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Severe neuroimaging anomalies are usually associated with random X inactivation in leucocytes circulating DNA in X-linked dominant Incontinentia Pigmenti

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ABSTRACT

Incontinentia Pigmenti (IP) is a skin disorder with neurological impairment in 30% of cases. The most common disease causing mutation is a deletion of exons 4-10 of the IKBKG gene, located on chromosome Xq28, with skewed X-chromosome inactivation in females, but few cases of random X-inactivation have been reported. We have correlated brain anomalies with X-chromosome inactivation status determined on leucocytes circulating DNA. We reviewed MRI of 18 girls with genetically proven IP. We found three patterns of MRI, normal MRI ($n = 5$), mild white matter abnormalities with cortical and corpus callosum atrophy ($n = 6$), and severe cortical abnormalities suggesting a vascular disease ($n = 7$). Most patients with severe abnormalities had random X-inactivation (6/7,86%), while 80% (4/5) of patients with normal MRI and 100% (6/6) of patients with mild white matter abnormalities had skewed inactivation. These results suggest that skewed chromosome X-inactivation may protect brain from damage, while in case of random inactivation, expression of the mutated *IKBKG* gene may lead to severe brain lesions.

1. Introduction

Incontinentia pigmenti (IP, OMIM#308300) is an X-linked dominant skin disorder characterized by a four stage skin eruption in heterozygote females, followed by Blaschko lines. IP is usually lethal in male fetuses. Tooth anomalies and ophthalmologic manifestations might be severe [1] and central nervous system manifestations occur in approximately 30% of cases [2]. The most frequent neurological anomalies include seizures, motor impairment and mental retardation [2,3]. In 2000, the International Incontinentia Pigmenti Consortium has ascribed IP to mutations of the inhibitor of the kappa B kinase gamma gene (*IKBKG, NEMO*), located on chromosome Xq28 [4–6]. The most common disease causing mutation is a deletion of exons 4-10 of the *IKBKG* gene located on chromosome Xq28 [4,5,7]. This mutation is associated with skewed X chromosome inactivation in females, but few cases of random X inactivation have been also reported [8]. Various patterns of brain MRI anomalies have been reported, including white matter T2-weighted hypersignal, corpus callosum and cortical atrophy, and rarely ischemic and hemorrhagic lesions [2,9]. To date, why some but not all IP patients develop neurological anomalies remains poorly understood.

We have questioned whether brain anomalies in IP could be associated with either particular *IKBKG* mutations or a specific pattern of X-chromosome inactivation.

Abbreviations: IP, Incontinentia Pigmenti; IKBKG, Inhibitor of the Kappa B Kinase Gamma gene.

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2. Methods

Brain MRIs of 18 IP patients seen in our hospital between 1997 and 2016 were included in the study (18 girls). Informed consent was waived for this retrospective observational study.

Nine IP girls had brain imaging for neurological symptoms, namely seizures (7/18), *status epilepticus* (5/18), hemiparesis (4/18) and mental retardation (4/18). Three adult patients and three affected girls with no overt neurological symptoms were also included in the study. Criteria for inclusion in the study were *i)* clinical evidence of IP, *ii)* molecular evidence of *IKBKG* mutation and *iii)* availability of brain MRI, performed either systematically or for various neurological symptoms (Table 1).

Genetic studies included the search for the most common deletion of exons 4-10 of the *IKBKG* gene [10] and, when negative, Sanger sequencing of *IKBKG* coding exons after specific long-range PCR amplification of the *IKBKG* gene, so as to eliminate the *IKBKGP1* pseudogene [11]. The pattern of chromosome X inactivation was determined on leucocytes circulating DNA at the HUMARA locus, and for uninformative patients, at the FMR1 locus using the Genescan software on an ABI 3130 sequencing analyzer [10]. X inactivation was considered skewed when more than 80% of a given allele was active/inactive.

