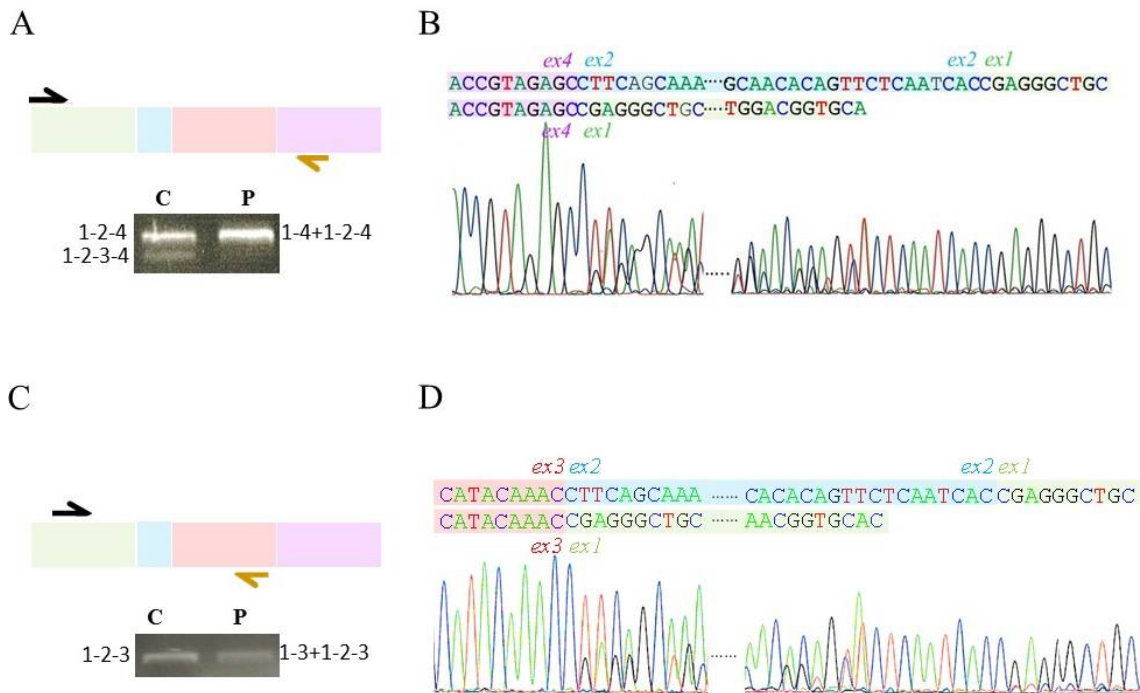




Supplementary Figure 1. *MCAT* intron 1 / exon 2 region encompassing pathological c.424-2A>G variant, and splicing score predictions. The consensus splice site predicted in the wildtype sequence (reference sequence) is lost in the sequence harbouring the c.424-2A>G change (Mutated sequence), according to the five prediction programs available through the Alamut software. This is predicted to result in the skipping of exon 2.



Supplementary Figure 2. RT-PCR and Sanger sequencing analysis of leukocyte mRNA from control and index case II-1. Simplified representations of four *MCAT* exons (green = exon 1; blue = exon 2; red = exon 3; purple = exon 4) and agarose gel images showing cDNA products amplified using forward and reverse primers (arrows) in (a) exons 1 and 4 and (c) exon 1 and 3 (C = control; P = case II-1). Exonic composition of cDNA products amplified from II-1 leukocytes was determined by Sanger sequencing using reverse primers (see gold arrows in left panels) in (b) exon 4 and (d) exon 3. Sequence electropherograms obtained from control cells are not shown.

p.E347K

Homo Sapiens	F	P	Q	T	F	E	V	G	P	G	R
Rattus norvegicus	F	P	S	T	F	E	V	G	P	G	Q
Mus musculus	F	P	S	T	Y	E	V	G	P	G	Q
Canis familiaris	F	P	K	T	F	E	V	G	P	G	K
Gallus gallus	F	P	Y	T	Y	E	V	G	P	G	K
Xenopus tropicalis	F	P	R	T	F	E	A	G	P	G	K
Tetraodon nigroviridis	F	P	Y	T	Y	E	V	G	P	G	K
Drosophila melanogaster	F	P	R	T	F	E	C	G	P	G	K
Caenorhabditis elegans	F	P	R	F	Y	E	V	G	A	G	R
Escherechia coli	A	T	D	F	T	E	C	G	P	G	A

Supplementary Figure 3. Alignment of MCAT sequence showing strict conservation of glutamic acid 347 across species, from human to bacteria. Numbering of residues refers to NM_173467.4.