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Pattern of *p63* Mutations and Their Phenotypes—Update

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Heterozygous mutations in the transcription factor gene *p63* cause at least six different syndromes with various combinations of ectodermal dysplasia, orofacial clefting and limb malformations. Here we will present an update of mutations in the *p63* gene together with a comprehensive overview of the associated clinical features in 227 patients. These data confirm the previously recognized genotype–phenotype associations. Moreover, we report that there is a large degree of clinical variability in each of the p63-associated disorders. This is illustrated by the different phenotypes that are seen for the five-hotspot mutations that explain almost 90% of all EEC syndrome patients. © 2006 Wiley-Liss, Inc.

Key words: EEC; AEC; Rapp–Hodgkin; Limb-mammary; ADULT; *p63*; ectrodactyly; ectodermal dysplasia; split hand; split foot

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INTRODUCTION

Ectodermal dysplasias (EDs) constitute a large and complex group of developmental syndromes, comprising more than 170 different clinical conditions. The combined incidence of ED is approximately seven in 10,000 births. Causative genes for EDs have been identified in approximately one fifth of EDs. One group of EDs is associated with orofacial clefting and split-hand/foot malformation (SHFM). Ectrodactyly, Ectodermal Dysplasia, and Cleft lip/palate syndrome (EEC, OMIM 604292) is the prototype of these syndromes, which is caused by heterozygous mutations in the *p63* gene. A number of EEC-like syndromes has been described, five of which are also caused by mutations in the transcription factor gene *p63*: Ankyloblepharon-Ectodermal defects-Cleft lip/palate (AEC, OMIM 106260), Limb Mammary Syndrome (LMS, OMIM 603543), Acro-Dermato-Ungual-Lacrimal-Tooth syndrome (ADULT, 103285), Rapp–Hodgkin Syndrome (RHS, OMIM 129400) and non-syndromic Split Hand/Foot Malformation (SHFM4, OMIM 605289). These syndromes share at least one of the three main phenotypic hallmarks with EEC syndrome (Fig. 1). Previously, syndrome-specific mutation patterns have been identified upon analysis of *p63* mutations in 78 families, comprising five different syndromes (RHS was not elucidated). Here we will provide an update of *p63* mutation patterns by providing detailed clinical overviews of 126 unrelated families in which a causative *p63* mutation has been identified. The combined data confirm the genotype–phenotype associations that have been previously uncovered. In addition, it is becoming clear that phenotypes differ for the five hotspot mutations that account for the large majority of patients with EEC syndrome.

EEC — PROTOTYPE OF P63 SYNDROMES

EEC syndrome comprises limb malformations, ED and orofacial clefting. Representative limb malformations are ectrodactyly and syndactyly. ED is seen as light colored, sparse hair and absence of eyelashes, eyebrows and alopecia can be observed. Skin is thin and dry, sometimes resembling dermatitis. Nails are usually dystrophic and have pits. Also dental changes are reported such as hypodontia or anodontia and teeth can be prone to caries, because of enamel defect and salivary gland malfunction.

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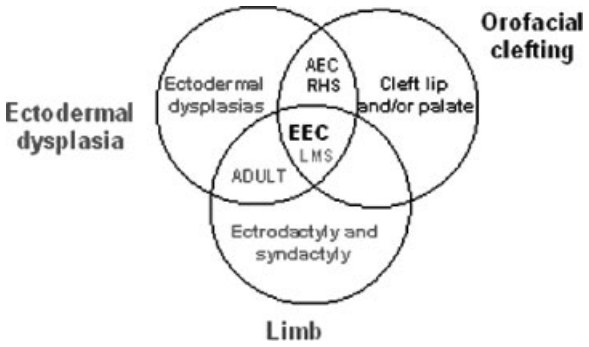

FIG. 1. Ectodermal dysplasia (ED) combined with orofacial clefting and limb malformations are the three hallmarks of p63 syndrome family. EEC, the prototype of these syndromes combines all three main symptoms, whereas the others lack mainly one of the hallmarks. LMS falls into a borderline expressing less ectodermal signs than EEC. ED and orofacial clefting are main symptoms in AEC and RHS, whereas limb malformations replace the orofacial clefting symptoms in ADULT syndrome.

Orofacial clefting is frequent, and facial maxillary and mild malar hypoplasia can be present. Hearing loss is uncommon. Choanal atresia is rare. Lacrimal duct stenosis contributes to keratitis, which is common in EEC patients, sometimes associated with photophobia. Genitourinary malformations are also part of the EEC: sometimes external and internal genitalia are abnormal, but more often malformation has affected kidney, ureters or bladder. Mental retardation is rare in EEC, and probably as common as in the general population. These are the main symptoms that can be generally observed in EEC syndrome. However, there is a large degree of clinical variability, as we will demonstrate below.

