# Screening for variants in $\mathbf{2 0}$ genes in $\mathbf{1 3 0}$ unrelated patients with cone-rod dystrophy 

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#### Abstract

Cone-rod dystrophy (CORD) is a hereditary retinal disorder with primary cone impairment and subsequent rod involvement. To date, mutations responsible for CORD have been reported in 24 genes. However, the systemic evaluation of variants in these genes in a cohort of patients is rare, particularly in East Asia. In this study, 58 coding exons from 20 CORD genes, including 35 exons with previously identified mutations in 17 genes and all 23 coding exons for the other 3 genes (GUCY2D, PRPH2 and KCNV2), were analyzed by cycle sequencing on 130 unrelated probands with CORD. Four heterozygous mutations, 1 novel and 3 known, were detected in $4 / 130$ patients, including c. $259 \mathrm{G}>\mathrm{A}$ (p.Asp87Asn) in UNC119, $\mathrm{c} .2512 \mathrm{C}>\mathrm{T}$ (p.Arg838Cys) and c.2513G $>\mathrm{A}$ (p.Arg838His) in GUCY2D and c.946T>G (p.Trp316Gly) in PRPH2. The result implies a comparatively low rate of mutations in these exons in Chinese patients. These data suggest that in Chinese patients, CORD may be caused by mutations in exons that have not yet been screened or in genes that have yet to be identified. Further analysis of these patients may provide clarification.


## Introduction

Cone-rod dystrophy (CORD) is a heterogeneous inherited retinal disease characterized by reduced visual acuity, photophobia and color vision defects. Fundus observation usually identifies temporal pallor of the optic disc, attenuation of retinal arterioles and macular atrophy. Recordings on an electroretinogram (ERG) usually reveal the predominant functional impairment of cones over rods during the early stages (1). The prevalence of CORD is approximately 1 in 40,000 individuals (2).

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The disease may be transmitted as an autosomal dominant, autosomal recessive or X-linked trait. At least 24 genes have been identified to be responsible for CORD (RetNet: https:// sph.uth.tmc.edu/Retnet/). The genes for autosomal dominant CORD are AIPLI (3), CRX (4), GUCAlA (5), GUCY2D (6), PITPNM3 (7), PROM1 (8), PRPH2 (9), RIMS1 (10), SEMA4A (11) and UNC119 (12). The genes for autosomal recessive CORD are ABCA4 (13), ADAM9 (14), CACNA2D4 (15), CDHR1 (16), CERKL (17), CNGB3 (18), CNNM4 (19), KCNV2 (20), PDE6C (21), RAX2 (22), RPGRIP1 (23) and RDH5 (24). The genes for X-linked CORD are CACNAIF (25) and $R P G R$ (26). Although studies on individual genes have been reported, the systemic analysis of these genes in a cohort of patients is rare, with the exception of a few studies on the genes for autosomal dominant CORD (27) or the genes for autosomal recessive CORD $(17,28)$. Extensive analysis may provide insight into the mutation frequency and spectrum of the majority of CORD-related genes (29). In this study, we comprehensively screened 58 exons in 20 genes for mutations in 130 unrelated Chinese patients with CORD, mostly on the coding regions with reported mutations.

## Materials and methods

Data from 130 unrelated patients with CORD were collected at the Pediatric and Genetic Eye Clinic, Eye Hospital of Zhongshan Ophthalmic Center, Guangzhou, China. Of the 130 patients, 111 were isolated cases, 8 had an autosomal dominant trait and 11 had an autosomal recessive trait. This study was performed in accordance with the guidelines set out in the Declaration of Helsinki and was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. Informed consent was obtained from all participants or their guardians prior to the collection of clinical data and genomic samples. Genomic DNA was extracted from the leukocytes of venous blood using previously reported methods (30).

