Recurrent *ATP2A2* p.(Pro602Leu) Mutation Differentiates Acrokeratosis Verruciformis of Hopf from the Allelic Condition Darier Disease.

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ABSTRACT

Darier disease and Acrokeratosis Verruciformis of Hopf (AKV) are rare disorders of keratinization with autosomal dominant inheritance and very distinct clinical pictures. Both have been shown to be caused by mutations in *ATP2A2* (ATPase, Ca^{++} transporting, cardiac muscle, slow-twitch) a gene encoding one of the SERCA (sarcoplasmic/endoplasmic reticulum calcium ATPase2) intracellular pumps with a crucial role in cell-to-cell adhesion in both skin and heart. While hundreds of different missense and nonsense mutations cause Darier disease, only one missense mutation, p.(Pro602Leu), has been identified in families with AKV. We report a family with AKV due to the p.(Pro602Leu) mutation and discuss implications for this recurrent mutation on knowledge of *ATP2A2* structure and function.

Keywords:

Darier Disease, Acrokeratosis Verruciformis, ATP2A2, desmosomes, keratinocytes, Allelic imbalance, genotype-phenotype correlation, human, heart failure.

INTRODUCTION

Darier disease and Acrokeratosis Verruciformis of Hopf (AKV) are rare genodermatoses which share features of hyperkeratosis and autosomal dominant inheritance. Darier disease is a severe generalized and debilitating disorder causing widespread greasy and malodorous warty plaques on the skin with flexural predominance. Acantholysis is a characteristic histological feature, reflected in skin and mucosal fragility. Reported associations include corneal damage and increased frequency of neuropsychiatric disorders. AKV is mild, limited to keratotic papules on the dorsum of the hands and feet in most, with nail and palmar involvement in some. Both conditions show completely penetrant expression in adults.

Despite decades of suspicion that these are related disorders they remain clinically distinct and in only rare exceptions have been found to co-exist in families. Acral papules may be an early manifestation in Darier disease however making this distinction difficult in childhood and sporadic cases [Dhitavat and others 2003]. Many families have a 'pure' AKV phenotype with no progression to Darier disease seen in many generations. The finding of causative mutations in the *ATP2A2* gene first in Darier disease [Sakuntabhai and others 1999] and shortly after in AKV [Dhitavat and others 2003] proved that these are allelic disorders. Mutation analysis of 2 large affected pedigrees with 'pure' AKV, one British and one Druze, identified the same *ATP2A2* p.(Pro602Leu) mutation in each [Bergman and others 2012; Dhitavat and others 2003]. A de novo p.(Ala698Val) variant in *ATP2A2* was reported in a simplex case of an 11 year old Afghani boy with no family history of AKV, but given his age and the lack of other affected relatives it remains to be proven that this was not early manifestation of Darier Disease [Bergman and others 2012; Berk and others 2010;

Dhitavat and others 2003]. One study of 2 Chinese families with AKV did not identify any mutation in *ATP2A2* [Wang and others 2006].

Recently a database of *ATP2A2* variants has been created using The Leiden Open Variation Database (LOVD) [Nellen and others 2016]. LOVD currently lists 277 unique *ATP2A2* mutations in 506 individuals. Mutations associated with Darier disease are distributed throughout the gene and mostly unique to one affected family. One sporadic individual with a phenotype previously described as Darier disease is listed here as 'AKV with Guttate Leucoderma' [Nellen and others 2015]. Four families with AKV are included, 2 previously reported and 2 new, and all have the same *ATP2A2* p.(Pro602Leu) variant [Bergman and others 2012; Dhitavat and others 2003; Nellen and others 2016]. The p.(Pro602Leu) variant was not seen in any families with a classic Darier disease phenotype [Nellen and others 2016].

We have identified the recurrent *ATP2A2* p.(Pro602Leu) mutation in a large Australian family with AKV. This means that the only five AKV-affected families known to have a genetic diagnosis carry the same missense mutation in the ATP-binding domain of the *ATP2A2* gene. *ATP2A2* is central to calcium transport involved in desmosomal cell-to-cell signaling and adhesion, not only in skin but also cardiac myocytes, significant in both causation and treatment options for heart failure [Prasad and others 2015]. We reframe the question around Darier disease and AKV from *are they the same?* to *why are they different?* and suggest avenues of further research to clarify the function of *ATP2A2* as it affects cellular adhesion.