MRI were performed in various radiology units on 1.5 T Tesla scanners (1 T for the earliest examinations). All patients underwent T1 and T2-weighted sequences (or Fluid Attenuated Inversion Recovery), 15/18 had DWI sequences and 5/18 had gradient echo T2-weighted imaging. Brain MRI were analyzed by two experienced neuro-radiologists blinded for the type of *IKBKG* mutation and pattern of chromosome X inactivation.

3. Results

Most patients carried the common exon 4-10 deletion in the *IKBKG* gene (15/18) but some had missense or nonsense mutations (3/18). All mutations but one (patient 17) have been previously reported as disease causing (Table 1). The heterozygote variation found in patient 17

Table 1
Clinical, MRI and genotype data.

Patient	Age	Neurological symptoms	White matter anomalies	Corpus callosum	Cortex	Diffusion-weighted imaging	T2*	IKBKG variation	X inactivation	Tested locus
Normal MRI										
Patient 1	37y	0	0	Normal	Normal	Normal	NA	del 4–10	Skewed (100%)	HUMARA
Patient 2	11y	0	0	Normal	Normal	Normal	NA	del 4–10	Skewed (96/4%)	HUMARA
Patient 3	74y	0	0	Normal	Normal	Normal	NA	del 4–10	Skewed (90/10%)	HUMARA
Patient 4	4 m	0	0	Normal	Normal	Normal	NA	del 4–10	Skewed (90/10%)	HUMARA
Patient 5	41y	0	0	Normal	Normal	Normal	NA	del 4–10	Random (70/30%)	HUMARA
Mild white matter abnormalities										
Patient 6	13 m	0	Periventricular	Thin	Frontal atrophy	Normal	NA	del 4–10	Skewed (100%)	FMR1
Patient 7	18y	SE, seizures, MR, hemiparesis	Periventricular	Thin	Central sulcus atrophy	NA	NA	c.454delG, p.Glu152fsX129	Skewed (100%)	FMR1
Patient 8	3y	SE, seizures, MR, hemiparesis	Periventricular	Atrophic	Central sulcus atrophy	Normal	NA	del 4–10	Skewed (99/1%)	HUMARA
Patient 9	24 m	NA	Periventricular	Normal	Normal	Normal	Normal	del 4–10	Skewed (94/6%)	HUMARA
Patient 10	11 m	NA	Periventricular	Thin	Frontal atrophy	NA	NA	del 4–10	Skewed (90/10%)	HUMARA
Patient 11	20y	NA	Periventricular	Normal	Normal	Normal	NA	del 4–10	Skewed (90/10%)	HUMARA
Severe abnormalities										
Patient 12	8y	Seizures	Periventricular and subcortical	Thin	Frontal ischemic sequelae	Normal	Normal	del 4–10	Skewed (85/15%)	HUMARA
Patient 13	2 m	Seizures	0	Normal	Normal	Multiple punctuated lesions	Multiple punctuated lesions (less than diffusion)	del 4–10	Random (76/24%)	HUMARA
Patient 14	3 m	SE	0	Normal	T2 hypersignal	Multiple asymmetrical punctuated lesions	Multiple punctuated lesions (less than diffusion)	del 4–10	Random (70/30%)	HUMARA
Patient 15	7y	Seizures, MR, hemiparesis	Periventricular and subcortical	Thin	Atrophy Frontal ischemic sequelae	NA	NA	del 4–10	Random (70/30%)	HUMARA
Patient 16	3d	SE, death	Periventricular and subcortical	Normal	T2 hypersignal	Multiple asymmetrical punctuated lesions	Multiple punctuated lesions (less than diffusion)	del 4–10	Random (60/40%)	HUMARA
Patient 17	2y	SE, seizures, MR, hemiparesis	Periventricular and subcortical, posterior fossa	Atrophic	Polymicrogyria (cerebrum and cerebellum), global atrophy	Normal	NA	c.227delG, p.C76fsX38	Random (60/40%)	FMR1
Patient 18	12 m	Seizures	Periventricular and subcortical	Normal	Frontal ischemic sequelae Contrast uptake (disrupted BBB)	Multiple punctuated lesions	NA	c.940G > C, p.Ala314Pro	Random (50/50%)	HUMARA

SE: Status Epilepticus. MR: Mental Retardation. NA: Not Available.

