Genotype–phenotype correlations in Darier disease: A focus on the neuropsychiatric phenotype

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Abstract
Darier disease (DD) is an autosomal dominant skin disorder caused by mutations in ATP2A2 encoding the sarco/endoplasmic reticulum Ca2+ ATPase Isoform 2 (SERCA2). Evidence of a population-level association between DD and psychiatric disorders suggests that mutations in ATP2A2 may have pleiotropic effects on the brain as well as skin. Evidence of genotype–phenotype relationships between ATP2A2 mutations and neuropsychiatric phenotypes would further support this suggestion. We investigated genotype–phenotype correlations between lifetime neuropsychiatric features and ATP2A2 mutation type (dichotomized into likely gene disrupting [LGD] or protein altering) in 75 unrelated individuals with DD. We also looked for evidence of clustering of mutations within SERCA2 according to neuropsychiatric features. Combining our data with the existing literature, the rate of LGD mutations was found to be significantly higher among DD cases/families with bipolar disorder, schizophrenia, or affective psychosis ($p = .011$). We also found a significant relationship between mutations located in the S4– M4 region of the protein and the presence of a severe neuropsychiatric phenotype ($p = .032$). Our findings add support to the hypothesis that Darier-causing mutations in ATP2A2 confer susceptibility to neuropsychiatric dysfunction, in particular severe psychiatric illness. This, together with evidence from research on common polymorphisms confirms ATP2A2 as a gene at which variation influences susceptibility to major psychiatric illness.

KEYWORDS
Darier disease, genotype–phenotype correlations, neuropsychiatric features

1 | INTRODUCTION

Darier disease (DD) is a rare autosomal dominant inherited skin disorder with an estimated average prevalence of between 1 in 100,000 and 30,000 (Svendsen & Albrectsen, 1959; Tavadia, Mortimer, & Munro, 2002) and usually presents in the second decade with no sex difference (Burge & Wilkinson, 1992). It is characterized by hyperkeratotic papules in seborrheic areas, palmo-plantar pits, and nail dystrophy. DD is inherited with a high penetrance although the phenotypic expression is variable (Munro, 1992).

The disease is caused by mutations in the gene ATP2A2 (Sakuntabhai, Ruiz-Perez, et al., 1999) which encodes the sarco/endoplasmic reticulum (ER) Ca2+ ATPase Isoform 2 (SERCA2), a calcium pump located in the ER membrane which plays a key role in Ca2+ homeostasis. The protein contains five major domains which include 11 transmembrane helices (M1–M11), 5 stalks (S1–S5), and 3 cytoplasmic domains: the actuator (A) domain, the nucleotide ATP-binding (N) domain, and the phosphorylation (P) domain. To date, well over 200 different Darier-causing mutations have been identified throughout ATP2A2 including missense, nonsense, substitutions, and both frame-shift and in-frame insertions and deletions (Human Gene Mutation Database http://www.hgmd.org). Generally, these mutations do not seem to cluster within potential “hot-spot” regions throughout the primary sequence of the SERCAb molecule and most are unique within individual families.

The co-occurrence of neuropsychiatric features, including depression, bipolar disorder, epilepsy, and learning difficulties, with DD has frequently been reported (Burge & Wilkinson, 1992; Cederlöf et al., 2015; Cederlöf et al., 2015; Denicoff, Lehman, Rubinow, Schmidt, & Peck, 1990; Dodiuk-Gad et al., 2014, 2016; Gordon-Smith et al., 2010; Medansky & Woloshin, 1961; Ringpfeil et al., 2001). The nature of this co-occurrence has long been debated in the literature with a number of potential explanations being put forward. A seemingly plausible explanation is that the high psychiatric morbidity observed in DD is a direct psychological consequence of having a chronic skin disorder. However, our previous investigations into the neuropsychiatric phenotype in DD and the work of others have not found any significant relationships between psychiatric phenotypes and DD clinical features including disease severity, suggesting this argument alone cannot account for the association (Dodiuk-Gad et al., 2016; Gordon-Smith et al., 2010).

In recent years, studies have found evidence of a population-level association between DD and psychiatric disorders—specifically mood disorders, including bipolar disorder, and schizophrenia (Cederlöf, Bergen, et al., 2015; Dodiuk-Gad et al., 2016; Gordon-Smith et al., 2010). This included a matched cohort study based on Swedish national registers that found individuals with DD had a 4.3 and 2.3 times higher risk of bipolar disorder and schizophrenia, respectively, than individuals in the general population (Cederlöf, Bergen, et al., 2015). These findings suggest that mutations in ATP2A2 have pleiotropic effects in the skin and brain and confer susceptibility to neuropsychiatric features. This theory would be strongly supported by evidence of genotype–phenotype relationships between ATP2A2 mutation type and co-occurrence of neuropsychiatric phenotypes.

