



Supporting Online Material for

Human Hair Growth Deficiency Is Linked to a Genetic Defect in the Phospholipase Gene *LIPH*

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Published 17 November 2006, *Science* **314**, 982 (2006)
DOI: 10.1126/science.1133276

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Supporting Online Material

(1) Materials and Methods

Epidemiologic analysis and families

We obtained genetic information about the affected individuals during a genetic epidemiological study performed in the Mari El and Chuvash populations (Russia). A detailed description of the methodology applied in the epidemiologic analysis was previously published (*S1*). Briefly, the study of the distribution of hereditary diseases in each population involved three main steps. First, special registration cards that included a catalog of hereditary disorders (*S2*) were distributed among local medical assistants, physicians and District Specialists (ophthalmologist, neurologist, dermatologist, etc). The data collected by the local medical personnel were combined with data collected from other medical sources about handicapped patients (e.g., specialized institutional schools for deaf and blind children). Second, all families were visited by clinical geneticists and those with a clearly nonhereditary pathology were excluded from the study. Finally, clinical evaluations were performed by neurologists, ophthalmologists, orthopedic surgeons, dermatologists, pediatricians and clinical geneticists. The collected data were subjected to segregation analysis.

In total, 350,222 individuals living in seven geographic areas in Mari and six geographic areas in the Chuvash Republics were screened. There was no significant difference in the frequency of the total hypotrichosis phenotype between the Mari and Chuvash populations ($\chi^2=1.25$, $p>0.05$). An autosomal-recessive type of inheritance of this disorder was suggested ($q=0.32\pm 0.06$, $p=0.89$). The frequency of the mutant gene was predicted to be at least 1.0-2.0% or higher in these populations. We selected 14 families from Mari and 36 families from Chuvash for molecular genetic analysis. No relationships were found between any of the pedigrees (Fig. *S2*). In four families, the clinical presentations of hypotrichosis were distinct from the hypotrichosis phenotype observed in all other families and no evidence for linkage to chromosome 3q27 was found in these families. Examples of families used for mapping and gene identification are shown in Figs. *S2*, *S5*.

Genotyping, sequencing and mutation analysis.

Multiple STR (simple tandem repeats/microsatellites) markers across the human genome and from chromosome 3q26-27 were selected from the Marshfield and Decode databases(<http://research.marshfieldclinic.org/genetics>; <http://www.decode.com/nrg1/markers/>). In addition, new STRs were identified with the Tandem Repeats Finder (<http://tandem.bu.edu/trf/trf.submit.options.html>) and tested for population polymorphisms. The PCR primers for these STRs are shown in Table *S1*.

PCR was performed in a total volume of 20 μ l with 20-50 ng of genomic DNA and AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA) under the following conditions: initial denaturation 94°C 8 min, 5 cycles consisting of 94°C 30 s, 58°C 30 s, 72°C 1 min, 30 cycles with 94°C 30 s, 55°C 30 s, 72°C 1 min, final extension step on 72°C 15 min. The STRs were tested on ABI371 or ABI3730 (Applied Biosystems, Foster City, CA) and examined on GeneMapper Software V4.0 (Applied Biosystems, Foster City, CA) by microsatellite analyses.

The sequencing of genes for mutational screening was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit from Applied Biosystems, Foster City, CA. To screen for mutations in candidate genes on 3q27, we designed intronic primer oligonucleotides for each exon of *C3orf65* (previous name *LOC651498*), *MAGEF1*, *ETV5* and *MAP3K13*, *TMEM41A*, *LIPH*, *SEN2* genes. The PCR amplification of *LIPH* using intronic primers flanking exon 4 failed in samples from affected individuals. Eight different pairs of PCR primers were designed for the *LIPH* genomic region to identify the boundaries of deletion of exon 4. Interspersed repeats and low complexity DNA sequences were identified by Repeat Masker web resource (<http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker>).

Linkage Analysis

Two-point linkage analysis was calculated between each marker and the disorder using Superlink version 1.5 (<http://bioinfo.cs.technin.ac.il/superlink/>) and Allegro Version 2.0 (S3) (<http://www.decode.com/software/allegro>) and multi-point linkage analysis was performed using Allegro software. We assumed an equal frequency of STR alleles, autosomal-recessive inheritance of the disorder, full penetrance of the phenotype and a common mutation founder for all families analyzed. The frequencies for wild-type and mutant alleles were considered to be 0.99 and 0.01, respectively. Also, since *de novo* mutations of STRs could be misinterpreted as a recombination event, we tested several closely located STRs in key putative recombination spots distal and proximal to the D3S1530-CA5 and D3S1530-D3S3592 intervals.

Identification of deletion breakpoints

To identify intronic boundaries for the *LIPH* deletion, we examined the genomic sequence between exon 3 and exon 5 (9609 bp). The region contains multiple SINE-retrotransposons. To determine the deletion breakpoints, we performed PCR using a series of primers in the breakpoint region on DNA samples from parents and affected individuals. PCR with primers Del D and Del R (Fig. S10) yielded PCR products for mutant deletion allele and wild type allele (Fig. S5). The PCR fragments corresponding to wild type and *LIPH* deletion alleles were separated by 1% agarose gel, purified via QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA) and subjected to sequencing analysis (Fig. S10).

RNA preparation, Real-Time PCR and semi-quantitative PCR

The cDNA panel from human tissues was obtained from Clontech (Human MTC™ Panel I, Clontech laboratories, Mountain View, CA). Human hair follicles were obtained and dissected to retrieve the epithelial portions and dermal papilla as previously described (S4, S5). Briefly, fresh samples of adult human scalp skin from plastic surgery procedures were obtained from the University of Massachusetts Cancer Center Tissue Bank. The skin (1 X 2 cm) was treated with 4 mg/ml Dispase (Sigma-Aldrich, St. Louis, MO) in DMEM (Invitrogen-Gibco, Carlsbad, CA) overnight at 4°C. Using forceps, hair follicles were plucked from the Dispase-treated skin, and segregated into telogen club hairs based on their morphology under a dissecting microscope. Plucked anagen follicles were

dissected to remove the upper outer root sheath corresponding to the bulge region as well as the matrix area (bulb region). The keratinocytes from these regions were cultured on a feeder layer of 3T3 fibroblasts as previously described (S4, S5). Dermal papilla cells were isolated from fresh untreated skin samples and cultured in Chang's media for up to two weeks (Irvine Scientific, Santa Ana, CA).

Total RNA was isolated from the tissue fragments and primary cell cultures using the RNeasy kit (QIAGEN, Valencia, CA), according to the manufacturer's instructions. As indicated in Fig. 3 and Fig. S8 the following specimens were used: *skin* - adult scalp skin, *matrix* - matrix area of plucked follicles, *an. follicle* - whole plucked follicle on anagen stage, *an. bulge* - dissected bulge region of anagen follicles which contains stem cells, *bulb* - bulb region of plucked follicles which contains matrix cells, *telogen* - plucked telogen follicles which contains stem cell region, *DP*-dermal papilla cells after 0, 1 or 12 days of culturing; the samples marked as *PO* are primary cultured keratinocytes from corresponding follicle areas.

First-strand DNA was synthesized using High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) and RNase Inhibitor at a final concentration of 1.0 U/ μ l using 10ng/ μ l final RNA concentration in the volume of 50 μ l. Reverse transcription was carried out under the standard conditions (25°C 10 min, 37°C 120 min). Semi-quantitative RT-PCR was performed with the following primers:

KRT15 forward primer, 5'-GGGTTTTGGTGGTGGCTTTG-3',
KRT15 reverse primer, 5'-TCGTGGTTCTTCTTCAGGTAGGC-3';
GAPDH forward primer, 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3', *GAPDH*
reverse primer, 5'-CATGTGGCCATGAGGTCCACCAC-3' (*GAPDH* gene, Human
MTC™ Panel I, Clontech Laboratories, Inc.);
LIPH forward primer, 5'-CCATGGTCTTGAAGGAATTT-3',
LIPH reverse primer, 5'-ACAGACCTCTGGTGGTCACA-3'.
LysoPLD forward primer, 5'-GGACCCATGGAAGTTTGAAT-3',
LysoPLD reverse primer, 5'-GGAATCCGTAGGACATCTGC-3';
LIP1 forward primer, 5'-GTGTGAGCTTAGGGGCTCAT-3',
LIP1 reverse primer, 5'-CAGCCAGGTTGTTTATTTCCCTC-3';
PS-PLA1 forward primer, 5'-GACGCTGTCTGGATTGCTTT-3',
PS-PLA1 reverse primer, 5'-TGGTTTATCTGGCATTGTGG-3';
LPA1 forward primer, 5'-ATGGCACCCCTCTACAGTGA-3',
LPA1 reverse primer, 5'-GCAGCAGAGGATCTGCCTAA-3';
LPA2 forward primer, 5'-TTGTCTTCCTGCTCATGGTG-3',
LPA2 reverse primer, 5'-CTCGGCAAGAGTACACAGCA-3';
LPA3 forward primer, 5'-CAACGTCTTGTCTCCGCATA-3',
LPA3 reverse primer, 5'-CACCTTTTCACATGCTGCAC-3';
SIRP1 forward primer, 5'-CGAGAGCACTACGCAGTCAG-3',
SIRP1 reverse primer, 5'-ACGTAGTCAGAGACCGAGCTG-3';
SIRP2 forward primer, 5'-GCCGGCCTAGCCAGTTCT-3',
SIRP2 reverse primer, 5'-AGGTCGTCTCCTGCGTTTC-3';
SIRP3 forward primer, 5'-CCAGCCCATCTGGCATTTC-3',
SIRP3 reverse primer, 5'-AGCTCCAAAATCCACGAGAG-3'.

PCR was performed in a final volume of 15 μ l with 10-50 ng of cDNA, Taq DNA Polymerase (New England Biolabs, Ipswich, MA) under the following conditions: 94°C

3 min, subsequent PCR cycles with 94°C 30 s, 57°C 30 s, 72°C 40 s, final extension 72°C 3 min.

Since certain housekeeping genes may have different expression patterns in hair follicles than in other tissues, we used several housekeeping genes for normalization (e.g., *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* and *ubiquitin C (UBC)*) (Fig. S8) in semi-quantitative RT-PCR and real-time PCR experiments.

The expression level of *LIPH* and *PS-PLA1* mRNA in the human tissue panel and hair follicle samples was examined by quantitative real-time PCR under the following conditions. PCR was carried out on an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA) with TaqMan Universal PCR Master Mix using TaqMan Gene Expression Assays for *LIPH* (Hs00373748_m1) and *UBC* (Hs00824723_m1) in a total reaction volume of 25 μ l. PCR cycling conditions were as follows: 95°C for 10 min, 95°C for 15 s and 60°C for 1 min (40 cycles). The plate contained replicates for each tissue cDNA sample and a no-template water control. To evaluate the expression level of *LIPH* mRNA, we used the comparative threshold cycle (Ct) method. We determined the relative amounts of *LIPH* mRNA by normalizing to endogenous control gene (*UBC*) and relative to a calibrator sample (pancreas), and then calculating the $2^{-\Delta\Delta C_t}$ for each sample.

Histopathology

We obtained punch biopsies of the scalp from three affected individuals. The biopsy samples were fixed in formalin, paraffin embedded and stained with hematoxylin and eosin. Multiple sections of each biopsy were examined under a light microscope by a dermatopathologist (S.L.).

cDNA sequences and gene expression constructs

To determine nucleotide sequence for complete encoding region of *LIPH*, we produced RT-PCR products for *LIPH* wild type (wt) and *LIPH* mutant type (with exon 4 deletion, Δ Ex 4) using pancreas RNA or RNA extracted from hair follicle bulge keratinocytes of affected individual. In addition, sequences for all exons of *LIPH* were determined using PCR products generated from genomic DNA.

A full length cDNA clone for wild type *LIPH* gene was obtained from Open Biosystems (Huntsville, AL, USA) and was subcloned into the BamHI and XhoI sites of pcDNA6/V5-His A and pcDNA4/V5-His A (Invitrogen, Carlsbad, CA) using primers BamHI-d 5'-TTTTGGATCCTGTGAGCAAATCCCACAGT-3', XhoI-r 5'-TTTTCTCGAGCAACTGCAACTCTGGGCAAAG-3'.

A cDNA clone of *LIPH* with deleted exon 4 (Δ Ex 4) was produced as following: 1) Two PCR products were obtained using *LIPH* wt - pcDNA4/V5-His A as a template and two pairs of primers:

BamHI-d, Δ ex4R1 5'-CTCCTTGTAGCCCAGTGCTGTAATTCTCCCCAGC-3

and Δ ex4D2 5'-GCTGGGGAGAATTACAGCACTGGGCTACAAGGAG-3', XhoI-r.

2) Second round of PCR was performed on a mixture of gel-purified PCR products from the first round PCR using primers BamHI-d and XhoI-r.

3) The product of the second round PCR was subcloned into the PflMI - BspEI sites of *LIPH* wt- pcDNA4/V5-His A, by substituting the wild type fragment with the Δ Ex 4

fragment and resulting in LIPH-ΔEx 4 pcDNA4/V5-His A. The gene constructs were verified by DNA sequencing.

