Hum Genet (1999) 105 : 489–495
Digital Object Identifier (DOI) 10.1007/s004399900146

ORIGINAL INVESTIGATION

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Structure of the human *Lanosterol Synthase* gene and its analysis as a candidate for holoprosencephaly (*HPE1*)

Received: 1 June 1999 / Accepted: 16 August 1999 / Published online: 26 October 1999

Abstract Holoprosencephaly (HPE) is the most common birth defect of the brain in humans. It involves various degrees of incomplete separation of the cerebrum into distinct left and right halves, and it is frequently accompanied by craniofacial anomalies. The *HPE1* locus in human chromosome 21q22.3 is one of a dozen putative genetic loci implicated in causing HPE. Here, we report the complete gene structure of the human *lanosterol synthase* (*LS*) gene, which is located in this interval, and present its mutational analysis in HPE patients. We considered *LS* an excellent candidate HPE gene because of the requirement for cholesterol modification of the Sonic Hedgehog protein for the correct patterning activity of this HPE-associated protein. Despite extensive pedigree analysis of numerous polymorphisms, as well as complementation studies in yeast on one of the missense mutations, we find no evidence that the *LS* gene is in fact *HPE1*, implicating another gene located in this chromosomal region in HPE pathogenesis.

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Introduction

Holoprosencephaly (HPE) is a clinically heterogeneous brain malformation, which can be caused by genetic and/or environmental factors (Ming et al. 1998a; Roessler and Muenke 1998). In cyclopia, the most severe form, there is a single eye, a superiorly placed nasal structure called a proboscis, and the lack of separation of the cerebral hemispheres into discrete left and right halves (Cohen 1989). The clinical HPE spectrum is variable even in autosomal dominant families segregating mutations in a single HPE gene such as *Sonic Hedgehog* (Ming and Muenke 1998). Craniofacial findings of microcephaly, hypotelorism, or a single central incisor can be clinical clues to underlying brain anomalies comprising HPE (DeMyer et al. 1964). Recently, several genes have been identified with a role in HPE pathogenesis, including *Sonic Hedgehog* (*HPE3*) (Belloni et al. 1996; Roessler et al. 1996, 1997a, 1997b), the transcription factor *ZIC2* (Brown et al. 1998a, 1998b), *SIX3* (*HPE2*) (Wallis et al. 1999), and several others currently under investigation (Gripp et al. 1998; Ming et al. 1998b). The *HPE1* locus on human chromosome 21q22.3 is one of several named HPE loci (Frézal and Schinzel 1991) and is defined by a set of three overlapping cytogenetic deletions (Muenke et al. 1995).

The lanosterol synthase (LS) enzyme catalyzes the cyclization step in the biosynthesis of cholesterol. Among

the essential functions of cholesterol in preserving the integrity of cell membranes and, as the substrate for the synthesis of sterols and hormones, there is a novel role recently described for cholesterol as an adduct to signaling molecules such as Sonic hedgehog (Porter et al. 1996a, 1996b). Undermodification of the Sonic Hedgehog protein is suspected to be a contributing factor in the pathogenesis of Smith-Lemli-Opitz (SLO) syndrome, which in turn is associated with an increased incidence of HPE (Kelley et al. 1996). SLO has been shown to result from the biochemical deficiency of cholesterol during fetal development (Cunnif et al. 1997) and is associated with mutations in the enzyme that catalyzes the penultimate step in cholesterol biosynthesis, 7-delta sterol reductase (Fitzky et al. 1998; Moebius et al. 1998; Wassif et al. 1998; Waterham et al. 1998). We speculated that mutations in the human *LS* gene might lead to similar patterning deficiencies of the Sonic Hedgehog protein and contribute to brain malformations within the HPE spectrum. Now, we report on the gene structure of the human *LS* gene and a strategy to amplify each coding exon for mutational analysis. Here, we show the mutational analysis of the human *LS* gene (Young et al. 1996), which maps within the 5 cM minimal critical region for *HPE1* (Muenke et al. 1995).

