Supplementary material

Quality Control Procedures

Following quality-control procedures, samples yielded between 5.3 and 7.4 Gb of high quality, aligned data. This amount of data represented mean target coverage between 44.2–62.3×, percentages of targets covered at greater than or equal to 10× of 48–57%. Sequence alignment and variant calling were performed against the reference human genome (UCSC hg19) using bwa(20) and the Genome Analysis Toolkit(21). PCR duplicates were removed prior to variant calling using the Picard software (http://picard.sourceforge.net/index.shtml).

Molecular analysis of the *CECR1* gene

All exons were amplified and Sanger sequenced. The PCR reaction consisted of 0.8x FastStart (FS) PCR Master mix (Roche, Welwyn Garden City, UK) and 0.4 µM primers each in a total of 23 µl. For three primer pairs, we added 4% DMSO (Table e-1).

The PCR cycle consisted of a touchdown (TD) PCR cycle comprising an initial denaturation step at 95°C for 5 min; 20 cycles of 95°C for 15 s, of 65°C for 30 s that decreased 0.5°C for every cycle, and 72°C for 30 s or 45 s depending on the primers (see Table e-1); extra 10 cycles of 95°C for 15 s, of 55°C for 30 s, and 72°C for 30 s or 45 s; and a final extension step at 72°C for 5 min. The three primer pairs amplified with DMSO ran in a constant (CT) PCR cycle consisting of an initial denaturation step at 94°C for 5 min; 30 cycles of 94°C for 30 s, constant annealing temperature at 59°C for 30 s, and 72°C for 30 s; and a final extension step at 72°C for 5 min. Table e-1 summarizes the PCR conditions used for each primer set. We increased the PCR cycles from 30 to 40 cycles for three samples that did not amplify the first time for exons 2, 3, 4, 8 and 9.

Amplification was confirmed by electrophoresis of the PCR products using a 1.5% agarose gel. After confirmation, PCR products were cleaned-up with a mixture of 0.2 U Fast-AP (Thermo Scientific, Leicestershire, UK) and 1 U Exonuclease I (Thermo Scientific, Leicestershire, UK) in water. Sequencing PCR consisted of 0.9x Sequencing Buffer, 0.12x of BigDye Terminator v3.1 Ready Reaction mix (ABI, Bleiswijk, Netherlands), 0.9 µM of one of the primers (all exons were sequenced using the forward and reverse primers) and 5 µl of PCR product in a total of 11 µl. Purification of the

sequencing PCR was performed with the Dye Terminator Removal Kit (Thermo Scientific, Leicestershire, UK) as per manufacturer's instructions. Samples were run in a 3730xl DNA Analyzer and Sanger sequencing results were analyzed with Sequencher™ (Gene Codes Comporation, Ann Arbor, USA).

ENST00000399837.6 was used for primer design (Table e-1) and as the reference for sequence analysis and the protein reference used was NP_001269154.1.

Table s-1: PCR conditions used for the molecular analysis of the *CECR1* gene

Exon	Forward primer	Reverse primer	Reaction	PCR cycle	Extension
2	CCTTCAATATCTTGCCTTCTGG	TGAGTGAGGCTTTCTCTGCTC	FS	TD	45 sec
3	CCCTCCTTTGTCCCCAAG	ACCAAGGGAGACACCTACCC	FS + DMSO	CT	30 sec
4	GGATATGCAAGGTGGGTAGC	TCCATTTCAGGTTTCCTTGC	FS + DMSO	CT	30 sec
5	GTGGCGTCTCTCACTGCTC	GTAGCTCTGCCTGCATCCC	FS	TD	30 sec
6	GAAACGGGACCTGCACAC	ATGTCAGGGTACCAACAGGC	FS	TD	30 sec
7	AGGCTGTGTGACAAAGGTCC	CAGGAAAGGGCTCTGGAAAC	FS	TD	45 sec
8	TATTTGAGGATGGTCGGGG	AAAGGCCCCTCCTGAATAAC	FS	TD	45 sec
9	TGATGGGAAGATGAAGGGAG	GCCAGCAGCAAGGACTAAAC	FS + DMSO	CT	30 sec
10	GTTTAGTCCTTGCTGCTGGC	CAGCCAAGTGCTTCTCACAG	FS	TD	30 sec

Table s-2: Number of individuals carrying *CECR1* mutations in the cohort studied.

CECR1 mutation	Bi-allelic mutation	Heterozygous mutation
NM_001282225.1:c.752C>T:p.Pro251Leu	2	1
NM_177405:c.[355A>G];[424G>A], p.[(T119A);(G142S)]	4	6

The Pro251Leu was identified in homozygosity in an Iraqi/Iranian family[14]. The p.[(T119A);(G142S)] was found in compound heterozygosity in a Portuguese family[6].
Abbreviations: *CECR1*: Cat Eye Syndrome Chromosome Region Candidate 1 gene

Table s-3. Coding variants identified in the individuals without *CECR1* mutations (n=26).

Variant	Position	Nucleotide change	rs ID	N (het/hom)	MAF in gnomAD
p.Leu46Leu	22:17690430	C/G	rs7289141	3/0	0.15
p.Asn53Asn	22:17690409	G/A	rs362129	15/7	0.43
p.His335Arg	22:17669306	T/C	rs2231495	9/2	0.34
p.Tyr453Tyr	22:17662793	A/G	rs7289170	14/4	0.26
p.Glu489Gln	22:17662444	C/G	rs45497794	1/0	$1.30x10^{-4}$

Variants according to NM_001282225.1; Position - Chromosomal position of the variant according to GRCh37; Rs ID - Reference SNP cluster ID; N (het/hom) – number of samples heterozygous or homozygous for each variant in the cohort studied; MAF in gnomAD – frequency of the alternative allele in the general population in gnomAD (http://gnomad.broadinstitute.org/), including 123,136 exome sequences and 15,496 whole-genome sequences from unrelated individuals.

Supplementary Data:

Material and Methods

Table s-1: PCR conditions used for the molecular analysis of the *CECR1* gene

Table s-2. Number of individuals carrying *CECR1* mutations in the cohort studied.

Table s-3. Coding variants identified in the individuals without *CECR1* mutations (n=26).