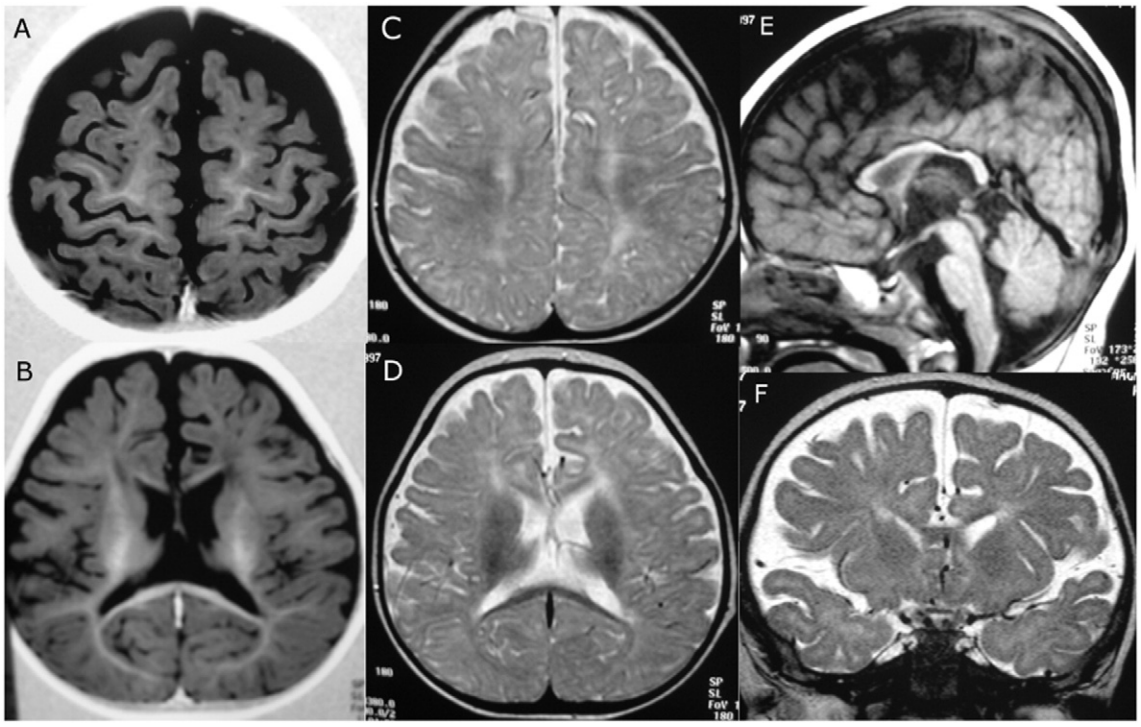

Fig. 1. MRI of patient 10 (11 months-old girl). (A) (B) Axial T1-weighted images, displaying mild atrophy predominating in frontal lobe. (C) (D) Axial T2-weighted images, displaying periventricular white matter hypersignal. (E) Sagittal T1-weighted image on the midline, displaying corpus callosum atrophy. (F) Coronal T2-weighted images, showing atrophy and white matter hypersignal.