Expression of gene constructs in cultured cells

Human keratinocyte stem cells isolated from the hair follicle bulge region were maintained in complete keratinocyte medium (KCM) at 37°C, 5% CO₂ (S4, S5). Cell transfections with gene constructs were performed using Lipofectamine and Plus Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. 24 hours after transfection, cell lysates were prepared using modified Radioimmunoprecipitation (RIPA) Buffer, containing 50 mM Tris HCl, pH 7.4, 150 mM NaCl, 0.25% Sodium Deoxycholate, 1 mM EDTA, 1% NP-40, and complete Protease Inhibitor Cocktail (Roche Applied Science, Indianapolis, IN). Membranes were probed with primary monoclonal anti-V5 antibody (Invitrogen, Carlsbad, CA) and anti-mouse horse-radish-conjugated secondary antibody (Pierce Biotechnology, Rockford, IL) incubated with ECL-Plus Reagent (Amersham Biosciences, Buckinghamshire, UK) and exposed to X-Ray film.

(2) Supporting text

***LIPH* mutation in populations of the Volga-Ural region.**

The actual frequency of *LIPH* mutation can be estimated by direct genotyping of the population samples collected regardless of the pathology. The frequency of mutation in the Mari population was 0.030 (tested in 100 individuals) and in the Chuvash population was 0.033 (tested in 122 individuals). There are no religious or ethnic restrictions regarding marriages between different ethnic groups in the Chuvash and Mari El republics and based on the standard assumptions of the Hardy-Weinberg distribution of genotypes, we can expect the following numbers of heterozygous carriers and affected homozygous individuals (the demographics are according to the 1989 and 2002 Censuses) (<http://www.perepis2002.ru/>).

In the Chuvash republics (total population, 1,346,000; 889,268 ethnic Chuvash), the number of heterozygous mutant carriers should be 88,262 and 1,447 affected homozygous individuals (956 of them ethnic Chuvash). The total population of ethnic Chuvash (which includes compact groups living outside the Chuvash republic) is 906,922. Thus the number of all ethnic Chuvash heterozygous individuals should be 59,470 with 975 affected individuals.

In the Mari El republic (total population, 727,979; 312,178 ethnic Mari), the number of heterozygous mutant carriers should be 43,679 with 655 homozygous affected individuals (281 ethnic Mari). Calculations of the total ethnic Mari (~643,700), including those living outside the Mari El republic, indicate that the number of heterozygous individuals should be 38,622 and homozygous affected individuals should be 579. In addition, we have divided the Chuvash and Mari republics into several geographic regions and found disease in all regions across the republics (data are available upon request). Thus, the interpretation of the calculations of total Chuvash and Mari populations seems reasonable.

In summary, the calculations suggest that there are more than 98,000 heterozygous

carriers and 1, 500 homozygous individuals of Mari and Chuvash ethnicity and, perhaps, more than 2, 000 affected individuals in total in this geographic region.

We carried out additional analysis of ethnically unrelated 405 individuals of Russian ethnicity including Russians from Moscow (219 individuals) and the Southern (Rostov) and Central (Kostroma) provinces of Russia (186 individuals). We did not find the mutation in these Russian populations. Next, we tested a Finno-Ugric group (174 individuals from Udmurt population) from the Volga-Ural region and also found no mutation. Interestingly, however, when we genotyped 345 individuals from a large population of Baskortostan (Volga-Ural region, 4,104,336 inhabitants including Turk-linguistic groups of Bashkirs and Tatars, Finno-Ugric Mari and Russians), we found heterozygous individuals and mutation with a frequency of ~0.004. Thus, the frequency of the mutation accumulated in ethnic groups of the Volga-Ural region appears to be relatively high for an autosomal-recessive disorder. In this regard, the distribution of inherited diseases (S6) and the history of Chuvash population might be of interest. The origin of Chuvash is believed to be related to the history of ancient Bulgars who moved to the Volga region. The Bulgar-Suvar groups were nomadic people and Chuvash may have a Hunnish heritage. The Chuvash language is a distant form of Turk and is the only surviving group of Altaic languages spoken by the Huns (S7). The Asian origin of the Hun tribes and the invasion of Europe by Attila the Hun potentially might contribute to the flow of mutant genes that are found in Chuvash to other populations. Indeed, other rare autosomal-recessive disease, Chuvash polycythemia (MIM 263400), is caused by C598T (Arg200T) mutation in VHL gene (S8). The C598T mutation was found with frequency of 0.0167 among Chuvash and 0.0087 among Mari (our own data). Surprisingly the C598T mutation was found on the “Chuvash” like- haplotype chromosome also in several families of Asian and European ethnic origin and also accumulated on the Southern Italian Island of Ischia (S9, S10) with no evidence of recent contact of Chuvash with other populations. It would be of interest to elucidate further the incidence of Mari-Chuvash hypotrichosis mutation in geographically distant ethnic populations.

Expression of genes for enzymes regulating synthesis of LPA

Several lines of evidence have indicated that membrane associated or secreted phospholipases may hydrolyze phosphatidic acid (PA) associated with membranes (S11-S14). LPA can be produced from PA by LIPH (mPA-PLA₁α) as well as by highly homologous LIPI (mPA-PLA₁β) and PS-PLA1. In addition, Lysophospholipase D (LysoPLD, identical to autotaxin) converts lysophospholipids to LPA and is the most important enzyme for LPA production in blood. Since these enzymes have overlapping functions and might be expected to compensate for the loss of one of them, we compared the expression patterns for *LIPH*, *PS-PLA1* and *LysoPLD* in human somatic tissues and dissected regions from human hair follicles. We found that *LysoPLD* is highly expressed in many tissues tested, except the anagen hair follicle bulge. *LIPI* and *PS-PLA1* also have no detectable expression in follicle bulge, but are expressed in testis and other somatic tissues. Thus, among these phospholipases producing LPA only *LIPH* is sufficiently expressed in the hair follicle bulge region where hair follicle stem cells are located. These molecular data support the genetic findings and help explain why mutations in *LIPH* produce a hair-specific phenotype.

In order to determine if LPA receptors are also expressed in the hair follicle bulge, we have analyzed the levels of mRNA for six members of G- protein-coupled receptors (GPCR), and found that *LPA₁*, *LPA₂* and *LPA₃* are expressed in this hair follicle compartment (Fig. S8C).

(3) Supporting figures

Fig. S1. Hypotrichosis in (A) a child (B) a female and (C) a male and baldness (alopecia) in (D) an elderly female and (E) an elderly male individuals. (F) The eyebrows are sparse or absent in the child, but in many cases visible in adults. (G) Axillary hairs and (H) body hairs (external part of forearm shown) are absent or sparse in affected individuals. (I, J) The biopsies of affected individuals (I) show slightly decreased numbers of large terminal follicles in comparison to normal individuals (J). No significant inflammatory infiltrate or scarring is seen in the affected subjects. Bar scale = 200 microns

Fig. S2. Linkage of hypotrichosis and alopecia to chromosome 3q27. (A) Examples of pedigrees with hypotrichosis in Mari and Chuvash populations. (B) The STR genotyping of nuclear families with hypotrichosis. The haplotypes of affected individuals denoted in box show a genomic interval for homozygosity.

Fig. S3. Parametric LOD Scores. (A) Two-point LOD scores for the linkage of hypotrichosis to STR markers on chromosome 3q27 (B) Multipoint LOD scores in homozygous interval and in flanking genomic regions calculated for Mari families. High Multiple Lod Scores were obtained when summarized data for all Mari and Chuvash families in the homozygosity CA4-D3S1530 interval (Multiple Lod Scores: 17.040-18.027).

Fig. S4. Positional cloning of the mutant gene. (A) Representative haplotypes for the STR markers showing linkage to hypotrichosis mutation in families from Mari and Chuvash populations. The Mari and Chuvash affected individuals from unrelated families share the common haplotypes associated with the disorder. Two rare recombination events in Mari and Chuvash individuals defined the minimal genomic interval for the mutant gene between D3S1530 and CA5 markers. (B) Genetic and physical map of the chromosome 3q27 locus showing linkage to the hypotrichosis. The black bars are the centromeric to telomeric intervals defined by critical recombination events in Mari and Chuvash populations. STRs in black indicate markers isolated in this study.

Fig. S5. Identification of deletion in homozygous affected individuals and heterozygous carriers. PCR with primer oligonucleotides DEL d, 5'-CTGGCCTGACCAGTGAGTTT-3' and DEL r, 5'-TTAGCCTGTCCTTCATTGAGC-3' were used for detection of the *LIPH* intragenic deletion. Filled symbols indicate affected homozygous individuals and semi-filled symbols indicate heterozygous carriers. (A) Panel of samples from Mari population; (B) panel of samples from Chuvash population. The bottom gel in panel B shows in comparison (1) the PCR products produced by the DEL d and DEL r primers

and (2) the PCR products obtained with intronic primers adjusted to exon 4 within the deletion region. No PCR product is detected with the primers adjusted to exon 4 in affected homozygous individuals.

Fig. S6. Expression of mutant *LIPH* bearing a deletion of exon 4. (A) RT-PCR analysis for mRNA extracted from keratinocytes of follicle bulge region from three individuals with hypotrichosis (1,2,3) and normal individual (4). The lane 5 is a negative (no template) control. (B) Expression of cDNA3.1 constructs of wild-type and mutant *LIPH* (fused to V5-tag) in cultured hair follicle keratinocytes. Western blot detects efficient expression of LIPH protein (Δ Ex 4) omitting 34 amino acid sequence of exon 4. The proteins were detected with primary monoclonal anti-V5 antibodies. (C) Sequencing of *LIPH* cDNA generated from mRNA of affected individual showed deletion of exon 4 and fusion of exon 3 and exon 5 in mRNA transcript with no alteration of predicted open reading frame. These data confirm the results of genomic sequencing for the *LIPH* deletion allele (Fig. S10).

Fig. S7. Multiple sequencing alignment of the human LIPH with orthologous members of LIPH family from the following vertebrate species: *H.sap.* - *Homo sapiens* NP_640341; *P.trog.* - *Pan troglodytes* XP_516924; *B.taur.* - *Bos taurus* XP_589466; *C.fam.* - *Canis familiaris* XP_545236; *M.mus.* - *Mus musculus* AAM18804; *X.trop.* - *Xenopus tropicalis* NP_001011098; *D.rer.* - *Danio rerio* XP_687645. LIPH proteins have only partial β 9 loop and a short Lid domain (indicated in boxes). These structural characteristics may determine the substrate selectivity of phospholipases (*S11*, *S15*). The evolutionarily conserved LIPH domain deleted in hypotrichosis patients contains catalytic Asp-178 residue and other evolutionary invariant amino acid residues. ClustalW program was used for multiple sequence alignment and GeneDoc program was used for shading. Amino acids of 100% identity are marked by red, 80% - green and 60% - yellow. The hairless protein that is transcriptional factor regulating hair growth and inactivated in alopecia universalis (*S16*) contains JmJC domain frequently observed in DNA-binding proteins and identified in truncated form in Phospholipase A2 β protein (*S17*). However, we found no evidence for JmJC domain in the LIPH (Conserved Domain Database (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>), NCBI).

Fig. S8. Expression of genes involved in LPA production and signaling in human tissues. (A) Real-time RT-PCR revealed mRNA transcripts for *LIPH* but not for *PS-PLA1* in hair follicle bulge and showed that these two homologous genes have overlapping mRNA expression in other tissues. The *ubiquitin C (UBC)* housekeeping gene was used for normalization. (B) Semi-quantitative RT-PCR analysis demonstrated no detectable mRNA for *LysoPLD*, *PS-PLA1* and *LIPI* within hair follicles or the hair follicle bulge, but showed the expression of these mRNAs in other tissues. *LIPI* expression is restricted to testis, consistent with the data obtained by others (*S11*, *S18*) (description of specimens is also in Material and Methods S). (C) RT-PCR analysis of members of GPCR family demonstrated expression of LPA receptors in hair follicles. For comparison, the level of mRNA in pancreas tissue is shown.

Fig. S9. The predicted catalytic role of LIPH in the production of bioactive LPA should be abolished in affected individuals. LPA with either saturated and unsaturated fatty acids may be produced by different biochemical mechanisms. LPA can be generated from phosphatic acid (PA) by LIPH (mPA-PLA1 α) and other phospholipases (*SI4*). We speculate that the deletion of catalytic domain (loss-of function) of LIPH reduces or abolishes the production of LPA mediators in hair follicles and, thereby, affects the normal function of keratinocytes culminating in arrest of hair growth.

Fig. S10. Sequencing analysis of genomic region containing exon 4 and flanking introns of *LIPH* gene. The sequence for wild type allele (A) and mutant allele caring the exon 4 deletion (B) are shown. The sequences for PCR primers (Del D and Del R) used to detect the deletion and predicted regions of recombination between two ALU-elements are indicated on the wild type allele sequence.

