Darier's disease (keratosis follicularis) A local survey, study of life impact, mutation analysis of the ATP2A2 gene and review

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1) Abstract

This is a study which consists of two parts. Part I is a multi-clinic, 20-year, retrospective study to review the clinico-pathological features, life impact, treatment and disease outcomes of patients with Darier's disease in the Social Hygiene Service, Hong Kong Special Administrative Region, China. Part II is a genetic study on the patterns of mutation of ATP2A2 gene in the aforesaid patients. Data were collected from 32 affected patients (15 males and 17 females with approximately equal sex ratio) and the mean age of onset was 15. Twenty patients had positive family history. The estimated incidence of DD in Hong Kong is 0.025 per 100,000 per year. The life impact of DD on our patients which was measured by the DLQI score was found to be low in our locality. The average DLQI score was 6.43 with the majority (27/28=96%) scoring less than 15. Most patients lived with the disease without major problems. The major symptom experienced was itching. Keratotic papules were invariably present in our patients and mixed pattern with seborrhoeic plus flexural involvement was the commonest phenotypic variation. All of them had acral signs; 38% had oral mucosal lesions. Lesions were most frequently found on face, neck, front chest and ears. There was no full remission. Focal acantholytic dyskeratosis with corps ronds and grains were the usual histopathological findings. Systemic retinoid was the most effective therapy for our patients although minor side effects were common. Mutation analysis in the ATP2A2 gene on chromosome 12 had been performed in 28 Chinese patients with DD. These patients had mild to severe skin symptoms. DNA extraction followed by polymerase chain reaction (PCR) amplification of the exons and flanking regions of the ATP2A2 gene were performed. Mutation detection strategies included heteroduplex scanning by denaturing high performance liquid chromatography (DHPLC) and nucleotide sequencing. We found four distinct mutations, all of which were novel. DHPLC in normal controls further verified the mutations. Three sisters within a family had a new T insertion at nucleotide 3022 in exon 20 resulting in a premature termination codon (PTC) at codon 1008. This mutation only affected SERCA2b. A 68-year-old gentleman with moderately severe DD had a novel T deletion at nucleotide 216 resulting in a PTC at codon 72. Another 22-year-old gentleman with mild disease had a new frameshift deletion mutation (2918-2920delCCT) in exon 20 resulting in deletion of serine at position 973. A further novel altered splice site mutation IVS10+1G>T resulting in exon skipping PTC was detected in a 35-year-old pregnant woman who had severe DD. Conclusions: Darier's disease is a rare dermatosis in HKSAR. Systemic retinoid is the most effective therapy and well tolerated in our patients despite minor side effects are common. Most DD patients can live with minimal life impact. Four pathogenic mutations in the ATP2A2 gene were identified in 19 Chinese pedigress. All of these mutations are private with nonsense type being the commonest. No mutation hotspots and clear-cut genotype-phenotype association could be identified.

Keywords: Darier's disease; Keratosis follicularis; ATP2A2 gene mutation; Hong Kong; Chinese

2) Abbreviations

ATP: Adenosine triphosphate
Ca^{2+}: Calcium
CDLQI: Children's Dermatology Life Quality Index
DD: Darier's disease
DHPLC: Denaturing high performance liquid chromotography
DLQI: Dermatology Life Quality Index
DNA: Deoxyribonuleic acid
Dp: Desmoplakin
Dsc: Desmocollin
Dsg: Desmoglein
ER: Endoplasmic reticulum
FAD: Focal acantholytic dyskeratosis
HK: Hong Kong
HKSAR: Hong Kong Special Administrative Region
IP_3: Inositol triphosphate
PCR: Polymerase chain reaction
Pg: Plakoglobin
PTC: Premature termination codon
SD: Standard deviation
SERCA: Sarco-endoplasmic reticulum Ca^{2+} ATPase
SHS: Social Hygiene Service, Departement of Health, HKSAR
UK: United Kingdom

3) Darier’s disease: A local survey and study of life impact

3.1) Preface

Darier's disease (DD; keratosis follicularis; OMIM 124200) is a rare cutaneous disease with an autosomal dominant mode of inheritance. Greasy papules and plaques arise on the seborrhoeic areas and in the flexures and almost all patients have nail abnormalities. Itch, disfigurement, secondary infection (bacterial, viral, fungal) and malodour are the most common disturbing symptoms. Acantholysis and dyskeratosis are the typical histological findings. Recent studies showed that the underlying defect is a result of mutations in the ATP2A2 gene on chromosome 12q23-24 [1-5] that encodes for a sarco/endoplasmic reticulum calcium ATPase pump (SERCA 2) expressed on human skin and mucosa.[6-8] Oral retinoid is the treatment of choice for severe disease but their adverse effects are troublesome.[9] Topical retinoids, topical corticosteroids, dermabrasion and laser surgery have their advocates but evidence on their efficacy is sparse.[9]

Epidemiological study on Darier's disease were carried out in different parts of the world including the United Kingdom,[10-12] Denmark,[13] Slovenia.[14] There is no data in Hong Kong on these aspects.

3.2) Materials and methods

A) Patient selection

Skin biopsy records were reviewed in the following 9 Social Hygiene Clinics in Hong Kong from 1983 to 31.3.2003. They include:-

1. Cheung Sha Wan Dermatology Clinic
2. Yaumatei Dermatology Clinic
3. Yung Fung Shee Dermatology and Social Hygiene Clinics
4. South Kwai Chung Social Hygiene Clinic
5. Chai Wan Social Hygiene Clinic
6. Lek Yuen Social Hygiene Clinic
7. Sai Ying Pun Dermatology Clinic
8. Tuen Mun social Hygiene Clinic
9. Wan Chai Social Hygiene Clinic

All the cases with the following keywords in the biopsy records or computer were noted:-

1. Darier's disease
2. Keratosis follicularis
3. Acantholytic dyskeratosis

Thirty-four biopsy reports bearing one or more of the above mentioned keywords were found. Subsequently, 32 corresponding clinical records could be retrieved. Two clinical records were either lost or cancelled because patients had defaulted follow-up.

Each of these 32 clinical records were reviewed thoroughly. Four cases were finally excluded from this study. These included three patients with Hailey-Hailey disease confirmed by subsequent skin biopsies and one patient with incompatible clinical features despite skin biopsy suggested DD.

B) Cases review

Each of the 28 recruited DD patients were invited via phone to attend an interview conducted by author and telephone interview was carried out if patient declined invitation. All the patients were informed that they were involved in a study and verbal consent from each of them had been obtained.

Formal permission from the ethical committee of the Department of Health (HKSAR) for the study was obtained. Information sheet concerning the aim, nature, social impact study with DLQI and blood check for mutation was given to each patient attending the interview and informed written consent was then obtained. Careful history taking and physical examination including fundal examination for any retinal anomalies were carried out by the author during the interview. Patients' clinical features were classified as mild, moderate and severe according to the criteria (Table 3.1) set up by Sakuntabhai et al. [15] Clinical photos were taken at the same time.

First degree relatives of index cases were invited to attend a physical assessment for any features of Darier's disease. Skin biopsy was performed for suspected lesions to confirm the diagnosis. Four more cases of DD (Patient 2, 26, 27, 31) were recruited in this way. So, there were 32 DD cases in our cohort altogether

Figure 3.1: 32 cases of Darier's disease in this study

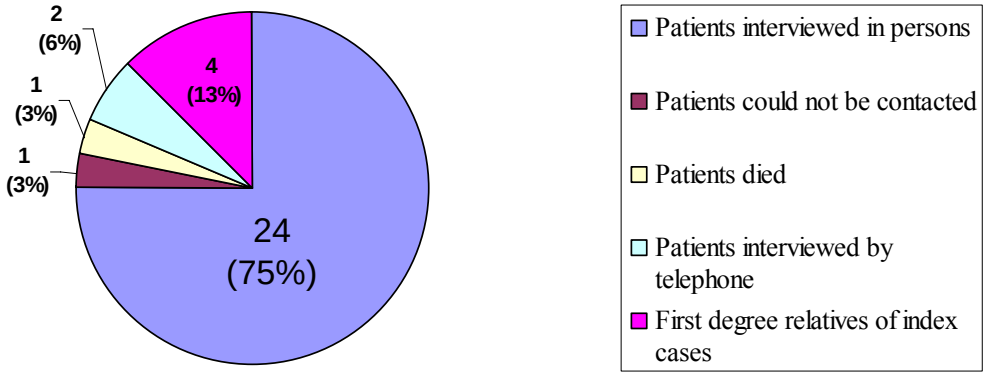


Table 3.1: Classification of clinical severity of Darier's disease

Severity	Description
Mild	Keratotoic papules scattered sparsely over the trunk or flexures or disease limited to one or two areas
Moderate	More extensive papular lesions or localized verrucous plaques
Severe	Coalescent verrucous plaques involving most of the trunk or grossly hypertrophic flexured disease

C) A study of life impact with DLQI & CDLQI

Cantonese version of DLQI and CDLQI were used to study the social impact of DD on our patients. Formal permission had been obtained from the copyright holder of DLQI and CDLQI – Professor A Y Finlay. Every DD patient was invited to complete the questionnaire well before the interview. Each questionnaire was then scored according to the instructions by Professor A Y Finlay and DLQI was calculated subsequently. Detailed analysis of DLQI under the six headings i.e. "Symptoms and feelings", "Daily activities", "Leisure", "Work and school", "Personal relationships", "Treatment" were also performed.

N.B.

1. The DLQI questionnaire is a simple practical measure used to study the impact of skin diseases and their treatment on patients' lives. It was developed by Professor A.Y. Finlay and G.K Khan, Department of Dermatology, University of Wales College of Medicine, Cardiff, UK and designed for use in adults, i.e. patients over the age of 16.
2. The DLQI is calculated by summing the score of each question resulting in a maximum of 30 and a minimum of 0. The higher the score, the more quality of life is impaired. The DLQI can also be expressed as a percentage of the maximum possible score of 30.
3. The CDLQI questionnaire was designed for use in children, i.e. patients from age 5 to age 16. It can be handed to the patient who is asked to fill it in within the help of the child's parent or guardian.
4. The CDLQI is calculated by summing the score of each question resulting in a maximum of 30 and a minimum of 0. The higher the score, the more quality of life is impaired. The CDLQI can also be expressed as a percentage of the maximum possible score of 30.
5. Formal validation of DLQI and CDLQI in English version had been performed in the United Kingdom.

3.3) Results

A) Basic demographic data

Thirty-two patients from 22 families were studied (Pedigrees shown in Appendix 9). The age range was 11-86 years (mean 41.84). There were 15 male and 17 female patients with approximately equal sex ratio (p=0.671, Fisher's exact test). Twenty patients (62%) had a positive family history of Darier's disease whilst the others were sporadic cases. The mode of inheritance was probably autosomal dorminant as there was no skip of generation and equal sex distribution. There was no statistical difference for the mean age of onset between male and female patients (p=0.884, Mann-Whitney U test). The disease began before 20 years in 25 patients (78%) and the peak age of onset was at 11-20 . All of the patients were Chinese. The distribution of age of onset of our patients is shown in Figure 3.2.

B) Clinical features

B1) Morphology and distribution of lesions (Table 3.2 & Figure 3.3-3.9)

The commonest morphology of lesions found in our patients were greasy hyperkeratotic papules followed by verrucous plaques. Face (78%), neck (75%), front chest (75%), axilla (66%), scalp (53%) and ears (53%) were the six commonest areas affected whilst buttocks (3%), perineum (3%) and genitalia (6%) were the least frequently involved. Oral mucosal involvement with cobblestone appearance over upper palate or buccal mucosa was found in 38% of our patients. The pattern of disease was recorded according to the distribution of lesions. It was found that 57% of them had a mixed pattern with seborrhoeic, flexural involvement. Pure seborrhoeic form and pure flexural form occurred in 28% and 9% cases respectively

B2) Site of onset (Table 3.3)

All but one of our patients could recall their site of onset. The disease most commonly started on the face (52%) which was followed by the neck (13%), ears (9%) and hands (9%).

B3) Clinical severity (Figure 3.10)

Thirteen percent (3 men and 1 woman) had mild disease, thirty-eight percent (5 men and 7 women) had moderate disease and forty-nine percent (7 men and 9 women) had severe disease. There was no statistical difference in the clinical severity between male and female patients in this study. (p=0.505, Mann-Whitney U test). The presence of family history also did not result in any statistically significant difference in clinical severity.

B4) Symptoms and aggravating factors

Seventy-five percent of the patients complained of itch while six percent experienced pain as the major irritating symptom. Twenty-eight percent were bordered by the malodour. Bacterial infection was fairly common in our patients (66%) necessitating topical or systemic antibiotics therapy. Fungal infection and herpes simplex infection were found in 19% and 9% respectively.

