A mutation of the p63 gene in non-syndromic cleft lip

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Cleft lip with or without cleft palate (CL/P)

- is the most common craniofacial anomaly
- 1 in 500–2500 live births
- 70% of cases of CL/P occur as an isolated abnormality
- non-syndromic CL/P
- several genes and environmental factors involved

monogenic disorders

- *MSX1*
- *IRF6*

mendelian clefting syndromes include

- ectrodactyly
- ectodermal dysplasia
- ankyloblepharon

three syndromes are allelic disorders caused by mutations in the *p63* gene.

- EEC(ectrodactyly-ectodermal dysplasia-clefting syndrome)
- AEC (ankyloblepharon-ectodermal dysplasia-clefting syndrome)
- RHS(Rapp-Hodgkin syndrome)

p63

- This gene has several isoforms with two different transcription initiation sites
- TA
- TA isotypes
- N isotypes

The participants in the study :

- 88 sporadic cases of non-syndromic CL/P
- 12 additional cases with a positive family history

Table 1 Characteristics of the patients with nonsyndromic cleft lip with or without cleft palate

	Cleft lip only	Cleft lip with cleft palate	Total
No. of probands	37	63	100
Sporadic	31	57	88
Familial	6	6	12
Laterality			
Right side cleft	9	15	24
Left side cleft	26	32	58
Bilateral cleft	2	16	18
Severity			
Complete cleft	19	61	80
Incomplete cleft	18	2	20
Sex			
Male	16	42	58
Female	21	21	42

PCR

- Genomic DNA was isolated from peripheral blood
- amplify fragments encompassing each of exons 1–15 and exon 3' of the *p63* gene
- PCR reactions were carried out in a 20 µl volume containing 50 ng genomic DNA,1X PCR buffer, 1.5 mmol/l MgCl2, 0.2 mmol/l dNTPs, 0.2 µmol/l of each primers and 0.5 U *Taq* polymerase, using the following parameters: 35 cycles
- 30 seconds at 94°C, 30 seconds at the appropriate annealing temperature

Table 2 Oligonucleotides and PCR conditions for *p63* mutation analysis

Exon	Forward	Reverse	Product size (bp)	AT (C°
1	CCCTATTGCTTTTAGCCTCC	ACTGTGCTGACTAAACAAGG	281	55
2	CTACATATATACCTGCATGG	AAAAACATGCCCTAGTAAGC	344	52
3	AGCCTTGCTGACTTTGAAGC	CACATGACTGAAAAGACAGG	317	55
3'	CATATTGTAAGGGTCTCAGAGG	GACCGAGAACCGCAAATACG	223	62
4	ATGCATTCACCCATGGATGC	GAATCGCTAAACTGGGAAGG	437	55
5	GTAAACAGGCAGCATGCAGC	AGTCTGAATCAGGTAGGTGG	401	55
6	CACCAACATCCTGTTCATGC	GCTAGAAACATCCCTGTTGC	296	55
7	AGAGGGAAGAACTGAGAAGG	CAGCCACGATTTCACTTTGC	256	55
8	GGAAGTGGTAGATCTTCAGG	GCAGCTTCTCCAATATCACC	294	55
9	GTGTTGCTGGTACTACTGTC	GACTAAGACACCTCCTTTCC	334	55
10	ACTTCTAACAGTTCTACAGC	CTCATCAATCACCCTATTG	275	52
11	CCATGTTTTAAACAGAGACC	CACAGAGTCTTGTCCTAAGC	313	52
12	TTAACCAGACAAGATGGACC	CCCTTCCAACTGTTTTATGG	321	52
13	CTTATCTCGCCAATGCAGTT	TACAAGGCGGTTGTCATCAG	238	62
14	GGAATGATAGGATGCTGTGG	GCAGGAGTGCGCAGGAGTGC	450	55
15	CAGGCACTCTATTCTGTCTA	GGAAATACAACACACACACT	280	62

RESULTS

- In 100 DNA samples from subjects with non-syndromic CL/P,
- 21 variant sites were identified.
- All were single nucleotide changes,
- comprising 14 transitions (five in coding regions)
- seven transversions (one in a coding region)

- The coding regions of *p63* contained six different variants, three synonymous and three non-synonymous.
- The three non-synonymous variants, 269C T, 937A G, and 1690G C, have not been reported previously.
- One non-synonymous variant was found in each of three patients;
- all were sporadic cases, with no anomalies besides the oral clefts, normal radiographs of hands and feet (data not shown), no consanguinity, normal development, and different geographic origins.

