SUPPLEMENTAL MATERIAL

Meuwissen et al., http://www.jem.org/cgi/content/full/jem.20151529/DC1

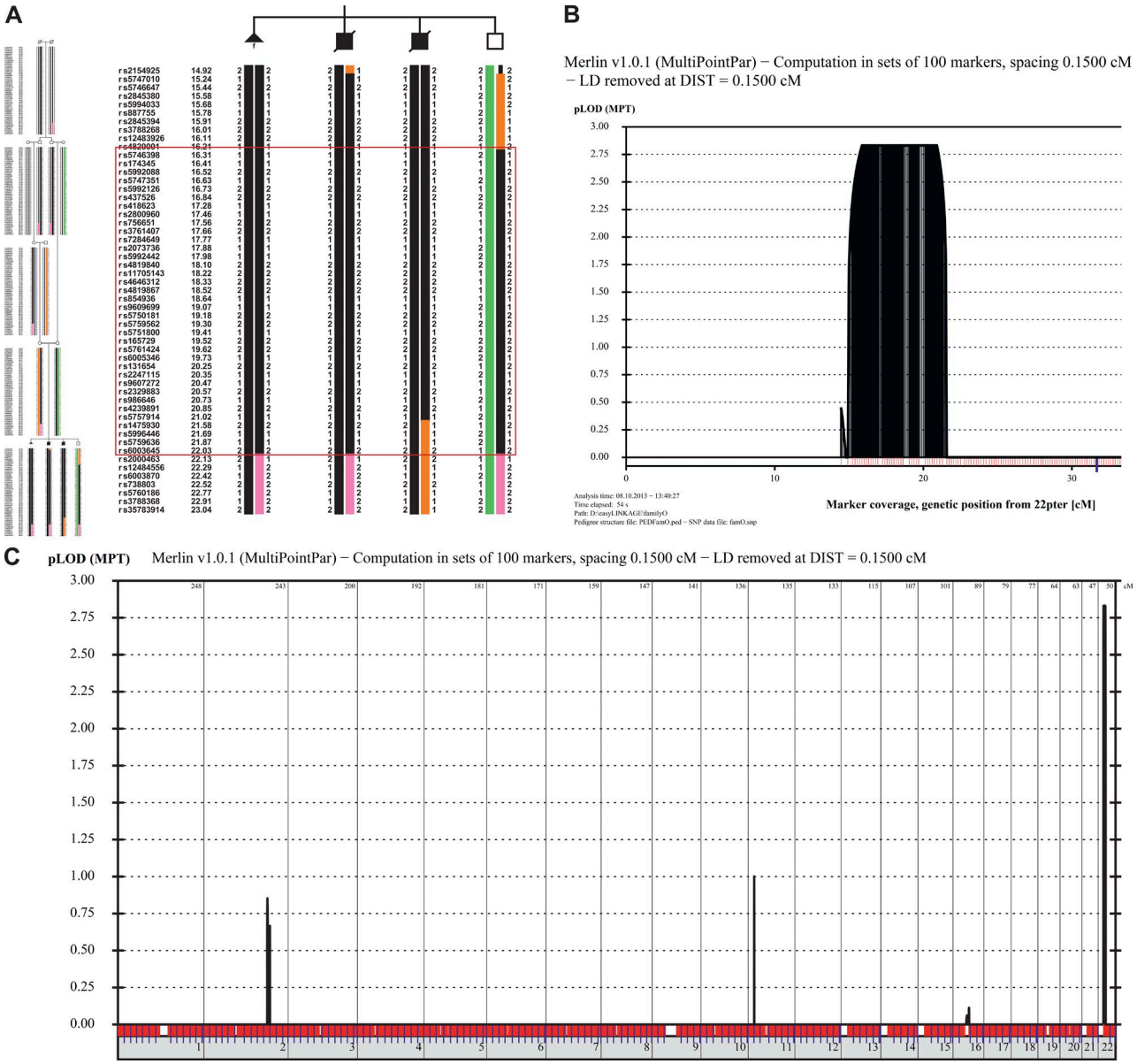

Figure S1. **Linkage data and haplotyping.** (A) Haplotype analysis of family A; the haplotype shared by the affected individuals is located in the red box (A, part of it is included in Fig. 2 A). (B) The linked area on chromosome 22 with a LOD score of 2.83. (C) Genome-wide linkage, confirming that the linked area on chromosome 22 is the only linkage area with a significant LOD score.

