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SHORT REPORT

A novel locus for autosomal dominant cone-rod dystrophy maps to chromosome 10q

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Here we report recruitment of a three-generation Romani (Gypsy) family with autosomal dominant cone-rod dystrophy (adCORD). Involvement of known *adCORD* genes was excluded by microsatellite (STR) genotyping and linkage analysis. Subsequently, two independent total-genome scans using STR markers and single-nucleotide polymorphisms (SNPs) were performed. Haplotype analysis revealed a single 6.7-Mb novel locus between markers D10S1757 and D10S1782 linked to the disease phenotype on chromosome 10q26. Linkage analysis gave a maximum LOD score of 3.31 for five fully informative STR markers within the linked interval corresponding to the expected maximum in the family. Multipoint linkage analysis of SNP genotypes yielded a maximum parametric linkage score of 2.71 with markers located in the same chromosomal interval. There is no previously mapped CORD locus in this interval, and therefore the data reported here is novel and likely to identify a new gene that may eventually contribute to new knowledge on the pathogenesis of this condition. Sequencing of several candidate genes within the mapped interval led to negative findings in terms of the underlying molecular pathogenesis of the disease in the family. Analysis by comparative genomic hybridization excluded large chromosomal aberrations as causative of adCORD in the pedigree.
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INTRODUCTION

Cone-rod dystrophies (CORDs) are a group of clinically and genetically heterogeneous retinal disorders with prevalence of 1/40 000 and characterized by primary degeneration of cone photoreceptors, followed by loss of rod photoreceptors. Affected individuals initially show symptoms of decreased visual acuity and abnormalities of color vision, visual field loss and a variable degree of nystagmus and photophobia. Central or paracentral scotomas can also be present upon visual testing. The fundus examination often reveals pigment deposits and retinal atrophy in the macular region. Patients with CORD develop additional rod system abnormalities that lead to night blindness later in the disease process.[1]

Autosomal dominant, autosomal recessive and X-linked (Xl) inheritance patterns are seen in CORD families.[2] Currently, ten genes, including *AIPL1* (17p13.2), *CRX* (19q13.32), *GUCA1A* (6p21.1), *GUCY2D* (17p13.1), *PITPNM3* (17p13.2), *PROM1* (4p15.32), *PRPH2* (6p21.2), *RIMS1* (6q13), *SEMA4A* (1q22) and *UNC119* (17q11.2) and three loci, *CORD4* (17q), *RCD1* (6q25-q26) and *CORD16* (2q24.2-2q33.1) have been identified as responsible for autosomal dominant cone-rod dystrophy (adCORD) (http://sph.uth.tmc.edu/Retnet). The products of these genes are associated either with photoreceptor structure, cellular function including the phototransduction cascade, or synaptic transmission.

Here, we describe a three-generation Bulgarian family of Romani origin segregating adCORD (Figure 1) and provide evidence for a novel, still unidentified locus implicated in this condition. Romani are known as a genetically isolated founder population of Indian origin that settled in the Balkans in 13th–14th century and rest of Europe by the end of the 15th century.[3,4] A large group of the initial migrant population, called 'Balkan Gypsies,' settled permanently in the Balkans, south of the Danube in Bulgaria. Interestingly the various geographically, socially and linguistically divergent Romani groups have been shown to share common Mendelian disorders and founder mutations due to their common origin.[3]

MATERIALS AND METHODS

Phenotyping

A three-generation autosomal dominant family of Romani origin with non-syndromic CORD was identified. A total of 6 affected and 15 unaffected individuals were clinically ascertained. Diagnosis of CORD was based on the following criteria: reduced visual acuity and photophobia evident in the second decade of life, impairment of color vision, fundoscopic evidence of maculopathy with peripheral retinopathy and the demonstration of an abnormal cone-rod ERG.

