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Evidence of a founder effect for four cathepsin C gene mutations in Papillon-Lefèvre syndrome patients

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Abstract

We describe a mutation and haplotype analysis of Papillon-Lefèvre syndrome probands that provides evidence of a founder effect for four separate cathepsin C mutations. A total of 25 different cathepsin C mutations have been reported in 32 families with Papillon-Lefèvre syndrome (PLS) and associated conditions. A characteristic of these findings is the diversity of different cathepsin C mutations that have been identified. To evaluate the generality of cathepsin C mutations, PLS probands representative of five reportedly unrelated Saudi Arabian families were evaluated by mutational and haplotype analyses. Sequence analysis identified two cathepsin C gene mutations: a novel exon 7 G300D mutation was found in the proband from one family, while probands from four families shared a common R272P mutation in exon 6. The R272P mutation has been previously reported in two other non-Saudi families. The presence of the R272P mutation in probands from these four Saudi families makes this the most frequently reported cathepsin C mutation. To distinguish between the presence of a possible founder effect or a mutational hot spot for the R272P mutation, we performed haplotype analysis using six novel DNA polymorphisms that span a 165 kb interval containing the cathepsin C gene. Results of haplotype analysis for genetic polymorphisms within and flanking the cathepsin C gene are consistent with inheritance of the R272P mutation "identical by descent" from a common ancestor in these four Saudi families. Haplotype analysis of multiple PLS probands homozygous for other cathepsin C mutations (W249X, Q286X, and T153I) also supports inheritance of each of these mutations from common ancestors. These data suggest that four of the more frequently reported cathepsin C mutations have been inherited from common ancestors and provide the first direct evidence for a founder effect for cathepsin C gene mutations in PLS. Identification of these six short tandem repeat polymorphisms that span the cathepsin C gene will permit haplotype analyses to determine other founder haplotypes of cathepsin C mutations in additional PLS families.

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Keywords: Papillon-Lefèvre syndrome; cathepsin C; founder effect; chromosome 11q14

Papillon-Lefèvre syndrome (PLS) is a type IV palmoplantar keratosis (PPK).[1 2] While the palmoplantar keratodermas share some features of palmoplantar keratosis, they are aetiologically heterogeneous. PLS differs from other PPK by the presence of severe, early onset periodontitis. PLS (MIM 245000) is transmitted as an autosomal recessive trait, and an increased prevalence of parental consanguinity has been reported in PLS patients.[3–5] Although several different immunological findings have been reported in PLS affected subjects, these findings did not help to identify the underlying genetic defect. The genetic basis for most PLS cases appears to be mutations affecting both alleles of the cathepsin C gene (*CTSC*), located on chromosome 11q14. The majority of patients with PLS are reported to be homozygous for the same cathepsin C mutation.[6–8] Given the increased prevalence of parental consanguinity in PLS affected subjects, this finding is not unexpected. Biochemical assays of cathepsin C indicate that *CTSC* mutations associated with PLS and related conditions dramatically reduce its enzymatic activity. Heterozygous carriers of the mutation have approximately 50% of enzymatic activity, while subjects in whom both cathepsin C alleles are mutated show less than 10% of normal activity in hydrolysis of the synthetic substrate glycyl-L-arginine-7-amino-4-methylcoumarin.[7 8]

A causative role for *CTSC* mutations in PLS has been established for most (~80%), but not all cases of PLS. Several PLS cases exist in which mutations of the coding regions of *CTSC* have not been identified, although several of these families are consistent with genetic linkage to the interval of chromosome 11q14 that contains the cathepsin C gene locus.[8] While cathepsin C mutations have been identified in a range of ethnic groups, they have not been reported in PLS affected subjects from Saudi Arabia, even though PLS is well documented in this population.[9–13] To determine the generality of cathepsin C mutations, five PLS probands from reportedly unrelated families were ascertained from a dental clinic in Saudi Arabia. These subjects presented for treatment of severe periodontitis associated with PLS.