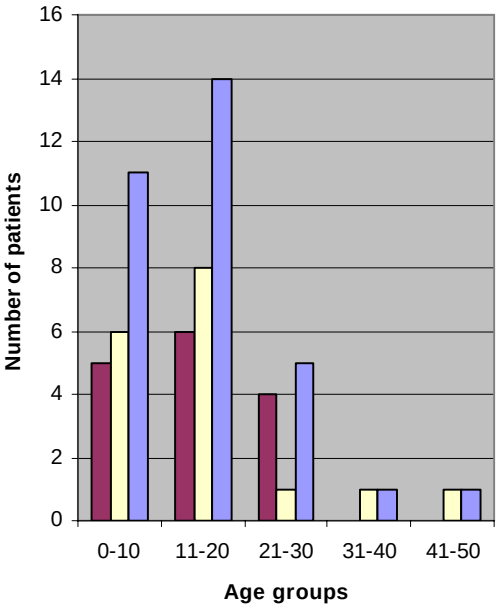
There were no associated disorders such as salivary gland obstruction, bone cysts, psychiatric illness found in our patients

Heat, sweating and sunlight were claimed to aggravate their disease in more than ninety percent of patients. Other exacerbating factors included stress (22%), menstruation (22% female patients), high humidity (16%) had also been mentioned. None of our patients could recall any episodes of drug induced exacerbation. Three female patients (Patient 2,12,28) claimed there was an overall improvement of their disease after menopause.

B5) Acral involvement (Table 3.4)

All the patients had hand involvements. Common findings included palmar pits (53%), V-shaped nicks (47%), longitudinal ridging (38%) and brittle nails (28%). Toenails involvement was found in 9% patients only. Only two patients (6%) had the pathognomic nail triad. i.e. red streaks, white streaks and V-shaped notch

Figure 3.2: Age of onset and sex distribution of patients



	Mean Age of onset	Median age of onset	Range	SD
Total	**14.75**	**13**	**0-42**	**9.98**
Male	**13.93**	**13**	**0-30**	**9.48**
Female	**15.47**	**13**	**1-42**	**10.65**

Table 3.2: Distribution of lesions

Area	No. of Patients (%)
Face	25 (78.13)
Front chest	24 (75.00)
Neck	24 (75.00)
Axilla	21 (65.63)
Ears	17 (53.13)
Scalp	17 (53.13)
Back	14 (43.75)
Groin	14 (43.75)
Feet	10 (31.25)
Hands	10 (31.25)
Abdomen	9 (28.13)
Forearms	7 (21.88)
Upper arms	7 (21.88)
Thigh	5 (15.63)
Fingers	4 (12.50)
Lower legs	4 (12.50)
Toes	3 (9.38)
Genitalia	2 (6.25)
Buttock	1 (3.13)
Perineum	1 (3.13)

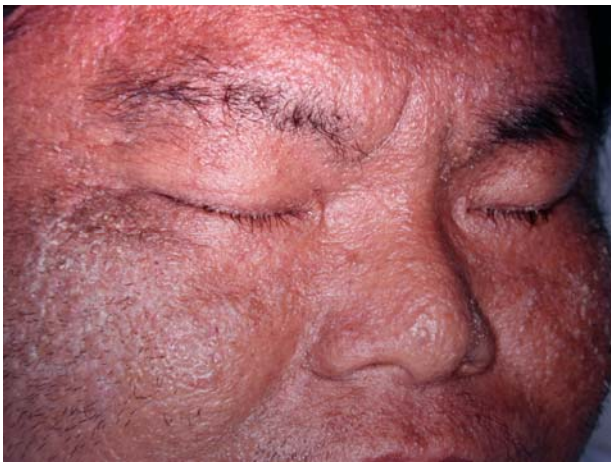


Figure 3.3
Hyperkeratotic papules coalesce to form extensive flesh-coloured plaques over face of DD patient (Patient 16)

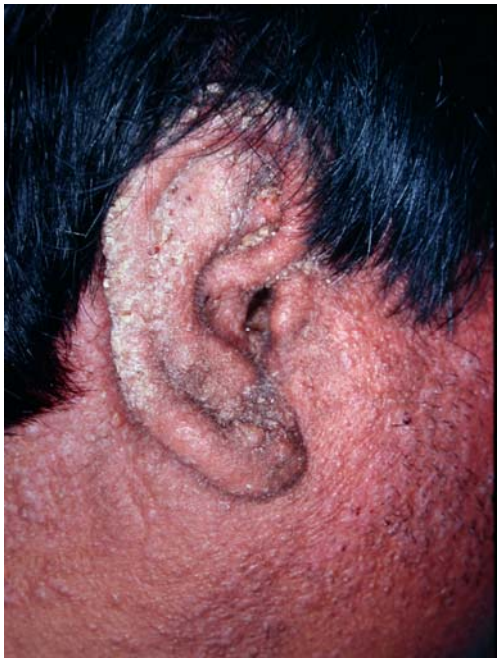


Figure 3.4
Numerous hyperkeratotic papules with overlying greasy scaling over ears and periauricular region (Patient16)

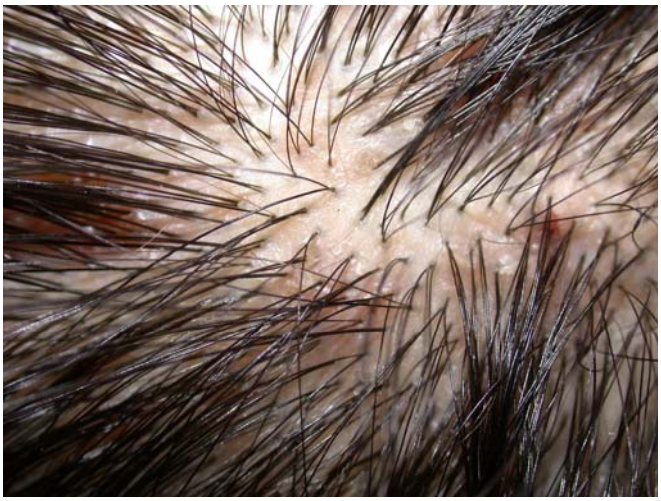


Figure 3.5
Greasy hyperkeratotic papules over the scalp (Patient 21)

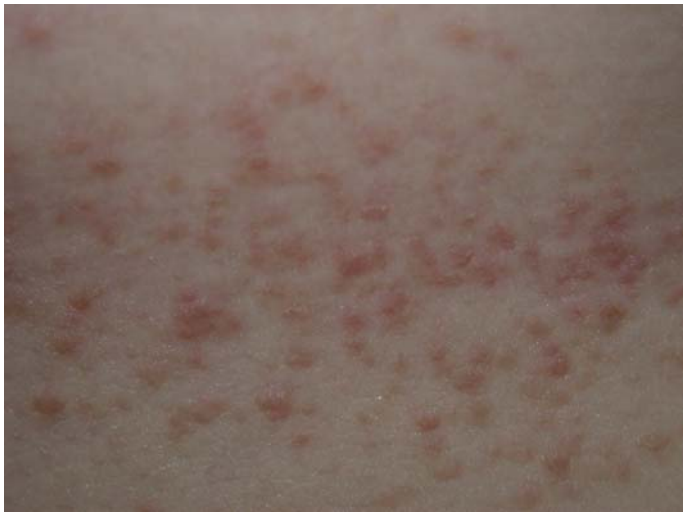


Figure 3.6
Erythematous and flesh-coloured hyperkeratotic papules over the neck
(Patient 21)

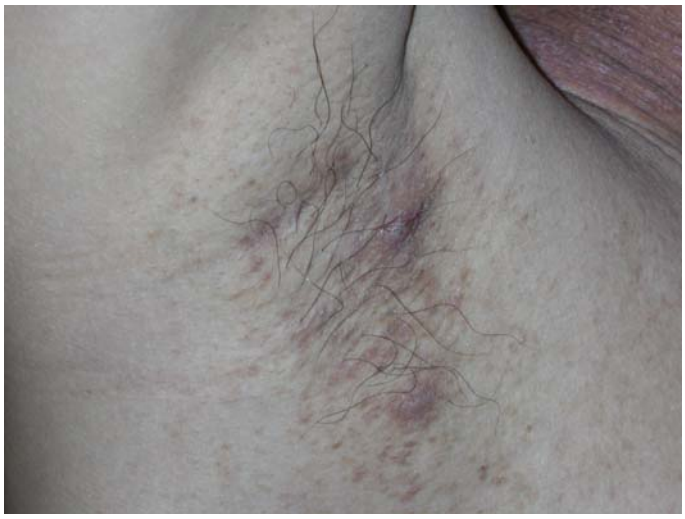


Figure 3.7
Greasy erythematous papules and plaques over right axilla
(Patient 19)

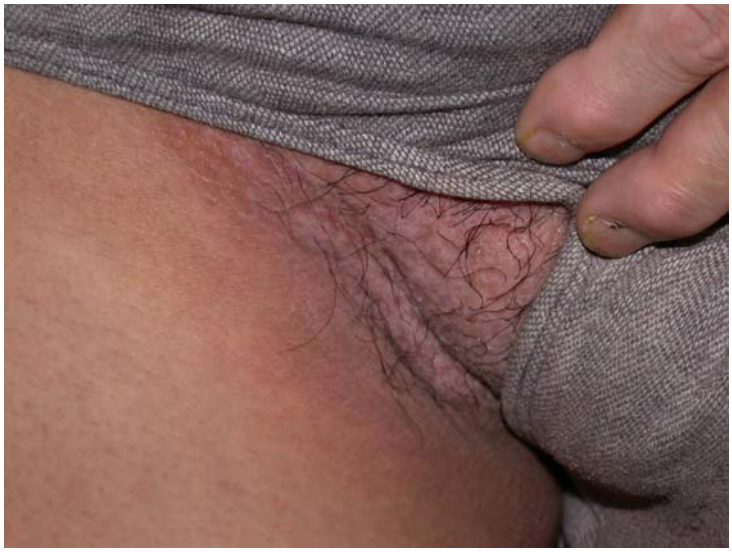


Figure 3.8
Greasy erythematous plaque over right groin
(Patient 16)

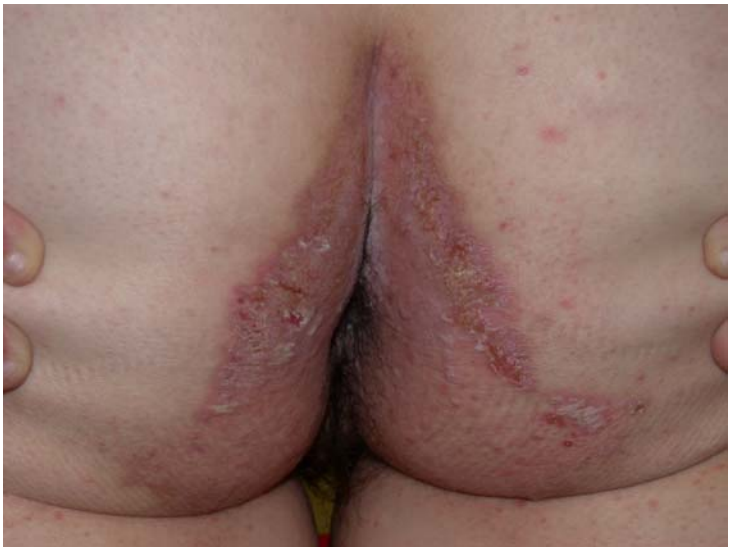


Figure 3.9
Greasy erythematous plaques with erosion at the edges over natal cleft
(Patient 21)

Table 3.3: Site of onset

Initial site of onset	No. of patients (%)
Face	16 (52)
Neck	4 (13)
Ears	3 (9)
Hands	3 (9)
Axilla	1 (3)
Back	1 (3)
Feet	1 (3)
Lower limbs	1 (3)
Upper limbs	1 (3)
Unknown	1 (3)

Figure 3.10: Clinical severity of DD cases

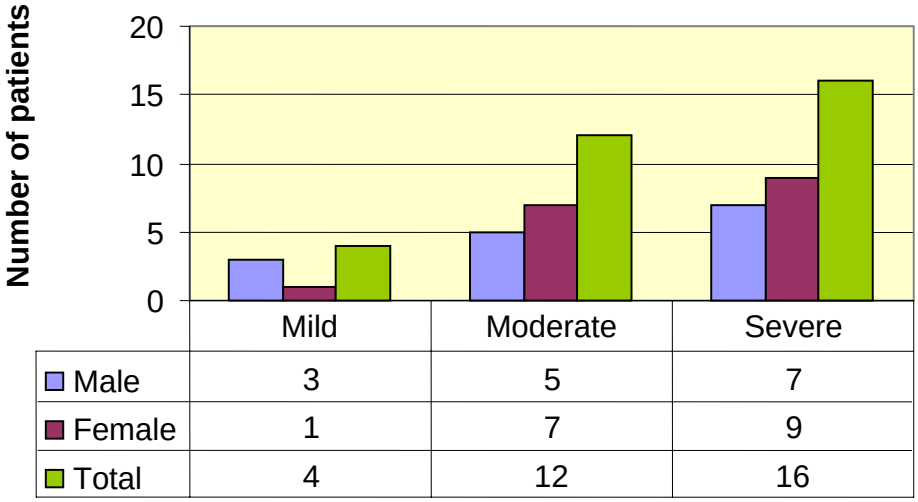
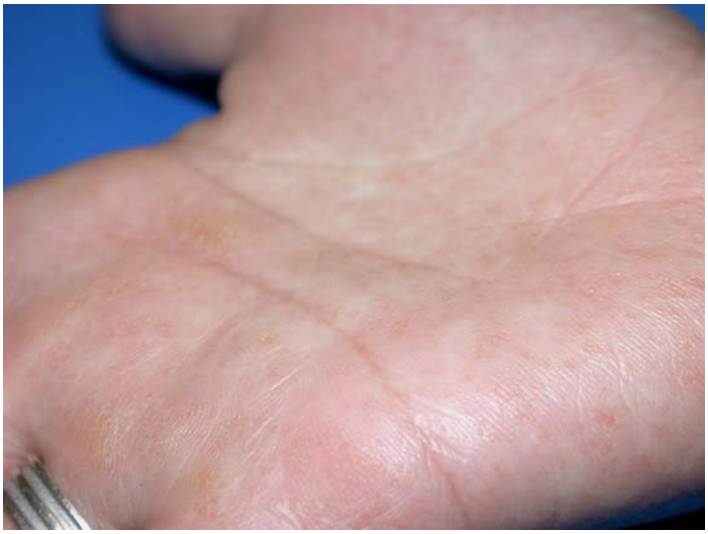