Genotyping, linkage analysis and mutation screening

After informed consent, blood samples were obtained and genomic DNA extracted from peripheral blood lymphocytes according to the standard protocols. In total, 460 fluorescently labeled microsatellite (STRs) markers from the ABI PRISM Linkage Mapping Set V2.5 (Applied Biosystems Incorporated, Foster City, CA, USA) were used for genotyping of 21 members

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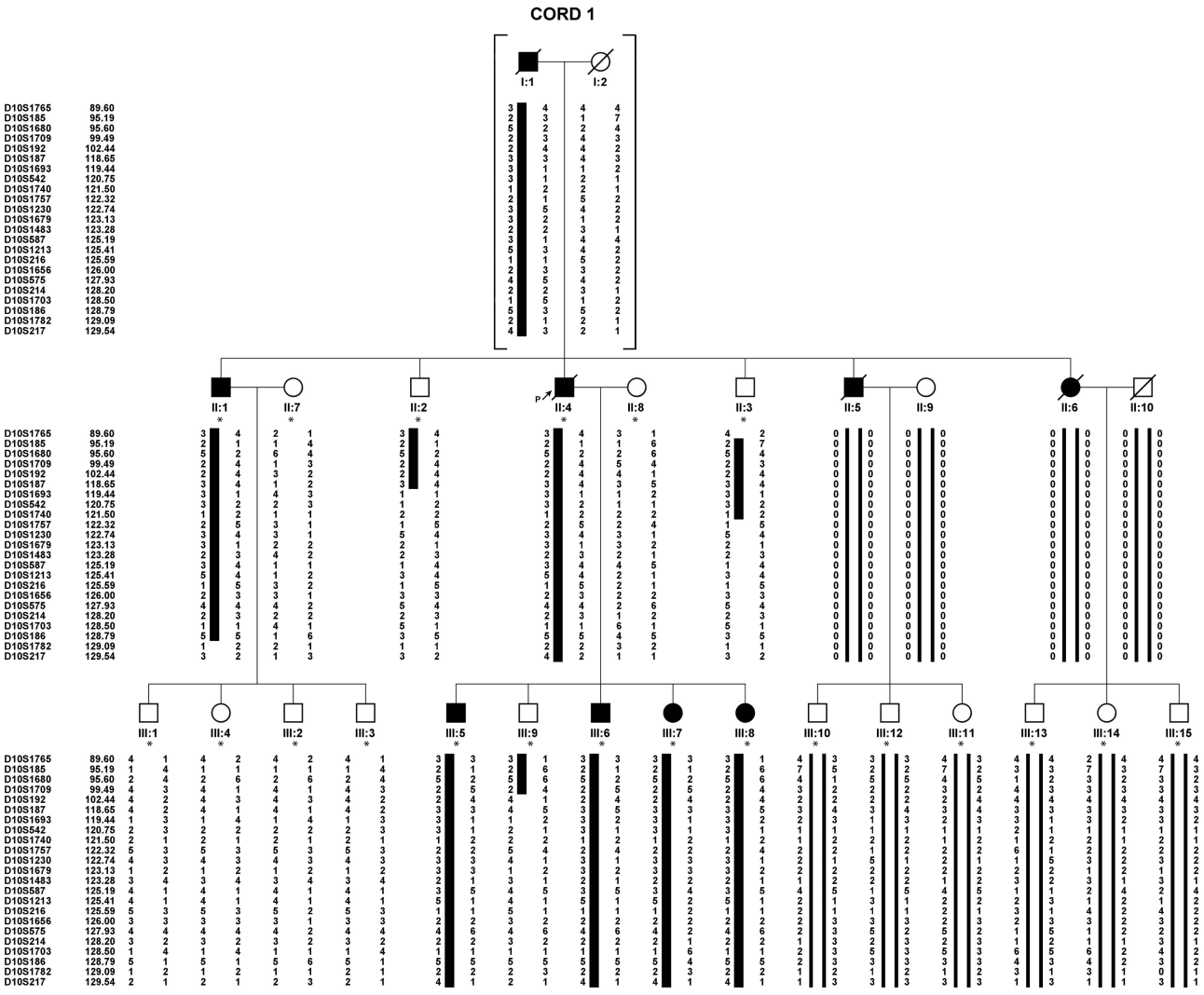

Figure 1 Pedigree of the family is shown with haplotypes of STR markers spanning the linked interval on chromosome 10. Markers and their physical positions (Mb) are indicated at the left of each row. Open symbols represent unaffected and filled symbols represent affected individuals; symbols with diagonal slash represent deceased members; arrow indicates the proband; black vertical bars represent haplotype segregating with the disease in the family. Twenty-one members indicated with asterisks were genotyped. Predicted haplotypes for individuals I:1 and I:2 are enclosed in brackets. Haplotypes were created with Genehunter and viewed on Haplopainter.[15]

of this family. Multiplex microsatellite genotyping was performed using 3–5 markers per reaction using QPCR mix (ABgene, Epsom, Surrey, UK) and subsequently analyzed on an ABI3730xl genetic analyzer (Applied Biosystems). Data collection and allele identification were performed using GeneScan and GeneMapper v4.0 software (Applied Biosystems). All regions with suggestive LOD scores (>1.5) were saturated with additional highly polymorphic markers designed according to the information obtained from Marshfield, GDB Human Genome Database (http://research.marshfieldclinic.org) and Ensembl genome data resources (http://www.ensembl.org).