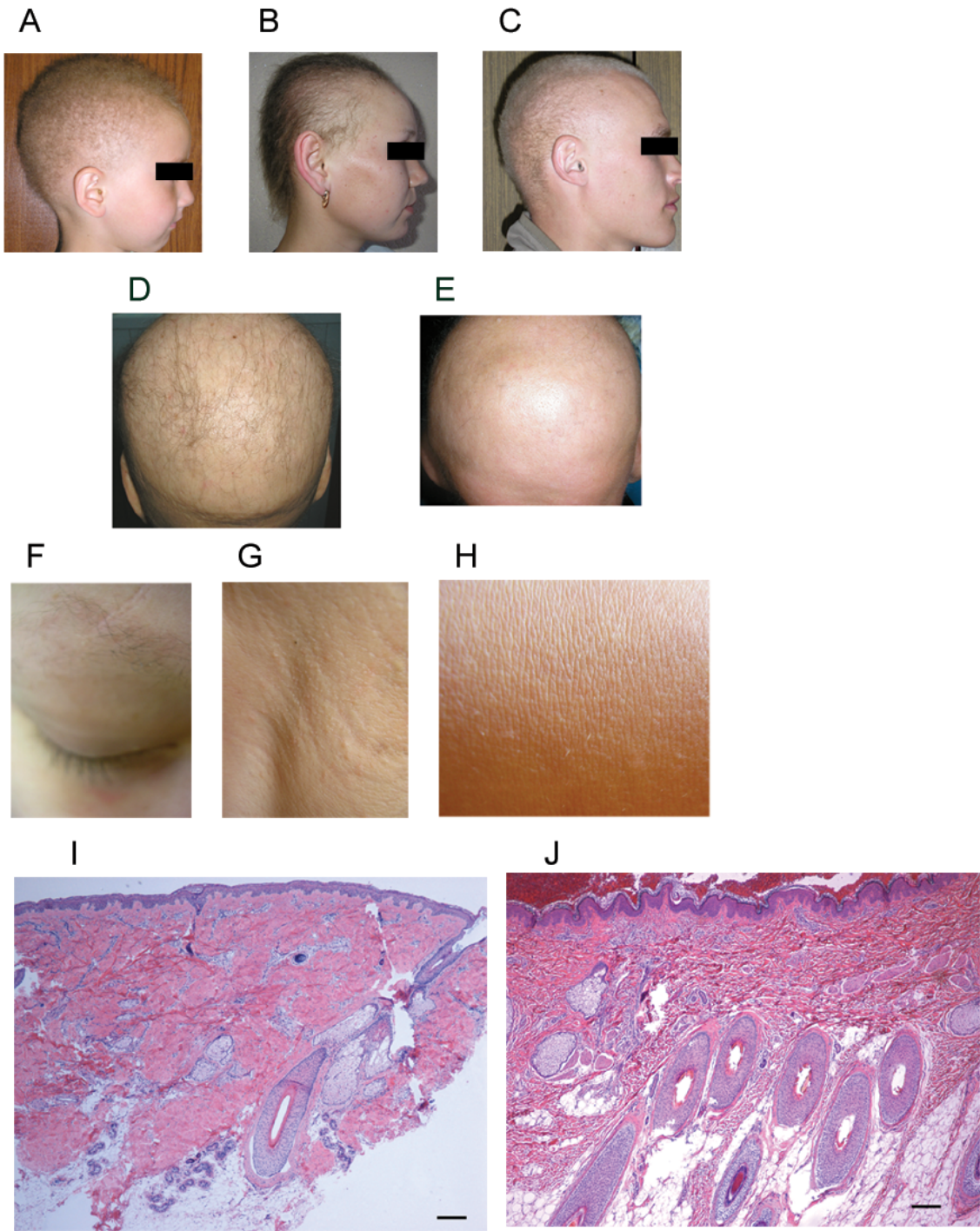


Fig. S1

Mari Pedigrees (selected)

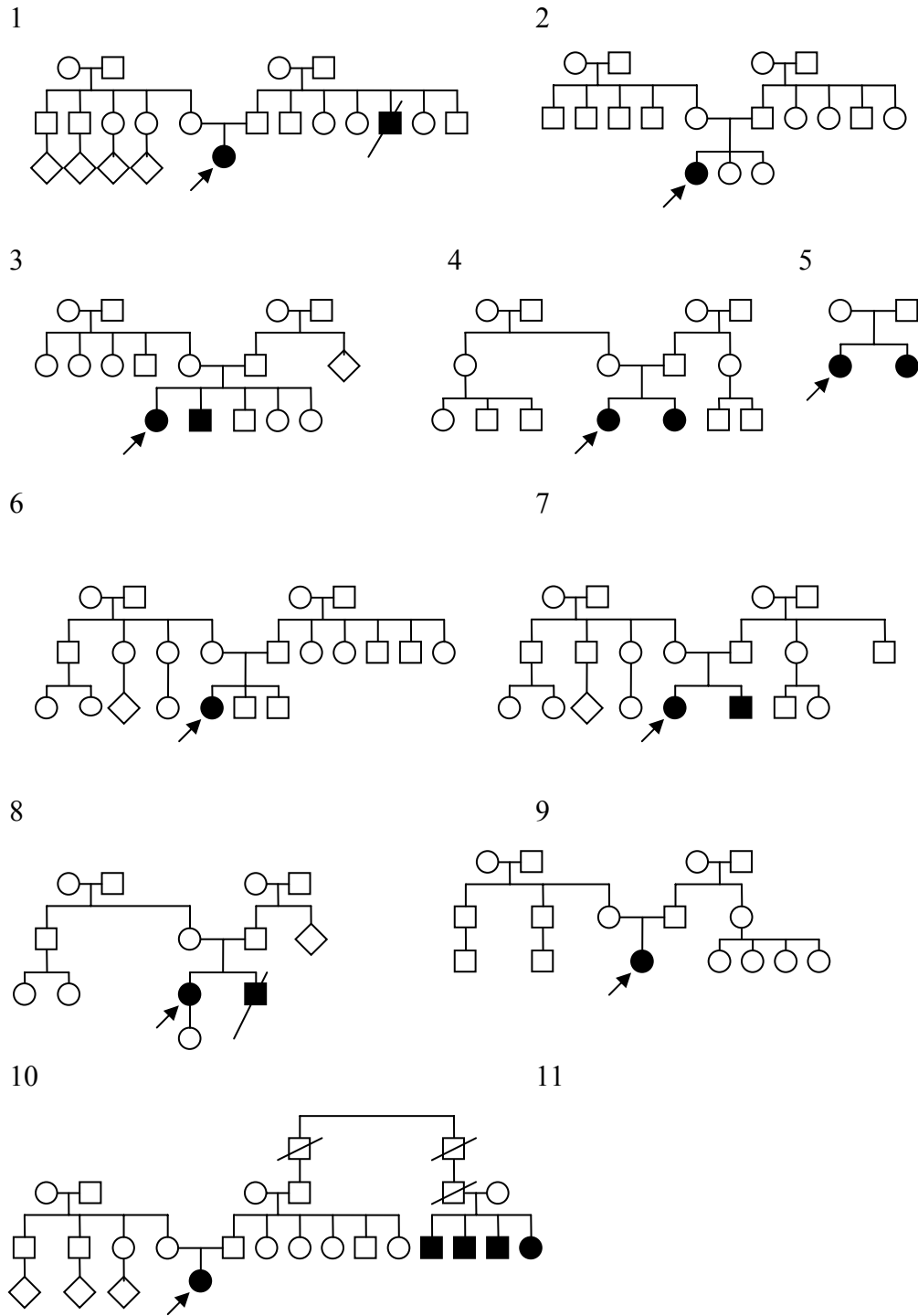


Fig. S2 (A)

Chuvash Pedigrees (selected)