Of the 24 genes responsible for CORD, 4 genes, $C R X$, GUCA1A, CACNA1F and RDH5, were not analyzed in this study, as they already have been analyzed in independent studies [(31) and unpublished data]. When this study was initiated in April 2011, all coding exons with a previously reported mutation in the 20 genes (Table I) were selected as targets for

Table I. The genes and targeted exons analyzed in this study.

| Genes | OMIM | cDNA | Trait | Total coding exons ${ }^{\text {a }}$ | Exons for sequencing $^{\text {b }}$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| GUCY2D | 600179 | NM_000180.3 | AD | 18 | $1-18^{\text {c }}$ |
| PRPH2 | 179605 | NM_000322.4 | AD | 3 | $1-3$ |
| RIMS1 | 606629 | NM_014989.4 | AD | 34 | 6,34 |
| AIPL1 | 604392 | NM_014336.3 | AD | 6 | 5,6 |
| PITPNM3 | 608921 | NM_031220.3 | AD | 20 | 9,14 |
| UNC119 | 604011 | NM_005148.3 | AD | 5 | 1,2 |
| SEMA4A | 607292 | NM_022367.3 | AD | 14 | 9 |
| PROM1 | 604365 | NM_006017.2 | AD | 26 | 11,13 |
| ADAM9 | 602713 | NM_003816.2 | AR | 22 | $6,9,12$ |
| CNGB3 | 605080 | NM_019098.4 | AR | 18 | $6,8,11$ |
| KCNV2 | 607604 | NM_133497.3 | AR | 2 | 1,2 |
| PDE6C | 600827 | NM_006204.3 | AR | 22 | 1 |
| CDHR1 | 609502 | NM_033100.2 | AR | 17 | 6 |
| CACNA2D4 | 608171 | NM_172364.4 | AR | 38 | 25,30 |
| RPGRIP1 | 605446 | NM_020366.3 | AR | 24 | 13,16 |
| RAX2 | 610362 | NM_032753.3 | AR | 2 | 2 |
| ABCA4 | 601691 | NM_000350.2 | AR | 50 | 6 |
| CERKL | 608381 | NM_201548.4 | AR | 10 | $1,2,6,8$ |
| CNNM4 | 607805 | NM_020184.3 | AR | 7 | $1,4,7$ |
| RPGR | 312610 | NM_000328.2 | X-LINKED | 19 | $4,6,7$ |
| Total |  |  |  | 357 | 58 |

${ }^{\text {a }}$ All coding exons were referred to NCBI (http://www.ncbi.nlm.nih.gov/). ${ }^{\text {b }}$ Sequenced exons were referred to HGMD (http://www.hgmd.org/). ${ }^{\text {a }}$ The majority of CORD-associated mutations in $G U C Y 2 D$ were reported in exon 12 . AD , autosomal dominant; AR , autosomal recessive.


Figure 1. Pedigrees and sequence chromatography. (Left column) Four sequence changes detected in the probands with cone-rod dystrophy (CORD). (Right column) Corresponding normal sequences. For the pedigrees, black-filled symbols represent the individuals in each family affected by CORD. Arrow indicates the proband in each family.
Table II. Mutations detected in 130 unrelated cone-rod dystrophy (CORD) patients and 192 healthy controls.

| Family | Gene | Changes |  | Description |  |  | Computational prediction |  |  |  | Cases | Controls | Refs |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | DNA | Protein | State | Cons | Blosum62 ${ }^{\text {a }}$ | PolyPhen-2 | SIFT | Pmut | PANTHER ${ }^{\text {b }}$ |  |  |  |
|  | GUCY2D | c. $2512 \mathrm{C}>\mathrm{T}$ | p.Arg838Cys | Hetero | Yes | 8 | PD | D | PA | -8.7 | 1/130 | ND | (38) |
|  | GUCY2D | c. $2513 \mathrm{G}>\mathrm{A}$ | p.Arg838His | Hetero | Yes | 5 | PD | D | PA | -5.5 | 1/130 | ND | (39) |
| 3 | PRPH2 | c. $946 \mathrm{~T}>\mathrm{G}$ | p.Trp316Gly | Hetero | Yes | 13 | Benign | D | PA | NA | 1/130 | ND | (40) |
| 4 | UNC119 | c. $259 \mathrm{G}>\mathrm{A}$ | p.Asp87Asn | Hetero | Yes | 5 | PD | Tolerated | Neutral | -3.2 | 1/130 | 0/192 | This study |