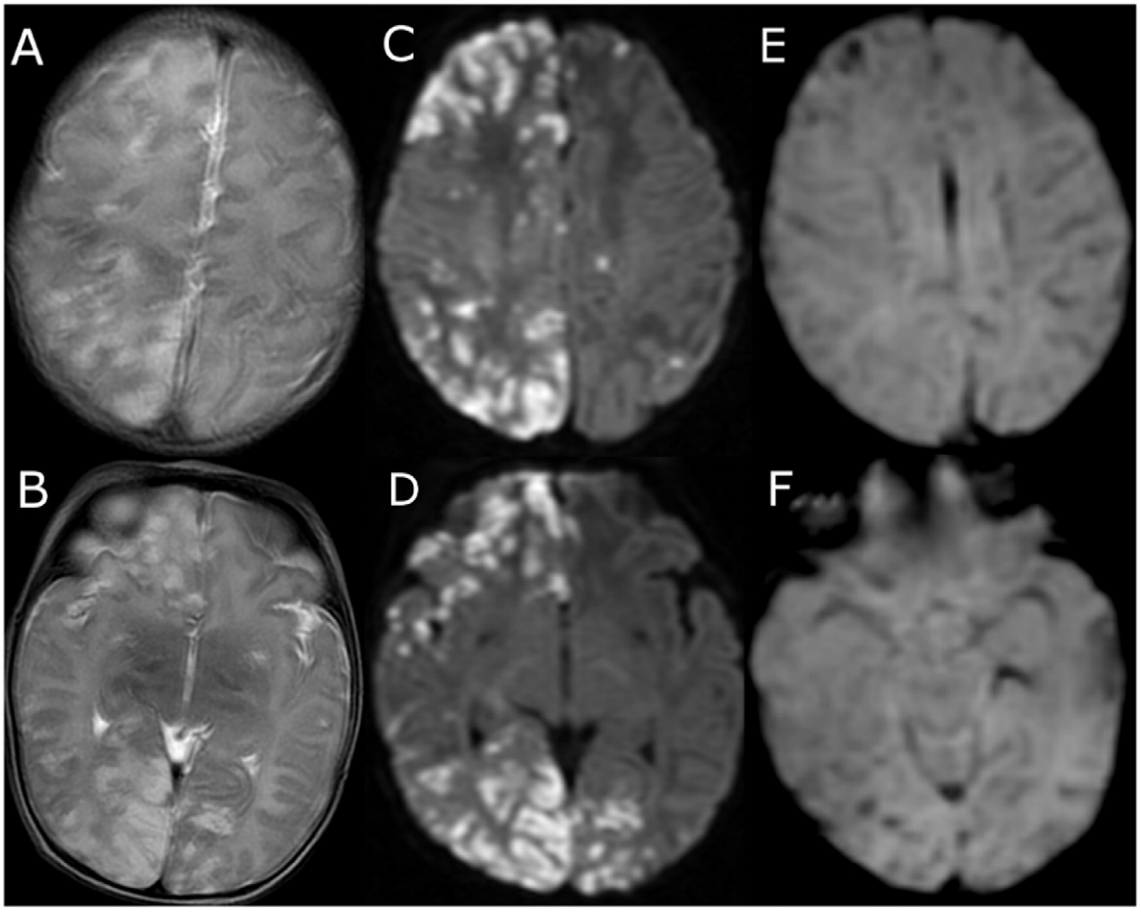

Fig. 2. MRI of patient 16 (3-days-old girl). (A)(B) Spin echo T2-weighted axial images (C)(D) Difussion-weighted axial images with b = 1000 (E)(F) Gradient echo T2-weighted axial images. This figure shows multiple bilateral ischemic lesions, without arterial territory, and few hemorrhagic lesions.

(c.227delG) was considered pathogenic as it caused a frameshift and a premature termination of translation.

Three patterns of brain MR imaging were found in our study, namely *i)* normal MRI in three adults and two girls with no neurological symptoms (5/18); *ii)* mild anomalies of the periventricular white matter with T2-weighted hyper-signal in 7/18 patients, occasionally associated with mild cortical atrophy (5/7) and atrophy of the *corpus callosum* (5/7, Fig. 1), and *iii)* severe cortical anomalies suggestive of a vascular disease in 7/18 patients, including multiple areas of acute diffusion restriction without vascular territory, contrast uptake, micro-bleeds (Fig. 2), severe atrophy and images consistent with ischemic sequelae (focal cortical atrophy, ulegyria, see Fig. 3). Interestingly, all affected patients in this last group had severe neurological involvement including one neonatal death following *status epilepticus* (patient 16).

