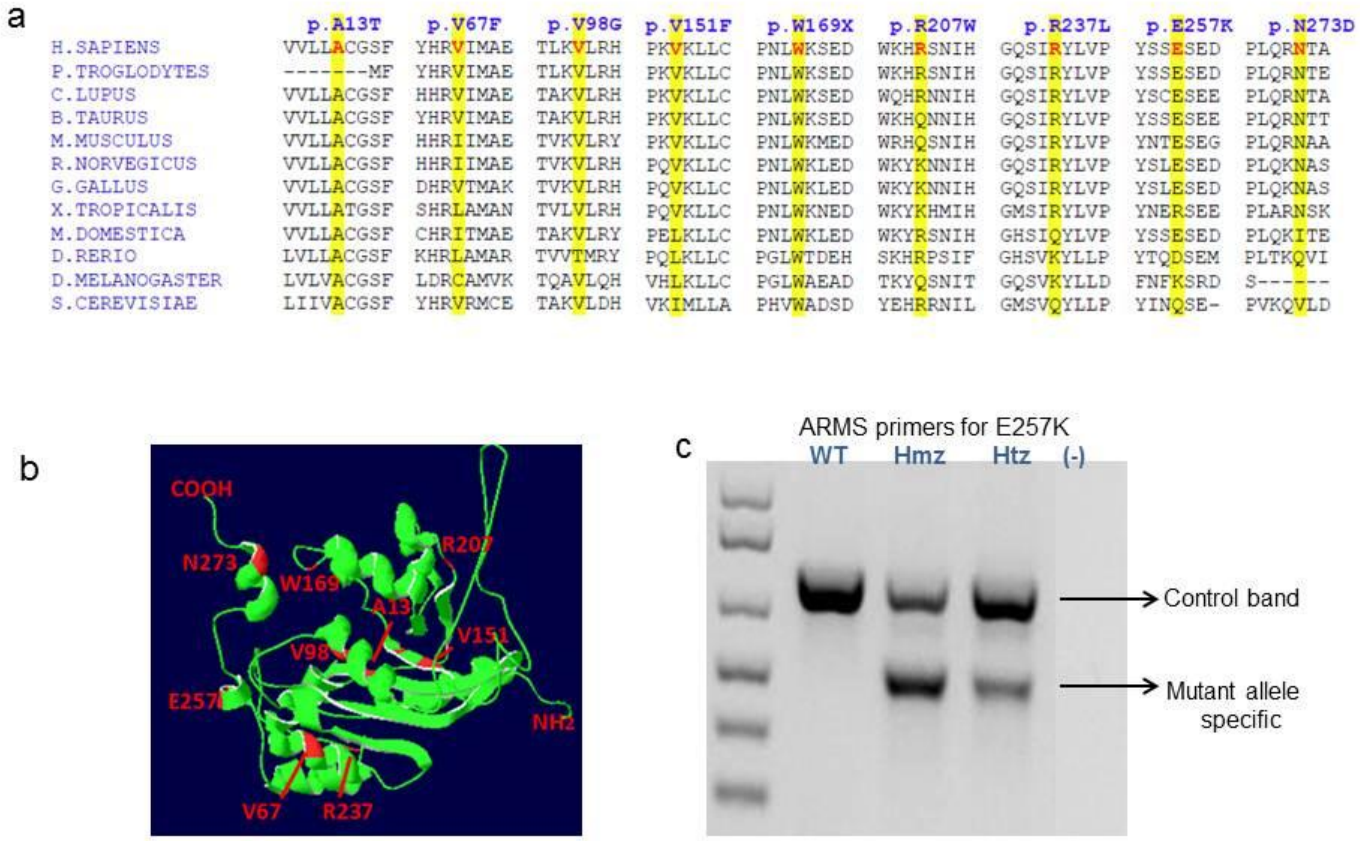
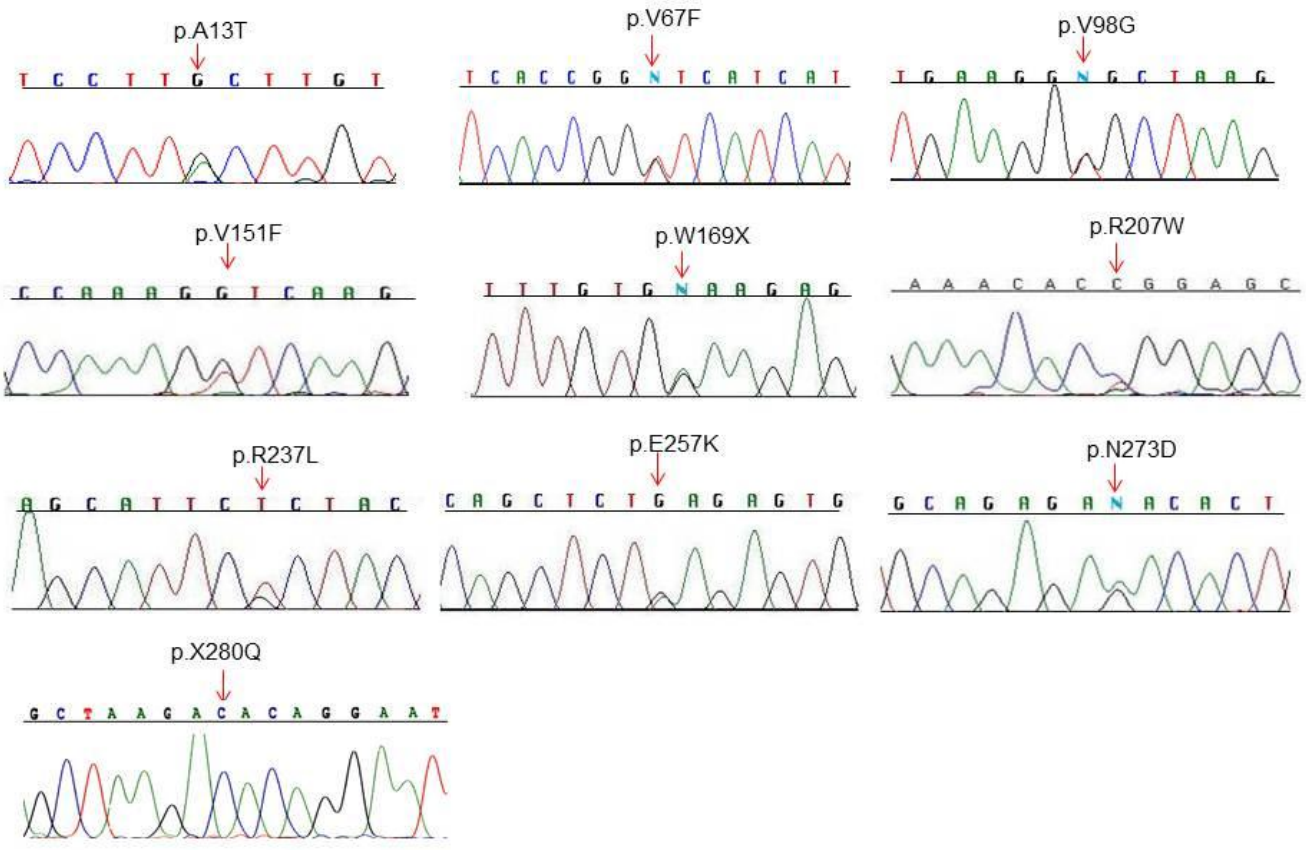