Table 3.4: Acral involvement

Acral involvement	No. of patients (%)
Palmar pits	17 (53.13)
V shaped nicks	15 (46.88)
Longitudinal ridging	12 (37.5)
Brittle and thin nails	9 (28.13)
Subungual hyperkeratosis	7 (21.88)
Warty papules	7 (21.88)
White streaks	5 (15.63)
Red streaks	3 (9.68)
Punctate keratosis	2 (6.25)

Figure 3.11
Palmar pits and punctate keratosis over right hand
(Patient 21)



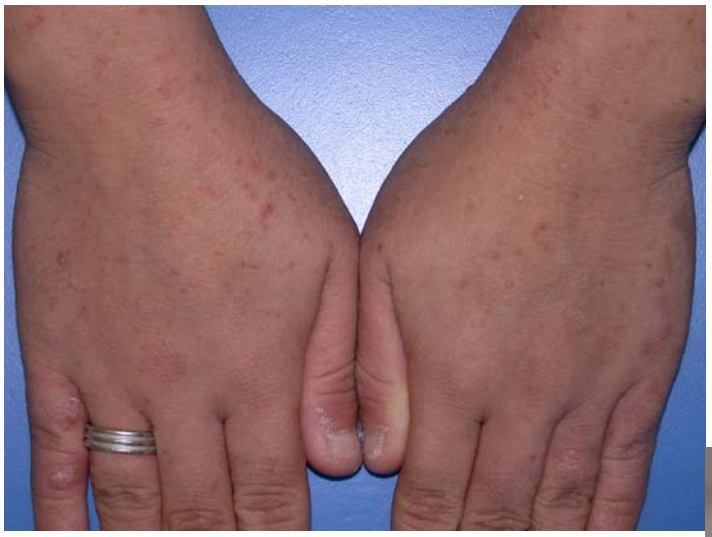


Figure 3.12
Punctate papules over dorsum of hands (Patient 21)

Figure 3.13
Brittle nails and V-shaped nicks of fingernails
(Patient 16)

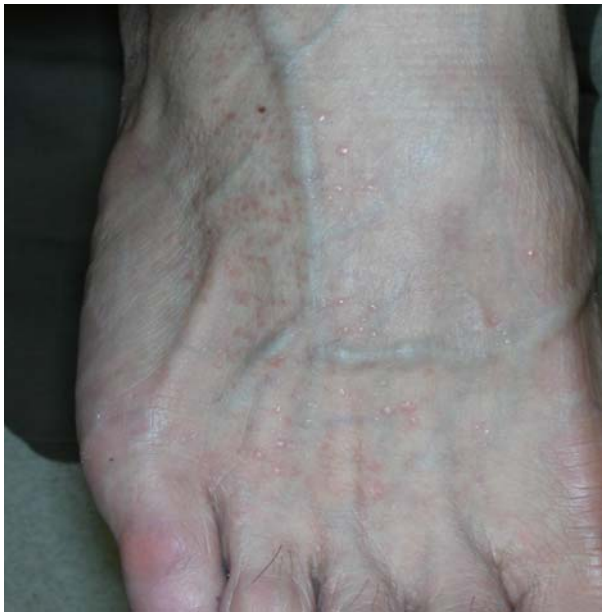


Figure 3.14
Erythematous papules over dorsum of right foot
(Patient 11)

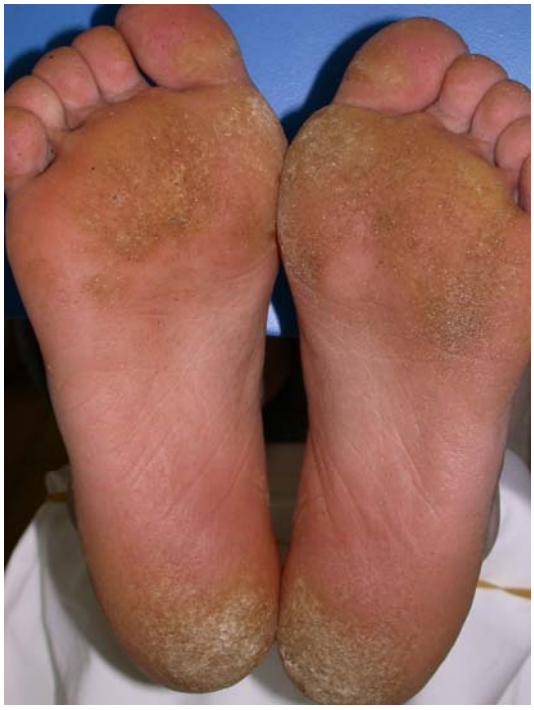


Figure 3.15
Punctate keratosis and pitting over soles
(Patient 21)

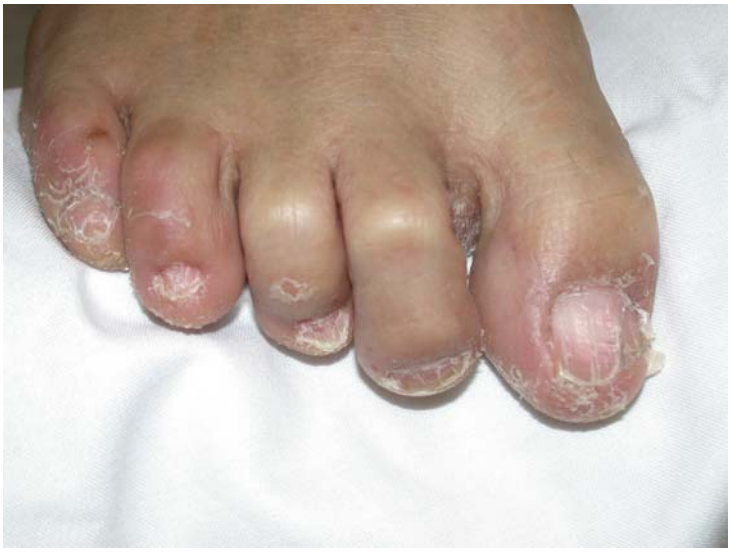


Figure 3.16
Nail ridging and splitting of toenails (Patient 18)

C) Histological features (Figure 3.17-3.19)

Histology slides of 28 DD patients were reviewed by the author and Dr. K.C. Lee, Institute of Pathology, Princess Margaret Hospital, Dr. W.Y. Lam, Institute of Pathology, Tuen Mun Hospital, New Territories, Dr. K.C. Lee, Institute of Pathology, Queen Elizabeth Hospital, Kowloon and Dr. K.C. Yau, Institute of Pathology, Department of Health (HKSAR).

A total of 28 skin biopsies were available for histological review by the author and dermatopathologists. Figure 20 is a summary of frequency of occurrence of different histological findings in these biopsies. Acantholysis, dyskeratosis, grains and suprabasal clefting were the four commonest features identified and they were all present in more than 90% cases. The feature of focal acantholytic dyskeratosis was present in 100% cases in our cohort whilst 72% cases have all the classical tetrad of DD. i.e. Focal acantholysis, dyskeratosis, corps ronds and grains

Table 3.5: Histological features of DD skin biopsies

Bx finding	No. of patients (%)
Acantholysis	29 (100)
Dyskeratosis	29 (100)
Suprabasal clefting	28 (97)
Grains	27 (93)
Hyperkeratosis	25 (86)
Acanthosis	23 (79)
Corps ronds	22 (76)
Papillomatosis	21 (72)
Parakeratosis	19 (66)
Perivascular infiltrate	13 (45)

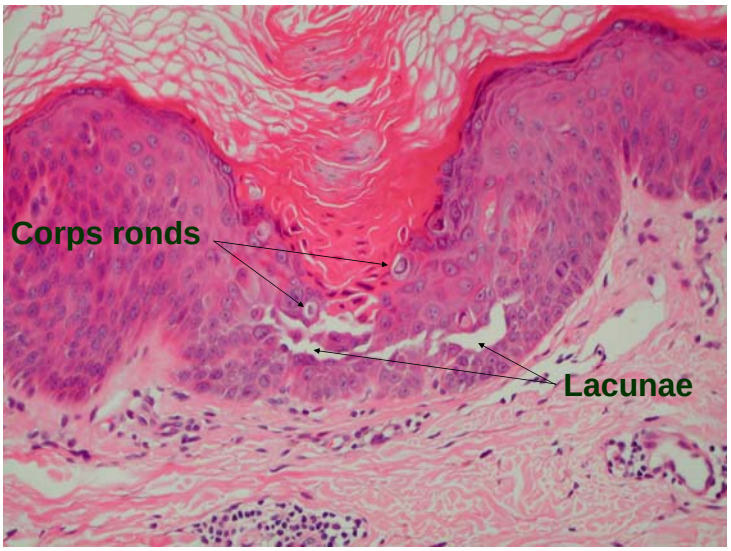


Figure 3.17
Characteristic case of DD with acantholytic dyskeratosis, conspicuous corps ronds and grains. There is also parakeratosis and mild degree of perivascular lymphocyte infiltrate.

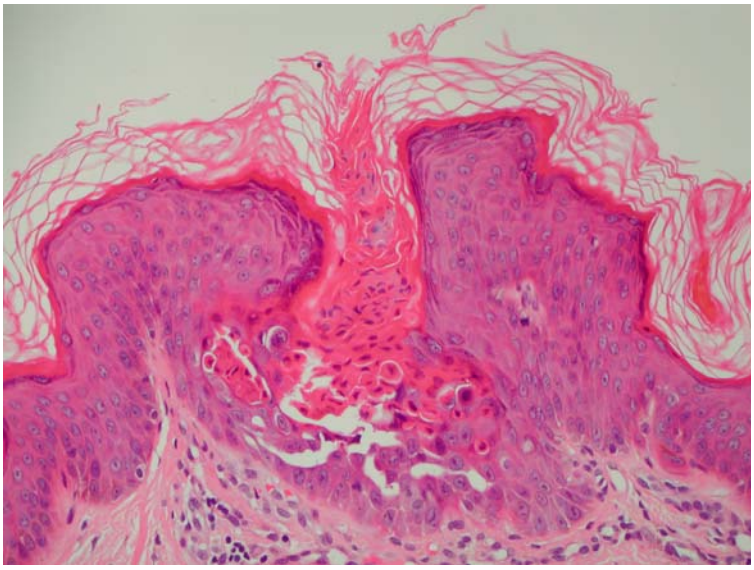


Figure 3.18
DD case with prominent suprabasal acantholysis. Marked dyskeratotic keratinocytes with orangophilic cytoplasm are seen with numerous corps ronds and grains. Hyperkeratosis and acanthosis are also noted

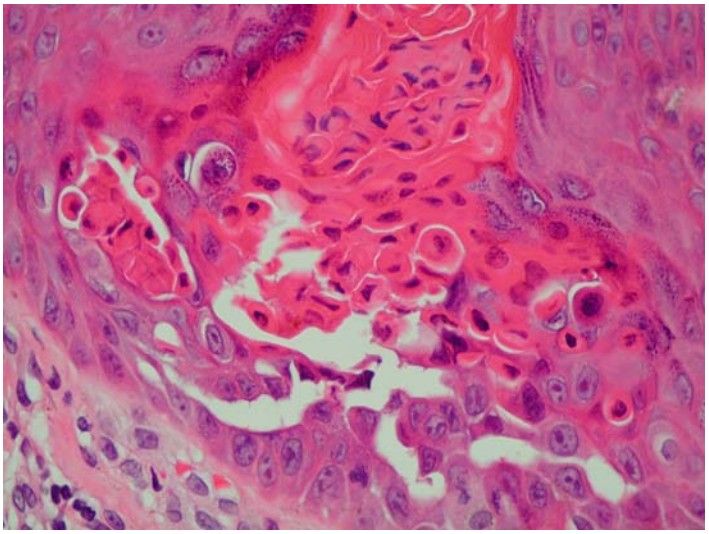


Figure 3.19
Close up view shows the acantholysis, corps ronds and grains in detail

D) Study of life impact with DLQI and CDLQI

D1) DLQI score (Table 3.6)

Twenty-seven out of 28 patients (96%) in our study had DLQI score less than 16. Fifty percent cases had DLQI score less than 6 and none of them had the score more than 20. These indicated that most of our patients did not consider DD casting major influence on their usual daily lives.

D2) DLQI subscore (Table 3.7-3.8)

For adults, detailed analysis of DLQI showed that the highest score related to the domain of symptoms and feelings whilst detailed analysis of CDLQI showed that the highest score related to the domain of treatment.

D3) Relationship of DLQI with clinical severity (Figure 3.20)

Gender, age groups and presence of family history did not confer statistically significant effect on the DLQI score of our DD patients. ($p=0.945$, 0.702, 0.427, Mann-Whitney U test). There was a trend of higher DLQI score for more severe clinical presentation. However, this finding was statistically insignificant ($p=0.354$, Kruskal-Wallis non-parametric one-way analysis of variance).