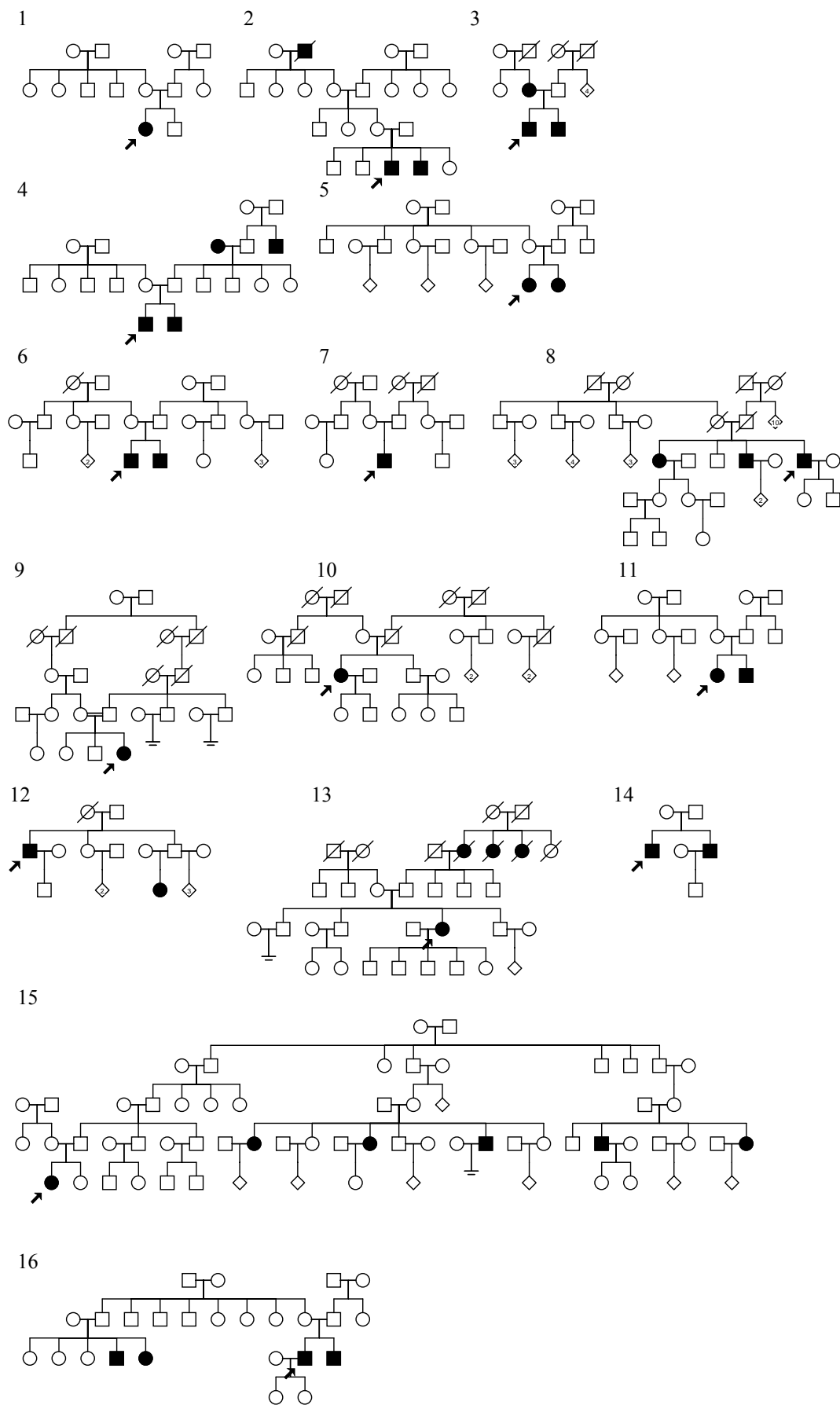


Fig. S2 (A), continued

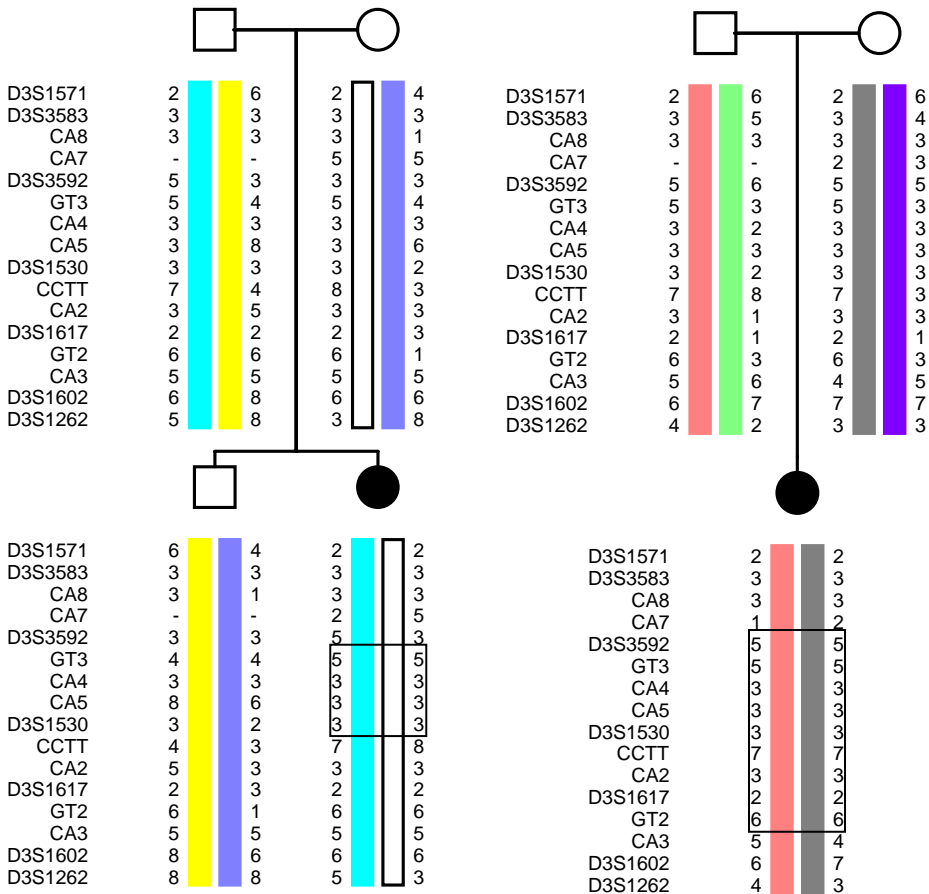
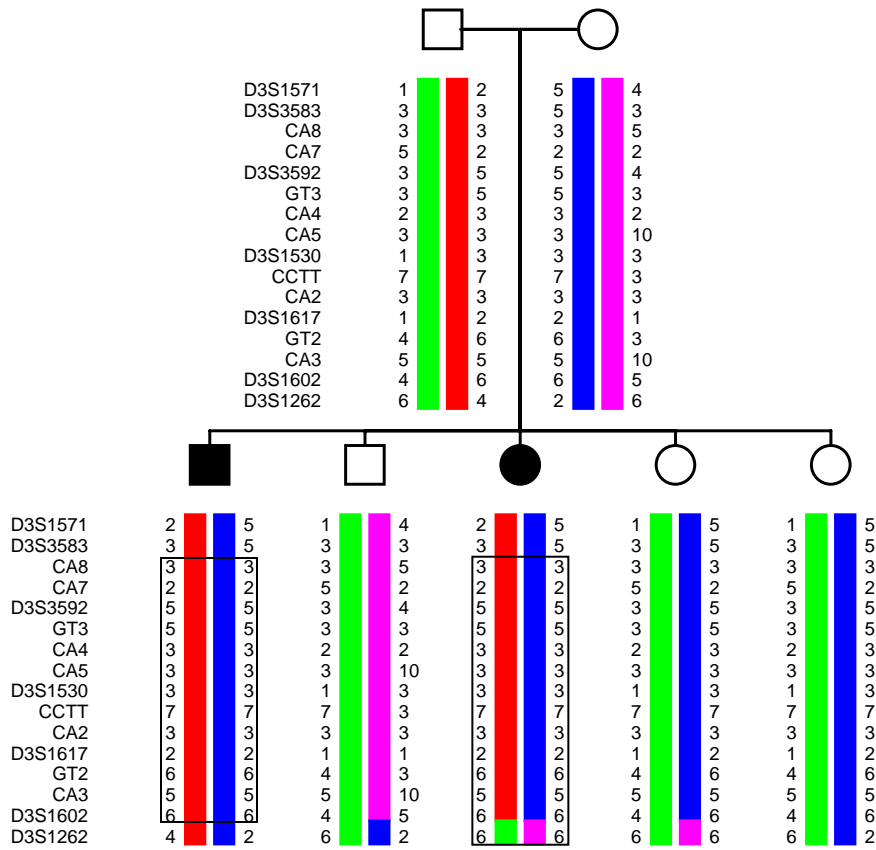


Fig. S2 (B)

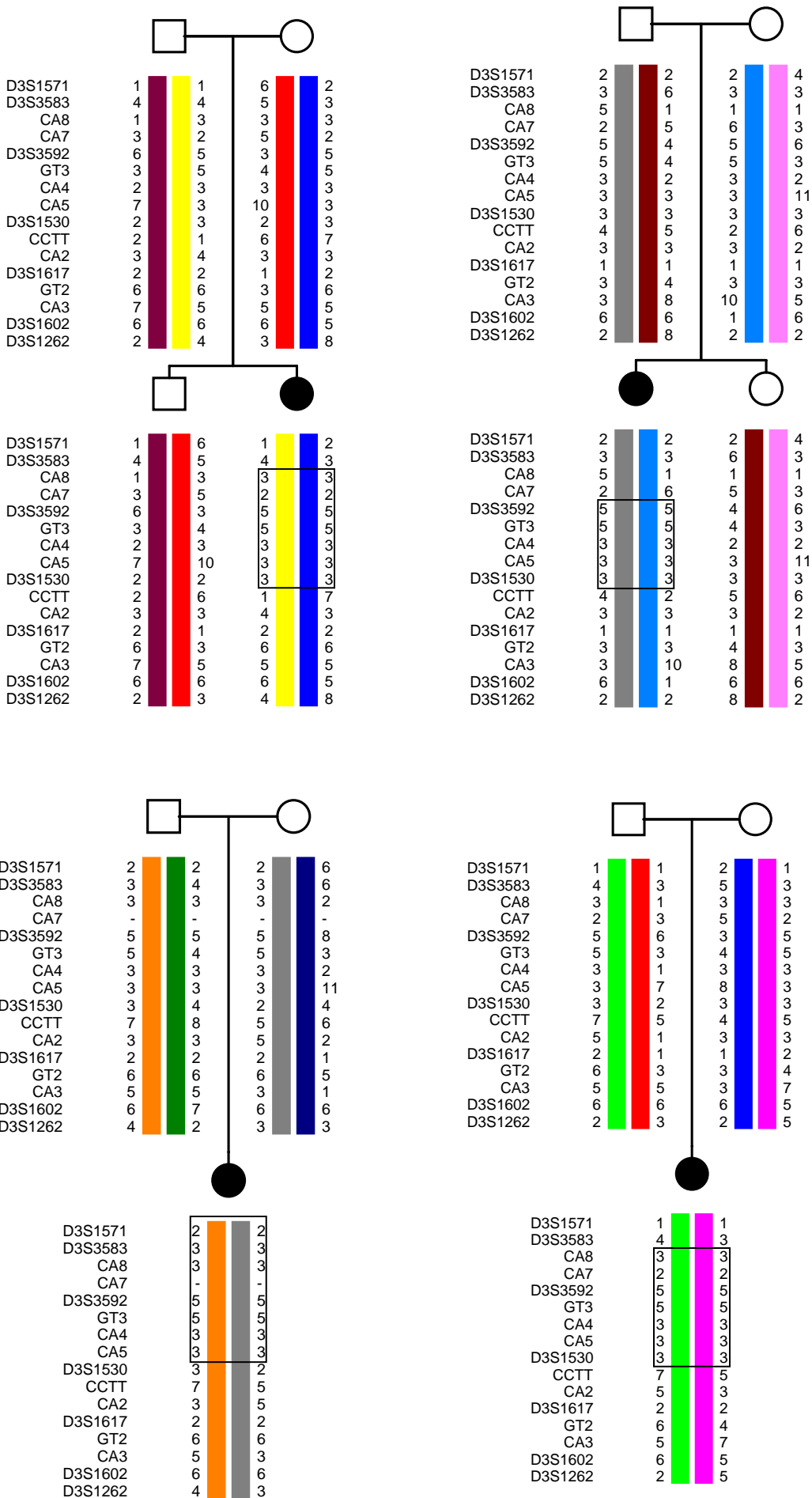


Fig. S2 (B) (continued)

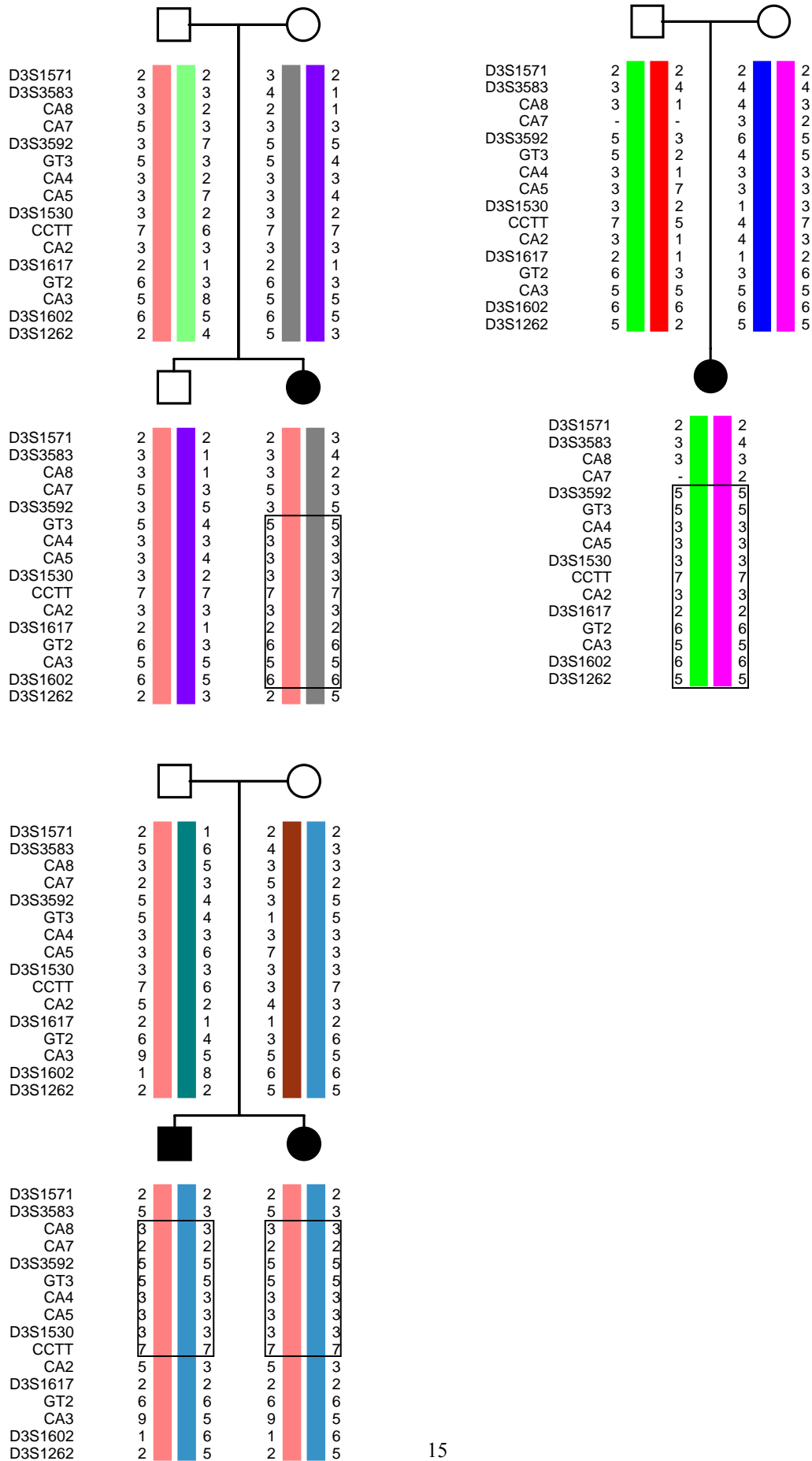


Fig. S2 (B) (continued)

Mari population

		Two-Point LOD score					
MARKER	POS cM	Recombination fractions					
		0	0.05	0.1	0.2	0.3	0.4
D3S1571	189.46	0.2243	0.8893	0.9520	0.7086	0.3623	0.0942
D3S3583	190.37	0.4617	0.9673	0.9032	0.5903	0.2851	0.0781
D3S3592	190.89	2.9547	2.5638	2.1620	1.3754	0.6961	0.2178
GT3	191.11	3.8130	3.2278	2.6539	1.6043	0.7740	0.2311
CA5	191.50	3.1211	2.6554	2.1989	1.3601	0.6884	0.2346
D3S1530	191.78	2.6793	2.2632	1.8646	1.1508	0.5870	0.1990
D3S1617	192.63	3.4746	2.9022	2.3593	1.3997	0.6632	0.1933
CA3	193.56	0.6304	0.9647	0.9108	0.6059	0.2900	0.0759
D3S1262	194.33	-Inf	-1.5668	-0.8339	-0.2987	-0.1322	-0.0636

Mari + Chuvash populations

		Two-Point LOD score					
MARKER	POS cM	Recombination fractions					
		0	0.05	0.1	0.2	0.3	0.4
CA4	191.28	7.9324	6.8986	5.7717	3.5962	1.8090	0.5991
CA5	191.50	11.9837	10.3155	8.6545	5.5050	2.8616	0.9995
D3S1530	191.78	9.0923	7.6757	6.3166	3.8794	1.9679	0.6769

Fig. S3 (A)

