Epidermolysis bullosa simplex with *KLHL24* mutations is associated with dilated cardiomyopathy

Short Title

Cardiomyopathy in Epidermolysis bullosa KLHL24

Authors

Schwieger-Briel A*[1,2], Fuentes I[3,4]*, Castiglia D[5], Barbato A[6], Greutmann M[7], Leppert J[2], Duchatelet S[8], Hovnanian A[8], Burattini S[3], Yubero MJ[3,9], Ibañez-Arenas R[3,9,10], Rebolledo-Jaramillo B[4], Gräni C[11], Ott H[12], Theiler M[1,13], Weibel,L[1,13], Paller AS[14], Zambruno G[5], Fischer J[15], Palisson F[3,9,16], Has C[2]

Author details: [1]Department of Pediatric Dermatology, University Children's Hospital Zurich, Zurich, Switzerland, [2]Epidermolysis bullosa Centre, Department of Dermatology, Medical Center – University of Freiburg, Freiburg, Germany; [3] Fundación DEBRA Chile, [4]Centro de Genética y Genómica, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo, Santiago, Chile, [5]Istituto Dermopatico dell'Immacolata, IRCCS, Rome, Italy; [6] Emergency Medicine and Hypertension Unit, Department of Clinical Medicine and Surgery, Federico II University Medical School, Naples, Italy; [7]Department of Cardiology, University Hospital Zurich, Zurich Switzerland; [8]INSERM, Laboratory of Genetic Skin Diseases, Imagine Institute and Paris Descartes University, Paris, France; [9]Facultad de Medicina, Clínica Alemana Universidad del Desarrollo, Santiago, Chile; [10]Department of Cardiology, Clínica Alemana Universidad del Desarrollo, Santiago, Chile; [11]Department of Nuclear Medicine, Cardiac Imaging, University Hospital Zurich, Switzerland; [12]Division of Pediatric Dermatology and Allergology, Children's Hospital Auf der Bult, Hanover, Germany, [13]Department of Dermatology, University Hospital Zurich, Zurich, Switzerland, and [14]Departments of Dermatology and Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [15]Department of Human Genetics, Medical Center – University of Freiburg, Germany; [16]Dermatology Department, Clínica Alemana Universidad del Desarrollo, Santiago, Chile.

* Equal contribution

Key words: Epidermolysis bullosa simplex, KLHL24, dilated cardiomyopathy, NT-Pro-BNP

Correspondence
Cristina Has, MD and A. Schwieger-Briel, MD
Department of Dermatology
University of Freiburg
Hauptstrasse 7
79104 Freiburg, Germany
Telephone: +49 – 761–27069920
Fax: +49 – 761–27067200
E-mail: cristina.has@uniklinik-freiburg.de, agnes.schwieger@kispi.uzh.ch

To the Editor

Inherited epidermolysis bullosa (EB) comprises rare heterogeneous disorders characterized by cutaneous and mucosal fragility. Most of the 20 proteins affected have structural functions. Recently, a novel type of EB simplex (EBS), caused by gain-of-function mutations in *KLHL24*, encoding the kelch-like family member 24 (KLHL24) has been identified (He et al. 2016; Lin et al. 2016). This protein seems to be involved in protein ubiquitination. Patients carrying monoallelic mutations in the translation initiation codon of *KLHL24* have a characteristic clinical phenotype, demonstrating skin defects as well as blistering at birth and unusual stellate scarring, skin fragility, and whorled or macular hyper- or hypopigmentation in childhood (**Fig1a-e**). While skin fragility improves by adulthood, nail dystrophy, anetoderma, and hair loss may occur (**Fig1f-h**).

Given that KLHL24 is widely expressed, we investigated extra-cutaneous features in a cohort comprising families from Chile, Germany, Italy, Switzerland, and USA (**Supplementary Material**).

Eighteen patients with EBS-KLHL24 from nine families (9 males, 9 females) were examined (**Table 1**). The mean patient age was 21.7 years, with nine children (2-13 years old) and nine adults (24-46 years old). Patients 1-10 have previously been described (He et al. 2016), whereas patients 11-18 are newly reported. Two additional individuals (#19 and #20) belong to family 9; they had skin fragility and succumbed to dilated cardiomyopathy (DCM) at the ages of 39 and 54 years. In all examined patients, pathogenic variants in the translation initiation codon of *KLHL24* were found (**Table 1**). WES and multi-gene panel data revealed no pathogenic variants in genes known to be associated with DCM (Burke et al. 2016).

