

(CTLA4A<sup>17</sup>) and that this results in inefficient glycosylation and decreased cell-surface expression (Anjos et al., 2002). Our association results show that rs231775 was also strongly associated with AA in our sample although the *P*-values were less significant and the ORs were lower than those for rs3087243. Furthermore, conditional analysis revealed that rs1427678 explained the entire association signal at the locus.

In conclusion, our results provide strong support for the hypothesis that *CTLA4* is a susceptibility gene for AA, and they also suggest that it has the strongest effect in patients with a severe form of the disorder. Given the low *P*-values observed in our study and the genome-wide association study by Petukhova et al. (2010), we consider *CTLA4* a proven susceptibility gene for AA.

#### CONFLICT OF INTEREST

The authors state no conflict of interest.

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**Karsten K.-G. John<sup>1,12</sup>,  
Felix F. Brockschmidt<sup>1,2,12</sup>,  
Silke Redler<sup>1</sup>, Christine Herold<sup>3</sup>,  
Sandra Hanneken<sup>4</sup>, Sibylle  
Eigelshoven<sup>4</sup>, Kathrin A. Giehl<sup>5</sup>,  
Jozef De Weert<sup>6</sup>, Gerhard Lutz<sup>7</sup>,**

**Roland Kruse<sup>8</sup>, Hans Wolff<sup>5</sup>,  
Bettina Blaumeiser<sup>9</sup>, Markus Böhm<sup>10</sup>,  
Tim Becker<sup>3,11</sup>, Markus M. Nöthen<sup>1,2</sup>  
and Regina C. Betz<sup>1</sup>**

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany; <sup>2</sup>Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; <sup>3</sup>Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany;

<sup>4</sup>Department of Dermatology, University of Düsseldorf, Düsseldorf, Germany;

<sup>5</sup>Department of Dermatology, University of Munich, Munich, Germany; <sup>6</sup>Department of Dermatology, University Hospital of Gent, Gent, Belgium; <sup>7</sup>Hair & Nail, Wesseling, Germany; <sup>8</sup>Dermatological Practice, Paderborn, Germany; <sup>9</sup>Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; <sup>10</sup>Department of Dermatology, University of Münster, Münster, Germany and <sup>11</sup>German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany  
E-mail: regina.betz@uni-bonn.de

<sup>12</sup>These authors contributed equally to this work.

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# Erythropoietic Uroporphyrin Associated with Myeloid Malignancy Is Likely Distinct from Autosomal Recessive Congenital Erythropoietic Porphyria

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#### TO THE EDITOR

Congenital erythropoietic porphyria (CEP; MIM 263700) is a rare autosomal

recessive disease caused by mutations in uroporphyrinogen III synthase (UROS) or, rarely, in *GATA1* genes,

leading to UROS deficiency (Fritsch et al., 1997; de Verneuil et al., 2003; Phillips et al., 2007). The resulting overproduction of type I porphyrin isomers by erythroid cells causes severe photosensitivity and hemolytic anemia.

Abbreviations: BFU, burst-forming unit; CEP, congenital erythropoietic porphyria; MDS, myelodysplastic syndrome; UROS, uroporphyrinogen III synthase

It usually presents in infancy; however, 13 patients have been reported who did not present with the disease until adulthood (Fritsch *et al.*, 1997; Kontos *et al.*, 2003; de Verneuil *et al.*, 2003). Some of them had mild late-onset autosomal recessive CEP (de Verneuil *et al.*, 2003); however, five of the seven presenting with the disease after the age of 50 years had an associated myeloid malignancy, usually myelodysplastic syndrome (MDS; Kontos *et al.*, 2003).

We describe three new patients (patients 1–3) aged 64–75 years with a preexisting myeloid malignancy (Supplementary Table S1 online), who subsequently developed CEP (Table 1). Archived blood samples were also available from a previously reported patient (patient 4; Murphy *et al.*, 1995). We show that these patients have a disorder that, although similar to CEP, is likely distinct, with biochemical and molecular features that define it as a separate condition, which we term “erythropoietic uroporphyrin secondary to myeloid malignancy”. All patients gave informed consent to participate in the investigation. Institutional approval for the study was

obtained as required. The study was conducted in accordance with the Declaration of Helsinki principles.

All four patients had bullae on the back of their hands and produced excess porphyrins in the characteristic CEP pattern, but porphyrin concentrations were lower than that in childhood-onset CEP (Table 1). Unlike hereditary CEP, erythrocyte UROS activity was normal (Table 1). We sequenced *UROS* genomic DNA from peripheral blood obtained from these patients and from 24 patients with childhood-onset CEP (Supplementary Materials and Methods online). *UROS* mutations were identified in 19 of the 24 childhood-onset cases but in none of our four MDS patients ( $P \leq 0.01$ ), implying that MDS-associated uroporphyrin is not caused by a germline *UROS* mutation. Deletion of one *UROS* allele was also excluded by demonstrating heterozygosity for a single-nucleotide polymorphism in intron 8 (rs2281955; patients 1–3) and for single-nucleotide polymorphisms in exon 1, intron 5, and intron 8 (rs4256900, rs10901444, rs2281954, and rs3740179; patients 2 and 3).