Methods and results

Following informed consent, periodontal tissues were surgically resected. DNA was isolated using the QiaAmp blood DNA purification kit with modification. After homogenisation in phosphate balanced saline, gingival

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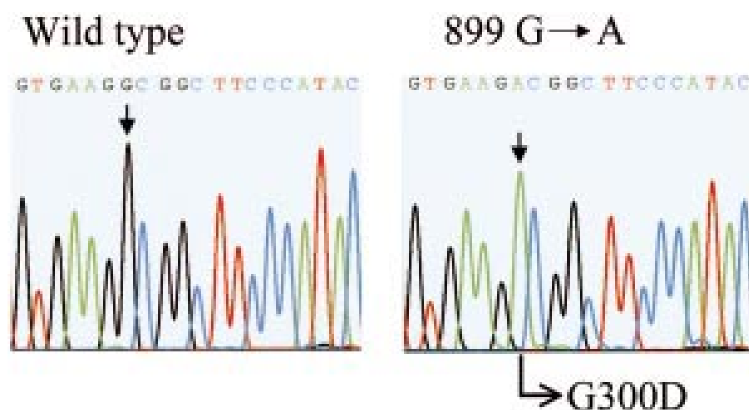

Figure 1 Novel mutation in CTSC. Sequencing of exon 7 showed a G to A transition at nucleotide 899 which results in substitution of glycine at position 300 by aspartic acid identified in proband x768 from Saudi Arabia.

tissue was incubated in a solution of AL buffer (Qiagen Inc) and protease for two hours at 56°C. All seven exons and associated splice site junctions of the cathepsin C gene were PCR amplified using oligonucleotide primers as previously described.[7 8] Following amplification, PCR products were purified using a Qiagen PCR Clean-Up kit. The sense and antisense strands of each PCR product were directly sequenced on an ABI 310 Genetic Analyzer using four dye terminator chemistry. Sequencing data were automatically collected and analysed by the ABI Sequence Analysis software. DNA sequences were compared with *CTSC* genomic sequences in GenBank (GI1947070) using the BLAST search program.[14] Intron-exon junctions were manually compared with the reported wild type *CTSC* consensus sequence.[7 8] Sequence analysis of the *CTSC* gene in these probands identified a nucleotide 815 mutation (G to C) in exon 6, resulting in a R272P change in four of the probands, and a nucleotide 899 mutation (G to A) in exon 7 resulting in a novel G300D change in the fifth proband (fig 1).

The coding region of the *CTSC* gene spans approximately 46 000 kb, consisting of seven exons that code for a 463 amino acid polypeptide. To date, 25 different *CTSC* mutations have been identified in probands from 32 different families with PLS and related conditions. A remarkable finding has been the diversity of cathepsin C gene mutations identified. Mutations have been identified in exons 2-7 of the cathepsin C gene.[7 8] While the majority of *CTSC* mutations reported have been unique, several have been reported in two or more families, including T153I (three families), Q286X (two families), G301S (two families), Y347C (two families), R339C (three families), and W429X (three families).[7 8] The R272P mutation identified in four Saudi families in this report has also been previously reported in two additional families.[7 8] In the homozygous state, the R272P *CTSC* mutation is associated with minimal enzymatic activity of cathepsin C.[8] The presence of the R272P mutation in six PLS families makes this the most frequently reported *CTSC* mutation, occurring in approximately 15% of PLS probands. Several possibilities exist for the relatively high frequency of *CTSC* R272P mutations, including a possible mutational hot spot or a founder effect for this mutation. Haplotype studies could help to determine if a specific mutation appears to occur on a common genetic background suggesting it has been inherited from a common ancestor, or if the shared mutation appears to have arisen independently multiple times. Unfortunately, the available genetic polymorphisms that were used to localise the gene responsible for PLS to chromosome 11q14 are not adequate for such haplotyping studies. The contiguous DNA sequence is not known for the genetic interval spanning the STRP markers used in linkage analyses for PLS, and the distance between these markers and their exact order is not known with certainty.[15–17] Additionally, current physical maps of the interval place the *CTSC* gene at three possible locations relative to STRP loci, in a 4.5 cM interval flanked by D11S1887 and D11S1311 (Genbridge 4 Radiation Hybrid map; www.ncbi.nlm.nih.gov/genemap99). In previous studies we have placed the *CTSC* gene within a 3 cM interval flanked by D11S1367 and D11S931, but this localisation cannot be confirmed without a contiguous primary sequence of the interval.[18] As a result, use of these STRPs to perform haplotype analyses is inadequate. This genetic interval is too large to make assumptions of inheritance of mutations "identical by descent" (IBD) for subjects who may be separated over many generations.

