Appendix 1

Supplemental Methods

Microsatellite and markers analysis

To detect a possible deletion in chromosome 2, twenty-three chromosome markers were analyzed in all the members of family PCG-47. Markers were amplified from genomic DNA (primers and conditions available upon request). For the markers D2S367, D2S2230, D2S177, D2S1346, D2S2726, D2S2974, D2S2238 and D2S2259, fragments were amplified by PCR and analyzed in 8% polyacrylamide gel electrophoresis. The presence of one or two bands indicated that the sample contained one or two alleles, respectively. When the band resolution was inconclusive, PCR fragments were sequenced to identify the presence of one or two alleles. The markers SHGC-100880, SHGC-148149, WIAF-1508, D2S2571 and SHGC-68716 were analyzed by quantitative PCR with Lightcycler FastStart DNA Master Plus SYBR Green Kit to determine the gene dose of marker. Additionally, we designed ten primer pairs (primers and PCR conditions upon request) to determine the exact breakpoint of the deletion by analyzing the gene dosage with Lightcycler FastStart DNA Master Plus SYBR Green Kit.

Long-Range PCR

To determine the extent of the deletion in chromosome 2, long-range PCR was performed using the primers FAM-DEL1-F and C2O58-DEL1-R (primers and conditions available upon request), and TaKaRa LA PCR Amplification Kit Version 2.1 (Takara Bio, Millipore, Spain) according to the manufacturer's instructions. PCR reactions were performed in a total volume of 50 μl using 1X LA Buffer II (Mg2+ plus), 400 μM dNTP mixture, 0.5 μM of each primer and 2 μl of template DNA. Thermal cycling consisted of 30 cycles of denaturation at 94ºC for 30 seconds and annealing and extension at 68ºC for 15 minutes. PCR products were analyzed with a 0.8% agarose gel. A 10 kb-PCR product obtained in alleles with the deletion was sequenced using the "primer-walking" technique performed by Stab Vida (Oeiras, Portugal)

Gene dose assessment by real-time PCR

For the quantification of the *CYP1B1* gene copy number we amplified four fragments representing the three exons of *CYP1B1* in the LightCycler System, using primers and probes from Universal Probe Library (Roche), and the LightCycler Taqman Master (Roche). qPCR were set up in duplicate in a total volume of 20 μl per capillary. One reaction mixture contained 4 μl of Master Mix, 0.5 μM of each primer (forward and reverse), 0.1 μM of probe and 5 μl of DNA. (primers, probes and PCR conditions available upon request).

To normalize results, quantification of a single copy gene (B-globin) was done in all the samples using primers for the B-globin gene and Lightcycler FastStart DNA Master Plus SYBR Green Kit (Roche). PCR reactions were performed in a total volume of 20 μl per capillary. One reaction mixture contained 4 μl of Master Mix, 0.5 μM of each primer (forward and reverse) and 5 μl of DNA.

All the experiments were also performed in a control sample known to have the two alleles for *CYP1B1*.

The gene dose was calculated as follows:

$$\text{Ratio} = \frac{\text{Conc. target gene (sample)}}{\text{Conc. B} - \text{globin (sample)}} : \frac{\text{Conc. target gene (control)}}{\text{Conc. B - globin (control)}}$$

We considered:

- When ratio= 0.5 [0.38-0.59] → Deletion of one allele
- When ratio= 1 [0.83-1.35] → No deletion

Results

Table S1. Single nucleotide polymorphisms of *MYOC* and *CYP1B1* identified in the cohort studied

Gene	Location	Nucleotide change	Amino acid change	SIFT prediction	PolyPhen prediction
MYOC	Exon 1	c.227G>A	p.Arg76Lys	Damaging	Benign
MYOC	Exon 1	c.366C>T	p.Gly122Gly	Tolerated	-
MYOC	Exon 1	c.425C>T	p.Ala141Ala	Tolerated	-
MYOC	Exon 2	c.855G>T	p.Thr285Thr	Tolerated	-
MYOC	Exon 3	c.906G>T	p.Asp302Asp	Tolerated	-
MYOC	Exon 3	c.975G>A	p.Thr325Thr	Tolerated	-
MYOC	Exon 3	c.1041T>C	p.Tyr347Tyr	Tolerated	-
MYOC	Exon 3	c.1193 A>G	p.Lys398Arg	Tolerated	Benign
MYOC	Exon 3	c.1278C>T	p.Val426Val	Tolerated	-
CYP1B1	Exon 2	c.46C>T	p.Leu16Leu	Tolerated	-
CYP1B1	Exon 2	c.142C>G	p.Arg48Gly	Tolerated	Benign
CYP1B1	Exon 2	c.355G>T	p.Ala119Ser	Damaging	Benign
CYP1B1	Exon 2	c.564C>A	p.Gly188Gly	Tolerated	-
CYP1B1	Exon 2	c.729G>C	p.Val243Val	Tolerated	-
CYP1B1	Exon 3	c.1294G>C	p.Leu432Val	Tolerated	Benign
CYP1B1	Exon 3	c.1347T>C	p.Asp449Asp	Tolerated	-
CYP1B1	Exon 3	c.1358A>G	p.Asn453Ser	Damaging	Possibly Damaging

Table S2. Mutations of *MYOC* and *CYP1B1* identified in the cohort studied

Gene	Mutation	SIFT prediction	PolyPhen prediction	1000Genomes frequency	EVS frequency
MYOC	p.Ala427Thr	Damaging	Probably damaging	0.0%	0.0%
MYOC	p.Gln368Stop	n/a*	n/a*	0.0%	0.1%
MYOC	p.Glu218Lys	Tolerated	Probably damaging	0.0%	0.0%
MYOC	p.Glu352Lys	Damaging	Probably damaging	0.0%	0.0%
MYOC	p.Lys39Arg	Tolerated	Benign	0.0%	0.0%
MYOC	p.Thr293Lys	Tolerated	Benign	0.0%	0.1%
MYOC	p.Tyr479His	Damaging	Probably damaging	0.0%	0.0%
MYOC	p.Val426Phe	Damaging	Probably damaging	0.0%	0.0%
CYP1B1	p.Ala443Gly	Tolerated	Benign	0.3%	1.9%
CYP1B1	p.Arg355fsX69	n/a*	n/a*	0.0%	0.0%
CYP1B1	p.Arg368His	Damaging	Probably damaging	0.3%	0.2%
CYP1B1	p.Arg469Trp	Damaging	Probably damaging	0.0%	0.0%
CYP1B1	p.Asp449fsX6	n/a*	n/a*	0.0%	0.0%
CYP1B1	p.Glu229Lys	Damaging	Possibly damaging	0.5%	0.4%
CYP1B1	p.Gly61Glu	Damaging	Probably damaging	0.0%	0.0%
CYP1B1	p.Thr404fsX30	n/a*	n/a*	0.0%	0.0%
CYP1B1	p.Tyr81Asn	Damaging	Probably damaging	0.7%	0.3%

*n.a.: Not available; EVS: Exome Variant Server