Mosaicism

and PI3K-AKT genetics

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Definition

In genetics, mosaicism denotes the presence of two or more populations of cells with different genotypes in an individual who developed from a single fertilized egg

Research Article

A Quantitative Measurement of the Human Somatic Mutation Rate

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Abstract

The mutation rate (μ) is a key biological feature of somatic cells that determines risk for malignant transformation, and it has been exceedingly difficult to measure in human cells. For this purpose, a potential sentinel is the X-linked *PIG-A* gene, because its inactivation causes lack of glycosylphosphatidylinositol-linked membrane proteins. We previously found that the frequency (f) of *PIG-A* mutant cells can be measured accurately by flow cytometry, even when f is very low. Here we measure both f and μ by culturing B-lymphoblastoid cell lines and first eliminating preexisting *PIG-A* mutants by flow sorting. After expansion in culture, the frequency of *new* mutants is determined by flow cytometry using antibodies specific for glycosylphosphatidylinositol-linked proteins (e.g., CD48, CD55, and CD59). The mutation rate is then calculated by the formula $\mu = f/d$, where d is the number of cell divisions occurring in culture. The mean μ in cells from normal donors was 10.6×10^{-7} mutations per cell division (range 2.4 to 29.6×10^{-7}). The mean μ was elevated >30-fold in cells from patients with Fanconi anemia ($P < 0.0001$), and μ varied widely in ataxia-telangiectasia with a mean 4-fold elevation ($P = 0.002$). In contrast, μ was not significantly different from normal in cells from patients with Nijmegen breakage syndrome. Differences in μ could not be attributed to variations in plating efficiency. The mutation rate in man can now be measured routinely in B-lymphoblastoid cell lines, and it is elevated in cancer predisposition syndromes. This system should be useful in evaluating cancer risk and in the design of preventive strategies. (Cancer Res 2005; 65(18): 8111-7)

Introduction

In microbial organisms, the rate of mutation can be measured by Luria-Delbrück fluctuation analysis (1) or in continuous cultures (2, 3). By such methods, the rates of inactivating (forward) mutations in *lacI* in *Escherichia coli* and *CAN1* in *Saccharomyces*

division (μ_s) and the number of cell divisions (d) that have occurred since embryogenesis, this expression can be written as = $\mu_1 d \cdot \mu_2 d \cdot \mu_3 d \ldots \mu_n d$. Using μ as the geometric mean of the mutation rates for the genes involved, and k as a constant <1, to account for cell death, the probability (P) of a cell becoming malignant can be expressed as $P = k(\mu d)^n$. Thus, P increases as a power function of both μ and d. Because d increases with age, this formula is consistent with the general increase in cancer rates with age (8). Although μ is well recognized as a critical variable (see discussion in ref. 9), in the 60 years since Luria and Delbrück's landmark study, measuring μ in man remains quite challenging.

We previously described a technique for determining f for the X-linked *PIG-A* gene in humans (10). The *PIG-A* gene encodes one of the subunits of an enzyme essential for an early step in the biosynthesis of glycosylphosphatidylinositol (GPI; refs. 11, 12). Deficiency of GPI-linked proteins from the surface of blood cells is characteristic of the human disease paroxysmal nocturnal hemoglobinuria (PNH), and it results from somatic mutations of *PIG-A* (13). Spontaneously arising *PIG-A* mutations have been identified in a broad range of cell types (10, 14–16). Mutant cells cannot express the set of proteins that require GPI for attachment to the cell surface (the "GPI-anchored" proteins)—resulting in cells with a "GPI-negative phenotype"—which can coexist with normal cells in stable proportions over a prolonged period of time in humans (17) and mice (18). We now use these properties of *PIG-A* to directly measure μ in human cells.

Materials and Methods

Cell lines. Normal B-lymphoblastoid cell lines (BLCL) were a gift from Dr. Gunther Koehn and Dr. Nathan Ellis (Memorial Sloan-Kettering Cancer Center, New York, NY). Cell lines from patients with cancer predisposition syndromes are summarized in Table 1. BLCLs from patients with PNH (19) were generated as previously described (20). Patients with PNH are somatic cell mosaics, or "somatic chimeras" and harbor GPI$^+$ and GPI$^-$ subpopulations of hematopoietic cells: sometimes both can be

The mutation rate is then calculated by the formula $\mu = f/d$, where d is the number of cell divisions occurring in culture. The mean μ in cells from normal donors was 10.6×10^{-7} mutations per cell division (range 2.4 to 29.6×10^{-7}).

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ORIGINAL ARTICLE

An estimation of the number of cells in the human body

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Abstract

Background: All living organisms are made of individual and identifiable cells, whose number, together with their size and type, ultimately defines the structure and functions of an organism. While the total cell number of lower organisms is often known, it has not yet been defined in higher organisms. In particular, the reported total cell number of a human being ranges between 10^{12} and 10^{16} and it is widely mentioned without a proper reference.
Aim: To study and discuss the theoretical issue of the total number of cells that compose the standard human adult organism.
Subjects and methods: A systematic calculation of the total cell number of the whole human body and of the single organs was carried out using bibliographical and/or mathematical approaches.
Results: A current estimation of human total cell number calculated for a variety of organs and cell types is presented. These partial data correspond to a total number of 3.72×10^{13}.
Conclusions: Knowing the total cell number of the human body as well as of individual organs is important from a cultural, biological, medical and comparative modelling point of view. The presented cell count could be a starting point for a common effort to complete the total calculation.

Keywords

Cell size, human cell number, organ, total cell count, theoretical issue

History

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Introduction

Since Schleiden's and Schwann's first formulations of cell theory (Mazzarello, 1999), it has been known that all living organisms are made of individual and identifiable cells, whose size, number and type ultimately define the structure and functions and

Cell size (ploidy): for haploid one salamander s der, althoug have the sam salamander c (Conlon & R of relationsh DNA conten there is a m because ther

translation rates. Since proteins have a limited lifetime, there is a maximal amount of protein-associated cell mass that can be supported by a genome (Gomer, 2001). In size-control mechanisms, even cell communication has a role in defining specific organs, as reported by Depaepe et al. (2005).

Therefore, since the mean weight of a mammalian cell has been estimated to be 1 ng (Makarieva et al., 2008), for a standard body weight of 70 kg (Irving, 2007), there would be 7×10^{13} cells.

The mean volume of a mammalian cell has been estimated to be 4×10^{-9} cm^3 (Alberts et al., 2002) (i.e. 4 pL).

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limiting proliferation. Examples are myostatin, which regulates skeletal muscle mass; leptin, which regulates the amount of adipose tissue; growth hormone and insulin-like growth

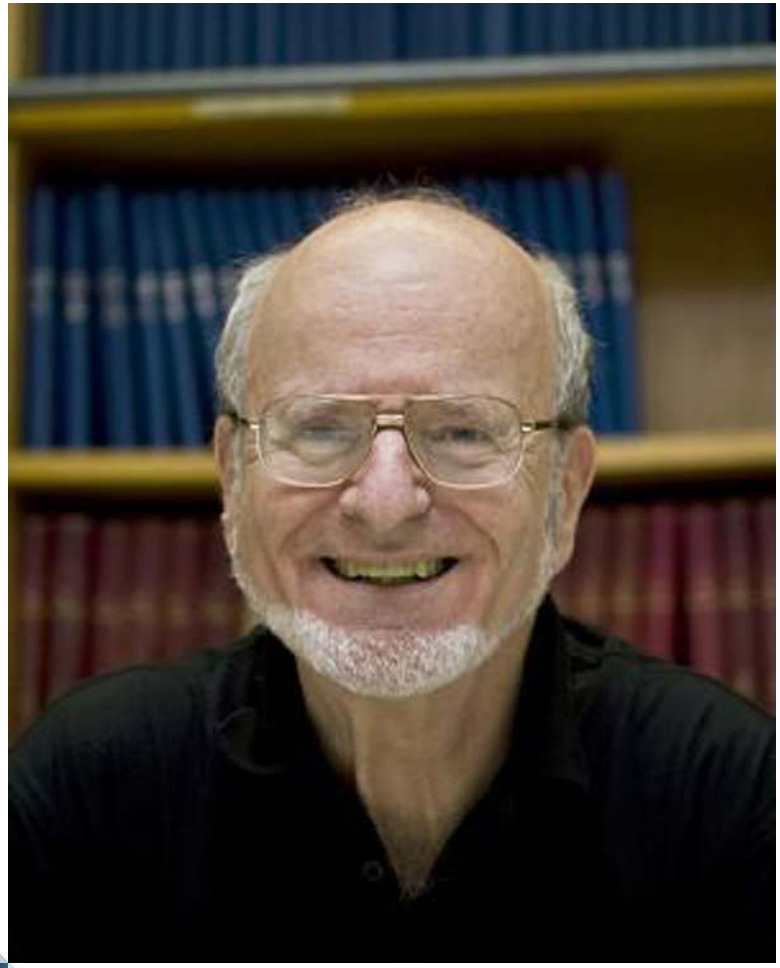
Postzygotic mutations are common

Time	Stage	Cells	Genes	Mutation rate	Mutations
7 days	Blastula	100	20,000	$10^{-7} – 10^{-6}$	
14 days		10,000	20,000	$10^{-7} – 10^{-6}$	20-200
15 days	Gastrula	15-18,000	20,000	$10^{-7} – 10^{-6}$	30-300
21 weeks	Fetus (21g)	10^{9}	20,000	$10^{-7} – 10^{-6}$	$10^{6} – 10^{7}$
40 weeks	Newborn	$10^{12\text{-}12.5}$	20,000	$10^{-7} – 10^{-6}$	$10^{9} – 10^{10}$

- Mutation N at 14 days gestations (10,000 cell pre-gastrula)
  - $(10^4$ cells$)(2 \times 10^4$ genes$)(10^{-7}$ mutations/gene/cell division$) = 20$ mutations
  - $(10^4$ cells$)(2 \times 10^4$ genes$)(10^{-6}$ mutations/gene/cell division$) = 200$ mutations

- Exponential growth of mutant clones
  - 1 mutant cell at 15 days (gastrula) expands to **10^8 cells** at term

The Happle hypothesis | theorem



mosaic

Commentary

Lethal genes surviving by mosaicism: A possible explanation for sporadic birth defects involving the skin

Rudolf Happle, M.D. *Münster, Federal Republic of Germany*

A genetic concept is advanced to explain the origin of several sporadic syndromes characterized by a mosaic distribution of skin defects. It is postulated that these disorders are due to the action of a lethal gene surviving by mosaicism. The presence of the mutation in the zygote will lead to death of the embryo at an early stage of development. Cells bearing the mutation can survive only in a mosaic state, in close proximity with normal cells. The mosaic may arise either from a gametic half chromatid mutation or from an early somatic mutation. This concept of origin is proposed to apply to the Schimmelpenning-Feuerstein-Mims syndrome, the McCune-Albright syndrome, the Klippel-Trenaunay syndrome, the Sturge-Weber syndrome, and neurocutaneous melanosis. Moreover, this etiologic hypothesis may apply to two other birth defects that have recently been delineated, the Proteus syndrome (partial gigantism of hands or feet, hemihypertrophy, macrocephaly, linear papillomatous epidermal nevus, subcutaneous hemangiomas and lipomas, accelerated growth, and visceral anomalies), and the Delleman-Oorthuys syndrome (orbital cyst, porencephaly, periorbital appendages, and focal aplasia of the skin). (J Am Acad Dermatol 1987;16:899-906.)

Among the congenital disorders affecting the skin, there are several syndromes for which no evidence of a mendelian basis can be found because they always occur sporadically. Some of these disorders display a mosaic pattern of involvement of the skin and other organs. For example, there is no observation of familial occurrence of the Schimmelpenning-Feuerstein-Mims syndrome, which is characterized by a systematized linear sebaceous nevus associated with ocular and cerebral defects.[1] Recently it has been proposed that these sporadic syndromes are due to the action of an autosomal "dominant" lethal gene surviving by mosaicism.[2] The disorders cannot be transmitted to another individual because the underlying gene, when present in the zygote, leads to early death of the embryo. Cells bearing the mutation can survive only when they are intermingled with normal cells. This mosaic may arise from either of two different mutational events. In the first event, a half chromatid mutation may occur before fertilization in one of the two gametes forming the zygote, leading to mosaicism already present at the two-cell stage.[3,4] In the second event, a somatic mutation may occur after fertilization during early embryogenesis. So far, this etiologic concept has been applied to the Schimmelpenning-Feuerstein-Mims syndrome, the McCune-

Happle rules

- Type 1
  - Postzygotic mutation – HET
- Type 2
  - Germline mutation – HET
  - Postzygotic mutation – HOMO

BRIDGING THE LABORATORY AND CLINIC

A Rule Concerning the Segmental Manifestation of Autosomal Dominant Skin Disorders

Review of Clinical Examples Providing Evidence for Dichotomous Types of Severity

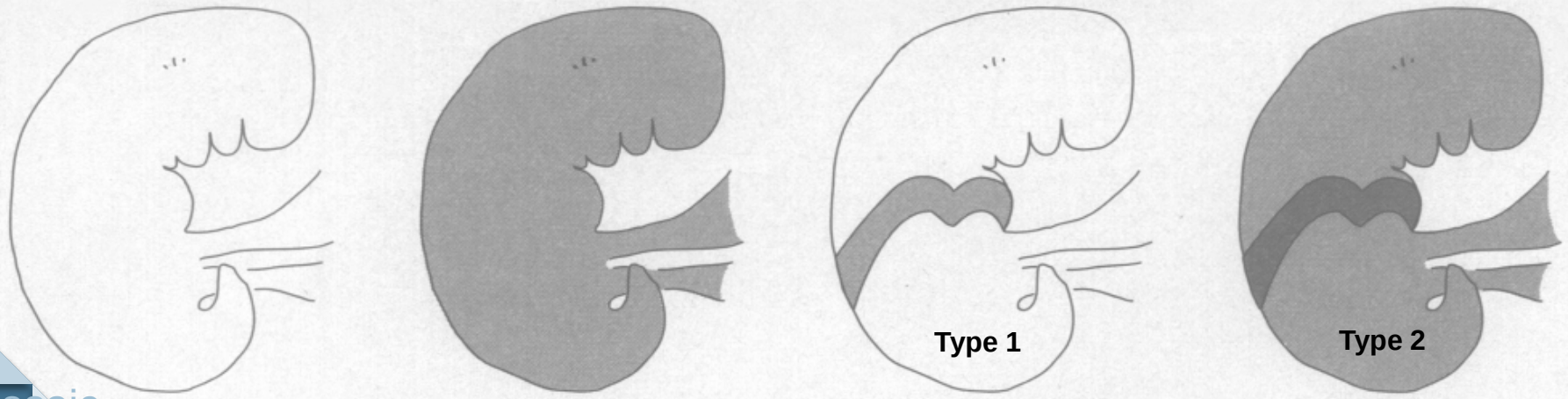
Rudolf Happle, MD

It is well known that autosomal dominant skin disorders may sometimes become manifest in a mosaic form, involving the body in a linear, patchy, or otherwise circumscribed arrangement. Such cases can be explained by an early postzygotic mutation. The segmental lesions usually show the same degree of severity as that found in the corresponding nonmosaic trait. Occasionally, however, the intensity of involvement observed in the circumscribed area is far more pronounced. This phenomenon can be explained by delineating a rule of dichotomous segmental manifestations reflecting different states of zygosity. Heterozygosity for the mutation results in severity corresponding to that in the nonsegmental phenotype; loss of heterozygosity for the same allele causes markedly more severe involvement.