To date, a small number of studies have examined genotype– phenotype correlations with the neuropsychiatric phenotypes observed in DD (Bchetnia et al., 2009; Dodiuk-Gad et al., 2016; Jacobsen et al., 1999; Nellen et al., 2016; Ringpfeil et al., 2001; Ruiz- Perez et al., 1999; Sakuntabhai, Burge, Monk, & Hovnanian, 1999) with no clear and consistent correlations being identified. Difficulties in establishing these relationships may be because of a number of reasons including small sample sizes (ranging from 8 to 49 cases) and the use of diverse methods to measure neuropsychiatric phenotypes. The lack of consistent application of any standardized method of grouping the types of mutations has also been a major methodological impediment. A recent review of the literature divided reported Dariercausing mutations into two categories: (a) likely gene disrupting (LGD) mutations (frameshift, splice site, nonsense, gain of stop codon, or loss of start codon) and (b) protein altering (PA) mutations (missense or inframe-insertion/deletions; Nakamura et al., 2016). This study found significantly higher rates of LGD mutations in Darier cases with reported co-occurring neuropsychiatric features than in those without such features. A single study has reported a nonrandom clustering of mutations in the last half of ATP2A2 and a neuropsychiatric phenotype (in a sample of 19 unrelated individuals with DD; Jacobsen et al., 1999). However, no other genotype–phenotype associations between mutation location along the primary structure of the gene and the presence of neuropsychiatric features have been reported in other samples (Dodiuk-Gad et al., 2016; Nellen et al., 2016; Ringpfeil et al., 2001) including the recent literature survey (Nakamura et al., 2016). The authors of the survey noted that this lack of association would be reasonable as LGD mutations in any exon could result in similar molecular consequences.

We have previously reported a systematic investigation of the neuropsychiatric characteristics in a large U.K. sample of unrelated individuals with DD (Gordon-Smith et al., 2010), and more recently reported the disease causing sequence variants of ATP2A2 within this large sample (Green et al., 2013). Although these mutations were included in the recent analysis by Nakamura et al. (2016), the associated neuropsychiatric phenotypes were unknown to the authors at the time. In the current study, we investigated potential correlations between mutation type (LGD vs. PA) and neuropsychiatric phenotypes among 75 unrelated individuals with DD. This is the largest such study to date. This large sample has also enabled us to look for evidence of clustering of mutations within the SERCA2 protein among individuals with similar neuropsychiatric phenotypes and we report that here. Using this clustering approach may be a more useful way of examining genotype–phenotype correlations with neuropsychiatric phenotypes in DD given the likely complex nature of the associations. Finally, we combined our sample with previously reported DD cases/families in the literature to date to establish whether any genotype–phenotype correlations observed were enriched in the combined dataset.

2 | MATERIALS AND METHODS

2.1 | Recruitment of participants

A detailed description of the sample has previously been published (Gordon-Smith et al., 2010). In summary, 100 unrelated individuals with a diagnosis of DD were recruited throughout the United Kingdom mainly via dermatology services and the U.K. Darier Support Group. The study was approved by the Multi-Centre Research Ethics Committee for Wales.

2.2 | Neuropsychiatric assessment

Neuropsychiatric assessments were conducted in a single session by a trained research psychologist (KGS). Psychiatric symptomatology, including history of suicidal thoughts/attempts, was measured using an adapted version of Schedules for Clinical Assessment in Neuropsychiatry interview (Wing et al., 1990). This information was supplemented by psychiatric notes and/or general practice case-notes. Lifetimeever psychiatric diagnoses were made according to the Diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV; American Psychiatric Association, 2000). Three experienced research psychologists/ psychiatrists (KGS, LJ, and NC) made the psychiatric ratings independently, using written case vignettes, and consensus was reached. Inter-rater reliability was high with mean kappa statistics of 0.86 and 0.93 for DSM-IV diagnoses and suicidal ideation, respectively. Lifetime history of neurological symptoms and disorders was assessed using a brief interview checklist, supplemented by hospital and/or general practice case-notes.

2.3 | ATP2A2 variant identification

A DNA sample was obtained from 95 individuals. Sixty-six potentially pathogenic mutations in ATP2A2 were identified in 74 individuals. No potentially pathogenic variant in ATP2A2 was identified in the remaining 21 individuals. One of the five remaining individuals from whom a sample was not collected had taken part in a previous study (Sakuntabhai, Ruiz-Perez, et al., 1999) from which the potential pathogenic variant was supplied. For further details on the identification of mutations, see our previous publication (Green et al., 2013).

2.4 | Genotype–phenotype correlations

According to the definitions used in a recent study (Nakamura et al., 2016), mutations were classified as either LGD (frameshift insertions/ deletions, mutations predicted to alter a splice codon, nonsense mutations, gain of stop codon, or loss of start codon) or PA (missense or inframe-insertion/deletions).