Table 3 Variant sites of *P63* found in 100 Thai patients with nonsyndromic CL/P

Nucleotide position(	Exon/intron	Nucleotide change	Expected amino acid change	Hetero-zygotes	Homo-zygotes
261	Exon 4	C T	N87N	1	0
269	Exon 4	C T	S90L	1	0
462+39	Intron 4	T A	–	6	0
463–49	Intron 4	T A	–	1	0
463–42	Intron 4	G A	–	1	0
463–41	Intron 4	G A	–	1	0
463–40	Intron 4	T A	–	1	0
649+42	Intron 5	G A	–	9	0
742	Exon 6	C T	L248L	7	0
875+51	Intron 7	G A	–	1	0
937	Exon 8	A G	R313G	1	0
1013–22	Intron 8	A G	–	23	1
1095+79	Intron 9	A G	–	41	11
1218	Exon 10	C T	H406H	1	0

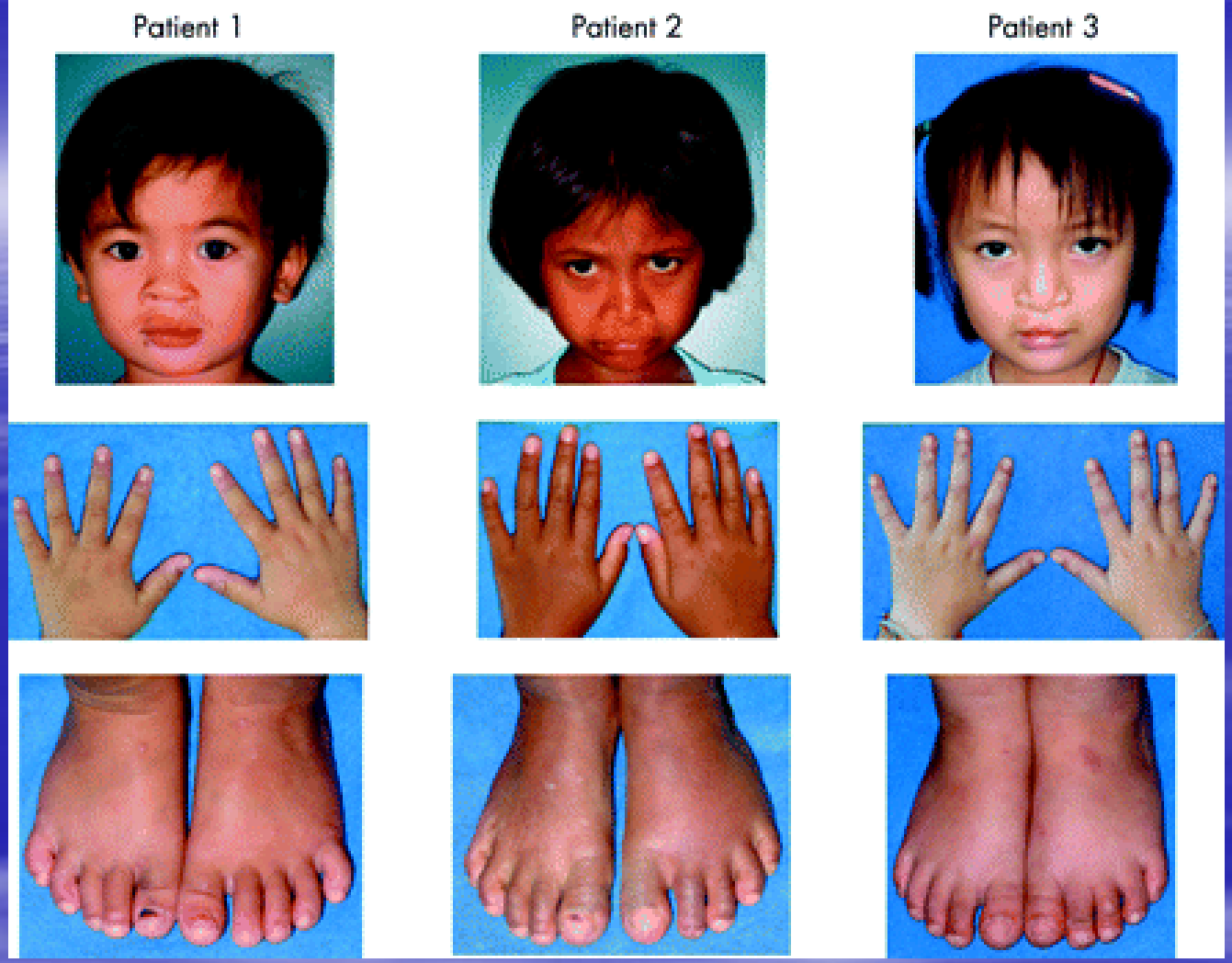

Figure 1 Clinical features of patients with non-synonymous variants. The left, middle, and right panels relate to patients 1, 2, and 3, respectively. Note that there were no other dysmorphic features besides oral clefts in all three patients. Written consents were obtained from the patients' legal guardians for publication of the images.