EEC syndrome has been localized to three different chromosomes, although only one causative gene has been found [Celli et al., 1999]. EEC1 has been mapped to chromosome location 7q21 both by the identification of chromosomal abnormalities, translocations and deletions in syndromic SHFM patients and by linkage analysis in a single large EEC1 family [Haberlandt et al., 2001; Tackels-Horne et al., 2001]. However the chromosome 7 phenotype differs from classical EEC in that the patients have fewer ectodermal defects and present sensorineural deafness and ear malformations. EEC2 denotes linkage to chromosome 19 in one large EEC family [Maas et al., 1996; O'Quinn et al., 1998]. EEC3 on 3q27, is characterized by mutations of the *p63* gene [Celli et al., 1999]. Interestingly, a common *p63* mutation, typical for EEC syndrome (R227Q) was also found in the EEC2 family, indicating that the linkage to chromosome 19 was a spurious finding. Indeed, *p63* mutations have been demonstrated in 98% of patients with a classical EEC phenotype [van Bokhoven et al., 2001 and our own unpublished data]. Mutations in *p63*-derived EEC syndrome (EEC3) have been reported previously in 152 cases. These comprise 26 families and 60 sporadic cases [Wessagowit et al., 2000; Kosaki et al., 2001; van Bokhoven et al., 2001; Barrow et al., 2002; Hamada et al., 2002; South et al., 2002; Akahoshi et al., 2003; Dianzani et al., 2003; de Mollerat et al., 2003; Berdon-Zapata et al., 2004; Pozo et al., 2004; Ray et al., 2004; Lehmann et al., 2005]. All mutations are point mutations clustered in the DNA binding domain, except for one frameshift mutation, which is located in the Sterile Alpha Motif (SAM) domain [Celli et al., 1999]. The most frequently mutated amino acids residues are R204, R227, R279, R280, and R304, which cover 86.8% of all EEC syndrome cases. These mutation hotspots are the same as previously reported [van Bokhoven and Brunner, 2002] and mimics p53 mutation hotspots entirely, except for the R227 residue. All of the hotspot missense mutations are C to T transitions at CpG islands.

A compilation of the manifestations of the 152 p63-associated EEC patients provides a more complete picture of the main characteristics observed in patients, the mutation patterns and mutation-specific phenotypes (Table I). Ectodermal tissues, such as hair, teeth, nails, and lacrimal ducts are affected in about one half of the EEC patients, whereas abnormal skin was reported only in one third of the patients. Clefting of lip and palate is present in approximately 40% of the patients. Isolated cleft lip or cleft palate was observed only in rare instances (two and four cases, respectively). Limb malformations are a main component of the syndrome with ectrodactyly in two thirds of EEC cases and syndactyly less frequent in about 40% of patients. Minor characteristics in EEC are mammary gland and/or nipple hypoplasia, which are present in approximately 15% of the cases. Urinary and kidney problems may cause serious morbidity, and these were reported in 15% (Table I).

EEC HOTSPOT MUTATIONS DEMONSTRATE MUTATION-SPECIFIC PHENOTYPE PATTERNS

Each of the five EEC hotspot mutations was found in 24–31 patients. Remarkable phenotypic differences can be observed for these different mutations. Amino acid R204 is located in the beginning of the DNA binding domain and was mutated from arginine to tryptophan, glutamine or leucine. All patients were clinically diagnosed as EEC except for one patient with R204Q who was considered to have the LMS, apparently because of mammary gland hypoplasia (Table I). The R204 mutation phenotype is very similar to the overall EEC syndrome phenotype. Exceptions are a lower frequency of hypohidrosis and orofacial clefting (Fig. 2B).

Amino acid R227 is known to be mutated from arginine to glutamine, and was observed in 25 EEC patients and one with LMS. Mutations of this amino acid are rarely associated with orofacial clefting. Only two patients had clefts and these involved only the palate. This is striking since approximately 40% of

TABLE I. Phenotypic Characteristics of Five Human Ectodermal Dysplasias Associated With *p63* Mutations

	Families	Patients	Hair	Lacrimal duct	Nail	Skin	Teeth	Cleft Lip	Cleft Palate	Ectrodactyly	Syndactyly	Hypohydrosis	Mammary gland/nipple hypoplasia	Ankylo-blepharon	Urinary/ Kidney	Hearing impairment	Reference
EEC																	
L162P	1	2	2	0	1	0	2	1	1	2	2	0	0	0	0	0	Authors' unpublished data
Y163C	1	1	1	1	1	1	1	0	1	0	1	1	1	0	0	0	van Bokhoven and Brunner [2002]
V202M	2	2	1	1	1	1	1	1	1	2	0	0	0	0	0	0	van Bokhoven and Brunner [2002]; Pozo et al. [2004]
R204L/Q/W	16	26[a]	16	13	15	9	11	7	7	17	11	1	3	0	4	2	van Bokhoven and Brunner [2002]; Celli et al. [1999]; van Bokhoven et al. [2001]; de Mollerat et al. [2003]; Berdon-Zapata et al. [2004]
H208Y	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	Authors' unpublished data
R227Q	6	25	17	19	14	6	18	0	1	11	0	8	7	0	8	0	van Bokhoven and Brunner [2002]
C269Y	1	2	2	1	1	0	0	2	2	2	2	0	0	0	0	0	van Bokhoven et al. [2001]
S272N	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	Celli et al. [1999]
C273Y	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0	0	van Bokhoven and Brunner [2002]
R279C/H/Q	16	23[b]	11	13	9	6	11	11	11	18	7	4	4	2	2	1	van Bokhoven and Brunner [2002]; Celli et al. [1999]; de Mollerat et al. [2003]; Berdon-Zapata et al. [2004]; South et al. [2002]; Dianzani et al. [2003]; Kosaki et al. [2001]
R280C/H/S	9	31	20	8	10	12	13	8	8	23	17	0	2	0	0	0	van Bokhoven and Brunner [2002]; van Bokhoven et al. [2001]; Barrow et al. [2002]; Ray et al. [2004]
R304P/Q/W	21	27	16	20	16	7	12	22	22	20	16	1	1	0	7	5	Celli et al. [1999]; van Bokhoven et al. [2001]; de Mollerat et al. [2003]; Dianzani et al. [2003]; Wessagowit et al. [2000]; Hamada et al. [2002]
C306R/Y	2	2	2	1	2	2	1	2	1	2	2	1	1	0	1	0	Celli et al. [1999]; Lehmann et al. [2005]
C308S/Y	2	2	2	2	2	2	1	0	0	0	2	0	1	0	1	0	van Bokhoven et al. [2001]
P309S	1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	van Bokhoven et al. [2001]
D312G/H/N	3	3	3	3	2	3	3	3	3	3	2	1	0	0	0	1	van Bokhoven et al. [2001]; Akahoshi et al. [2003]
1572InsA	1	1	0	1	0	0	1	1	1	1	0	0	1	0	0	1	Celli et al. [1999]
L563P	1	1	1	0	1	0	1	0	0	0	1	0	0	0	0	0	Authors' unpublished data
Total	86	152	98	86	77	51	79	60	61	104	66	17	22	2	23	11	
Percentage			66	57	52	34	53	39	40	68	43	11	14	1	15	7	
ADULT																	
N6H	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	0	Amiel et al. [2001]
R298G/Q	4	14	8	10	14	14	14	0	0	9	8	1	11	0	0	0	Propping and Zerres [1993]; Propping et al. [2000]; Duijf et al. [2002]; Chan et al. [2004]; [Rinne et al., in preparation]
Total	5	15	8	10	15	14	15	0	0	9	9	1	12	0	0	0	
Percentage			53	67	100	93	100	0	0	60	60	7	80	0	0	0	