No correlation between mutation type (deletion vs missense/nonsense mutations) and MRI presentation was found. Most interestingly however, patients with severe MRI abnormalities had random X inactivation (6/7, 86%), while patients with no or mild MRI abnormalities had skewed X inactivation (10/11, 91%,).

4. Discussion

Here we show that severe brain MRI anomalies were usually associated with random X inactivation and severe neurological anomalies, while skewed X inactivation was associated with no or mild brain MRI anomalies. These results suggest that skewed chromosome X inactivation may protect brain from damage, while in case of random inactivation, expression of the mutated *IKBKG* gene may lead to severe brain lesions.

While several studies have reported abnormal brain MRIs in IP patients, the present study reports what we believe to be the first evidence of a correlation between severe neurological/brain MRI anomalies and a random pattern of chromosome X inactivation in IP. The most frequent brain MRI anomalies reported to date included white matter T2-

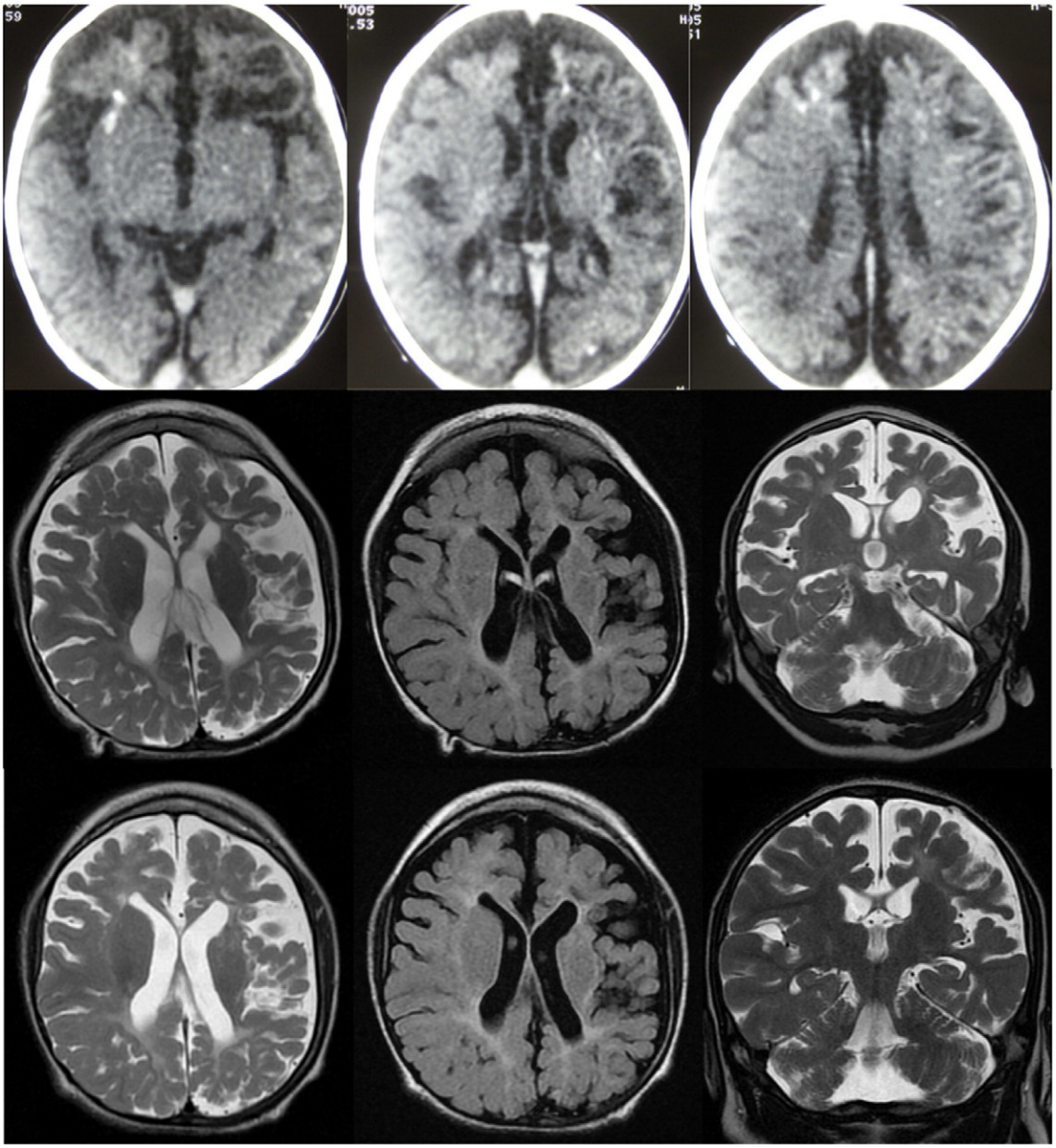

Fig. 3. Imaging of patient 17. Neonatal CT scan (first line, 3 days-old) showed cortical and subcortical ischemic lesions, without vascular territory, predominantly in the left hemisphere, and cortical calcification. Repeated MRI (2 years-old on second line, 7-year-old on third line) showed stable cortical atrophy with diffuse polymicrogyria, predominantly in the left hemisphere, consistent with sequelae of prenatal ischemic lesions.

weighted hyper-signals and mild cortical and corpus callosum atrophy [2,12–15]. Several authors also reported on ischemic and hemorrhagic diffuse lesions, always in neonates [13,16,17]. Consistent with our findings, Soltirovska Salamon et al. recently reported on ischemic or hemorrhagic changes in 7/8 IP neonates [9]. Other studies reported acute ischemic lesions in older children [13,18], yet within systematized vascular territories of large vessel occlusion, suggestive of a different pathophysiological mechanism. Our findings of non-systematized lesions suggest microvascular anomaly rather than macrovascular mechanism.