For the purposes of haplotype analyses, the most important interval is that containing and immediately spanning the *CTSC* gene. Using computational methods, we have identified and ordered fragments from 11 BACs to create a contig that contains the *CTSC* gene. Using the BLAST program algorithm, we searched the non-redundant human database and the high throughput genome sequence to identify a BAC clone (RP11-292E14) that contained sequence homology to the *CTSC* gene (AC011088).[14] We then used the fragmented pieces of this BAC to identify additional BACs that shared >95% sequence homology. With this strategy, we identified 10 additional fragmented BACs (listed in fig 2), which were then aligned and ordered using the Sequencher program (GeneCodes Corp). With this approach we were able to develop a BAC contig (fig 2), which spans ~380 000 base pairs, containing the complete coding region as well as the immediate 5' promoter region and 3' UTR of the *CTSC* gene. In order to identify polymorphic loci for haplotyping, we screened this contig for short tandem repeat polymorphisms (STRPs) using the Tandem Repeat Finder at http://c3.biomath.mssm.edu/trf.html.[19] With this approach, we identified six novel STRPs that span the *CTSC* gene contig. Oligonucleotide primers flanking each STRP were designed using Oligo 4.0 based on the BAC contig fragments listed in fig 2. Fluorescence labelled primers were used in the amplification of specific products. PCR products were detected by an ABI377 fluorescent sequencer and analysed by GENESCAN 2.1 (Applied Biosystems Inc).

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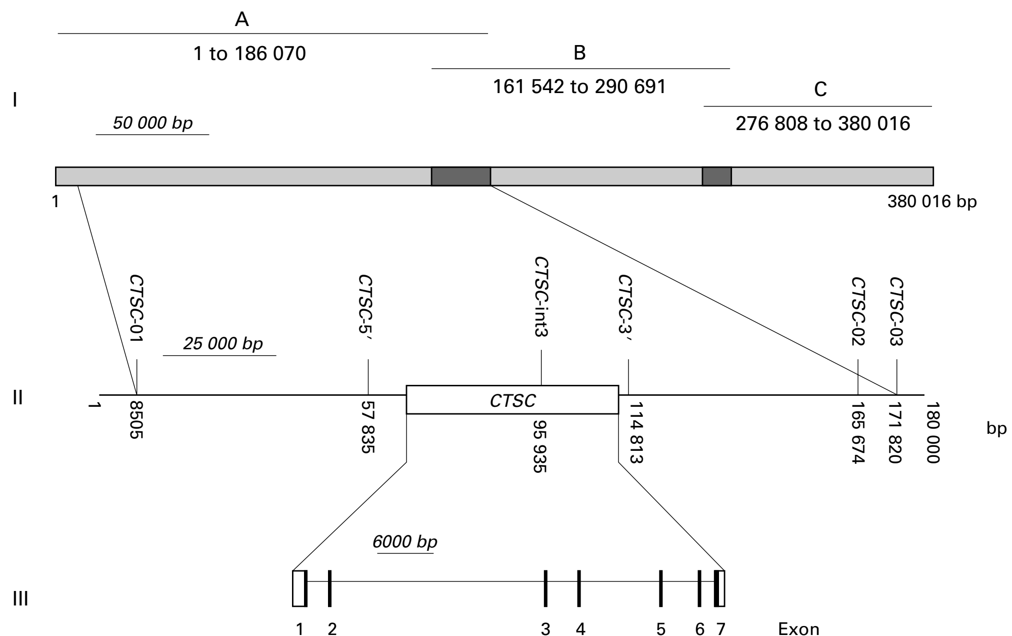

Figure 2 BAC contig spanning the CTSC gene. The BAC contig was constructed from 11 BAC clones as described in the text. Fragmented BACs which were incorporated into contig are: RP11-428B3 (Accession No AC009885) fragments 1, 2, 3, 4, and 6; RP11-114M5 (Accession No AC009927) fragments 2 and 3; complete RP11-292E14 (Accession No AC011088); RP11-334M8 (Accession No AC011629) fragments 3, 4, 5, 7, 8, 10, 12, 13, 14, 15, 16, 17, and 19; RP11-22C16 (Accession No AC018775) fragments 3, 7, 9, and 12; RP11-588K15 (Accession No AC023888) fragments 5 and 7; RP11-876F8 (Accession No AP000795) fragments 1, 5, 6, and 9; RP11-685B24 (Accession No AP001642) fragments 1, 2, 3, 4, 5, 7, and 8; RP11-113K4 (Accession No AP002362) fragments 1, 3, 4, 5, 6, 7, and 8; RP11-2544N1 (Accession No AP002497) fragments 2, 3, 5, 6, 7, 10, 11, 12, 14, 15, 16, 17, 20, 21, 22, 23, 24, and 25; and RP11-2232N20 (Accession No AP002510) fragments 1, 2, 4, 5, 6. (I) The BAC contig was first constructed as three overlapping fragments (A, B, and C) as shown above the complete contig. The overlapped sequences of fragments A and B and B and C are shown in hatched boxes. The first nucleotide of the contig was considered to be No 1. (II) Location of novel STRP markers spanning the CTSC gene. Each of the STRP markers and the starting nucleotide number at the 5' end of the forward primer are shown. The location of the CTSC gene is shown in a box. (III) The genomic structure of the CTSC gene with intron distances drawn to scale and exons depicted as vertical lines. The 5' and 3' untranslated regions are shown as open boxes. A base pair scale (bp) is provided for each section.