MATERIALS AND METHODS

A 53-year-old Australian woman of English and possibly Portuguese descent was referred to the cardiac genetic service in Newcastle NSW for investigation of Long QT syndrome. It was noticed that she had multiple skin-colored keratotic lesions over the dorsum of both hands and feet with longitudinally ridged, brittle fingernails (Figure 1). The skin lesions had been present from childhood and responded partially to topical salicylic acid applications but were not a major problem for her. Palmar skin was thickened and dry which she related to occupational use of cleaning fluids. There were also small keratotic patches of skin on the medial knees and thighs. Two years previously she had undergone a partially successful corneal transplant for unilateral corneal keratosis secondary to keratoconus. Between the ages of 1 and 4 years she had recurrent short seizures which were not all related to known fevers. She had no current neurological or psychiatric problems.

Other family members including mother, maternal aunts, 2 brothers, nephew and niece were reported to have identical lesions on the dorsum of the hands, with only her mother also having nail involvement. No other relatives had corneal keratosis requiring treatment. No family member for at least 4 generations was known to have had more widespread disease or features resembling Darier disease. There were no significant neuropsychiatric disorders in the affected family members. Two adult brothers with AKV had childhood febrile convulsions but no seizures since then.

RESULTS

Dermatological review suggested the diagnosis of Acrokeratosis verruciformis of Hopf (AKV) and 2 punch biopsies were taken from one hand and one foot. Histopathology showed hyperkeratosis, 'church-spire' papillomatosis, hypergranulosis and moderate acanthosis diagnostic of AKV (Figure 2). There was no evidence of acantholysis suggestive of Darier disease.

Sequencing of the *ATP2A2* gene including coding regions of all transcripts of the gene and their flanking regions (approximately 20bp from each side) performed by Gene by Gene Laboratories (Houston, Texas, U.S.A.) identified a heterozygous p.(Pro602Leu) missense mutation in exon 14. This mutation has previously been shown to lead to complete loss of calcium transport activity [Dhitavat and others 2003].

No genetic cause for Long QT syndrome was identified. In light of the known association between keratinizing dermatoses and Arrhythmogenic Right Ventricular Cardiomyopathy an echocardiogram was performed which was normal.

DISCUSSION

This report describes the fifth family with a 'pure' AKV phenotype for whom a genetic diagnosis has been made. All 5 families have the recurrent p.(Pro602Leu) missense mutation causing loss of function of the *ATP2A2* gene, and this variant has not been reported in any individual with classic Darier disease. In keeping with the purely AKV phenotype seen with this variant, our family had no features of Darier disease in 4 generations. Our patient also suffered significant corneal keratosis which has been reported in Darier disease [Blackman and others 1980]. However this was clearly linked to a co-existing ophthalmological diagnosis of bilateral keratoconus.

The *ATP2A2* gene is one of a family of genes encoding ATP-coupled calcium ion pumps situated in the sarcoplasmic/endoplasmic reticulum membrane (SERCAs). Their action is to actively transport Ca(2+) into the sarcoplasmic reticulum, believed to play a key role in the signaling pathway regulating cell-to-cell adhesion and differentiation of the epidermis [Sakuntabhai and others 1999], but also in protecting cardiac muscle from failure, particularly under load stress [Prasad and others 2015; Savignac and others 2014]. Four specific SERCA2 isoforms result from alternate splicing, with isoform 2b expressed particularly in keratinocytes, and isoforms 2a and 2c in heart muscle. SERCA2 has a large intramembranous domain while the 'ATP-binding' domain crucial to activation of Ca(2+) transport is located in the cytosol. The p.(Pro602Leu) substitution found in AKV families lies in this ATP-binding domain.

Pathogenic *ATP2A2* variants in Darier disease include non-sense and missense mutations both affecting the ATP-binding domain and spread throughout the gene. At least 75% are 'private' or unique to the family affected, with some others reported in 2 to 4 families [Green and others 2013] and many are *de novo*. Attempts at genotype-phenotype correlation have been unsuccessful, with attempts to associate neuropsychiatric complications with 3' missense mutations [Jacobsen and others 1999] being refuted by more recent comprehensive reviews [Nellen and others 2016]. Some families have been reported to have a 'mixed' phenotype of AKV and Darier disease in various members, and in these families *ATP2A2* variants have been identified which lie outside the ATP-binding domain [Nellen and others 2016].

However the distinctive clinical picture of AKV as evidenced by non-progression to Darier disease over many generations appears to be associated only with one mutation, p.(Pro602Leu), located in the ATP-binding domain. Review of both literature and online mutation databases did not identify any case of Darier disease caused by the p.(Pro602Leu) mutation.

Functional studies to date suggest haploinsufficiency of *ATP2A2* as the basis for both Darier disease and AKV, with decreased Ca(2+) transport, binding or feedback sensitivity in most mutants including p.(Pro602Leu) [Dhitavat and others 2003; Nellen and others 2016]. However one study, which did not include the p.(Pro602Leu) variant, identified that mutant SERCA2 inhibited wild-type protein activity, possibly through formation of less active heterodimers, suggesting a possible 'dominant negative' effect [Ahn and others 2003].