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TABLE I. (*Continued*)

	Families	Patients	Hair	Lacrimal duct	Nail	Skin	Teeth	Cleft Lip	Cleft Palate	Ectrodactyly	Syndactyly	Hypohydrosis	Mammary gland/nipple hypoplasia	Ankylo-blepharon	Urinary/ Kidney	Hearing impairment	Reference
LMS																	
G76W	1	27	0 of 21	7 of 15	7 of 21	0 of 21	3 of 21	0 of 27	6 of 22	21 of 27		7 of 21	27 of 27				van Bokhoven and Brunner [2002]; van Bokhoven et al. [1999]
R204Q[c]	1	1	0	1	1	0	1	0	0	0	1	0	1	0	0	0	Authors' unpublished data
R227Q[c]	1	3	3	3	3	3	2	0	0	0	0	0	3	0	3	1	Authors' unpublished data
1576-1577DelTT	1	1	0	0	0	0	1	0	1	1	0	0	1	0	0	0	van Bokhoven and Brunner [2002]
K632X	1	1	0	1	1	0	1	1	1	1	1	1	1	0	0	0	Authors' unpublished data
1743-1744DelAA	1	1	1	1	1	1	0	0	1	1	1	0	1	0	0	0	van Bokhoven et al. [2001]
Total	6	34	4	13	13	4	8	1	9	24	3	8	34	0	3	1	
Percentage			14	59	46	14	29	3	31	71	43	29	100	0	43	14	
RHS																	
R279H[c]	1	1	1	1	1	1	1	0	0	0	1	1	0	0	1	0	Bougeard et al. [2003]
I510T (+P472T)	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	Bertola et al. [2004]
S541P/Y	2	2	2	2	2	1	2	2	2	0	1	0	1	0	0	1	Kantaputra et al. [2003]; Shotelersuk et al. [2005]
1709DelA	1	1	1	0	1	0	1	0	1	0	0	1	0	0	1	0	Bougeard et al. [2003]
1721DelC	1	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	Authors' unpublished data
1787DelG	1	1	1	1	0	1	1	0	1	0	0	0	0	0	0	1	Chan et al. [2005]
1859DelA	1	3	3	3	0	0	3	1	2	0	0	0	0	1	0	0	Dianzani et al. [2003]
Total	8	10	10	8	6	4	9	3	7	0	3	3	1	1	2	2	
Percentage			100	80	60	40	90	30	70	0	30	30	10	10	20	20	
AEC																	
3′ ss intron 10/ exon 11	1	3	3	1	3	3	3	0	1	0	0	0	0	0	3	0	Barrow et al. [2002]
I510T	1	1	1	1	1	1	1	0	0	0	1	0	0	1	0	0	Bertola et al. [2004]; Bertola et al. [2000]
L514F/V	2	2	2	2	2	2	1	1	2	0	0	0	0	2	1	2	McGrath et al. [2001]
C522G/W	2	2	1	0	0	2	1	2	2	0	1	0	1	0	1	1	McGrath et al. [2001]
G530V	1	1	1	0	0	1	1	1	1	0	1	0	0	0	0	1	McGrath et al. [2001]
T533P	1	2	2	2	2	0	2	0	2	0	1	0	1	2	0	0	McGrath et al. [2001]
534-535InsTTC(F)	1	1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	Tsutsui et al. [2003]
Q536L	1	1	1	0	1	0	1	0	1	0	0	0	0	0	0	0	McGrath et al. [2001]
I537T	2	2	2	1	2	2	2	1	2	0	0	1	0	2	1	1	van Bokhoven and Brunner [2002]; McGrath et al. [2001]
1742DelC	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	1	van Bokhoven and Brunner [2002]
Total	13	16	15	8	13	13	13	7	13	0	4	2	2	7	6	6	
Percentage			94	50	81	81	81	44	81	0	25	13	13	44	38	38	

[a]ED of two patients not described in details, not taking to account in percentage.
[b]ED of one patient not described in details, not taking to account in percentage.
[c]Typical EEC syndrome mutation.

