ATP2A2 mutations in Darier’s disease and their relationship to neuropsychiatric phenotypes


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Darier’s disease (DD) is a rare, dominantly inherited disorder that affects the skin producing a variety of types of lesion. Close examination of lesional DD skin shows the presence of abnormal keratinization (epidermal differentiation) and acantholysis (loss of cohesion) of keratinocytes. A number of clinical studies have described the co-occurrence of various neurological and psychiatric symptoms with DD, including mood disorders, epilepsy, mental retardation and a slowly progressive encephalopathy. A single locus for DD has been mapped to chromosome 12q23–q24.1, and a variety of missense, nonsense, frameshift and splicing mutations in the ATP2A2 gene have been described recently in families with DD. This gene encodes the sarcoplasmic/endoplasmic reticulum calcium-pumping ATPase SERCA2, which has a central role in intracellular calcium signalling. In this study, we performed mutation analysis on ATP2A2 in 19 unrelated DD patients, of whom 10 had neuropsychiatric phenotypes. We identified and verified 17 novel mutations predicting conservative and non-conservative amino acid changes, potential premature translation terminations and potential altered splicing. Our findings confirm that mutations in ATP2A2 are associated with DD. In neuropsychiatric cases, there was a non-random clustering of mutations in the 3’ end of the gene (P = 0.01), and a predominance of the missense type (70% versus 38% in DD patients). This supports the hypothesis that the DD gene has pleiotropic effects in brain and that mutations in SERCA2 are implicated in the pathogenesis of neuropsychiatric disorders.

INTRODUCTION

Darier’s disease (DD), also known as Darier–White disease or keratosis follicularis (OMIM 124200), is a dominantly inherited disorder predominantly affecting the skin (1). It is characterized by warty papules and plaques present in seborrheic areas (i.e. the central trunk, flexures, scalp and forehead), palmo-plantar pits and distinctive nail dystrophy. DD has an estimated prevalence of 1 in 55 000 (2). Age of onset is typically within the second decade (1) peaking between 11 and 15 years of age, after which there is little or no remission. Penetrance is high in adults although expressivity is variable (2), with disease severity ranging from mild, which is characterized by sparsely scattered keratotic papules, or lesions limited to one or two areas (e.g. the hands), to severe, characterized by verrucous plaques or grossly hypertrophic flexural disease.

A single locus for DD has been mapped to chromosome 12q23–q24.1 (3–7). A variety of missense, nonsense, frameshift and splicing mutations in the ATP2A2 gene have been described recently in families with DD (8). This gene encodes the sarcoplasmic/endoplasmic reticulum calcium-pumping ATPase SERCA2, which has a central role in intracellular calcium signalling (9), together with related pumps SERCA1 and SERCA3 encoded by ATP2A1 and ATP2A3, respectively. These pumps couple ATP hydrolysis with the transport of calcium ions from the cytosol into the sarcoplasmic/endoplasmic reticulum, thus regulating cytosolic calcium concentrations (10,11).

Clinical experience suggests an association between neuropsychiatric abnormalities (in particular mood disorders, epilepsy and mental retardation) and DD and, although there have been no formal epidemiological studies, this impression receives support from a number of reports of cases and families in the literature. Epilepsy has been described at increased prevalence in DD patients compared with the general population (1), although the association does not appear to be confined to a specific subtype of epilepsy. Mental retardation, usually of mild–moderate severity, has been reported repeatedly in asso-
There have been several reports of mood disorder phenotypes associated with DD, including bipolar disorder (1,6–8), affective psychosis (14,19), major depression (20,21) and suicidal ideation and behaviour (13,19,22). Of particular interest is the recent report of a family, pedigree 324, in which DD co-occurs with severe mood disorder, including bipolar disorder (1,16–18), affective psychosis (14,19), major depression (20,21) and suicidal ideation and behaviour (13,19,22). The most likely cause of co-segregation is genetic linkage between the DD gene and a susceptibility gene for bipolar disorder (maximum lod score = 2.1). Supporting evidence for a relationship between mutation type or position and the occurrence of neuropsychiatric abnormalities.

RESULTS

We identified 17 distinct mutations in affected individuals (Table 1), none of which was found in 50 controls, showing that these mutations are unlikely to be rare polymorphisms, and all segregated with disease where family members were available. The mutations identified predict conservative and non-conservative amino acid changes, premature translation termination and potentially altered splicing mutations. Conservative amino acid changes were observed in patients 6 (N767S), 7 and 8 (V843F), 10 (S920Y) and 11 (N39T). Non-conservative amino acid changes (27–29) leading to changes in intracellular calcium concentration, distribution and signalling (30).

