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Setleis syndrome: Clinical, molecular and structural studies of the first TWIST2 missense mutation

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Abstract

Setleis syndrome is characterized by bitemporal scar-like lesions and other characteristic facial features. It results from recessive mutations that truncate critical functional domains in the basic helix-loop-helix (bHLH) transcription factor, *TWIST2*, which regulates expression of genes for facial development. To date, only four nonsense or small deletion mutations have been reported. In the current report, the clinical findings in a consanguineous Turkish family were characterized. Three affected siblings had the characteristic features of Setleis syndrome. Homozygosity for the first *TWIST2* missense mutation, c.326T>C (p.Leu109Pro), was identified in the patients. *In silico* analyses predicted that the secondary structure of the mutant protein was sustained, but the empirical force field energy increased to an unfavorable level with the proline substitution (p.Leu109Pro). On a crystallographically generated dimer, p.Leu109 lies near the dimer interface,

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and the proline substitution is predicted to hinder dimer formation. Therefore, p.Leu109Pro-TWIST2 alters the three dimensional structure and is unable to dimerize, thereby hindering the binding of TWIST2 to its target genes involved in facial development.

Keywords

bHLH domain; facial development; inborn error of development; missense mutation; molecular modeling; Setleis syndrome; *TWIST2*

INTRODUCTION

The Focal Facial Dermal Dysplasias (FFDDs) are a group of developmental syndromes in which the hallmark abnormalities are the bitemporal (FFDD Types I-III) or peri-auricular (FFDD Type IV) scar-like depressions that resemble forceps marks or cutis aplasia, respectively (1, 2). Among these disorders, FFDD Type III or Setleis syndrome (OMIM # 227260) have the characteristic bitemporal scar-like lesions as well as other facial manifestations, including a low frontal hairline, sparse lateral and upslanting eyebrows, multiple rows of eyelashes on the upper eyelid (distichiasis), the absence of eyelashes on the lower eye lid, periorbital puffiness, flattened nasal bridge, bulbous nasal tip, and prominent upper lip. Subsequently, affected sibs and sporadic cases were reported who had the classic findings as well as a variety of other eye, nose, and ear manifestations and developmental delay (3).

In 2010, Tukel et al. used positional cloning techniques to identify *TWIST2* at 2q37.3 as a cause of Setleis syndrome (4). TWIST2 is a member of the basic helix-loop-helix (bHLH) transcription factor family that is highly conserved in evolution and which primarily functions as an inhibitor of expression of target genes (5-7), including genes involved in dermal and bone development during mammalian embryogenesis (4, 8, 9). The gene encodes 160 amino acids including nuclear localization signals (residues 29-32 and 52-56), a basic region (residues 66-77) adjacent to the HLH domain (residues 78-118) and a twist box at the C-terminus (residues 141-160) (Fig. 1). To date, two homozygous nonsense mutations, c.486C>T (p.Gln119*) and c.324C>T (p.Gln65*), were detected in the affected members of a large Puerto Rican family and a consanguineous Arab family, respectively (4). The p.Gln119* mutation predicted premature termination of the TWIST2 polypeptide including the twist box, while the p.Gln65* lesion deleted the twist box as well as the entire bHLH region (Fig. 1).

More recently, homozygous single nucleotide deletions, c.168delC (p.Ser57Alafs*45) and c. 91delC (p.Arg31Glyfs*71), were identified in two affected sibs from a consanguineous Mexican Nahua family (3) and a female infant from a consanguineous Indian family (10), respectively. These frameshift mutations are predicted to truncate the TWIST2 polypeptides deleting both the bHLH and the twist box domains (Fig. 1). Thus, all mutations to date have truncated the transcription factor, deleting at least the terminal portion of its bHLH domain and the twist box. Sequencing of the *TWIST2* gene in five other unrelated patients with Setleis syndrome did not detect mutations, suggesting that Setleis syndrome was genetically heterogeneous (4).

Here, we describe the first homozygous missense mutation in the *TWIST2* gene in three affected siblings from a Turkish consanguineous family, all having the typical phenotype of Setleis syndrome. *In silico* analyses of the effect of the protein substitution predicted a significant alteration of the bHLH domain, and loss of the transcription factor's function.