Materials and methods

Determination of the LS gene structure

Having previously identified a cosmid 7G8 (Young et al. 1996) that contained the 5' end of the *LS* gene, we used the primer pairs LSH2830F (5' ggcaggtagtgtgcatccgt 3') and HSLS3000R (5' gacttgggatgttccatgacagac 3'), derived from the 3' UTR sequence, as a probe to screen a chromosome-21-specific cosmid library (LL2INCO2-Q, Soeda et al. 1995) for the 3' end of the gene. A single cosmid, 72H3, was chosen for further analysis, since it completed the contig for the entire *LS* gene. Putative exon-specific primers based on the cDNA sequence and the initial 5 trapped exons (Young et al. 1996) were then used to sequence the cosmids and to determine the first set of intron/exon boundaries. Additional primers were then synthesized to determine the sequence of both DNA strands adjacent to the intron/exon boundaries as each new set was empirically determined. Note that the initiator methionine is in exon 2. Genbank numbers are as follows: accession no. AJ239021 (exons 2, 3, and 4), AJ239022 (exons 5 and 6), AJ23903 (exon 7), AJ239024 (exons 8, 9, and 10), AJ239025 (exon 11), AJ239026 (exon 12), AJ239027 (exons 13 and 14), AJ239028 (exons 15, 16, 17, and 18), AJ239029 (exons 19 and 20), AJ239030 (exon 21), AJ239031 (exons 22 and 23). Sequencing was performed on an ABI PRISM 377 automated sequencer.

Patients and DNA preparation

Patient samples were obtained after being given informed consent according to the guidelines of the Children's Hospital of Philadelphia Institutional Review Board, and DNA samples were prepared from blood or lymphoblastoid cells lines using routine methods. Three HPE patients had various deletions, including the distal long arm of chromosome 21. Two of these were previously published: 46,XX,del(21)(q22.3->qter) and 46,XY,-21,+der(21)t(10;21)(p11.2;q22.3)mat (Muenke et al. 1995). A third patient with semilobar HPE had the following karyotype: 46,XY,r(21).

A total of 30 HPE patients from 20 unrelated families were chosen for single-strand conformational polymorphism (SSCP) analysis. These HPE families were chosen for their apparent autosomal recessive pedigrees with clinically normal parents and multiple affected children. These families included the typical range of HPE phenotypes. When SSCP band shifts were detected, these samples were sequenced to determine the potential mutations. Where possible, the sequence variations were examined in the parents and siblings to determine the pattern of transmission and also examined in a panel of normal controls to determine any correlation with the disease state or uniqueness of the sequence variations. All families were too small for effective linkage analysis. As has been shown, some of these families could also be consistent with germline mosaicism for a dominant HPE gene, such as *SHH* (Roessler et al. 1996, 1997a) or *SIX3* (Wallis et al. 1999).

Reverse-transcriptase polymerase chain reaction analysis

Poly-A selected RNA was isolated from lymphoblastoid cell lines (FastTrack, InVitrogen), synthesized into complementary DNA using random primers (cDNA cycle kit, InVitrogen), and amplified with the three primer sets as depicted in Fig. 1. The first primer pair is LSF2 (5' cccaagcttaggggctctgaacgggatgaca 3') and LSR2 (5' cccaagcttcgatgctgatgctcttggtgaat 3'), which overlaps with the second primer pair LSF3 (5' ggggaattcccgacgaccgattcaccaa 3') and LSR3 (5' cccaagcttaggagcagcacagccttcaa 3'), which overlaps with the last amplicon LSF4 (5' ggggaattccccagataaccctcccgactacc 3') and LSR4 (5' cccaagcttttcctcacccaagcccgacaagc 3'). Amplicons were directionally subcloned into Bluescript KS$^-$ (Stratagene) using the *Hin*dIII or *Eco*RI sites nested within them and pools of at least 20 clones were subjected to automated sequencing. Candidate sequence changes were confirmed by individual exon amplification using patient genomic DNA and re-sequencing. The reverse-transcription polymerase chain reaction (RT-PCR) products were sufficient to screen exons 5 to 23; the first three coding exons (2, 3 and 4) were analyzed by direct amplification and sequencing of the patient genomic DNA.

Mutational analysis

Amplification of genomic DNA was performed in a 15-µl reaction volume, using 60–100 ng DNA template, 200 µM each of dATP, dGTP, and dTTP, 125 µM dCTP, 3.5 µCi α^{32}P-dCTP (800 Ci/mmol [10 mCi/ml]), 30 pmol each primer, 1.5 µl 10× PCR buffer (Gibco), 1.25 µl 10× PCR Enhancer (Gibco), 1.5 mM $MgSO_4$ (Gibco), and 1 U *Amplitaq* polymerase (Perkin Elmer). All of the PCR reactions were performed in a PTC-100 thermal cycler (MJ Research, Inc.).