Rates of LGD and PA mutations were compared between individuals stratified according to the presence or absence of each of the following key lifetime neuropsychiatric features:

1. Any neuropsychiatric phenotype.
2. Any psychiatric disorder meeting DSM-IV criteria.
3. Contact with a neurologist or neurological procedure.
4. Suicide attempt.
5. Severe neuropsychiatric phenotype (bipolar disorder, schizophrenia, or affective psychosis).

All mutations detected were mapped onto a schematic diagram of the SERCA2b protein to look for evidence of possible clustering of mutations according to occurrence within individuals of similar neuropsychiatric features.

2.5 | Combined analysis with existing literature

We combined our sample with the previously reported DD cases/families in the literature with an identified Darier causing mutation (n = 384 including the current sample).
Within the combined sample, we

1. Compared rates of LGD and PA mutations according to the absence or presence of a reported severe neuropsychiatric phenotype.

2. Compared the rates of mutations located in specific functional domains of the SERCA2 protein according to the absence or presence of a reported severe neuropsychiatric phenotype.

A severe neuropsychiatric phenotype was rated as present if the index case and/or a family member with DD was reported as having a diagnosis of bipolar disorder, schizophrenia, or affective psychosis. All members of the same family will have the same DD causing mutation and therefore a shared diathesis. Within this analysis, we also included an unpublished missense mutation (P312R) identified in an individual with DD and bipolar disorder seen by our research group as part of our ongoing mood disorders research program who was not included in the current study as they were identified on the basis of having bipolar disorder rather than on the basis of having DD.

2.6 | Statistical analyses

Groups were compared using Chi-Square Tests or Fisher's Exact Tests where 20% or more of the cells in a Chi-square table had an expected count of <5. For significant findings ($p < .05$), odds ratios with 95% confidence intervals (95% CI) were calculated. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) for Windows.

3 | RESULTS

3.1 | Current sample (75 cases)

Table 1 summarizes the DD clinical and neuropsychiatric features in the sample along with the type and location of ATP2A2 mutation identified and mutation classification according to the LGD and PA groupings.

In our 75 cases, we found a consistent nonsignificant trend for a higher prevalence of LGD mutations among individuals with a lifetime occurrence of each of the neuropsychiatric phenotypes compared with individuals with the absence of the neuropsychiatric phenotype (any neuropsychiatric phenotype 45.5% vs. 35%, $p = .418$; any psychiatric disorder meeting DSM-IV criteria 46% vs. 38%, $p = .480$; contact with a neurologist or neurological procedure 53% vs. 36%, $p = .127$; suicide attempt 50% vs. 41%, $p = .733$; severe neuropsychiatric phenotype 75% vs. 41%, $p = .307$; Figure 1; Table 2).

We observed possible clustering of mutations according to individuals with similar neuropsychiatric phenotypes (Figure 2), but none reached statistical significance:

1. All four individuals with mutations located in the functional "A" domain between the Stalk 2 and Stalk 3 domains of the protein had a history of a psychiatric disorder severe enough to warrant contact with psychiatric services (IDs 11, 12, 13, and 14).
2. Three individuals with the same missense mutation at one of the seven Ca2+ binding sites in the M5 domain all had a DSM-IV diagnosis of a mood disorder (IDs 54, 55, and 56).
3. Two individuals with frameshift mutations one base pair apart in the S4 (Stalk 4) domain of the protein both had a history of suicide attempts in addition to having idiopathic epilepsy (ID 19) and an extensive psychiatric history including major depressive disorder and investigations for a blackout (ID 20).

3.2 | Analysis of combined data with existing literature (384 cases)

In the combined analysis with existing literature, the rate of LGD mutations was significantly higher where a history of a severe neuropsychiatric phenotype (bipolar disorder, schizophrenia, or affective psychosis) was reported in either the index case and/or family member with DD compared those without a reported history; 68% versus 40.5%, $p = .011$ (OR 3.15, 95% CI 1.25–7.91; Table 3). This remained significant when we only included the 14 cases of bipolar disorder; 71.4% versus 41.0%, $p = .024$ (OR 3.44, 95% CI 1.10–10.76). With respect to mutation location, no significant genotype–phenotype correlations were found with mutations located in the "A" domain of the SERCA2 protein or calcium binding sites. However, the rate of mutations located between the Stalk 4 and transmembrane Helix 4 (S4– M4) region was significantly higher where a history of a severe neuropsychiatric phenotype was reported compared to where not; 14% versus 3%, Fisher's = 0.032 (OR 5.66, 95% CI 1.41–21.88). When this analysis was narrowed down further to only include PA,

mutations became more significant: 43% versus 4%, Fisher's = 0.003 (OR 19.22, 95% CI 3.67–100.6). A summary of all mutations identified by our research group and those in the literature located within the S4–M4 region is presented in Figure 3 along with brief descriptions of all known neuropsychiatric features reported among individuals/ families with DD the mutations. This includes two families with bipolar disorder and an individual with schizophrenia (Figure 3).