The location of these STRP loci on the contig and their relation to the cathepsin C gene is shown in fig 2. Two loci, *CTSC*-01 and *CTSC*-5', are in the region 5' to the *CTSC* start codon, the *CTSC*-int3 locus is located within intron 3, and three loci, *CTSC*-3', *CTSC*-02, and *CTSC*-03, span an interval 3' to the gene. Oligonucleotide primers and annealing temperatures used to amplify each of these STRPs are given in table 1.

The six novel STRPs identified span the *CTSC* gene and are localised within an interval of less than 164 kb. The expected recombination frequency across this interval is <0.002. Existing genetic maps that include genetic markers used in linkage studies to localise the PLS gene locus (D11S931, D11S1311, D11S1358, D11S1887) span 4.5 cM and do not precisely localise the cathepsin C locus (GenBridge 4). Using data obtained from the Human Genome Project Working Draft UCSC (http://genome.UCSC.edu), we estimated that the cathepsin C gene is 1187 kb from D11S1780 and 760 kb from D11S1367. The positions and distances of each of the six newly identified STRPs from the cathepsin C gene are known with certainty. The genetic distance between these new STRP markers is less than 10% of the distance between the closest markers used for linkage. None of the genetic markers used in previous PLS linkage studies are located on this BAC contig, indicating that they are outside this interval spanning the cathepsin C gene. These new STRPs are therefore much better markers to haplotype the interval and to evaluate evidence for a common or conserved genetic haplotype for PLS than any markers on existing physical maps of the interval. To determine allele frequencies for these novel STRP markers, we genotyped 112 chromosomes from unrelated, normal Turkish controls. PCR amplification of these STRPs was performed using 5 ng of genomic DNA in a total volume of 7.5 μl using *Taq*Gold as described by the manufacturer for a total of 40 cycles with the annealing temperatures shown in table 1. Allele sizes and relative allele frequencies of each STRP observed for these controls are shown in table 1.

For haplotype analyses, we genotyped polymorphisms for the five Saudi PLS probands as well as an additional 22 PLS probands from different families, reported to be unrelated, for whom we have identified cathepsin C mutations. All subjects were genotyped for the six new STRPs that span the *CTSC* gene contig. They were all also genotyped for six DNA polymorphisms used in previous PLS linkage studies. Two markers, D11S937 and D11S4147, were from the Perkin Elmer 10 cM linkage panel (Applied Biosystems Inc). The oligonucleotide primers for four other markers (D11S1887, D11S1780, D11S1367, and D11S1358) were redesigned using the Oligo 4.0 program, to permit more optimal PCR amplification. The modified oligonucleotide

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Table 1 STRP markers for human cathespin C gene interval