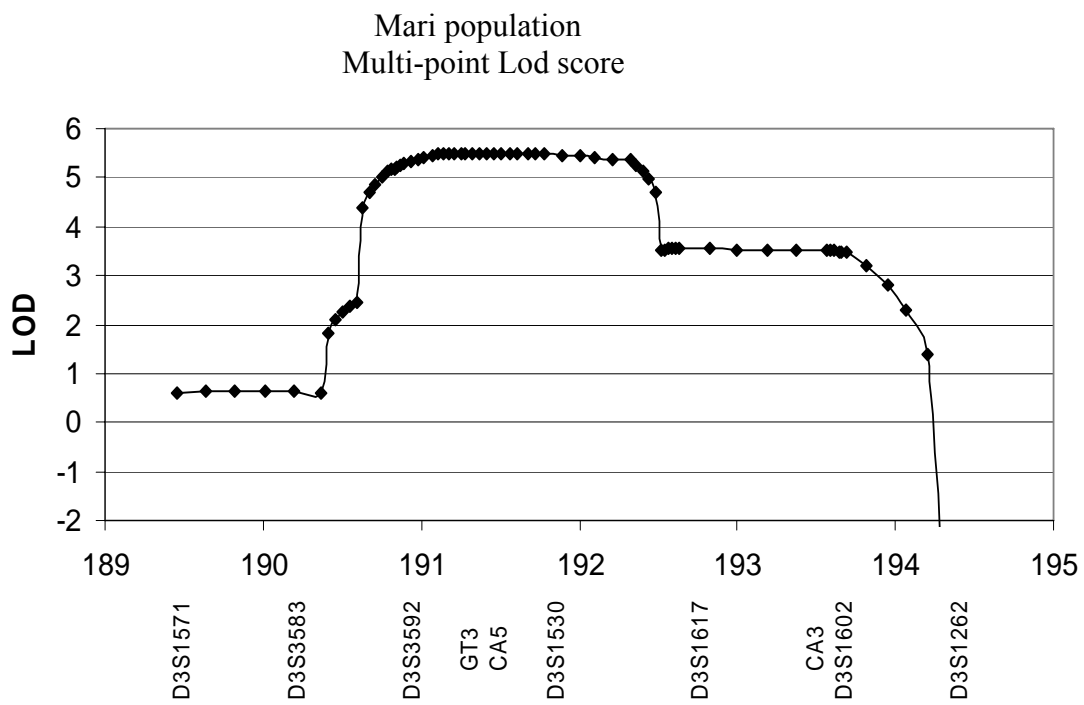


Fig. S3 (B)

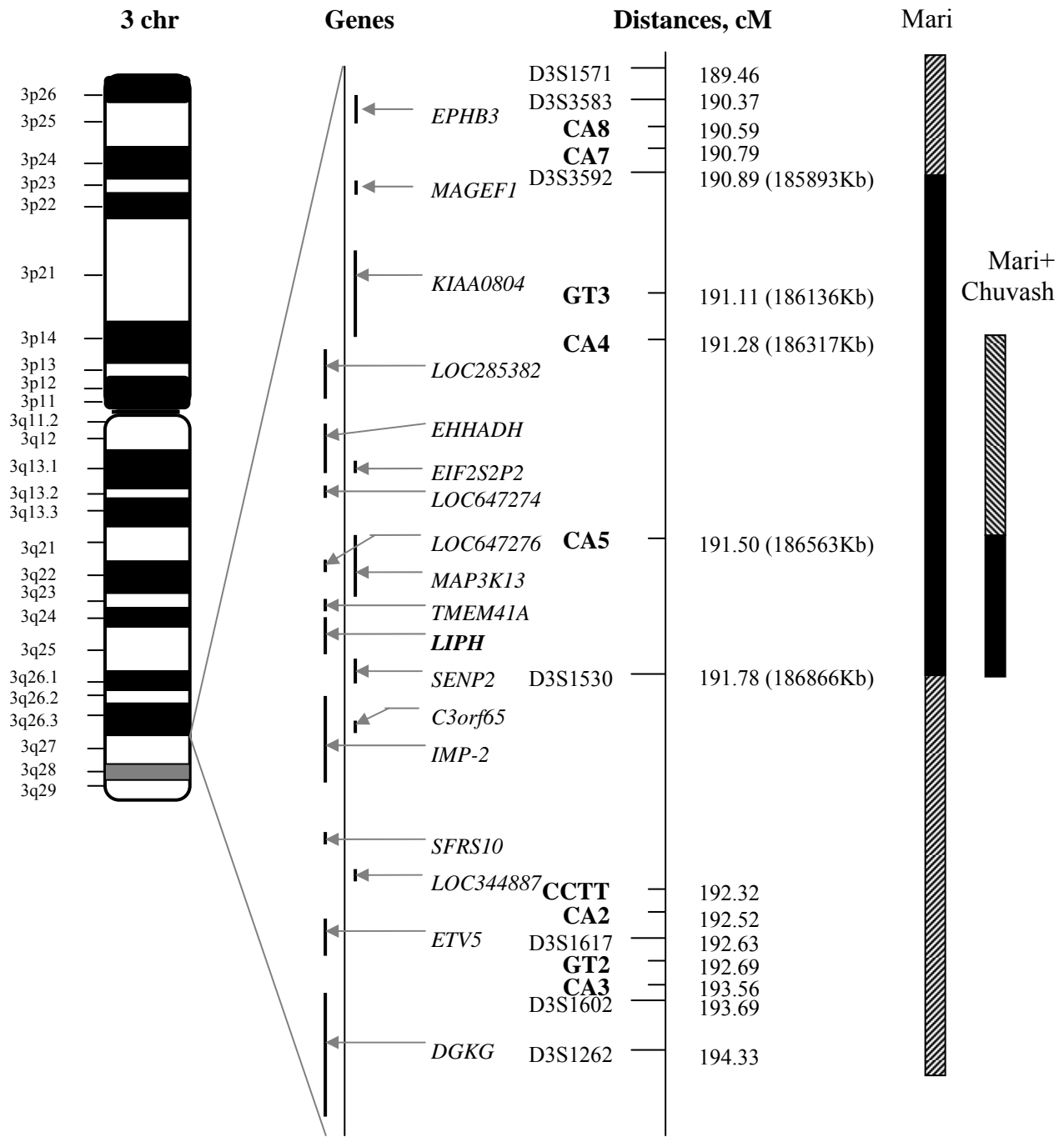


Fig. S4 (B)

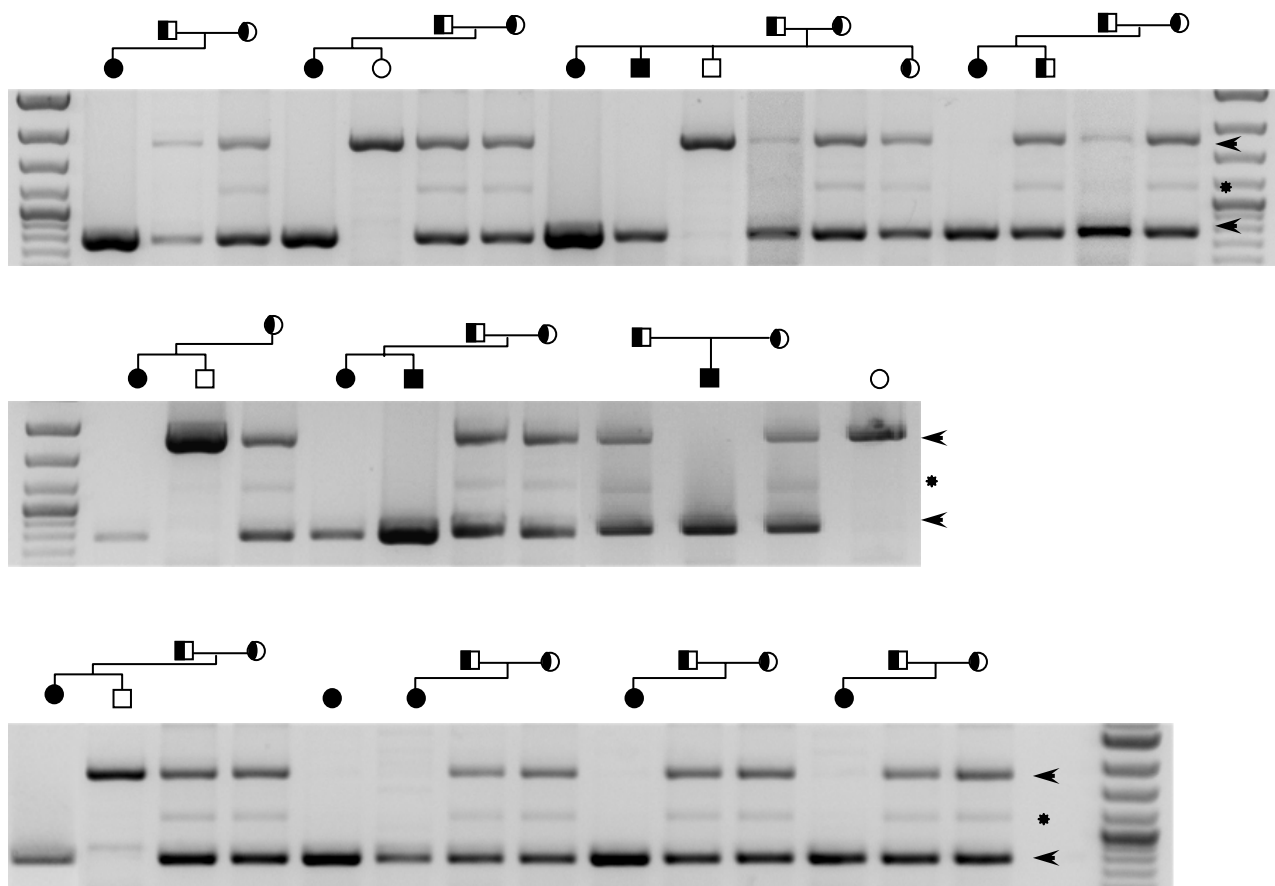


Fig. S5 (A)

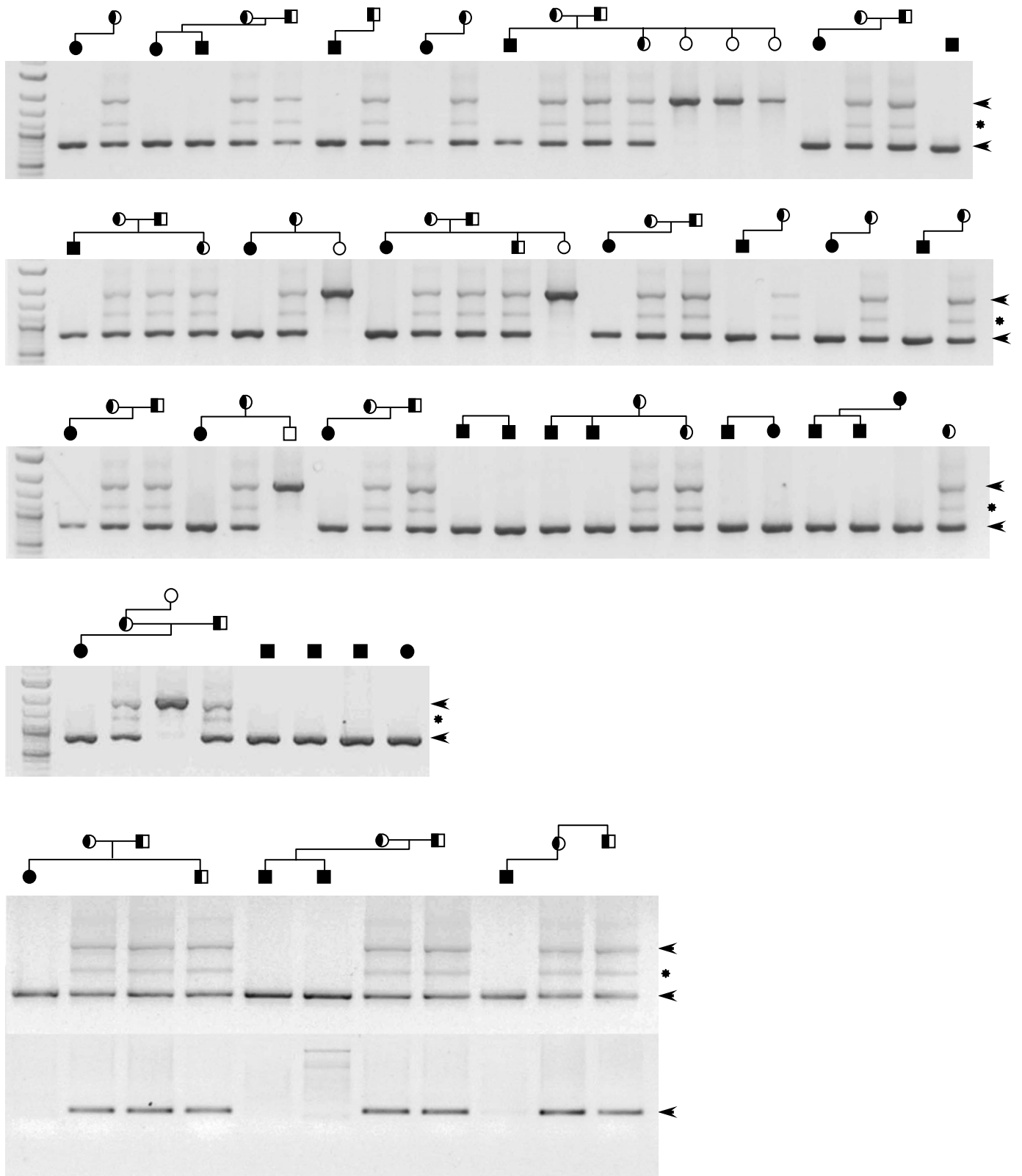


Fig. S5 (B)