Arch Dermatol. 1997;133:1505-1509

When an autosomal dominant skin disorder occurs in a mosaic arrangement, the circumscribed lesions are sometimes particularly severe and, remarkably, the segmental involvement may be superimposed on a milder, diffuse manifestation of the same disease. In the past, this phenomenon has neither been recognized nor clarified, but recently a concept of dichotomous forms of segmental manifestation has

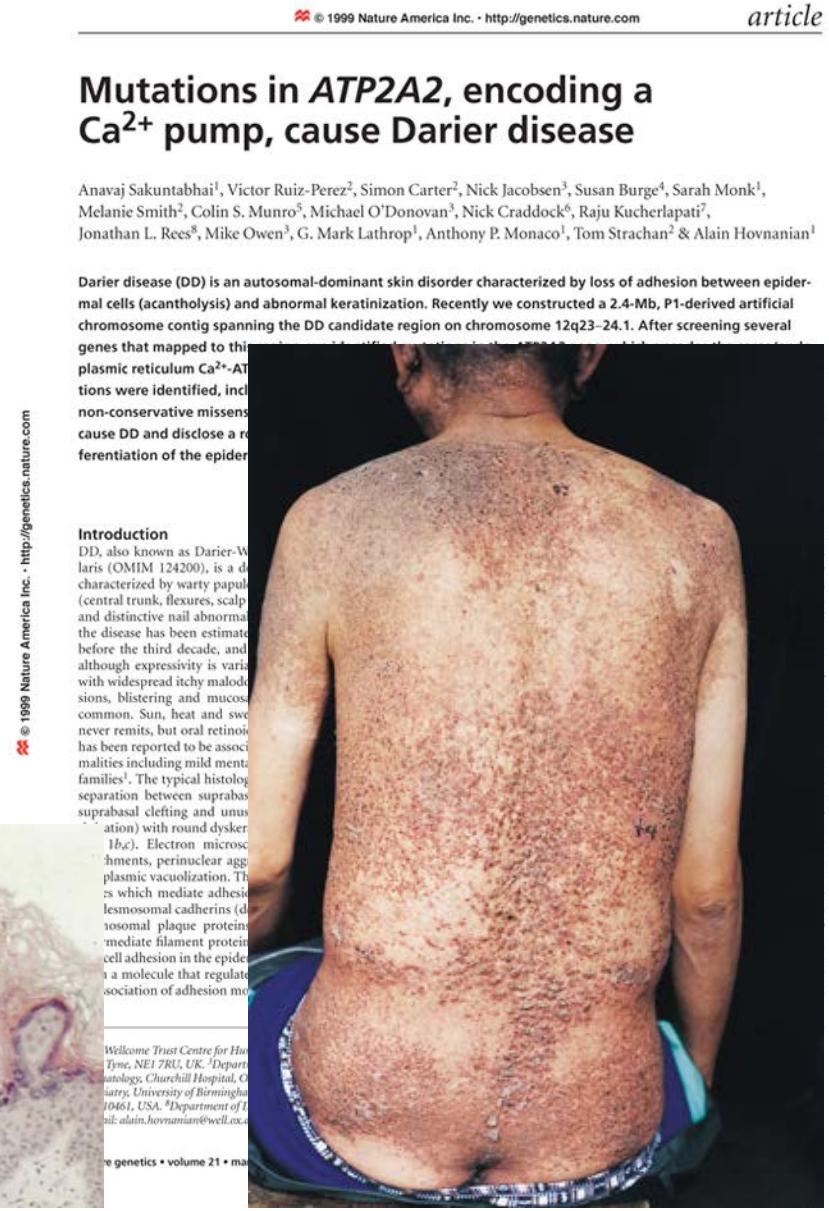
such as mitotic recombination, nondisjunction, or deletion would give rise to a population of cells either homozygous (**Figure 2**) or hemizygous (**Figure 3**) for the underlying gene. As a characteristic feature, the segmental type 2 is superimposed on a milder, nonsegmental, heterozygous manifestation of the same trait. In some cases, however, this diffuse manifestation may be absent because of a re-



mosaic

Darier disease

- Darier disease
  - a.k.a. dyskeratosis follicularis
  - AD skin disorder
  - Onset in the teens-20s
  - Dark crusty patches (warty papules and plaques) in seborrheic areas
  - Poorly formed and fragile fingernails with vertical striations
  - Short stature



Mutations in *ATP2A2*, encoding a Ca^{2+} pump, cause Darier disease

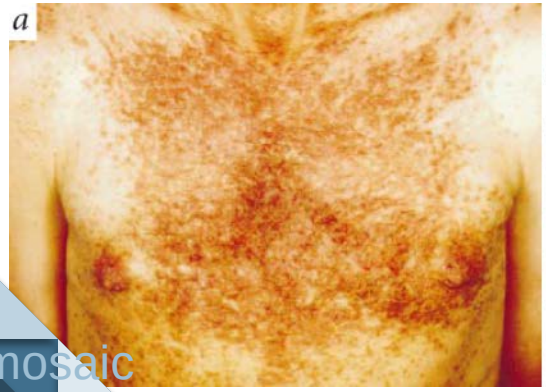
Anavaj Sakuntabhai[1], Victor Ruiz-Perez[2], Simon Carter[2], Nick Jacobsen[3], Susan Burge[4], Sarah Monk[1], Melanie Smith[2], Colin S. Munro[5], Michael O'Donovan[3], Nick Craddock[6], Raju Kucherlapati[7], Jonathan L. Rees[8], Mike Owen[3], G. Mark Lathrop[1], Anthony P. Monaco[1], Tom Strachan[2] & Alain Hovnanian[1]

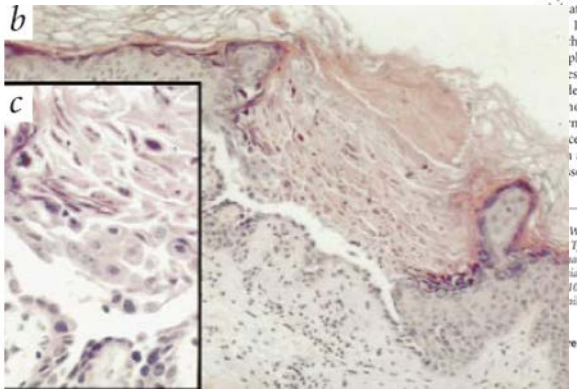
Darier disease (DD) is an autosomal-dominant skin disorder characterized by loss of adhesion between epidermal cells (acantholysis) and abnormal keratinization. Recently we constructed a 2.4-Mb, P1-derived artificial chromosome contig spanning the DD candidate region on chromosome 12q23–24.1. After screening several genes that mapped to thi… plasmic reticulum Ca^{2+}-AT… tions were identified, incl… non-conservative missens… cause DD and disclose a r… ferentiation of the epider…

Introduction

DD, also known as Darier-W… laris (OMIM 124200), is a d… characterized by warty papul… (central trunk, flexures, scalp… and distinctive nail abnormal… the disease has been estimate… before the third decade, and… although expressivity is vari… with widespread itchy malod… sions, blistering and mucos… common. Sun, heat and swe… never remits, but oral retinoi… has been reported to be associ… malities including mild menta… families[1]. The typical histolog… separation between suprabas… suprabasal clefting and unus… ation) with round dysker… 1b,c). Electron microsc… hments, perinuclear agg… plasmic vacuolization. Th… s which mediate adhesi… lesmosomal cadherins (d… iosomal plaque protein… mediate filament protein… cell adhesion in the epide… a molecule that regulate… sociation of adhesion m…

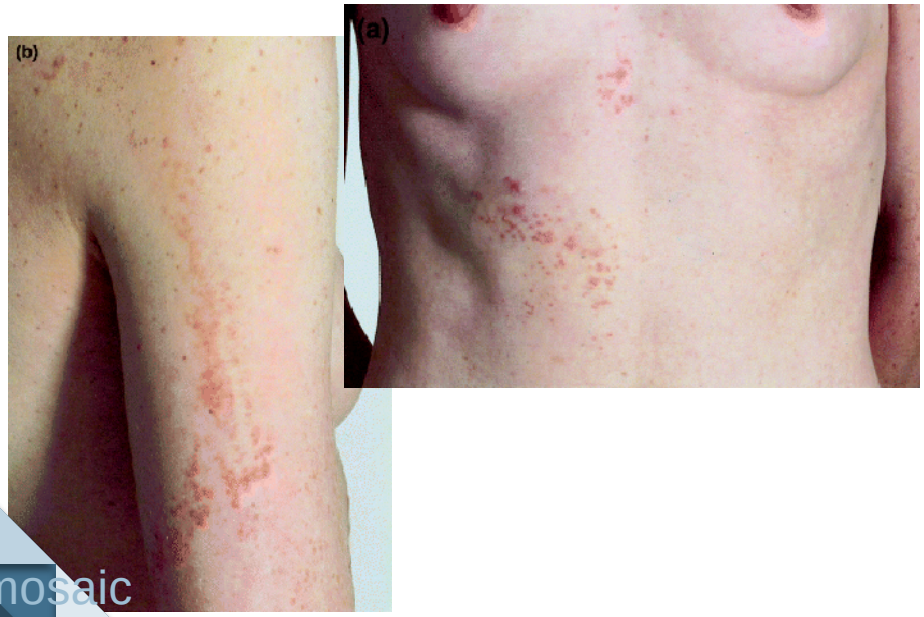
Wellcome Trust Centre for Hu… Tyne, NE1 7RU, UK. [3]Depart… atology, Churchill Hospital, O… iatry, University of Birmingha… 10461, USA. [8]Department of … il: alain.hovnanian@well.ox.…





Darier disease

- Darier disease
  - Segmental involvement of typical warty patches
  - Heterozygous mosaic mutation
  - **Type 1 mosaicism**



mosaic

MUTATION REPORT

Mosaicism for *ATP2A2* Mutations Causes Segmental Darier's Disease

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Epidermal naevi are localized malformations of the epidermis consisting of verrucoid scaly papules and plaques following Blaschko's lines. Genetic mosaicism has been proposed to underlie the development of linear epidermal naevi. Rarely, epidermal naevi show acantholytic histology similar to Darier's disease, a dominantly inherited skin condition characterized by widespread warty papules. As patients with acantholytic dyskeratotic naevi often give a history of worsening after sun exposure and the lesions are typical of Darier's disease, numerous authors have proposed that these patients have segmental Darier's disease. The postulated relationship has not been proven, however. Recently, we identified *ATP2A2*, which encodes the sarco/endoplasmic reticulum Ca^{2+} ATPase isoform 2 as the defective gene in Darier's disease. In this report, we investigated the involvement of *ATP2A2* in acantholytic dyskeratotic naevi following Blaschko's lines in two patients. We identified a nonsense mutation (Y894X) in the first patient and a nonconservative glycine to arginine mutation at codon 769 (G769R) in the other patient. These mutations were present in affected skin, and were not detected in unaffected skin or in leukocytes. We conclude that acantholytic dyskeratotic naevi can arise from a somatic mutation in *ATP2A2*. These individuals are mosaics for the mutation, but the risk of transmission of generalized Darier's disease will depend on whether the germline is affected. Our findings provide further evidence that Blaschko's lines do reflect genetic mosaicism and that the term acantholytic dyskeratotic naevus might be replaced in the future by segmental Darier's disease induced by postzygotic mosaicism.

***Key words:** acantholytic dyskeratotic naevi/Blaschko's lines/genetic mosaicism/SERCA2. J Invest Dermatol 115:1144–1147, 2000*

Epidermal naevi are localized malformations of the epidermis consisting of verrucoid scaly papules and plaques, which tend to follow Blaschko's lines. In most epidermal naevi the epidermis is hyperkeratotic and acanthotic although a number of less common histologic patterns have been described including epidermolytic hyperkeratosis, acantholytic dyskeratosis, and nevus comedonicus (Su, 1982). Genetic mosaicism has been proposed to underlie the development of linear epidermal naevi (Moss, 1999). Evidence to support this concept was first provided by a study of three individuals affected by widespread epidermal naevi of the epidermolytic type and four of their offspring with generalized epidermolytic hyperkeratosis (a widespread dominantly inherited blistering disorder arising from mutations in the keratin genes, K1 and K10). In these patients point mutations were present in 50% of the K10 alleles in the epidermal naevi, whereas no mutation was found in uninvolved skin or blood (Paller *et al*, 1994). Offspring with epidermolytic hyperkeratosis had the same mutations in 50% of the K10 alleles from all cell types examined. Subsequently, a K10 mutation was identified in the keratinocytes from a child with linear epidermal naevi of the epidermolytic type but not in the normal epidermis (Moss *et al*, 1995). Mosaicism was also demonstrated in a patient with a linear comedonal naevus. Epidermal scrapings showed a mutation in the gene for fibroblast growth factor receptor 2. Mutations in this gene cause Apert syndrome, a complex congenital malformation, characterized by craniosynostosis, syndactyly, and generalized acne. This mutation was not found in peripheral blood lymphocytes nor in follicular keratoses on the other arm (Munro and Wilkie, 1998).

Epidermal naevi with an acantholytic dyskeratotic histology show clinical and histologic similarities to a dominantly inherited skin condition, Darier's disease (Burge and Wilkinson, 1991). Patients with Darier's disease develop widespread warty papules often around the second and third decades. Heat, sweating, and sunburn exacerbate the condition. Similarly, many of these unusual epidermal naevi present around the same age and may be exacerbated by the same conditions. The histologic hallmark of both Darier's disease and this form of epidermal naevus is acantholytic dyskeratosis, i.e., loss of adhesion between keratinocytes (acantholysis) with abnormal keratinization (dyskeratosis) (Weedon, 1992). On the basis of these clinical and histologic similarities, linear epidermal naevi with an acantholytic dyskeratotic histology are often classified as localized (linear, naevoid, unilateral, or segmental) Darier's disease (Starink and Woerdeman, 1981; Moore and Schosser, 1985; Munro and Cox, 1992; Cambiaghi *et al*, 1995; O'Malley *et al*, 1997). The prevalence of this variant is

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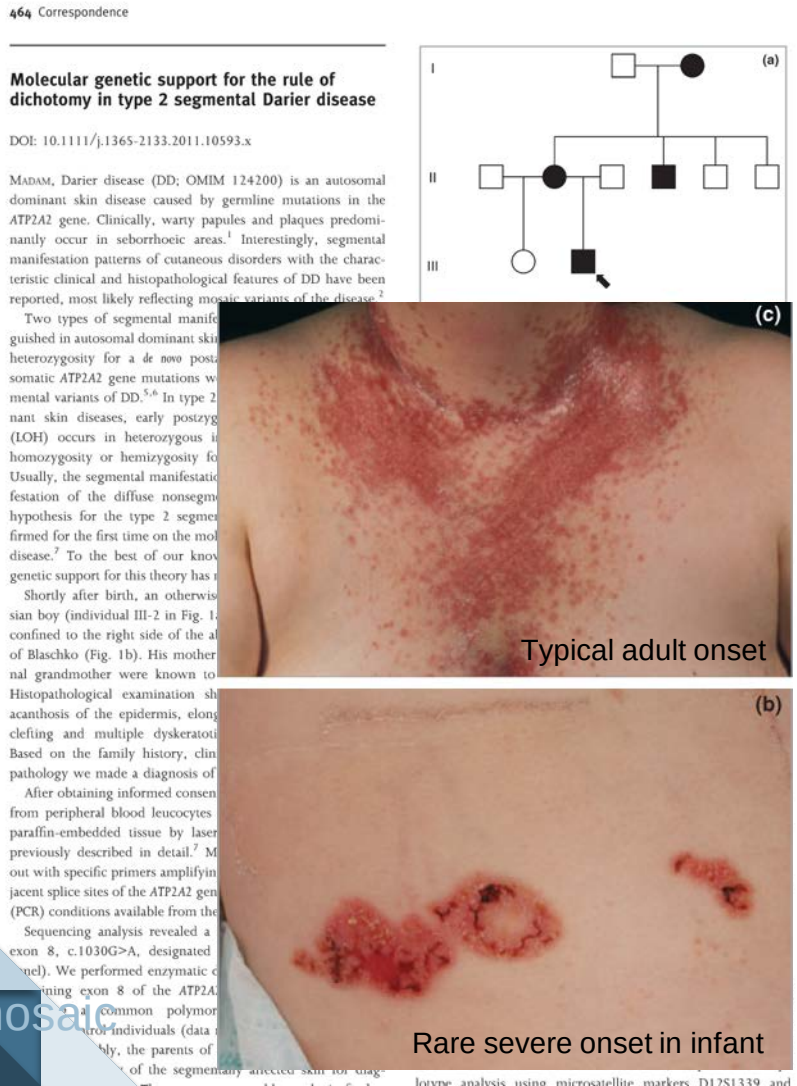
Abbreviations: CSGE, conformation-sensitive gel electrophoresis; SERCA2, sarco/endoplasmic reticulum Ca^{2+} ATPase isoform 2.

[1]These authors contributed equally to the work.

Darier disease



464 Correspondence

Molecular genetic support for the rule of dichotomy in type 2 segmental Darier disease

DOI: 10.1111/j.1365-2133.2011.10593.x

MADAM, Darier disease (DD; OMIM 124200) is an autosomal dominant skin disease caused by germline mutations in the *ATP2A2* gene. Clinically, warty papules and plaques predominantly occur in seborrhoeic areas.[1] Interestingly, segmental manifestation patterns of cutaneous disorders with the characteristic clinical and histopathological features of DD have been reported, most likely reflecting mosaic variants of the disease.[2]

Two types of segmental manif
guished in autosomal dominant ski
heterozygosity for a *de novo* post
somatic *ATP2A2* gene mutations w
mental variants of DD.[5,6] In type 2
nant skin diseases, early postzyg
(LOH) occurs in heterozygous i
homozygosity or hemizygosity fo
Usually, the segmental manifestatio
festation of the diffuse nonsegm
hypothesis for the type 2 segmen
firmed for the first time on the mo
disease.[7] To the best of our kno
genetic support for this theory has

Shortly after birth, an otherwis
sian boy (individual III-2 in Fig. 1
confined to the right side of the a
of Blaschko (Fig. 1b). His mother
nal grandmother were known to
Histopathological examination sh
acanthosis of the epidermis, elong
clefting and multiple dyskeratoti
Based on the family history, clin
pathology we made a diagnosis of

