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EEC Syndrome Type 3 With a Heterozygous Germline Mutation in the *P63* Gene and B Cell Lymphoma

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Lines of evidence have recently indicated a relationship between mutations in the *P63* gene and ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome type 3 (EEC3). The p63 gene (*P63*) has homology to *P53* known as a tumor-suppressor gene, but biological function of its protein has not yet been known well. There have been two reported patients who had EEC syndrome associated with malignant lymphoma. However, they did not undergo sequencing analysis of *P63*. Here, we present with a Japanese girl who had EEC3 and developed diffuse large B-cell type non-Hodgkin lymphoma. In this patient, we documented a heterozygous germline mutation, Asp312Gly, in *P63*. We speculated that p63 may exert a biological function as a tumor suppressor. Malignant lymphoma should be considered as an important complication of EEC3.

KEY WORDS: EEC syndrome; p63; B-cell lymphoma; apoptosis

INTRODUCTION

Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome is a group of autosomal dominant disorders characterized by ectrodactyly, ectodermal dysplasia, and facial clefts [Penchaszadeh and de Negrotti, 1976]. EEC syndrome is a model of the disturbance of apoptosis, because split hands and feet are caused by a failure of cell death between fingers and toes, while urogenital anomalies are due to abnormal regression of Wolfian or Müllerian duct, and facial clefts are attributed the abnormal elimination of excess cells during fusion of the archetypal palate. At least three types of EEC syndrome and their respective gene loci have been identified. EEC syndrome type 1 (EEC1, MIM *129900) was assigned to 7q11.2-q21.3 [Qumsiyeh, 1992; Fukushima et al., 1993], type 2 (EEC2, MIM *602077) to chromosome 19 [O'Quinn et al., 1997], and type 3 (EEC3, MIM #604292) to 3q27 [Celli et al., 1999].

Recently heterozygous mutations in the DNA-binding domain of the p63 gene (*P63*) at 3q27 have been indicated as the molecular basis for EEC3 [Celli et al., 1999; Ianakiev et al., 2000; Wessagowit et al., 2000; Kosaki et al., 2001]. The p63 protein is a member of the p53 family and is implicated in apoptosis rather than tumor suppression [Levrero et al., 2000; Flores et al., 2002]. Increased susceptibility for cancer development has not been shown in patients with EEC syndrome.

Here we report on a 16-year-old Japanese girl with EEC syndrome due to a *P63* mutation, who developed non-Hodgkin B-cell lymphoma. A causal relationship of p63 as a tumor suppressor with the development of B-cell lymphoma is discussed.

CLINICAL REPORT

A Japanese girl was born at full-term following a normal pregnancy and delivery with a birth weight of 2,650 g. Both of her non-consanguineous parents are healthy, whereas her elder sister has epilepsy. At birth, the patient had lobster-claw hands and feet, cleft palate and lip, and scalp defects showing general burn-like skin with erosions and bullae. A diagnosis of EEC syndrome was made. She underwent several operations for repair of her cleft palate and lip, skin grafting to the head, and bilateral corneal transplantation.

At the age of 16 years, her height was 125.3 cm (−6.3 SD), weight 26 kg (−3.7 SD), and OFC 51 cm (−3.4 SD) (Fig. 1). She had thin and hypopigmented skin, a decrease of sweat and sebaceous glands, absent lateral eyebrows, sparse and fair eyelashes, hypoplasia of lacrimal ducts; narrow auditory canals, a flat and low nasal bridge, a short philtrum, a thin upper lip and cleft