TABLE 1 Clinical and neuropsychiatric features in 75 individuals with Darier disease and type and location of *ATP2A2* mutations

ID	Sex	Severity of DD	Neuropsychiatric phenotype	Location	Nucleotide and alteration	Amino acid alteration	Type	Protein domain	LGD or PA mutation
1	F	Moderate		Exon 1	1A>G	Met1V	Missense	Start codon	LGD
2	M	Moderate		Exon 1	34+57ins	V29Met ins repeat of previous 19 amino acids	In-frame insertion	A domain	PA
3	M	Moderate	Investigations for hearing problems.	Exon 1	48 del14/ins11	V18X	Frameshift (PTC+1aa)	A domain	LGD
4	M	Mild		Exon 1	94C>T	L32F	Missense	A domain	PA
5	M	Mild	Epilepsy.	Intron 2	137-3C>G		Splice site		LGD
6	F	Moderate	Bipolar I disorder[a]. Investigations for periods of unconsciousness.	Exon 3	142 in. 18		Frameshift (PTC+14aa)	S1	LGD
7	F	Mild	Major depressive disorder[a].	Exon 3	167A>G	Q56R	Missense	M1	PA
8	F	Severe	Major depressive disorder.	Exon 3	194T>C	L65S	Missense	M1	PA
9	F	Moderate	Dysthymic disorder.	Intron 3	219+5 insAA		Splice site		LGD
10	M	Moderate	Investigations for hearing problems.	Intron 5	325-2A>G		Splice site	M2	LGD
11	F	Moderate	Bipolar I disorder[a].	Intron 6	464-1A>C		Splice site	A domain	LGD
12	F	Moderate	Major depressive disorder[a].	Exon 6	490A>G	R164G	Missense	A domain	PA
13	F	Moderate	Major depressive disorder[a].	Exon 6	543delA		Frameshift (PTC+42aa)	A domain	LGD
14	M	Moderate	Depression NOS[a]. Suicide attempt.	Exon 8	698G>T	G233V	Missense	A domain	PA
15	F	Moderate		Exon 8	826 del15	Del276-300 (IGHFN)	In-frame deletion	M3–M4 lumenal	PA
16	F	Severe	Depression NOS.	Exon 8	923C>A	P308H	Missense	M4	PA
17	M	Moderate	Treatment for hearing problems.	Exon 8	925G>A	E309K	Missense	M4	PA
18	F	Severe		Exon 8	929G>T	G310V	Missense	M4	PA
19	F	Moderate	Psychiatric disorder NOS[a]. Suicide attempt. Epilepsy.	Exon 8	948delC		Frameshift (PTC+65aa)	S4	LGD
20	F	Moderate	Major depressive disorder[a]. Suicide attempt. Investigations for a blackout.	Exon 8	949del7		Frameshift (PTC+65aa)	S4	LGD
21	F	Mild		Exon 8	958G>C	A320P	Missense	S4	PA
22	F	Moderate	Headaches requiring investigation.	Exon 8	1000C>T	R334X	Nonsense	P domain	LGD
23	M	Severe		Exon 8	1000C>T	R334X	Nonsense	P domain	LGD
24	M	Severe		Exon 8	1043T>C	I348T	Missense	P domain	PA
25	F	Mild		Exon 8	1070C>G	T357R	Missense	P domain	PA
26	M	Moderate	Major depressive disorder. Investigations for hearing problems.	Exon 8	1070C>A	T357K	Missense	P domain	PA
27	F	Moderate	Major depressive disorder. Investigations for suspected epileptic seizure.	Exon 8	1095+1G>C		Splice site	P domain	LGD
28	F	Moderate	Major depressive disorder[a]. Suicide attempt. Headaches requiring investigation.	Exon/ intron 10	1228 del 86		Deletion	N domain	LGD
29	M	Moderate	Investigations for fainting episodes.	Exon 11	1321A>C	T441P	Missense	N domain	PA
30	F	Moderate	Major depressive disorder[a]. Multiple suicide attempts. Investigations for blackouts.	Exon 11	1413C>A	C471X	Nonsense	N domain	LGD
31	F	Moderate	Investigations for fainting	Exon 11	1419 del GA		Frameshift	N domain	LGD