Two- and multipoint parametric linkage analysis was performed with FastLink and Genehunter, respectively, from easyLINKAGE-Plus package.[5] PedCheck was used for detection of Mendelian errors,[6] and non-Mendelian errors were identified with Merlin.[7] The phenotype was analyzed as an autosomal dominant trait with complete penetrance (0.9990) and a frequency of 0.0001 for the affected allele. Because of the computational limitations of Genehunter and the size of the family, the pedigree was split into two halves for LOD score analysis.

To confirm the STR linkage, a second genome-wide scan was performed using DNA samples from 15 family members genotyped on the Affymetrix 10K SNP panel (AROS Applied Biotechnologies, Aarhus, Denmark). This version of microarray (v.Xba142 2.0) comprises a total of 10 204 single-nucleotide polymorphisms (SNPs) with a mean intermarker distance of 258 kb, equivalent to 0.36 cM and average heterozygosity of 0.38 predicting a spacing of three fully informative markers per 1 Mb. Genome-wide linkage analysis via the Genehunter program in the easyLINKAGE plus platform v5.08[8] was used to validate the original STR linkage. The call rate, defined as the percentage of successful genotype calls among subjects, was used as a measure of data quality. The results reported in this study are based on 95% call rate. Uninformative SNPs were removed from the data by Merlin.[7]

Sequence analysis of the coding exons, intron–exon junctions and 5′ and 3′ untranslated regions of all genes within the linkage region, was performed on ABI3730xl genetic analyzer (Applied Biosystems) according to the manufacturer's protocol. All novel sequence variants were tested using control individuals from Human Random Control DNA Panels (European Collection of Cell Cultures).

Whole-genome and chromosome 10 tiling comparative genomic hybridization (CGH) arrays (Roche NimbleGen, Incorporated, Madison, WI, USA) were undertaken using the 385K version. Fluorescence intensities and log2 intensity ratios for the test *versus* control sample were calculated using the SignalMap software (Roche NimbleGen).

RESULTS

We first excluded all previously known loci for adCORD using selected microsatellite markers closely linked to these loci. Non-segregation of the marker haplotypes within the family for a given adCORD locus ruled out its involvement in the pathogenesis of the disease. Total-genome scan using highly informative STRs was then performed and the generated negative or non-significant LOD scores for all genotyped loci allowed exclusion of all regions, except for eight different intervals on chromosome 1 (one interval of 74 Mb between D1S435 and D1S2878), chromosome 5 (one interval of 49 Mb between D5S471 and D5S400), chromosome 10 (one interval of 40 Mb between D10S1765 and D10S217), chromosome 14 (three intervals of 10, 13 and 38 Mb between D14S972 and D14S70, D14S288 and D14S980 and D14S274 and D14S1054, respectively), chromosome 17 (one interval of 5.8 Mb between D17S785 and D17S928) and chromosome 20 (one interval of 9.6 Mb between D20S115 and D20S112). Consequently, additional densely spaced microsatellite markers spanning these regions were used and haplotypes were constructed. The segregation of different haplotypes in affected individuals and the negative LOD scores obtained allowed exclusion of all regions except a 6.7-Mb interval on chromosome 10q26, which could not be further refined. Recombination in unaffected individual II:3 at D10S1757 (122 324 738 bp) defined the proximal boundary and similarly, recombination event in affected individual II:1 at D10S1782 (129 086 934 bp) defined the distal boundary of the region (Figure 1). Thus, the linked interval on chromosome 10q26 (122 324 738–129 086 934 bp) was reduced from 40 to 6.7 Mb, and therefore the number of the genes needed to be screened for mutation was greatly decreased from approximately 200 to only 46. Affected individuals shared a common haplotype tested with a number of microsatellite markers spanning the linked region and generating positive LOD score. Five fully informative markers, D10S1757 (122 324 738 bp), D10S1230 (122 742 692 bp), D10S1679 (123 133 534 bp), D10S587 (125 188 640 bp) and D10S1213 (125 406 637 bp), within this interval yielded maximum LOD score of 3.31 at $\theta = 0.0$ (Table 1).