STRP	Location*	Allele size (bp)	Primer	Allele frequency	T_{an} (°C)
CTSC-01	8505–8811	306–322	F 5'-TAGGGCAAAATGTGTCTGAAGAGT-3'† R 5'-TACCCATGAGATGGCTTTCATCAG-3'	306(.10); 308(.20); 310(.34); 312(.04); 314(.08); 318(.17); 320(.04); 322(.03)‡	58
CTSC-5'	57835–58180	332–364	F 5'-GCAGCACAGGACGGCTTTGAATAC-3'† R 5'-GATGGTGAAACCCTGTCTCTACTA-3'	332(.01); 334(.02); 336(.07); 338(.01); 340(.02); 342(.04); 344(.01); 346(.10); 348(.08); 350(.07); 352(.07); 354(.07); 356(.23); 358(.01); 360(.15); 362(.01); 364(.03)‡	58
CTSC-int3	95935–96181	239–251	F 5'-TATGGAAGAGTGCTCACAACTATA-3'† R 5'-TCTCATGCCACCATTCAGAGGTAA-3'	239(.02); 241(.15); 243(.06); 245(.10); 247(.62); 249(.03); 251(.02)‡	60
CTSC-3'	114813–115158	347–381	F 5'-CTAAACTCAGGCCCTTCATCATTC-3' R 5'-ACCCCTCATAAGCCCTTCTCTATC-3'†	347(.43); 353(.02); 355(.01); 361(.02); 363(.24); 365(.06); 367(.02); 369(.05); 371(.04); 373(.10); 381(.01)‡	58
CTSC-02	165674–165835	148–168	F 5'-TAGCAGAGAAGAAGCAAGATGTGT-3' R 5'-AAGCTGGACAAGGAGACTGTATAG-3'†	148(.04); 150(.03); 152(.04); 154(.28); 156(.05); 158(.06); 160(.25); 162(.07); 164(.15); 166(.02); 168(.01)‡	58
CTSC-03	171820–172020	193–213	F 5'-CTCCCTATCCATTCTCAGTATCTA-3'† R 5'-CCAGAGTCACAGCAAATCTCAGAG-3'	193(.05); 195(.17); 197(.22); 199(.09); 201(.18); 203(.11); 205(.12); 207(.02); 209(.03); 213(.01)‡	58
D11S1887M	93	237–257	F 5'-CAGGCTTCAAAGGCTTAATGTGAA-3' R 5'-CCTACCTGCAACATGAGATGAGTG-3'†	237(.33); 239(.15); 241(.11); 243(.11); 245(.07); 249(.04); 251(.04); 253(.11); 257(.04)§	58
D11S1780M	94.6	263–289	F 5'-TTCAGGGATCTGCAGCAATTACTT-3' R 5'-TCTGAGATTACAGGCGTGAGTCAC-3'†	263(.04); 271(.28); 273(.14); 275(.04); 281(.32); 287(.04); 289(.14)¶	58
D11S1367M	95–96	229–254	F 5'-TTAAATCTTGGTAGCCAGAACTAT-3'† R 5'-ATTGTGCTAGGCTCTCTAAATACA-3'	221(.03); 229(.20); 233(.26); 237(.10); 241(.17); 245(0.10); 249(.07); 253(.07)**	58
D11S1358M	96.3	273–283	F 5'-AGCTCAAGTGCCTGTCAACAGATG-3'† R 5'-TGCGATTCTCCTGGCTCTTAGAAC-3'	273(.21); 275(.04); 277(.13); 279(.33); 281(.25); 283(.04)††	58

*The location of the CTSC specific marker is based on the BAC contig spanning the cathepsin C gene (fig 2). For the modified (M) D11S markers, the cM location is the distance from the telomeric end of the short arm on chromosome 11 based on GB4 map (www.ncbi.nlm.nih.gov/genemap99) and the physical map of 30 000 human genes.[20]
†Location of the fluorescent label for each primer set.
‡Based on 112 chromosomes of Turkish controls.
§¶**††Based on the PLS patients in this study. The founder effects of each haplotype were taken in consideration with these patients based on 25, 22, 30, and 24 chromosomes, respectively. T_{an}: annealing temperature for PCR.

primers and annealing temperatures used to amplify these polymorphic loci (D11S1887*M*, D11S1780*M*, D11S1367*M*, and D11S1358*M*) are shown in table 1.

Results of the haplotype analysis for the 27 PLS probands are shown in table 2. Although all of the newly identified STRPs from the *CTSC* contig were highly polymorphic in the control population with a minimum of seven alleles detected for each locus, not all markers were equally useful for haplotype analysis. The relatively high frequency of specific alleles for several markers (*CTSC*-01, *CTSC*-int3, and *CTSC*-02) potentially limits their use in drawing inferences in some cases. Nonetheless, the composite haplotype data are compelling and are consistent with a shared haplotype for the four Saudi probands with the R272P mutation. These subjects are each homozygous at seven syntenic STRPs from D11S1887 to *CTSC*-02 (bold in table 2). These four subjects (x766, x767, x769, and x770) all share the same genetic haplotype for the interval spanning the *CTSC* gene. This haplotype was not present in the Saudi proband (x768) with the novel exon 7 G300D mutation, suggesting that this haplotype is not simply a highly conserved haplotype in the Saudi population. A Turkish proband (x235) with an R272P mutation shares the same allele at the flanking 5' marker in *CTSC* intron 3 (*CTSC*-int3). This allele occurred with a frequency of 15% in the Turkish control population. With these data, we cannot determine if subject x235 shares a common haplotype with the Saudi patients or if the haplotype similarity represents the serendipitous sharing of common alleles.