For SSCP analysis, following amplification, 5 µl of each sample was diluted with 10 µl stop solution [9.5 ml deionized formamide (Fluka), 20 mM ethylene diamine tetraacetic acid (EDTA), 0.5% (w/v) bromophenol blue, and 0.5% (w/v) xylene cyanol], denatured for 10 min at 94°C, then immediately chilled on ice. The samples were run either with or without 5% glycerol at room temperature on 0.5× MDE gel solution (FMC Bioproducts) sequencing-grade polyacrylamide gels. Sequencing of the amplicons demonstrating SSCP band shifts was performed by the Protein and DNA Core Facility of the Children's Hospital of Philadelphia on an ABI Prism 377 analyzer.

Functional analysis of LS activity

The yeast *LS* cDNA (Corey et al. 1994) was modified by site-directed mutagenesis (Ausubel et al. 1999) to include the corresponding K625Q mutation and was expressed in a yeast mutant for functional complementation analysis. The yeast LS mutant SMY8 was transformed using this construct, and the resultant strain's ability to grow on media without supplemental ergosterol was tested as described previously (Corey et al. 1996).

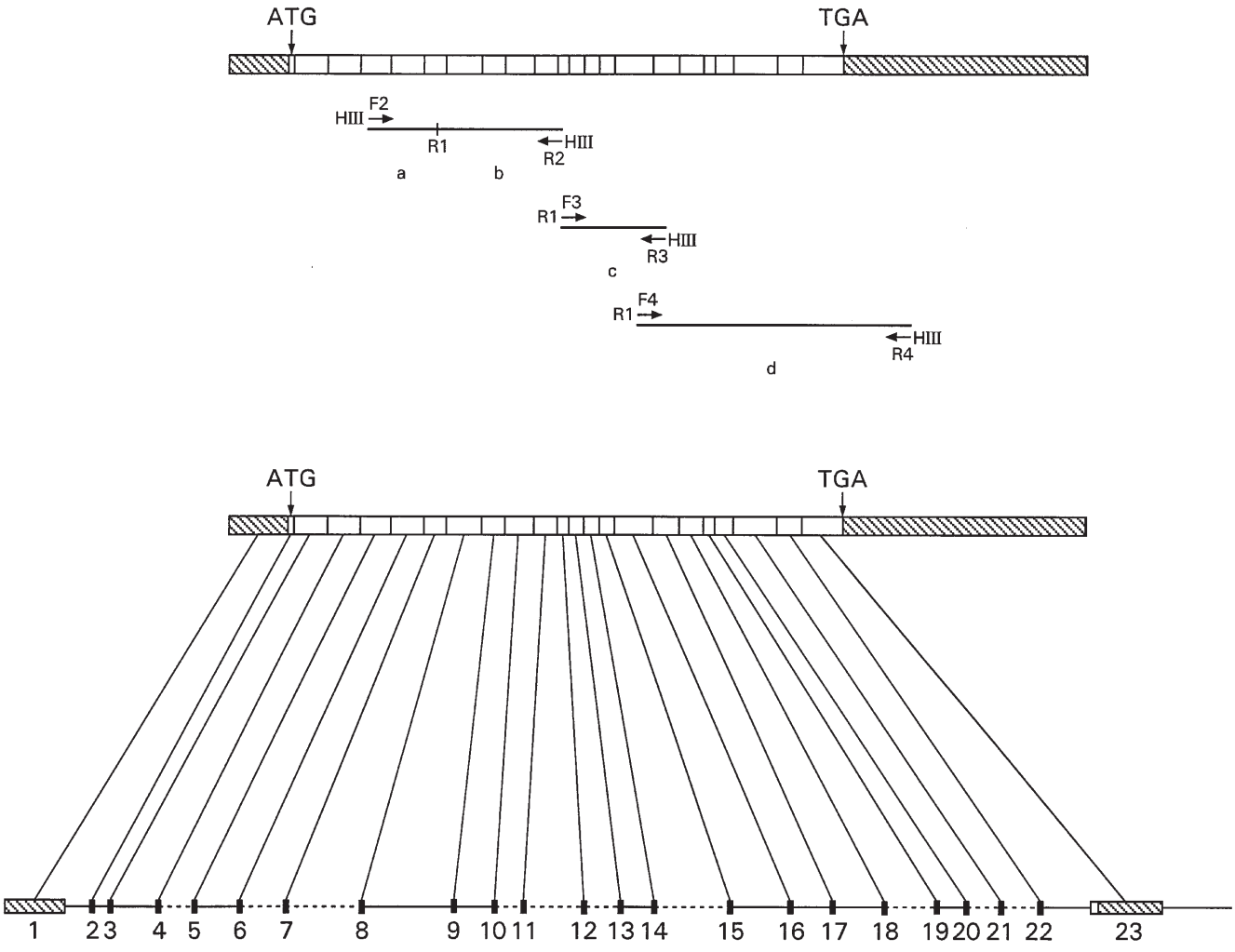

Fig. 1 In the *top panel*, a diagram of the *lanosterol synthase* (*LS*) cDNA is shown aligned with a set of three amplicons used in reverse-transcription polymerase chain reaction (RT-PCR) experiments to subclone the *Eco*RI (R1) and *Hin*dIII (HIII) fragments a, b, c, and d for sequencing. In the *bottom panel*, the same *LS* cDNA diagram and its relationship to the genomic structure of individual exons is shown. Note that the coding exons extend from exon 2 to 23

Results

Structure of the human LS gene