When subdivided into disease severity, only daily activities domain score and work and school domain score indicated a pattern of increase with disease severity. However, statistical analysis indicated that such increases were not significant ($P>0.05$, Kruskal-Wallis non-parametric one-way analysis of variance) for these two domains.The other domain scores did not follow a parallel trend with clinical severity of DD.

Table 3.6: DLQI score of DD patients

DLQI score	No. of patients (%)
0-5	14 (50)
6-10	7 (25)
11-15	6 (21)
16-20	1 (4)
>20	0 (0)

Table 3.7: DLQI statistical analysis

DLQI score and subscore (n=28)	Mean (±SD*)	Median** (lower, upper quartiles***)
Age	41.84±18.36	39 (30.25,52)
Symptoms and Feeling score	2.48 ± 1.69	2 (1,3)
Daily acitivities score	1.12 ± 1.24	1 (0,2)
Leisure score	1.0 ± 1.29	0 (0,2)
Work and School	0.88 ± 1.09	1 (0,1)
Personal relationships score	0.4 ± 0.76	0 (0,1)
Treatment score	0.92 ± 0.91	1 (0,1)
DLQI total score	6.8 ± 4.97	6 (3,11)

* SD: A parameter to measure the degree of dispersion of data about the mean

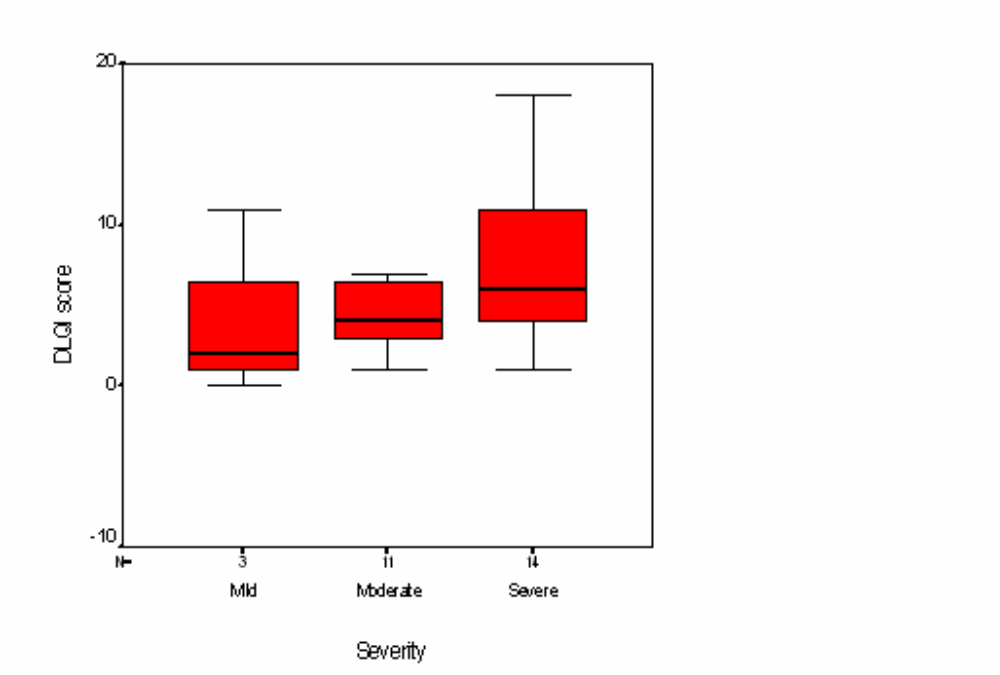
** Median: median is the middle of a distribution: half the scores are above the median and half are below the median. The median is less sensitive to extreme scores than the mean and this makes it a better measure than the mean for highly skewed distributions

*** Lower, upper quartiles: Quartile is another term referred to in percentile measure. The total of 100% is broken into four equal parts: 25%, 50%, 75%, 100%. Lower quartile is the 25th percentile and upper quartile is the 75th percentile

Table 3.8: CDLQI statistical analysis

CDLQI score and subscore (n=3)	Mean (±SD)	Median (lower, upper quartiles)
Symptoms and feelings score	1.33 ± 0.58	1 (1,2)
Leisure score	0.33 ± 0.58	0 (0,1)
School or holidays score	0 ± 0	0 (0,0)
Personal relationships score	0.33 ± 0.58	0 (0,1)
Sleep score	0.33 ± 0.58	0 (0,1)
Treatment score	1.0 ± 0	1 (1,1)
CDLQI total score	3.33 ± 1.53	3 (2,5)

Figure 3.20: Relationship of DLQI score and clinical severity



E) Treatment

E1) Topical treatment

Topical therapy which include emollient, steroid, keratolytics, antibiotics and antifungal were prescribed regularly during the course of the disease. Topical steroid had ever been used in 97% patients and was claimed by most patients to be effective in relieving pruritus and erythema associated with DD. In our study, emollient was used on a long term basis in 91% patients and most patients found it an useful measure to decrease "rough feeling" of their skin.

Topical tretinoin had also been tried in 50% patients but all of them stopped the therapy finally either because of its irritating effect on skin or because of unremarkable improvement after a long period of application.

E2) Systemic retinoid

Systemic retinoid – either acitretin or isotretinoin was used. It was prescribed in 14 (44%) out of 32 of our patients at the dose of 0.33-0.5mg/kg. All of these patients had improvement either in form of flattening of keratotic papules or reduction in skin pruritus and malodour. However, most lesions persisted and there was never a complete remission for all the patients.

Side effects of oral retinoid (Table 3.9) were experienced in a significant number of our patients. Xerosis (92%), hands and feet desquamation (92%), lip dryness and dermatitis were the four most common minor side effects reported. Hyperlipidaemia complicated the treatment of one patient (Patient 29) only and was managed by low fat diet and lipid lowering agent. None of these 14 cases developed liver dysfunction during the course of retinoid therapy. Despite of these undesirable effects, all our patients on systemic retinoid therapy preferred to continue the therapy.

F) Treatment outcome & course (Figure 3.21)

Among our DD cases, 14 (44%) were on topical therapy whilst 14 (44%) were on systemic therapy with oral retinoid. In contrast to many overseas studies, our DD patients on systemic therapy tend to continue such therapy despite its side effects as most of them found oral retinoid improved their skin condition in the sense of flattening of papules and plaques plus reduced erythema. The dose of retinoid used was between 0.33-0.5mg/kg/day. Initial effect was noted at 4-6weeks (mean: 4.71weeks) whilst maximal effect occurred by 8-12weeks (mean: 9.71weeks).

All the 32 DD patients claimed there had never been full remission of their skin disease and they all made the comments of " Wax and Wane and never go away completely" during interview.

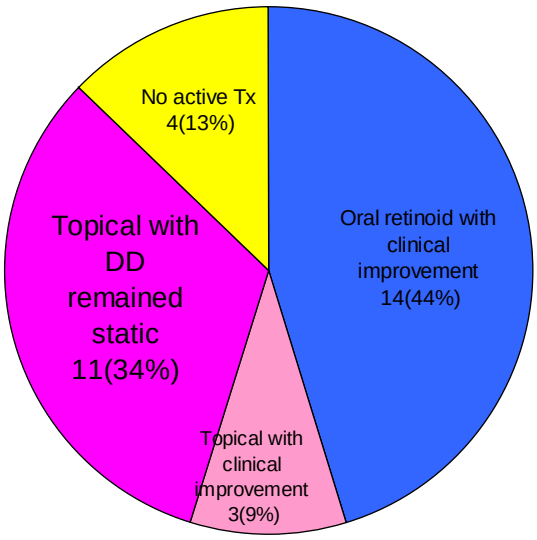
None of our DD cases (excluding the 4 cases who were not currently under our care) claimed their disease got worse with time. 15 patients found their skin disease

remained static on topical therapies with or without systemic retinoid therapy whilst 13 patients claimed there were clinical improvement on treatment (10 of them (77%) were on oral retinoid therapy).

Table 3.9: Adverse effects of oral retinoid therapy

Tx complication	No. of patients (%)
Palmar and sole scaling	92.31
Xerosis	92.31
Dermatitis and erythema	76.92
Lip dryness	76.92
Skin pruritus	61.54
Alopecia	53.58
Joint symptoms such as back pain and large joint pain	46.15
Myalgia	38.46
Paronychia	38.46
Conjunctivitis	23.08
Frequent headache	23.08
Cheilitis	15.38
Hyperlipidaemia	7.69
Epistaxis	0
Liver dysfunction	0

Figure 3.21: Treatment outcome



N=32

3.4) Discussion

Darier's disease (DD) or keratosis follicularis was first described by Darier and White in 1889.[16-17] DD is a rare dermatosis with autosomal dorminant inheritance with high penetrance. There were DD series which indicated 100% penetrance [12, 18-19] but cases with no family history are common and represented a third of cases reported in a large series. [10] Possible explanations for this finding may include incomplete penetrance, high rate of spontaneous mutation, gene may not always be fully expressed and non-paternity. [12] It rarely skips a generation but expressivity of DD is highly variable with relatives of affected patients may have very mild symptoms of DD which make them unaware of their skin disease. [12] Marked inter- and intrafamilial phenotypic variablility of DD illustrate the considerable diversity of ATP2A2 mutations causing DD and suggest that additional (environmental) factors are important contributors to the clinical phenotype. [20]

A) Epidemiology

The prevalence of DD is estimated to be 1 in 100,000 in Denmark,[13] 1 in 45,000 in Slovenia,[14] 1 in 55,000 in central England[11] and 1 in 36,000 in North East England.[12] The prevalence in HK was estimated to be 1 in 220,000 in the present study which was much lower than that in Europe. This can partially be explained by the fact that these European studies involved multiple dermatology centres within their state or country.

The numbers of newly diagnosed DD cases in SHS each year are shown in Table 3.10 and the deducted incidence in HK are shown in Figure 3.22 respectively.

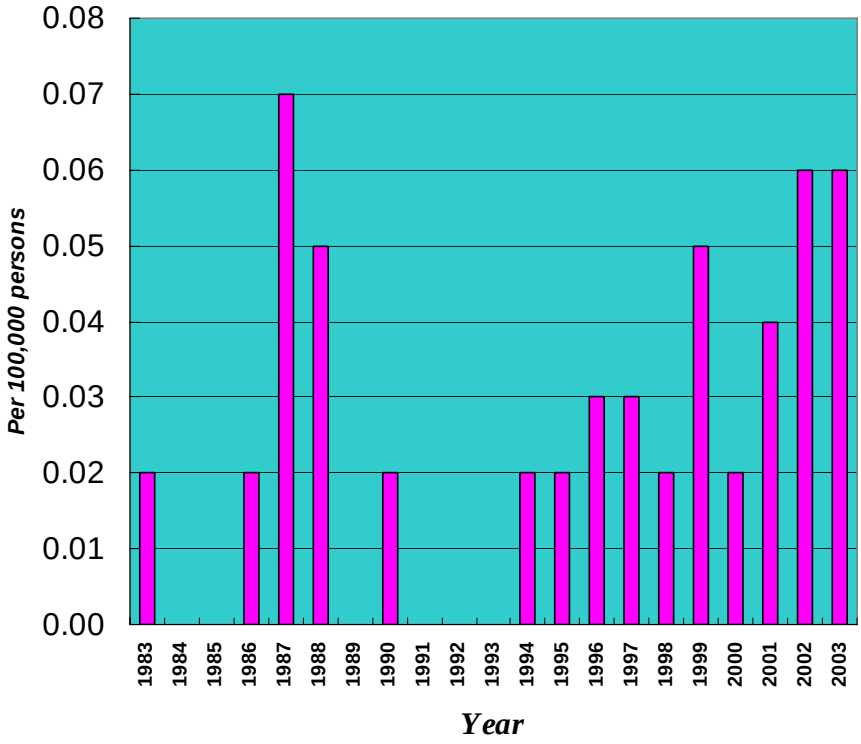
Except the study by Svendsen and Albrechtsen in Denmark which showed a male to female ratio of 1.6:1,[13] other large-scale epidemiologic studies indicated an equal sex distribution of DD.[10,12] In the present study, the male to female ratio was around 1:1.

The peak age of onset of DD was found to be in the second decade in three epidemiologic studies[10,12,14] whilst the Denmark series by Svendsen and Albrechtsen indicated an earlier onset in the first decade of life[13]. In our cohort, mean and median age of onset were 14.75 and 13 respectively with peak in the second decade.