further analysis, with the exception of $A B C A 4$, in which a large number of variations have previously been identified both in patients and controls (32). Furthermore, in 3 genes, $G U C Y 2 D$, PRPH2 and KCNV2, all exons were analyzed, as mutations in GUCY2D and PRPH2 are frequently observed in patients with CORD $(27,33)$, while mutations in both exons of $K C N V 2$ have been reported (20). In this study, a total of 58 exons in 20 genes were analyzed (Table I). For the 58 coding exons, DNA fragments encompassing individual exons were amplified by PCR using corresponding primer pairs (available upon request). The sequences of amplicons were determined by Sanger sequencing using an ABI BigDye Terminator Cycle Sequencing kit v3.1 on an ABI 3130 Genetic analyzer (Applied Biosystems, Foster City, CA, USA). The results from the patients were aligned with the reference sequences from the NCBI database using SeqManII (DNAstar, Madison, WI, USA) to determine the variations. Each variant was bidirectionally sequenced and any novel variant was further evaluated using 192 normal controls ( 384 chromosomes). The mutation descriptions are in accordance with the recommendations from the Human Genomic Variation Society (http://www. hgvs.org/mutnomen/).

Four online computational algorithms (34-37), PANTHER (http://www.pantherdb.org/),PMut(http://mmb2.pcb.ub.es:8080/ PMut/), SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http:// genetics.bwh.harvard.edu/pph2/), respectively, were used to predict the functional impact of the detected missense mutations.

## Results

Upon the sequencing analysis of 58 exons in 20 genes, 4 mutations, 1 novel and 3 known ( $38-40$ ), in 3 genes were discovered in $4 / 130$ unrelated probands $(4 / 130=3.08 \%)$ (Table II). All 4 mutations were heterozygous and detected in genes known to cause autosomal dominant CORD: $\mathrm{c} .259 \mathrm{G}>\mathrm{A}$ (p.Asp87Asn) in UNC119, c.2512C $>$ T (p.Arg838Cys) and $\mathrm{c} .2513 \mathrm{G}>\mathrm{A}$ (p.Arg838His) in $G U C Y 2 D$ and c.946T $>\mathrm{G}$ (p.Trp316Gly) in PRPH2 (Fig. 1). In addition to the 4 mutations, a number of possible non-pathogenic variants were also detected in KCNV2, CERKL, PITPNM3, RPGRIP1, AIPL1, RPGR, ABCA4, RIMS1, CNGB3, PDE6C, CDHR1, RAX2, CNNM4, GUCY2D and PRPH2 (Table III).

The clinical data of the 4 patients with a mutation in GUCY2D, PRPH2 or UNC119 are summarized in Table IV. Affected members had poor vision, photophobia or nystagmus as initial symptoms. The onset age varied from the first few months after birth to 16 years of age. Fundus examination revealed attenuated vessels, macular atrophy and temporal pallor of the optic disc. ERG recordings revealed severely reduced or extinguished cone responses accompanied by normal to mildly reduced rod responses in 3 patients with these mutations.

## Discussion

In this study, 4 mutations in 58 exons from 20 genes were detected in $4 / 130$ patients with CORD, which suggests that the frequency of mutations in these regions is rare in Chinese patients. All coding exons of GUCY2D and PRPH2 were
Table III. Polymorphisms detected in 130 unrelated patients.