A contig of two cosmids (7G8 and 72H3) was analyzed to identify 23 individual exons spanning 40–50 kb of genomic DNA for the *LS* gene as diagramed in Fig. 1. The genomic structure shown in Fig. 1, predicts a total of 22 coding exons and is in agreement with the cDNA sequence (Baker et al. 1995). Note that the 5' untranslated region represented as exon 1 has not been completely characterized. Also note that the sequence variants seen in the hemizygous probands (see below) were detected as expressed transcripts as well as from genomic DNA, indicating that this is a functional gene. The relevant sequences of the intron/exon boundaries of the *LS* gene are shown in Table 1.

RT-PCR analysis of HPE patients with deletions of 21q22.3

Poly-A RNA was isolated from an affected member of each of the three different 21q22.3 hemizygous deletions (these unrelated families are denoted here as families A, B, and C), which served to define the *HPE1* locus. In all three cases, the deletion includes the human *LS* gene based on the comparison of the somatic cell hybrid data defining *HPE1* (Muenke et al. 1995) and the genetic position of the YACs and cosmids containing the *LS* gene (Young et al. 1996). We reasoned that these would be the most likely HPE individuals to have a loss of function mutation in the remaining *LS* allele. As shown in Table 2 (number 6), we found a sequence variation 863C>G in family A, predicting no change in the coding sequence (P288P) and this change was also noted in four additional HPE families (G, H, M, P) as a common polymorphism that failed to segregate with the disease. This sequence variant was also readily detectable in either the heterozygous or homozygous state in the normal control population. In family A, we also noted a 1949T>C sequence variation in exon 20, predicting no change in the coding sequence (H650H). This variation was seen as a homozy-