Two examined patients had been diagnosed with DCM prior to our study (#9 and #14). The latter had a positive family history with cardiac death in a brother and mother, both of whom also had skin fragility (#19 and #20).

Three unrelated patients had neurological involvement: patient #4 with delay of cognitive and motor development, patient #2 with speech delay, and patient #11 with seizures of unknown origin (**Table 1**). As missense and pathologic in-frame mutations of *KLHL24* were recently linked to neurological disease (Anazi et al. 2017) and as KLHL24 is involved in the regulation of the function of kainate receptors (Laezza et al. 2008), these results might also be of significance. This is supported by a recent case report (Yenamandra et al. 2018).

Laboratory examinations revealed normal values for kidney, liver, pancreas, and thyroid parameters.

In 12 of 16 tested patients, NT-pro-BNP, a specific and prognostic biomarker for heart disease (Zile et al. 2016) was increased. All adult patients with more than two-fold elevated NT-pro-BNP levels (n=5) had significant dysfunction on echocardiography and/or cardiac MRI (**Fig 1i-l**). CK muscle band was elevated in seven of the 16 patients. Fifteen patients were evaluated by cardiology, of whom six were diagnosed with DCM (40%); the youngest was 25 years old (#15). None of the newly diagnosed patients (#1, #5, #15, #16) had reported cardiac symptoms (**Table 1**).

Taken together, in this cohort of 20 patients with EBS-KLHL24, 17 (85%) had evidence of cardiac involvement with either elevated cardiac biomarkers or documented DCM (8/20 patients, 40%), leading to death at an early age in two of them.

DCM is defined as ventricular dilatation with impaired ventricular systolic function (left ventricular ejection fraction <50%) in the absence of features of different phenotypes of cardiomyopathy or evidence of acquired heart disease (e.g. coronary artery disease). Early, even pre-symptomatic intervention improves outcome (Burke et al. 2016).

DCM occurs as a familial disease in 20–35% of cases with more than 30 genes identified. Onset of familial DCM tends to be earlier in life than average. There is significant variability explained by modifying genetic and acquired risk factors (Burke et al. 2016).

DCM in EB may occur as a primary feature when due to *PLEC, JUP* or *DSP* mutations (Fine et al. 2014) or as secondary complication in severe dystrophic and junctional EB (Lara-Corrales et al. 2010). KLHL24 is expressed at similar levels in keratinocytes and cardiomyocytes (Supplementary figure). As in the skin, cardiac KLHL24 may regulate the degradation of structural cytoskeletal proteins involved in mechanical resilience. KLHL24 was also identified to interact with components of the constitutive photomorphogenic 9 (COP9) signalosome, a critical regulator of cullin-RING family of ubiquitin ligases activity (Bennett et al. 2010; Sowa et al. 2009). The dysfunction of COP9 signalosome has been associated with DCM in mice (Su et al. 2013; Su et al. 2011).

Here we provide evidence that patients with EBS-KLHL24 display potentially life-threatening DCM in early adulthood. While dilated cardiomyopathy may be a final common pathway of a variety of acquired and inherited cardiac conditions, none of our patients with DCM demonstrated a different cardiac phenotype (e.g. hypertrophic cardiomyopathy or restrictive cardiomyopathy) or heart disease (e.g. coronary artery disease, cardiac arrhythmias).

Our data are supported by two other reports. Recently severe cardiomyopathy was reported in a single case of EBS-KLHL24 (Yenamandra et al. 2018), and homozygous *KLHL24* loss of function mutations were found in two siblings with hypertrophic cardiomyopathy with glycogen accumulation in cardiomyocytes (Hedberg-Oldfors C. et al. 2016).

DCM in EBS-KLHL24 may be missed because skin fragility and blistering improve with age, leading to infrequent follow-up evaluations. Moreover, because of reduced physical activity due to skin fragility and slow development of DCM, patients may not experience early cardiac symptoms. Thus, regular screening for cardiac involvement to identify pre-symptomatic DCM is required. Given its high sensitivity, serial measurements of NT-pro-BNP-levels along with careful history taking may be the optimal screening tool (Zile et al. 2016). In case of cardiomyopathy, timely implementation of preventive measures, such as heart failure medication or device therapy may likely improve outcomes. Of note, this study

was likely crucial for survival of patient #1 who initially denied echocardiogram for lack of clinical symptoms. His ejection fraction turned out to be 27%. If EBS-KLHL24 is clinically suspected, genetic testing and cardiac monitoring are mandatory.