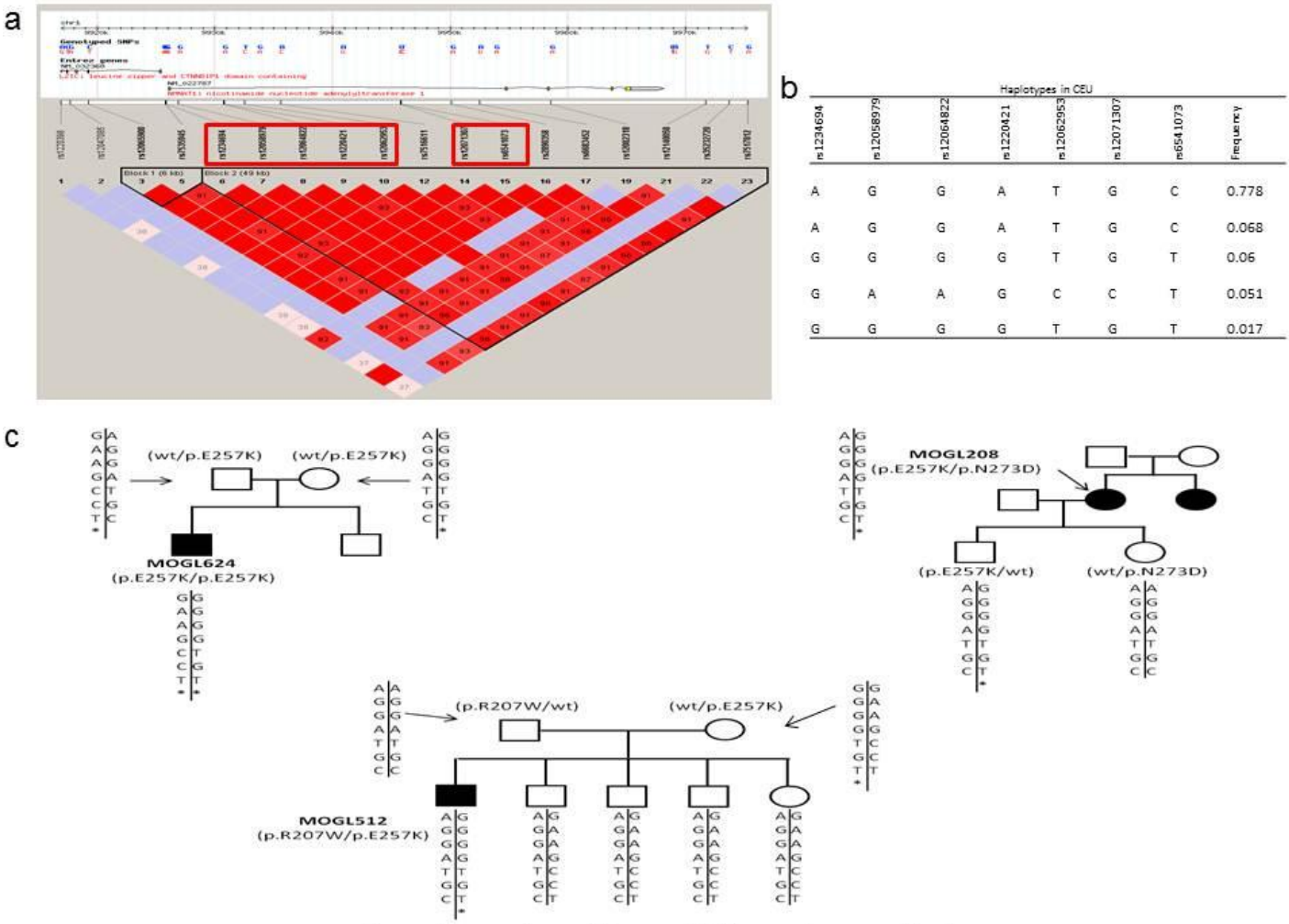
Supplementary Figure 1 Koenekoop *et al*