Table 3.10: No. of new cases of DD per year in SHS

Year	DD new cases	SHS new cases	Per 10,000 new skin cases
1983	1	19017	0.53
1984	0	19386	0.00
1985	0	18530	0.00
1986	1	17946	0.56
1987	4	16412	2.44
1988	3	12680	2.37
1989	0	13726	0.00
1990	1	13089	0.76
1991	0	12570	0.00
1992	0	13310	0.00
1993	0	12756	0.00
1994	1	14640	0.68
1995	1	14569	0.69
1996	2	15520	1.29
1997	2	14977	1.34
1998	1	15235	0.66
1999	3	20536	1.46
2000	1	19167	0.52
2001	3	17373	1.73
2002	4	27178	1.47
2003	4	27937	1.43

Figure 3.22: Incidence of DD in HK



B) Aetiology and pathogenesis (Figure 3.23)

The ATP2A2 gene encodes for a sarco/endoplasmic reticulum calcium adenosine triphosphate pump—SERCA 2.[4,6] SERCA 1,2,3 are all P-type cation pumps which couple ATP hydrolysis with cation transport across cell membranes. They are widely expressed and highly conserved across all species. [21]

SERCA2 are Calcium ion (Ca^{2+}) pumps involved in intracellular Ca^{2+} signaling. When ligands bind to plasma membrane receptors (G protein-coupled or tyrosin kinase receptors) Inositol triphosphate (IP_3) acts as a second messenger, triggering Ca^{2+} release form the endoplasmic reticulum (ER) into the cytoplasm, increasing cytosolic Ca^{2+}. SERCA2 counterbalance this by actively transporting Ca^{2+} back into the ER lumen, restoring low cytosolic Ca^{2+}. (Figure 3.24) Repeated cycles of Ca^{2+} release and reuptake generate complex fluctuations in intracellular Ca^{2+}, which influence gene expression and cellular responses. [6] The predicted secondary structure of SERCA2 consists of 10 transmembranous domains and three globular cytoplasmic domains separated by a stalk sector. The cytoplasmic domains contain a beta-strand, a phosphorylation domain and an ATP-binding domain. The ATP-binding domain is linked to the stalk sector 5 by the hinge region. Four of the transmembrane domains (M4, M5, M6, M8) contain Ca^{2+} binding sites. [15] (Figure 3.25) SERCA 2 is expressed in many tissues and has two isoforms: SERCA2a and SERCA2b. SERCA2b isoform has a higher Ca^{2+} affinity but lower turnover than SERCA2a. [25] This can be explained by the presence of an extra transmembrane domain (M11) containing a hydrophobic sequence in SERCA2b. [21] SERCA 2a isoform is mainly found in cardiac muscle and slow twitch skeletal muscle whereas SERCA 2b isoform is predominant in keratinocytes especially in epidermis including pilosebaceous units and sweat glands.[22,23] Tavadia et al studied the cardiac and platelet function in patients with DD and concluded that only skin is sensitive to defects in SERCA2 function to which other systems remained robust.[23]

Desmosomes are the cell-cell adhesion junctions in the epidermis. They include desmosomal cadherins such as desmoglein (Dsg) and desmocollins (Dsc), plaque proteins desmoplakins (Dp) and plakoglobin (Pg) and plaque associated proteins such as plakophilin 1. Recent work suggests Dp, Pg and plakophilin 1 link cytoplasmic domains of desmosomal cadherins to the keratin intermediate filaments network. The assembly of desmosomes in epithelial cells in vitro is initiated through an increase in the extracellular Ca^{2+} concentration. [4] This process begins with the trafficking of Dsc to the cell surface, followed by Dsg and Pg with the final stages involving Dp. [24] In DD, Burge et al found there was decreased desmoplakin 1 and 2 around the basaloid cells in the buds at the base of the lesion with Dp and Dsg distributed in a ring around the nucleus or diffusely in the cytoplasm of DD acantholytic cells. These findings indicated internalization of half desmosomes associated with loss of intercellular adhesion. [25-26]

The relationship between SERCA and desomomal protein assembly was supported by the finding that inhibition of SERCA by thapsigargin in normal human keratinocytes

impairs the trafficking of the Dp, Dg and Dsc to the cell surface. However, only the trafficking of Dp is significantly inhibited whilst transmembrane proteins Dsg and Dsc and Pg are efficiently transported to the cell surface in DD.[24] This may be explained by the fact that thapsigargin inhibited SERCA 1,2,3 whilst DD patients have anomaly in SERCA2b only.

The possible role of decreased expression of Bcl-2, Bax and Bcl-x (antiapoptotic proteins) in the pathogenesis of DD was advocated by Bongiorno and Arico .[27] In their study, reduced immunoreactivity for Bcl-2, Bax and Bcl-x were found in epidermal keratinocytes in DD lesions. They proposed such decrease in antiapoptotic proteins increases the proapoptotic pathway in keratinocytes of the lesional epidermis. Hakuno et al found that there was P-cadherin expression not only in basal cells but also in the suprabasal cells in lesional skin of acantholytic disorders such as DD, Hailey-Hailey disease, pemphigus vulgaris and foliaceus. [28] Proposed explanations for this finding include a high proliferation activity in DD lesions, suprabasal cells in diseased skin have delayed differentiation, and an alteration in Ca^{2+} in the lesional skin.

In a recent study by Ahn et al, mutations in DD gene was found to affect protein expression, degradation and activity of SERCA2b pump. [8] The resultant mutant SERCA either have reduced expression or enhanced cellular proteasome-mediated degradation or reduced Ca^{2+} transport activity. [21] There was evidence that several DD-associated mutants inhibit the activity of the native and the expressed wild type pumps by the formation of dimeric complexes.[8,21] These changes will cause an alteration of cytosolic Ca^{2+} oscillations and thus trigger a cascade of events involving the phosphorylation of target proteins, the regulation of gene transcription and most importantly, the transport of desmosomal proteins to the plasma membrane. Impaired desmosome assembly and altered anchorage of cytokeratin filaments to the desmosomal plaque result. [4] Desmosomal breakage will occur which give rise to loss of cohesion between keratinocytes and acantholysis.[25-26]

Figure 3.23: Pathomechanism of Darier's disease

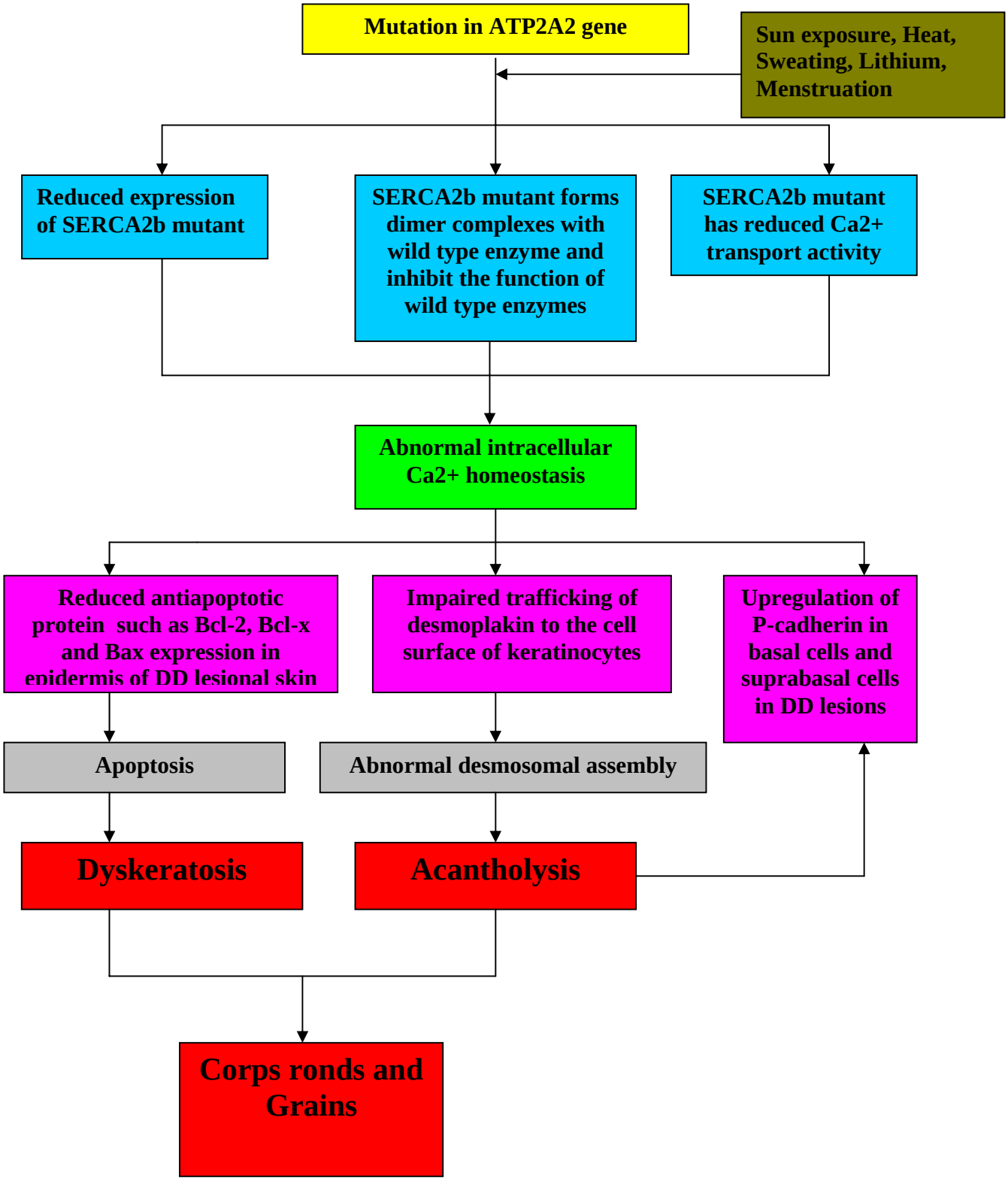


Figure 3.24: Function of SERCA

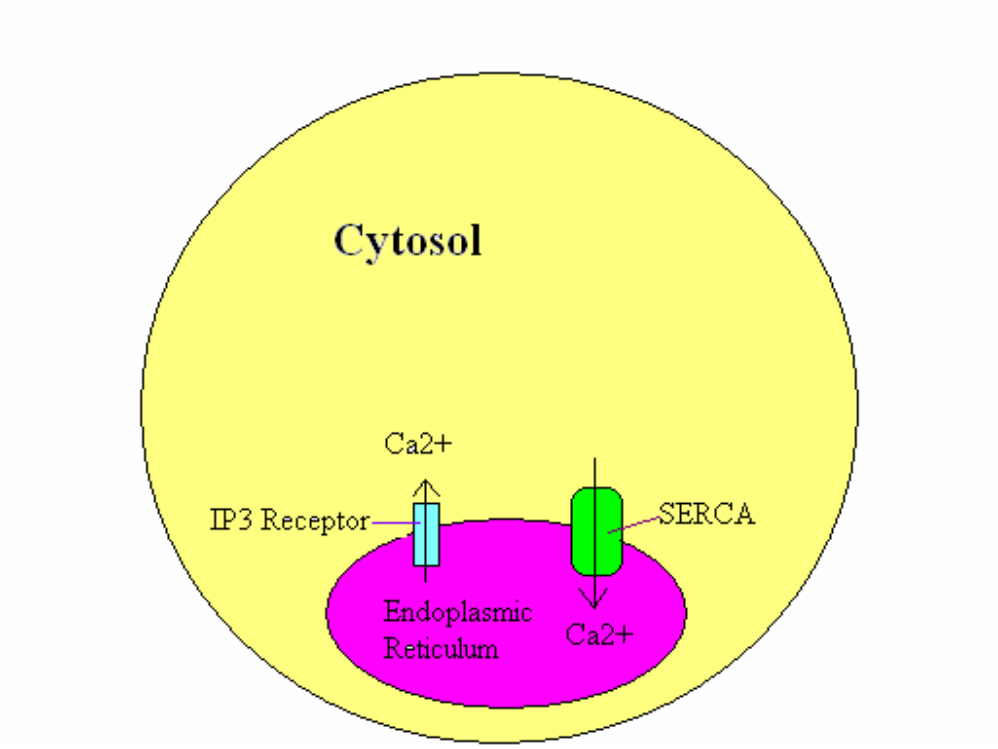
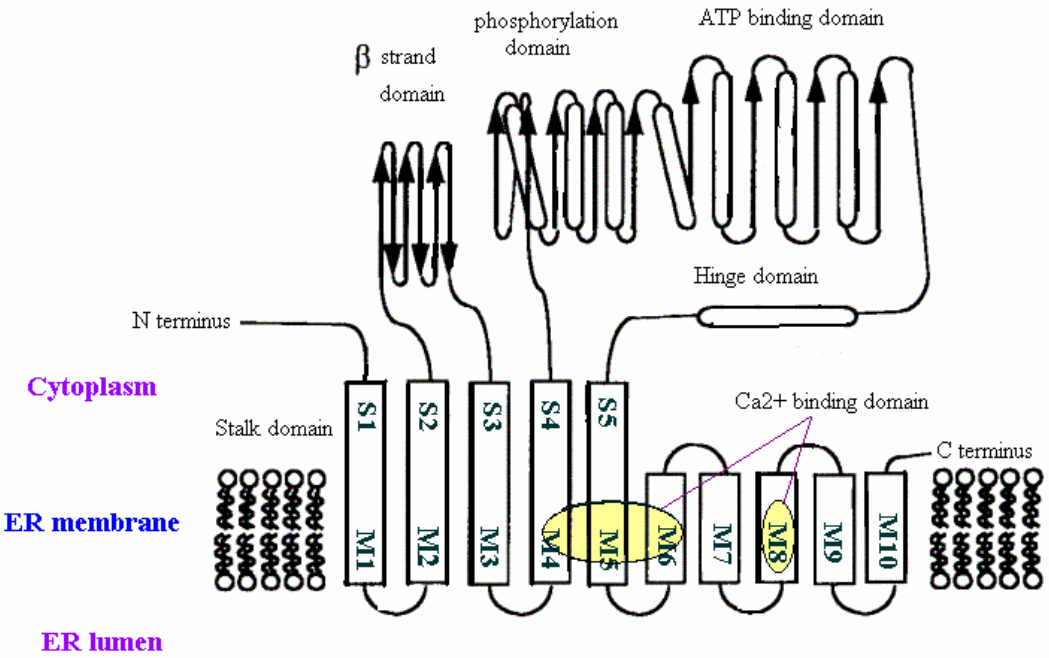


Figure 3.25: Structure of SERCA



C) Clinical aspects of Darier's disease

DD usually present with multiple greasy, hyperkeratotic, firm skin coloured to brown papules over seborrhoeic areas, flexures, behind the ears, neck and acral areas. But lesions may occur on extensor aspects of limbs. These papules may coalesce into plaques. DD can be classified in the seborrhoeic, flexural, acral or mixed pattern according to the major areas affected.[10] In contrast to the study by Burge and Wilkinson which denoted a predominance of seborrhoeic pattern of DD (92%) in England, the present study showed that mixed pattern (seborrhoeic plus flexural) was more common (57%) in Hong Kong.