Table 1 Sequences of the lanosterol synthase (LS) intron/exon boundaries

Exon	Splice donor	Size bp (Intron)	Splice acceptor	Size bp (Exon)
1	5'UTR	> 300 (1)		> 133 (1)
2	ACGGAGGGCAC gtgagt	130 (2)	ccggtggcctcag GTGTCTGCGG	29 (2)
3	GGCTGGACACC gtaagt	730 (3)	cgatctctttgcag AAGAATTACT	166 (3)
4	CCTCCTGCCAG gtagga	> 480 (4)	ctcctgccacacag GCCTCCTGAT	139 (4)
5	GGCTGGGGCCT gtgagt	650 (5)	ctctgctcctgtag GCACATTGAG	109 (5)
6	CACAAGAAAG gtacgg	> 970 (6)	ctctccccgctcag GTGGTGCTGT	121 (6)
7	CAGAGATGTG gtatgt	> 900 (7)	tgctgttgccccag GCTGTTTCCT	97 (7)
8	GCCTCCGCCAG gtagga	600 (8)	actgcccccaccag GAGCTCTATG	136 (8)
9	CGTGGTATATG gtgcac	390 (9)	cctgtcccctgtag CGCTCCTCAA	109 (9)
10	GCATCGGCCCG gtcagt	> 850 (10)	tgtctctcctgcag ATCTCGAAAA	119 (10)
11	GACTATCTCTG gtgagt	> 540 (11)	ttttctcctcacag GATGGGCCTT	98 (11)
12	TGAAAATGCAG gtaagg	> 500 (12)	tcctttgtgtgtag GGCACCAACG	28 (12)
13	CTCTGCTTGAG gttcgt	1060 (13)	gtctctctcttccag GCGGGCGGGC	57 (13)
14	GGCTCTCACAG gtgagg	> 920 (14)	tcgatgctgcccag GTCCCAGATA	72 (14)
15	AGATGCGCAAG gtatgc	670 (15)	cttgtgctctgcag GGTGGCTTCT	51 (15)
16	CTGTGGCTGTG gtaagg	660 (16)	ctctgtgatttcag CTGCTGAACA	150 (16)
17	GGAGGTCTTCG gtgagt	730 (17)	cctccctttcccag GGGACATCAT	97 (17)
18	GCGGAGATCCG gtaagg	760 (18)	atctcgtgttccag GGAGACCCTC	106 (18)
19	TCCTGGGAAGG gtgagt	450 (19)	ctgtttccctccag CTCCTGGGGA	66 (19)
20	TACCGAGATGG gtgagt	> 430 (20)	tctggtctttgcag GACTGCCTGT	81 (20)
21	ATGGCCGTTCG gtgggg	> 450 (21)	ttctcttccaccag GCATCCTGAC	170 (21)
22	ACTGGCCGCAG gtatgc	640 (22)	ctgtgttcttttag GAAAACATTG	79 (22)
23	TGA codon at position 130–132			> 960 (23)

Table 2 Sequence variations in the human *lanosterol synthase* (*LS*) gene. For the normal controls, the first number is the number of individuals who were heterozygous for the normal and the sequence variation, followed by the number of individuals homozygous for the sequence variation, followed by the number of individuals studied by means of single-strand conformational polymorphism (SSCP). All band-shift patterns detected in the normal controls were verified by sequencing. *N.D.* not detected under these gel conditions. Notation follows the recommendations of Antonarakis and the Nomenclature Workshop Group (1998)

Sequence variation	Intron	Exon	Codon	Position	Family	Normal controls hetero/homo/total
1. GC**C** to GC**T**		4	A121A	362C>T	D, E, F	12/2/56
2. C**G**A to C**A**A		5	R175Q	523G>A	D, E, F	10/5/54
3. cccc(**c/g**)ctcag	5		Acceptor	IVS5–5C>G	K, M	16/12/54
4. cccc(**c/t**)accag	7		Acceptor	IVS7–6C>T	E[b], F, G, H, M, P	13/13/53
5. CC**T** to CC**C**		8	P282P	845T>C	G	N.D.
6. CC**C** to CC**G**		8	P288P	863C>G	A[a], G, H, M[b], P	14/17/53
7. cccctgtc(**c/t**)cctgtag	8		Acceptor	IVS8–8C>T	M	N.D.
8. C**A**C to C**G**C		9	H310R	928A>G	D, E, F	11/1/50
9. ttgc(**a/g**)cct	10		None	IVS10–29A>G	D, E, F	6/0/50
10. acgt(**a/g**)agc	12		None	IVS12+32A>G	L, O	18/16/52
11. agatgca(**c/t**)ggg	18		None	IVS18+36C>T	D[b], G[b], I	28/12/52
12. atcca(**c/g**)aag	18		None	IVS18+45C>G	E[b], F[b], H[b], I[b]	22/20/52
13. C**T**G to C**G**G		20	W576R	1728T>G	K, R[b]	N.D.
14. C**G**G to C**A**G		20	R622Q	1864G>A	C[a]	2/0/103
15. **T**TG to **G**TG		20	L641V	1923T>G	B[a], E[b], F[b], K[b], P	19/20/53
16. CA**T** to CA**C**		20	H650H	1949T>C	A[a], F[b], K[b]	N.D.
17. C**C**G to C**T**G		21	P688L	2062C>T	O	0/0/57

[a]Hemizygous
[b]Homozygous variant: individuals were detected in the holoprosencephaly (HPE) families who were homozygous for the rare allele without clear correlation with HPE

gous variant in clinically normal individuals in families F and K, although, it was not readily detected in the analysis of the normal group of patients either because the band shift was minor under these gel conditions or that it was indistinguishable from the other band shifts in exon 20. There were no other sequence changes noted in either the cDNA fragments or the genomic amplicons for exons 2, 3 or 4 in family A.