After obtaining informed consen
from peripheral blood leucocytes
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previously described in detail.[7] M
out with specific primers amplifyin
jacent splice sites of the *ATP2A2* gen
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Sequencing analysis revealed a
exon 8, c.1030G>A, designated
nel). We performed enzymatic c
ining exon 8 of the *ATP2A2*
common polymor
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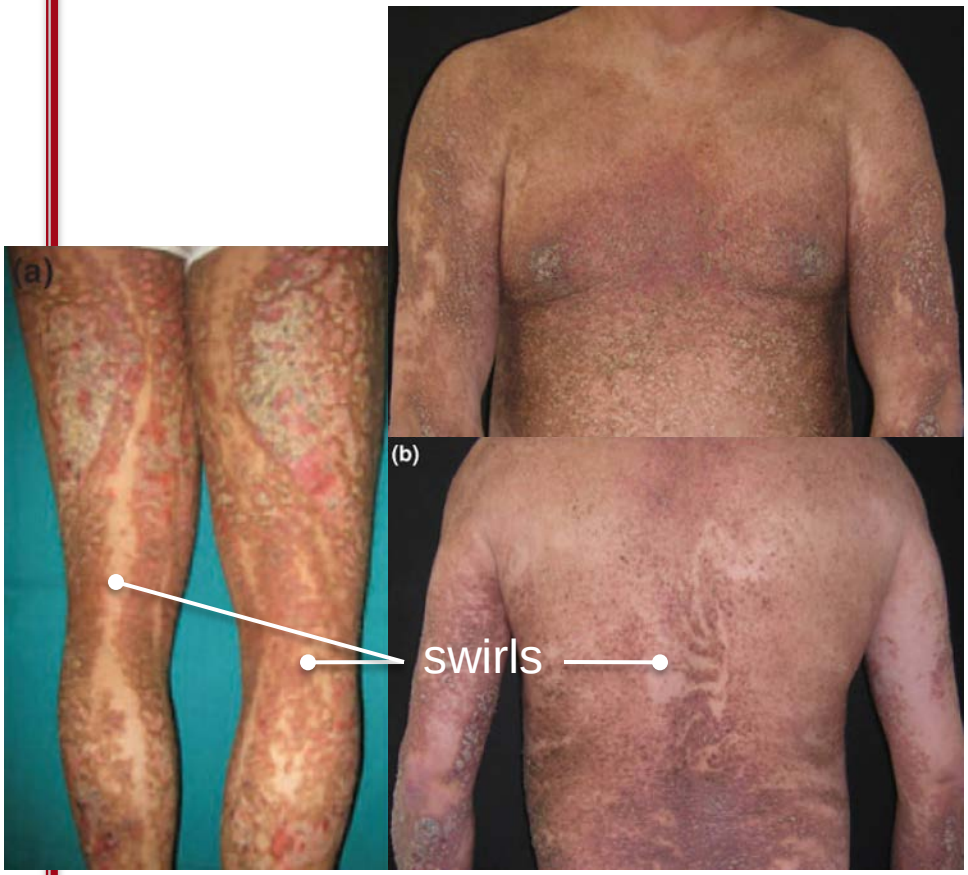
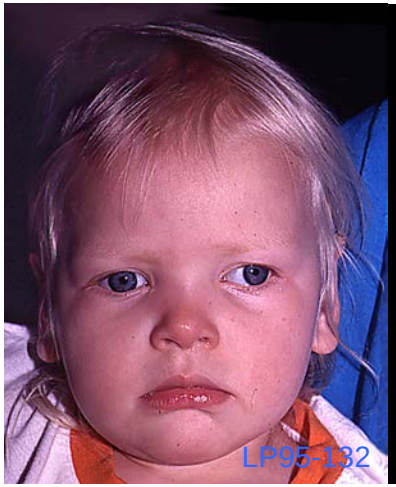
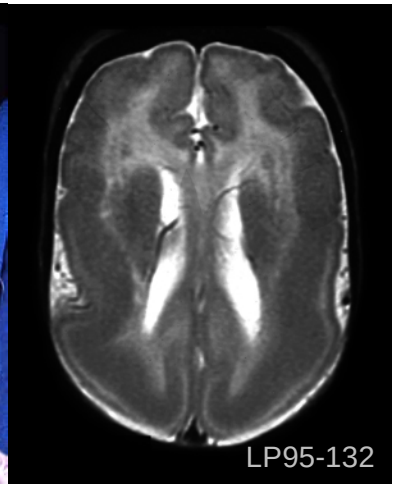
- Darier disease
  - Generalized typical warty patches
  - Heterozygous germline mutation plus second mosaic mutation
  - **Type 2 mosaicism**



mosaic

Lissencephaly





CME

Mosaic mutations of the *LIS1* gene cause subcortical band heterotopia

F. Sicca, MD; A. Kelemen, MD; P. Genton, MD; S. Das, PhD; D. Mei, MT; F. Moro, PhD; W.B. Dobyns, MD; and R. Guerrini, MD

Abstract—*Background:* Subcortical band heterotopia (SBH) is a neuronal migration disorder. *DCX* mutations are responsible for almost all familial cases, 80% of sporadic female cases, and 25% of sporadic male cases of SBH, and are associated with more severe gyral and migration abnormality over the anterior brain regions. Somatic mosaicism has previously been hypothesized in a patient with posteriorly predominant SBH and a mutation of the *LIS1* gene, which is usually mutated in patients with severe lissencephaly. The authors identified mosaic mutations of *LIS1* in two patients (Patients 1 and 2) with predominantly posterior SBH. *Methods:* After ruling out *DCX* mutations, the authors performed sequencing of the *LIS1* gene in lymphocyte DNA. Because sequence peaks in both patients were suggestive of mosaic mutations, they followed up with denaturing high-pressure liquid chromatography analysis on blood and hair root DNA and compared the areas of heteroduplex and homoduplex peaks. A third patient showing the same mutation as Patient 2 but with no evidence of mosaicism was used for comparing the phenotype of mosaic vs full mutation. *Results:* The two patients with posterior SBH harbored a missense (Arg241Pro) and a nonsense (R8X) mosaic mutation of *LIS1*. The rate of mosaicism in Patient 1 was 18% in the blood and 21% in the hair roots, whereas in Patient 2 it was 24% and 31% in the same tissues. The patient with a full R8X mutation of *LIS1* had severe lissencephaly. *Conclusions:* Subcortical band heterotopia can occur with mosaic mutations of the *LIS1* gene. Mutation analysis of *LIS1*, using highly sensitive techniques such as denaturing high-pressure liquid chromatography, should be considered for patients with posteriorly predominant subcortical band heterotopia and pachygyria.

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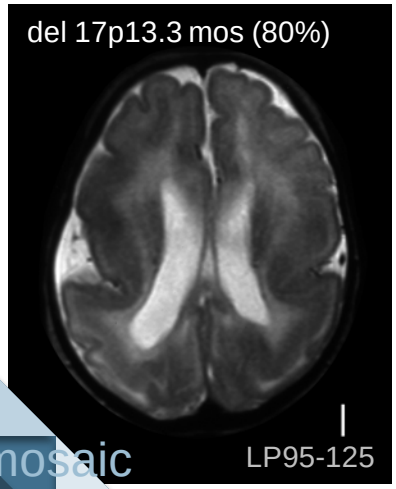
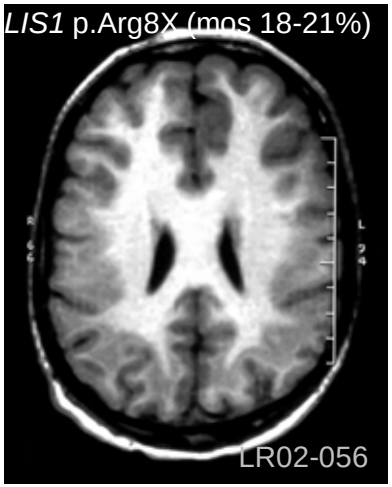
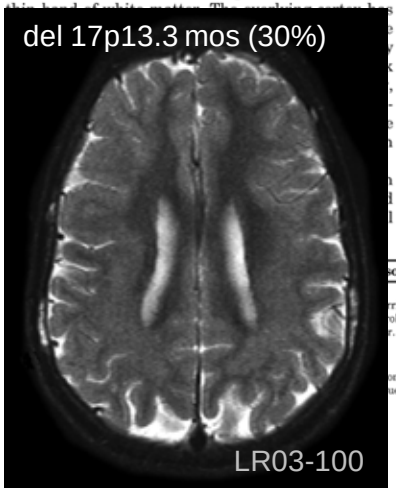
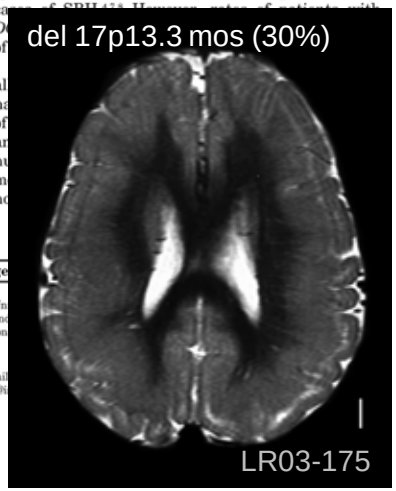
Subcortical band heterotopia (SBH) is a disorder of cortical development caused by the failure of a subpopulation of migrating neurons to reach the developing cortical plate. Heterotopic neurons form a bilateral, symmetric band of gray matter located below the cortical ribbon and separated from it by a

migration disorder, in hemizygous male patients.[5,6] Studies on SBH suggest that mutations of the *DCX* gene are found in almost all familial cases, including families in which female patients have SBH and male patients have LIS, and in approximately 80% of sporadic female cases and 25% of sporadic male



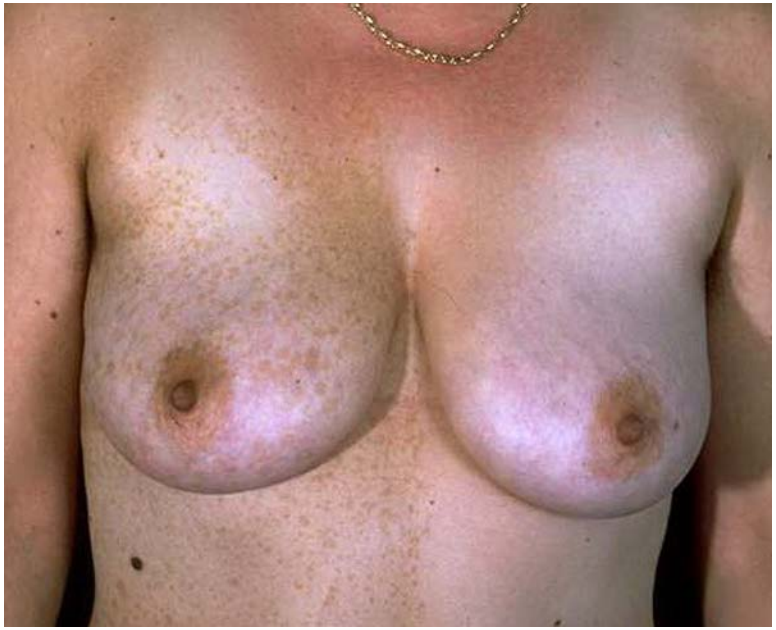


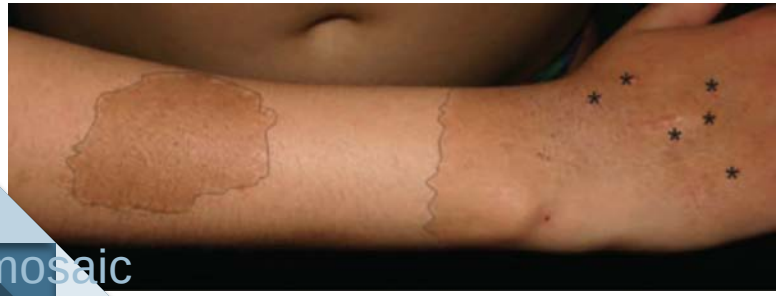




mosaic

NF1 segmental





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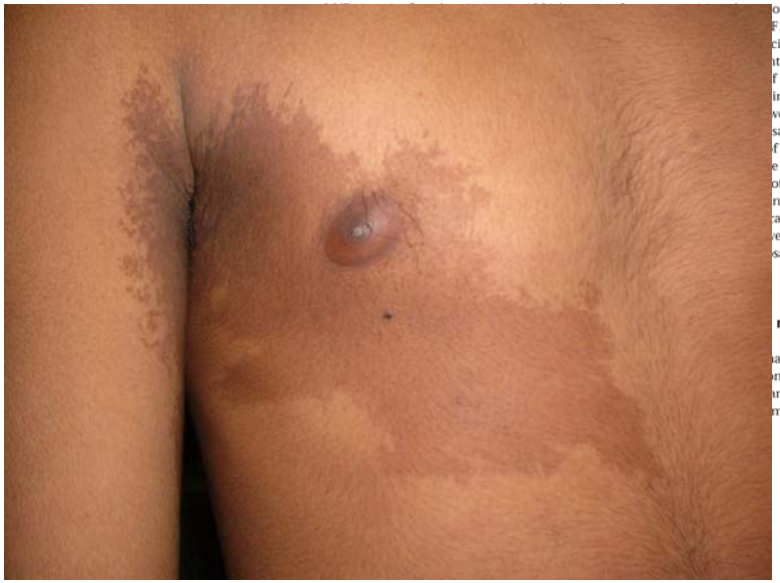

European Journal of Human Genetics (2000) 8, 455–459

www.nature.com/ejhg

ARTICLE

Segmental neurofibromatosis is caused by somatic mutation of the neurofibromatosis type 1 (*NF1*) gene

Sigrid Tinschert[1], Ilka Naumann[1], Elisabeth Stegmann[2], Annegret Buske[1], Dieter Kaufmann[3], Gundula Thiel[1] and Dieter E Jenne[2]

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Segmental neurofibromatosis (NF) is generally thought to result from a postzygotic *NF1* (neurofibromatosis type 1) gene mutation. However, this has not yet been demonstrated at the molecular level. Using fluorescence *in situ* hybridisation (FISH) we identified an *NF1* microdeletion in a patient with segmental NF in whom café-au-lait spots and freckles are limited to a single body region. The mutant allele was present in a mosaic pattern in cultured fibroblasts from a café-au-lait spot lesion, but was absent in fibroblasts from normal skin as well as in peripheral blood leukocytes. These findings prove the hypothesis that the molecular basis of segmental cutaneous NF is a mutation in the *NF1* gene and that the regional distribution of manifestations reflects different cell clones, commensurate with the concept of somatic mosaicism. *European Journal of Human Genetics* (2000) **8**, 455–459.

Keywords: Segmental neurofibromatosis; FISH; *NF1* gene deletion; microdeletion; somatic mosaicism

Introduction

Despite the number of articles dealing with localised cuta-

bromatoses. Segmental NF, defined by regionally limited cutaneous signs of NF1, is placed in the category of alternate onditions with some of the respec-
F1 or NF2.
cism explains the various clinical
ital NF. The phenotype may be
f the *NF1* mutation, the type of the
ineage and the viability of the cells
well as by the body region involved.
saicism explains the few reports on
f children with generalized NF1.[8–12]
e parents the postzygotic mutation
otent precursor cell[13] and results in
nadal cell mosaics.[14] However, the
ases was not elucidated.
e offer the first molecular demon-
saicism as the cause of segmental

methods

as a clearly demarcated area of
minantly on the left side of the
nd arm associated with café-au-lait
mm and seven of 5 up to 15 mm in

Commentary

Lethal genes surviving by mosaicism: A possible explanation for sporadic birth defects involving the skin

Rudolf Happle, M.D. *Münster, Federal Republic of Germany*

A genetic concept is advanced to explain the origin of several sporadic syndromes characterized by a mosaic distribution of skin defects. It is postulated that these disorders are due to the action of a lethal gene surviving by mosaicism. The presence of the mutation in the zygote will lead to death of the embryo at an early stage of development. Cells bearing the mutation can survive only in a mosaic state, in close proximity with normal cells. The mosaic may arise either from a gametic half chromatid mutation or from an early somatic mutation. This concept of origin is proposed to apply to the Schimmelpenning-Feuerstein-Mims syndrome, the McCune-Albright syndrome, the Klippel-Trenaunay syndrome, the Sturge-Weber syndrome, and neurocutaneous melanosis. Moreover, this etiologic hypothesis may apply to two other birth defects that have recently been delineated, the Proteus syndrome (partial gigantism of hands or feet, hemihypertrophy, macrocephaly, linear papillomatous epidermal nevus, subcutaneous hemangiomas and lipomas, accelerated growth, and visceral anomalies), and the Delleman-Oorthuys syndrome (orbital cyst, porencephaly, periorbital appendages, and

Happle R. 1987. J Am Acad Dermatol 16(4):899-906

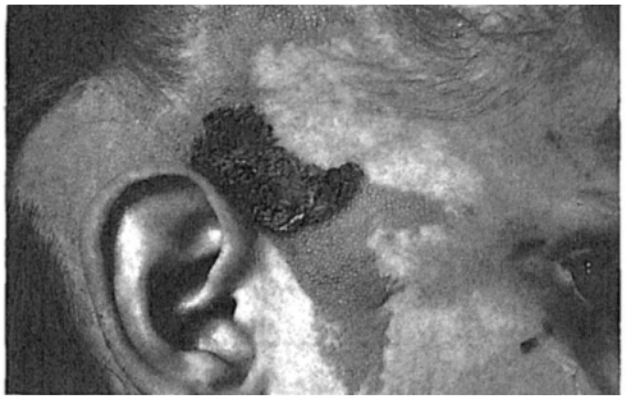
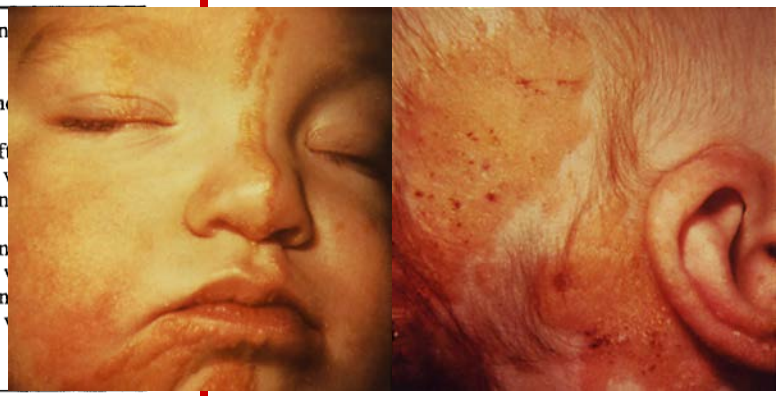


Fig. 1. Systematized linear sebaceous nevus in the Schimmelpenning-Feuerstein-Mims syndrome. Diffuse involvement of the entire skin never occurs.