ID	Sex	Severity of DD	Neuropsychiatric phenotype	Location	Nucleotide and alteration	Amino acid alteration	Type	Protein domain	LGD or PA mutatio
32	F	Moderate	Major depressive disorder.	Exon 12	1484C>T	S495L	Missense	N domain	PA
33	F	Mild	Major depressive disorder. Suicide attempt.	Exon 12	1484C>T	S495L	Missense	N domain	PA
34	F	Mild	Panic disorder.	Exon 12	1508del C		Frameshift (PTC+5aa)	N domain	LGD
35	F	Mild	Treatment for viral encephalitis.	Exon 13	1628_1630delAGA	del K543	In-frame deletion	N domain	PA
36	F	Moderate	Bipolar I disorder[a]. Suicide attempt. Investigations following an episode of loss of consciousness.	Exon 13	1697dupA		Frameshift (PTC+1aa)	N domain	LGD
37	F	Moderate	Major depressive disorder[a].	Exon 13	1713delAA		Frameshift (PTC+4aa)	N domain	LGD
38	M	Mild		Intron 13	1762-1G>C		Splice site	N domain	LGD
39	M	Mild		Exon 14	1919insT		Frameshift (PTC+4aa)	P domain	LGD
40	F	Moderate	Major depressive disorder. Investigations for fainting episodes.	Exon 14	2017del C		Frameshift (PTC+14aa)	P domain	LGD
41	M	Moderate	Anxiety disorder NOS.	Exon 14	2046 in. C		Frameshift (PTC+2aa)	P domain	LGD
42	F	Moderate	Investigations for blackouts.	Exon 14	2048A>T	K683M	Missense	P domain	PA
43	F	Moderate		Intron 14	2098-2A>C		Splice site	P domain	LGD
44	F	Moderate	Dysthymic disorder[a]. Investigations for headaches.	Exon 15	2104G>A	D702N	Missense	P domain	PA
45	M	Moderate	Major depressive disorder[a]. Suicide attempt.	Exon 15	2116G>A	D706N	Missense	P domain	PA
46	M	Moderate		Exon 15	2116G>A	D706N	Missense	P domain	PA
47	F	Moderate	Investigations for hearing problems.	Exon15	2123C>A	P708H	Missense	P domain	PA
48	M	Moderate	Major depressive disorder.	Exon 15	2249G>A	R750Q	Missense	S5	PA
49[b]	F	Moderate		Exon 15	2258del3bp		In-frame deletion	S5	PA
50	M	Moderate	Depression NOS.	Exon 15	2287C>G	L763V	Missense	S5	PA
51	M	Moderate		Exon 15	2294C>T	S765L	Missense	M5	PA
52	M	Moderate	Investigations for loss of feeling in lower limbs.	Exon 15	2294C>T	S765L	Missense	M5	PA
53	M	Moderate	Treatment for hearing problems. Investigations for poor memory.	Exon 15	2300A>G	N767S	Missense	M5	PA
54	F	Moderate	Major depressive disorder[a]. Investigations for blackouts.	Exon 15	2300A>G	N767S	Missense	M5	PA
55	F	Moderate	Major depressive disorder. Investigations for hearing problems.	Exon 15	2300A>G	N767S	Missense	M5	PA
56	F	Mild	Depression NOS. Investigations for hearing problems.	Exon 15	2300A>G	N767S	Missense	M5	PA
57	M	Mild		Exon 15	2317T>C	C773R	Missense	M5	PA
58	F	Moderate	Depression NOS. Investigations for headache and fainting episodes.	Intron 15	2319-1G>A		Splice site		LGD
59	F	Moderate	Anxiety disorder NOS.	Exon 16	2384A>G	N795S	Missense	M6	PA
60	F	Moderate	Bipolar I disorder[a].	Exon 16	2405C>G	P802R	Missense	M6	PA
61	F	Moderate	Major depressive disorder.	Exon 16	2417T>G	L806R	Missense	M6	PA
62	F	Severe	Major depressive disorder[a]. Suicide attempt.	Exon 17	2527G>T	V843F	Missense	M7	PA

ID	Sex	Severity of DD	Neuropsychiatric phenotype	Location	Nucleotide and alteration	Amino acid alteration	Type	Protein domain	LGD or PA mutation
63	M	Moderate		Exon 17	2584insG		Frameshift (PTC+14aa)	M7–M8 lumenal	LGD
64	F	Moderate	Major depressive disorder[a].	Exon 18	2620C>T	Q874X	Nonsense	M7–M8 lumenal	LGD
65	F	Mild	Major depressive disorder.	Exon 18	2678dupC		Frameshift (PTC+16aa)	M7–M8 lumenal	LGD
66	M	Moderate	Depression NOS. Suicide attempt.	Exon 18	2684C>T	P895L	Missense	M7–M8 lumenal	PA
67	F	Moderate		Exon18	2709 del 6bp	del V904 & T905	In-frame deletion	M8	PA
68	F	Moderate	Depression NOS.	Exon 18	2730 in. C		Frameshift (PTC+71aa)	M8	LGD
69	F	Moderate		Exon 18	2741+1G>T		Splice site		LGD
70	F	Severe	Major depressive disorder. Investigations for blackouts.	Exon 18	2741+5G>C		Splice site		LGD
71	F	Moderate	Depression NOS.	Exon 19	2759C>T	S920F	Missense	M8–M9 cytoplasmic	PA
72	F	Severe	Investigations for one-sided weakness.	Exon 19	2759C>A	S920Y	Missense	M8–M9 cytoplasmic	PA
73	M	Severe	Medical notes report "adjustment reaction" to relapse in DD[a,c].	Exon 19	2759C>A	S920Y	Missense	M8–M9 cytoplasmic	PA
74	F	Mild	Cyclothymic disorder[a].	Exon 19	2777C>G	P926R	Missense	M8–M9 cytoplasmic	PA
75	F	Moderate	Treatment for meningitis (unknown type).	Exon 20a	2965del 7 in. 9 & 2983del A		Frameshift (PTC+42aa)	M10–M11 cytoplasmic	LGD