Fluorescence microscopic studies of bone marrow aspirates from patients 1 and 2 showed that only ~25% of normoblasts had the characteristic red nuclear fluorescence of uroporphyrin (Figure 1a and b). We cultured burst-forming unit-erythroid colonies from the peripheral blood of patient 2; of seven colonies picked at random, only one showed red porphyrin fluorescence (Figure 1c and d). We conclude that the porphyrin defect is present in only a minority of hemopoietic cells.

Genomic instability of myeloid progenitors in MDS causes gross chromosomal abnormalities and point mutations. Bone marrow karyotyping showed that patient 2 had a partial deletion of chromosome 11q (breakpoint in band q1.4), whereas patient 1 had no abnormalities. No deletions of chromosome 10, which carries *UROS* (q25.2–q26.3), were observed. No *UROS* mutations were identified by sequencing *UROS* genomic DNA (patients 1 and 2) or complementary DNA (patient 1) from unfractionated bone marrow cells. Both patients showed the same single-nucleotide polymorphism heterozygosity pattern in genomic DNA

**Table 1. Metabolite and enzyme measurements in patients with uroporphyrin**

Patient	Age at onset of myeloid disorder (years)	Age at onset of bullous skin lesions (years)	Total urine porphyrin (nmol l <sup>-1</sup> )	Total fecal porphyrin (nmol g <sup>-1</sup> )	Plasma porphyrin (nmol l <sup>-1</sup> )	Erythrocyte porphyrin (μmol l <sup>-1</sup> )	Erythrocyte UROS activity (% mean control)
1	71	72	3,278 (79%)	718 (86%)	150 <sup>1</sup>	2.4 <sup>2</sup> (58%)	98
2	74	75	19,332 (82%)	1,413 (79%)	Increased	5.3 (52%)	112
3	57	64	12,330 (69%)	2,628 (76%)	543	4.7 <sup>3</sup>	—
4 <sup>4</sup>	60	65	12,280 (97%)	1,655	—	1.9	—
Childhood-onset CEP	No myeloid disorder	<5	44,000 (7)	6,111 (7)	1,121 (15)	22.1 (15)	16–26 (3)
			11,986–123,249	1,797–11,687	232–5,125	7.2–125.1	
			(NS) <sup>5</sup>	( $P=0.02$ ) <sup>5</sup>		( $P=0.003$ ) <sup>5</sup>	
Reference range			20–320	10–200	<11.2	0.4–1.7	

Abbreviations: CEP, congenital erythropoietic porphyrin; UROS, uroporphyrinogen III synthase.

Urinary and fecal porphyrin concentrations for childhood-onset CEP are for adults. Figures in parentheses are percent isomer I for uroporphyrin (urine) or coproporphyrin (feces), percent zinc-protoporphyrin for erythrocyte porphyrin or, for CEP, number of patients. For CEP, figures are medians and ranges. Fluorescence emission maxima for total plasma porphyrins were 617–618 nm in all cases.

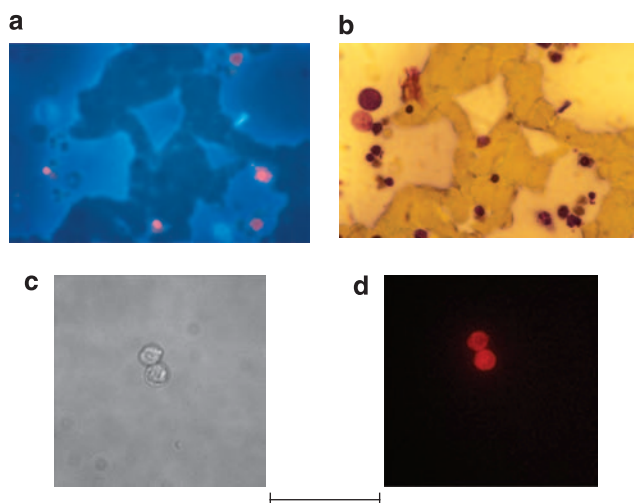
<sup>1</sup>Median of 12 measurements over 64 months; range 89–1,818 nmol l<sup>-1</sup>.

<sup>2</sup>Median of 8 measurements over 64 months; range 1.3–14.3 μmol l<sup>-1</sup>.

<sup>3</sup>Mainly zinc protoporphyrin.

<sup>4</sup>Data from the study by Murphy *et al.*, 1995.

<sup>5</sup>Significance of difference between patient (patients 1–4) and CEP groups: NS, not significant. For methods, see Supplementary Materials and Methods online.