Table II. Sporadic syndromes that may be due to survival of a lethal gene by mosaicism

	No. of entries in McKusick's catalog[9]	Cutaneous anomaly	Cutaneous pattern of distribution	Other organs affected in a mosaic pattern
Schimmelpenning-Feuerstein-Mims syndrome	16,320	Sebaceous nevus	Lines of Blaschko	Eye, brain
McCune-Albright syndrome	17,480	Nevoid hyperpigmentation	Lines of Blaschko	Bones, en
Proteus syndrome	Not yet registered	Epidermal nevus of papillomatous type	Lines of Blaschko	Brain, soft bones,
Delleman-Oorthuys syndrome	16,418	Nevoid lesions of hypoplasia and aplasia	Linear (lines of Blaschko?)	Eye, brain
Klippel-Trenaunay syndrome	14,900	Telangiectatic nevus	Nonlinear, with sharp midline separation	Eye, brain bones,
Sturge-Weber syndrome	18,530	Telangiectatic nevus	Nonlinear, with sharp midline separation	Eye, brain bones,
Neurocutaneous melanosis	24,940	Melanocytic nevus	Nonlinear, without midline separation	Brain

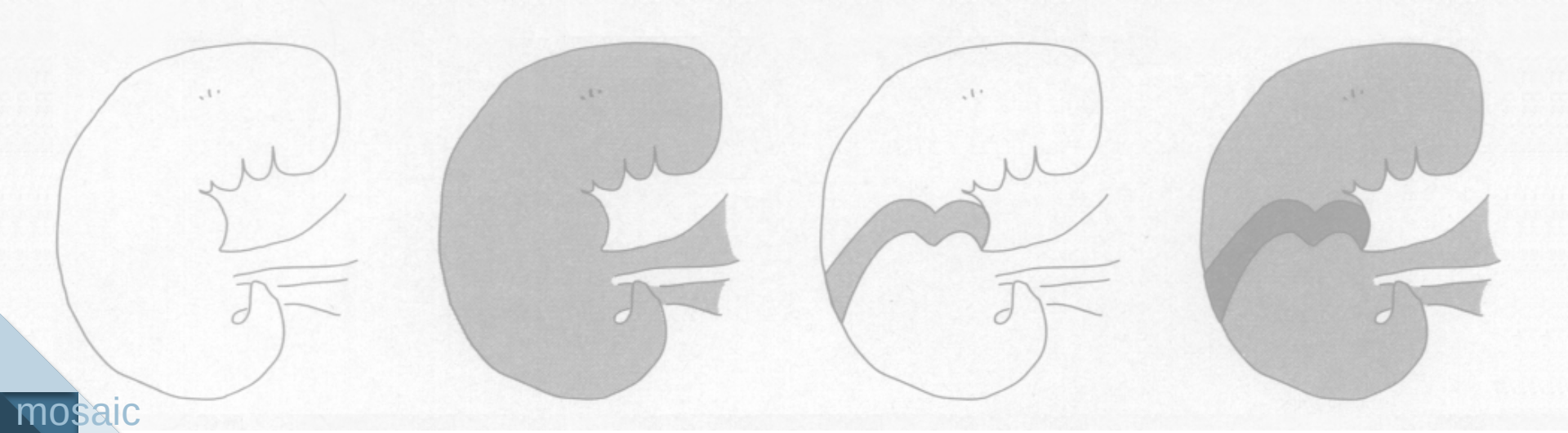


mosaic

The Happle hypothesis | theorem

- Certain developmental disorders are caused by action of an autosomal dominant lethal gene surviving by mosaicism.

- The presence of the mutation in the zygote will lead to the death of the embryo at an early state of development. Cells bearing the mutation can survive only in the mosaic state, in close proximity with normal cells [Happle, 1986; Happle, 1987].



mosaic

Check-up on Happle 1987, Hall 1988

Disorder	Mosaic	Gene
Delleman-Oorthuys (OCCS)		
Encephalo-cranio-cutaneous [illegible] (ECCL)		
Klippel-Trenaunay syn[illegible]		
Maffucci syndrome	✚	*IDH1, IDH2*
McCune-Albright s[illegible]	✚	*GNAS1*
Neurocutaneous [illegible]	✚	*NRAS*
Ollier disease	✚	*IDH1, IDH2*
Proteus syndrome[illegible]	✚	*AKT1*
Schimmelpenning-[illegible]	✚	*HRAS, KRAS*
Sturge-Weber syndro[illegible]	✚	*GNAQ*

70%

Mosaicism 1991

Pathogenesis. The pathogenesis of ENS thus appears to involve hypertrophy, hyperplasia, and dysplasia of multiple tissues originating from different germ layers although ectodermal tissues clearly predominate. While the cause is unknown, repeated observations of (1) striking abnormalities in cellular growth and differentiation, (2) striking lateralization, (3) predisposition for tumor development, and (4) lack of genetic transmission strongly suggest a somatic, probably autosomal dominant, mutation. This is further supported by rare reports of autosomal dominant transmission of epidermal nevi.[22]

Epidermal nevus syndrome:
A neurologic variant with hemimegalencephaly, gyral malformation, mental retardation, seizures, and facial hemihypertrophy

L. Pavone, MD; P. Curatolo, MD; R. Rizzo, MD; G. Micali, MD; G. Incorpora, MD; B.P. Garg, MB, BS; D.W. Dunn, MD; and W.B. Dobyns, MD

Article abstract—The epidermal nevus syndrome (ENS) is a sporadic neurocutaneous disorder that consists of epidermal nevi and congenital anomalies involving the brain and other systems. From among over 60 patients with ENS presenting with stations, we identified 17 who had hemimegalencephaly based on pathologic or radiologic studies. Associated gic abnormalities included gyral malformations in 12 of 12, mental retardation in 13 of 14, seizures in 16 of 17 infantile spasms), and contralateral hemiparesis in 7 of 12. All had ipsilateral epidermal nevi of the head, and teral facial hemihypertrophy. We concluded that these abnormalities comprise a recognizable *neurologic* at we believe represents the full expression of primary brain involvement. Several patients also had evidence of ions such as infarcts, atrophy, porencephaly, and calcifications, which are best explained by prior ischemia or n repeated observations of blood vessel anomalies in ENS patients, we hypothesize that underlying vascular oses to these acquired lesions. The same cause may be invoked to explain the wide variety of neurologic d in ENS patients without hemimegalencephaly. While the cause of ENS remains unknown, several observa- matic mutation.

NEUROLOGY 1991;41:266-271

are congenital skin lesions that consist ovoid or linear plaques. In infants, they -colored and velvety. As time passes, ore verrucous, and orange or darker Smaller lesions occur most frequently lp, and neck, while larger lesions may There is often a predilection for the t 7 different types have been described, nevus sebaceous (of Jadassohn), nevus ichthyosis hystrix. Under current use, a "epidermal nevi" encompasses all of e studies show hyperplasia of both epi- mis in all types, while involvement of s may vary. Malignant transformation l nevi occurs, usually after puberty, but unknown.[1]

al nevus syndrome (ENS) is a sporadic disorder (although it is listed as MIM Kusick[2]) that consists of epidermal nevi ty of congenital anomalies of the brain, d other systems. Our observations of 4 recognizable neurologic syndrome con- l epidermal nevus, ipsilateral hemi- y and gyral malformation, mental ures (especially infantile spasms), and sometimes facial hemihypertrophy prompted this review of the neurologic manifestations of ENS.

Methods. For this study, we gathered data on 63 patients with ENS (4 from our practices and 59 from the literature) who presented with severe neurologic abnormalities. From among these, we selected 17 patients for further review based on (1) availability of adequate clinical description and pathologic or radiologic studies and (2) presence of hemimegalencephaly. The studies included autopsy or surgical biopsy results in 4 patients, cranial CT in 12, and both pneumoencephalogram and angiogram in one.

Case reports. Patient 1. This boy was the 1st of 2 children born to unrelated Sicilian parents. Two earlier pregnancies ended in miscarriage; his younger brother is healthy. The father was 52 years old and the mother 37 at the time of his birth. Family history was not significant. Pregnancy and delivery were uncomplicated. Birth weight was 3.3 kg, length 50 cm, and occipitofrontal circumference (OFC) 35 cm (all 50th percentile). He had a large swelling of the right cheek that soon extended to involve the entire right face, hypotonia, and a weak cry. Subsequent development was delayed. At 1.5 months, he had a brief generalized seizure. Infantile spasms began at 3 months, and were more prominent on the left side. At 3 months, weight was 5.8 kg, length 57 cm, and OFC 42 cm (50th percentile). Examination showed an asymmetric skull,

From the Pediatric (Drs. Pavone, Rizzo, and Incorpora) and Dermatology (Dr. Micali) Clinics, University of Catania, Catania, Italy; the Pediatric Neurology Clinic, University of Rome (Dr. Curatolo); and the Departments of Neurology (Drs. Garg, Dunn, and Dobyns), Pediatrics (Dr. Dunn), and Medical Genetics (Dr. Dobyns), Indiana University School of Medicine, Indianapolis, IN.

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Happle, 1987

Table I. Clinical criteria suggesting a lethal gene surviving by mosaicism

- Birth defects always occur sporadically
- Lesions are distributed in a mosaic pattern
- Extent of involvement is variable
- Diffuse involvement of the entire body or an entire organ system does not occur
- Sex ratio is 1:1

TABLE IV. Overview of Publications on Monozygotic Twin Pairs of Whom Only One Twin Is Considered to Have a (Possibly) Mendelian Disorder

Disorder	MIM[a]	Refs.
Aglossia-adactylia	103300	Robinow et al. [1978]
Aicardi syndrome	%304050	Costa et al. [1997]
Asplenia syndrome	%208530	Hwang et al. [2006], Wilkinson et al. [1979]
Bladder exstrophy	%600057	Bugge [1981], Reutter et al. [2003]
Body stalk anomaly	230750	Daskalakis and Nicolaides [2002], Vidaeff et al. [2005]
Beckwith–Wiedemann syndrome	#122470	Berry et al. [1980], Bose et al. [1985], Chien et al. [1990], Clayton-Smith et al. [1992], Leonard et al. [1996], Litz et al. [1988], Olney et al. [1988], Orstavik et al. [1995], Weksberg et al. [2002], Bliek et al. [2009]
Caudal duplication anomaly	#607864	Kroes et al. [2002], Oates et al. [2006]
Congenital hypothyroidism	#275200/#218700	Rettig et al. [1980]
Colorblindness	+303800	Jorgensen et al. [1992]
Complete congenital heartblock	234700	Cooley et al. [1997]
Congenital bilateral perisylvian syndrome	#300388	Lenti and Triulzi [1996]
Cornelia de Lange syndrome	#122470	Carakushannsky and Berthier [1976], Carakushansky et al. [1996]
Darrier's disease	#124200	Sakuntabhai et al. [1999]
Duane's retraction	#604356, +%126800	Kaufman et al. [1989], Rosenbaum and Weiss [1978]
Duchenne muscular dystrophy	#310200	Burn et al. [1986], Chutkow et al. [1987], Gomez et al. [1977], Lupski [illegible], Pena et al. [1987], Richards et al. [1990], Tremblay et al. [1993], Zo[illegible] et al. [1993], Bonilla et al. [1990]
Epilepsy with structural brain abnormalities		Briellmann et al. [illegible], Brodtkorb et al. [2000], Kuzniecky et al. [1995], Sisodiya et al. [illegible]1999], Supprian et al. [2000]
Fabry disease	#301500	Redonnet-Vernhet et al. [illegible]
Fragile X syndrome	#300624	Kruyer et al. [1994], Tucken[illegible] et al. [1985]
Frontonasal dysplasia	136760	Mohammed et al. [2004], Wu et[illegible] [2007]
Facioscapulohumeral mu[illegible]ular dystrophy [FSHD]	%158900	Griggs et al. [1995], Tawil et al. [illegible]93]
Goldenhar syndrome	%164210	Boles et al. [1987], Stoll et al. [1984], Touliatou et al. [2006], Verona et al. [2006], Wieczorek et al. [200[illegible]
Growth hormone de[illegible]iency	+139250	Simpson et al. [1999]
Hemihypertrophy	%235000	West et al. [2003]
Hemophilia A	+306700	[illegible] et al. [illegible]
Hemophilia B	#306900	[illegible]chens [1987], Reeves[illegible] al. [1972]
Holoprosencephal[illegible]	[illegible]	[illegible], P[illegible] et al. [2007]
Humero-radial syn[illegible]stosis	#236400/143050	[illegible]redieu [illegible]
Hunter syndrome		Winchest[illegible] et al. [1992]
Idiopathic torsion [illegible]ystonia (ITD	#128100/%602124	[illegible]
Kabuki syndrome	%147920	[illegible]
Kearns–Sayre synd[illegible]me	#530000	Blakely et al. [2004]
Klippel–Feil syndrom[illegible]	%118100	Toyoshima et al. [2006]
Klippel–Trenaunay sy[illegible]rome	%149000	Hofer et al. [2005], Oduber et al. [20[illegible]]
Landau–Kleffner syndr[illegible]e	245570	Feekery et al. [1993]
Leber's hereditairy optic[illegible]trophy	#535000	Biousse et al. [1997], Johns et al. [illegible]1993]
Lesch–Nyhan syndrome	#300322	De et al. [2005]
Mayer Rokitansky Küster sy[illegible]me	%277000	Heidenreich et al. [1977], D[illegible]and Laufer [2009]
McCune–Albright syndrome	#174800	Peleg et al. [2009]
Ocular cicatricial pemphigoid	164185	Bhol et al. [1995]
Oculo-oto-radial (IVIC) syndrome	#147750	Elcioglu and Bern[illegible]997]
Oto-palato-digital syndrome	#311300	Robertson et[illegible]2006]
Oral–facial–digital syndrome	#[illegible]	[illegible]uk et al. [1999]
Primary lateral sclerosis	%611637	Sorenson [2006]
Primary progressive aphasia	#607485	Doran and Larner [2004]
Proteus syndrome	%176920	Brockmann et al. [2008]
Retinitis pigmentosa	#180100 + others	Bernstein and Aptsiauri [2003]
Rett syndrome	#312750	Carter et al. [2008], Migeon et al. [1995], Subramaniam et a[illegible]
Rubinstein–Taybi syndrome	#180849	Kajii et al. [1981]
[illegible]y syndrome	181180	Ashton-Prolla and Felix [1997]

[Co[illegible]

N=59

REVIEW ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Identical But Not the Same: The Value of Discordant Monozygotic Twins in Genetic Research

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Received 6 November 2008; Accepted 4 March 2010

Monozygotic (MZ) twins show remarkable resemblance in many aspects of behavior, health, and disease. Until recently, MZ twins were usually called "genetically identical"; however, evidence for genetic and epigenetic differences within rare MZ twin pairs has accumulated. Here, we summarize the literature on MZ twins discordant for Mendelian inherited disorders and chromosomal abnormalities. A systematic literature search for English articles on discordant MZ twin pairs was performed in Web of Science and PubMed. A total number of 2,016 publications were retrieved and reviewed and 439 reports were retained. Discordant MZ twin pairs are informative in respect to variability of phenotypic expression, pathogenetic mechanisms, epigenetics, and post-zygotic mutagenesis and may serve as a model for research on genetic defects. The analysis of single discordant MZ twin pairs may represent an elegant approach to identify genes in inherited disorders. © 2010 Wiley-Liss, Inc.

Key words: twins; monozygotic; twin studies; discordant; chromosome disorders; genetic diseases; inborn

MZ twins

How to Cite thi[illegible]
Zwijnenburg PJG,[illegible]
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Same: The Value of[illegible]
Twins in Genetic Res[illegible]
Am J Med Genet Part B[illegible]

TWINNING

Twins and multiples always have intrigued humans, as illustrated by numerous legends, myths, tales, and anecdotes in which twins play a principal part.

Twins can result from a single ovum, fertilized by one sperm, and Hoekstra et al., 2008]. Within Europe and the United States, twinning rates also can vary considerably across time and place [Hoekstra et al., 2008]. As MZ twinning generally occurs at a constant rate of 3–4 per 1,000 maternities around the world [Tong et al., 1997; Hall, 2003], the variation in twinning rates is generally accepted as being the result of variation in DZ twinning rates. The rates of DZ, and possibly also MZ twinning, have increased the past decades, in part due to assisted reproductive technologies (ART) [Chang et al., 2009] and due to increases in maternal age.

The causes of MZ twinning in humans are unknown. Until recently, the process of MZ twinning was considered to represent a random process. However, new data indicate that (epi)genetic mechanisms may be involved in the phenomenon of splitting of the zygote [Shur, 2009]. An excess of females over males has been observed in spontaneous, surviving, MZ twins. This female pre-[illegible]dominance is observed in particular in mono-chorionic dizygotic[illegible]

TABLE IV. (*Continued*)

Disorder	MIM[a]	Refs.
Schimmelpenning–Feuerstein–Mims syndrome	%163200	Schworm et al. [1996]
Silver-Russell syndrome	%180860	Bailey et al. [1995], Sagot et al. [1996], Samn et al. [1990], Yamazawa et al. [2008]
Sotos syndrome	#117550	Brown et al. [1998]
Spondylocostal dysostosis	#277300	Van Thienen and Van der Auwera [1994]
Thumb polydactyly	#174400	Peterson and Rayan [2004]
VACTERL	192350	Becker et al. [2005], Camacho et al. [2008]
Van der Woude syndrome	#119300	Kondo et al. [2002]
X-linked adrenoleukodystrophy	#300100	Korenke et al. [1996]
X-linked hypophosphataemic rickets	#307800	Owen et al. [2009]

Symbols preceding a MIM number: #, a descriptive phenotype, not necessarily representing a unique locus; +, description of a gene of known sequence and a phenotype; %, a confirmed Mendelian phenotype or phenotypic locus for which the underlying molecular basis is not known; no symbol before a MIM number generally indicates a description of a phenotype for which the Mendelian basis, although suspected, has not been clearly established. [a]Mendelian Inheritance in Man [MIM] numbers correspond to Online Mendelian Inheritance in Man, OMIM [TM]. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University [Baltimore, MD] and National Center for Biotechnology Information, National Library of Medicine [Bethesda, MD] [November 1, 2009]. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/.

mosaic

Guidelines for mosaicism

- **Nil recurrence risk in families**
  - Or very low risk (due to genetic heterogeneity)
- **Asymmetry or focal / multifocal anomalies**
  - Variable expressivity
  - No diffuse involvement
- **Dysplastic growth especially overgrowth**
- **Predisposition for tumors**
- **Discordant monozygotic twins**

PI3K-AKT pathway

1997 MCAP

American Journal of Medical Genetics 70:67–73 (1997)

Macrocephaly-Cutis Marmorata Telangiectatica Congenita: A Distinct Disorder With Developmental Delay and Connective Tissue Abnormalities

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We describe 13 unrelated children with abnormalities of somatic growth, face, brain, and connective tissue including vasculature. Although the condition in these children falls under the general group of disorders known as cutis marmorata telangiectatica congenita (CMTC), the constellation of abnormalities appears to constitute a distinct and easily recognizable phenotype within this general group. In contrast to most children reported with CMTC, children in this subgroup have a high risk for neurologic abnormalities, including developmental delay, mental retardation, megalencephaly, and hydrocephalus. Early recognition of this condition is important for appropriate surveillance for known complications and parental counseling. Am. J. Med. Genet. 70:67–73, 1997.