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all EEC patients present clefting, almost always both cleft lip and cleft palate. Patients with R227 mutations also have fewer limb defects. Ectrodactyly was present in 40% (11/28) versus 68% for the EEC group as a whole. Syndactyly was never reported. On the other hand, kidney and urinary problems are quite common in R227 mutation carriers occurring in 11/28 patients (40% vs. 12% for EEC patients with other mutations). Hypohidrosis is also common, whereas no hearing impairment is detected (Fig. 2C). These characteristics indicate that this amino acid R227 differs importantly from the other hotspot mutations

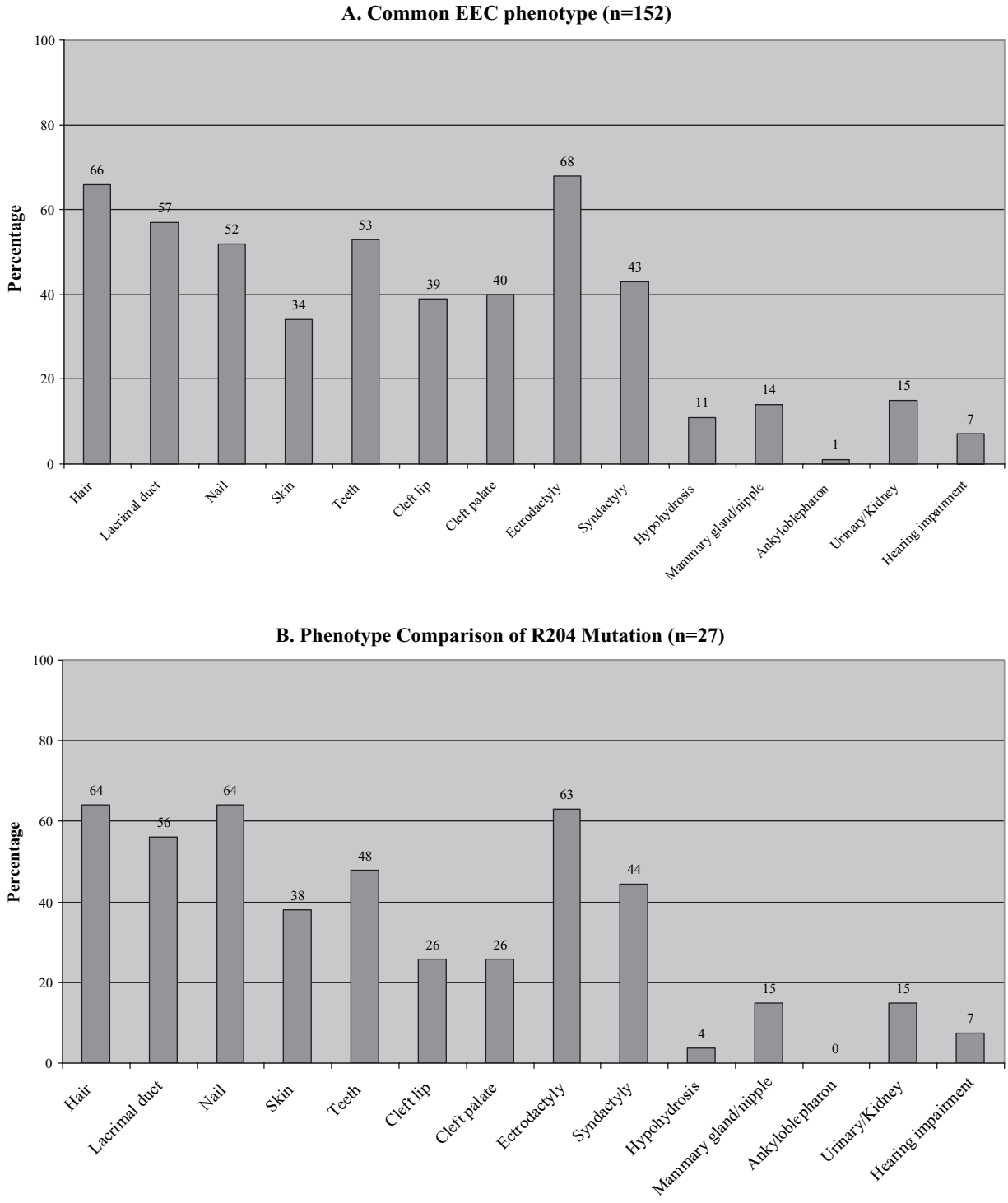


Fig. 2. EEC hotspot mutations allow the specific phenotype delineation. Striking differences especially between orofacial clefting and limb malformations.