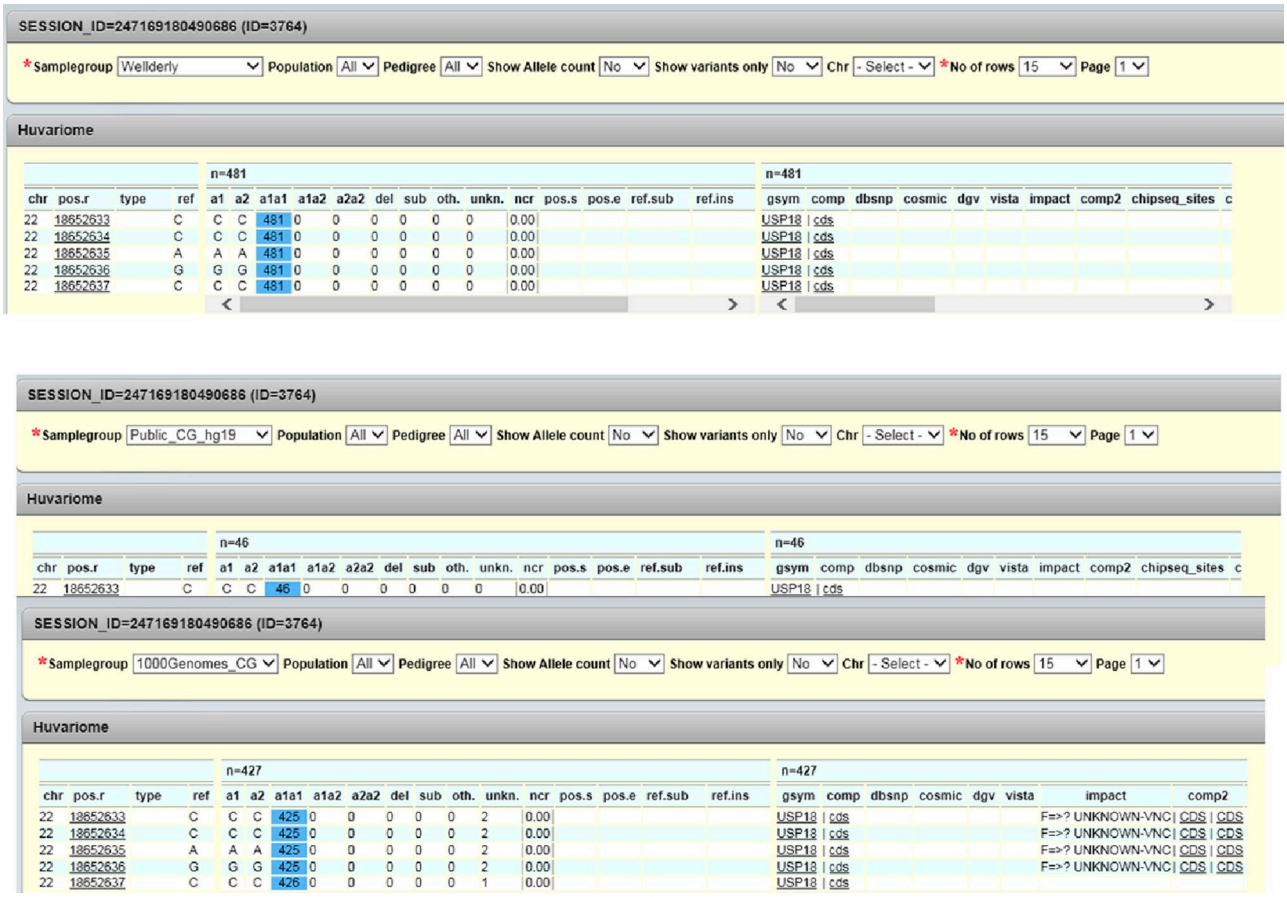

Figure S2. **Complete Genomics control alleles.** Analysis of 952 DNA samples sequenced by CG (whole genome sequencing, containing 481 samples of Wellderly-aged Americans cohort, 46 public CG data from in-house database of the Erasmus Medical Center, and 425 samples from the 1,000 genomes project), which provide full coverage of USP18 shows no c.652C>T variant.