In the present study, itch was the most frequent complaint (75%) followed by body odour and pain. Hands involvement may take the form of palmar pits, punctate papules or plaques over dorsum of hands whilst nail involvement include white or red lines, longitudinal ridges, V-shaped nicks and subungual hyperkeratosis. The combination of a red and white sandwich of streaks associated with a V-shaped notch is the pathognomonic nail sign. Acral haemorrhagic macules occur less frequently but was found to be associated with missense mutations.[21]

Oral involvement usually appear as fine granular or coarse pebblestone lesions over the palate and less commonly, the tongue and buccal mucosa.[29] Most of these lesions are asymptomatic and require no treatment. Parotid gland swelling due to metaplasia of parotid ductal lining with secondary obstruction had been reported.[29-30] In the present study, 38% patients had oral involvement. This proportion is lower than that reported by Svendsen et al[13] and Macleod et al[24] which were both around 50%.

The most common site of onset was the face (56%) in the present study while chest, shoulders and back were the most common in UK population.[10]

Malodour, secondary bacterial and fungal infection can cause significant disturbance to the usual daily living of DD patients and lead to social embarrassment.[9] In the present study, bacterial infection was the most common complication encountered by our DD cases (66%) which was followed by malodour (28%), fungal infection (19%) and herpes simplex infection (9%).

Various forms of DD are recognized including vesicobullous,[31] cornifying,[32] acral,[33-34] comedonal,[35] linear,[36-38] haemorrhagic[39] and hypopigmented or depigmented variants.[40-43] Linear form of DD is localized disease following Blaschko's lines. It represents genetic mosaicism resulting from a post-zygotic mutation in the DD gene.[38] None of the these DD variants were present in the current study.

There is no significant association between DD and other medical disease. However, there seems to be an excess of neuropsychiatric disorders such as mental retardation,[10] schizophrenia,[43] bipolar affective disorder[44-46] and epilepsy in a number of families with DD. In spite of repeated attempts to identify the corresponding mutation pattern which predispose to such increase in neuropsychiatric disorders, no conclusive finding had been

obtained yet. There was also report suggesting possible association between DD and retinitis pigmentosa.[47] In the present study, no association between DD and the above mentioned disorders was found.

Reported exacerbating factors for DD include heat and sweating,[10] sunlight,[48] emotional stress, menstruation,[10] pregnancy, drugs such as lithium.[49] More than 90% of our DD cases considered heat, sunlight and sweating as the major exacerbating factors for their skin condition. Some female patients also complain of premenstrual flare.

Differential diagnoses of DD include eczema, Hailey-Hailey disease, Grover's disease for seborrhoeic or flexural disease, acantholytic epidermal nevus for linear or segmental lesions,[50] acrokeratosis verruciformis of Hopf [51] and plane wart for acral lesions.

D) Histological features

The histological findings reviewed in this series conformed to classic description of DD in many literature. The key features in diagnosing Darier's disease were focal acantholytic dyskeratosis (FAD) with corps ronds and grains plus the absence of other histological features such as spongiosis, extensive blisters formation, and positive immunofluorescence study. Both corps ronds and grains are abnormal keratinocytes. Corps ronds are large keratinocytes with brightly eosinophilic cytoplasm and a large nucleus surrounded by a clear halo usually located in stratum spinosum and granulosum while grains are oval cells with elongated cigar-shaped nuclei and abundant keratohyaline granules usually located in stratum corneum. However, FAD is not specific for Darier's disease. FAD can also be found in Grover's disease, warty dyskeratoma, epidermal naevi and even malignant melanoma.

E) Life impact

Dermatology Life Quality Index (DLQI) and Children's Dermatology Life Quality Index (CDLQI) were simple, compact uniform assessment tools for assessment of the psychosocial impact of chronic skin disease on patients.[52-53] In this study, we used DLQI questionnaire (Validated Cantonese version) to determine the level of psychosocial impairment in DD patients.

DLQI survey in this study revealed that more than 95% of DD cases had DLQI score less than 16 and none of them had score higher than 20. This indicated that most DD

cases adjusted well to their own skin disease. This finding was consistent with the work m by Harris et al in 1996.[54]

F) Treatment (Table 3.10)

The management of DD can be divided into general advice, genetic counseling, topical remedies, systemic retinoid and surgical treatment. The main aims are symptomatic relief and treatment of complications.[50]

F1) General advice

Cotton clothing, sunblock and sunprotection for those with history of photoaggravation, avoidance of sweaty exercise, use of emollients and soap substitutes are common advice given to DD patients.

F2) Topical therapy

Mild or moderately potent topical steroid sometimes controls inflammation, but generally the impact of such therapy is disappointing.[55] Bacterial colonization can be reduced with antiseptics or topical steroid combined with an antibiotics.[55]

Topical retinoid such as isotretinoin gel, tretinoin cream and tazarotene gel are shown in small studies and case reports to be effective in reducing hyperkeratosis in DD[56-59] but skin irritation is the main drawback. This may, however, be overcome by alternative day regimen or in combination with topical steroid.[55] In the present study, 50% DD patients were ever prescribed topical retinoid but all of them stopped such therapy finally because of its irritation and poor efficacy.

Topical 1% 5-Fluorouracil was reported to be useful in two patients with refractory DD.[60] Complete clearance was achieved within 3 weeks and remission lasted for 2-6 months. However, both of these 2 patients were also taking oral retinoids. Topical calcipotriol has been proved to be ineffective in DD.[61] None of the 32 DD cases in this study were ever prescribed topical 5-Fluorouracil or topical calcipotriol and thus, no data on their efficacy in our population was available.

F3) Systemic therapy

Oral retinoid therapy is the single most effective therapy in DD.[9] At the dose of 0.33-0.5mg/kg. It can reduce hyperkeratosis and thus induce flattening of the papules. Most patients on retinoid therapy will experience improvement in disease severity and extent after 6-8weeks. Acitretin,[62-63] isotretinoin and etretinate[63] are all effective at the dose of 0.5-1mg/kg/day but isotretinoin is recommended for young females because pregnancy

need only be avoided for 1-2months after ceasation of therapy. Dose related side effects such as cheilitis, alopecia, pruritus, xerosis, dermatitis, paronychia, mucosal dryness, epistaxis, conjunctivitis are common. Informed consent should be obtained and adequate patient education on such adverse reactions plus emphasis on the use of emollient and fluid intake should be given prior to start of therapy. In a review of 163 DD cases by Burge and Wilkinson, 91% of those prescribed oral retinoid had clinical improvement although 23% of them finally stopped the therapy because of adverse effects.[10] In the present study, 14 DD patients received systemic retinoid therapy at the dose of 0.33-0.5mg/kg/day. Clinical improvement was noted at 4-6weeks (mean: 4.71weeks) and the maximal effect occurred at 6-12weeks (mean: 9.71weeks). Only one patient (patient 29) developed hyperlipidaemia after initiation of retinoid therapy and was managed by low fat diet and lipid lowering agent. On the other hand, most of them experienced minor side effects such as xerosis, palm and sole scaling, erythema and skin pruritus. But they preferred to continue retinoid therapy despite of these adverse effects as they all considered retinoid effective in reducing the severity of DD.

F4) Treatment of infection

Systemic antibiotics were needed in case of superimposed bacterial infection. Herpes simplex virus can cause painful exacerbations in DD and should be treated aggressively with systemic acyclovir.

F5) Treatment of "eczematized" DD

Systemic steroid[64] and cyclosporine[65] were reported to be effective in reducing the inflammation in "eczematous" DD but papules and erosions persist.

F6) Surgical management

The role of surgical approaches in the DD had not been supported by any controlled trials or large scale studies. Electrosurgery was reported to be useful in two patients with DD refractory to etretinate therapy.[66] Surgical excision with skin grafting was advocated for hypertrophic DD.[67] Dermabrasion up to the whole thickness of papillary dermis had been reported to be beneficial in five patients with severe DD.[68-69] Laser therapy such as carbon dioxide laser[70] and Ebrium Nd:YAG laser[71] were found to be effective in isolated cases.

F7) Photodynamic light therapy

Photodynamic therapy using topical 5-aminolaevulinic acid as a photosensitizer was commented as a potential adjuvant therapy to oral retinoid in one small uncontrolled study. [72]

Table 3.10: Treatment modalities for DD

General advice:	Evidence level*
•Sunscreen and sun-avoidance	
•Avoidance of excessive sweating	
•Avoidance of lithium	
•Cotton clothing	IV
Topical therapy:	
• Steroid	
• Retinoid	III
Systemic therapy:	
• Acitretin	IIb
• Isotretinoin	IIb
• Tretinoin	IIb
Surgical management:	
• Electrosurgical excision	IV
• Dermabrasion	IV
• Surgical excision	IV
• Laser ablation	IV
Genetic counseling	

*Evidence level: [73]
Ia Evidence obtained from meta-analysis of RCTs
Ib Evidence obtained from at least one RCT
IIa Evidence obtained from at least one well designed controlled study without randomisation
IIb Evidence obtained from at least one other type of well-designed quasi-experimental study
III Evidence obtained from well-designed non-experimental descriptive studies, e.g. case series, cross sectional studies.
IV Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities.

G) Prognosis

In the present study, symptoms and signs wax and wane in our patients and none of them had full remission. This is consistent with findings in the European series.[10,12-14] However, most DD patients lead a normal life with minimal psychological distress in spite of the unsightly skin lesions, malodour of flexural lesions and repeated bacterial and fungal infections.[9-10]

3.5) Reference

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4) Mutational analysis of ATP2A2 gene

4.1) Preface

The underlying gene defect of DD has been mapped to chromosome 12q23-24.1.[1,2,3] This gene—ATP2A2 spans 21 exons that range between 15 and 466 base pairs and has 2 splice variants: ATP2A2a and ATP2A2b. It encode for a sarco/endoplasmic reticulum calcium adenosine triphosphate pump—SERCA 2.[4,6] Mutation analysis of the ATP2A2 gene on chromosome 12 of patients with Darier's disease were carried out in different parts of the world including the United Kingdom,[5,7-11] Taiwan,[12] China[13] and Japan.[14] This is the first set of data on mutational analysis of the ATP2A2 gene in Hong Kong Chinese patients..

4.2) Methods

All probands and affected family members were invited to participate in a genetic study which detected the presence of mutation of ATP2A2 gene on chromosome 12 and studied the pattern of such mutation if present. Informed written consent were obtained from all participating subjects before five ml blood samples were collected. Blood samples were sent to Department of Chemical Pathology of the Prince of Wales Hospital for mutation analysis.

A) DNA extraction

Genomic DNA of subjects was extracted from whole blood samples by a QIAamp blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction.

B) Polymerase chain reaction

The exons and flanking regions of the ATP2A2 gene were amplified by polymerase chain reaction (PCR) with specific primers. (Table 4.2.2.1)

C) Heteroduplex and denaturing high performance liquid chromatography

Heteroduplexes are formed by mixing wild-type and mutant DNA amplified by PCR. The samples are denatured and 're-annealed' (usually by heating and cooling). Four distinct species are generated by this reassortment: wild-type homoduplex, mutant homoduplex, and two heteroduplexes. Heteroduplexes can also be formed during standard PCR if the DNA has two different alleles.

Heteroduplex analysis of PCR-amplified DNA was performed on WAVE denaturing high performance liquid chromatography instrument (Transgenomic Inc., San Jose, California, USA) to scan for any mutation on ATP2A2 gene. The stationary phase consists of 2-μm non-porous alkylated poly(styrene-divinylbenzene) particles packed into a 50X4.6mm ID column (DNASep column, Transgenomic Inc., San Jose, California, USA). Ten microlitres of crude PCR product was loaded onto the column and was eluted from the column by an acetonitrile gradient in 0.1mol/L triethylammonium acetate buffer (TEAA), pH 7.0, at a constant flow rate of 0.9mL/min. The standard buffers are prepared from tEAA buffer concentrate to give A=0.1mol/L TEAA, B=0.1mol/L TEAA and 25% acetonitrile. The gradient was created by mixing eluents A and B. The recommended gradient for mutation detection is a slope of 2% increase in buffer B per minute. Eluted DNA fragments were detected with ultraviolet absorption at wavelength 260nm. The WAVE utility software helps determine the correct temperature for mutation scanning Recognition of mutations is based on the separation of homo- and heteroduplex species by the elution of PCR amplified DNA through the mixture of eluents which result in characteristic peak patterns both for homozygous and heterozygous samples.