KEY WORDS: telangiectasia; congenital overgrowth; hemihypertrophy; megalencephaly

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Received 8 April 1996; Accepted 8 July 1996

INTRODUCTION

Cutis marmorata telangiectatica congenit
is a condition characterized by cutis marmor
is congenital generalized or segmental "m
"mottled" skin appearance caused by promi
laries and veins, and vascular lesions chara
telangiectasias, which resemble spider angi
venous dilatation or phlebectasias. Other v
sions, such as capillary and cavernous hem
nevus flammeus, and varicose veins, can
[Cohen and Zalar, 1988]. The cause of CM
known and likely heterogeneous. Way et al.
viewed 41 cases of CMTC (38 previously publ
found the incidence of associated anomalies
More recently, Pehr and Moroz [1993] rev
affected individuals and reported that 68%
additional congenital anomaly; however,
minor or of questionable causal association
common associated manifestations include
try, localized limb defects, other vascular
and glaucoma. Other anomalies such as ma
and developmental delay or mental retardat
incidence findings in most series [Stephan e
Pehr and Moroz, 1993].

In this report we summarize the clinical
13 patients, ages 11 months to 17 years (
females), with a distinct combination of CM
mal growth patterns, minor craniofacial ar
anomalies, central nervous malformation,
mal connective tissue and vasculature.

Clinical Dysmorphology **6**, 291–302 (1997)

Macrocephaly with cutis marmorata, haemangioma and syndactyly – a distinctive overgrowth syndrome

J. CLAYTON-SMITH[1]*, B. KERR[2], H. BRUNNER[3], L. TRANEBJAERG[4], A. MAGEE[5], R.C.M. HENNEKAM[6], R.F. MUELLER[7], L. BRUETON[8], M. SUPER[2], J. STEEN-JOHNSEN[9] and D. DONNAI[1]

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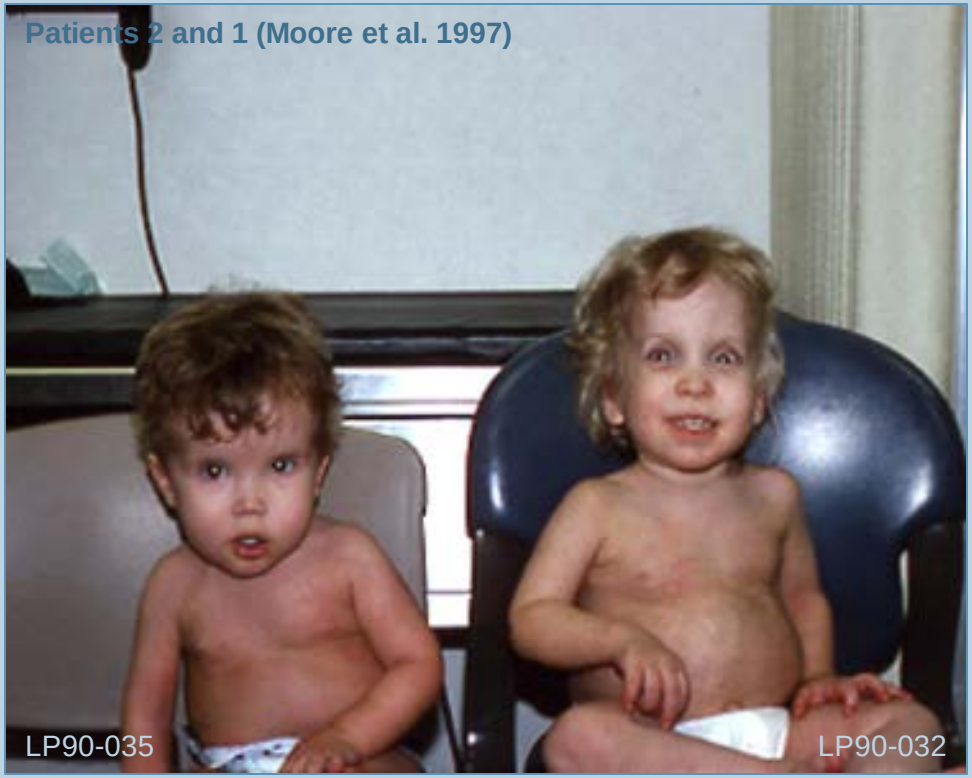
Patients 2 and 1 (Moore et al. 1997)



mosaic

2004 MPPH

G. Mirzaa[1]
N. N. Dodge[2]
I. Glass[3]
C. Day[4]
K. Gripp[5]
L. Nicholson[5]
V. Straub[6]
T. Voit[6]
W. B. Dobyns[7]

Megalencephaly and Perisylvian Polymicrogyria with Postaxial Polydactyly and Hydrocephalus: A Rare Brain Malformation Syndrome Associated with Mental Retardation and Seizures

Original Article

Abstract

Background and Objective: Megalencephaly (MEG) or enlarged brain occurs as a mild familial variant with normal brain structure, but otherwise is an uncommon human brain malformation that may be associated with significant developmental and neurological problems. It has been classified into anatomic and metabolic subtypes. The clinical findings associated with anatomic megalencephaly have been variable and few distinct subtypes have been described. We report five unrelated children with severe congenital MEG associated with polymicrogyria (PMG), postaxial polydactyly (POLY) and hydrocephalus (HYD).
Methods: The clinical records and brain MRI of five patients have been reviewed.
Results: All patients had striking MEG that was symmetric in three of the five patients, and mildly asymmetric in two. The birth OFC was between +2 and +4 SD. The gyral pattern was irregular with microgyri typical of PMG, which was most severe in the perisylvian region in all five patients. Four of the five had hydrocephalus treated with a shunt. Subsequently, one of the shunted patients had small ventricles while the others had mildly to moderately enlarged lateral ventricles. Three of the five patients had postaxial pol
callosum was dysmorphic
and genu, and intact altho
were abnormally thick. All patients had severe mental retardation; three had seizures and another had an epileptiform EEG.
Conclusion: We believe this constellation of findings (MEG-PMG-POLY-HYD) comprises a new and distinct malformation syndrome that we designate the MPPH syndrome.

Key words
Megalencephaly · polymicrogyria · postaxial polydactyly · hydrocephalus · MPPH syndrome

Introduction

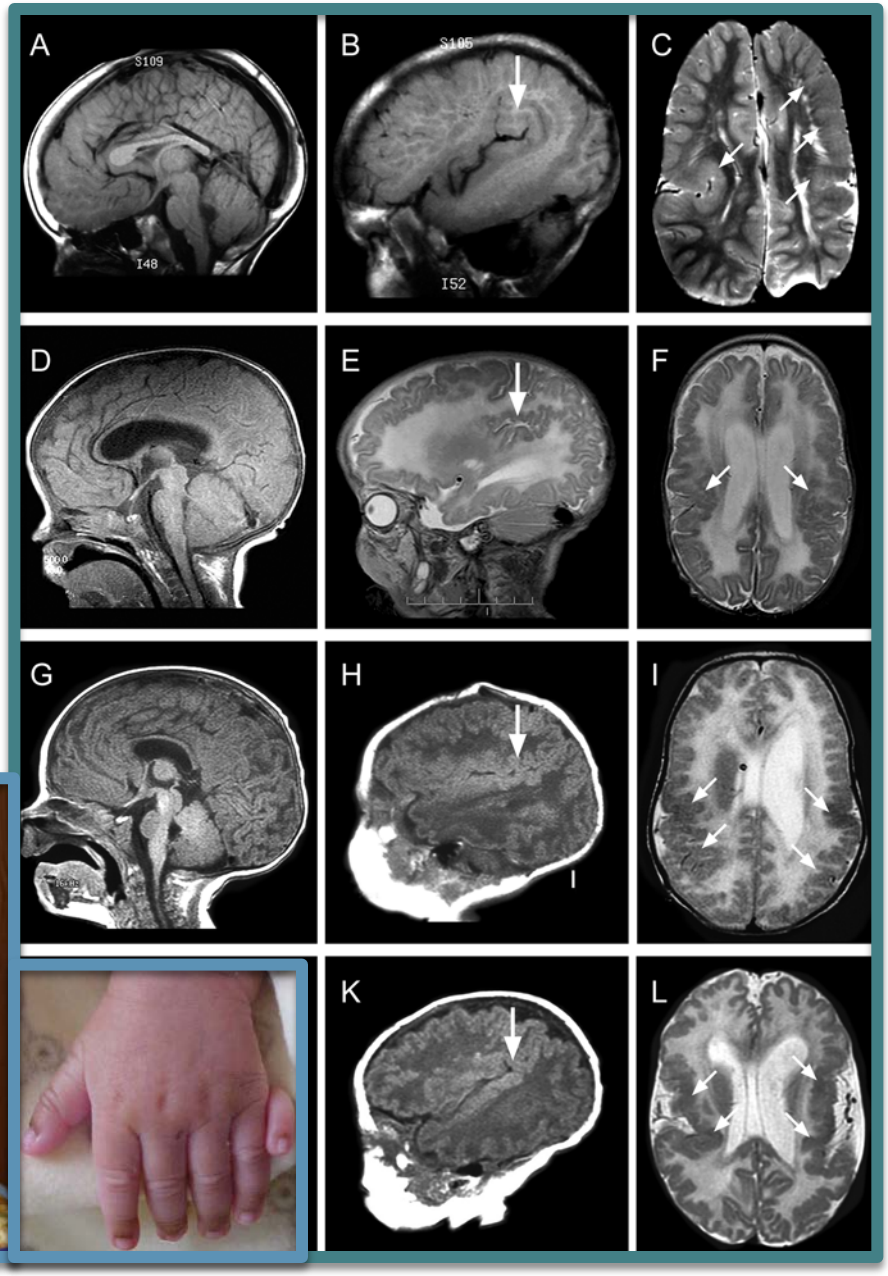
Megalencephaly (MEG) or enlarged brain occurs as a mild familial variant with normal brain structure, but otherwise is an uncommon human brain malformation that may be associated with significant developmental and neurological problems. It has been divided into anatomic and metabolic subtypes [5]. The clinical findings associated with anatomic megalencephaly have been variable. Apart from the mild familial type, anatomic megalencephaly has been found in many different disorders includ-

353

[1] Arabian G
[3] Department of Pediatrics,
[4] Division
[6] Department
[7] Departments of Human Ge

William B. Dobyns, MD · The
920 E. 58th

Neuroped



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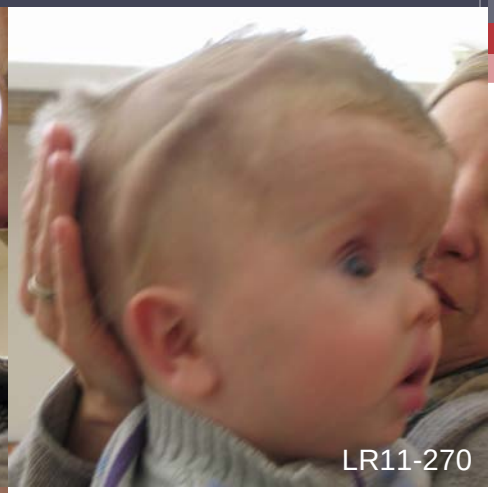
MCAP


LR11-270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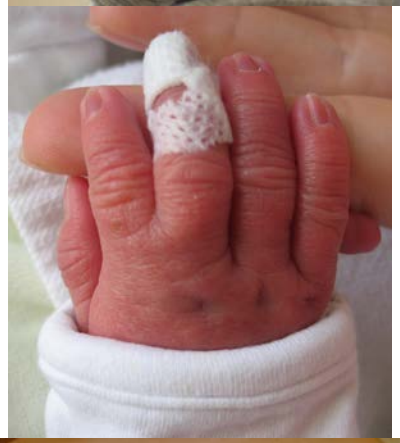


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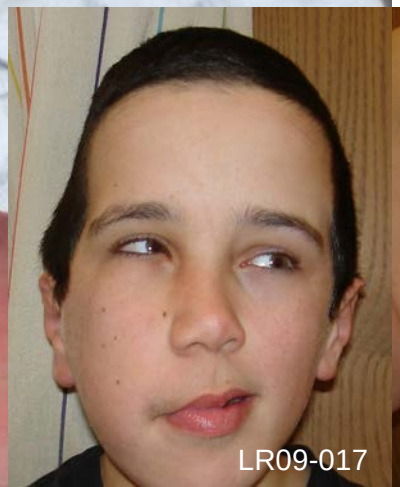
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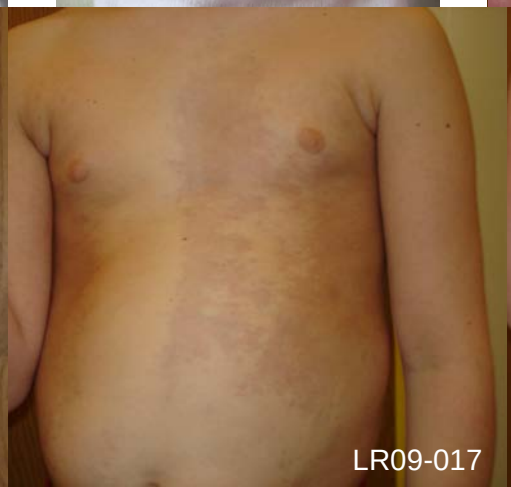
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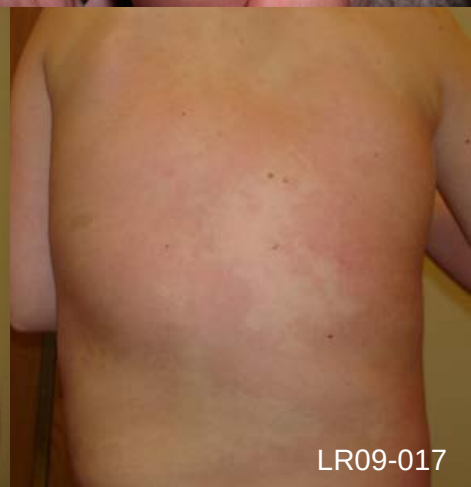
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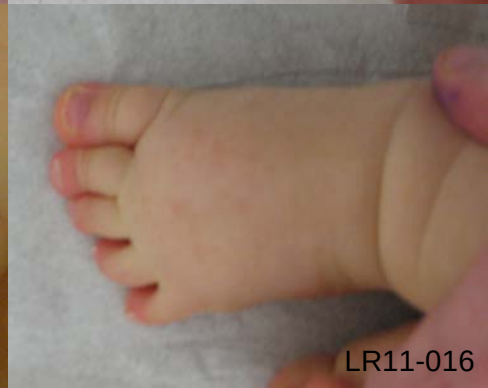
MPPH


LR11-016


LR11-016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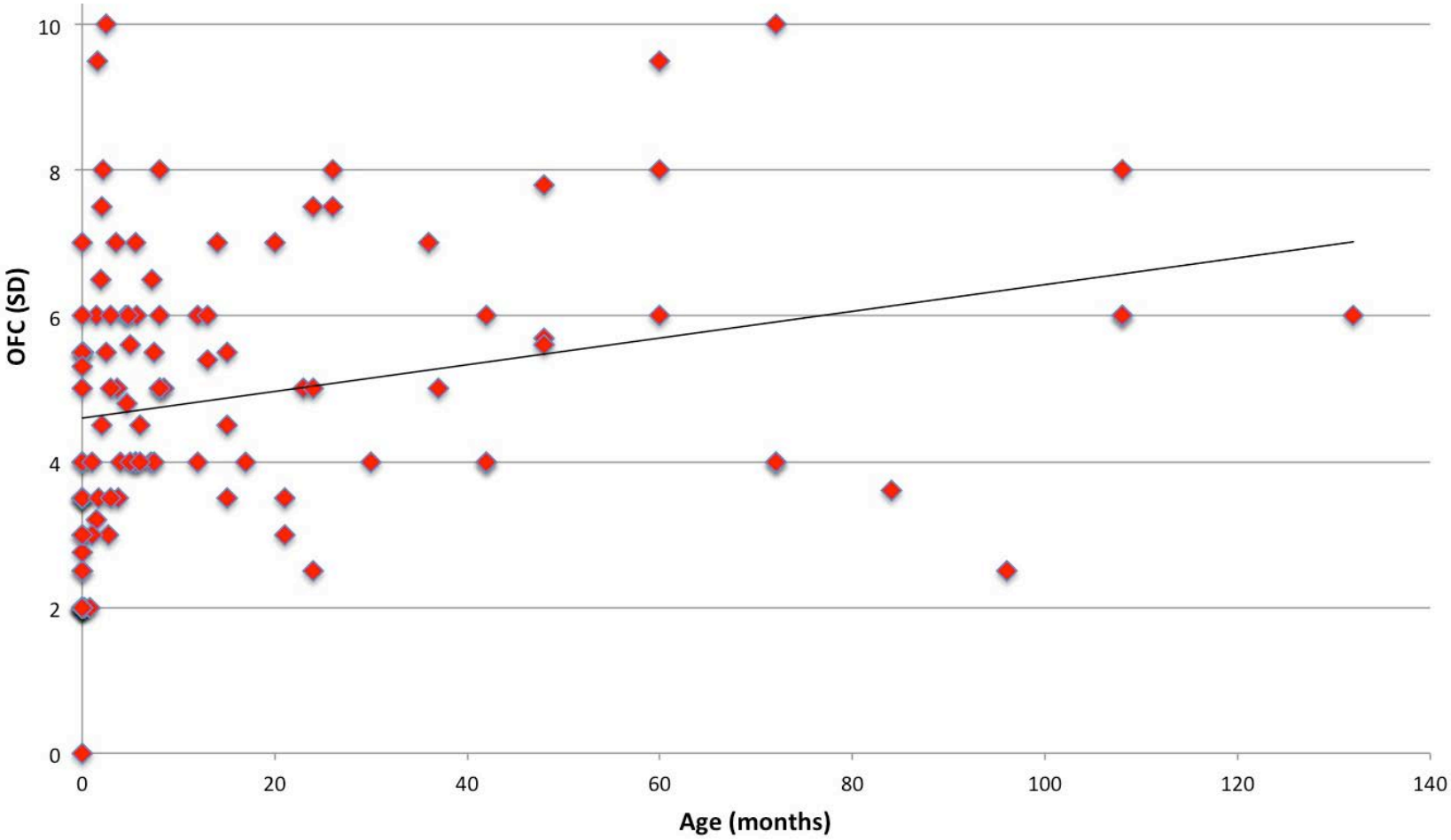
LR11-016