Table 3. Sequences of the primers used in this study

Primer	5' to 3' Sequence	Exon	Amplicon size (bp)	Annealing temperature (°C)
HSLSF1	gccagcattagagcactgcagcag	1 to 2	212	62
LSH200R	gcgggctcggtcttgtagg	1 to 2		
065F	cccctacaagaccgagcccgcc	2	195	63
066R	ctcacccctcagggtcccgag	2		
111F	ggctgtatgtgaagagggttcct	3	259	58
112R	gatcccaagggtgatccagggtg	3		
067F	aggaaggggcctggtcttagcag	4	210	61
068R	catgtgcagtgacacaggggcagg	4		
114F	ggtttctcacctgtcctctgctcc	5	178	58
115R	gcccagcacatgctgcacattcc	5		
069F	gctgacaggaagcagatgacgtgg	6	174	63
070R	cgacccaacaacccaatcaacagc	6		
071F	gcaggcttgagtcttggggtctcc	7	214	61
072R	ccttcactttgttccctgatgagg	7		
073F	gaggccacccctcctggtttgagc	8	235	63
074R	accctgaccctgaccctgggctgc	8		
075F	agtgtcatggtgtctggcatccac	9	219	61
076R	cactttcggacctcaacccccagg	9		
160F	ggtctggagcggagccttgacagg	10	191	60
161R	ctcggttccagtcttcgtgagagg	10		
162F	cctgttttccatagagtgtggc	11	230	59
163R	gcagccttacctgcattttcat	11		
061F	gcatctgctgctgtaacacc	12	199	56
062R	gaggagtggcaagtgtgtgg	12		
077F	cctctctgtcctgctgtctcttcc	13	200	56
078R	tagggcagggtggaggtgaggtgg	13		
204F	tgtcctctgcttccacatttgc	14	120	58
205R	cgggacagggatggggctggctcc	14		
079F	ctgtggggaggttctgagaactgg	15	259	61
080R	ggtatggacggggctgctgggacc	15		
121F	gtgtgtgctcagcacatgggc	16	172	58
122R	cgcgcgagtgctggccgacc	16		
081F	tgcttgttgtggctttgcctgag	17	216	56
082R	acactgaatggctgagaccctcc	17		
145F	ttcactggaccaatctcgtgttcc	18	162	59
146R	acgcagtgtgtgagagcagaaacc	18		
123F	ggtggaagggctgaggctctccc	19	167	58
124R	acagagcattgggttgagaacc	19		
125F	tgtgatggtgtctcctgtctctgg	20	239	60
126R	acccaggctcagggacggtcc	20		
147F	gggtgtgtttcagccttctcttcc	21	160	60
148R	cacgctggaggtcagtgctgg	21		
127F	aggctcacccacaagccagccaagc	22	268	60
128R	acagcgggcacccagcaggtaggc	22		

Similarly, for family B, we identified only one missense mutation in exon 20, predicting a conservative L641V change, that was also seen in four additional pedigrees (E, F, K, P); this 1923T>G mutation segregated as a common polymorphism and not with the affected status in these families (data not shown). This mutation is also commonly seen in the control population.

In family C, we identified a potentially significant missense mutation (Table 2, number 14) in exon 20. This 1864G>A sequence variation was not observed initially in over 100 normal chromosomes and predicts the substitution of a Q for an R residue at a position conserved as the positively charged residues R or K in the family of *LS* genes. When we tested the ability of this mutation to complement the ability of a LS mutant yeast strain to grow on media lacking ergosterol, the mutant form of the yeast protein allowed the transformed strain to grow normally (data not shown). This demonstrates that the mutant enzyme retains catalytic function. Subsequent analysis of 53 additional normal control individuals revealed that this is a rare polymorphism, since it was eventually detected in two normal control individuals.

SSCP analysis

We examined the entire coding region of the *LS* gene by means of SSCP in a total of 30 additional patients from 20 unrelated families with apparent autosomal recessive inheritance of HPE and present the results in Table 2. The primers used for the amplification are described in Table 3. We identified a total of 17 different sequence variations, which we number sequentially for clarity. The majority of these sequence variations were also detected in the normal control population (1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 14, and 15). For the remainder, either the analysis of the pedigree transmission and/or the benign nature of the sequence change predicts no correlation with the HPE phenotype.