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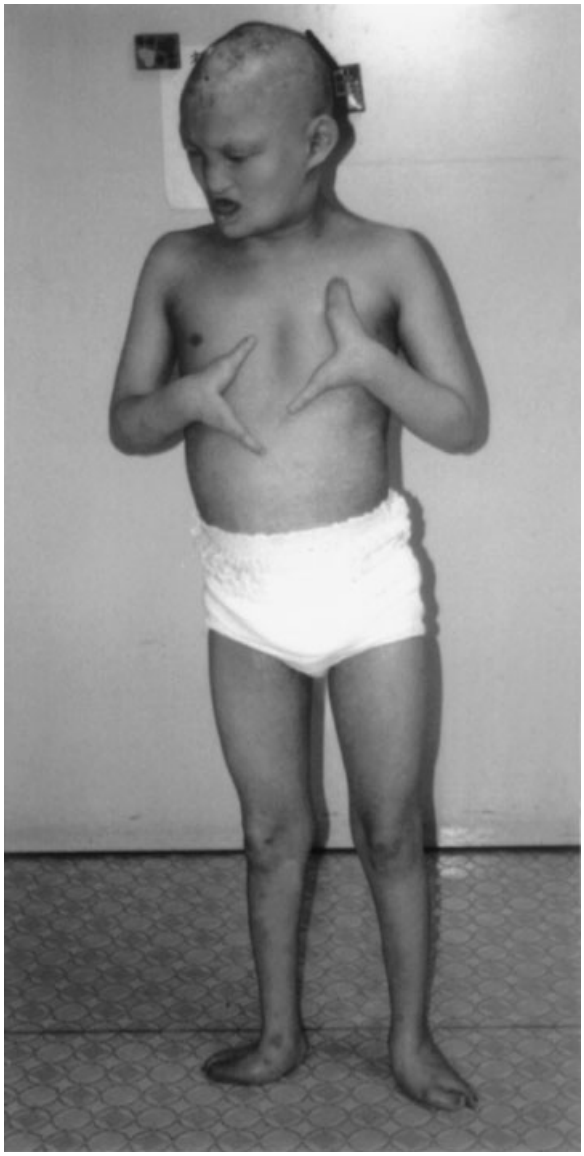

Fig. 1. The girl at the age of 16 years.

palate, maxillary hypoplasia, total anodontia, a thick and prominent tongue, split hands (syndactyly between left fingers 1 and 2, and ectrodactyly of left fingers 3–4 and of right fingers 2–4), split feet (ectrodactyly of bilateral fingers 2–4), and nail dysplasia. She was severely mentally retarded and could not say any specific words. She was mild hearing loss and almost completely blind. She never developed secondary sexual characteristics, and her uterus was found to be small on CT scanning. Routine laboratory tests showed no abnormalities, except decreased serum levels of IgG, IgA, and IgM.

At the age of 16 years, she developed a high fever, generalized lymphadenopathy, and hepatosplenomegaly. Diffuse, large B-cell non-Hodgkin lymphoma (stage III) was diagnosed by examination of a lymph-node biopsy specimen (Fig. 2A,B). She received chemotherapy, but died of sepsis 8 months later.

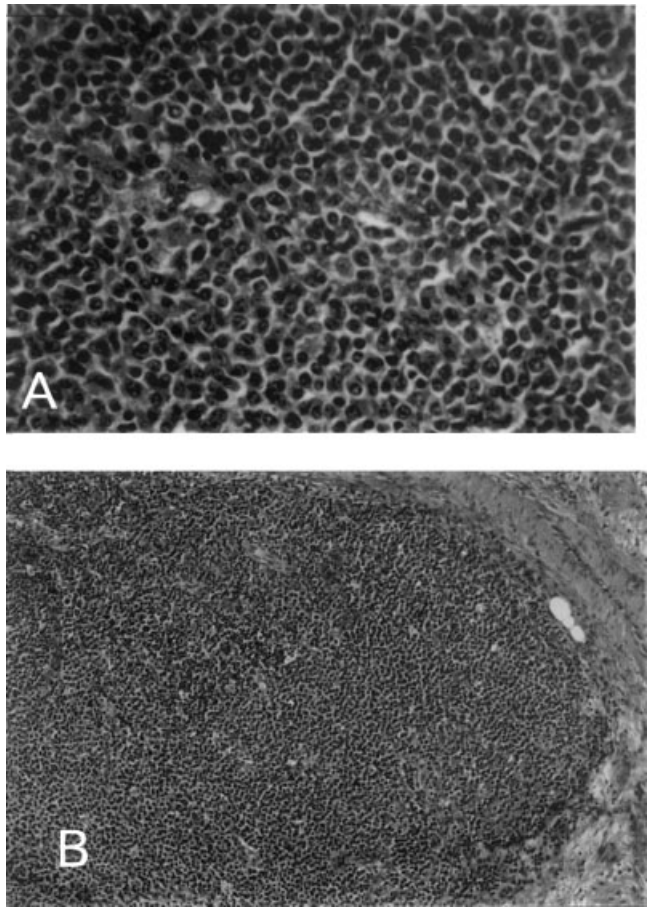

Fig. 2. Histopathological findings of a lymphnode, showing diffuse proliferation of L-26 (B cell marker) positive large cells in the lymphnode.