Haplotype analyses are consistent with a common founder for three other cathepsin C mutations. The W429X mutation is shared by three Turkish probands, who are all homozygous for alleles at six loci spanning the cathepsin C gene (*CTSC*-01 to *CTSC*-03), consistent with inheritance of the W429X mutation from a common ancestor. Two Turkish probands share the same Q286X mutation, and these subjects are both homozygous for alleles at 11 loci spanning the cathepsin C gene locus. These findings are supportive of inheritance of the Q286X mutation identical by descent from a common ancestor. Two probands with T153I mutations were studied. X914, an ethnic Turk, is homozygous for the mutation. The other (p-T), of Scottish and Chinese heritage, is a compound heterozygote with the T153I mutation and an exon 2 deletion (nt199-222 deletion resulting in deletion of amino acids 67-74, table 2). Based on haplotype data for the parents of this subject, we can determine the haplotypes transmitted with each mutation. The T153I mutation carried by subject p-T appears as part of a four locus haplotype shared with x914. This shared haplotype (*CSTC*-5' - *CSTC*-02) spans the cathepsin C gene. Although these patients are reported to be of diverse ethnic background, the shared cathepsin C haplotype is consistent with inheritance of the T153I mutation identi-

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Table 2 Haplotype of genetic interval spanning the cathepsin C gene locus

(A) Haplotype associated with recurrent CTSC mutations

	ID													
STRP	*x766*	*x767*	*x769*	*x770*	*x235*	*p7*	*p22*	*p701*	*p29*	*p78*	*p-T**	*x914*	*p70*	*p53*
D11S937	171/173	159/159	159/159	161/169	163/177	163/163	159/159	146/146	161/173	173/173	159/165	163/163	159/169	159/175
D11S4147	245/245	245/245	245/245	237/237	239/241	243/243	239/239	241/241	**243/243**	**243/243**	227/237	237/237	239/247	241/243
D11S1887	**237/237**	**237/237**	**237/237**	**237/237**	253/257	239/239	241/241	245/245	**237/237**	**237/237**	237/243	241/241	237/237	245/245
D11S1780	**281/281**	**281/281**	**281/281**	**281/281**	289/289	271/271	271/271	281/281	**289/289**	**289/289**	271/281	281/281	289/289	289/289
CTSC-01	**310/310**	**310/310**	**310/310**	**310/310**	310/310	**310/310**	**310/310**	**310/310**	**306/306**	**306/306**	310/310	314/314	318/318	310/310
CTSC-5'	**360/360**	**360/360**	**360/360**	**360/360**	346/346	**352/352**	**352/352**	**352/352**	**336/336**	**336/336**	344/**352**	**352/352**	356/360	360/360
CTSC-Int3	**241/241**	**241/241**	**241/241**	**241/241**	**241/241**	**241/241**	**241/241**	**241/241**	**245/245**	**245/245**	247/**247**	**247/247**	247/247	243/243
CTSC mutation	**R272P/ R272P**	**R272P/ R272P**	**R272P/ R272P**	**R272P/ R272P**	**R272P/ R272P**	**W429X/ W429X**	**W429X/ W429X**	**W429X/ W429X**	**Q286X/ Q286X**	**Q286X/ Q286X**	del in ex2/**T153I**	**T153I/ T153I**	R339C/ R339C	R339C/ R339C
CTSC-3'	**373/373**	**373/373**	**373/373**	**373/373**	371/371	**363/363**	**363/363**	**363/363**	**363/363**	**363/363**	347/**373**	**373/373**	377/377	369/369
CTSC-02	**160/160**	**160/160**	**160/160**	**160/160**	160/160	**160/160**	**160/160**	**160/160**	**154/154**	**154/154**	154/**160**	**160/160**	160/160	150/150
CTSC-03	195/195	193/193	193/193	195/195	195/195	**205/205**	**205/205**	**205/205**	**195/195**	**195/195**	195/195	201/201	203/203	209/209
D11S1367	249/253	249/249	245/249	241/245	233/237	233/233	233/233	233/241	**233/233**	**233/233**	233/233	229/241	237/237	245/245
D11S1358	279/279	279/279	273/273	279/279	275/275	277/277	277/277	277/277	**277/277**	**277/277**	279/281	279/279	273/273	283/283