The SERCA2 molecule consists of a number of functional domains, a calcium-binding domain (stalk region) consisting of five α-helices, a β-strand domain, a nucleotide-binding domain, a phosphorylation domain, a hinge domain and a transmembrane domain consisting of 10 α-helices in the case of SERCA2a and a potential eleventh α-helix in SERCA2b. Potentially form the sides of the ion channel lumen. The ATP2A2 gene spans 76 kb of genomic DNA (8) and consists of 21 exons.

The aim of the current study was twofold: first, to confirm the presence of SERCA2 mutations in DD and, second, to seek evidence for a relationship between mutation type or position and the occurrence of neuropsychiatric abnormalities.

### Table 1. ATP2A2 mutations in patients with DD with neuropsychiatric phenotypes and in patients with DD only

<table>
<thead>
<tr>
<th>Family/patient</th>
<th>Location</th>
<th>Mutationa</th>
<th>Nucleotide changea</th>
<th>Consequenceb</th>
<th>Protein domainb</th>
<th>Verification method</th>
</tr>
</thead>
<tbody>
<tr>
<td>家系/患者</td>
<td>位置</td>
<td>突变</td>
<td>核苷酸改变</td>
<td>后果</td>
<td>蛋白质域</td>
<td>验证方法</td>
</tr>
<tr>
<td>DD and neuropsychiatric phenotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Exon 13</td>
<td>1626delG</td>
<td>CAGAAG→CAAAG</td>
<td>Frameshift (PTC + 27 aa)</td>
<td>ATP binding</td>
<td>SSCP</td>
</tr>
<tr>
<td>2</td>
<td>Exon 13</td>
<td>C560R</td>
<td>1678T→C</td>
<td>Missense</td>
<td>ATP binding</td>
<td>HpaI</td>
</tr>
<tr>
<td>3</td>
<td>Exon 13</td>
<td>C560R</td>
<td>1678T→C</td>
<td>Missense</td>
<td>ATP binding</td>
<td>HpaI</td>
</tr>
<tr>
<td>4</td>
<td>Exon 14</td>
<td>2025insG</td>
<td>TTTGCT→TTTGCT</td>
<td>Frameshift (PTC + 3 aa)</td>
<td>Hinge</td>
<td>MwoI</td>
</tr>
<tr>
<td>5</td>
<td>Exon 14</td>
<td>K683E</td>
<td>2047A→G</td>
<td>Missense</td>
<td>Hinge</td>
<td>HindII</td>
</tr>
<tr>
<td>6</td>
<td>Exon 15</td>
<td>N767S</td>
<td>2300A→G</td>
<td>Missense</td>
<td>M5</td>
<td>Tail</td>
</tr>
<tr>
<td>7</td>
<td>Exon 17</td>
<td>V843F</td>
<td>2527G→T</td>
<td>Missense</td>
<td>M7</td>
<td>Tail</td>
</tr>
<tr>
<td>8</td>
<td>Exon 17</td>
<td>V843F</td>
<td>2527G→T</td>
<td>Missense</td>
<td>M7</td>
<td>Tail</td>
</tr>
<tr>
<td>9</td>
<td>Exon 18</td>
<td>Y894X</td>
<td>2682C→A</td>
<td>Nonsense</td>
<td>M7, loop, M8</td>
<td>Real</td>
</tr>
<tr>
<td>10</td>
<td>Exon 19</td>
<td>S920Y</td>
<td>2759C→A</td>
<td>Missense</td>
<td>M8, loop, M9</td>
<td>Scal</td>
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<tr>
<td>DD only</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>11</td>
<td>Exon 1</td>
<td>N39T</td>
<td>116A→C</td>
<td>Missense</td>
<td>Upstream S1</td>
<td>SSCP</td>
</tr>
<tr>
<td>12</td>
<td>Intron 1</td>
<td>118+1G→C</td>
<td>ACCgt→ACCgt</td>
<td>Altered splicing</td>
<td>Upstream S1</td>
<td>SSCP</td>
</tr>
<tr>
<td>13</td>
<td>Exon 8</td>
<td>G310V</td>
<td>929G→A</td>
<td>Missense</td>
<td>S4</td>
<td>SSCP</td>
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<tr>
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<td>1097insT</td>
<td>GATGTT→GATGTT</td>
<td>Frameshift (PTC + 9 aa)</td>
<td>Phosphorylation</td>
<td>BseGI</td>
</tr>
<tr>
<td>15</td>
<td>Intron 12</td>
<td>1543–10A→G</td>
<td>gtagcat→gtagcat</td>
<td>Altered splicing</td>
<td>ATP binding</td>
<td>MaeIII</td>
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<tr>
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<td>E695X</td>
<td>2083G→T</td>
<td>Nonsense</td>
<td>Hinge</td>
<td>SSCP</td>
</tr>
<tr>
<td>17</td>
<td>Exon 15</td>
<td>S765L</td>
<td>2294C→T</td>
<td>Missense</td>
<td>M5</td>
<td>SSCP</td>
</tr>
<tr>
<td>18</td>
<td>Exon 16</td>
<td>A803T</td>
<td>2407G→A</td>
<td>Missense</td>
<td>M6</td>
<td>Cca81</td>
</tr>
<tr>
<td>19</td>
<td>Exon 19</td>
<td>E917X</td>
<td>2749G→T</td>
<td>Nonsense</td>
<td>M8, loop, M9</td>
<td>SSCP</td>
</tr>
</tbody>
</table>