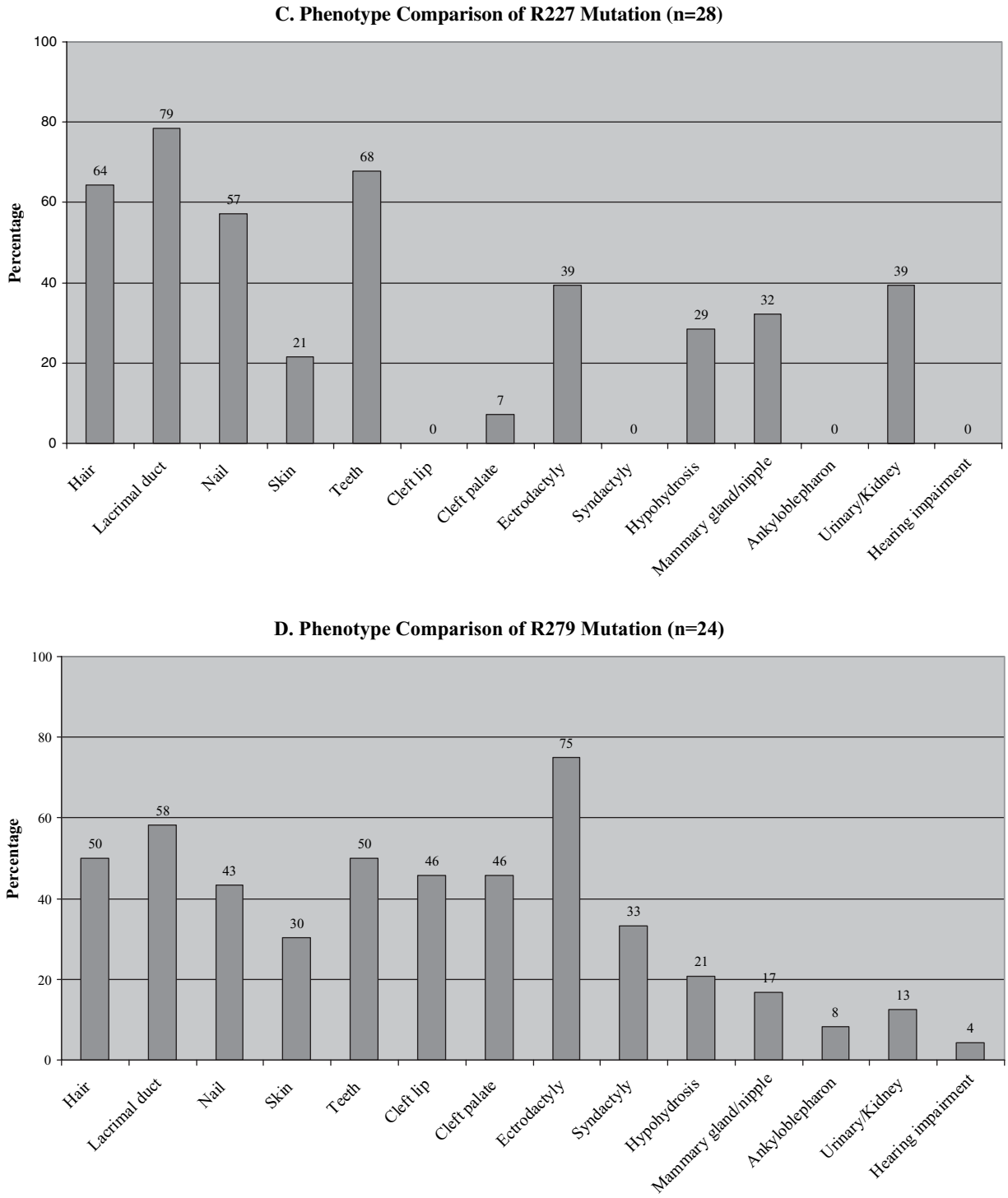
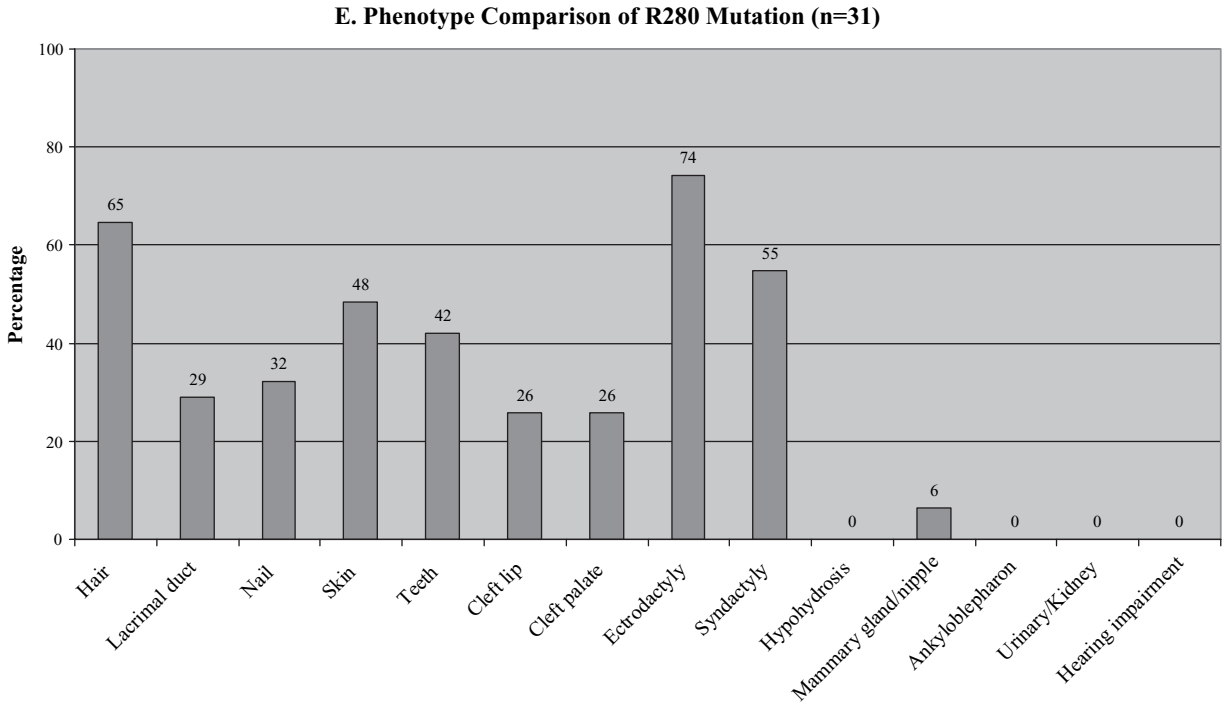