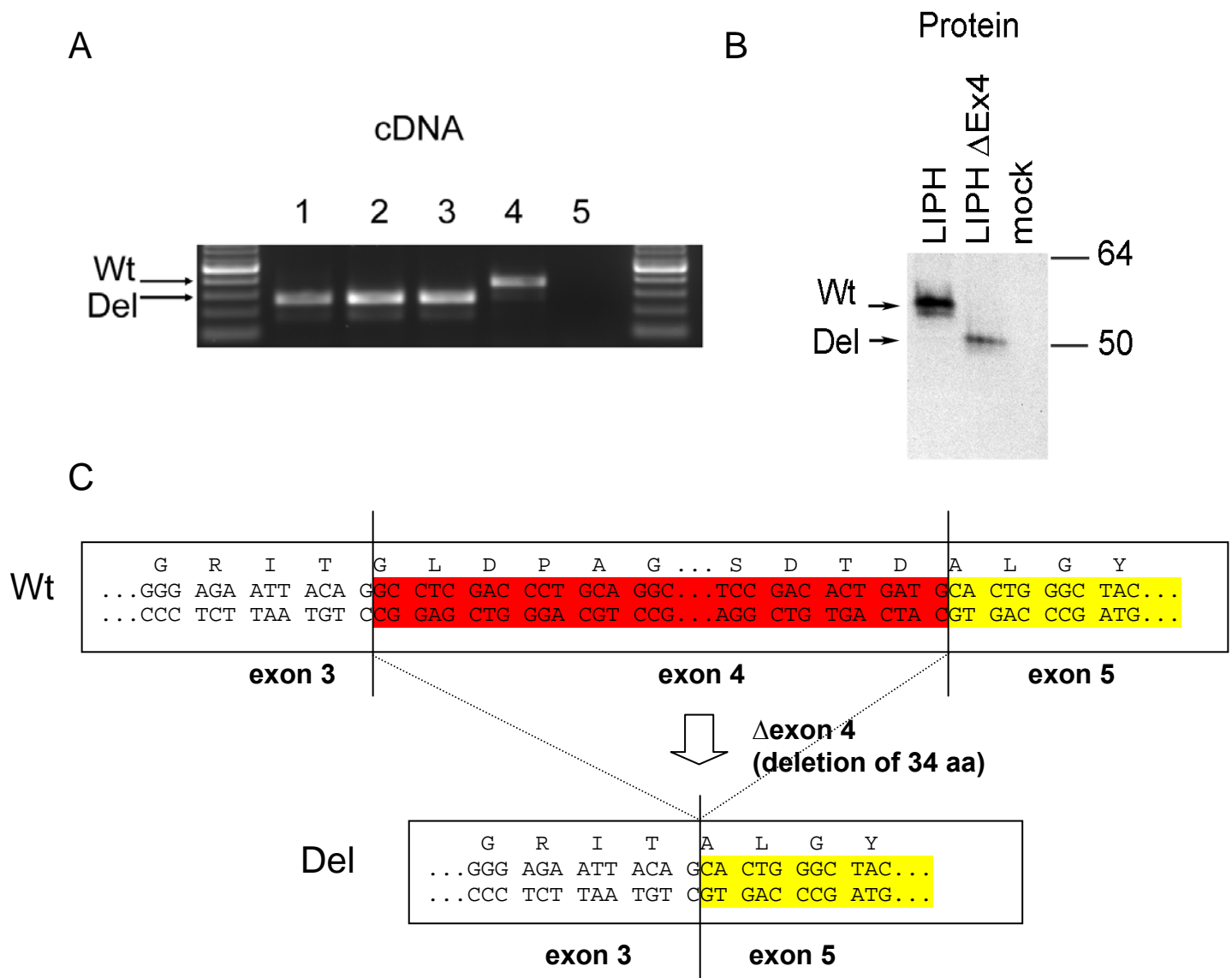


Fig. S6

H. sap. : -----MLRFYLFISLLCLSRSD-----AEETCPSTRLSF : 30
P. trog. : -----MCLSRSD-----AEETCPSTRLSF : 20
B. taur. : MEAADCEDGVRPQSQTSRIMATMRLRRGKEGSRPRAFRGIMTLRAPGFQSSGLQNYTDEETCPSTKLSF : 70
C. fam. : MHPCNVFMLLSMSYRGLGVLECKTVLKAGESRN-----PDEETCPSTRLSF : 46
M. mus. : -----MLRLCFFISFMCLVKSD-----TDEETCPSTRLSF : 30
X. trop. : -----MLLRFYFNGLLFVGCLLSWGRSD-----TEEQCHTFTDLNI : 36
D. rer. : -----MLAVHEDVNSE-----DNDCDNFIDLDF : 23

H. sap. : HSAVVGTGLNVRLMLYTRKNLTCQAQTIN---SSAFGNLNVTKKTTFFIVHGFRPTGSPVWMDLWKGLLS : 97
P. trog. : HSAVVGTGLNVRLMLYTRKNLTCQAQTIN---SSAFGNLNVTKKTTFFIVHGFRPTGSPVWMDLWKGLLS : 87
B. taur. : HSAVVGTGLNVKLMYTRRNPTCAQAIN---STVLGNLNVTKKTTFFVHGFRPTGSAVWVIEDLVEGLLS : 137
C. fam. : HSAVVGTGLNVKLMYTRKNQTCQAQTIN---STVFGNLMNTKKTTFFIVHGFRPTGSPVWVMDLVQGLLD : 113
M. mus. : HSAVVGTGLSVRLMLYTQRDQTCQAQTIN---STALGSLNVTKKTTFFIVHGFRPTGSPVWVIEELVQSLIS : 97
X. trop. : HNSLIGTGLKVLQLLYTRENPKCAQDLNVDNSTGFQYLVNTRRTVFIHGYRPTGSPVWVDDIVKFFLD : 106
D. rer. : HESFVGTNLYVRLLYTRANLECGQELPHHNFITQQLPLFNVTRPTTFVIVHGFRPTGAPPIWVNHIVHLIAA : 93

Putative catalytic residue (**Ser-154**)

H. sap. : VEDMNVVVVDWNRGATTLIYTHASSKTRKVAMVLKEFIDQMLAEGASLDDIYMGVSLGAHISGFVGEY : 167
P. trog. : VEDMNVVVVDWNRGATTLIYTHASSKTRKVAMVLKEFIDQMLAEGASLDDIYMGVSLGAHISGFVGEY : 157
B. taur. : VEDMNVVVDWNRGATTVMYNHASSKTRKVATVLKEFIDMLAEGASLDDIYIIGVSLGAHIAGFVGEY : 207
C. fam. : VEDMNVVVDWNRGATTVIYNHASSKTRKVANVLKEFIDQMLVKGASLDNIYIIGVSLGAHIAGFVGEY : 183
M. mus. : VQEMNVVVVDWNRGATTVIYPHASSKTRQVASTLKEFIDQMLVKGASLDNIYMGVSLGAHIAGFVGEY : 167
X. trop. : IQDFNVVVDWNRGATTVLYHMAAANTRKVADILKRFIDNMLSQGATLDSIYMGVSLGAHISGFVGEY : 176
D. rer. : QKDMNILLVVDWNRGANLNYLTAVANTRGTALNITRFIESMEKEGASLDSIHLIGVSLGAHVAGFVGEY : 163

Putative catalytic residue (**Asp-178**)

Deletion (34 aa)

LIPH exon 4

β9 loop

lid domain

H. sap. : DGWLGRITGLDPAGPLFNGKPHQDRIDPDAQFVDVIHSDTDALGYKEPLGNIDFYPNGGLDQPGCPKTI : 237
P. trog. : DGWLGRITGLDPAGPLFNGKPHQDRIDPDAQFVDVIHSDTDALGYKEPLGNIDFYPNGGLDQPGCPKTI : 227
B. taur. : DGQLGRITGLDPAGPLFNGRPPEDRLDPRDAQFVDVIHSDTDALGYKQQLGNIDFYPNGGLDQPGCPQTI : 277
C. fam. : NGQLGRITGLDPAGPLFNGRPPEDRLDPRDAQFVDVIHSDTDALGYREPLGNIDFYPNGGLDQPGCPKTI : 253
M. mus. : EGKLGRVITGLDPAGPLFNGRPPERLDPDAQFVDVIHSDTDALGYKEALGHIDFYPNGGLDQPGCPKTI : 237
X. trop. : NGSIGRITGLDPAGPLFNGKPEPERLHYTDAQFVDVHSDTDGLGYKESLGHIDFYPNGGLDQPGCPKTI : 246
D. rer. : GGRVGRITGLDPAGPMFASVSPERLDPDAQFVDVHTDMNSFGLRGTHGHIDFYANGGLDQPGCPKTI : 233

Putative catalytic residue (**His-248**)

lid domain

H. sap. : LGGFQYFKCDHORSVYLYLSSIRESCITITAYPCDSYQDYRNGKCVSCGTSQKESCPVLLGYYADNWKDHLR : 307
P. trog. : LGGFQYFKCDHORSVYLYLSSIRESCITITAYPCDSYQDYRNGKCVSCGTSQKESCPVLLGYYADNWKDHLR : 297
B. taur. : FGGMQYFKCDHORSVYLYLSSIRENCTITITAYPCDSYQDYRNGKCVINCGISQTEPCVLLGYYADNWKDYLR : 347
C. fam. : LGGLKYFKCDHORSVYLYLSSIRENCSITITAYPCDSYRDYRNGKCVNCGIPIQKESCPVLLGYYADNWKKEYLK : 323
M. mus. : FGGIKYFKCDHQMVSVYLYLASLQNNCSITITAYPCDSYRDYRNGKCVSCGAGQIVPCPRVGYADSWKEYLW : 307
X. trop. : LAGSEYFKCDHORSVFLYIASLTKSCDLVAFPCKSYRDYRICNCTDCKEFLPLSCPVLGFYADKWKDHLV : 316
D. rer. : FSGKSYFVCDHORSVFLYLCSLNRTCSLITGYPCSSYSDHLSCQCLQCETFKPPASCPVLLGYDLSQWRDKLL : 303

Fig. S7

H. sap. : GKDP^YPM^YTKA^YFFDTA^YEES^YPF^YCMYHYFVDIITW^YNKNVRRGDIITIK^YLRDKAGN^YTESKINHEPTTFOKYHOVS : 377
P. trog. : GKDP^YPM^YMKA^YFFDTA^YEES^YPF^YCMYHYFVDIITW^YNKNVRRGDIITIK^YLRDKAGN^YTESKINHEPTAFOKYHOVS : 367
B. taur. : EGD^YPM^YIKA^YFFDTA^YE^YEK^YPF^YCIYHYFVDIITW^YNKNVRRGSIITIK^YLKDKAGN^YTESKINHEPATFOKYHOVS : 417
C. fam. : QRNE^YPM^YTKA^YFFDTA^YE^YEK^YPY^YCIYHYFVDIITW^YNKNIRRGSIITIK^YLKDKAGN^YTESKINHEPAIFOKYHOVS : 393
M. mus. : DRDP^YPM^YTKA^YFFDTA^YE^YTK^YPY^YCMYHYFVDIVSW^YNK^YSVRRGFITIK^YLRGEGDN^YTESKIDHEPSAFEKYHOVS : 377
X. trop. : KRNH^YPG^YTTA^YFFDTA^YAK^YDPY^YCLFHYYLDFMTW^YSSQIRRGYIITIK^YLTSLDGNVTE^YSKLDKDAAVFEQYKEES : 386
D. rer. : RLG--QT^YRAYF^YST^YTAEL^YPY^YSKTSYRVDLVTW^YNQFLRWGVVILRLHN-GKNVTE^YAHL^YDNKLMRFEQY^YTSTR : 370

H. sap. : LLARFN^YQDL^YDKVAA^YISLM^YFSTGSLIG^YPRYKLRILRM^YKLRSLAHPERPOLCRYDVL^YLMENVETV^YFQ^YPILCP : 447
P. trog. : LLARFN^YQDL^YDKVAA^YISLM^YFSTGSLIG^YPRYKLRILRM^YKLRSLAHPERPOLCRYDVL^YLMENVETV^YFQ^YPILCP : 437
B. taur. : LLARFN^YQDL^YDKVEA^YISLV^YFSTG^YSVIG^YPRYKLRILRM^YKLRSLGHPERPOLCRYDVL^YLMENVETV^YFQ^YPILCP : 487
C. fam. : LLARFN^YQDL^YDKVST^YISLV^YFSTG^YSVIG^YPKYKLRVLRM^YKLRSLAHPERPOLCRYDVL^YLMENIEN^YVFQ^YPILCP : 463
M. mus. : LLARFN^YRDL^YDKVAA^YISLM^YFSTG^YSVV^YGPKYKLRVLRM^YKLRSLAHPERDRPHLCRYDVL^YLMENVET^YSFQ^YPILCS : 447
X. trop. : LLAK^YFDQDM^YPI^YSRI^YSVTE^YFTG^YSVIG^YPKYKLRVLRM^YRLRPFTN^YRNR^YPI^YLCRYD^YFV^YLLEN^YIET^YBE^YIP^YICE : 456
D. rer. : LLAQ^YFD^YEDLY^YPI^YQK^YISL^YRIV^YTGN^YVIG^YPRYKIRLL^YRIR^YISL^YE^YK^YY^YRSL^YMCRYDI^YIV^YEEN^YAE^YVSE^YK^YPI^YCH : 440

H. sap. : EL^YQL^Y* : 451
P. trog. : EL^YQL^Y* : 441
B. taur. : KL^YQM^Y* : 491
C. fam. : KL^YQM^Y* : 467
M. mus. : QQ^YQM^Y* : 451
X. trop. : DT^YNL^Y* : 460
D. rer. : ----* : -