Distinct diseases caused by variants in the same gene, known as 'allelic disorders' are well recognized. Mechanisms for the differing phenotypes may be evident from examination of the variants involved or functional studies. Thus Kennedy disease is caused by a CAG triplet repeat expansion and Androgen Insensitivity syndrome by point mutations in the same gene *SMAX1*. In the more similar *GJB2*-associated disorders KID and Vohwinkel syndromes difference in severity may be due to truncating mutations in the former and missense in the latter condition. Gain and loss of function variants in *SCN5A* cause Long QT syndrome and Brugada syndrome respectively. Alteration of a specific functional domain or binding site may lead to distinct phenotypes as seen in the spectrum of *RET* gene mutations, with those causing enhanced dimerization associated with Multiple Endocrine Neoplasia (MEN) type 2A, altered substrate specificity leading to MEN type 2B, and others causing

Hirschprung disease. Much can be learned about the function of a gene by observation of these disparities, whether they are seen as 'allelic disorders' or 'genotype-phenotype correlations'.

It is quite unique however that one recurrent missense mutation causes a distinct inherited phenotype, as in the *ATP2A2* p.(Pro602Leu) variant causing AKV, with a different disorder, Darier disease also caused by missense mutations including those in the same functional domain of the gene in close proximity to p.(Pro602Leu). AKV is obviously related to Darier disease in pathology, but does not show the characteristic acantholysis in the suprabasal layer of the epidermis causing skin fragility in Darier disease, and remains a mild skin disorder limited to the extremities. Extensive inter- and intrafamilial variation is seen in Darier Disease, but not in AKV. Stress caused by emotion, heat, mechanical stress and UV radiation is known to worsen Darier disease while AKV is relatively stable and non-progressive. They are very distinct conditions, to the extent that AKV has probably avoided diagnosis and research attention due to its very mild presentation.

Functional studies to date do not provide the cause of this phenotypic distinction. Missense mutations at the ATP binding domain are involved in both conditions. Although p.(Pro602Leu) occurs at a highly conserved site in all human SERCA1, 2 and 3 isoforms, loss of Ca(2+) transport has been demonstrated with other ATP-binding site mutations as well as this variant [Dhitavat and others 2003; Nellen and others 2016]. It is not obvious that the AKV-associated p.(Pro602Leu) mutation should have a significantly different effect on known binding sites than the adjacent p.(Arg603Ile) mutation identified in a family with Darier disease. It is still possible that a dominant-negative effect of mutant SERCA2 protein

associated with Darier disease is somehow avoided with the p.(Pro602Leu) variant as this has not been studied. Cell-to-cell adhesion is more severely and widely affected in Darier disease than AKV, where upregulation of keratinocyte differentiation rather than acantholysis may be the only response to local stress and skin trauma. The specific consequences of missense variants on calcium homeostasis at intracellular versus endoplasmic reticulum level and the flow-on effects to desmosome and cell adherence have been studied for many mutations, but not specifically the p.(Pro602Leu) variant to our knowledge. It may be at this cellular level where the difference is crucial. Expression of SERCA2 in basal, suprabasal and acantholytic epidermal keratinocytes was reported to be increased in cultured Darier disease cells compared to normal cells, [Sheridan and others 2002], but again this association has not been tested with the specific p.(Pro602Leu) variant seen in familial AKV.

This question may not just be of interest to dermatologists. The desmosome and the mechanisms of cell-to-cell adhesion are critical in maintenance of myocardial contractility and strength. Calcium channel and desmosomal gene mutations cause Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) [Tiso and others 2001]. While *ATP2A2* has not been associated with ARVC it is known to play a key role in Ca(2+) homeostasis in the heart and inhibition is associated with decreased contractility and heart failure [Prasad and others 2015].

Discussion has long centered around whether AKV and Darier disease are truly 'allelic disorders'. There can be no doubt now that they are closely related through their proven associations with the *ATP2A2* gene. We propose that there is enough evidence

through these AKV families to direct future functional studies in this area to comparison consequences of the p.(Pro602Leu) variant on calcium homeostasis and cell signaling/ adhesion with those variants causing Darier disease. This highly specific genotype-phenotype relationship could reveal much of clinical importance about maintenance of the desmosome.

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Conflict of Interest: Nil

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FIGURE LEGENDS

Figure 1. Characteristic discrete keratotic macules of AKV phenotype on feet. Identical lesions were seen on dorsum of both hands.

Figure 2. Punch biopsy from right hand showing characteristic 'church-spire' papillomatosis with hyperkeratosis, hypergranulosis and moderate acanthosis. (Haematoxylin and eosin, X 100)