Acknowledgements

We thank the patients and their families for the participation in this study. Gabriele Grüninger is acknowledged for expert technical assistance. Most of this study was supported by Debra International (C.H.) and the Chilean WES data analysis by the CONICYT FONDEQUIP EQM150093.

Legends to figures

Figure 1. Cutaneous and cardiac features of EBS-KLHL24. (a) Congenital skin defects and blistering, (b) characteristic stellate scarring, and (c-e) macular or whorled hypo- and hyperpigmentation in children, (f-h) mild skin fragility, anetoderma, diffuse alopecia, and nail dystrophy in adults. (i-j) Echocardiography of patient #1: 4-chamber view at end-diastole (i) and end-systole (j) with severe dilatation of LV and LA and severely impaired LV ejection fraction. (k-l). Magnetic resonance imaging (CMR): cine sequence (end-diastole) in the short axis view at basal level (k): wall thinning of inferoseptal and inferior LV wall segments (white arrows). (l) Extensive Late-Gadolinium Enhancement in all segments: transmural fibrosis, corresponding to the thinned inferior and inferolateral wall of the LV (little arrows), non-transmural mid wall fibrosis visible in the septum (large arrows) and to a lesser extent, the lateral, anterior and septal segments of the LV and diffuse fibrosis of the RV.

Abbreviations: LV: Left ventricle; RV: Right ventricle; LA: Left atrium; RA: Right atrium

References

Anazi S, Maddirevula S, Salpietro V, Asi YT, Alsahli S, Alhashem A, et al. Expanding the genetic heterogeneity of intellectual disability. Hum. Genet. 2017;136(11–12):1419–29

Bennett EJ, Rush J, Gygi SP, Harper JW. Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. Cell. 2010;143(6):951–65

Burke MA, Cook SA, Seidman JG, Seidman CE. Clinical and Mechanistic Insights Into the Genetics of Cardiomyopathy. J. Am. Coll. Cardiol. 2016;68(25):2871–86

Fine J-D, Bruckner-Tuderman L, Eady RAJ, Bauer EA, Bauer JW, Has C, et al. Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. J. Am. Acad. Dermatol. 2014;70(6):1103–26

He Y, Maier K, Leppert J, Hausser I, Schwieger-Briel A, Weibel L, et al. Monoallelic Mutations in the Translation Initiation Codon of KLHL24 Cause Skin Fragility. Am. J. Hum. Genet. 2016;99(6):1395–404

Hedberg-Oldfors C., Danielsson O., Hübbert L., Nennesmo I., Abramsson A., Banite R., et al. Hypertrophic cardiomyopathy and abnormal glycogen storage in heart and skeletal muscle associated with inactivation of KLHL24. Neuromuscula Disord. 2016;26(Sup 2):S152

Laezza F, Wilding TJ, Sequeira S, Craig AM, Huettner JE. The BTB/kelch protein, KRIP6, modulates the interaction of PICK1 with GluR6 kainate receptors. Neuropharmacology. 2008;55(7):1131–9

Lara-Corrales I, Mellerio JE, Martinez AE, Green A, Lucky AW, Azizkhan RG, et al. Dilated cardiomyopathy in epidermolysis bullosa: a retrospective, multicenter study. Pediatr Dermatol. 2010;27(3):238–43

Lin Z, Li S, Feng C, Yang S, Wang H, Ma D, et al. Stabilizing mutations of KLHL24 ubiquitin ligase cause loss of keratin 14 and human skin fragility. Nat. Genet. 2016;48(12):1508–16

Sowa ME, Bennett EJ, Gygi SP, Harper JW. Defining the human deubiquitinating enzyme interaction landscape. Cell. 2009;138(2):389–403

Su H, Li J, Menon S, Liu J, Kumarapeli AR, Wei N, et al. Perturbation of cullin deneddylation via conditional Csn8 ablation impairs the ubiquitin-proteasome system and causes cardiomyocyte necrosis and dilated cardiomyopathy in mice. Circ. Res. 2011;108(1):40–50

Su H, Li J, Osinska H, Li F, Robbins J, Liu J, et al. The COP9 signalosome is required for autophagy, proteasome-mediated proteolysis, and cardiomyocyte survival in adult mice. Circ. Heart Fail. 2013;6(5):1049–57