A second genome scan using the 10K SNP array revealed a 16-cM (7.3 Mb) genomic interval on chromosome 10q26 suggestive for linkage (LOD score 2.71), containing the STR marker-detected region as described above. A second, minor linkage peak generating a LOD score of 1.78 was observed on chromosome 1p13.3 in the region between 117 and 118 cM (Figure 2). Genotyping and linkage analysis using highly informative microsatellite markers resulted in negative LOD scores, thereby excluding 1p13.3 as a potential locus for CORD in our family. Haplotype analysis combining the data from two independent genome-wide scans restricted the shared interval to a 6.7-Mb region on chromosome 10q26 between markers D10S1757 (122.32 Mb) and D10S1782 (129.08 Mb).

Direct exonic sequencing of 46 genes, including splice site junctions, 5′ and 3′ untranslated regions as well as 4 microRNAs, 5 miscellaneous RNAs, 1 ribosomal RNA, 2 spliceosomal RNAs and 1 small nucleolar RNA, from the critical 6.7-Mb interval was subsequently performed, but this led to negative findings in terms of the underlying molecular pathogenesis of CORD in our pedigree. Analysis of the entire coding sequences of all positional genes detected only known polymorphisms. All novel sequence variants were also observed in one or more individuals in our panel of 192 controls. A list of the genes and non-coding RNAs mapping to the critical region on 10q26 is given in Supplementary Table 1.

Initially array CGH (aCGH) technology was used to evaluate the whole genome followed by the finer analysis of chromosome 10 rearrangements (Supplementary Figure 1). Automated segmentation

Table 1 Two-point LOD scores for linkage between the CORD locus and 10q26 markers

		LOD score[a]							
		Two-point							*Multi-point*
Marker	*Position (Mb)*[b]	$\Theta = 0.00$	$\Theta = 0.05$	$\Theta = 0.10$	$\Theta = 0.30$	$\Theta = 0.50$	Z_{max}	Θ_{max}	$\Theta = 0.00$
D10S1765	89.60	0.47	0.81	0.90	0.64	0.00	0.90	0.15	−4.83
D10S185	95.19	0.80	0.94	0.94	0.45	0.00	0.94	0.10	−5.48
D10S1680	95.60	0.80	0.94	0.94	0.45	0.00	0.94	0.10	−3.81
D10S1709	99.49	0.80	0.94	0.94	0.45	0.00	0.94	0.10	−0.21
D10S192	102.44	2.34	2.13	1.93	0.98	0.00	2.34	0.00	0.81
D10S187	118.65	2.33	2.13	1.93	0.98	0.00	2.33	0.00	0.11
D10S1693	119.44	0.81	0.97	0.98	0.42	0.00	0.98	0.10	−2.32
D10S542	120.75	1.19	1.26	1.19	0.50	0.00	1.26	0.05	−3.52
D10S1740	121.50	2.15	2.07	1.92	0.98	0.00	2.15	0.00	−2.21
D10S1757	122.32	3.31	3.02	2.72	1.31	0.00	**3.31**	0.00	2.71
D10S1230	122.74	3.31	3.02	2.72	1.31	0.00	**3.31**	0.00	2.71
D10S1679	123.13	3.31	3.02	2.72	1.31	0.00	**3.31**	0.00	2.71
D10S1483	123.28	2.33	2.13	1.93	0.98	0.00	2.33	0.00	2.71
D10S587	125.19	3.31	3.02	2.72	1.31	0.00	**3.31**	0.00	2.71
D10S1213	125.41	3.31	3.02	2.72	1.31	0.00	**3.31**	0.00	2.71
D10S216	125.59	2.35	2.15	1.94	0.98	0.00	2.35	0.00	2.71
D10S1656	126.00	3.27	2.98	2.68	1.27	0.00	3.27	0.00	2.71
D10S575	127.93	0.64	0.57	0.49	0.17	0.00	0.64	0.00	2.71
D10S214	128.20	2.38	2.18	1.97	0.99	0.00	2.38	0.00	2.71
D10S1703	128.50	0.65	0.57	0.49	0.17	0.00	0.65	0.00	2.71
D10S186	128.79	0.65	0.57	0.49	0.17	0.00	0.65	0.00	2.71
D10S1782	129.50	1.72	1.72	1.61	0.69	0.00	1.72	0.05	infinity

[a]Significant LOD scores are shown in bold.
[b]Mb locations based on NCBI map, build 37/hg19.

