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PLACK syndrome resulting from a new homozygous insertion mutation in *CAST*

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Inherited abnormalities of proteases or protease inhibitors have been shown to underlie a spectrum of skin barrier genodermatoses that lead to variable degrees of fragility, scaling and inflammation (reviewed in de Veer et al. [1]). One recent addition to this group is the autosomal recessive disorder, PLACK syndrome (OMIM616295). In 2015, Lin *et al.* [2] reported homozygous loss-of-function mutations in *CAST* (encoding calpastatin) in three families from different ethnic backgrounds (Chinese, Nepalese and European). Clinically, the signs comprised peeling skin, leukonychia, acral keratoses, cheilitis, and knuckle pads, hence the derivation of the acronym, PLACK syndrome. Calpastatin is an endogenous specific inhibitor of calpain, a calcium-dependent cysteine protease [3]. It is expressed in most tissues (except brain) with high expression of *CAST* noted in stratified squamous epithelia, including skin [3]. Loss of calpastatin in the affected individuals led to defective keratinocyte adhesion as well as increased keratinocyte apoptosis [2]. Three different homozygous mutations were reported: c.607dup (p.Ile203Asnfs*8), c.424A>T (p.Lys142*), and c.1750delG (p.Val584Trpfs*37) [2]. However, no further reports of PLACK syndrome have been documented thereafter.

Here, we outline the case of a 10 year old Tunisian boy, born to consanguineous parents, who presented with fragile blistering skin since birth. The parents described superficial post-traumatic or sometimes spontaneous superficial blisters mainly on his limbs. Typically these lesions healed completely within few days with mild superficial desquamation but without scarring. Furthermore, he also had chronic fissuring of the lips and around the mouth, as well as increasing pallor affecting all 20 nails. He has a younger sister with normal skin but three third-degree cousins have been noted to have similar skin blistering (no further details available).

Although the history raised the possibility of an intra-epidermal form of epidermolysis bullosa simplex [4], clinical examination demonstrated the full characteristic features of PLACK syndrome (Fig. 1). The punctate keratoses were most noticeable along the margins of the feet (Fig. 1c), but were also seen embedded within the knuckle pads on the fingers (Fig 1d). No mucous membrane involvement or hair abnormality was noted. The only other clinical finding was an abnormal gait: since the age of 4 years he had tended to walk on his toes. Full neurologic assessment by a pediatric neurologist revealed a slight weakness of the

reflexes and the proximal muscular strength of lower limbs, but the boy failed to attend for subsequent investigations or follow-up. The etiology of the walking anomaly is not known but is not thought to have any direct connection to the PLACK syndrome, although this cannot be completely discounted since some members of the calpain family have been implicated in muscular dystrophies [3, 5]. Of note, however, muscular dystrophy was not a feature present in the previous cases of PLACK syndrome [2].

Following informed consent, Sanger sequencing of the coding exons and flanking introns of *CAST* was performed using peripheral blood genomic DNA template sampled from the affected individual, which revealed a new homozygous 4-base pair insertion, c.461dupGCAT (p.Ser154Cysfs*6) (Fig. 2a, b). This mutation does not appear in lists of polymorphic variants, such as the ExAC browser (http://exac.broadinstitute.org/). Similarly to the previously published data, this new mutation is sited within one of the calpain inhibitor domains of the protein (Fig. 2c). Unfortunately, parental DNA samples were not available for analysis.

Calpains represent a well-conserved family of calcium-dependent cysteine proteases with multiple ubiquitous and tissue specific isoforms identified [3, 5, 6]. Calpastatin is the natural endogenous inhibitor of calpain activity, specifically inhibiting calpain and not other cysteine proteases. Disruption of the calpain-calpastatin proteolytic system influences several aspects of cell biology, including migration, adhesion, proliferation and apoptosis, with clinical relevance already demonstrated in muscular dystrophy, cancer, Alzheimer's disease, neurological injury, ischemia/reperfusion injury, atherosclerosis, diabetes mellitus, cataract formation, and in PLACK syndrome [2, 3, 5, 6]. Knockout calpastatin mouse models display an unusual phenotype with decreased locomotor activity in stressful environments and a decreased auditory startle response, but abnormal movements or gait disturbances are not observed [7]. Different mouse models looking at calpastatin overexpression reveal delayed wound healing with reduced proliferation and re-epithelialization, particularly in the early phases of wound healing [8]. In contrast, all four human loss-of-function mutations in *CAST* (including this report) lead to a constellation of readily identifiable ectodermal anomalies and the clinical