Figure S3. **Alignment of USP18 and USP41.** The sequences of *USP18* and *USP41* were aligned using NCBI Blast. The resulting alignment highlights the extensive similarity of these sequences. The Sanger sequencing of *USP18* identified several SNPs that could be mapped to the *USP41* gene and which were therefore not regarded as possible pathogenic *USP18* variants. We classified *USP41* as a pseudogene because the data in the Ensembl database indicated an absence of UTRs.

Table S1. **Exome sequencing data**

Data	P03	Mother	P01	P02	Father
Raw data (bases)	7548982198	6097723500	6870304618	5113423960	7913013872
Raw data (Gb)	7.55	6.10	6.87	5.11	7.91
Raw data (n)	74742398	60373500	68022818	50627960	78346672
Duplicate reads (n)	26999908	17033872	21416278	14531319	30411386
Mapped reads (n)	68345462	55487730	62369119	46790969	71743856
Data mapped to target region (Gb)					
RefSeq (CDS)					
Data mapped to refseq Coding Segments (bases)	3147011420	2528692389	2798328026	2126940432	3276055579
Data mapped to refseq Coding Segments (Gb)	3.15	2.53	2.80	2.13	3.28
Bases in refseq	33327179	33327179	33327179	33327179	33327179
Bases with 1X coverage	32647651	32568203	32622555	32521253	32634218
Bases with 10X coverage	31088779	30802989	30852060	30052398	31281767
Bases with 20X coverage	29045082	28158446	28464365	26501230	29542937
% with 1X coverage	97.96%	97.72%	97.89%	97.58%	97.92%
% with 10X coverage	93.28%	92.43%	92.57%	90.17%	93.86%
% with 20X coverage	87.15%	84.49%	85.41%	79.52%	88.65%
Mean coverage	94.43	75.87	83.97	63.82	98.30

Table S2. **Filtering steps**

Filtering of variants in the exome sequencing data for family A	
Total variants in each family member	~100,000
Removal of low-quality and non-coding variants	~20,000
Application of homozygous recessive model	78
Variants in linkage region	14
Removal of common variants (<0.01)	1[a]