MATERIALS and METHODS

Patients

The proband, his affected siblings and parents were examined and their clinical characteristics were recorded. This study was approved by Ethics Committee of Istanbul University, Istanbul, Turkey and written informed consent was obtained for each subject. In addition, this study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai, New York, NY.

Molecular and structural analysis

Genomic DNA was isolated from venous blood obtained from each subject. The *TWIST2* coding region (NM_057179.2) was amplified and sequenced as previously described (4). Genomic DNAs from 50 unrelated and unidentified Turkish individuals were used for a restriction fragment length polymorphism (RFLP) assay to determine if the p.Leu109Pro mutation was a common sequence variant. For the structural analysis of the TWIST2 mutant protein, the web-based homology modeling tool, SWISS-MODEL (http://swissmodel.expasy.org) was used and the empirical force field energy (GROMOS) and the atomic empirical mean force potential (ANOLEA) were calculated. Two *in silico* programs, SIFT (http://sift.jcvi.org) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), were used to predict the effect of the mutation on the protein's function. In addition, a model of the mutant TWIST2 dimer docked on the DNA was developed.

RESULTS

Clinical description

Three affected sibs, the offspring of Turkish parents who were first cousins, had the characteristic features of Setleis syndrome (Table S1). The oldest (Fig. S1, IV:4), a male, was the product of a normal pregnancy, labor, and delivery; his childhood and adolescent medical history were uneventful, except for the surgical procedures noted below. At age 18, when examined, his growth, development, and intellect were normal. He had a low frontal hairline, slight frontal bossing, sparse fronto-temporal hair and eyebrows, a flat nasal bridge, a protuberant columella, full lips, a prominent jaw and chin, and small ears (Fig. S2A). He had bitemporal scars from a previous attempt to repair his bilateral lesions cosmetically. He also had surgical scars in both eyebrow regions, the result of previous hair transplants. At 15 years old, he had a right inguinal hernia repair; his abdominal ultrasound and echocardiograph were normal. A biopsy taken from the bitemporal lesions revealed hypoplastic dermis along with an absence of subcutaneous fat, typical of that previously described in affected individuals (1, 11, 12). His neurological evaluation was within normal limits, and he graduated from high school with honors.

The second affected sib, a male (Fig. S1, IV:5), was 13 years old when examined. He had the classic bitemporal scar-like lesions (Fig. S2B), as well as a low frontal hairline, sparse fronto-temporal hair and eyebrows, a flat nasal bridge, prominent columella, full lips, a prominent jaw and chin, and small ears. He also had downward slanting palpebral fissures and a simian crease of the left hand. His physical and neurological examinations were normal, and his academic performance was normal.

The third affected sib, a female (Fig. S1, IV:6), was examined at age 11 and had the same facial findings as her affected brothers (Fig. S2C). Like her brother IV:5, on her upper eyelid she had multiple rows of eyelashes, bilaterally, and on her lower eyelids, her eyelashes were absent. She had periorbital puffiness and wrinkling of the facial skin (Fig. S2D). Her systemic examination was normal. Her parents reported that in her first year of school, she had mild difficulty in learning to read and write, however, her subsequent performance was satisfactory. Her Wechsler Intelligence Scale for Children (WISC – R) score was 85.

The mother of the patients also carried similar facial characteristics such as a prominent jaw and chin, laterally sparsed eyebrows, redundant facial skin and a long face. She did not have any temporal scarring or any additional abnormal hair growth or development. The father did not have any manifestations of Setleis syndrome.

Molecular and structural Studies

Genomic DNA was isolated from venous blood obtained from the parents and two affected sibs (Fig. S1 III-1, III-2, IV-2, IV-4). The *TWIST2* coding region (NM_057179.2) was analyzed in subject IV-4 and a homozygous c.326T>C missense variant was identified, substituting proline for the evolutionarily conserved leucine, located in the bHLH domain of the protein (NP_476527.1, p.Leu109Pro) (Fig. 2A, 2C and 2F). This alteration was not reported in the 1000 genome database (www.1000genomes.org), and evaluation of genomic DNAs from 50 unrelated Turkish individuals, performed by a RFLP assay, did not detect this lesion (Fig. 2B). The parents, along with one unaffected and otherwise healthy 20-year-old son were heterozygous carriers of the missense mutation.