entity of PLACK syndrome. Precisely how an imbalance in calpain-calpastatin proteolysis results in this particular syndrome, or what the phenotypic extent of this syndrome might be (e.g. additional muscular pathology) as further cases are reported, is uncertain. Nevertheless, we present the fourth example of PLACK syndrome to highlight the clinical and molecular features of this relatively newly described disorder.

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Figure Legends

Fig. 1. Clinical features of PLACK syndrome. (a) Peeling skin and superficial blistering of the lower limbs; (b) Cheilitis; (c) Acral punctate keratoses and superficial peeling on the sole; (d) Leukonychia and Knuckle pads.

Fig. 2. *CAST* molecular pathology in PLACK syndrome. (a) Sanger sequencing reveals a homozygous 4-bp duplication in *CAST* (c.461dupGCAT; p.Ser154Cysfs*6). (b) Database of known mutations in *CAST* in PLACK syndrome. Previously described gene mutations are indicated in blue and the new mutation is depicted in green.

Fig. 1

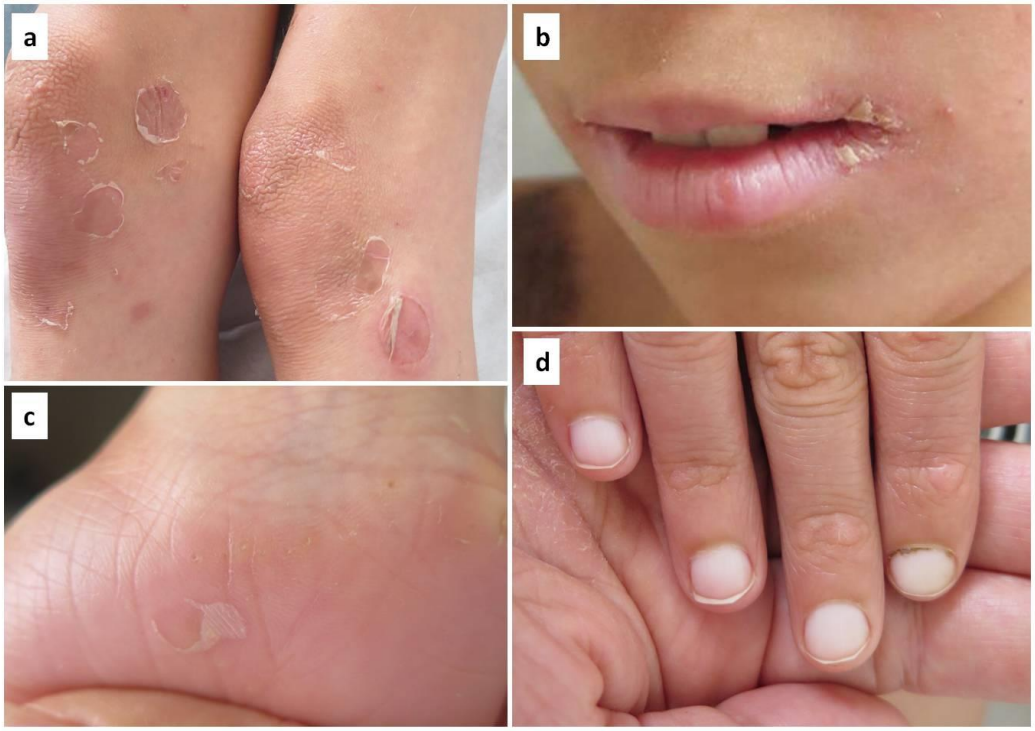

Fig. 2