One may hypothesize that skewed X-inactivation is a result of death and elimination of cells expressing the mutated IKBKG gene. *IKBKG* is known to be actively expressed in endothelial brain cells [19], and Ridder et al. reported that neurological involvement in IP might be due to endothelial cell death, rarefaction of brain micro-vessels, cerebral hypo-perfusion and disruption of the blood-brain barrier, triggered by *IKBKG* mutations [19]. Therefore, late X-chromosome inactivation and consecutive endothelial cell death may explain severe neurological lesions in "random" patients, while "skewed" patients, whose endothelial cell death may have occurred earlier during embryogenesis are more likely to have normal MRIs. The hypothesis that skewed chromosome X inactivation could protect brain cortex from vascular damage has been previously considered to account for variability of retinal involvement in IP [20]. To demonstrate this hypothesis, evaluation of X-inactivation directly in the brain would be necessary, but is clinically non-feasible. Therefore, we must hypothesize that blood cell X-inactivation pattern reflects X-inactivation pattern in the brain.

A limit of our study is that patients within the "severe abnormalities" group are globally younger than those in the other groups, while skewing may sometimes develop with age. Unfortunately, it is ethically impossible to perform MRI in IP neonates without neurological symptoms, and therefore impossible to compare imaging features of neonate patients with and without skewing. Monitoring X-inactivation pattern in blood cells of IP patients over time is an alternative to demonstrate the association between MRI/severe neurological anomalies with random X inactivation, but it requires repeated blood-sampling. Nevertheless, we report in the "severe abnormalities" group two older patients (2 years-old and 7 years-old) with random X inactivation and ischemic sequelae, who may be compared with patients of the same age in the other groups.