D) Nucleotide sequencing

Sequence analysis on purified DNA fragments obtained by polymerase chain reaction amplification was then performed. Firstly, PCR products were purified by Microspin S300-HR columns (Amersham Pharmacia, Uppsala, Sweden). Both strands of the homologous DNA were then sequenced using the amplification primers as sequencing primers and BigDyeDeoxy terminator cycle sequencing reagents, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Products of sequencing reactions were purified by Centri-Sep spin columns (Princeton Separations, Adelphia, NJ). Purified sequencing fragments were separated by capillary electrophoresis and detected via laser-induced fluorescence on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Sequencing results were compared to the established human ATP2A2 sequence (GenBank accession nos M23115 and M23114) and any mutations detected were designated according to the recommendations by Dunnen and Antonarakis.

E) Exclusion of rare polymorphism

Mutations identified in ATP2A2 fragments were analysed by denaturing high-performance liquid chromatography (DHPLC) in a cohort of 50 unrelated normal controls to exclude the possibility of rare polymorphisms.

Table 4.1: PCR Primers for amplication of ATP2A2 from genomic DNA

Exon	Primer(5[prime]->3[prime])	Product size (bp)	Annealing temp (degree C)
ATP2A2exon1F	GCAAGAGGAGGAGGGGAGA	450	
ATP2A2exon1R	CCCGAGAAGCGAAGAGGT		
ATP2A2exon2F	GACCTCAGGCCATTGATTACA	411	60
ATP2A2exon2R	CATGCTGCCAGTAATAAAATCC		
ATP2A2exon3F	GGTCTGTGTTTTAAAGATATTGATGCT	418	58
ATP2A2exon3R	TTCACCCAATGGACATCATTT		
ATP2A2exon4F	AGGTGATCGCCTGCCTTG	421	62
ATP2A2exon4R	AGAGTGAGACTGTGTCAGAAAACA		
ATP2A2exon5F	TCCTTGTGTCTGTTGCCTTAGA	438	56
ATP2A2exon5R	GGATTTGTATGAATGGCAATAAAA		
ATP2A2exon6F	CCAAGATAGGTTGATCACTTTGC	400	60
ATP2A2exon6R	GGCAATGGAGCGAGACTAAA		
ATP2A2exon7F	CCTGACACCCTTTGTTCTGG	439	58
ATP2A2exon7R	TGTTACTGCCATATTCTGACACC		
ATP2A2exon8F	GGTGCCCTAGTCAAAAACCA	623	62
ATP2A2exon8R	TTTGATGGCATGAAGGCATA		
ATP2A2exon9F	GTGATGGGCGAAGCAGTCTA	410	62
ATP2A2exon9R	TGACTTGAGCCACATTCAGA		
ATP2A2exon10F	TGGCATCAAATTGTTTGGAA	431	62
ATP2A2exon10R	AAGGCTCTCAGCTTTCTTTGG		

ATP2A2exon11F	GGGTCACCTGTTTCAGAGGA	429	60
ATP2A2exon11R	CCTGAGAGTGAATATGGGGAAG		
ATP2A2exon12F	TCTCCCAAAATAGGGGACAA	422	58
ATP2A2exon12R	ATGTGGGTGCACCTGTCAAT		
ATP2A2exon13F	GTTAAGATCCCGGTGAACCA	415	56
ATP2A2exon13R	TGGCAGGCAGAAAAACAAAT		
ATP2A2exon14F	GGGCAACAAGAGCGAAACT	662	56
ATP2A2exon14R	TCTTCCCTGCAAGTAGCACA		
ATP2A2exon15F	CCTTTGTCCCACTTCGGTAA	512	60
ATP2A2exon15R	AAGACCAAAGCTGCTGCAAT		
ATP2A2exon16F	TGGCCTCAGTCATCTGAATTT	487	62
ATP2A2exon16R	AAACACGCTTTTAAGGGATGAA		
ATP2A2exon17F	CCCTGTCCTTCAGAAACCAG	425	60
ATP2A2exon17R	AAGCCCTGGTCCTTCAGAAT		
ATP2A2exon18F	GAGGCCTTGACCTTTCTGTG	455	62
ATP2A2exon18R	TGATCCTCAATCAACCGTGA		
ATP2A2exon19F	GAGGTAGGTCAGCGGATGGT	400	60
ATP2A2exon19R	ATCACGGGCAAGGAGATTTT		
ATP2A2exon20F	TCGAACCCTTGCCAGTAAGT	482	60
ATP2A2exon20R	ACCTCCATCACCAGCCAGTA		

4.3) Results

In our study, mutational analyses were performed in 28 patients. There were 4 novel mutations (table 4.3.1) identified. None of these mutations were found in 50 controls which indicated that these mutations are unlikely to be rare polymorphisms. The first nonsense mutation (S1031X[†]) affected 3 sisters (Patient 7, 8, 9) within a family. All of them had severe DD. Another novel nonsense mutation [*] (V89X[††]) was detected in a 68-year-old gentleman with moderately severe DD. One frameshift deletion mutation (2918-2920delCCT) was found in a 22-year-old gentleman with mild DD. A further splice site mutation (IVS10+1G>T [**]) was occurred in a 35-year-old pregnant lady with severe DD. In view of finding two novel mutations on exon 20 of ATP2A2 gene in our patients, nucleotide sequencing of exon 20 of every patient (except patient3) was performed but no more mutation was detected. Thus, there was no mutation hotspot [***]detected in our cohort. The mutation detection rate in our study was around 18% and most mutations in the ATP2A2 gene in our locality are of the non-sense type (2/4=50%).

* Nonsense mutation: Mutation that change a codon or an amino acid to a termination or a stop codon and leads to premature termination of translation

** IVS10+1G>T: Guanine (G) to thymidine (T) substitution at nucleotide +1 of intron 10

*** Mutation hotspot: Sites of mutation that recur significantly more often than expected by chance.

† S1031X: Amino acid 1031 (Serine, S) is changed to a stop codon (X)

†† V89X: Amino acid 89 (Valine, V) is changed to a stop codon (X)

Table 4.2: 4 Novel ATP2A2 mutations detected among 28 DD patients from 22 families

Study no.	Location	Mutation	Nucleotide change	Consequence	Amino acid	Functional or protein domain	Verification method
Patient3	Exon3	216delT	216delT	Frameshift, Premature termination	V89X	M1	All DNA Sequencing
Patient7	Exon 20	3022insT	3022insT	Frameshift, Premature termination	S1031X	M11	SERCA2b-specific C-terminal DNA sequencing
Patient8	Exon 20	3022insT	3022insT	Frameshift, Premature termination	S1031X	M11	SERCA2b-specific C-terminal DNA sequencing
Patient9	Exon 20	3022insT	3022insT	Frameshift, Premature termination	S1031X	M11	SERCA2b-specific C-terminal DNA sequencing
Patient23	Exon 20	2918delCCT	2918delCCT	in-frame, deletion of serine at position 973	ΔS973	M11	SERCA2b-specific C-terminus DNA sequencing
Patient32	Intron	IVS10G>T	IVS10G>T	Exon skipping premature termination			All except domain A and M1-M4 DNA sequencing

4.4) Discussion

In our cohort, the mutation detection rate was 18% which was relatively low when compared with Caucasian series (50-96%), [5,7] Taiwan series (86%),[12] Japan series (40%).[14] It might be related to characteristics of the DNA or possibly due to technical reasons. DD patients with no mutations detected had been described in other studies. [5, 8, 15] It is possible that these patients harbour mutations in an unscreened region of the ATP2A2 gene such as the promoter region, intronic sequences or 3[prime]-untranslated region which could affect the expression and / or function of the SERCA2 protein or even in other location of the human genome. A linkage analysis which involves the identification of genetic markers lying close to the diseased gene and narrow down the possible location of such mutation in the human genome may be carried out in our locality. A variety of DD mutations had been reported and they can be categorized into 3 groups according to their predicted consequence on the SERCA2 protein: Nonsense mutations which result in premature termination codon and thus absence of protein synthesis through nonsense mRNA decay, missense and splice shift mutations which result in the synthesis of shortened or elongated SERCA2 protein, and the third group result in non-conservative amino acid substitutions.[5, 12-14, 16] These mutations were spread throughout the ATP2A2 gene and no clustering or hotspots of mutations were identified so far. [4, 8, 12, 16, 17] Except acral haemorrhagic variant of DD which was found to be associated with missense mutations, there is no clear-cut genotype-phenotype association reported. [8, 16, 17] It has been proposed that DD is an example of haploinsufficiency* where mutations disrupt important domains of the SERCA2 molecule and result in complete or partial loss of function of the mutated pumps. Compensation by the normal SERCA2 pumps and by other systems involved in intracellular Ca^{2+} homeostasis is not sufficient in adult skin especially in the presence of exacerbating factors such as heat, sweating and sun exposure which results in abnormal Ca^{2+} signaling in the epidermis. [12] These may explain the onset of DD in the second decade of life and precipitation of DD by its known aggravating factors. Most mutations in the ATP2A2 gene in our cohort are of the non-sense type (50%). This is in contrast to the findings in Taiwan[12] and Japan[14] where mis-sense mutations accounted for 67% and 70% of all mutations detected.

***** Haploinsufficiency: A situation in which the protein produced by a single copy of an otherwise normal gene is not sufficient to assure normal function.

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5) Relevance in SHS and Hong Kong

As the only local study on the clinical and genetic aspects of Darier's disease, this dissertation is important in unraveling the genetic defect of this rare dermatosis in our population and providing a thorough review on DD including its local epidemiological data, characteristic presentations and local treatment experience. The discovery of four novel mutations that had not been reported before and the predominance of non-sense mutations in this study suggest a unique pattern of ATP2A2 gene mutations in our locality. The relatively low detection rate of ATP2A2 mutations in our DD cases may also indicate the possibility that DD in HK being caused by mutation somewhere else in the human genome other than ATP2A2. It is hoped that our findings can provide a better understanding of DD in Hong Kong and function as a stimulus for subsequent larger studies (both clinical and genetic). Only then the enigma of genotype-phenotype association of DD can be solved. From our data, mutations for DD in Chinese may occur in regions other than chromosome 12q23-24.1, a linkage analysis which involves the identification of genetic markers lying close to the diseased gene and narrow down the possible location of such mutation in the human genome may be carried out in our locality.

6) Limitations of the present study

Although this is the largest series of DD that had ever been reported in Hong Kong, the whole story is not completely revealed yet. The main drawbacks lie in the study include:

1. DD cases may be managed by dermatologists in the private sector.
2. Patient with mild disease may neglect their own skin lesions and not seek medical attention.
3. The small sample size of this study rendered statistical analyses less powerful and imposed difficulty in clarifying the exact genotype-phenotype association of DD.
4. Limitations in DD cases recruitment:

-Failure to include all DD patients in the SHS due to errors or incomplete information in the clinic biopsy books or computer

-Failure to retrieve all patient records either due to record loss or incorrect patient data

5. Limitations in the clinical review of DD cases:

-Incomplete records notes

-Failure to reach all index patients for interview, either due to loss of contact or patient reluctance.

6. Limitations in the study of social impact of DD on our patients with DLQI

-DLQI has not been validated for use in Hong Kong

7. Limitations in the genetic study of DD cases:

-Technical errors (either machine or person)

-Limitation of funding as this study did not receive funding from any organizations.

7) Conclusion

This study has reviewed 32 DD patients, 15 males and 17 females, in the SHS. The male to female ratio was around 1:1. The mean age of onset was 14.75 with peak in the second decade for male and female cases. DD was rare in Hong Kong. There average incidence of DD in SHS was 0.825 cases per 10,000 new skin cases per year from 1983 to 2003 and the corresponding average incidence of DD in Hong Kong per year from 1983 to 2003 was 0.025 cases per 100,000 persons.

Greasy hyperkeratotic papules were the commonest morphology in our patients followed by verrucous plaques. Face, neck, front chest and axilla were the most commonly affected whilst buttocks and perineum were usually spared. The clinical patterns of involvement could be summarized as seborrhoeic, flexural, acral and mixed. 57% of our DD cases had a mixed pattern with combined seborrhoeic and flexural involvement. DD usually started on the face, neck and ears. Most of our patients were in the severe group with diffuse involvement and plaques formation. Itch was the most frequent complaint followed by pain. Majority of them considered heat, sweating and sunlight as exacerbating factors for their skin disorders All our DD patients had hand involvement. Palmar pits, V-shaped notching, longitudinal ridging and brittle nails were the 4 most commonly seen. Oral mucosal involvement was present in 38%. DD cases in HK were not associated with disorders such as salivary gland obstruction, bone cysts and psychiatric illness.

In SHS, the use of oral retinoid at the dose of 0.33-0.5mg/kg in DD patients resulted in marked clinical improvement in 40% cases and all of these patients preferred to continue systemic retinoid therapy despite its side effects such as xerosis, hands and feet desquamation, lip dryness and dermatitis. Systemic retinoid was the single most effective therapy for our DD cases. Emollient, topical steroid, topical antibiotics and antifungals were prescribed in most of our DD patients but none of these modalities altered the natural course of the disease. Topical retinoid was generally considered to be ineffective and irritable.

DLQI survey in this study indicated that most DD cases adjusted well to their own skin disease. Adult patients were most bothered by the symptoms of DD and resultant social embarrassment whilst adolescent patients were most bothered by its treatment. There was a poor correlation between clinical severity of DD and social impairment perceived by DD patients.