Supplementary Figure 1 Summary of *NMNAT1* substitutions. (a) The conservation of mutations across different species. (b) Crystal structure of NMNAT1. All variants found in LCA patients are indicated. (c) PCR experiment with ARMS primers to identify the p. Glu257Lys allele in *NMNAT1*. We developed ARMs primers and show here the WT and mutant alleles. Primers: flanking primers forward: CCCTCATCCTTGAAAAGCAC, reverse: TCCCAAAGTGCTGGGATTAC, allele specific primer: AGCATTCCTGTCTTCACTCTT. Conditions are available upon request.



Supplementary Figure 2 Koenekoop *et al*

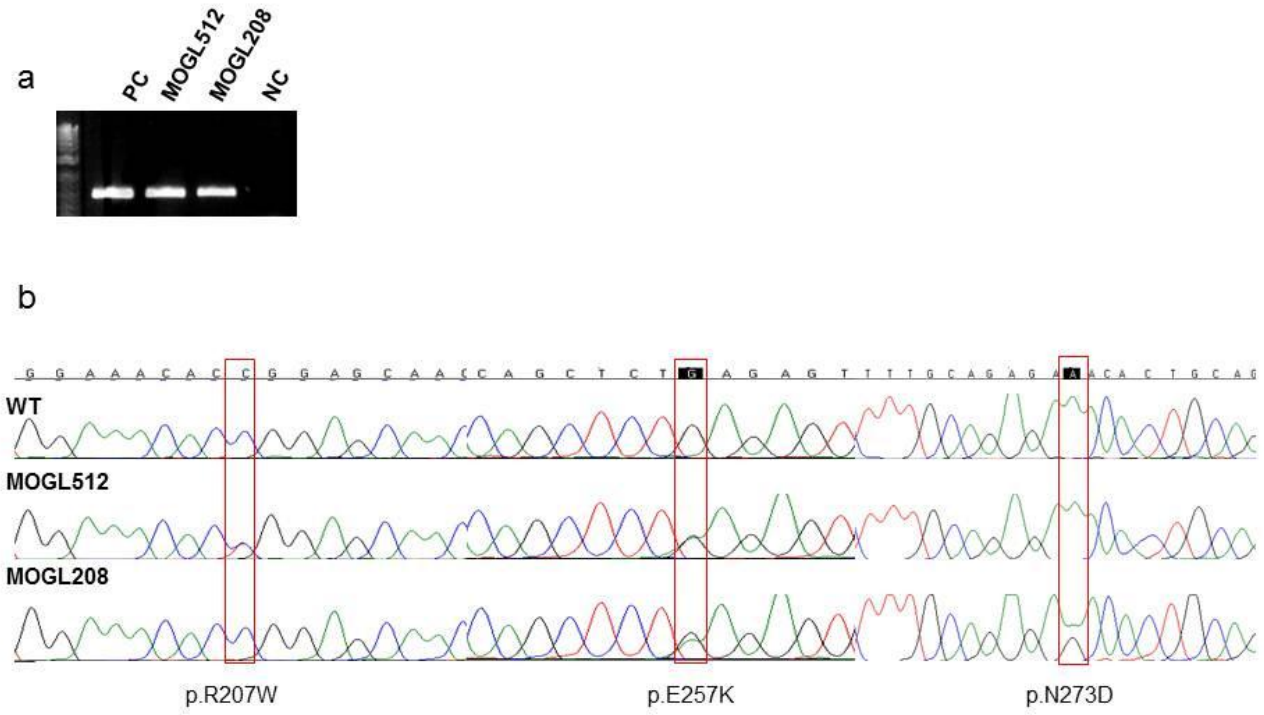
Supplementary Figure 2 Sanger sequencing chromatograms showing all mutations found in this study.