OFC in MEG: Z-scores



Diagnosis of MCAP vs MPPH

MCAP

- MEG
- 2nd major abnormality
  - Growth
  - Vasc
  - Syn
  - PM
- Associa
  - Hydroce BTE
  - Polydactyly
- Genetics
  - NO familial recurrence
  - Discordant MZ twins (x1)

mosaic

2 trios

MPPH

- MEG
- 2nd major abnormality
  - P
- A ties
  - CBTE
  - Poly
- Genetics
  - Rare familial recurrence x1

1 trio +1

Exome SEQ in MPPH

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Significant Overlap and Possible Identity of Macrocephaly Capillary Malformation and Megalencephaly Polymicrogyria-Polydactyly Hydrocephalus Syndromes

Karen W. Gripp,[1]* Elizabeth Hopkins,[1] Chana Vinkler,[2] Dorit Lev,[2] Gustavo Malinger,[3] Tally Lerman-Sagie,[4] and William B. Dobyns[5]

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[2]Institute of Clinical Genetics, Wolfson Medical Center, Holon, Israel
[3]Fetal Neurology Clinic, Wolfson Medical Center, Holon, Israel
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Received 18 November 2008; Accepted 17 December 2008

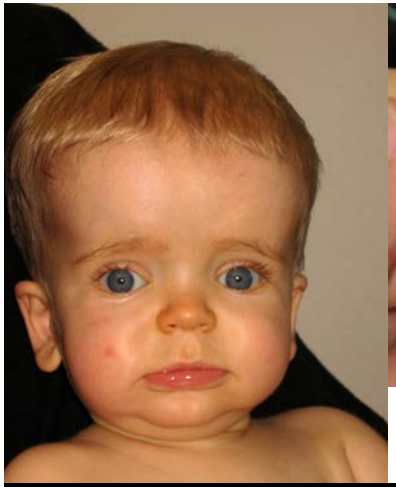
We report on three patients with macrocephaly and polymicrogyria, and additional anomalies seen in megalencephaly polymicrogyria-polydactyly hydrocephalus (MPPH) and macrocephaly capillary malformation (MCM) syndromes. Based on their characteristic brain malformations they were originally diagnosed with MPPH. In one patient the phenotype evolved during early infancy, and ultimately resulted in a diagnosis of MCM. A second was prenatally diagnosed with MPPH, but postnatally visualized capillary malformations led to a diagnosis of MCM. In a third, the original MPPH diagnosis was reconsidered after a critical review revealed additional subtle findings suggestive of MCM. Characteristic brain malformations are thought to distinguish between MPPH with perisylvian polymicrogyria, and MCM with megalencephaly with Chiari 1 malformation. However, polymicrogyria was reported in a significant number of patients with MCM. Conversely, upon review of imaging studies of patients with MPPH, we noted progressive crowding of the posterior fossa and acquired tonsillar herniation, a process deemed characteristic for MCM. Thus, neither polymicrogyria nor acquired tonsillar herniation are distinguishing features, and occur in both disorders. In addition to brain abnormalities, shared findings include cognitive impairment, coarse facial features and postaxial polydactyly. Facial [illegible] and cutis marmorata are most noticeable in [illegible] ligamentous laxity and redundant soft tissue are

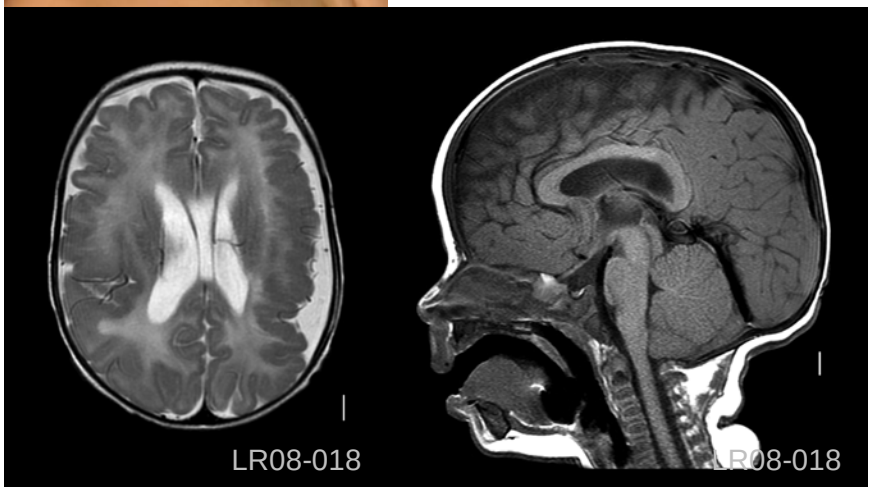
How to Cite this Article:
Gripp KW, Hopkins E, Vinkler C, Lev D, Malinger G, Lerman-Sagie T, Dobyns WB. 2009. Significant overlap and possible identity of macrocephaly capillary malformation and megalencephaly polymicrogyria-polydactyly hydrocephalus syndromes.
Am J Med Genet Part A 149A:868–876.

INTRODUCTION

The macrocephaly capillary malformation (MCM) syndrome, previously known as macrocephaly cutis marmorata teleangiectatica congenita [Toriello and Mulliken, 2007] combines a greatly enlarged head size with a striking marbled appearance of the skin that resembles cutis marmorata. More than 100 patients with MCM have been reported [Clayton-Smith et al., 1997; Moore et al., 1997; Vogels et al., 1998; Thong et al., 1999; Franceschini et al., 2000; Robertson et al., 2000; Giuliano et al., 2004; Lapunzina et al., 2004; Canham and Holder, 2008], and its neuroimaging findings were reviewed by Garavelli et al. [2005] and Conway et al.

***AKT3* p.Asn229Ser**





Del 1q43q44

Patient	*AKT3*	Birth OFC	Later OFC	Malformations
Gentile et al. 2003	Deleted	**–2 SD**	**–4 SD at 8 months**	MIC, partial ACC
Van Bever et al. 2005	Deleted	**–3 SD**	**NA**	MIC, partial ACC, CVH
LR04-249	Deleted	**–4 SD**	**NA**	MIC, ACC, CVH
LR05-202	Deleted	**–3 SD**	**–5 SD at 2 years**	MIC, ACC, CVH
LR02-409	Deleted	**–5 SD (4mo)**	**–7 SD by 5 years**	MIC, partial ACC, CVH
LR06-076	Deleted	**–2 SD (4mo)**	**–3.5 SD at 4 years**	Mild MIC, partial ACC
LR05-101	Disrupted	**50th %**	**–2.5 SD at 2 years**	MIC, ACC
De Vries et al. 2001a	Not tested	**NA**	**–5 SD at 2.5 years**	MIC, partial ACC
De Vries et al. 2001b	Not tested	**–2 SD**	**–5 SD at 1 year**	MIC, partial ACC

ARTICLE

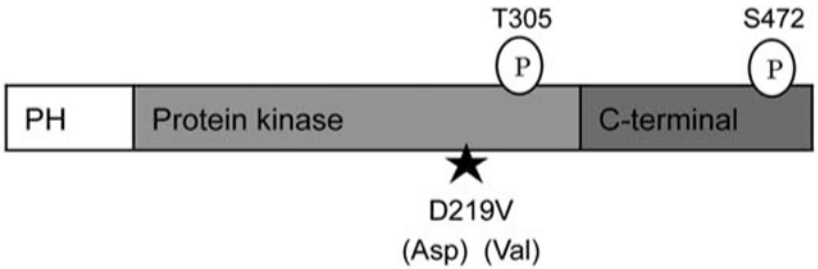
Mapping of Deletion and Translocation Breakpoints in 1q44 Implicates the Serine/Threonine Kinase AKT3 in Postnatal Microcephaly and Agenesis of the Corpus Callosum

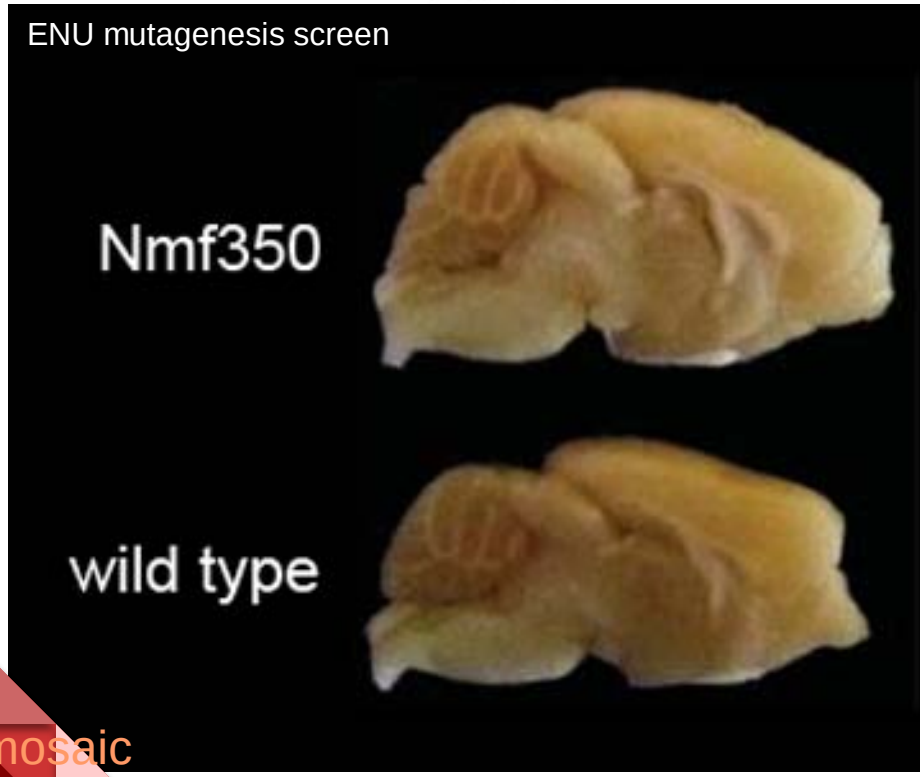
Elena Boland, Jill Clayton-Smith, Victoria G. Woo, Shane McKee, Forbes D. C. Manson, Livija Medne, Elaine Zackai, Eric A. Swanson, David Fitzpatrick, Kathleen J. Millen, Elliott H. Sherr, William B. Dobyns, and Graeme C. M. Black

Deletions of chromosome 1q42-q44 have been reported in a variety of developmental abnormalities of the brain, including microcephaly (MIC) and agenesis of the corpus callosum (ACC). Here, we describe detailed mapping studies of patients with unbalanced structural rearrangements of distal 1q4. These define a 3.5-Mb critical region extending from RP11-

Am. J. Hum. Genet. 2007;81:292–303. 0002-9297/2007/8102-0010$15.00
DOI: 10.1086/519999

Akt3$^{+/Nmf350}$





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Human Molecular Genetics, 2011, Vol. 20, No. 5 **988–999**
doi:10.1093/hmg/ddq544
Advance Access published on December 15, 2010

A novel *Akt3* mutation associated with enhanced kinase activity and seizure susceptibility in mice

Satoko Tokuda[1], Connie L. Mahaffey[1], Bobby Monks[2], Christian R. Faulkner[3], Morris J. Birnbaum[2], Steve C. Danzer[3] and Wayne N. Frankel[1,*]

[1]The Jackson Laboratory, Bar Harbor, ME 04609, USA, [2]Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA and [3]Department of Anesthesia, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

Received July 23, 2010; Revised November 22, 2010; Accepted December 9, 2010

In a phenotype-driven mutagenesis screen, a novel, dominant mouse mutation, *Nmf350*, caused low seizure threshold, sporadic tonic–clonic seizures, brain enlargement and ectopic neurons in the dentate hilus and molecular layer of the hippocampus. Genetic mapping implicated *Akt3*, one of four candidates within the critical interval. Sequencing analysis revealed that mutants have a missense mutation in *Akt3* (encoding one of three AKT/protein kinase B molecules), leading to a non-synonymous amino acid substitution in the highly conserved protein kinase domain. Previous knockout studies showed that *Akt3* is pivotal in postnatal brain development, including a smaller brain, although seizures were not observed. In contrast to *Akt3*^{Nmf350}, we find that *Akt3* null mice exhibit an elevated seizure threshold. An *in vitro* kinase assay revealed that *Akt3*^{Nmf350} confers higher enzymatic activity, suggesting that *Akt3*^{Nmf350} might enhance AKT signaling in the brain. In the dentate gyrus of *Akt3*^{Nmf350} homozygotes, we also observed a modest increase in immunoreactivity of phosphorylated ribosomal protein S6, an AKT pathway downstream target. Together these findings suggest that *Akt3*^{Nmf350} confers an increase of AKT3 activity in specific neuronal populations in the brain, and a unique dominant phenotype. *Akt3*^{Nmf350} mice provide a new tool for studying physiological roles of AKT signaling in the brain, and potentially novel mechanisms for epilepsy.

INTRODUCTION

Epilepsy, defined by recurrent seizures resulting from abnormal, synchronized neuronal discharges in the brain, affects up to 1% among the population. A genetic contribution to the disease has been estimated for about 40% of epilepsy patients (1). In recent years, advanced genetic approaches, such as association studies of candidate genes and genome-wide linkage studies with patients or family cases, have provided a better understanding of the genetic basis of idiopathic generalized epilepsies, with the identification of more than 20 variants involved in these disorders (2). The majority of responsible genes, however, remain unidentified because of the multifactorial etiology and genetic heterogeneity of the disease.

Phenotype-based chemical mutagenesis with ethylnitrosourea (ENU) has been performed with high success and efficiency to obtain new animal models of various neurological disorders and to discover human disease genes (3–7). The electroconvulsive threshold (ECT) test in mice has been used extensively to advance the study of human seizures, and is an integral part of the ongoing program for development of antiepileptic drugs. We adopted ECT as a robust screening tool to find seizure-prone or resistant genes following ENU mutagenesis. Using this approach, we previously discovered that a dominant mutation, *Szt1*, lowers the seizure threshold in mice without spontaneous seizures (8). Interestingly, genetic analysis revealed that the *Szt1* mutation was a spontaneous deletion that included the *Kcnq2* gene, the human orthologue of which is mutated in human epilepsy families. This provided proof of principle that ECT can be a relevant screening tool to develop genetic models that are relevant for human epilepsy research.

Upstream and downstream molecules involved in serine/threonine kinase AKT pathways have been implicated in mechanisms of epilepsy (9–13). AKT, also known as

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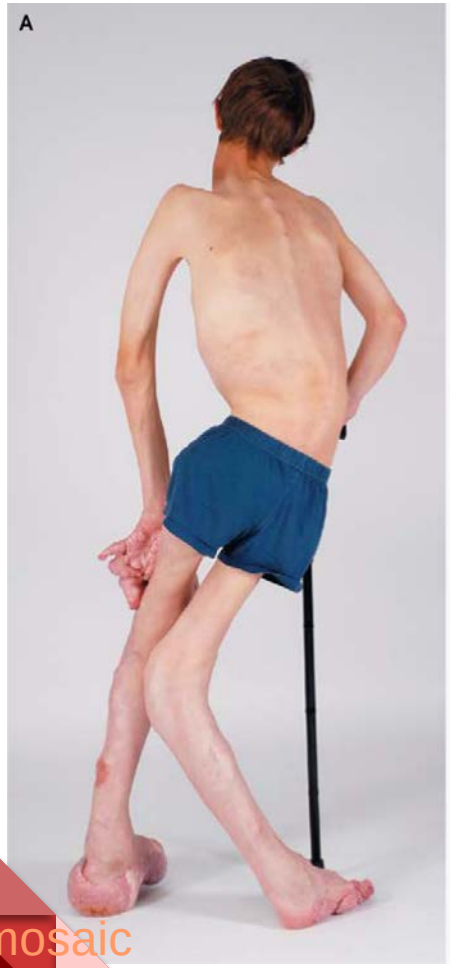
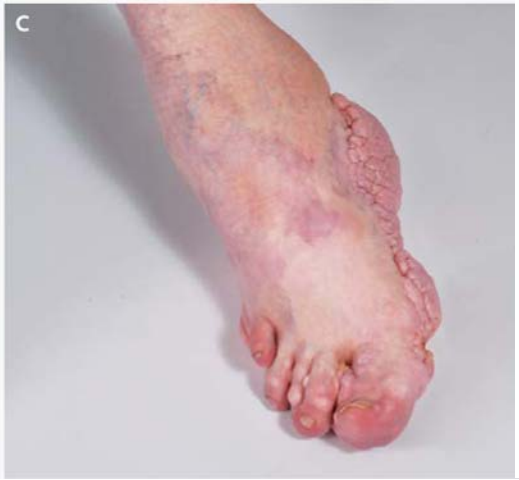
***Akt3* p.Asp219Val**

Proteus syndrome





mosaic

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Mosaic Activating Mutation in *AKT1* Associated with the Proteus Syndrome

Marjorie J. Lindhurst, Ph.D., Julie C. Sapp, Sc.M., Jamie K. Teer, Ph.D., Jennifer J. Johnston, Ph.D., Erin M. Finn, B.A., Kathryn Peters, M.S., Joyce Turner, M.S., Jennifer L. Cannons, Ph.D., David Bick, M.D., Laurel Blakemore, M.D., Catherine Blumhorst, M.S.N., Knut Brockmann, M.D., Peter Calder, M.B., B.S., Natasha Cherman, Ph.D., Matthew A. Deardorff, M.D., Ph.D., David B. Everman, M.D., Gretchen Golas, M.S., Robert M. Greenstein, M.D., B. Maya Kato, M.D., Kim M. Keppler-Noreuil, M.D., Sergei A. Kuznetsov, Ph.D., Richard T. Miyamoto, M.D., Kurt Newman, M.D., David Ng, M.D., Kevin O'Brien, M.S., Steven Rothenberg, M.D., Douglas J. Schwartzentruber, M.D., Virender Singhal, M.D., M.B.A., Roberto Tirabosco, M.D., Joseph Upton, M.D., Shlomo Wientroub, M.D., Elaine H. Zackai, M.D., Kimberly Hoag, Tracey Whitewood-Neal, Pamela G. Robey, Ph.D., Pamela L. Schwartzberg, M.D., Ph.D., Thomas N. Darling, M.D., Ph.D., Laura L. Tosi, M.D., James C. Mullikin, Ph.D., and Leslie G. Biesecker, M.D.