Based on our results, we suggest giving consideration to the pattern of chromosome X inactivation as a prognostic factor of neurological involvement in IP. Further studies will hopefully confirm these results and help elucidating the putative role of other factors in onset of neurological/brain MRI anomalies in IP.

Conflict of interest statement

None of the authors have any conflict of interest to disclose.

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References

[1] F. Fusco, M. Paciolla, M.I. Conte, A. Pescatore, E. Esposito, P. Mirabelli, M.B. Lioi, M.V. Ursini, Incontinentia pigmenti: report on data from 2000 to 2013, Orphanet J. Rare Dis. 9 (2014) 1–5.

[2] M.E.C. Meuwissen, G.M.S. Mancini, Neurological findings in incontinentia pigmenti; a review, Eur. J. Med. Genet. 55 (2012) 323–331, http://dx.doi.org/10.1016/j.ejmg.2012.04.007.

[3] S. Minić, D. Trpinac, M. Obradović, Systematic review of central nervous system anomalies in incontinentia pigmenti, Orphanet J. Rare Dis. 8 (2013) 25–35.

[4] A. Smahi, G. Courtois, P. Vabres, S. Yamaoka, S. Heuertz, A. Munnich, A. Israël, N.S. Heiss, S.M. Klauck, P. Kioschis, S. Wiemann, A. Poustka, T. Esposito, T. Bardaro, F. Gianfrancesco, A. Ciccodicola, M. D'Urso, H. Woffendin, T. Jakins, D. Donnai, H. Stewart, S.J. Kenwrick, S. Aradhya, T. Yamagata, M. Levy, R.A. Lewis, D.L. Nelson, Genomic rearrangement in NEMO impairs NF-kappaB activation and is a cause of incontinentia pigmenti. The International Incontinentia Pigmenti (IP) Consortium, Nature 405 (2000) 466–472, http://dx.doi.org/10.1038/35013114.

[5] S. Aradhya, H. Woffendin, T. Jakins, T. Bardaro, T. Esposito, A. Smahi, C. Shaw, M. Levy, A. Munnich, M. D'Urso, R.A. Lewis, S. Kenwrick, D.L. Nelson, A recurrent deletion in the ubiquitously expressed NEMO (IKK-gamma) gene accounts for the vast majority of incontinentia pigmenti mutations, Hum. Mol. Genet. 10 (2001) 2171–2179.

[6] A. Smahi, G. Courtois, S.H. Rabia, R. Döffinger, C. Bodemer, A. Munnich, J.-L. Casanova, A. Israël, The NF-κB signalling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes, Hum. Mol. Genet. 11 (2002) 2371–2375.

[7] G. Courtois, A. Smahi, A. Israël, NEMO/IKKγ: linking NF-κB to human disease, Trends Mol. Med. 7 (2001) 427–430.

[8] S. Aradhya, G. Courtois, A. Rajkovic, R.A. Lewis, M. Levy, A. Israël, D.L. Nelson, Atypical forms of incontinentia pigmenti in male individuals result from mutations of a cytosine tract in exon 10 of NEMO (IKK-gamma), Am. J. Hum. Genet. 68 (2001) 765–771, http://dx.doi.org/10.1086/318806.

[9] A. Soltirovska Salamon, K. Lichtenbelt, F.M. Cowan, A. Casaer, J. Dudink, A. Dereymaeker, D. Paro-Panjan, F. Groenendaal, L.S. de Vries, Clinical presentation and spectrum of neuroimaging findings in newborn infants with incontinentia pigmenti, Dev. Med. Child Neurol. (2016)http://dx.doi.org/10.1111/dmcn.13140.