[a]No functional effect of this variant could be demonstrated

Table S3. **Primers used for the Sanger sequencing of missing exons**

Sanger sequencing primers for exons with low coverage in the linked area				
Gene	**Exon**	**OMIM gene**	**Forward primer**	**Reverse primer**
POTEH	1,2,3,4,5,6,7,8,9,10,11	yes		
OR11H1	1	no		
CCT8L2	1	no		
TPTEP1	2,3,4,5,6,7,8,9	no		
ANKRD62P1-PARP4P3	1,3,4,5,6,7,9	no		
XKR3	1	yes		
CECR7	1,4	yes		
CECR3	1,2,3	no		
MICAL3	19	yes		
FLJ41941	1,2	no		
USP18	5,7,8,9,10,11	yes		
GGT3P	whole gene	no		
DGCR5	3	no		
(NT_002733)	4			
DGCR9	1	no		
DGCR10	1	no		
DGCR14	1	yes	ATAGCATCCTCCCCGCCACC	CCACAGACGTCTTCCTCTGCCC
LOC150185	1	no		
TBX1 (NM_080646)	3	yes	CCCGCGGGTCATGATCTCCG	GGTGTTAGGAGGGGAGCGCC
RANBP1	1	yes	GAGTGTCCGCCTCCTGAGCC	CAAACGCCCCAACTCCGC
LOC284865	1,2,3,4	no		
LOC150197	1	no		
LOC729444	1,2,3,4,5,6,7,8,9,10,11,12	no		
TMEM191B	1,2,3,4,5,6,8,9	no		
PI4KAP1	1,3,4,6,8,9,10,11,12,13,14,15	no		
RIMBP3	1	yes		
POM121L4P	1	no		
TMEM191A	1,3,4,5,8	no		
PI4KA (NM_002650)	33	yes	CGGGTTGAACAGAAGGCAGCC	AAGACGCTGTGGTGGTGGGG
	34		GCCAGTCAGCCCAGAACCCC	GCCCCGGACCCAGTTAGAGC
	35		TGTGTACAGTGTGTCATGGGGCG	GGGGTTTGGGGCGATGGAGC
	36		–	–
	45		–	–
	46, 47		TGGAAACTGACTCTGGCTCACC	AGGGATCTGACTGAGTGAGGGC
	48		TGGGGTTTTGAAGAAGTGATCCCC	AGATTGGACTCTGGCGGGCC
	49		–	–
	50		–	–
	51		GCCCACTTCCACAGAGAGCCC	GGCCAGGCACCCTCTACAGC
	52		GCTCCTTCCTCCACTTCCTCCC	GGGCAGGATCGTGGGGAAGG
	54		–	–
	55		CACGGATGGAAGCGGTTTGGC	TTGTCGCTGCAGTCCATGGC
TUBA3FP	1,2,3	no		
LOC400891	ex3,4,7,9,10,11	no		
BRCP2	ex1,2,3,4,6	yes		
POM121L8P	ex1,2,3,4,5,6,7	no		
RIMBP3C	ex1	yes		
TMEM191C	ex1,2,3,4,5,6,7,8,9	no		
PI4KAP2	ex1,2,3,4,5,6,7,9,10,11,12,13,14,15,17	no		
RIMBP3B	ex1	yes	–	–
UBE2L3	ex3	yes	AGTTTTGTTCCCGGATTAGCTGCC	CGGTGGCACGTGTCAATTTCC
TOP3B	ex18	yes	TGTGGCCCAGTGACATGAGG	CAGGGGACGGAGAAGTGTGG
	ex18 internal primer 1		GGGACGAGGGATCCAGGAGC	CGACCCTGCCTTCTCCCTGG
	ex18 internal primer 2		GGGCTGCGTCTTTTGTGACC	ATGCAGGCTGAAGGGAGACG
LOC96610	ex1,2,3,4,5,6,7,8,10	no		
LOC648691	ex3	no		
POM121L1P	ex1,2,3,4,5,6,8,9,10	no		
GGTLC2	1	yes	ACCTGTGTCCCCTCCCCACC	CACCTGACCTTGCCTGGCCC
	2		CTGGGTGGGAAAGGGCCAGG	TGCTTCCCTGTGGCCGATGG
	3		TTGGCTCCAAGGTCCGCTCC	ACGGCCAGGGAGAAAAGGGG
	4		GCCGCTCTCGTCAATGTGCC	ACTCTGTGATGATCCAGGCCTCC

Table S3. **Primers used for the Sanger sequencing of missing exons** *(Continued)*

Sanger sequencing primers for exons with low coverage in the linked area				
Gene	**Exon**	**OMIM gene**	**Forward primer**	**Reverse primer**
	5		GGCCCCAACCTGCTCTTCC	AGTGGGGAGACAGGGCAGGG
BCR (NM_004327)	17	yes		
	18			
	19			
	20		CGCACAGTGGTCAGCATGGC	GCTCGGTTCTGGTCCCCTGC
	22		AACTCCAGCACAGCCCAGCC	AGAAGATGGGCAGGACTGGGG
HIC2	ex2,3	yes		

Table S4. **Primers used for the genomic Sanger sequencing (intron and exon primers), qRT-PCR, RT-PCR and 3′ RACE of *USP18***