Note. LGD = likely gene disrupting; PA = protein-altering; NOS = not otherwise specified; Sn = stalk domains; Mn = transmembrane domains; A domain actuator domain; N domain = nucleotide binding; P domain = phosphorylation domain.

[a] Requiring contact with psychiatric services.

[b] Mutation identified in previous study (Sakuntabhai, Ruiz-Perez, et al., 1999).

[c] Classified as a maladjustment reaction as the patient was referred and seen by secondary psychiatric services.

4 | DISCUSSION

Our genotype–phenotype investigations have been carried out in the largest sample of individuals with DD to date. Consistent with there cent prior report (Nakamura et al., 2016), we found that LGD mutations were relatively more common in those DD individuals with neuropsychiatric phenotypes than those without such phenotypes. We had only four cases in our sample that met the prior report's definition of "psychosis" (bipolar disorder, schizophrenia, and affective psychosis). Of these four cases, three had LGD mutations and one had a PA mutation. Because of the small numbers, this difference compared to those with DD without "psychosis" does not meet statistical significance. However, when we add our new data to the existing literature, the statistical significance of the finding that the rate of LGD mutations is higher among cases/families with this severely defined neuropsychiatric phenotype is strengthened ($p = .011$, compared with the previously reported $p = .026$ [Nakamura et al., 2016] which although, included our cases the neuropsychiatric features associated with the mutations were unknown to the authors), and this association remains significant even when the phenotype definition is narrowed to include only bipolar disorder. We also observed in our sample that mutations in individuals with similar neuropsychiatric phenotypes tended to cluster in certain locations within the SERCA2 protein but none reached statistical significance. In our combined analysis, we did however find a significant relationship with mutations located in the S4–M4 region of the protein and the presence of a severe neuropsychiatric phenotype. Furthermore, when this analysis was repeated with only PA mutations the association remained significant. This finding has not previously been reported.

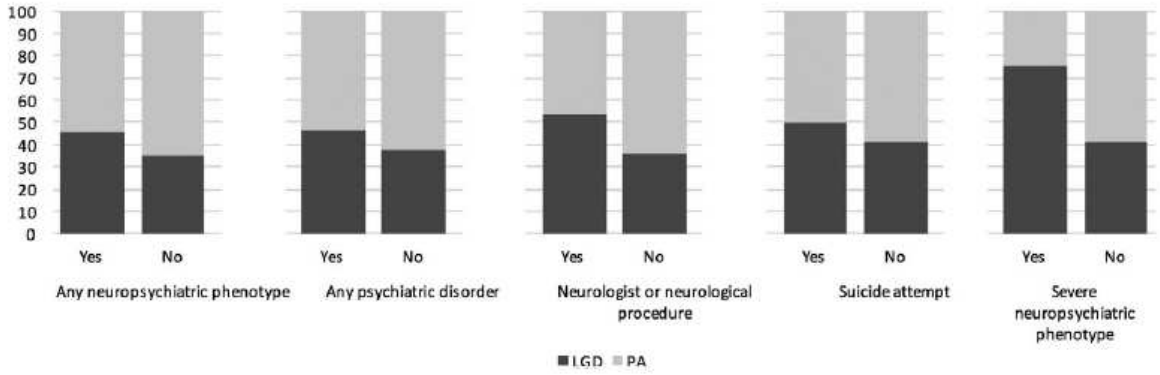


FIGURE 1 Comparison of prevalence of likely gene-disrupting (LGD) and protein-altering (PA) mutations among 75 individuals with and without the lifetime occurrence of neuropsychiatric phenotypes

TABLE 2 Likely gene-disrupting (LGD) and protein-altering (PA) mutations among 75 individuals with and without the lifetime occurrence of neuropsychiatric phenotypes

	Any neuropsychiatric phenotype				Any psychiatric disorder				Neurologist or neurological procedure				Suicide attempt				Severe neuropsychiatric phenotype			
	Yes		No		Yes		No		Yes		No		Yes		No		Yes		No	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
LGD	25	45.5	7	35	19	46	13	38	16	53	16	36	5	50	25	41	3	75	29	41
PA	30	54.5	13	65	22	54	21	62	14	47	29	64	5	50	36	59	1	25	42	59
Total N[a]	55		20		41		34		30		45		10		61		6		69	
	$\chi^2 = 0.655$, df = 1, $p = .418$				$\chi^2 = 0.499$, df = 1, $p = .480$				$\chi^2 = 2.326$, df = 1, $p = .127$				Fisher's, $p = .733$				Fisher's $p = .307$			

[a] N's vary because of unknown data.