| Gene | Exon | Variations |  | Status | Bioinformation analysis |  |  | Frequency in cases/controls ${ }^{\text {a }}$ | Refs ${ }^{\text {c }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Nucleotide | Amino acid |  | Conservation | PolyPhen-2 | Splice site |  |  |
| KCNV2 | 1 | c. $612 \mathrm{G}>\mathrm{A}$ | p.(=) | Hetero/Homo | Yes | NA | No change | 11/4 | This study |
|  | 1 | c. $645 \mathrm{G}>\mathrm{C}$ | p.Lys215Asp | Hetero/Homo | Yes | PD | No change | 8/6 | This study |
|  | 1 | c. $920 \mathrm{~T}>\mathrm{G}$ | p.Met307Arg | Hetero | No | Benign | No change | 5/8 | This study |
|  | 1 | c. $759 \mathrm{~A}>\mathrm{G}$ | p.(=) | Hetero | Yes | NA | No change | 2/14 | rs 10967709 |
|  | 1 | c.795C>G | p.(=) | Hetero/Homo | No | NA | No change | 91/170 | rs 12237048 |
|  | 2 | c. $1513 \mathrm{G}>\mathrm{T}$ | p.Ala505Ser | Hetero | Yes | Benign | No change | 1/1 | This study |
|  | 2 | c. $1638+6 \mathrm{~T}>\mathrm{C}$ | - | Hetero | NA | NA | Change | 8/NA | rs41306094 |
|  | 2 | c. $1386 \mathrm{C}>\mathrm{T}$ | p.(=) | Hetero | Yes | NA | No change | 8/NA | rs41312842 |
|  | 2 | c. $1597 \mathrm{C}>\mathrm{G}$ | p.Val533Leu | Hetero | No | Benign | No change | 8/NA | rs 12352254 |
| CERKL | 2 | c. $242 \mathrm{~A}>\mathrm{C}$ | p.Asp81Ala | Hetero/Homo | No | Benign | No change | 43/NA | rs61750041 |
|  | 2 | c. $239-12 \mathrm{~T}>\mathrm{A}$ | - | Hetero/Homo | NA | NA | No change | 27/NA | rs6433923 |
|  | 2 | c. $313 \mathrm{C}>\mathrm{T}$ | p.Arg 105 Trp | Hetero | Yes | PD | No change | 2/NA | rs149078111 |
| PITPNM3 | 9 | c. $901-10 \mathrm{G}>\mathrm{C}$ | - | Hetero/Homo | NA | NA | Change | 34/44 | rs77580616 |
|  | 9 | c. $1016 \mathrm{C}>\mathrm{G}$ | p.Pro339Arg | Hetero | Yes | Benign | No change | 1/1 | This study |
| RPGRIP1 | 16 | c. $2592 \mathrm{~T}>\mathrm{C}$ | p.(=) | Hetero | Yes | NA | No change | 1/NA | This study |
| AIPL1 | 5 | c. $726 \mathrm{G}>\mathrm{A}$ | p.(=) | Hetero | Yes | NA | No change | 9/NA | This study |
|  | 5 | c. $784+18 \mathrm{G}>\mathrm{A}$ | - | Hetero | NA | NA | No change | 6/NA | rs7222126 |
| RPGR | 7 | c. $732 \mathrm{G}>\mathrm{A}$ | p.(=) | Hetero | No | NA | No change | 1/NA | This study |
|  | 7 | c. $762 \mathrm{~T}>\mathrm{C}$ | p.(=) | Hetero | No | NA | No change | 1/NA | This study |
| ABCA4 | 6 | c. $635 \mathrm{G}>\mathrm{A}$ | p.Arg212His | Hetero | Yes | PD | Change | 10/7 | This study |
|  | 6 | c. $673 \mathrm{G}>\mathrm{A}$ | p.Val225Met | Hetero | Yes | PD | Change | 2/1 | This study |
|  | 6 | c. $634 \mathrm{C}>\mathrm{T}$ | p.Arg212Cys | Hetero | Yes | PD | No change | 1/1 | This study |
| RIMS1 | 6 | c. $942 \mathrm{G}>\mathrm{A}$ | p.(=) | Hetero | No | NA | No change | 2/NA | This study |
|  | 6 | c. $1209 \mathrm{G}>\mathrm{A}$ | p.(=) | Hetero | No | NA | No change | 28/NA | This study |
|  | 6 | c. $1311 \mathrm{G}>\mathrm{A}$ | p.(=) | Hetero | No | NA | No change | 1/NA | This study |
| CNGB3 | 8 | c. $919 \mathrm{~A}>\mathrm{G}$ | p.Val307Ile | Hetero | No | Benign | Change | 14/NA | rs13265557 |
|  | 8 | c. $912 \mathrm{C}>\mathrm{T}$ | p.(=) | Hetero | No | NA | No change | 1/NA | rs117806701 |
| PDE6C | 1 | c. $252 \mathrm{G}>\mathrm{A}$ | p.(=) | Hetero | Yes | NA | No change | 26/NA | rs1131978 |
|  | 1 | c. $471 \mathrm{~T}>\mathrm{G}$ | p.Asp157Glu | Hetero/Homo | Yes | PD | No change | 5/NA | rs76999928 |
| CDHR1 | 6 | c. $477 \mathrm{~A}>\mathrm{G}$ | $\mathrm{p} .(=)$ | Hetero/Homo | Yes | NA | No change | 22/NA | rs4933975 |
| RAX2 | 2 | c. $282 \mathrm{C}>\mathrm{T}$ | p.(=) | Hetero | No | NA | No change | 3/NA | This study |
|  | 2 | c. 217-8C>T | - | Hetero | NA | NA | No change | 3/NA | rs79588413 |