To analyze the effect of this substitution on the protein structure, wild-type (Q8WVJ9) and the predicted p.Leu109Pro-TWIST2 protein sequences were evaluated using SWISS-MODEL (http://swissmodel.expasy.org). The model builds on a single chain of the bHLH domain of *TWIST2* polypeptide residues 67 to 123, based on template 2qI2B of *mus musculus*, which had 48% amino acid identity. The Z-score of the estimated model was −1.243 (13-15). Although the bHLH structure of the mutant protein was sustained, the empirical force field energy (GROMOS) of leucine 109 (p.Leu109) in the normal protein structure increased from a favorable value to an unfavorable level when substituted by proline (p.Leu109Pro), further modifying the atomic empirical mean force potential (ANOLEA) of the proline residue at position 97 (p.97Pro) in the loop region of the domain (Fig. 2D, 2E, 2G). In addition, two *in silico* programs, SIFT (http://sift.jcvi.org) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), predicted this missense mutation significantly altered TWIST2 function.

Fig. 3 shows a model of *TWIST2* bHLH dimer docked against DNA. The model is based on the superposition of the SWISS-MODEL generated *TWIST2* bHLH model on a crystallographically generated dimer of the Max/DNA complex (PDB code 1AN2) (16). The *TWIST2* bHLH domain is composed of two long α-helices (H1 and H2) separated by a loop. Helix H1 lies in the major groove of the DNA, while helix H2 forms a coiled coil with the symmetry related helix from the two-fold related monomer. Leu109 lies near the start of helix H2, close to the dimer interface (Fig. 3).

Discussion

Here, we describe three affected sibs from a consanguineous Turkish family that had the characteristic features of Setleis syndrome. Analysis of the *TWIST2* gene in this family revealed the first missense mutation causing the disease, c.326T>C (p.Leu109Pro). Consistent with the autosomal recessive inheritance of the syndrome, three affected sibs were homozygous for the mutation, for which their parents and a healthy brother were heterozygous. Opthalmologic and certain facial features other than the bitemporal lesions have been observed in a few heterozygous carriers as in the patients' mother reported here (3, 17). The p.Leu109Pro substitution was not present in either 100 Turkish chromosomes or in the 1000 Genome database. Moreover, p.Leu109 is highly evolutionally conserved and its substitution by proline is predicted to alter the structural integrity of the polypeptide as indicated by *in silico* analyses. From our model of TWIST2 bHLH domain, the proline substitution at residue 109 is located in helix H2 near the dimer interface. By molecular modeling of the wild-type and p.Leu109Pro bHLH structures, we predict that, although the secondary structure of the polypeptide is maintained, the three dimensional structure may be altered due to the unfavorable force field energy of the proline residue. This alteration renders the bHLH unstable and unable to dimerize, thereby hindering the binding of TWIST2 to its target genes involved in the developmental process and causing Setleis syndrome.

Pathologically, the bitemporal lesions were shown to be a mesodermal dysplasia with near absence of subcutaneous fat and with skeletal muscle almost contiguous with the epidermis, suggesting insufficient migration of neural crest cells into the frontonasal process and the first branchial arch (1, 4, 11). Although the specific developmental genes regulated by TWIST2 have not been systematically characterized, Franco et al. demonstrated that periostin, a cell adhesion protein involved in connective tissue development and maintenance, was transactivated by TWIST2 and that improper regulation of periostin by a mutant form of *TWIST2* may explain some of the soft tissue abnormalities seen in these patients (18). Clearly other genes involved in facial development, related or regulated by TWIST2, may be responsible for the genetic heterogeneity underlying Setleis syndrome. To date, high-quality antibodies to TWIST2 are unavailable for chromatin-immunoprecipitation studies to identify the genes subject to TWIST binding and regulatory inhibition.