**Figure 1. Fluorescence microscopic study of bone marrow and cells from burst-forming unit (BFU)-erythroid colonies.** Top: Bone marrow smear from patient 1 showing the same field under fluorescence microscope (a) and stained with Wright-Giemsa stain to identify erythroid cells (b). Bottom: Cells from BFU-erythroid colonies cultured from peripheral blood of patient 2. (c) Light microscopy. (d) Fluorescence microscopy showing red porphyrin fluorescence. Cells from two different colonies are shown. BFU-erythroid colonies were cultured from peripheral blood mononuclear cells (Supplementary Materials and Methods online). For fluorescence microscopy, bone marrow smears or cells from BFU-erythroid colonies were mounted in vectashield (Alpha Laboratories, Eastleigh, Hampshire, UK). Photomicrographs were taken with a Zeiss Axioplan microscope (Carl Zeiss, Welwyn Garden City, UK) using a tetramethylrhodamine isothiocyanate filter at 200 $\times$  total magnification using a 20 $\times$ /0.50 objective lens. Bar = 0.1 mm.

from bone marrow as in peripheral blood cells, excluding deletion of one allele in the majority of marrow cell nuclei. Mutations in *GATA-1* on the X chromosome may also cause CEP. Karyotyping showed no X chromosome abnormalities, and no *GATA-1* mutations were identified in genomic DNA from peripheral blood (all four patients) or from bone marrow (patient 1) by sequencing or by denaturing high-performance liquid chromatography (Supplementary Methods online). Similar to the fluorescence microscopic results, these data suggest that any acquired *UROS* or *GATA1* mutation causing the porphyria is unlikely to be present in a high proportion of hemopoietic cells.

Our three patients bring the total number of reported cases of erythropoietic uroporphyrin associated with myeloid malignancy to eight. All were male patients, had a preexisting myeloid disorder, and presented with fragility and blistering in exposed skin after the age of 50 years. Hemorrhagic bullae in our four patients and in others presumably reflect thrombocytopenia (Kontos *et al.*, 2003). None had erythrodontia, a feature of childhood CEP

also absent from the adult-onset hereditary cases (Deybach *et al.*, 1981). None had a family history of CEP.

We have shown that these patients have lower erythrocyte porphyrin concentrations compared with childhood (Table 1) or adult-onset CEP (Deybach *et al.*, 1981) and that erythrocyte *UROS* activity is normal (Table 1) in contrast to the reduced activity in the hereditary disease (Deybach *et al.*, 1981; Desnick and Astrin, 2002; de Verneuil *et al.*, 2003). We did not find germline mutations in *UROS* or *GATA1*, in contrast to hereditary CEP (Desnick and Astrin, 2002; de Verneuil *et al.*, 2003; Phillips *et al.*, 2007). These clinical and pathological findings identify “uroporphyrin associated with myeloid malignancy” as a syndrome distinct from hereditary CEP.

The low erythrocyte porphyrin and normal *UROS* activity in our patients suggest that only a small proportion of circulating red cells are uroporphyrin and are consistent with the evidence from bone marrow microscopy and cell culture (Figure 1) that erythroid cells in uroporphyrin associated with MDS are a mosaic of normal and uroporphyrin

cells, with the former predominating. Acquired forms of  $\alpha$ -thalassemia (Steensma *et al.*, 2005) and erythropoietic protoporphyria (Goodwin *et al.*, 2006) occurring in association with MDS result from somatic mutations in clones of myelodysplastic cells. It seems probable that the minor clone of uroporphyrin erythropoietic cells in our patients similarly contains an acquired somatic mutation but one that leads to *UROS* deficiency.

The cause of *UROS* deficiency in uroporphyrin cells remains to be identified. Our inability to find *UROS* or *GATA1* mutations may be explained by the difficulty experienced in detecting mosaicism when the proportion of abnormal cells is low; the techniques we used were unlikely to detect less than  $\sim 15\%$  of mutant DNA. Alternatively, the abnormality may be in another gene that determines *UROS* activity.

#### CONFLICT OF INTEREST

The authors state no conflict of interest.

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**Robert P.E. Sarkany<sup>1</sup>, Sally H. Ibbotson<sup>2</sup>, Sharon D. Whatley<sup>3</sup>, Clifford M. Lawrence<sup>4</sup>, Pamela Gover<sup>5</sup>, Ghulam J. Mufti<sup>6</sup>, Gillian M. Murphy<sup>7</sup>, Gillian S. Masters<sup>8</sup>, Michael N. Badminton<sup>3</sup> and George H. Elder<sup>3</sup>**

<sup>1</sup>Photodermatology Unit, St John's Institute of Dermatology, London, UK; <sup>2</sup>Photobiology Unit, Ninewells Hospital and Medical School, Dundee, UK; <sup>3</sup>Department of Medical Biochemistry and Immunology, University Hospital of Wales and School of Medicine, Cardiff University, Cardiff, UK; <sup>4</sup>Department of Dermatology, Royal Victoria Infirmary, Newcastle Upon Tyne, UK; <sup>5</sup>Department of Haematology, Eastbourne District General Hospital, Eastbourne, UK; <sup>6</sup>Department of Haematological Medicine, King's College London, London, UK; <sup>7</sup>Photobiology Unit,

Beaumont Hospital, Dublin, Ireland and  
<sup>8</sup>Department of Haematology, University  
 Hospital of Wales and School of Medicine,  
 Cardiff University, Cardiff, UK  
 E-mail: Robert.Sarkany@gstt.nhs.uk

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#### SUPPLEMENTARY MATERIAL

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