ABSTRACT

BACKGROUND

The Proteus syndrome is characterized by the overgrowth of skin, connective tissue, brain, and other tissues. It has been hypothesized that the syndrome is caused by somatic mosaicism for a mutation that is lethal in the nonmosaic state.

METHODS

We performed exome sequencing of DNA from biopsy samples obtained from patients with the Proteus syndrome and compared the resultant DNA sequences with those of unaffected tissues obtained from the same patients. We confirmed and extended an observed association, using a custom restriction-enzyme assay to analyze the DNA in 158 samples from 29 patients with the Proteus syndrome. We then assayed activation of the AKT protein in affected tissues, using phosphorylation-specific antibodies on Western blots.

RESULTS

Of 29 patients with the Proteus syndrome, 26 had a somatic activating mutation (c.49G→A, p.Glu17Lys) in the oncogene *AKT1*, encoding the AKT1 kinase, an enzyme known to mediate processes such as cell proliferation and apoptosis. Tissues and cell lines from patients with the Proteus syndrome harbored admixtures of mutant alleles that ranged from 1% to approximately 50%. Mutant cell lines showed greater AKT phosphorylation than did control cell lines. A pair of single-cell clones that were established from the same starting culture and differed with respect to their mutation status had different levels of AKT phosphorylation.

CONCLUSIONS

The Proteus syndrome is caused by a somatic activating mutation in *AKT1*, proving the hypothesis of somatic mosaicism and implicating activation of the PI3K–AKT pathway in the characteristic clinical findings of overgrowth and tumor susceptibility in this disorder. (Funded by the Intramural Research Program of the National Human Genome Research

The authors' affiliations are listed in the Appendix. Address reprint requests to Dr. Biesecker at the Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bldg. 49, Rm. 4A56, Bethesda, MD 20892, or at leslieb@helix.nih.gov.

This article (10.1056/NEJMoa1104017) was published on July 27, 2011, at NEJM.org.

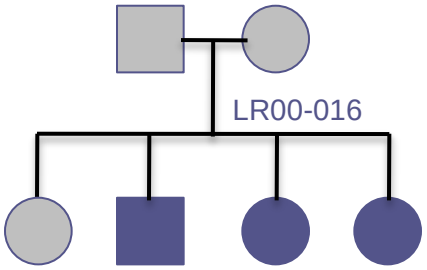
N Engl J Med 2011.

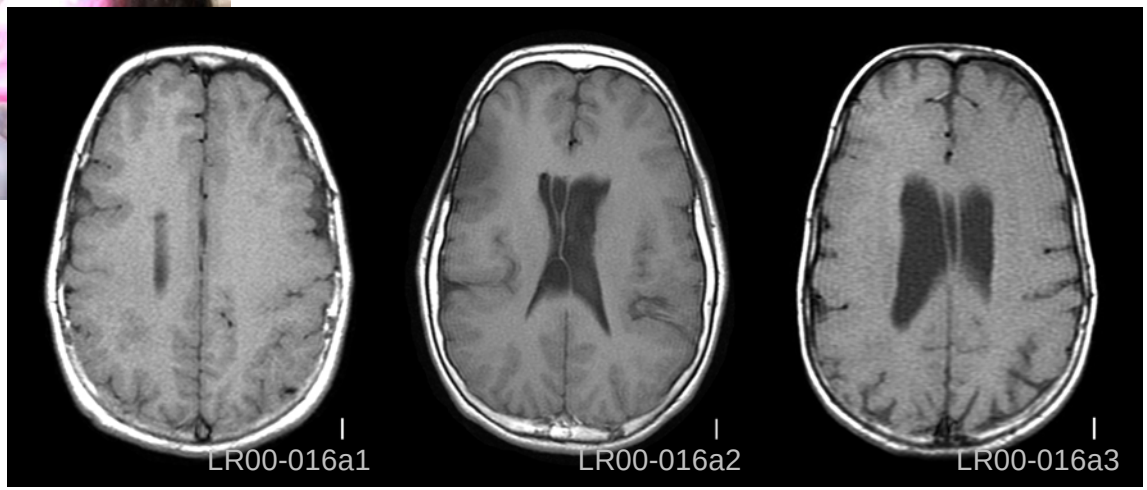
***AKT1* p.Glu17Lys**

Exome SEQ in MPPH

PIK3R2 p.Gly373Arg

- Brother
  - HYD, Chiari 1, no seizures or scoliosis
- Sister 1
  - HYD, seizures, Chiari 1, scoliosis
- Sister 2
  - Intractable seizures, Chiari 1, scoliosis

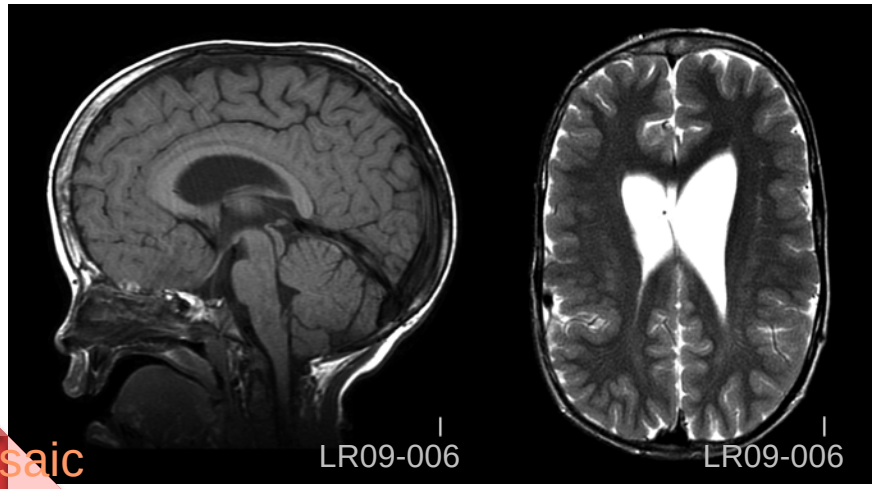


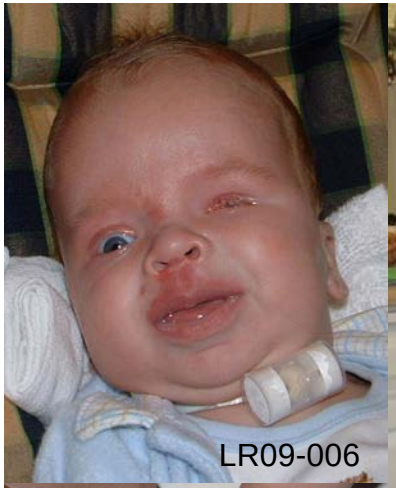


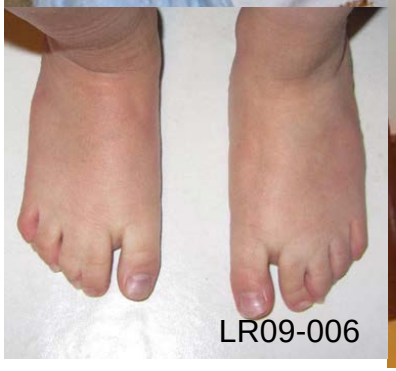
Exome SEQ in MCAP

PIK3CA **p.Gly914Arg**

- Growth
  - Megalencephaly
- Skin
  - Capillary malformation
- Skeletal
  - Polydactyly (L) and syndactyly
- Brain
  - Hydrocephalus







nature genetics

PIK3CA levels of mosaicism

De novo germline and postzygotic mutations in *AKT3*, *PIK3R2* and *PIK3CA* cause a spectrum of related megalencephaly syndromes

Jean-Baptiste Rivière[1], Ghayda M Mi… [2], Robert L Conway[6], Judith St-Onge[1], Thea Worthylake[4], Christopher T Su… Beate Albrecht[11], Christine M Armo… Beth A Drolet[16,17], A Micheil Innes[1… Wendy S Meschino[20], James D Reggi… Rosanna Weksberg[15], Birgit Zirn[25], … Consortium[26], Jacek Majewski[27], De… Kym M Boycott[4,9] & William B Doby…

**Megalencephaly-capillary malformation (… megalencephaly-polymicrogyria-polydact… (MPPH) syndromes are sporadic overgrow… associated with markedly enlarged brain s… recognizable features[1–5]. We performed e… 3 families with MCAP or MPPH, and our in… confirmed in exomes from 7 individuals w… control individuals, as well as in 40 additi… megalencephaly, using a combination of S… restriction enzyme assays and targeted de… We identified *de novo* germline or postzy… three core components of the phosphatidy… (PI3K)-AKT pathway. These include 2 mut… 1 recurrent mutation in *PIK3R2* in 11 unr… MPPH and 15 mostly postzygotic mutatio… individuals with MCAP and 1 with MPPH. … the central role of PI3K-AKT signaling in v… brain development and emphasize the po… parallel sequencing in a challenging conte… genetic heterogeneity combined with pos…

As described in our recent clinical analysis in … or MPPH[4], the former consists of megalen… hemimegalencephaly), associated growth dys… asymmetry, developmental vascular anomalie… tions (syndactyly and polydactyly), variable co… a mild connective tissue dysplasia (**Fig. 1** and … and **2**). The patchy skin vascular malformatio… growth seen in MCAP meet the criteria for …

A full list of affiliations appears at the end of the pape…

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Heterozygous, so . . .
% mutant reads x2 = % mutant cells

Table 2 ***PIK3CA*** **mutations identified or confirmed by deep sequencing of five mutation sites**

				Megalencephaly patients			Controls	
Subject ID	Source of DNA	Mutation coordinates (hg19)	Amino-acid change	Total reads[a]	Mutant reads[a]	Percent mutant allele	Mean mutant allele freq.[a,b] (× 10^{-4})	Max mutant allele freq.[a,c] (× 10^{-4})
Confirmed *PIK3CA* mutations								
LR06-220	Blood	Chr. 3: g.178916876G>A	p.Arg88Gln	149,513	64,260	43	1.7	2.9
44735	Blood	Chr. 3: g.178922364G>A	p.Cys378Tyr	285,251	84,600	30	0.4	1.1
LR08-261	Blood	Chr. 3: g.178938934G>A	p.Glu726Lys	293,540	35,865	12	0.5	0.7
	Buccal swab	Chr. 3: g.178938934G>A	p.Glu726Lys	5,087	2,110	41	0.5	0.7
LR06-333	LCL	Chr. 3: g.178938934G>A	p.Glu726Lys	260,333	36,348	14	0.5	0.7
	Saliva	Chr. 3: g.178938934G>A	p.Glu726Lys	125,336	19,195	15	0.5	0.7
LR09-006	Blood	Chr. 3: g.178947865G>A	p.Gly914Arg	392,036	61,427	16	19	23
LR11-070	LCL	Chr. 3: g.178947865G>A	p.Gly914Arg	663,398	102,811	15	19	23
	Saliva	Chr. 3: g.178947865G>A	p.Gly914Arg	327,763	56,313	17	19	23
LR06-341	Blood	Chr. 3: g.178947865G>A	p.Gly914Arg	728,792	112,818	15	19	23
	Saliva	Chr. 3: g.178947865G>A	p.Gly914Arg	538,366	103,171	19	19	23
LR11-069	LCL	Chr. 3: g.178952018A>G	p.Thr1025Ala	49,105	11,856	24	18	19
	Saliva	Chr. 3: g.178952018A>G	p.Thr1025Ala	12,126	1,440	12	18	19
New *PIK3CA* mutations								
LR06-342	Saliva	Chr. 3: g.178916854G>A	p.Glu81Lys	51,268	4,227	8	0.4	0.5
LR11-068	Blood	Chr. 3: g.178916876G>A	p.Arg88Gln	117,487	2,921	2	1.7	2.9
Validation of new *PIK3CA* mutations								
LR06-342	LCL	Chr. 3: g.178916854G>A	p.Glu81Lys	135,801	993	1	0.2	0.2
	Saliva	Chr. 3: g.178916854G>A	p.Glu81Lys	82,864	2,783	3	0.2	0.2
LR11-068	Blood	Chr. 3: g.178916876G>A	p.Arg88Gln	282,771	3,378	1	0.2	0.3
	Saliva	Chr. 3: g.178916876G>A	p.Arg88Gln	45,578	1,714	4	0.2	0.3

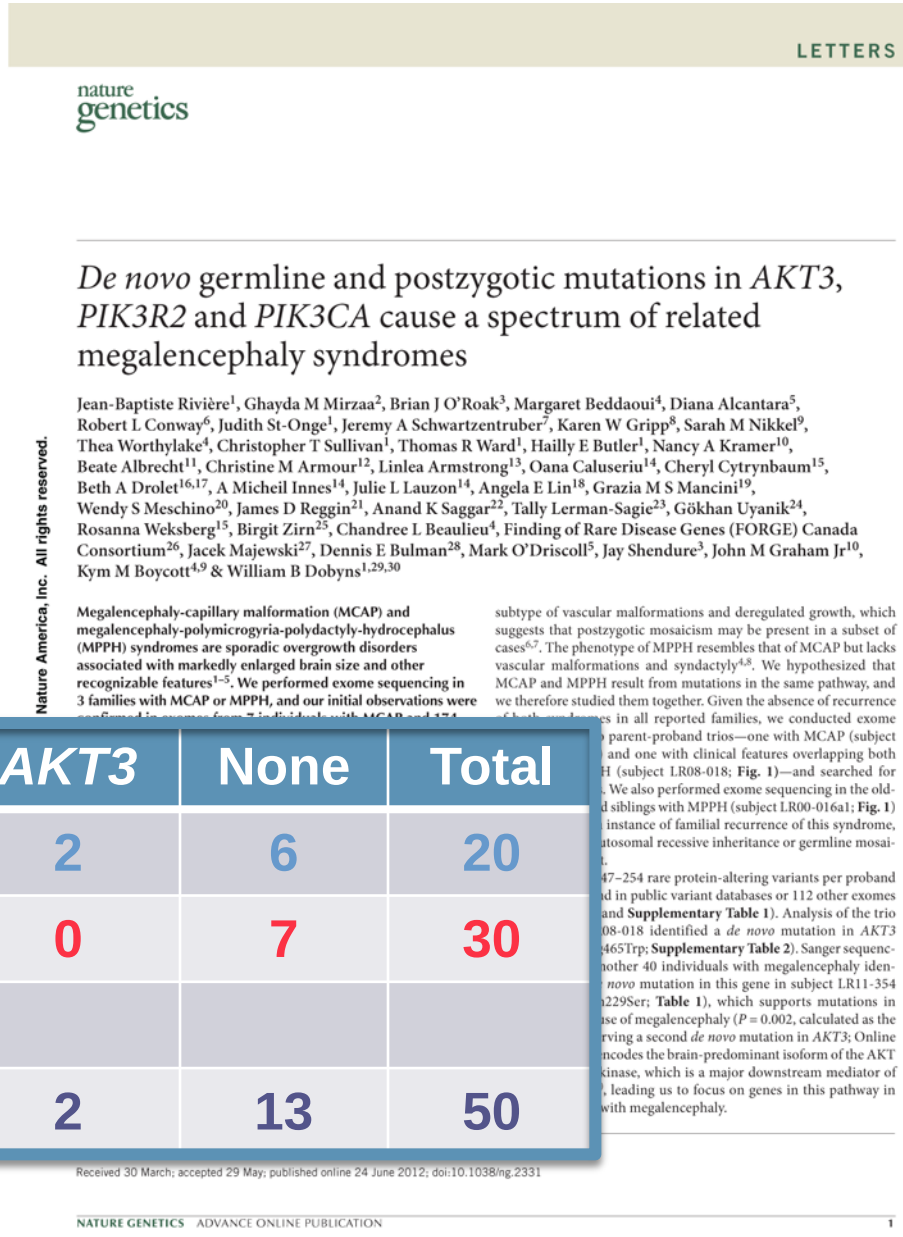
LCL, lymphoblastoid cell line; chr., chromosome; freq., frequency.
[a]Only bases with base quality of ≥20 were considered. [b]Average frequency of the mutant allele in control individuals. [c]Highest mutant allele frequency in control individuals.

mosaic

Megalencephaly

37/50 = 74%

	PIK3CA	PIK3R2	AKT3	None	Total
MPPH	1	11	2	6	20
MCAP	23	0	0	7	30
TOTAL	24	11	2	13	50



LETTERS

nature genetics

De novo germline and postzygotic mutations in *AKT3*, *PIK3R2* and *PIK3CA* cause a spectrum of related megalencephaly syndromes

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Megalencephaly-capillary malformation (MCAP) and megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndromes are sporadic overgrowth disorders associated with markedly enlarged brain size and other recognizable features[1–5]. We performed exome sequencing in 3 families with MCAP or MPPH, and our initial observations were

subtype of vascular malformations and deregulated growth, which suggests that postzygotic mosaicism may be present in a subset of cases[6,7]. The phenotype of MPPH resembles that of MCAP but lacks vascular malformations and syndactyly[4,8]. We hypothesized that MCAP and MPPH result from mutations in the same pathway, and we therefore studied them together. Given the absence of recurrence