In conclusion, five mutations have been described that cause Setleis syndrome, all in unrelated consanguineous families (3, 4, 10). All these mutations either altered or deleted the bHLH and twist domains of TWIST2, hindering its proper function. As reported previously, Setleis syndrome is genetically heterogeneous, as other patients with the classic

syndrome do not have *TWIST2* mutations (4). Exome and/or genome sequencing may reveal other gene defects causing Setleis syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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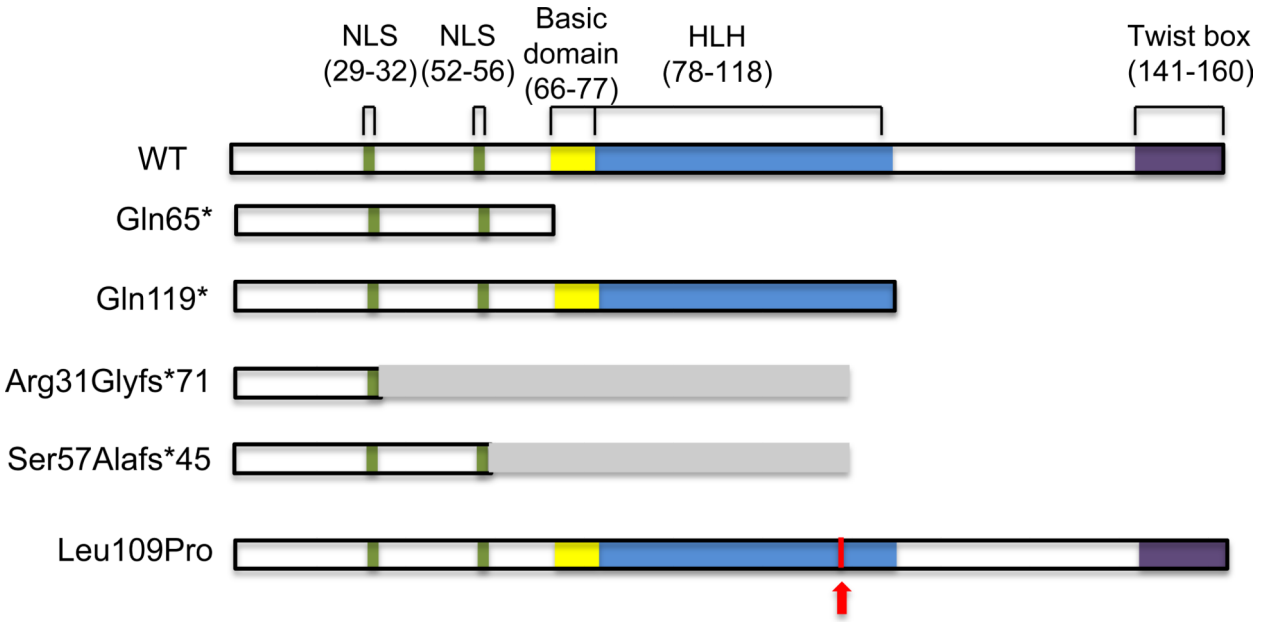

Fig. 1.

The five TWIST2 proteins and the predicted effects of the known mutations on the protein. The locations of the functional domains are indicated. The gray bar represents the aberrant peptides after a single base-pair deletion.