Yenamandra VK, van den Akker PC, Lemmink HH, Jan SZ, Diercks GFH, Vermeer M, et al. Cardiomyopathy in epidermolysis bullosa simplex patients with mutations in the KLHL24 gene. Br. J. Dermatol. 2018;

Zile MR, Claggett BL, Prescott MF, McMurray JJV, Packer M, Rouleau JL, et al. Prognostic Implications of Changes in N-Terminal Pro-B-Type Natriuretic Peptide in Patients With Heart Failure. J. Am. Coll. Cardiol. 2016;68(22):2425–36

8

Table 1) Patient descriptions with genetic, clinical, and laboratory findings

Patient Nº	Family Nº	Age (y) (Sex)	Country of origin	Mutation *KLHL24*	Cardiac symptoms/ clinical features	CK (U/l) (normal)	CKMB (ng/mL) (normal)	Pro-BNP (pg/ml) (normal)	ECG/ Echocardiogram/ MRI	Other clinical features / symptoms
1	1	34 (M)	Swiss-Italian	c.[1A>G]	None/ BP 105/80	**237** (<168)	**32** (<25)	**1221** (<120)	ECG: non-specific changes --- Echo: Severe biventricular DCM, EF of 27% --- MRI: Transmural fibrosis, corresponding to the thinned inferior and inferolateral wall of the LV. Non-transmural mid wall fibrosis in the septum; the lateral, anterior and septal segments of the LV show non-transmural fibrosis. Diffuse fibrosis in the RV.	Chronic protein- and hematuria Xerosis cutis, palmoplantar keratoderma, nail dystrophy
2	1	6 (F)	Swiss-Italian	c.[1A>G]	None	121 (<147)	2.3 (<6)	333 (<390)	ECG: n/d --- Echo: Normal	Diffuse alopecia, nail dystrophy, xerosis cutis. Speech delay
3	1	9 (F)	Swiss-Italian	c.[1A>G]	None	**526** (<147)	**23.7** (<6)	**874** (<390)	ECG: n/d --- Echo: Normal	Mild diffuse alopecia, xerosis cutis, palmo-plantar keratoderma, nail dystrophy
4	2	6 (M)	Swiss-German	c.[2T>C]	Tachycardia, Extra-systoles	**182** (<168)	**7.7** (<6)	82 (<390)	ECG: Normal --- Echo: Normal	Developmental and fine motor skill delay
5	3	46 (F)	Germany	c.[1A>G]	None	66 (<170)	15 (<25)	**695.7** (<169)	ECG: non-specific changes --- Echo: Mild systolic dysfunction with dilatation of left ventricle	Complete scalp and body hair loss
6	3	6 (F)	Germany	c.[1A>G]	None	95 (<149)	**64.9** (<24)	**60.1** (<40)	ECG: n/d --- Echo: n/d	
7	4	36 (F)	Germany	c.[1A>G]	None	74 (<168)	19 (<25)	**170** (<125)	ECG: n/d --- Echo: n/d	Complete scalp and body hair loss.
8	5	12 (M)	Germany	c.[1A>G]	None	74 (<152)	**30** (<25, 40,5% of CK)	**186,7** (<186)	ECG: n/d --- Echo: n/d	
9	6	39 (M)	Italy	c.[1A>G]	Occ. chest pain at night	n/d	n/d	n/d	ECG: Mean 50 bpm (bradycardia), sporadic ventr. extrasystoles ---	