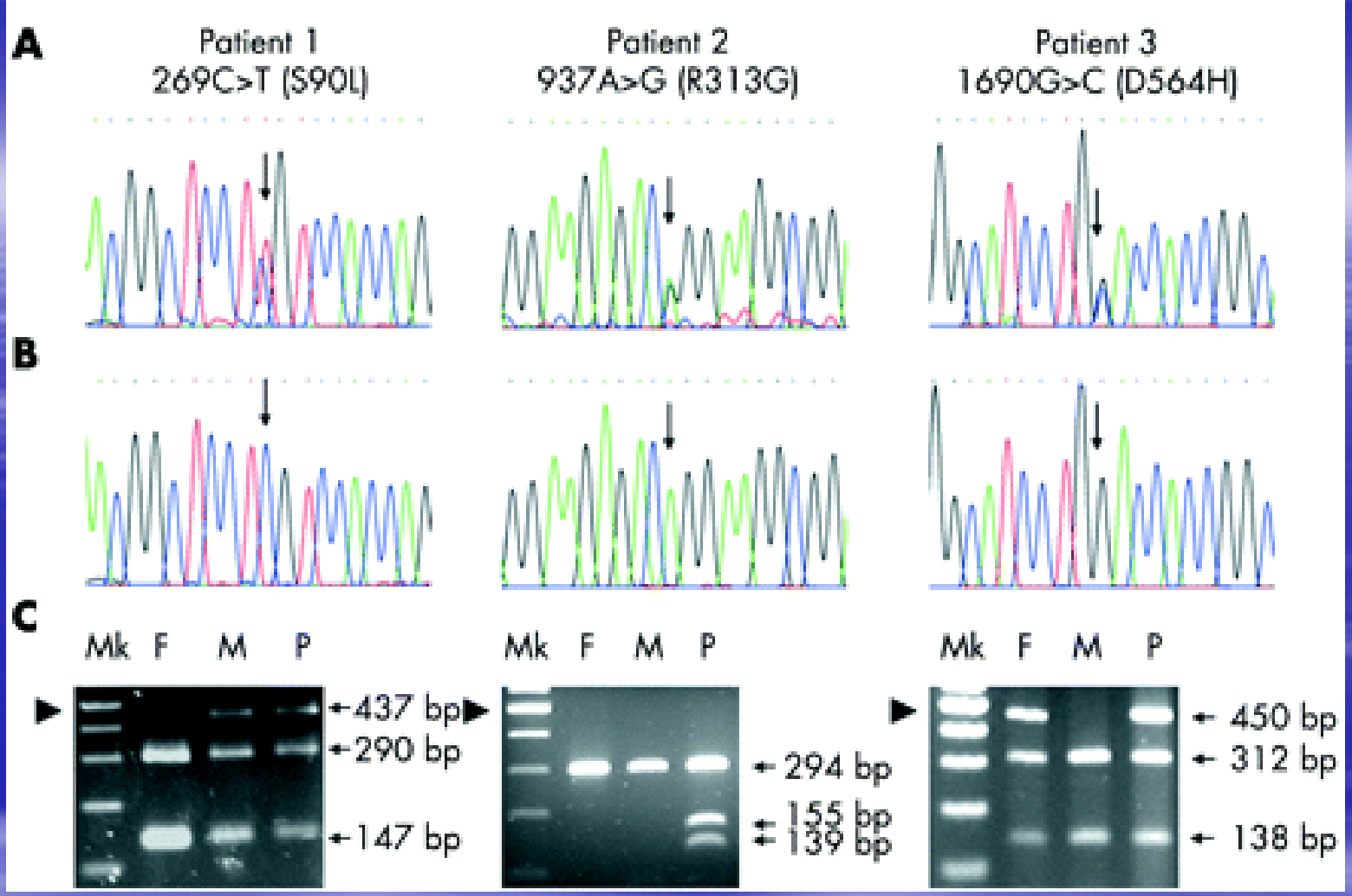

Figure 2 Mutation analysis. The left, middle, and right panels relate to patients 1, 2, and 3, respectively. Electropherograms of (A) patients, showing 269C T, 937A G, and 1690G C (arrows) in patients 1, 2, and 3, respectively; and (B) controls, showing normal genotypes at codons 269 as CC, 937 AA and 1690 GG (arrows).

DISCUSSION

- The variant is a nonconservative substitution, predicted to result in conversion of an arginine to glycine (R313G). Arginine is a polar, positively charged amino acid while glycine is non-polar.
- The arginine at codon 313 is evolutionarily conserved in all examined vertebrates, including rat, mouse, chicken, frog, and zebrafish.
- It is in the DB domain, a functionally important area, present in all isotypes of *p63*. A previously reported mutation in *p63*, D312H, has been found in patients with EEC syndrome and occurs just one amino acid N-terminal to the mutation found in our patient.
- PolyPhen predicted this variant to be probably damaging.
- The variant apparently arose de novo
- It was not identified in a cohort of 500 control individuals.

- the *p63* R313G mutation is associated with non-syndromic CL/P highlights the wide phenotypic spectrum of *p63* mutations

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