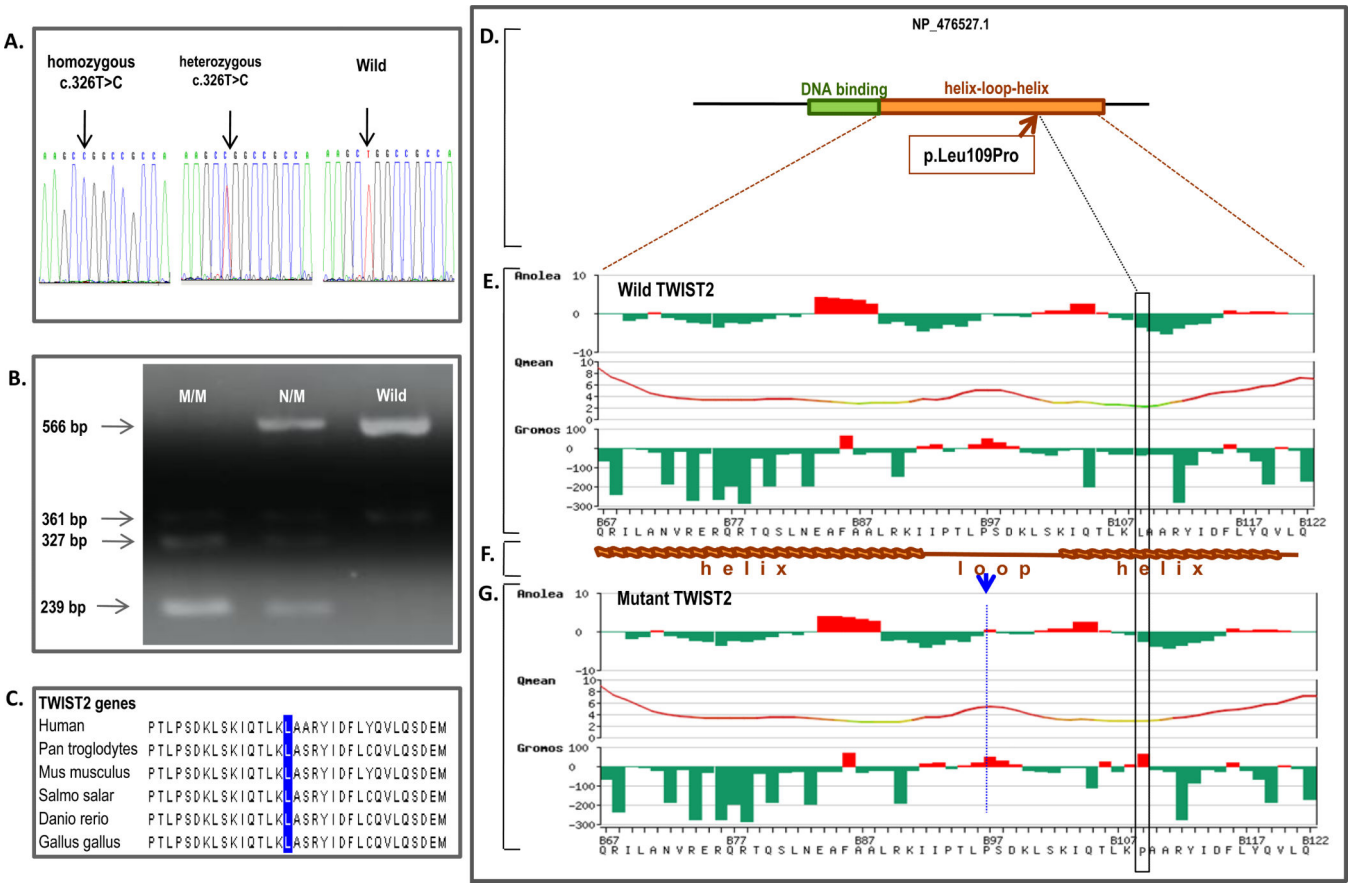

Fig. 2.

A. Electropherograms of the *TWIST2* amplicon showing the c.326T>C homozygous, heterozygous, and wild type sequences. **B.** Eco52I digestion of *TWIST2* gene amplification product of 927 bp, where the wild-type *TWIST2* amplicon was cleaved into 566 and 361 bp fragments, while digestion of the *TWIST2* amplicon from heterozygous individuals resulted in fragments of 566, 361, 327, and 239 bp, and that of affected homozygous individuals had fragments of 361, 327, and 239 bp. C. *TWIST2* amino acids p.Pro94 to p.Met126 of human and other species including the chimpanzee, mouse, Atlantic salmon, zebrafish, and chicken. **D.** Features of TWIST2 protein as designated in Uniprot Q8WVJ9 (http://www.uniprot.org/uniprot/Q8WVJ9), pointing out the novel c.326T>C missense mutation reported in the Turkish family. **E.** and **G.** Local model quality estimation graphs of wild-type *TWIST2* and the *TWIST2* missense mutation, respectively, covering the regions of helix-loop-helix domains as aligned on the X axis, as presented in the SWISS-MODEL workspace window. Y axis of ANOLEA presents atomic mean force potentials. Y axis of QMEAN provides composite scores collected from the function for both the estimation of the global quality of the entire model as well as for the local per-residue analysis of different regions within the model. Y axis of GROMOS presents empirical force field energy for each amino acid of the protein chain. **F.** The cartoon representation of the helix-loop-helix domain. It is observed that p.Leu109 is located at the second helical region, and p.Pro97 is located in the loop region.