Seven of these sequence variations (3, 4, 7, 9–12) occur in non-coding intronic sequences. Only one of these intronic variants was not readily detected in the control population. This intronic variation, number 7, predicts the substitution of a T for C in the pyrimidine tract of the consensus splice acceptor. This change is likely to be functionally equivalent.

Of the ten remaining sequence variations, four are in the wobble position, and the codons are predicted not to alter the primary structure of the protein (1, 5, 6, 16). Of the six remaining missense mutations, one of these (number 14) has been excluded as a likely cause for HPE by functional studies in yeast; note also that we eventually detected this variant when we screened 53 additional normal controls. The sequence variant 13, the W576R substitution, is observed in an asymptomatic parent (family R) in the homozygous state. The identical mutation fails to segregate with HPE in a second family, K, where it is detected in a clinically normal mother and not either of her children with HPE. Therefore, this missense mutation is unlikely to exhibit a significant effect. The mutation 17 predicts a P688L change, which is observed in a normal parent and not his child with HPE. Although we did not detect this variant in the control population, the lack of a correlation with HPE makes this missense mutation unlikely to be responsible for HPE in this family.

The remaining three missense mutations are part of an interesting haplotype consisting of at least seven different sequence variations (1, 2, 4, 8, 9, 12, and 15), which are transmitted as a unit from parent to child in three different HPE families. All of these sequence variants are detectable as common variants in the normal control population. In one of these families, D, the haplotype is observed only in the phenotypically normal mother and not her son with HPE. This mother has mutations 1, 2, 8, and 9 by sequencing and the remaining changes seen in the other two families (E and F) were not detected using SSCP. Furthermore, in family E, the haplotype is present in only one of two affected sibs, suggesting that these changes are irrelevant to the HPE phenotype, despite the fact that this haplotype accounts for the final three missense mutations.

Discussion

The *Sonic Hedgehog* gene was the first of several HPE genes to be identified, and there is growing evidence for many additional genes (Roessler and Muenke 1998). We considered the *LS* gene as an excellent candidate since it was located within the *HPE1* minimal critical region in 21q22.3, and cholesterol is an essential adduct to the carboxy-terminus of the signaling domain of SHH-N. This modification serves to limit the diffusion of the patterning activity away from the site of synthesis. Furthermore, teratogenic agents, which inhibit the synthesis of cholesterol, are an effective means of producing HPE in experimental animals such as the rat (Roux et al. 1980; Lanoue et al. 1997). We further suspected that defects in *LS* would be at least as severe as those associated with SLO syndrome, which is caused by deficiency of the final step in cholesterol synthesis. This study did not provide any evidence for the *LS* gene in HPE pathogenesis, at least in live-born infants, which were the only samples available for analysis. Since we are aware that an incidence of HPE of 1:250 conceptuses (Matsunaga and Shiota 1977) differs from the 1:16,000 incidence at birth (Roach et al. 1975) by 50-fold, we cannot exclude a role for cholesterol-synthesis defects that might not be detectable in our sample of patients. Furthermore, in contrast to the SLO defect, complete loss of LS activity would severely limit sterol intermediates, suggesting that complete lack of LS activity might be lethal to the developing embryo. Although our premise was that LS defects would be recessively inherited, even modeling the mutations that we have identified as dominant is incompatible with the pedigree transmission and does not alter our conclusion that *LS* is not the *HPE1* gene.

Analysis of the functional domains of the Sonic Hedgehog protein (Hall et al. 1995, 1997) has led us to speculate that defective cholesterol modification of the protein due to intrinsic abnormalities could be one of several mechanisms causing HPE. In addition to structural mutations in SHH-N, we are attempting to identify a category of mutations in HPE patients that could affect cholesterol transferase activity. Experiments are in progress to substantiate this hypothesis. Therefore, despite the negative results in this report, there continues to be the possibility that examination of the cholesterol synthesis pathway may yet provide additional clues to HPE pathogenesis.

Acknowledgements We wish to thank the patients and their families for their participation in these studies. This work was supported by grants from the Swiss National Science Research Foundation 31.40500.94 and the European Union/OPES/PL 950302 (S.E.A), NIH grants AI41598 (S.P.T.M.), HD28732 and HD29862 (M.M), and by the Division of Intramural Research, National Human Genome Research Institute, NIH.

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