Fig. S7, continued

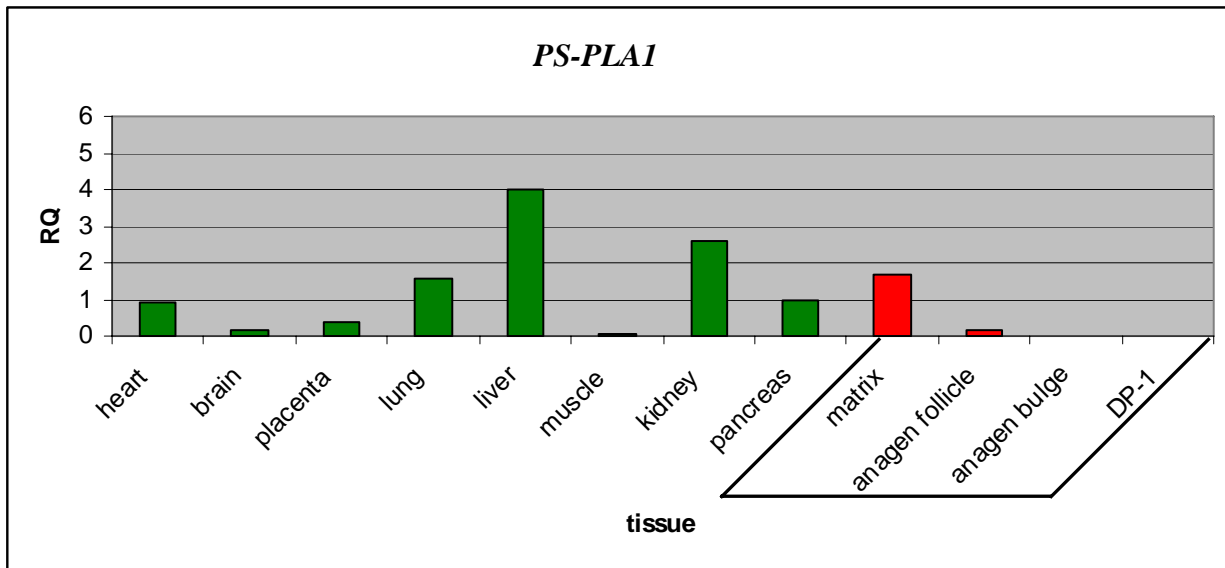
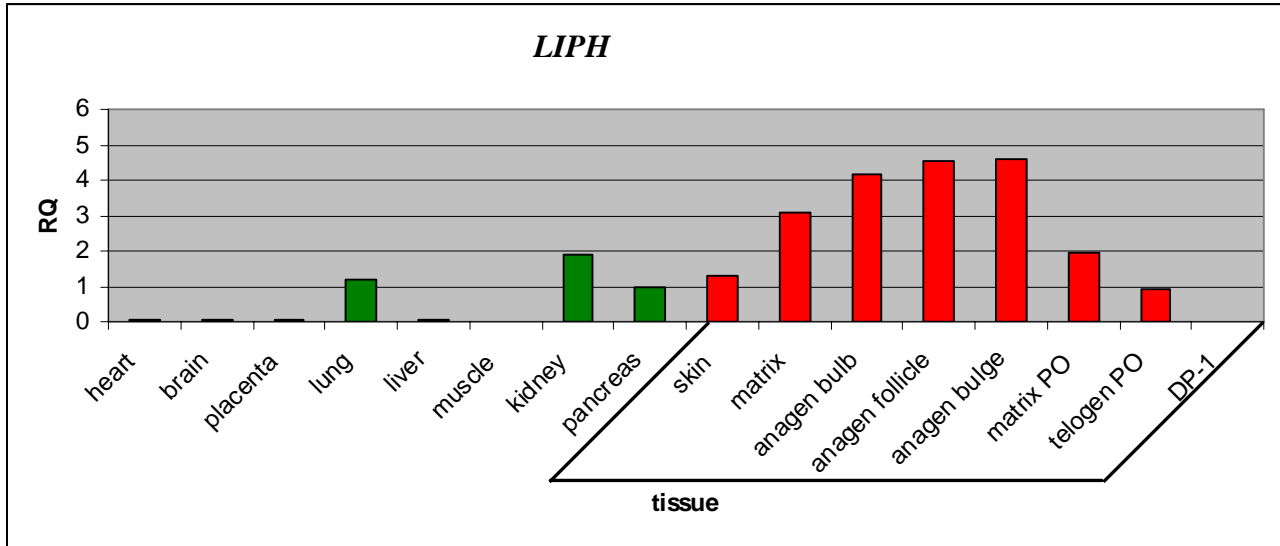


Fig. S8 (A)

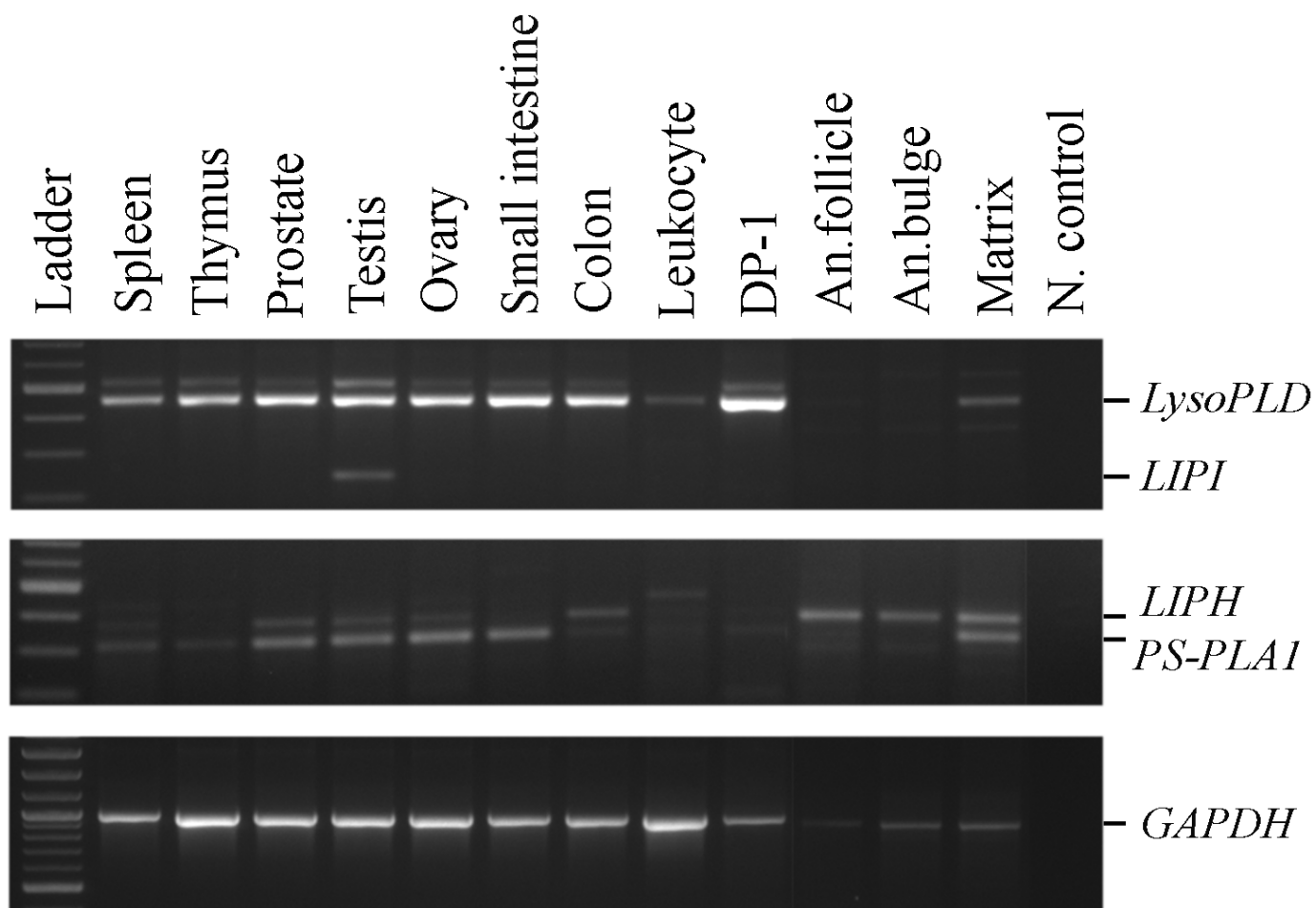


Fig. S8 (B)

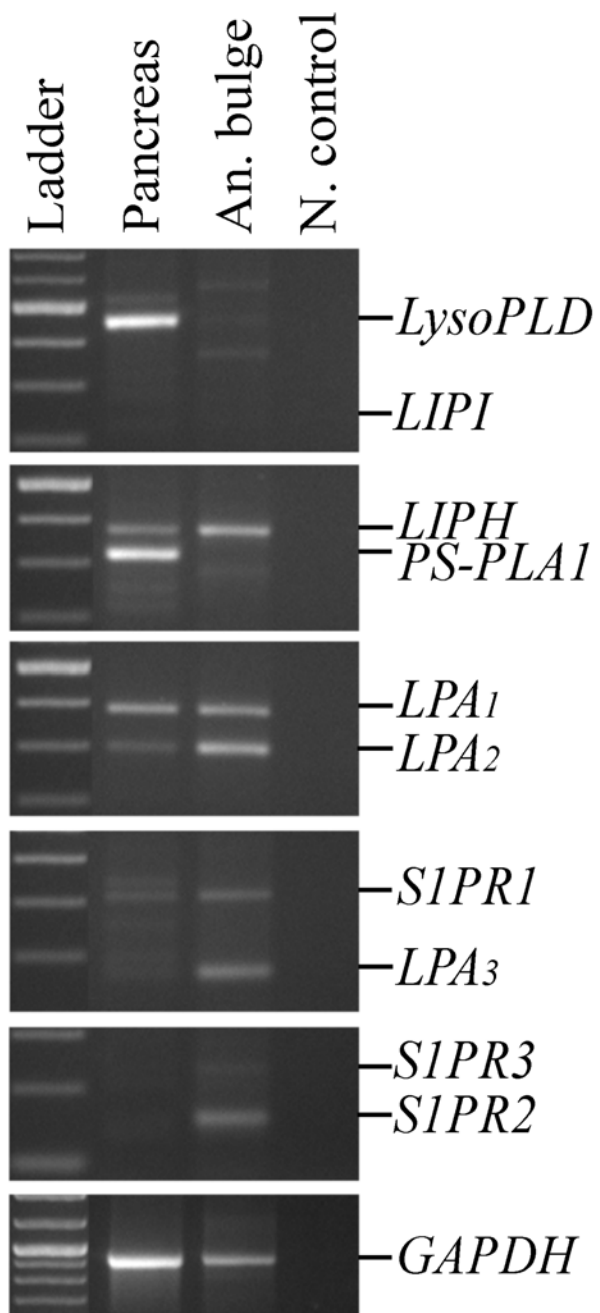


Fig. S8 (C)