Marker	CHR	cM	pLOD	NPL	npLOD	P val	INFO	Alpha	hLOD
1. SNPA1511982 – SNPA1519134	10	144.01	2.7093	1.6665	0.6031	0.0742	0.9436	1.0000	2.7093
2. SNPA1511982 – SNPA1519134	10	144.01	2.7093	1.6665	0.6031	0.0742	0.9436	1.0000	2.7093
3. SNPA1511982 – SNPA1519134	10	144.01	2.7093	1.6665	0.6031	0.0742	0.9436	1.0000	2.7093
4. SNPA1511982 – SNPA1519134	10	144.01	2.7093	1.6665	0.6031	0.0742	0.9436	1.0000	2.7093
5. SNPA1511942	10	144.01	2.7093	1.6665	0.6031	0.0742	0.9436	1.0000	2.7093

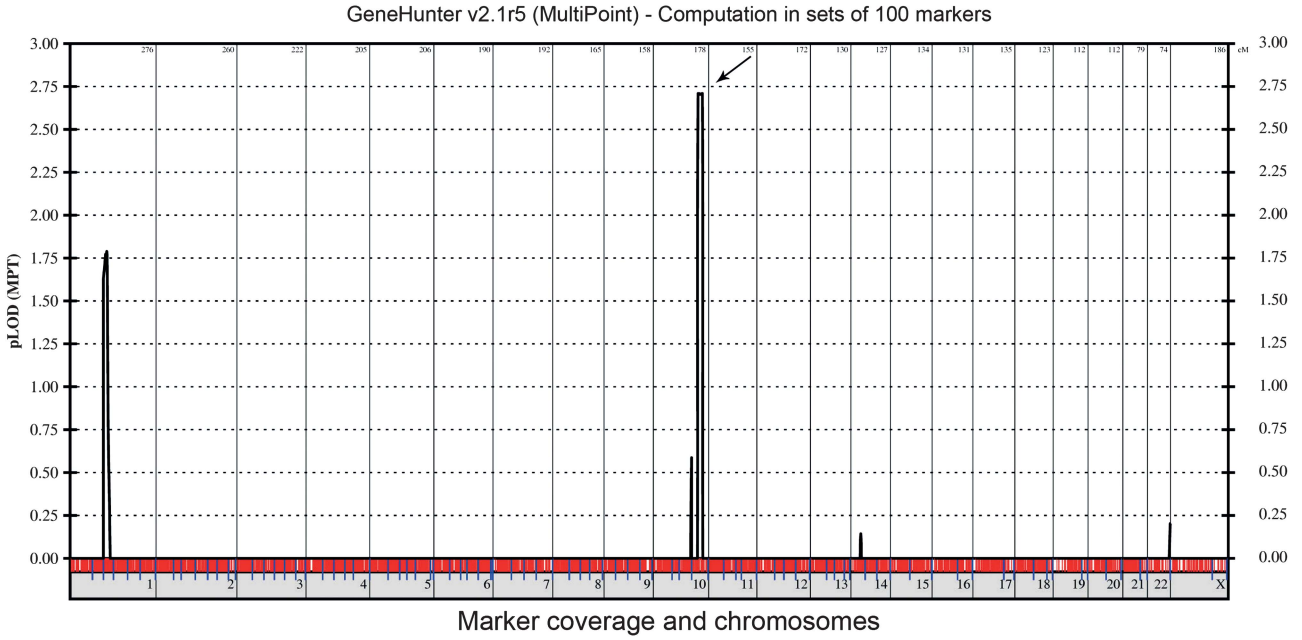

Figure 2 Genome-wide multipoint LOD score calculations using 10K SNP array genotyping data generated by Genehunter from the easyLINKAGE package.[5] Each chromosome on the *x*-axis is plotted against the LOD score on the *y*-axis. One clear peak, indicated by arrow, is visible on chromosome 10 with a genome-wide significant LOD score of 2.71.

analysis for deleted genomic areas predicted multiple segments with log2 intensity ratios in a wide range, from 0.15 to −0.57 along chromosome 10, but they were reported as a typical copy number variation in the human population (Database of Genomic Variants, http://projects.tcag.ca/variation/). Thus, the aCGH method did not detect any chromosomal aberration as disease-causing mutation in the pedigree.