FIG. 2. (*Continued*)

in terms of function. Interestingly, arginine 227 is the only *p63* mutation hotspot that does not have a homologous *p53* mutation hotspot as well [Celli et al., 1999].

At the end of the DNA binding domain there are three EEC mutation hotspots in close proximity: R279, R280, and R304. Each of these has its own typical phenotype pattern. R279 can mutate from arginine to histidine, cysteine or glutamine. All 24 mutations occurred in EEC syndrome patients, except for one patient who was diagnosed as Rapp–Hodgkin syndrome [Bougeard et al., 2003]. Interestingly this is the only EEC mutation, which can give rise to ankyloblepharon, as reported in two



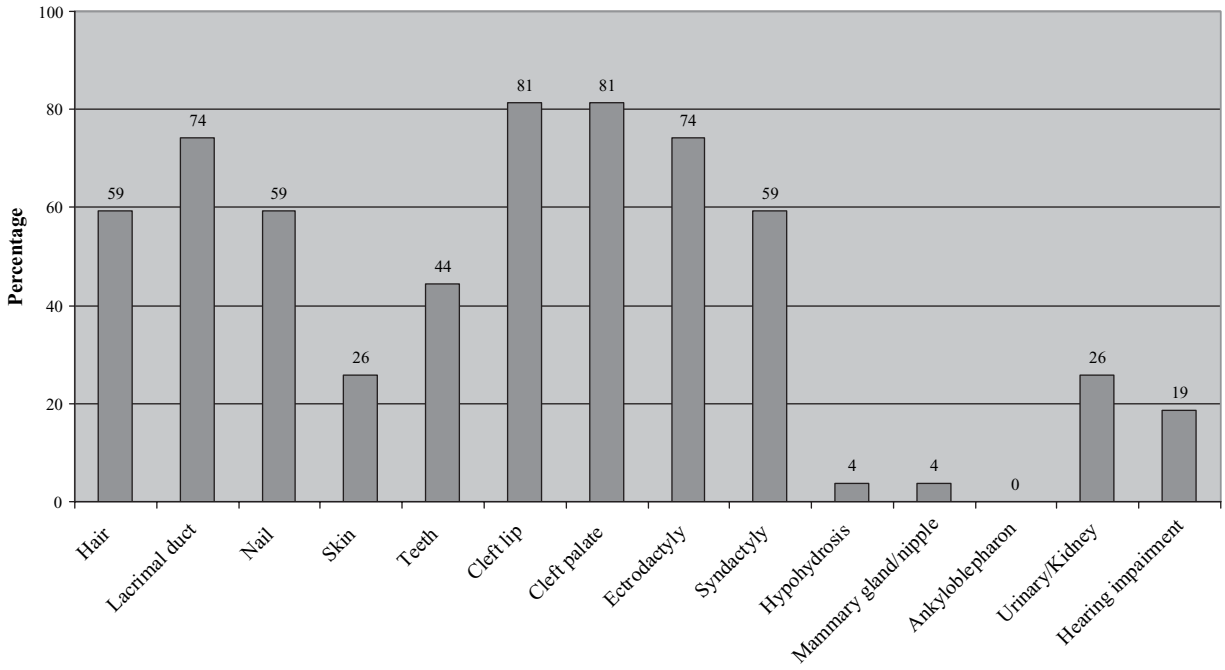


Fig. 2. (*Continued*)

cases, indicating limited overlap with AEC syndrome [Berdon-Zapata et al., 2004]. Other specific characteristics for the R279 mutation are a high incidence of ectrodactyly (18/23) and perhaps hypohidrosis (4/23) (Fig. 2D). The next amino acid R280 is known to be mutated from arginine to cysteine, histidine or serine. Symptoms mimic the common EEC pattern, with frequent skin signs and frequent syndactyly (17/27). Interestingly, no sweating, hearing or kidney problems were reported in patients with an R280 mutation (Fig. 2E). The last EEC hotspot mutation is at amino acid R304, which can be mutated to

tryptophan, glutamine or proline. The most striking finding in patients with this mutation is a very high percentage of orofacial clefting (22/27 patients), frequent occurrence of syndactyly and of hearing impairment (Fig. 2F).