Supplementary Figure 3 Koenekoop *et al*

Supplementary Figure 3 Haplotype analysis. (a) A 49kb block from rs1234694 to rs7517812 covers the NMNAT1 gene in European ancestry population (CEU). To test whether the p. Glu257Lys mutation is in a common haplotype shared by the patients, we selected 7 tagging SNPs near the 5' of p. Glu257Lys for genotyping. The 3' tagging SNPs were not selected because the excess amount of repetitive elements near those tagSNPs would cause non-specific PCR products. rs6541073 is the closest SNP to Glu257Lys , about 19kb away. CEU: Utah residents (CEPH) with Northern and Western European ancestry. (b) The haplotypes and their frequency in CEU population are listed. (c) The mutation p. Glu257Lys is linked with the GGGGTGT haplotype in family 512, 208 and 624. SNP genotype for each genotyped individual

is listed from top to bottom according to the sequential order on the chromosome from 5' end to 3' end. SNPs are phased to each chromosome according to the pedigree information. * represent the p. Glu257Lys mutation. Family 512 and 208 are French Canadian and family 624 is from Saudi Arabia. Family 512, 208 and 624 share the same GGGGTGT haplotype carrying p. Glu257Lys mutation. It is worth to note that there are two haplotypes carrying p. Glu257Lys in family 624 from Saudi Arabia.



Supplementary Figure 4 Koenekoop *et al*

Supplementary Figure 4 *NMNAT1* mutations do not affect transcription. *NMNAT1* transcription is examined in the peripheral blood of patient MOGL 208 and 512. (a) *NMNAT1* mutant alleles are transcribed in patients. RT-PCR was carried out using total RNA extracted

from patient blood samples and the DNA band specific for *NMANT1* is present in both patients as well as positive controls (PC) using placenta RNA. The band is absent in the negative control without RNA template. (b) To further confirm that both alleles are transcribed, RT-PCR bands were purified and sequenced by Sanger sequencing. Sequencing chromatograms of wild type control as well as MOGL208 and MOGL512 are shown. Base pair positions corresponding to mutations carried by these two patients are highlighted by red boxes. Consistent with the genotype of each patient, both p.Arg207Trp and p. Glu257Lys alleles are present in transcripts from MOGL512 and p. Glu257Lys and p.Asn273Asp alleles are present in transcript from MOGL208.

Supplementary Table 1 *In silico* analysis of *NMNAT1* mutations.

Mutation	Protein Change	Polyphen	Sift	GERP score	I-Mutant (Kcal/mol)	Mupro (Kcal/mol)	Blosum
c.37G>A	p.Ala13Thr	possibly damaging	affect protein function	3.92	-0.78	-1.02	0
c.199G>T	p.Val67Phe	possibly damaging	affect protein function	-0.53	-2.18	-0.93	-1
c.293T>G	p.Val98Gly	possibly damaging	affect protein function	4.93	-1.22	-2.22	-3
c.451G>T	p.Val151Phe	possibly damaging	affect protein function	2.04	-2.32	-1	-1
c.507G>A	p.Trp169Ter	stop	stop	5.16	N/A	N/A	
c.619C>T	p.Arg207Trp	probably damaging	affect protein function	2.95	-1.16	-1.3	-3
c.710G>T	p.Arg237Leu	probably damaging	affect protein function	3.1	-0.27	-1.03	-2
c.769G>A	p. Glu257Lys	benign	tolerated	5.01	-1.14	-1.49	1

c.817A>G	p.Asn273Asp	possibly damaging	tolerated	3.79	-0.45	-1.23	1
c.838T>C	p.Ter280Gln	read through	abnormal protein length	-	-	-	-

Supplementary Table 2 RT-PCR primer sequence.

Primer name	Sequence
Forward primer	tgactgtgatcaccagcagaa
Reverse primer	cccaaatgggaagtctgaaa