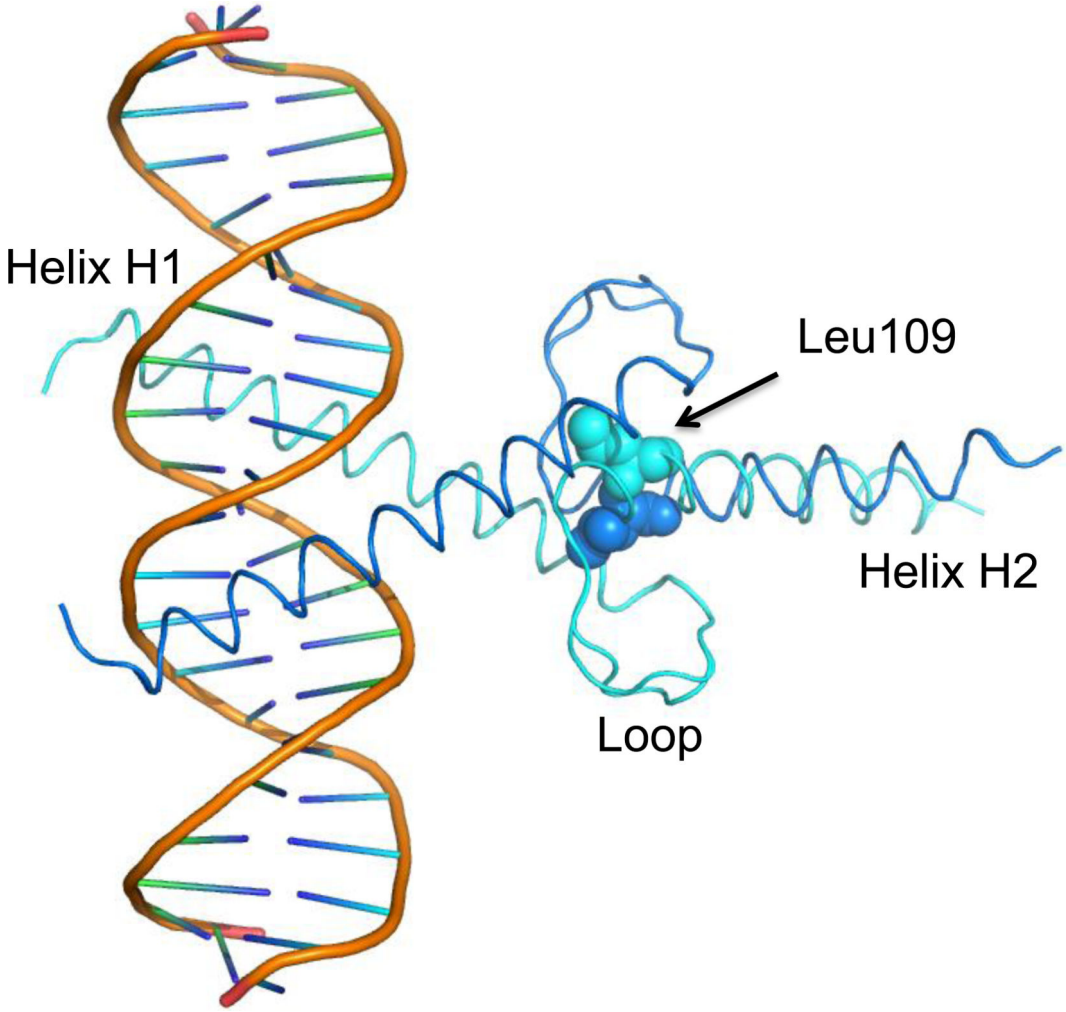

Fig. 3.

A model of TWIST2 bHLH dimer docked against DNA. The helices H1 and H2 and the loop region are labeled on one monomer. Leu109 is represented by van der Waals spheres.