Cytogenetic and Molecular Genetic Studies

GTG-banding chromosome analysis in the patient revealed a normal 46,XX karyotype. Genomic DNA was extracted from whole blood of the patient with QIAamp DNA blood Kit (QIA GEN, Hilden, Germany), according to the manufacturer's protocol. Blood samples of her parents were not available. Mutation analysis in *P63* was then performed with polymerase chain reaction (PCR) using primers designed by Celli et al. [1999], followed by direct sequencing. An A to G substitution at nucleotide position 1079 in exon 8 of *P63* was detected, the change predicting a heterozygous missense mutation, Asp312Gly (D312G) (Fig. 3). Since this mutant allele was not present among 100 normal control individuals, and another mutation (D312H) at the same nucleotide position has been reported in a patient with EEC syndrome [van Bokhoven et al., 2001], we concluded that the D312G mutation observed in our patient contributed to her EEC syndrome.

DISCUSSION

The findings that *P63* is highly expressed in embryonic ectoderm and in basal, regenerative layers of many

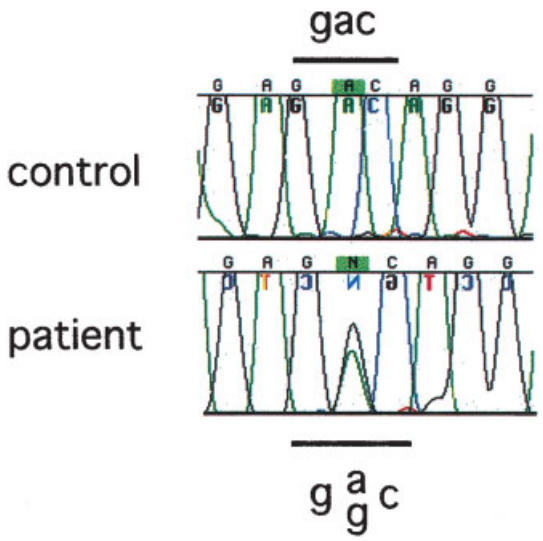

Fig. 3. A D312G mutation in the patient. A heterozygous "A" to "G" transition at nucleotide 1079 in exon 8, leading to an Asparagine-to-Glycine substitution at amino acid 312 within the DNA-biding domain.

epithelial tissues suggest its important role in the regulation of normal development [Mills et al., 1999]. In fact, heterozygous *P63* germline mutations have been shown to be associated with EEC3, as observed in our patient, and other split hand/split foot malformation syndromes [van Bokhoven et al., 2001]. These mutations may cause the malformations through both dominant-negative and gain-of-function mechanisms rather than loss-of-function haploinsufficiency [Yang et al., 1998; Brunner et al., 2002].

As p63 belongs to the p53 family [Levrero et al., 2000; Flores et al., 2002] and since patients with Li-Fraumeni syndrome (due to a *P53* mutation) develop malignant tumors [Srivastava et al., 1990; Birch et al., 1994], one can assume that EEC3 (due to a P63 mutation) may also be associated with malignancy. Two previous reports documented an association of EEC syndrome with malignant lymphoma [Gershoni-Baruch et al., 1997; Ogutcen-Toller et al., 2000]. Gershoni-Baruch et al. [1997] reported two brothers with EEC syndrome; one of them had mild primary hypothyroidism with a hypoplastic pituitary gland, developed Hodgkin disease, and died of the tumor at the age of 16 years. Ogutcen-Toller et al. [2000] described a 16-year-old boy with EEC syndrome and diffuse, large cell non-Hodgkin lymphoma. Although the patient described by Ogutcen-Toller et al. [2000] resembled our case, none of the three reported patients underwent mutation analysis for *P63*, and type of their EEC syndrome remained unknown. Thus, the girl we described is the first case of an association of EEC3 with malignancy, i.e., B-cell lymphoma. The following explanations are possible for the occurrence of non-Hodgkin lymphoma in our patient. First, p63 may have a tumor suppression activity as does p53. Secondly, abnormalities of cellular apoptosis due to a *P63* mutation may cause the progression of B-cell lymphoma. The arguments may be supported by the recent findings by Di Como et al. [2002] that *P63* is expressed not only in various tumor cells including a subset of B-cell lymphomas, but also in a subset of lymphocytes from the normal lymph node. It is likely that malignant lymphoma is an important complication of EEC3.

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