Four novel mutations of ATP2A2 gene were identified in our DD patients. They were 3022insT, 216delT, 2918delCCT and IVS10+1G>T. The first mutation (3022insT) was found in a family affecting 3 sisters whilst the other three were sporadic cases. The mutation detection rate in our study was around 18% and 50% mutations in the ATP2A2 gene in our locality are of the non-sense type. No mutation hotspots and clear-cut genotype-phenotype association could be identified.

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8) Acknowledgements

I must express my sincere gratitude to all the seniors, pathologists, nursing staff and patients who were involved in different stages of this study.

My former supervisor and current programme director Dr. Tang Yuk Ming and my current supervisor Dr. Ho King Man provided helpful guidance and support during the whole course of this study. Professor Wong Tze Wai of Department of Public Health of the Chinese University of Hong Kong and Dr. Lam Wai Sun gave me precious advice on the statistical methods adopted for data analysis. Dr. Su Chee Wai reviewed the manuscript before its submission.

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The last but not the least is my deep thankfulness to all my patients who participated in this study. I am really impressed by their eagerness in participating in the genetic study which help unraveling the enigma of ATP2A2 gene mutation of Darier's disease in this locality.

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11) Appendix

11.1) Patient proforma

<table>
<tr><td colspan="3">Demographic data and History</td></tr>
<tr><td>Name</td><td>Patient number</td><td>Duration of disease</td></tr>
<tr><td>Social hygiene clinic attended</td><td>Clinic no.</td><td>Disease activity at time of review
Active / Remission/ Controlled</td></tr>
<tr><td>Age</td><td>Sex
M / F</td><td>Initial site of onset</td></tr>
<tr><td colspan="2">Age of onset</td><td rowspan="2">Complication:
Bacterial infection
HSV infection
Other infection
Malodour</td></tr>
<tr><td colspan="2">Associated symptoms</td></tr>
<tr><td colspan="2">Family History: + / -
Other members clinic no.:</td><td rowspan="2">Associated disorders
Retinitis pigmentosa
Salivary gland obstruction
Renal and testicular agenesis
Epilepsy
Affective disorder or psychiatric illness</td></tr>
<tr><td colspan="2">Exacerbating factors:
Heat / Sunlight / High humidity/
Drugs</td></tr>
<tr><td colspan="2">Mode of onset
Acute / Chronic / Acute on chronic</td><td>Coexisting medical problems</td></tr>
<tr><td colspan="3">Pedigree:</td></tr>
</table>

<table>
<tr><td colspan="3">Clinical features and investigation</td></tr>
<tr><td rowspan="5">Areas involved:
Head: Scalp / Face / Ears
Trunk: Neck / Front chest / Back / Abdomen / Buttock / Groin / Perineum
Upper limb: Axilla / Upper arm / Forearms / Hands / Fingers
Lower limb: Thighs / Lower legs / Feet / Toes
Mucosal involvment: eyes vs oral vs genitalia vs anus
Nails</td><td colspan="2">Investigation</td></tr>
<tr><td>LFT</td><td rowspan="2">Others</td></tr>
<tr><td>Fasting Lipid</td></tr>
<tr><td colspan="2">Skin Biopsy: Biopsy no.</td></tr>
<tr><td colspan="2">Focal acantholytic dyskeratosis
Corps Ronds
Grains
Others</td></tr>
</table>

Detailed description of cutaneous lesions				
Cutaneous features	**Scalp**	**Palms and soles**	**Nail changes**	**Mucosal lesions**
Morphology of lesions: -Papules / Plaques / vesicobullous / hemorrhagic Distribution: Localised / Zosteriform / Generalised Colour: -Skin coloured / Brown / Erythematous Surface: Smooth / Greasy scaling and crusting / Hyperkeratotic	Alopecia	Palmar pits/ Punctate keratosis/ Flat warty papules	Thin and brittle (break distally)/ Longitudinal ridging/ Red streaks/ White streaks/ V shaped nicks/ Subungual hyperkeratosis	Cobberstone

Treatment										
Drug	Date started	Dose		Response			Duration		Side effect	Recurrence (Months from stop of Tx)
		Initial	Final	Yes		No	Till control (Month)	Total (Month)		
				Partial	Full					

Counselling on avoidance of precipitating factors Yes / No	Genetic counseling Yes / No	Surgery with or without skin graft Dermabrasion CO2 laser

11.2) Memorandum to patients

A) Memorandum to patients (Chinese version)

基因檢查備忘

毛囊角化病: 病歷回顧及基因檢查研究

我們正在進行關於毛囊角化病的基因異變之研究。 閣下已被確定爲毛囊角化病之患者。 故誠邀閣下參與此病之基因異變研究, 此項研究有助我們對本土(香港)之毛囊角化病者的基因異變有進一步的瞭解。

如果閣下願意參與是項研究, 我們將在選定的日子和地點抽取血液, 並於同日把血液樣本 (大概五毫升) 送交威爾斯病理部門作基因檢查。

在基因檢查完成後, 我們亦會給予閣下一份完整的基因檢查報告, 以便閣下對自己的基因異變有所認識。

你是否參加這項研究, 純屬自願, 你不會因爲拒絕參加而受到次等待遇

衛生署社會衛生科

B) Memorandum to patients (English version)

Memorandum on genetic study

Darier's disease: a clinical review and genetic study

We are conducting a study on the genetic mutation of Darier's disease. As you have been confirmed to be a case of Darier's disease, we would cordially invite you to participate in this genetic study. This study can facilitate the understanding of the mutational pattern of Darier's disease in our locality (Hong Kong SAR)

If you agree to participate in this genetic study, your blood specimen (about 5 ml) will be collected on a specified date & location and will be transferred to the Prince of Wales Hospital Pathology Department for gene analysis.

Upon completion of the genetic study, we will provide you a detailed report with explanation so that you can have an understanding of your own genetic mutation.

Your participation is absolutely voluntary and you can decline or withdraw from the study at any time without affecting your rights as a patient.

11.3) Consent forms for ATP2A2 gene mutation analysis

A) Consent (major) – Chinese version

毛囊角化病之基因突變研究

參與同意書

本人_____，身份証號碼:_____同意參加由社會衛生科進行的以上研究，並同意接受患處之臨床攝影，血液化驗。

我理解此項研究所得的資料只用於未來的學術報告和交流。我的私隱會受到保護，我的個人資料亦不會洩漏。我對這項研究的有關步驟及可能會出現的風險已經得到充份的了解。我是自願參與此項研究。

我理解我有權在研究過程中提出問題並在任何時候決定退出研究而不會受到任何不正常的待遇。

參加者簽署_____ 醫生簽署_____

參加者姓名_____ 醫生姓名_____

見証人簽署_____ 日期_____

B) Consent (major) – English verion

A study on genetic mutation of Darier's disease

Consent to participate in study

I _____, (ID no.: _____) hereby consent to participate in the above study and to have blood tests, and photographs related to Darier's disease.

I understand that all study materials will only be used for academia in the future.

I understand that my personal information will be protected and my particulars will not be disclosed.

I understand the study procedures and its associated risks have been explained to me. I take part in this study voluntarily.

I understand that I have the right to decline and withdraw from the study at any time without affecting my rights as a patient.

Participant's Signature_____Doctor's Signature_____

Participant's Name_____Doctor's Name _____

Signature of Witness _____ Date_____

C) Consent (minor) – Chinese version

毛囊角化病之基因突變研究

未成年病人參與研究
家長/監護人同意書

本人_____, 身份証號碼:
同意本人之_____
_____參加由社會衛生科進行的以上研究, 並同意接受患處之臨床攝影, 血液化驗。

我理解此項研究所得的資料只用於未來的學術報告和交流。我理解參與者的私隱會受到保護而參與者的個人資料亦不會洩漏。我對這項研究的有關步驟及可能會出現的風險已經得到充份的了解。我理解參與此項研究完全出於自願性質。

我理解我有權在研究過程中提出問題並在任何時候決定退出研究而不會受到任何不正常的待遇。

參加者簽署_____ 醫生簽署_____

參加者姓名_____ 醫生姓名_____

見証人簽署_____ 日期_____

D) Consent (minor) – English version

A study on genetic mutation of Darier's disease

Consent for minor

I _____, (ID no.: _____) hereby consent to submit my _____ _____ to participate in the above study and to have blood tests, and photographs related to Darier's disease.

I understand that all study materials will only be used for academia in the future.

I understand that personal information of my child will be protected and his/ her particulars will not be disclosed.

I understand the study procedures and its associated risks have been explained to me. I understand that participation in this research study is voluntary

I understand that I/ my child have the right to decline and withdraw from the study at any time without affecting his/ her rights as a patient.

Parent's/Guardian's Signature_____Doctor's Signature_____

Parent's/Guardian'sName_____Doctor's Name_____

Signature of Witness _____Date_____

11.4) DLQI SURVEY FORM

A) DLQI – Cantonese version

皮膚科生活質素指數
診所編號: 日期: 總分:
姓名:
呢份問卷調查嘅目的係量度係過去一個星期裏面, 你嘅皮膚問題對你生活嘅影響有幾大。請你係每一條問題嘅其中一個空格畫一個剔號。

1. 喺過去一個星期裏面, 你的皮膚痕癢、酸痛、 痛或者刺痛嘅程度點樣呢？
   非常嚴重 □
   嚴重 □
   些少 □
   無 □
2. 喺過去一個星期裏面, 你因爲皮膚問題而產生尷尬或者太注意自己嘅程度點樣呢？
   非常嚴重 □
   嚴重 □
   些少 □
   無 □
3. 喺過去一個星期裏面, 你喺行街買嘢、打理屋企或者花園嘅時候, 皮膚問題對你嘅影響有幾大？
   非常嚴重 □
   嚴重 □
   些少 □
   無 □
   無關 □
4. 喺過去一個星期裏面, 你嘅皮膚問題對你選擇着衫方面嘅影響有幾大？
   非常嚴重 □
   嚴重 □
   些少 □
   無 □
   無關 □
5. 喺過去一個星期裏面, 你嘅皮膚問題對你嘅社交或者休閒生活嘅影響有幾大？
   非常嚴重 □
   嚴重 □
   些少 □
   無 □
   無關 □

6. 喺過去一個星期裏面, 你嘅皮膚問題對你做運動構成嘅困難有幾大？

非常嚴重 □

嚴重 □

些少 □

無 □

無關 □

7. 喺過去一個星期裏面, 你嘅皮膚有冇令到你唔能夠做嘢或者讀書？

有 □

冇 □

無關 □

如困係「冇」, 嗷喺過去一個星期裏面, 你嘅皮膚問題喺你做嘢或者讀書方面造成嘅問題有幾大？

大 □

唔大 □

無 □

8. 喺過去一個星期裏面, 你嘅皮膚問題引起你同配偶或者同好朋友或者親戚之間嘅問題有幾大？

非常嚴重 □

嚴重 □

些少 □

無 □

無關 □

9. 喺過去一個星期裏面, 你嘅皮膚問題引起性方面嘅困難有幾大？

非常嚴重 □

嚴重 □

些少 □

無 □

無關 □

10. 喺過去一個星期裏面, 皮膚護理帶俾你嘅問題有幾大？ 譬如搞到屋企好亂或者用咗好多時間。

非常嚴重 □

嚴重 □

些少 □

無 □

無關 □

請你檢查你係唔係已經答哂所有問題. 。多謝。

此項調查所得的資料只用於未來的學術報告和交流

B) DLQI – English version

DERMATOLOGY LIFE QUALITY INDEX (DLQI)
Hospital No: Date: **DLQI SCORE:**
Name:
Address: Diagnosis:
The aim of this questionnaire is to measure how much your skin problem has affected your life OVER THE LAST WEEK. Please tick one box for each question.

1. Over the last week, how **itchy**, **sore**, **painful** or **stinging** has your skin been?
- Very much □
- A lot □
- A little □
- Not at all □

2. Over the last week, how **embarrassed** or **self conscious** have you been because of your skin?
- Very much □
- A lot □
- A little □
- Not at all □

3. Over the last week, how much has your skin interfered with you going **shopping** or looking after your **home** or **garden**?
- Very much □
- A lot □
- A little □
- Not at all □
- Not relevant □

4. Over the last week, how much has your skin influenced the **clothes** you wear?
- Very much □
- A lot □
- A little □
- Not at all □
- Not relevant □

5. Over the last week, how much has your skin affected any **social** or **leisure** activities?
- Very much □
- A lot □
- A little □
- Not at all □
- Not relevant □

6. Over the last week, how much has your skin made it difficult for you to do any **sport**?
- Very much □
- A lot □
- A little □
- Not at all □
- Not relevant □

7. Over the last week, has your skin prevented you from **working** or **studying**?

Yes □
No □
Not relevant □

8. Over the last week, how much has your skin created problems with your **partner** or any of your **close friends** or **relatives**?

Very much □
A lot □
A little □
Not at all □
Not relevant □

9. Over the last week, how much has your skin caused any **sexual difficulties**

Very much □
A lot □
A little □
Not at all □
Not relevant □

10. Over the last week, how much of a problem has the **treatment** for your skin been, for example by making your home messy, or by taking up time?

Very much □
A lot □
A little □
Not at all □
Not relevant □

Please check you have answered EVERY question. Thank you.

All the above data will only be used for academia in the future