DISCUSSION

In this study, we have described a three-generation Romani family segregating a novel locus for adCORD. The disease in the pedigree is best described as cone-rod dystrophy with slightly variable but early age of onset (around 10 years of age). Ophthalmoscopy disclosed typical signs of CORD including gradual visual impairment and photophobia, narrowing of retinal vessels, scattered bone-spicule pigmentation in the midperipheral retina, RPE (retinal pigment epithelium) atrophy and optic disk pallor. Electroretinogram showed reduced photopic and scotopic responses. Visual fields demonstrated central scotoma.

After the exclusion of known loci associated with adCORD, pedigree members were first genotyped with short tandem repeat markers and then with single-nucleotide polymorphism microarray chip. Advances in SNP identification and genotyping technology have extended the use of dense SNP maps for linkage purposes in place of STR marker panels.[9] It has been reported that the SNP microarrays has a greater than 99% chance in detecting heterozygosity, compared with a 70% chance in the case of STR markers, and consequently in the generation of better quality linkage data.[10] The use of high-density SNP genotyping platform has successfully identified disease loci in families with retinitis pigmentosa (RP) and Leber congenital amaurosis.[11,12] For this reason, we undertook a second genome scan using the 10K SNP array to verify the original STR-based linkage data for CORD in our pedigree.

Initially, we performed a total-genome search with the use of STR markers and identified a 6.7-Mb region on chromosome 10q26 for CORD between markers D10S1757 and D10S1782, with maximum LOD score of 3.31 in our pedigree. It represents the maximum value obtainable with this family, given its size, the number of informative meioses and the described genetic model. An independent genome-wide scan using SNP microarray defined a 7.3-Mb region on chromosome 10q26, spanning the 6.7-Mb region highlighted by the original STR markers, confirming the validity of the initial method. In addition, the lack of evidence for linkage to other genomic regions indicates strongly for a novel and only locus for CORD in this region of the human genome.

According to the human genome build 37 (hg19, 2009), the 10q26 region contains 46 annotated genes. We first sequenced the key positional candidate genes, including *PLEKHA1* (pleckstrin homology domain-containing family A, member 1; 10q25.3–q26.2), *ARMS2* (age-related maculopathy susceptibility, 10q26.13), *HTRA1* (HtrA serine peptidase 1; 10q26.13) and *OAT* (ornithine aminotransferase; 10q26), as well as *RGR* (retinal G protein-coupled receptor, 10q23.1) and *OPN4* (opsin 4, 10q23.2) mapping close to the linked interval. No mutation was found involving the exons, which suggests that intronic mutation spots acting as *cis*-regulatory elements, such as intronic splicing enhancers or silencers, could be involved in the pathogenesis of adCORD in our family. We then performed extensive screening of all genes (their coding region, splice site junctions,

5′ and 3′ untranslated regions) and 13 non-coding RNAs within the interval, but it failed to detect any pathogenic variant. However, mutations in other parts of the genes cannot be excluded. As previously reported, one of the most frequent causes of Leber congenital amaurosis (20% of the patients) is a deep intronic mutation, c.2991+1655A>G (p.Cys998X), which results in the insertion of a cryptic exon in the mRNA and introduces a premature stop codon in the CEP290 protein.[13]

High-density oligonucleotide-based aCGH technology was further applied to search for deletions/insertions as small as a few hundred base pair that may explain the genetic basis of the disease. No chromosomal aberration, deletion or amplification of a part or whole gene was detected in the pedigree, suggesting that a different mutation-detecting technology such as next-generation sequencing may need to be used.

In conclusion, here we report the mapping of a novel adCORD locus to the long arm of chromosome 10 in a Romani family. This is the first study of the molecular basis of CORD and only the second study of retinal degenerations in Romani families. Previously mutations in *PRPF31* and *RPGR* have been found to be causative for adRP and XlRP, respectively, in two Bulgarian Romani families.[14]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)