It is highly plausible that mutations in ATP2A2 could be involved in conferring susceptibility to neuropsychiatric illness because the gene is widely expressed in the brain. The dual role of the SERCA2b protein in intracellular Ca2+ signaling and in the synthesis and posttranslational modification of proteins within the ER also provides support for this suggestion. Intracellular Ca2+ signaling has been shown to play a role in a range of neuronal functions including neuronal excitability, neurotransmitter release, gene expression, neuronal growth, and synaptic plasticity (Berridge, 2002; Berridge, Bootman, & Lipp, 1998; Verkhratsky, 2005). Genome-wide association studies (GWAS) have suggested the role of calcium signaling and calcium-channel activity in the pathogenesis of a number of major psychiatric disorders, including bipolar disorder and schizophrenia (Cross-Disorder Group of the Psychiatric Genomics Consortium & Genetic Risk Outcome of Psychosis (GROUP) Consortium, 2013; Ferreira et al., 2008) with a more recent GWAS identifying ATP2A2 as a schizophrenia associated loci (Ripke et al., 2014).

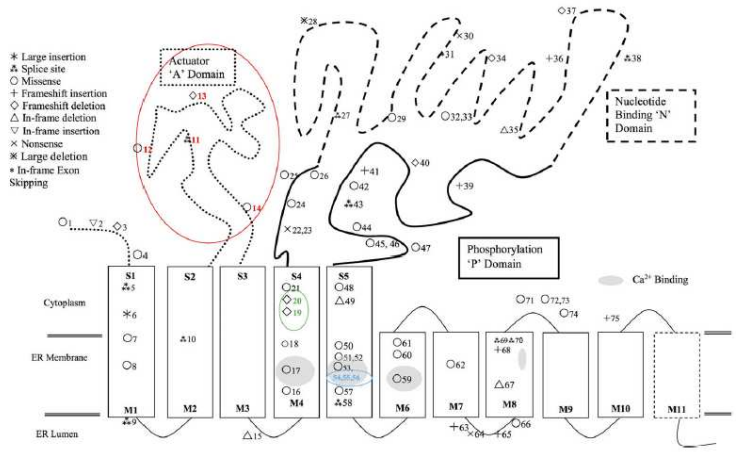
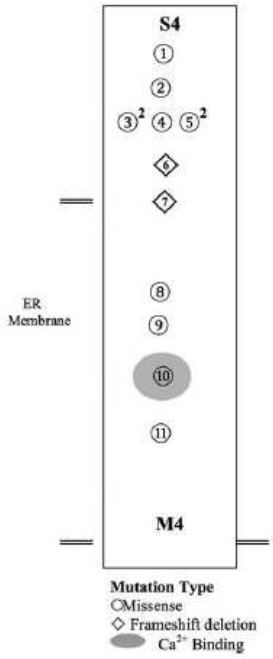


FIGURE 2 Key observations of evidence for possible clustering of mutations within the SERCA2b protein among 75 individuals with similar neuropsychiatric phenotypes [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Combined analysis with previously reported cases/families in the literature (n = 384): Comparison of mutation type and location within the SERCA2b protein according to the absence or presence of a reported severe neuropsychiatric phenotype (bipolar disorder, schizophrenia or affective psychosis)

	Reported severe neuropsychiatric phenotype in index case and/or family member with DD		
	Yes (n = 22)	No (n = 362)	
Likely gene-disrupting (LGD) mutation			
Yes n (%)	15 (68)	145 (40.5)	χ^2 = 6.514, df = 1, p = .011 OR 3.15, 95% CI 1.25–7.91
No n (%)	7 (32)	213 (59.5)	
Mutation located in actuator "A" domain			
Yes n (%)	1 (4.5)	73 (20)	Fisher's, p = .093
No n (%)	21 (95.5)	289 (80)	
Mutation located at calcium binding site			
Yes n (%)	2 (9)	18 (5)	Fisher's, p = .320
No n (%)	20 (91)	344(95)	
Mutation located between stalk 4 and transmembrane helix 4 (S4–M4)			
Yes n (%)	3 (14)	10 (3)	**Fisher's, p = .032 OR 5.66, 95% CI 1.41–21.88**
No n (%)	19 (86)	352 (97)	
Mutation located between stalk 4 and transmembrane helix 4 (S4–M4). Protein altering mutations only	Yes (n = 7)	No (n = 213)	
Yes n (%)	3 (43)	8 (4)	**Fisher's, p = .003 OR 19.22, 95% CI 3.67–100.6**
No n (%)	4 (57)	205 (96)	

Note. DD = Darier disease; OR = odds ratio; CI = confidence interval. n's vary because of four mutations in the literature that could not be classified according to type because of unknown function.