					(2014); improved with medication				Echo 2014: LV dilatation, EF 43%. Echo 2018: EF 43% by Simpson, global hypokinesia	
10	6	13 (M)	Italy	c.[1A>G]	None	209 (≤200)	5.0 (≤5)	**125.6** (<125)	ECG: Normal --- Echo: Normal	
11	7	35 (F)	USA	c.[1A>G]	None; episode with loss of consciousness	n/d	n/d	n/d	ECG: Normal --- Echo: Normal	Hair thinning in early adulthood, progressive hair loss. Seizure disorder of unknown origin.
12	8	28 (F)	Chile	c.[1A>G]	None Normal	60	14 (<25)	43 (<125)	ECG: Normal --- Echo: Normal	Moderate alopecia since 11 years of age. Fine and dry hair.
13	9	4 (M)	Chile	c.[2T>G]	None Normal	129 (<308)	**31 (**<25)	**89** (<125)	ECG: n/d --- Echo: Normal	Fine and dry hair. Mild follicular atrophoderma on arms.
14	9	33 (M)	Chile	c.[2T>G]	Heart murmur (I/IV)	75 (<308)	11 (<25)	**926** (<125)	ECG: Electric axis diverted to the left. Normal headsets, ventricular complexes and AV conduction. Intraventricular conduction altered by anterior left hemiblock. --- Echo: Severe DCM, diagnosed 2016: LV severely dilated in systole (83ml/m2) and diastole (112/m2), diffuse hypochinesia and severely depressed systolic function (LVEF 26%, est. by Simpson, 23% calculated by Heart Model)	Mild alopecia, dry hair. Moderate follicular atrophoderma on cheeks, arms and thighs.
15	9	25 (M)	Chile	c.[2T>G]	Heart murmur (II/IV), extra-systoles	85 (<308)	13 (<25)	**638** (<125)	ECG: Isolated ventricular extrasystoles, Nonspecific alteration of ventricular repolarization --- Echo: LV severely dilated, moderate to severe systolic dysfunction (LVEF 30%), mild to moderate functional mitral insufficiency, Carpentier III	Mild alopecia. Fine and dry hair. Mild follicular atrophoderma on thighs.
16	9	33(F)	Chile	c.[2T>G]	Heart murmur (I/VI)	104 (<308)	10 (<25)	**258** (<125)	ECG: Normal --- Echo: Mild systolic dysfunction of LV, LVEF 40% normal left atrium and right chambers	Moderate alopecia. Fine and dry hair. Moderate follicular atrophoderma on thighs and arms. Hip dysplasia and scoliosis
17	9	24 (F)	Chile	c.[2T>G]	None Normal	101 (<308)	9 (<25)	**152** (<125)	ECG: n/d --- Echo: Normal with normal/low systolic function	Dry hair and moderate alopecia since 13 years of age. Mild follicular atrophoderma on arms and thighs.
18	9	2 (M)	Chile	c.[2T>G]	None	141	**40** (<25)	**210** (<125)	ECG: n/d --- Echo: Normal	Mild alopecia, dry hair.

10

19	9	Death at age 39 (M)	Chile	c.[2T>G]	Cardiac death	-	-	-	DCM from history only	
20	9	Death at age 54 (F)	Chile	c.[2T>G]	Cardiac death	-	-	-	DCM from history only	

Legend: CK creatine kinase, **CKMB CK** muscle band, **DCM** dilated cardiomyopathy, **LV** left ventricle, **RV**, right ventricle, **EF** ejection fraction, **F** female, **M** male, **n/d** not done. Values in **bold** exceed reference values.