MAMMARY GLAND HYPOPLASIA IS COMMON IN BOTH LMS AND ADULT SYNDROME

LMS was the first p63 syndrome linked to chromosome region 3q27 [van Bokhoven et al., 1999; van Bokhoven et al., 2001; van Bokhoven and Brunner, 2002]. The LMS phenotype comprises malformations of the hands and/or feet and hypoplastic nipples and/or mammary glands. Ectodermal defects are much less prominent than in EEC syndrome and there are no hair and skin anomalies. Patients from six families have been described with this syndrome, four of these are sporadic cases making it difficult to establish whether they are real LMS patients or examples of variable expressivity of other p63 syndromes. Indeed, two cases diagnosed as LMS, have mutations that are typical of EEC syndrome: R204Q and R227Q. Nonetheless, the only large LMS family reported to date is clearly different from EEC [van Bokhoven et al., 1999], and this family and two further LMS patients have mutations that have never been observed in EEC. In contrast to EEC syndrome, LMS patients rarely have any hair and skin involvement and if clefting is present, it is always limited to the palate. Mammary gland and/or nipple hypoplasia or aplasia is more frequent in LMS than in EEC (100% and 29% of LMS cases; Table I). Hypohidrosis is relatively frequent (29%).

ADULT syndrome is clinically very similar to LMS, because mammary hypoplasia plays a main role in both syndromes. Nevertheless, there are clear differences. First of all, orofacial clefting has not been observed in ADULT patients, whereas nail, skin and teeth are affected in almost every case. Hypohidrosis is present in about 30% of LMS patients and was reported in ADULT syndrome in only 7% (1/14) (Table I).

ADULT syndrome is usually caused by "an ADULT syndrome hotspot" point mutation affecting amino acid R298 in exon 8 at the end of the DNA binding domain. There are five unrelated families with R298Q/G mutation each expressing more or less similar ADULT syndrome characteristics [Propping and Zerres, 1993; Propping et al., 2000; Duijf et al., 2002; Chan et al., 2004; Rinne et al., in press]. The first ADULT syndrome family was described to have intensive freckling [Propping and Zerres, 1993; Propping et al., 2000]. Other ADULT syndrome families did not show increased freckling and therefore, freckling does not seem to be a component of this syndrome. A further ADULT mutation N6H was reported in the alternative N-terminus [Amiel et al., 2001]. LMS and ADULT syndromes overlap, and differentiating them can be difficult, especially in sporadic cases [Rinne et al., in press].

AEC AND RAPP–HODGKIN SYNDROME

Two other syndromes belong to the p63 syndrome family. Hay–Wells syndrome, also known as Ankyloblepharon-Ectodermal defects Cleft lip/palate syndrome (AEC), which was first reported by Hay and Wells [1976]. Its main symptoms are ankyloblepharon (fusion of the eyelids), ectodermal defects and cleft lip and palate. About 75% of patients have severe skin erosions at birth, with some AEC patients reported to have up to 70% denuded skin, which resembles a second-degree burn [Tsutsui et al., 2003]. Normal neonatal skin is slowly recovered. By 4–5 years age erosions have usually disappeared except for the head and auricular region. Alopecia is also often linked to Hay–Wells syndrome as are absence of eyelashes and eyebrows. Clefting occurs approximately in 80% of AEC patients, mostly cleft palate or cleft lip and palate. It should be noted that the denominative ankyloblepharon occurs only in 44% of AEC cases. Hearing loss has been reported in about 40% of the patients. AEC patients have nail and teeth defects in about 75–80% of cases. About half of the patients have lacrimal duct atresia. Sweating abnormalities and mammary gland and/or nipple hypoplasias are rarely observed. One striking difference to the other p63 syndromes is the absence of limb malformations, which is almost complete, a few patients having only mild syndactyly. The clinical picture of AEC syndrome patients is thus unmistakably different from other p63-derived syndromes, with the exception of RHS (see below).

Rapp–Hodgkin Syndrome (RHS) was only recently shown to be a member of the p63 syndrome family. It was first described in 1968 by Rapp and Hodgkin [1968]. Kantaputra et al. [2003] linked this syndrome to defects in the *p63* gene. The ED in Rapp–Hodgkin syndrome manifests as sparse, fine hair with progressive alopecia, nail defects, hypodontia, lacrimal duct atresia and dry skin with a decreased number of sweat pores causing hypohidrosis. Milder skin symptoms in RHS are probably the main clinical distinction between Hay–Wells and Rapp–Hodgkin syndrome. Presence of ankyloblepharon supports a diagnosis of AEC. However, since more than half of all AEC patients have no ankyloblepharon, a lack of this anomaly should not be considered as distinguishing for RHS [Dianzani et al., 2003; Bertola et al., 2004]. Syndactyly has been reported in 30% of the RHS cases, whereas among AEC patients it has been described only once (1/16; or 6%) [McGrath et al., 2001]. In both syndromes clefting in lip and/or palate is equally frequent. Genitourinary defects are more common in RHS and AEC than in EEC syndrome, affecting every fourth patient. Also hearing impairment is more common in

RHS and AEC than it is in EEC syndrome, 20, 38, and 7% of patients being affected, respectively.

SHFM

Like EEC syndrome, non-syndromic Split Hand/Foot Malformation (SHFM) has been linked to several chromosomal loci in the human genome. In our experience, about 10% of SHFM patients have a *p63* mutation, referred to as SHFM4 [Ianakiev et al., 2000]. To date eight SHFM mutations have been described, which are dispersed along the *p63* gene. Various types of mutations are seen: splice-site mutation (3′ss intron 4), missense mutations (R58C, K193E, K194E, R280C/H) and stop-mutations (Q634X, Q639X) [Ianakiev et al., 2000; van Bokhoven et al., 2001; Zenteno et al., 2005].