	Neuropsychiatric features among index individual or family members with DD (number of individuals)	Reference/ Index ID in current study	Nucleotide and alteration	Amino acid change
①	Neuropsychiatric disorder and behaviour problems (n=2).	(Sato et al., 2004)	961G>T	L321F
②	Psychiatric hospital treatment for depression (n=1).	ID 21	958 G>C	A320P
③	Mild mental retardation and psychiatric abnormality (n=1).(Wang et al., 2006) None. (Ren et al., 2006)	(Ren et al., 2006; Wang et al., 2006)	953G>T	C318F
④	Schizophrenia and intellectual disability (n=1).	(Takeichi et al., 2016)	953G>A	C318Y
⑤	None.	(Onozuka, Sawamura, Yokota, & Shimizu, 2004; Sakuntabhai, Burge, et al., 1999)	952T>C	C318R
⑥	Major depressive disorder suicide attempt, investigations for a blackout (n=1). Treated by psychiatrist for depression (n=1).	ID 20	949 7bp del	
⑦	Psychiatric disorder NOS, suicide attempt, epilepsy (n=1).	ID19	948 del C	
⑧	Bipolar disorder (n=1). Treated by psychiatrist for depression (n=1).	(a)	935 C>G	P312R
⑨	(b)	ID 18	929 G>T	G310V
⑩	Bipolar disorder (n=1) Bipolar disorder not otherwise specified (n=1).	ID 17	925 G>A	E309K
⑪	Depression not otherwise specified (n=1).	ID 16	923 C>A	P308H

FIGURE 3 All known neuropsychiatric features in individuals/families with Darier disease causing mutations in the S4–M4 domain of SERCA2 including cases in current study. ER = endoplasmic reticulum, S = stalk domain, M = transmembrane domain, NOS = not otherwise specified. Mutations have been found to be unique to families, except for mutations marked [2] where mutations have been reported in two unrelated families. [a]Unpublished mutation identified in an individual with DD seen by our research group who was not included in the current study. [b]Parent of index was admitted to psychiatric hospital with "high mood" although neither parent was reported to have DD. All mutations are located in Exon 8

A recent study showing Darier keratinocytes display the hallmarks of constitutive ER stress with increased sensitivity to ER stressors lead the authors of the study to suggest DD should be classed as an ER stress related disease (Savignac, Simon, Edir, Guibbal, & Hovnanian, 2014). There is also evidence for the role of ER stress responses in neuropsychiatric disorders. Lymphoblastoid cell lines from individuals with bipolar disorder have showed an impaired response to ER stress (Hayashi et al., 2009; Pfaffenseller et al., 2014; So, Warsh, & Li, 2007).

Lithium is the main mood stabilizer used in the treatment of bipolar disorder and a recent study identified the response to ER stress as a lithium-regulated gene network (Breen et al., 2016). It is possible that the function of SERCA2b pumps may be more critical in the skin and the brain than in other tissues. Both tissues may have a particular susceptibility to a reduction in SERCA2b activity possibly relating to changes in ER Ca2+ concentration and ER functioning.

A limitation of the current analysis is that despite being the largest study to date, the small stratified group sizes in our genotype– phenotype analyses limits the power to detect significant relationships. Combining our sample with the existing literature enabled us to address this. However, previous studies of DD cases/families in the literature have not all recorded and/or reported neuropsychiatric features. Similarly, where neuropsychiatric features have been reported previously in the literature, in many cases only brief descriptions are provided. Ours and other studies have not included detailed assessments of the presence of neurological features such as hearing difficulties. Further studies systematically assessing neuropsychiatric features in individuals with DD are warranted including the administration of specific neurological tests such as audiograms.

We have found evidence to support the suggestion that mutations in ATP2A2 in addition to causing DD, confer susceptibility to neuropsychiatric features in individuals with DD. Given the complex nature of the disorders it is likely that the pleiotropic effects occur in association with other modifying factors. Our findings suggest that DD causing mutations as well as other genes encoding proteins in the same biological system as, and/or encoding proteins with a similar function to, SERCA2b would be good candidates for further investigations of potential involvement in predisposing individuals to developing severe neuropsychiatric illness.

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CONFLICT OF INTEREST
None.

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