Genomic *USP18* primers	
USP18ex2F	TCAGTCTCCCCAAACATTTATCTCC
USP18ex2R	CAGGCACAGGAAAGCATGAGC
USP18ex3F	ATGTTGGCCAGGCTGGTCTC
USP18ex3R	GGGAGGCAGAACTTGCAGTG
USP18ex4F	AACCCAGCATGTGCCTCTGC
USP18ex4R	CCTGCAGTTCCCTGACTGTGG
USP18ex4intF	TTCTTGTGTGGCAGGATCACG
USP18ex4intR	CGGCTGTCCTGCATCTTCTCC
USP18ex5F	CCTTGCAGTGGGGTGGAGGG
USP18ex5R	ACAGGGTTTCACTGTGTTAGCCAGG
USP18ex6F	TTGTGAGGAGCTTCTGTCTCTTGG
USP18ex6R	TGAGAGTTCCCACCAGCAAGC
USP18ex7F	GCTGCCATGCTTCCGGTT
USP18ex7R	CCCCATAGGCACGAGTTCCA
USP18ex8F	AACTCCGTGATGTCGCCCCG
USP18ex8R	GGGTGAAGGAAGGGAAAACAGAAAGG
USP18ex9F	CAGAGTCGGGCCTAGTGTGGG
USP18ex9R	CTCAGAGACCCACGCAGCCC
USP18ex10F	TCCAGGCTCTTGGGAGTTGC
USP18ex10R	AATCCATGGGCGTTTCATGC
USP18ex11F	CTGACGCTTGTCCTGTCACG
USP18ex11R	GGTCTGCTGGTGAAAGCATCC
qRT-PCR *USP18* primers	
USP18_qrt_e4-6F	AACGTGCCCTTGTTTGTCC
USP18_qrt_e4-6R	GCAGTCTCTCCACCAAGTGC
Intron primers	
USP18_intr9F	TGAACTGTCTCGTGCCTGTC
USP18_intr9R	AGTGGGGAGTTGGCCTAGAT
USP18_intr10_1F	GATGAGCTCACATAGGGTCCTTGG
USP18_intr10_1R	GAGGAGGAGCCAAGTGTAGTAGCC
USP18_intr10_2F	GATTCGTAGGGGCTTCTTGTATGG
USP18_intr10_2R	TGAGCTATGATCACACCACAGTGC
USP18_intr10_3F	ACCACACCTGGCCTTCTTATATGC
USP18_intr10_3R	AGAGCTTGCAATGAGCTGAGATCC
USP18_intr10_4F	GCTCTGCCTCGTGGGTTCAT
USP18_intr10_4R	TGCAACAGACACCGCACAGA
USP18_intr10_5F	CTGCCTCGTGGGTTCATGC
USP18_intr10_5R	TCCCACTGCCACTGCTCTCC
USP18_intr10_5i1F	CACCCAATCTTTGTCACTTTGTGG
USP18_intr10_5i1R	GGCACAATAATAGCTCACCACAGC
USP18_intr10_5i2F	GGAGGATCACTTCAAGCCAGGA
USP18_intr10_5i2R	TGCATTATGTTTTGTTTGGTTTTTGC
USP18_intr10_5i3F	CTGGGAGGCGGAGGTTGC
USP18_intr10_5i3R	CGTGACAGGACAAGCGTCAGC
USP18_intr10_5i4F	GGAGGGGTGAAAGGCCAACT
USP18_intr10_5i4R	CCTGAGGGGCCTCATGGTTA
Extra genomic primers *USP18*	
USP18_exon10uF	GTTGCGGTGAGCTGAGATCG
USP18_exon10uR	AAGAGGCACAAGGGCACAGG
USP18_9bF	CCTGGAGGGTGCCCACTG
USP18_9bR	GCAGAGCATGAGCCTAGAGCA
RT-PCR primers *USP18*	
USP18_cDNA_F	GTCCCGACGTGGAACTCAGC
USP18_cDNA_R	TCCCACTGCCACTGCTCTCC
USP18_cDNA_int1R	GCAGCAGAAGCATCTGGAAAGG
USP18_cDNA_int2F	GACAGACCTGCTGCCTTAACTCC
USP18_cDNA_int2R	TGTCTTCAGGGGCTTTGAGTCC
USP18_cDNA_int3F	CAGAAACAGCAGCATGCTCACC

Table S4. **Primers used for the genomic Sanger sequencing (intron and exon primers), qRT-PCR, RT-PCR and 3′ RACE of *USP18*** *(Continued)*

