of life.

Discussion

To define natural retinal disease history in nonsyndromic patients with biallelic mutations in the *CEP290* gene, we clinically characterized a large cohort of Italian patients with a multiyear follow-up. Although other studies reported exclusively on LCA cases associated with *CEP290* variants,[6,22,25] we describe a wider range of cases with inherited retinal dystrophy, including also EOSRD and RP.

The most frequent clinical diagnosis in our *CEP290* cohort was LCA/EOSRD (20/32; 62.5%). We also encountered a significant proportion of RP cases (12/32; 37.5%), more than double compared to a recently described German cohort.[26] Differently from other reports, we did not diagnose any case of cone-rod dystrophy.[25,26,43] The most common sign at disease onset was nystagmus or roving eye movements and the most frequent refractive error was hyperopia. The prevalence of these clinical features, typically associated with LCA,[44–46] was consistent with the high proportion of patients with LCA in our cohort.

In terms of visual performance, 12% of patients had no light perception in both eyes and 42% had severely reduced BCVA (i.e. hand motion or worse in the better-seeing eye), similarly to the cohort reported by Feldhaus et al.[26] The study by Sheck et al.[22] had reported severely reduced BCVA in a larger proportion of *CEP290* patients (62%). Longitudinal analysis showed limited changes in BCVA over the follow-up period, as previously reported,[22,26] with only five patients (20.8%) presenting a worsening of BCVA. Interocular asymmetry of the BCVA was also low.

Although severe nystagmus and patient compliance limited the quality of OCT imaging, we obtained acceptable quality SD-OCT scans for 12 patients, 6 of whom had nystagmus. SD-OCT imaging showed a slightly reduced CRT, in agreement with previous reports.[22,25,26] Furthermore, an EZ band with a mean width of approximately 2.000 µm was detected in a high proportion of analyzed patients (83.3%). These findings revealed a better preserved EZ band compared to the cohort described by Feldhaus et al.[26] with a mean width of about 1.000 µm. Furthermore, our data indicated a significant progression of the SD-OCT parameters with age. In particular, the EZ band width declined at a mean rate of about −2%/year, which is similar to the longitudinal estimates previously reported in RP cohorts (ranging from −4%/year to −9%/year).[47–49]

ERG examinations (available for 78% of patients) showed undetectable scotopic and photopic ERG responses in most cases (64.0%). Nevertheless, we observed detectable ERG with a rod-cone pattern in about 36% of cases, a percentage which is higher compared to other European cohorts that reported a range from 4% to 17.6%.[22,26] Moreover, by exploring the relationship with the other clinical parameters, we observed a significant association of preserved ERG response with absence of nystagmus, better BCVA, larger EZ band width, and RP fundus.

The vast majority of the variants in our cohort were presumably truncating, predicted to introduce premature termination codons. Missense variants represented 10.3% (6/58) of the alleles. This percentage is similar to that reported by Sheck et al. (12.5%), whereas in other *CEP290* cohorts missense variants were either not present[6,18,50] or very rare.[25,26] This is also in line with the small number of missense *CEP290* mutations reported in variant databases (Coppierters et al.[5] 4 missense out of 189 pathogenic variants deposited in ClinVar; http://clinvarminer.genetics.utah.edu/; accessed October 2020). The deep-intronic variant c.2991+1655A>G was the most recurrent mutation, albeit with a lower frequency (44%) compared to recently reported European cohorts (55% - 87.5%)[22,26] and a Brazilian cohort (50%).[51]

Considering the ongoing gene therapy clinical trials for patients carrying the c.2991+1655A>G variant,[52] we sought to better define the clinical phenotype associated with this mutation. The composition of our cohort limits the strength of any comparisons between subjects that were homozygotes ($n = 3$) or compound heterozygotes ($n = 10$) for the c.2991+1655A>G variant. Such comparisons were recently reported by Feldhaus et al. and Valkenburg et al. with discordant conclusions,[25,26] In particular, whereas Valkenburg et al.[25] found a mild phenotype in patients homozygous for the c.2991+1655A>G variant, consistently with the in vitro studies showing a residual CEP290 protein in either lymphoblastoid or fibroblast cells derived from patients with LCA homozygous for the deep-intronic mutation,[18,53,54] Feldhaus et al.[26] observed a more severe phenotype in homozygous patients. In our cohort, we observed phenotypic variability in the 3 patients homozygous for the deep-intronic variant c.2991+1655A>G (belonging to 2 unrelated families). Despite sharing the same *CEP290* genotype and the same age range (16–18 years), the 2 unrelated patients presented significant differences in BCVA. It remains to decipher the influence of modifier alleles[13,17] and environmental factors to the pleiotropy of *CEP290*-associated disease.

We found that compound heterozygous patients harboring the deep-intronic c.2991+1655A>G variant in trans with an LoF mutation had a worse BCVA compared to patients that either carried two bona fide LoF variants or were compound heterozygotes for an LoF mutation and a mild, nontruncating variant. Nevertheless, the limited size of the analyzed cohort and the heterogeneity of the genotypes grouped within each category demands further comparisons to strengthen the herein described association. Moreover, our classification is based on a conventional prediction of the effect of DNA mutations on protein function (i.e. variants resulting in a premature stop codon were classified as LoF mutations), although basal exon skipping and nonsense-associated altered splicing have been also evoked to account for the pleiotropy of *CEP290* disease.[16] Finally, evidence to support the hypomorphic character of truncating mutations is only available for a very small proportion of variants under investigation (hence could not be systematically considered for the entire cohort).[15–17,55]

This study presents some limitations mainly related to its retrospective design. First, the differences in follow-up time and visit frequency. Then, the fact that some exams, particularly SD-OCT, could not be performed due to severe nystagmus, poor visual acuity, and patient compliance. Finally, not all patients were willing to be re-examined in follow-up visits.

In conclusion, our study further delineates the natural course of nonsyndromic inherited retinal dystrophies due to *CEP290* mutations confirming the wide spectrum of retinal phenotypes, for the first time in an Italian cohort. In particular, we observed a subgroup of patients with a mild phenotype, characterized by absence of nystagmus, slightly delayed disease onset, better visual acuity, better preserved retinal morphology, and detectable photopic and scotopic ERG responses. Finally, in contrast to the evidences of in vitro studies[18,53,54] regarding the hypomorphic features of the deep intronic variant c.2991+1655A>G, our findings suggest that this variant is associated with a more severe phenotype. Therefore, further studies are needed to clarify the impact of the deep intronic variant c.2991+1655A>G on the clinical phenotype across different cohorts.

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