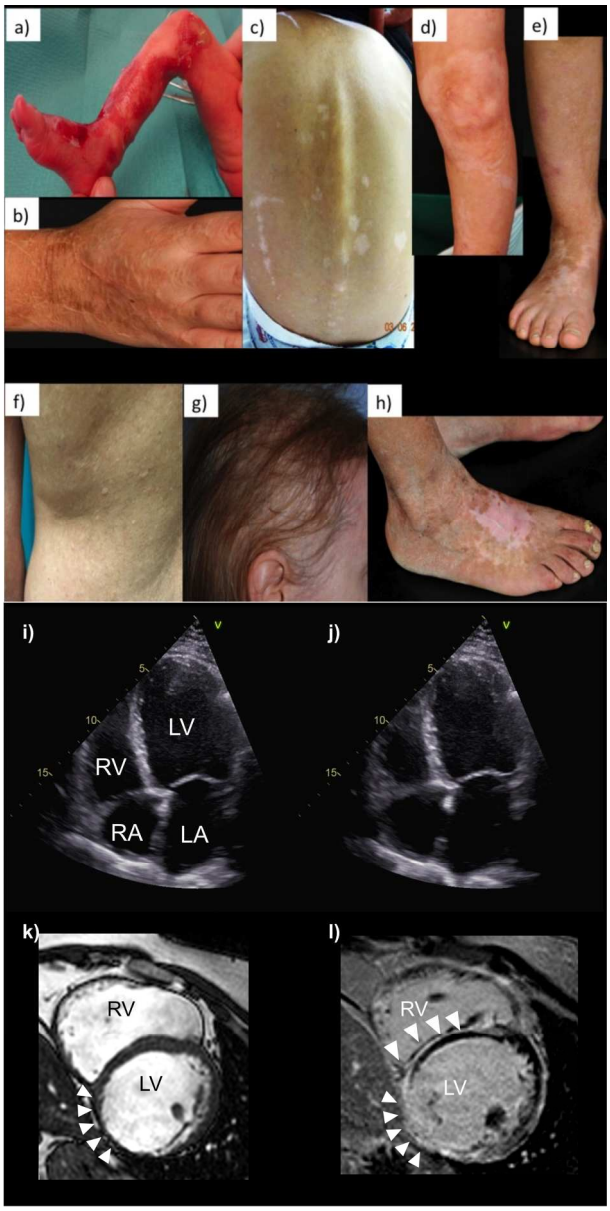


Figure 1. Cutaneous and cardiac features of EBS-KLHL24. (a) Congenital skin defects and blistering, (b) characteristic stellate scarring, and (c-e) macular or whorled hypo- and hyperpigmentation in children, (f-h) mild skin fragility, anetoderma, diffuse alopecia, and nail dystrophy in adults. (i-j) Echocardiography of patient #1: 4-chamber view at end-diastole (i) and end-systole (j) with severe dilatation of LV and LA and severely impaired LV ejection fraction. (k-l). Magnetic resonance imaging (CMR): cine sequence (end-diastole) in the short axis view at basal level (k): wall thinning of inferoseptal and inferior LV wall segments (white arrows). (l) Extensive Late-Gadolinium Enhancement in all segments: transmural fibrosis, corresponding to the thinned inferior and inferolateral wall of the LV (little arrows), non-transmural mid wall fibrosis visible in the septum (large arrows) and to a lesser extent, the lateral, anterior and septal segments of the LV and diffuse fibrosis of the RV.
Abbreviations: LV: Left ventricle; RV: Right ventricle; LA: Left atrium; RA: Right atrium

115x230mm (300 x 300 DPI)

Epidermolysis bullosa simplex with KLHL24 mutations is associated with dilated cardiomyopathy

Schwieger-Briel, Fuentes et al.

Supplementary Material

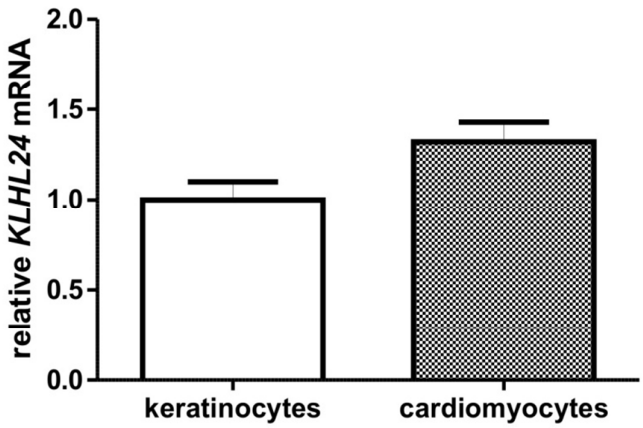
Material and methods

We performed dipstick urinalysis and the following blood assays: complete blood count, liver enzymes, thyroid hormones, creatinine, amylase, lipase, creatine kinase with muscle band (CKMB), and NT-proBNP. Mutation analysis was performed on genomic DNA extracted from EDTA-blood in all 18 patients by Sanger sequencing of exon 4 of *KLHL24*. Furthermore, whole exome sequencing (WES) was performed in individuals 1- 7 (He et al. 2016) and 12-13, and a multigene panel for 68 genes involved in cardiomyopathies was performed in patient 9.

Total RNA was extracted from primary human keratinocytes and cardiomyocytes (Promocell, Heidelberg, Germany) using the Qiagen totalRNA extraction kit. Quantitative real-time PCR was performed with primers published before (He et al. 2016) using iQTM SYBR® Green Supermix and Biorad CFX96 and the BioRad CFX Manager Software (version 1.5).

In patient 5 we performed next generation sequencing (NGS) using the Agilent HaloPlex technology with a custom designed gene panel containing 63 genes associated with cardiac diseases (available on request). The sequencing was performed on an Illumina MiSeq® sequencer using MISEQ REAGENT KIT V2 (2x150bp). Variant calling was performed using GATK (ver. 3.1-1-g07a4bf8) and the variants were annotated using ANNOVAR.

Supplementary Figure



Supplementary Figure. *KLHL24* expression in keratinocytes and cardiomyocytes.