[10] J. Steffann, V. Raclin, A. Smahi, H. Woffendin, A. Munnich, S.J. Kenwrick, A.-G. Grebille, A. Benachi, Y. Dumez, J.-P. Bonnefont, S. Hadj-Rabia, A novel PCR approach for prenatal detection of the common NEMO rearrangement in incontinentia pigmenti, Prenat. Diagn. 24 (2004) 384–388, http://dx.doi.org/10.1002/pd.889.

[11] M.I. Conte, A. Pescatore, M. Paciolla, E. Esposito, M.G. Miano, M.B. Lioi, M.A. McAleer, G. Giardino, C. Pignata, A.D. Irvine, A.E. Scheuerle, G. Royer, S. Hadj-Rabia, C. Bodemer, J.-P. Bonnefont, A. Munnich, A. Smahi, J. Steffann, F. Fusco, M.V. Ursini, Insight into *IKBKG*/*NEMO* locus: report of new mutations and complex genomic rearrangements leading to incontinentia pigmenti disease, Hum. Mutat. 35 (2014) 165–177, http://dx.doi.org/10.1002/humu.22483.

[12] S. Hadj-Rabia, D. Froidevaux, N. Bodak, D. Hamel-Teillac, A. Smahi, Y. Touil, S. Fraitag, Y. de Prost, C. Bodemer, Clinical study of 40 cases of incontinentia pigmenti, Arch. Dermatol. 139 (2003) 1163–1170, http://dx.doi.org/10.1001/archderm.139.9.1163.

[13] A.G. Lee, M.F. Goldberg, J.H. Gillard, P.B. Barker, R.N. Bryan, Intracranial assessment of incontinentia pigmenti using magnetic resonance imaging, angiography, and spectroscopic imaging, Arch. Pediatr. Adolesc. Med. 149 (1995) 573–580.

[14] S. Mangano, A. Barbagallo, Incontinentia pigmenti: clinical and neuroradiologic features, Brain Dev. 15 (1993) 362–366.

[15] M. Aydin, N. Hakan, N. Demirel, U. Deveci, A. Zenciroglu, N. Okumus, Neurological involvement in incontinentia pigmenti, Eur. J. Pediatr. 173 (2014) 547, http://dx.doi.org/10.1007/s00431-013-2167-4.

[16] N.I. Wolf, N. Krämer, I. Harting, A. Seitz, F. Ebinger, J. Pöschl, et al., Diffuse cortical necrosis in a neonate with incontinentia pigmenti and an encephalitis-like presentation, Am. J. Neuroradiol. 26 (2005) 1580–1582.

[17] S. Chatkupt, A.O. Gozo, L.J. Wolansky, S. Sun, Characteristic MR findings in a neonate with incontinentia pigmenti, AJR Am. J. Roentgenol. 160 (1993) 372–374.

[18] L. Fiorillo, D.B. Sinclair, M.L. O'Byrne, A.L. Krol, Bilateral cerebrovascular accidents in incontinentia pigmenti, Pediatr. Neurol. 29 (2003) 66–68, http://dx.doi.org/10.1016/S0887-8994(03)00144-9.

[19] D.A. Ridder, J. Wenzel, K. Müller, K. Töllner, X.-K. Tong, J.C. Assmann, S. Stroobants, T. Weber, C. Niturad, L. Fischer, B. Lembrich, H. Wolburg, M. Grand'Maison, P. Papadopoulos, E. Korpos, F. Truchetet, D. Rades, L.M. Sorokin, M. Schmidt-Supprian, B.J. Bedell, M. Pasparakis, D. Balschun, R. D'Hooge, W. Löscher, E. Hamel, M. Schwaninger, Brain endothelial TAK1 and NEMO safeguard the neurovascular unit, J. Exp. Med. 212 (2015) 1529–1549, http://dx.doi.org/10.1084/jem.20150165.

[20] C.J. Chen, I.C. Han, M.F. Goldberg, Variable expression of retinopathy in a pedigree of patients with incontinentia pigmenti, Retina Phila. Pa. (2015) http://dx.doi.org/10.1097/IAE.0000000000000615.