Table III. Continued.

| Gene | Exon | Variations |  | Status | Bioinformation analysis |  |  | Frequency in cases/controls ${ }^{\text {a }}$ | References ${ }^{\text {c }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Nucleotide | Amino acid |  | Conservation | PolyPhen-2 | Splice site |  |  |
| CNNM4 | 1 | c. $47 \mathrm{G}>\mathrm{A}^{\mathrm{b}}$ | p.Arg 16His | Hetero | Yes | Unknown | No change | 0/0 | This study |
| GUCY2D | 1 | c. $154 \mathrm{G}>\mathrm{T}$ | p.Ala52Ser | Hetero/Homo | No | Benign | No change | 80/NA | rs61749665 |
|  | 1 | c. $61 \mathrm{~T}>\mathrm{C}$ | p.Trp21Arg | Herero | No | PD | No change | 2/NA | rs9905402 |
|  | 1 | c. $164 \mathrm{C}>\mathrm{T}$ | p.Thr55Met | Hetero | Yes | PD | No change | 2/NA | rs201414567 |
|  | 1 | c. $340 \mathrm{G}>\mathrm{A}$ | p.Val114Met | Hetero | No | PD | No change | 1/0 | This study |
|  | 1 | c. $343 \mathrm{~T}>\mathrm{C}$ | p.Ser115Pro | Hetero/Homo | No | PD | No change | 2/3 | This study |
|  | 1 | c. 459 delC | p.Trp154GlyfsX12 | Hetero | NA | NA | NA | 1/0 | This study |
|  | 2 | c. $741 \mathrm{C}>\mathrm{T}$ | p.(=) | Hetero | Yes | NA | No change | 22/NA | rs3829789 |
|  | 9 | c. $2101 \mathrm{C}>\mathrm{T}$ | p.Pro701Ser | Hetero/Homo | No | Benign | No change | 38/NA | rs34598902 |
|  | 11 | c. $2282 \mathrm{G}>\mathrm{A}$ | p.Arg761Gln | Hetero | No | Benign | No change | 1/0 | This study |
| PRPH2 | 1 | c. $318 \mathrm{~T}>\mathrm{C}$ | $\mathrm{p} .(=)$ | Hetero/Homo | No | NA | No change | 106/NA | This study |
|  | 3 | c. $910 \mathrm{C}>\mathrm{G}$ | p.Gln304Glu | Hetero/Homo | No | Benign | No change | 116/NA | This study |
|  | 3 | c. $1013 \mathrm{~A}>\mathrm{G}$ | p.Asp338Gly | Hetero/Homo | No | Benign | No change | 116/NA | rs434102 |
|  | 3 | c. $1041+13 \mathrm{C}>\mathrm{T}$ | - | Hetero | NA | NA | No change | 40/NA | This study |

Hetero, heterogeneous; Homo, homogeneous; NA, not available; PD, probably damaging, ${ }^{\text {a Based }}$ on the analysis of 130 patients and 192 healthy individuals. ${ }^{\mathrm{b}}$ Mutation $\mathrm{c} .47 \mathrm{G}>\mathrm{A}$ in $C N N M 4$ was absent in the 192 normal controls but detected in his healthy father. ${ }^{\text {c The }}$ variations with a rs ID in this column were described in the dbSNP database.
Table IV. Clinical information of the cone-rod dystrophy (CORD) patients with mutations.