a Numbering of amino acids refers to the SERCA2a peptide sequence.
b Numbering of nucleotides refers to the ATP2A2 cDNA sequence with the first nucleotide of the ATG translation initiation codon being 1. Bases in exons are in upper case letters and those in introns in lower case letters. Altered bases are underlined.
c PTC + n aa indicates a premature termination codon at n amino acids downstream of the mutation.
d M, transmembrane domain; S, stalk domain.
were observed in patients 2 and 3 (C560R), 5 (K683E), 13 (G310V), 17 (S765L) and 18 (A803T). Single nucleotide deletions were identified in patients 1 and 4, and a single nucleotide insertion was identified in patient 14. These three mutations predicted frameshifts, potentially leading to premature translation termination.

The mutation identified in patient 12 (118+1G→C) occurs at the guanine of the conserved GT dinucleotide at the splice donor site of intron 1. It is possible that this mutation alters splicing of exon 1. A potential splice acceptor site mutation was identified in patient 15 (1543–10A→G) and may give rise to abnormal splicing of exon 13. In both potential splice site mutations, lack of a patient RNA source prevented verification of transcript structure and levels, although both mutations segregated correctly with disease.

Three distinct nonsense mutations were identified which lead to premature translation termination: patients 9 (Y894X), 16 (E695X) and 19 (E917X).

Figure 1 shows the SERCA2 molecule and the position of each mutation within the protein. Mutations identified in cases with neuropsychiatric phenotypes are numbered with patient IDs showing a predominance of missense mutations (70% versus 38% in DD patients). We performed a Fisher’s exact test (two-tailed) to compare exonic distributions of mutations between patient groups. Mutations showed non-random clustering within exons 13–19 of ATP2A2 in neuropsychiatric cases (P = 0.01).

DISCUSSION

In this study, we have confirmed that mutations in ATP2A2 are associated with DD. Seventeen novel mutations were identified. These were distributed throughout the gene in a number of functional domains thought to be crucial for the correct functioning of the SERCA2 molecule, by virtue of the conservation of such domains between a number of species (8). There
were no clear patterns of skin phenotype correlations with the mutations identified.

When we compared mutations identified in this study and those published by Sakuntabhai et al. (8), mutations in patients with DD alone were shown to be spread throughout \( ATP2A2 \) (from exons/introns 1–19), and there were a variety of forms, including missense, nonsense, frameshift, in-frame deletion, in-frame insertion and altered splicing type mutations. Interestingly, when DD patient groups were compared with those patients with neuropsychiatric phenotypes, a predominance of missense mutations was identified (at a frequency of 70% as opposed to 38% in DD sufferers), and all mutations were found to occur between exons 13 and 19, apart from an in-frame insertion mutation identified in exon 1 (8) in a patient known to have spinocerebellar dysfunction. Statistical comparison of the positions of mutations between both patient groups showed that there was a significant non-random distribution of mutations associated with neuropsychiatric phenotypes (\( P < 0.01 \)). However, this effect will require replication in a larger series of patients in order to give greater confidence in these observations. In addition, studies of the functional consequences of \( SERCA2 \) mutations in the brain will be required.