(B) Haplotype associated with unique CTSC mutations

	ID												
STRP	*x240*	*p706*	*IR1*	*p27*	*x768*	*p-P**	*IR19*	*IR8†*	*x236*	*IR11*	*p76*	*p5*	*p19*
D11S937	157/157	159/173	161/161	167/171	149/159	159/159	167/167	163/163	159/167	161/171	159/159	161/161	155/155
D11S4147	243/243	241/243	245/245	239/239	239/239	227/227	239/239	239/239	227/227	243/243	235/235	237/237	241/241
D11S1887	241/241	237/239	237/237	239/239	243/243	253/253	243/243	239/239	251/251	249/249	237/237	237/237	237/237
D11S1780	273/273	287/287	273/273	271/271	271/271	273/281‡	281/281	263/263	271/271	275/275	281/281	281/281	271/271
CTSC-01	308/308	306/306	308/308	310/310	310/310	314/318‡	310/310	310/310	310/310	310/310	310/310	314/314	320/320
CTSC-5'	346/346	332/332	342/342	356/356	357/357	350/354‡	352/352	362/362	346/346	354/354	348/348	352/352	360/360
CTSC-Int3	247/247	243/243	241/241	247/249	243/243	247/247	247/247	241/241	247/247	243/243	247/247	247/247	241/241
CTSC mutation	ins in ex3/ ins in ex3	ins in ex4/ ins in ex4	W235X/ W235X	R250X/ R250X	G300D/ G300D	G300S/ E447G	G301V/ G301V	G301S/ G301S	Y304N/ Y304N	E319G/ E319G	Y340C/ Y340C	del in ex7/ del in ex7	del in ex7/ del in ex7
CTSC-3'	349/349	365/365	363/363	347/347	361/361	347/347	347/347	363/363	363/363	347/347	347/347	375/375	369/369
CTSC-02	154/154	154/154	154/154	162/162	154/154	148/156‡	154/154	154/154	160/160	152/152	164/164	160/160	160/160
CTSC-03	201/201	197/197	201/201	205/205	197/197	197/205‡	199/199	213/213	205/205	195/195	207/207	201/201	203/203
D11S1367	237/237	233/233	229/241	221/221	249/249	229/229	233/253	245/245	229/229	241/241	233/233	229/229	233/233
D11S1358	281/281	279/279	279/279	279/279	281/281	279/281‡	273/273	281/281	281/281	277/277	273/273	279/279	273/273

*Compound heterozygous for *CTSC* mutation.
†Toomes *et al*[7] have also identified a family with this mutation.
‡The mutation specific haplotype not determined.
Syntenic regions of shared haplotypes are in bold.

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cal by descent from a common ancestor. In addition to the above shared mutations, one Turkish (p70) and one Egyptian (p53) proband are homozygous for the same R339C mutation without evidence to support inheritance from a common ancestor (table 2).

Discussion

Before this study, 25 different cathepsin C gene mutations had been reported in probands of PLS and related conditions from 32 reportedly unrelated families. While the majority of these *CTSC* mutations were reported in nuclear families or small kindreds, seven mutations were reported in two or more families that were not known to be related.[7 8] To determine if any of these mutations have been inherited from a common ancestor, or if they have arisen independently, we performed haplotype studies. A difficulty in this approach stemmed from the lack of a contiguous DNA sequence for the interval spanning the cathepsin C gene and polymorphic DNA markers used to establish linkage. To overcome this problem, we developed a BAC contig that contains the cathepsin C gene locus, and identified six new short tandem repeat polymorphisms that localise to this contig. Haplotyping of 27 PLS probands has provided evidence that four of the five *CTSC* mutations identified in more than one family, R272P, Q286X, W429X, and T153I, appear to have been inherited from a common ancestor in the families we studied. These findings suggest that the cathepsin C gene is not more prone to any specific mutation in the cases studied. The availability of these six new short tandem repeat polymorphisms spanning the cathepsin C gene should permit haplotype analyses in other PLS families to identify additional cases of *CTSC* mutations inherited from common ancestors.

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