| Family | Gene | Mutations | Gender | Age |  | Inheritance | First symptom | BCVA |  | Fundus changes | ERG responses |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Exam | Onset |  |  | OD | OS | OU | Rod | Cone |
| 1 | GUCY2D | c. $2512 \mathrm{C}>\mathrm{T}$ | M | 36 | 16 | Dominant | PV | 0.10 | 0.10 | APM, TDP | Normal | Extinguished |
| 2 | GUCY2D | c. $2513 \mathrm{G}>\mathrm{A}$ | M | 5 | EC | Isolated | PV, PP, NYS | 0.06 | 0.06 | AV | Mildly reduced | Extinguished |
| 3 | PRPH2 | c. $946 \mathrm{~T}>\mathrm{G}$ | M | 0.3 | FMB | Isolated | NYS | LP | LP | NA | Mildly reduced | Severely Reduced |
| 4 | UNC119 | c. $259 \mathrm{G}>\mathrm{A}$ | M | 3.5 | 3.25 | Isolated | PP | NA | NA | APM, AV | NA | NA |

M, male; NA, not available; BCVA, best corrected visual acuity; EC, early childhood; FMB, first few months after birth; PV, poor vision; NYS, nystagmus; PP, photophobia; LP, light perception; AV, attenuated vessels; APM, atrophy and pigmentation deposits of the central macula; TDP, temporal disc pallor.
analyzed in this study. The mutation frequency for $G U C Y 2 D$ was $1.54 \%$ (2/130), $0.77 \%$ for PRPH2 and $0.77 \%$ for UNC119.

The mutation spectrum and frequency for certain CORDrelated genes have previously been reported $(17,21,28,31,41)$. The systematic screening of 10 genes (AIPL1, CRX, GUCA1A, GUCY2D, PITPNM3, PROM1, PRPH2, RIMS1, SEMA4A and UNC119) responsible for autosomal dominant CORD identified mutations in 25/52 (48.1\%) families. The mutation frequency of individual genes in this cohort is as follows: GUCY2D (23.0\%), PRPH2 (11.0\%), GUCA1A (8.0\%), CRX (4.0\%) and PROM1 (2.0\%) (27). For individual gene analysis in different populations, the frequency of CORD-associated GUCY2D mutations has been detected in $11.0 \%$ of Japanese patients (42) and in $40.0 \%$ of European and American patients (33). Mutations in several other genes have been detected in a small proportion of patients with CORD, such as CNGB3 mutations in $5.0 \%$ of patients from the Netherlands (43), AIPL1 mutations in $3.6 \%$ of patients from the USA (3) and SEMA4A mutations in $8.0 \%$ of patients from Pakistan (11). However, the mutation spectrum and frequency for the majority of CORD-related genes have not been well evaluated. For a few genes, mutations have only been reported in 1 or 2 CORD families, such as the $\mathrm{c} .2459 \mathrm{G}>\mathrm{A}$ mutation of RIMS1 in a British family (44), the c.1878G $>\mathrm{C}$ mutation of PITPNM3 in 2 Swedish families (7) and the c.524dup1 mutation of CDHR1 in a family from the Faroe Islands (16). It is unclear as to whether this is due to the rare variants in these genes or a lack of subsequent studies. Comprehensive evaluation of these genes in various ethnic populations based on a large number of cases would provide a better overview of the mutation spectrum and frequency, which would be beneficial for use in personalized gene diagnosis and genetic counseling.

Using a similar strategy to this study, our previous study on Leber's congenital amaurosis (LCA) detected mutations in approximately half of the 87 families tested, based on Sanger sequencing of exons with reported mutations in 15 LCA-related genes (29); this correlated with other reports based on the individual analysis of one or several genes. However, in the present study, only 4 mutations were identified in 4/130 families with CORD, which is lower than previously reported. It is possible that the mutation spectrum and frequency of these genes may differ in Chinese patients than in those with different ethnic backgrounds, with frequent mutations in exons not covered in this study. It is also possible that the genetic causes of CORD in Chinese patients have not yet been identified. To answer these questions, additional comprehensive evaluation of these patients with other methods, such as exome sequencing, is required.

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