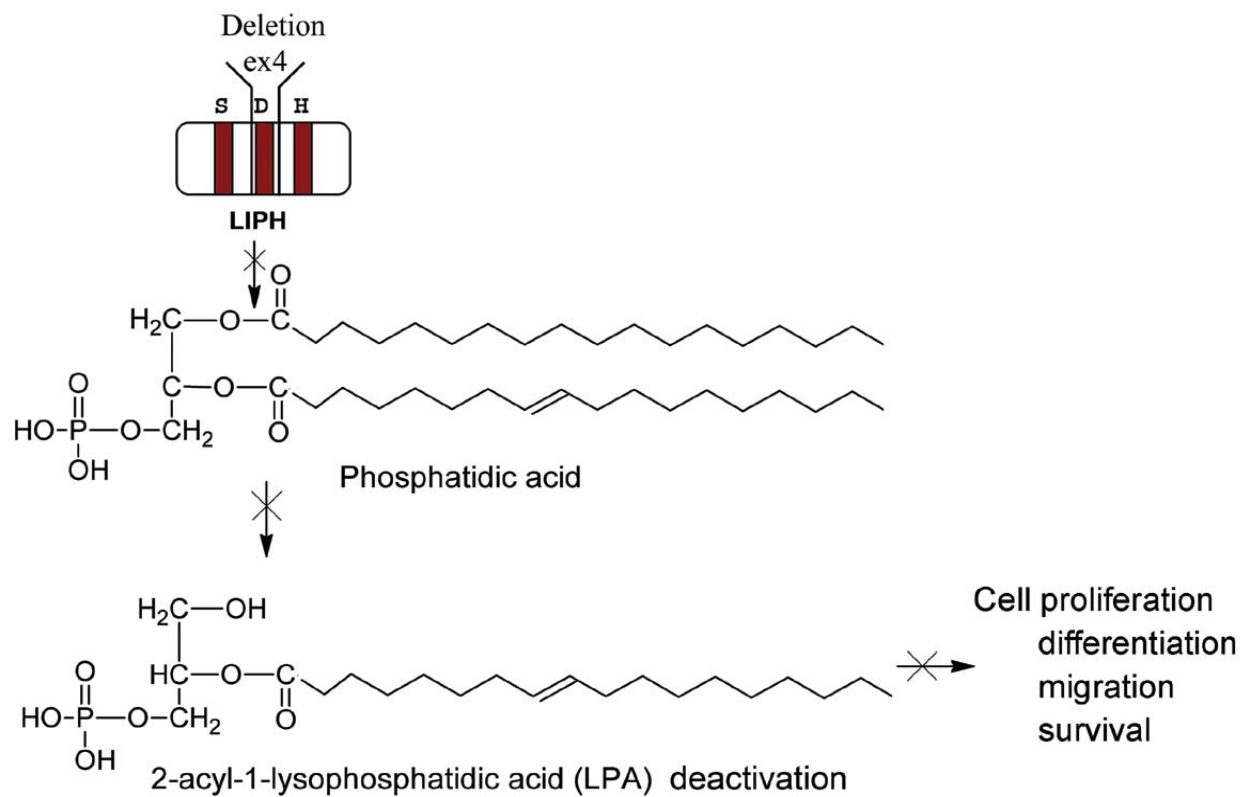


Fig. S9

Pr Del D

```

1 CTGATACCTG GCCTGACCAG TGAGTTTCGC CCTCTCCTGC CGAGCTTGCT AAACTCAGGG
                                     MIRb
61 CAGTGAAACC AAGAGTTCAA CAGATAGAAG TGGTTGCATT AAGAGGCTGT GTTCTGATCA
                                     MIRb
121 AGGCTCTTGA TCTTCCTGAA TCACAGTTCA TTCATCTGGA AAATAATAAT AGCTACCCTG
                                     MIRb
181 CCTCCCTTAC AGGTTTGCAA CATGGATCAA AGGGGCTAGC ATATATGAAT AAGAGTGGAA
241 GTTCTTTTATT TATTAACAAC AAAAAGGAAG GGCTGAGTGA GGTTCCTCGG TCATCTAAAG
301 TGGGATAAAG ATGAGTATAG CGCTTTCTTT CTTTCTTTCT TTCTTTCTTT CTTTCTTCTT
361 TCTTTCTTTC TTCCTCATCT AAAGTGGGAT AAAGATGAGT ATAGCATCTG CTTGCTTTCT
                                     AluSc
421 TTCTTTTTTTT CTTTCTCTCT CTCTCTCTCT CTCTCTCTTT CTTTCTTCGA GACAGAGCCT
                                     AluSc
481 TGCTCTGCCG CCAGGCTGGA ATGATCTTGG CTCACTGTAA CCTCTGCCTC CCGGGTTCAA
                                     AluSc
541 GCGATCCTCC TGCTCAGCC TCCCGAGTAG CTAGGACTAC AGGCACACAC CACCACGTCC
                                     AluSc
601 AGCTAATTTT TTTGTATTTT TGTAGAGACG GGATTTCAAC ATGTTGGCCA GGATGGTCTT

                                     Recombination region
                                     ~~~~~
                                     AluSc
661 GATCTCTTGA CCTCATGATC TGCCTGCCTC GGCCTCCCAA AGTGCTGGGA TTACAGGTGT
                                     AluSc
721 GAGCCACTGC GCCCAGGCTG CTCTTTTCTC TTTAATGTGC CCTTTGGTGC TTTTTTCTG
781 GGGACCGCCC GTTTATCCCC ACCCCTTGAA CCTCATAACAC GTGTACGTTT CCTACTGTTG
841 AGCTCTGCAT GACACATGCC CCTGATGGAG TCTGGATGCC ATTCTCAGGG GCACAGCCAG
                                     Pr 1D
901 GGTAACCGTC TCCACTGTGG GAACAAGCTG CAGAGCCATG GAAAGGAGTC CTGGCCAAGT
                                     Pr 2D
961 GGGACGGGAG TGTGAGCGCT CACCCACCTG AGGGTGCCCT GCAGCCTGGG AGCCACCCTC
                                     Pr 2D
1021 ATCCAGAAGG ATCAAAGGCC CGGCTGCAGG TGAATCATGG TGTGGGGCTG CCAAGTGACT
                                     Pr3 R
                                     ~~~~~
                                     ex4
1081 CTGTTTTTCTT TCCAGGCCTC GACCCTGCAG GCCCTTTATT CAACGGGAAA CCTCACCAAG

```

Fig. S10 (A)

Pr3 D
 ~~~~~  
 ex4  
 ~~~~~

1141 ACAGATTAGA TCCCAGTGAT GCGCAGTTTG TTGATGTCAT CCATTCCGAC ACTGATG GTA

Pr 2R
 ~~~~~

1201 ACGCTCCTTT CCTTGTGGGT CAGTGACACC GCCAGGCTCC TAAAGAGTGT CCCCTGGGGA

1261 GAGATAATCA TGTAGGAGCA GATCAGGTTC CTCTAGATTC TAACTTTTTTT TTCCAGGCAG

Pr 1R  
 ~~~~~

1321 GGTTCCAAAT CCACCGAAAT ATTGAAGGGA AGCTGGTTTA ATGTAGGCAC CCAGGACCTT
 ALuY

1381 GTGCTAGAGC GTGGATGATT ATCCCACGGT CTCTTCATAT TTTTATTTTT TTAATTTTTT
 ALuY

1441 TTGAGACAGA GTCTCGCTCT GTCGCCAGG CTGGAGTGCA GTGGCGCAAT CTCGACTCAC
 ALuY

1501 TGCAAGCTCT GCCTCCCAGG TTCTCGCCAT CCTCCCGCT CAGCCTCCCG AGTATCTGGG
 ALuY

1561 ATTATAGGCA CACGCCGCA CGCCTGGCTA ATTTTTTGTA TTTTATAGTAG AGACGGGGTT
 ALuY

1621 TCACCGTGTT AGCCAGGATG GTCTCTATCT CTGACCTTG TGATCCGCC GCCTCGGCCT

Recombination region
 ~~~~~

ALuY  
 ~~~~~

1681 CCCAAAGTGC TGGGATTACA GCGTGAGCC ACCGCGCCCG G CATATTTTT AAAGGAATCA
 Pr seq3 D

Pr Del R
 ~~~~~

1741 TCATGCTCAA TGAAGGACAG GCTAATTTTT TCAACATGAA GATAAGTGTT TTCCTATGTG

**Fig. S10 (A), continued**

Pr Del D

```

1   CTGATACCTG GCCTGACCAG TGAGTTTCGC CCTCTCCTGC CGAGCTTGCT AACTCAGGG
                                     MIRb
61  CAGTGAAACC AAGAGTTCAA CAGATAGAAG TGGTTGCATT AAGAGGCTGT GTTCTGATCA
                                     MIRb
121 AGGCTCTTGA TCTTCCTGAA TCACAGTTCA TTCATCTGGA AAATAATAAT AGCTACCCTG
                                     MIRb
181 CCTCCCTTAC AGGTTTGCAA CATGGATCAA AGGGGCTAGC ATATATGAAT AAGAGTGGAA
241 GTTCTTTTATT TATTAACAAC AAAAAGGAAG GGCTGAGTGA GTTTCTCGG TCATCTAAAG
301 TGGGATAAAG ATGAGTATAG CGCTTTCTTT CTTTCTTTCT TTCTTTCTTT CTTTCTTCTT
361 TCTTTCTTTC TTCCTCATCT AAAGTGGGAT AAAGATGAGT ATAGCATCTG CTTGCTTTCT
                                     AluSc
421 TTCTTTTTTTT CTTTCTCTCT CTCTCTCTCT CTCTCTCTTT CTTTCTTCGA GACAGAGCCT
                                     AluSc
481 TGCTCTGCCG CCAGGCTGGA ATGATCTTGG CTCACTGTAA CCTCTGCCTC CCGGGTTCAA
                                     AluSc
541 GCGATCCTCC TGCTCAGCC TCCCGAGTAG CTAGGACTAC AGGCACACAC CACCACGTCC
                                     AluSc
601 AGCTAATTTT TTTGTATTTT TGTAGAGACG GGATTTCAAC ATGTTGGCCA GGATGGTCTT
                                     Recombination region
                                     AluSc
661 GATCTCTTGA CCTCATGATC TGCTTGCCTC GGCCTCCCAA AGTGCTGGGA TTACAGGCGT
                                     ALuY
                                     Pr Del R
721 GAGCCACCGC GCCCGGCATA TTTTTAAAGG AATCATCATG CTCAATGAAG GACAGGCTAA
781 TTTTTTCAAC ATGAAGATAA GTGTTTTCTT

```

**Fig. S10 (B)**



#### (4) Supporting table

**Table S1.** PCR primers for genotyping with 3q27 STR markers

| <b>STR</b> | <b>Forward primer</b>           | <b>Reverse primer</b>          |
|------------|---------------------------------|--------------------------------|
| D3S1571    | /6-FAM/ACAGTGGCTGATGCCTTT       | CACAGGTGGGCACTACAT             |
| D3S3583    | /6-FAM/TGCAAAGTCACAGATGTCCA     | CGAGAGGCACCAGAGTGTT            |
| CA8        | GCCTGGCCCAGAAAATAGAT            | /6-FAM/TTCAAGGCCTACAACGTGATT   |
| CA7        | /6-FAM/ACCAATGTTCAGGGGATGTC     | GGTTGGGAGAGGAAGAGAGG           |
| D3S3592    | /6-FAM/GCAGTTCTGAGTGATTTACCA    | TCATCTGAGGTGCTGATTG            |
| GT3        | /6-FAM/TGGTGCAAAGTGATTGTGG      | GGGAGGATCTGTGGATTAGGA          |
| CA4        | /6-FAM/AAGCCATGCCATCACTTAG      | TGGAAGGCAAGGCTGTATCT           |
| CA5        | GCATTTTTGTTATCCTTGATTCC         | /6-FAM/CCAATAGGCTTCAGGCAGAC    |
| D3S1530    | /6-FAM/TTTAGCCTGGGTGACAGAGC     | AACCGCATAAGCCAGTTGTT           |
| GT1*       | /6-FAM/TCTGGTGTCCAGATTTTGAAAG   | CTTGGGTGCAGTTCTGGTCT           |
| CCTT       | /6-FAM/TGAGCAGGAAGGCAATAACA     | CCTGGGTGACAGAGCAAGAC           |
| CA2        | /6-FAM/AAGAAAGCCACCTCCCAAAC     | CCCTTTGATTGCTTTTCGAGT          |
| D3S1617    | /6-FAM/CTGGTACAAAGAACAACAGTTTCC | TCTGTGAAAACAACATGGGC           |
| GT2        | /6-FAM/TTAGGGTGACTTGCCCCTCT     | CAACCCATGCCTTAAAGGTG           |
| CA3        | TCATCTGCACCTCAGCCTTA            | /6-FAM/GGCTGGGTAAAGGTTTTTGC    |
| D3S1602    | AGAGCCTTCTATGGGTCTACAT          | /6-FAM/AGCTCAACCTTCAAACATACATT |
| D3S1262    | /6-FAM/GGCCCTAGGATATTTTCAAT     | AGTTTTTATGGACGGGGTTT           |

\* This STR marker, which has a low population heterozygosity, was not used for homozygous mapping of the critical genomic interval for the hypotrichosis locus.

## (5) Supporting References

- S1. E. K. Ginter *et al.*, Russian J. of Genetics (*Genetika*) **35**, 385 (1999).
- S2. V.A. McKusick , *Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders, 12th edn.* (Johns Hopkins Univ. Press, Baltimore, MD,1998).
- S3. D. F. Gudbjartsson, T. Thorvaldsson, A. Kong, G. Gunnarsson, A. Ingolfsdottir, *Nat. Genet.* **37**, 1015 (2005).
- S4. C. Roh, Q. Tao, S. Lyle, *Physiol. Genomics* **19**, 207 (2004).
- S5. C. Roh, Q. Tao, C. Photopoulos, S. Lyle, *J. Invest Dermatol.* **125**, 1099 (2005).
- S6. E. K. Ginter *et al.*, Russian J. of Genetics (*Genetika*) **37**, 692 (2001).
- S7. *Encyclopaedia Britannica*, <http://www.britannica.com/>.
- S8. S. O. Ang *et al.*, *Nat. Genet.* **32**, 614 (2002).
- S9. E. Liu *et al.*, *Blood* **103**, 1937 (2004).
- S10. S. Perrotta *et al.*, *Blood* **107**, 514 (2006).
- S11. T. Hiramatsu *et al.*, *J. Biol. Chem.* **278**, 49438 (2003).
- S12. H. Sonoda *et al.*, *J. Biol. Chem.* **277**, 34254 (2002).
- S13. F. Le Balle, M. F. Simon, S. Meijer, O. Fourcade, H. Chap, *Adv. Enzyme Regul.* **39**, 275 (1999).
- S14. J. Aoki *et al.*, *J. Biol. Chem.* **277**, 48737 (2002).
- S15. F. Carriere *et al.*, *Biochim. Biophys. Acta* **1376**, 417 (1998).
- S16. W. Ahmad *et al.*, *Science* **279**, 720 (1998).
- S17. P. M. Clissold, C. P. Ponting, *Trends Biochem. Sci.* **26**, 7 (2001).
- S18. X. Y. Wen *et al.*, *Hum. Mol. Genet.* **12**, 1131 (2003).