GENOTYPE–PHENOTYPE ASSOCIATIONS

Table I summarizes the clinical data on 227 patients with a causative *p63* mutation, allowing the delineation of the typical phenotypic appearance of each syndrome. Moreover, especially in EEC and AEC syndromes mutations cluster in specific protein domains, providing molecular support for their phenotypic distinction. EEC mutations are missense and are dispersed along the DNA binding domain with just five hotspots involving some >80% of patients. There are two mutations outside the DNA binding domain, a point mutation (L563P) at the end of Sterile Alfa Motif (SAM) domain and a frameshift mutation (1572InsA) at the beginning of the SAM domain. It has been predicted, that EEC mutations disturb the binding of the p63 protein to DNA, which leads to a loss of transactivation [Celli et al., 1999]. In contrast, missense mutations in the SAM domain are mainly found in AEC (12/16), although a splice-site mutation at the intron 10/exon 11 boundary and two frameshift mutations were also detected. A SAM domain is mainly found in developmental genes and is thought to participate in protein–protein interactions [Fomenkov et al., 2003]. Very likely AEC mutations in the SAM domain inhibit these specific protein–protein interactions.

CLINICAL VARIABILITY: ONE MUTATION CAN LEAD TO TWO SYNDROMES

Mutations in AEC and RHS are dispersed along SAM and transactivation inhibitory (TI) domains, and the same amino acid mutations have been reported in both syndromes. For instance amino acid residue I510 located at the beginning of the SAM domain was found to be mutated in two sporadic cases [Bertola et al., 2004]. The AEC patient had mild ankyloblepharon, severe erythrematous plaque on the scalp, which improved by age of 4 months, sparse hair which later became wiry, coarse, and curly, sparse eyebrows and eyelashes, hypodontia and decreased enamel, bilateral choanal atresia and absence of lacrimal puncta. He had mild cutaneous syndactyly and dysplastic nails, mild microretrognathia and asymmetric ears, intact palate and no history of heat intolerance [Bertola et al., 2000]. The other patient presented with persistent erythematous lesions on the scalp, back and genitalia, cleft palate, lacrimal duct atresia, syndactyly, nail dysplasia and hair defects and anterior displaced anus. Although their phenotypes are quite similar, they were diagnosed as having AEC and RHS, respectively. This distinction appears to have been based on the presence of ankyloblepharon in the first patient. Both patients were carrying the same mutation I510T, although the RHS patient also carries a p63 polymorphism P472T that was also in his healthy mother. It is presently unknown whether this rare P472T polymorphism has any effect on the phenotype.

A second mutated amino acid residue S541, has also been reported to cause either AEC or RHS. The S541P/Y mutation was seen in an RHS family and S541F in an AEC syndrome patient [Kantaputra et al., 2003; Bertola et al., 2004; Chan et al., 2005; Shotelersuk et al., 2005]. A third example is 1859delA occurred in a family where one individual had ankyloblepharon, cleft palate and ED, and two other affected members only ED with or without clefting [Dianzani et al., 2003]. Overall the data suggest that AEC and RHS are one single syndrome with clinical variability.

However, there is a good example of one *p63* mutation causing two distinct syndromes. R280 mutations to cysteine and histidine were described in EEC syndrome and in a large SHFM family [Ianakiev et al., 2000; van Bokhoven et al., 2001; Barrow et al., 2002; Ray et al., 2004]. So far, when arginine mutates to serine, it causes only EEC [van Bokhoven et al., 2001]. The phenotype is fully consistent within families carrying each of these R280 mutations. Either there is full-blown EEC syndrome or isolated SHFM. In the latter families, non-penetrance is observed [Ianakiev et al., 2000], which is never seen in EEC syndrome patients. This strikingly consistent phenotype within families suggests the involvement of a modifier allele close to the *p63* gene on chromosome 3. Perhaps a polymorphism within the *p63* gene itself is responsible for this effect.

CONCLUSIONS

In this study we present the results of mutation analysis in 227 patients with a p63-associated ED syndrome and their common phenotypic characteristics. An additional eight families are known with a causative *p63* mutation presenting with isolated SHFM. Our study documents 30 different mutations that can cause EEC syndrome, of which five are

responsible for 86% of the patients. Most of the mutations that were found in the other syndromes differ from the EEC mutations and are specific for each of the respective disorders. In addition, we show that there is a specific phenotype for each of the five *p63* hotspot mutations. For example, there is a complete lack of cleft lip/palate in patients with a R227 mutation, who instead have a high incidence of urogenital problems. Another example is the mutation R304, which gives rise to orofacial clefts in 80% of the cases, whereas this occurs in only 40% for EEC syndrome as a whole. This clinical variability may cause difficulties in arriving at the correct clinical diagnosis, especially in single patients. Yet, there are also clear examples where one mutation can cause two different disorders, which is most obvious in R280 mutations, which are seen in EEC syndrome and in non-syndromic SHFM. This study did not identify any clear differences between the AEC and RHS syndromes. Phenotypes of these syndromes resemble each other very much, the main difference being the more frequent, but certainly not consistent occurrence of ankyloblepharon in AEC syndrome. Because these syndromes can be due to same mutations [Bertola et al., 2004], we propose to group them into a single entity.

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