It is important to note that mutations that potentially can lead to premature translation termination can instead lead to accelerated mRNA decay, whereby truncated proteins are not made (32). It is possible, therefore, that the mutations occurring in patients 1 and 4 (mood disorder cases), patients 9, 14, 16, and possibly patients 12 and 15 result in accelerated mRNA decay. Mutagenesis studies have indicated that functional disruptions can occur when specific mutations are induced in all domains of \( SERCA2 \) (33). Such disruptions include loss of calcium affinity or calcium occlusion, reduction of ATP affinity, effects on phosphorylation by ATP and \( P_i \), effects on conformational shifts, blocking of dephosphorylation and uncoupling of calcium transport from ATP hydrolysis. Missense mutations that lead to these types of functional disruption in \( SERCA2 \) appear to localize to particular putative functional domains, although this relationship is not perfect. Certain defects in the protein may elicit specific changes in the cellular distribution or calcium signalling properties of the molecule. Precise calcium signalling is critically dependent on these factors (26).

It is of interest, therefore, that a unique mutation in two apparently unrelated individuals with mood disorders occurs within the ATP-binding domain of \( SERCA2 \). This may suggest that impaired function of this domain results in abnormalities of neuromal signalling events leading to susceptibility to mood disorders. This mutation is C560R and occurs in patient 2 (a sporadic case), and patient 3 from pedigree 324. Pedigree 324 originally was described by Craddock et al. (18) co-segregating DD and major affective disorder. Patient 2 also suffers a form of psychosis. Patient 1, with a mood disorder, also has a mutation within the ATP-binding domain, although this mutation is a premature translation termination. The C560R mutation is of particular interest since it is one of two distinct mutations (the other being V843F) that have occurred in more than one apparently unrelated DD patient or family with a neuropsychiatric phenotype. All other published mutations in patients and families with DD alone in this study and published by Sakuntabhai et al. (8) are unique.

Two apparently unrelated individuals possess the mutation V843F, which is localized to the seventh transmembrane \( \alpha \)-helix. Patient 7 has epilepsy and patient 8 has a history of blackouts of unknown cause, although a definitive diagnosis of epilepsy has not been made. There is also evidence for inconsistencies in supplementary phenotype between individuals with identical mutations. Although patient 1 (1626delG) has a mood disorder, the child of this patient has epilepsy as well as DD.

The \( SERCA2 \) pump is involved intimately in the regulation of cytosolic and endoplasmic reticulum calcium concentrations (10, 11). Defects in the control of such processes may lead to abnormal protein processing and transport in the endoplasmic reticulum or abnormal calcium-dependent signalling (26). It is possible that the disruptions caused by defective \( SERCA2 \) may, in some individuals, interact with secondary susceptibility factors thereby giving rise to neuropsychiatric phenotypes. These secondary susceptibility factors could consist of neuronal-specific proteins that possess functional polymorphisms, variants of which are particularly susceptible to anomalies in calcium regulation. Sensitivity to calcium concentration is exemplified by the A986S polymorphism of the calcium-sensing receptor (CASR) gene. CASR is involved in the sensing of extracellular calcium concentration, the regulation of which is achieved by parathyroid hormone. The A986S polymorphism is frequent in the general population but has been shown to have a significant effect on extracellular calcium levels (34).

In summary, our findings confirm the presence of mutations in \( ATP2A2 \) in DD, and we have identified and verified 17 novel mutations. We have found evidence to suggest that missense mutations in the 3′ half of \( ATP2A2 \) correlate with the presence of neuropsychiatric phenotypes, and more specifically that the ATP-binding domain may have relevance in mood disorders. It is possible that specific mutations within \( ATP2A2 \), or mutations coupled with the presence of secondary susceptibility factors, may be determining the presence of supplementary phenotypes in DD patients. We suggest that neuropsychiatric effects related to \( ATP2A2 \) mutations may be related to the presence of further susceptibility factors, but in some instances mutations might have high penetrance with respect to neuropsychiatric phenotypes. Clearly, functional data concerning the effect of \( SERCA2 \) mutation in brain will be required to verify these speculations. It will also be important to investigate this gene as a candidate for the bipolar susceptibility gene on chromosome 12q23–q24.1. To this end, we currently are analysing individuals with mood disorder for polymorphisms and mutations in \( ATP2A2 \).

**MATERIALS AND METHODS**

**Subjects**

Diagnosis of DD was made by a dermatologist with a research interest in the disorder (S. Burge, C. Munro or P.-Y. Kwok) on the basis of clinical examination and by histological examination of a skin biopsy. Diagnostic clinical features included keratotic papules or plaques on the trunk, nail dystrophy and/or palmar pits.