Genomic *USP18* primers	
USP18ex2F	TCAGTCTCCCCAAACATTTATCTCC
USP18_cDNA_int3R	TGACCGGAGTCTGCCATTCC
USP18_cDNA_int4F	GCGAGAGTCTTGTGATGCTGAGG
USP18_rt_stop1F	CGCCCTGCACTGCTTCTTCC
USP18_rt_stop_3R	GCTTGATAACTCCCTGGGCTG

Table S5. **Haplotypes of chromosome 22q11 in family A and B using Illumina cyto-snp-850 kb arrays**

Name	Chr	Position	Fam A p1	Fam B p4
rs450718	*22*	*18493980*	BB	AA
rs452579	22	18495470	BB	AB
rs390495	22	18504801	AA	AB
rs2034299	22	18505121	AA	AA
rs1867357	22	18507030	BB	BB
rs8139236	22	18512496	AA	AB
rs1076115	22	18518651	AA	AB
rs4819661	22	18518829	BB	BB
rs975826	22	18520582	BB	BB
rs450703	22	18522098	BB	AB
rs462904	22	18526789	AA	AA
rs466755	22	18530984	AA	AA
rs455758	22	18533335	BB	BB
rs458888	22	18533434	BB	AB
rs458480	22	18539370	AA	AB
rs460036	22	18539553	BB	BB
rs7288409	22	18542311	AA	AA
rs5992985	22	18545634	BB	BB
rs5992990	22	18547507	AA	AA
rs5992165	22	18549174	BB	BB
rs17742344	22	18549641	BB	BB
rs10483096	22	18551780	AA	AA
rs17809705	22	18552662	BB	BB
rs4819666	22	18555134	AA	AA
rs8139802	22	18558511	BB	BB
rs462055	22	18560570	BB	BB
rs12157958	22	18560611	AA	AA
kgp15096395	22	18560780	AA	AA
kgp10315947	22	18560825	AA	AA
kgp15024021	22	18562595	AA	AA
kgp12323098	22	18563052	BB	BB
rs361780	22	18565346	BB	BB
kgp14988429	22	18566702	BB	BB
rs17207360	22	18566917	BB	BB
kgp1211229	22	18567005	AA	AA
exm1584146	22	18567938	BB	BB
rs385130	22	18570175	BB	BB
kgp15048898	22	18570851	BB	NC
kgp1362953	22	18571008	AA	AA
kgp15050974	22	18571241	BB	BB
kgp10333452	22	18571595	AA	AA
kgp15077230	22	18571785	BB	BB
rs361807	22	18571828	BB	BB
kgp12284670	22	18572035	BB	BB
kgp15012795	22	18572274	BB	BB
kgp15085445	22	18572370	AA	AA
rs5992169	22	18572431	BB	BB
rs467998	22	18582650	AA	AA
rs467504	22	18583267	BB	BB
rs3827281	22	18584433	BB	BB
rs17809734	22	18584588	BB	BB
rs9617659	22	18586756	AA	AA
rs5992999	22	18590899	BB	BB
rs361893	22	18594931	AA	AA
rs9617661	22	18595352	AA	AB
rs362043	22	18596449	BB	BB
kgp1383642	22	18597404	BB	BB
rs464901	22	18597502	AA	AA
kgp15088499	22	18598859	BB	BB
rs361540	22	18601415	AA	AA
kgp10583533	22	18602653	BB	BB
kgp15048986	22	18606946	BB	BB
rs9618203	22	18607872	BB	BB

Table S5. **Haplotypes of chromosome 22q11 in family A and B using Illumina cyto-snp-850 kb arrays** *(Continued)*