Subjects with both DD and a neuropsychiatric disorder were identified by referral from a dermatologist or medical geneticist. Such individuals were all interviewed personally and examined by a trained research psychiatrist and, where possible, medical records were obtained and scrutinized. Diagnoses
Ten individuals (ID numbers 1–10) with DD and neuropsychiatric phenotypes, and nine individuals (ID numbers 11–19) colleagues in Oxford are preparing a report of new mutations also reported elsewhere (T. Strachan et al., submitted). Our colleagues in Oxford are preparing a report of new mutations (A. Hovnanian et al., in preparation). The Porton Down identification numbers of subjects referred by S. Burge are: patient 4, AH0032; and patient 10, AH0060.

### PCR amplification of ATP2A2 coding sequence

The ATP2A2 genomic DNA sequence was obtained from Sakuntabhai et al. (8) and by alignment of the cDNA sequences for ATP2A2a (SERCA2a) and ATP2A2b (SERCA2b) (accession nos M23114 and M23115) to the high throughput genomic sequence (HTGS) database using the BLAST program at the National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/).

Precise locations of each exon within the PAC clone 305120 (accession no. AC006088—updated frequently) were noted and the appropriate section of genomic sequence utilized for primer design. Oligonucleotide primers for PCR assays were designed for each exon from flanking non-exonic sequence. Alternatively, exons were split into two pieces with sufficient overlap. PCR primers were designed using the Primer3 program based at the Whitehead Institute Center for Genome Research (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). PCR analysis was carried out on high molecular weight genomic DNA isolated from lymphocytes or lymphoblasts by phenol–chloroform extraction. The analysis of each exon was carried out on the individuals described above.

PCR products were then amplified in an oil-free 25 µl volume containing 1× PCR buffer (Qiagen), 40 ng of genomic DNA, 2.5–10 pmol of each primer, 200 µM dNTPs and 1 U of Taq DNA polymerase (Qiagen). Each reaction began with a single denaturation step at 95°C for 5 min (or 15 min for HotStarTaq), followed by 35 cycles of 30 s at 94°C, 30 s at the appropriate primer pair annealing temperature and 30 s at 72°C. Reactions were completed with 5 min at 72°C.

### Denaturing high performance liquid chromatography (DHPLC) analysis of PCR-amplified products

Mutation detection was performed using DHPLC (36,37). In essence, the method depends upon detecting heteroduplexes in PCR products by HPLC. Sensitivity of the analysis is maximized by maintaining the HPLC column at a temperature that favours partial strand denaturation in the presence of base pair mismatching. The optimal temperature for the analysis was selected using DHPLCMelt (written by P. Oefner and N. Hansen, available at http://hardy-weinberg.stanford.edu/dhplc/melt.html). We have shown previously that the DHPLCMelt recommended temperature (RTm) supplemented by an extra run at 2°C above the RTm predicts DHPLC conditions that detect 103/103 mutations in blind analysis (38).

After PCR amplification, heteroduplexes were given the opportunity to form by heating the products to 95°C for 5 min and then cooling to 65°C over 30 min. DHPLC analysis was performed at the RTm using a WAVE DHPLC system (Transgenic, CA) and a DNASEp columns (Transgenic) using a linear triethylammonium acetate/acetonitrile gradient.

### DNA sequencing

Sequencing reactions were carried out on samples showing heteroduplexes upon DHPLC analysis. Sequencing analyses were performed on both strands of double-stranded PCR products using the appropriate forward and reverse PCR primers. Radioactive DNA cycle sequencing was performed using the Thermosequenase kit (Amersham) according to the manufacturer’s instructions.

### Mutation verification

Mutations identified in ATP2A2 fragments were analysed in family members (where available or appropriate) and 50 Caucasian controls to verify that they segregated with disease and were not rare polymorphisms. This was achieved using restriction endonucleases whose sites were either created or inactivated by the sequence variations observed (identified using the Lasergene DNASTar program), or by use of single-
stranded conformational polymorphism (SSCP) analysis. SSCP analysis was carried out by electrophoresis of 5 μl of PCR reaction through 10% non-denaturing polyacrylamide (49:1 acrylamide:bis; Sigma-Aldrich, Poole, UK) at 70 V for 16 h at 4°C. DNA was visualized by silver staining.

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