Name	Chr	Position	Fam A p1	Fam B p4
rs362133	22	18608627	AA	AA
rs2234329	22	18609065	BB	BB
rs2234331	22	18609128	BB	BB
rs361776	22	18609854	BB	BB
rs362249	22	18610110	AA	AA
rs10427839	22	18610146	BB	BB
rs7286465	22	18610798	AA	AA
kgp1376668	22	18611223	AA	AA
kgp10743826	22	18611596	BB	BB
rs8140197	22	18613045	BB	BB
kgp12096200	22	18614445	BB	BB
rs2540620	22	18614874	AA	AB
rs5747490	22	18624958	AA	AA
rs5993010	22	18626900	AA	AA
rs361534	22	18628715	BB	BB
rs5747494	22	18629028	BB	BB
rs7291885	22	18629153	BB	BB
rs9618216	22	18631365	BB	AB
rs5993013	**22**	**18633446**	BB	BB
rs5992185	**22**	**18633978**	AA	AB
rs2252257	**22**	**18640300**	BB	AB
rs3859817	22	18844632	BB	BB
rs2870978	22	18886915	BB	AB
rs2870982	22	18889490	AA	AA
rs454534	*22*	*18891398*	AA	BB

Analysis of 86 SNPs on Illumina cyto-snp-850K-B arrays covering 400-kb area across *USP18* (chr 22: 18483880-18891398, *USP18* spans bp 18632666-18660164) in P1 from family A and P4 from family B, shows a shared allele between the two families. The SNPs in *USP18* are shown in bold, the SNPs adjacent to the haplobloc are shown in italics.

Clinical description of family A

During the first pregnancy of the mother, a routine ultrasound scan at 22 wk of gestation (GW) demonstrated microcephaly, enlarged ventricles, and diffuse, irregular hyperechogenicity in the brain parenchyma (P1). Prenatal MRI at 22 5/7 GW confirmed these findings, in addition to periventricular and (sub)cortical calcifications and subcortical infarcts. The pregnancy was terminated at 23 5/7 GW. Postmortem pathology analysis showed the fetus to be female, with severe microcephaly and a brain weight <<p10 (35 g), hydrocephalus, subcortical infarcts, hemorrhages of the germinal matrix, choroid plexus and dentate nucleus, and calcifications located in the basal ganglia and cortical layers. In addition, the cortex was thin, equivalent in thickness to that of a fetus at 16 wk of gestation.

The second and third pregnancies were initially uneventful. However, at the thirtieth and 32thirty second weeks of gestation, respectively, a decrease in fetal movement was observed, with CTG decelerations, requiring emergency cesarean section in both pregnancies. Immediately after birth, the second child (P2), a boy, required intubation and mechanical ventilation because of respiratory failure. Brain imaging on day 1 showed massive hemorrhages, mostly in the basal ganglia, brain stem, and cerebellum. He developed myoclonic seizures, progressive hypotonia, and lethargy. The EEG showed a slow trace without epileptic activity. The third child (P3), also a boy, was initially able to breathe spontaneously, but he also required intubation and ventilation by the end of the first day. Brain imaging showed initial white matter and cortical abnormalities. Thalamic hemorrhage was subsequently noted. He became increasingly lethargic and the EEG showed a burst-suppression pattern. Liver function defects (high levels of ammonia, SGOT, and SGPT activity, low levels of albumin and clotting factors) were observed, with ascites, but were more severe in P3, and thrombocytopenia (between 14–62 x10E9/l, normal 144–449), with diffuse petechiae was observed in P2. Both had variable lactic acidosis. Ophthalmological abnormalities were observed only in P3, in the form of thin retinal vessels and retinal hemorrhage. Head circumference was normal in both boys. They both developed severe bradycardia and died at the ages of 7 and 17 d, respectively.

A differential diagnostic of AGS was considered for P1, leading to an analysis of the sequences of the TREX1, RNASEH2B (exons 2, 6, and 7), and RNASEH2C (exons 2 and 3) genes. No abnormalities of these genes were observed. Extensive screening was performed for mitochondrial disorders (POLG, TWINKLE, TP, TK2, ANT1, SCO2, SUCLA2, RRMB2, MPV17, DGK, and dGUOK), but normal results were obtained for both patients. In P2, Sanger sequencing of the COL4A1 and COL4A2 genes yielded normal results. Extensive metabolic screening and muscle biopsy results were normal for P2 and P3. In all patients, negative results were obtained for screening for TORCH and parvo B19 infections.