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Neuron
Report

Cell PRESS

Somatic Activation of *AKT3* Causes Hemispheric Developmental Brain Malformations

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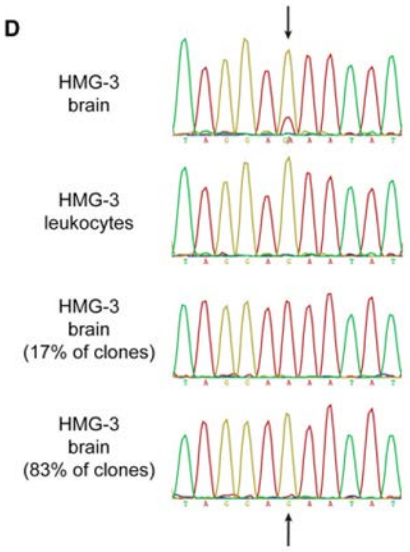

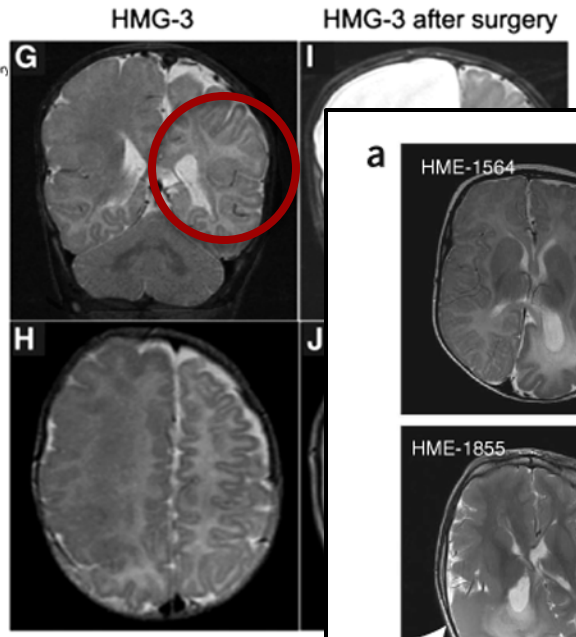

somatic overgrowth syndromes. We show that *AKT3* is the most abundant AKT paralog in the brain during neurogenesis and that phosphorylated AKT is abundant in cortical progenitor cells. Our data suggest that somatic mutations limited to the brain

essentially impossible to study potential roles of mutati
are limited to brain cells, because such mutations are b
tion absent from blood and other tissues typically avail
genetic study. Such somatic mutations could conceiva
important roles in complex neurogenetic disorders,
epilepsy, intellectual disability, and psychiatric dise

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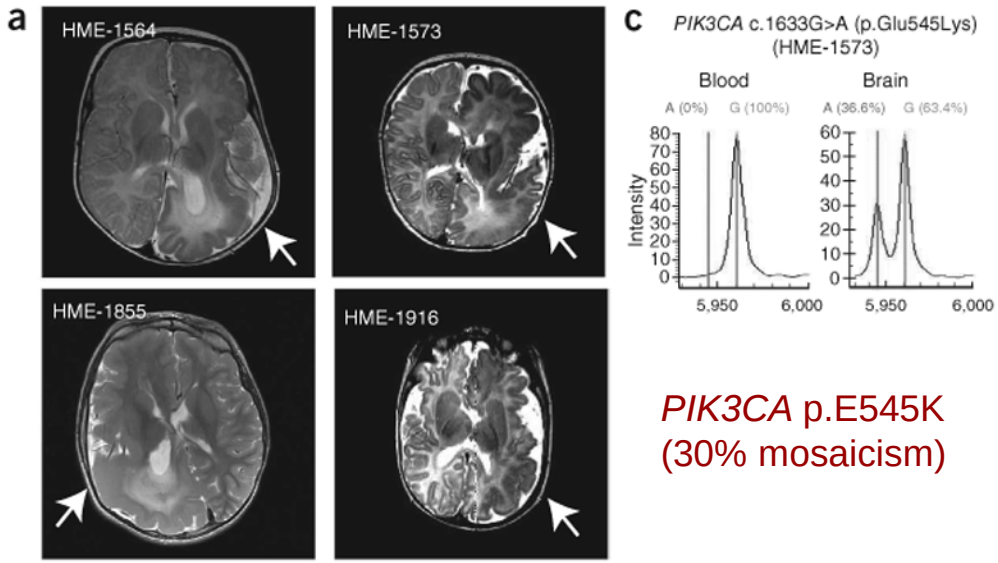
LETTERS

nature genetics

De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly

ıg Ho Lee[1,2,9], My Huynh[3,4], Jennifer L Silhavy[1,2], Sangwoo Kim[5], Tracy Dixon-Salazar[1,2], Andrew Heiberg[1,2], : Scott[1,2], Vineet Bafna[5], Kiley J Hill[1,2], Adrienne Collazo[1,2], Vincent Funari[6,7], Carsten Russ[8], :ey B Gabriel[8], Gary W Mathern[3,4,10] & Joseph G Gleeson[1,2,10]

ıovo somatic mutations in focal areas are well documented individuals display progressive and catastrophic epilepsy, which is



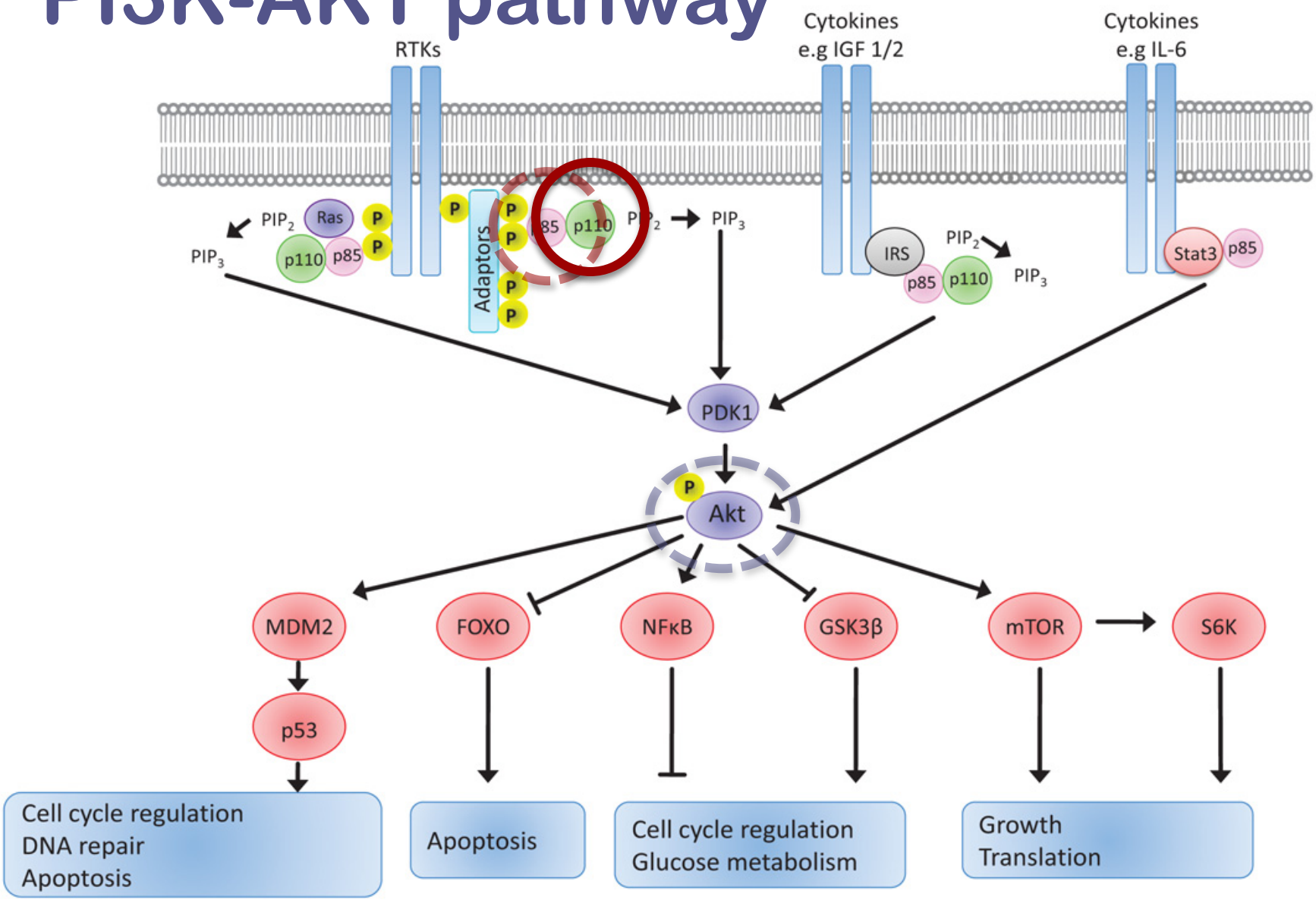
b

	Blood		Brain		
	Mut counts	Ref counts	Mut counts	Ref counts	Mut allele (%)
PIK3CA c.1633G>A (HME-1573)	0	121	9	47	16%

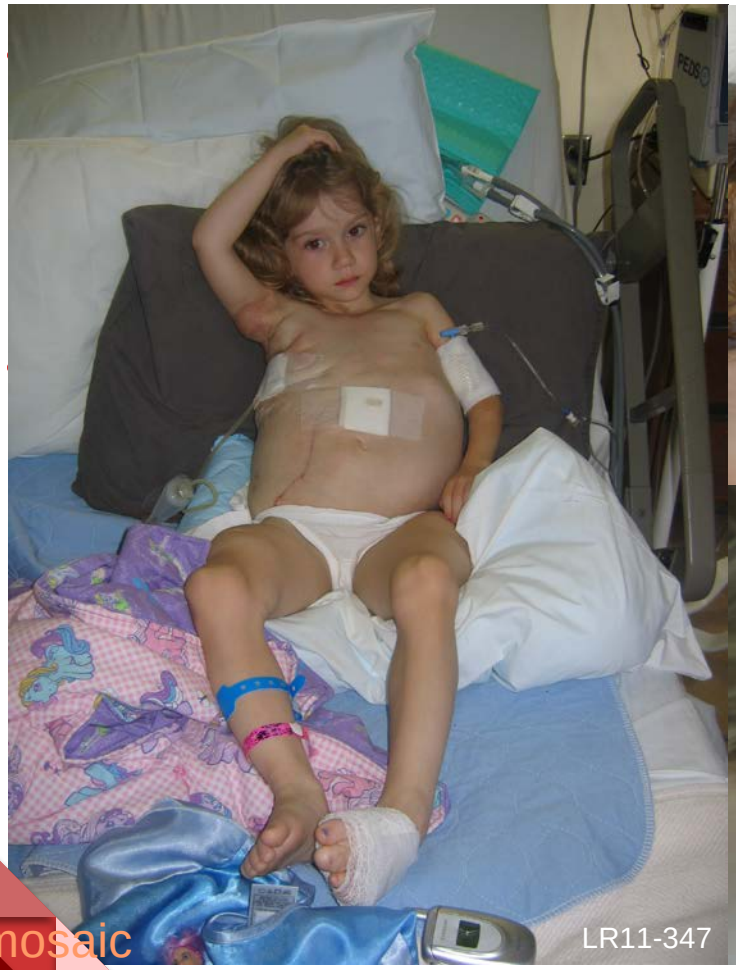
PIK3CA p.E545K
(30% mosaicism)

mosaic

PI3K-AKT pathway



CLOVES | *PIK3CA*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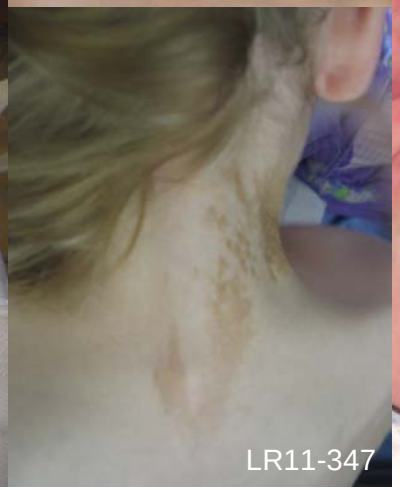
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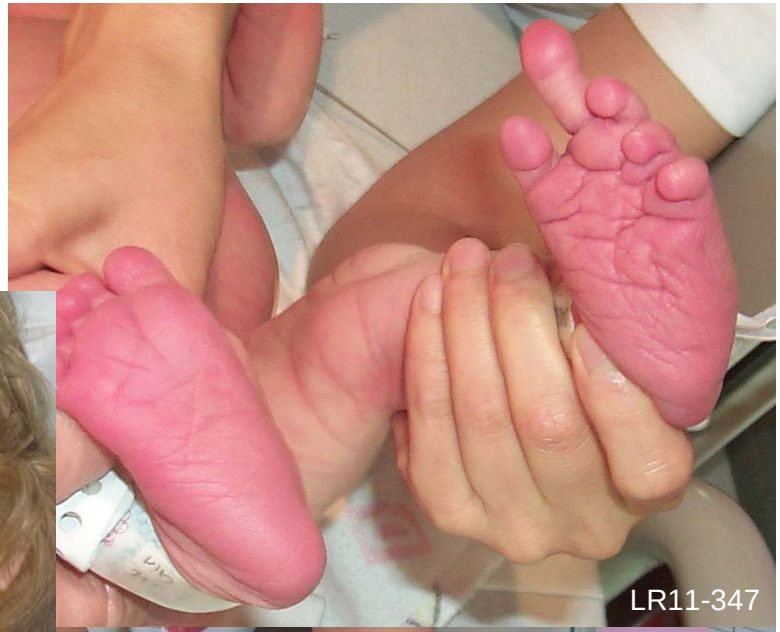
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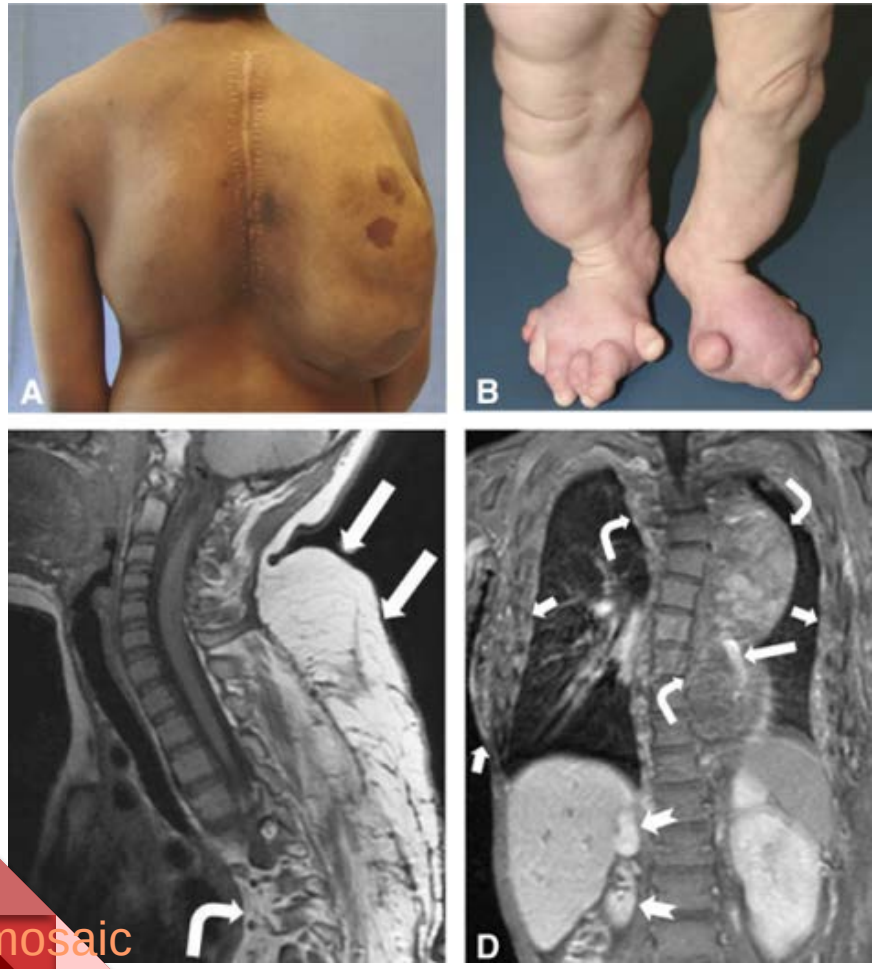
from John Graham LR11-347

CLOVES | *PIK3CA*



mosaic

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REPORT

Somatic Mosaic Activating Mutations in *PIK3CA* Cause CLOVES Syndrome

Kyle C. Kurek,[1] Valerie L. Luks,[2] Ugur M. Ayturk,[2,9] Ahmad I. Alomari,[3,6] Steven J. Fishman,[4,6] Samantha A. Spencer,[2,6] John B. Mulliken,[5,6] Margot E. Bowen,[2,9] Guilherme L. Yamamoto,[7] Harry P.W. Kozakewich,[1,6] and Matthew L. Warman[2,6,8,9,*]

Congenital lipomatous overgrowth with vascular, epidermal, and skeletal anomalies (CLOVES) is a sporadically occurring, nonhereditary disorder characterized by asymmetric somatic hypertrophy and anomalies in multiple organs. We hypothesized that CLOVES syndrome would be caused by a somatic mutation arising during early embryonic development. Therefore, we employed massively parallel sequencing to search for somatic mosaic mutations in fresh, frozen, or fixed archival tissue from six affected individuals. We identified mutations in *PIK3CA* in all six individuals, and mutant allele frequencies ranged from 3% to 30% in affected tissue from multiple embryonic lineages. Interestingly, these same mutations have been identified in cancer cells, in which they increase phosphoinositide-3-kinase activity. We conclude that CLOVES is caused by postzygotic activating mutations in *PIK3CA*. The application of similar sequencing strategies will probably identify additional genetic causes for sporadically occurring, nonheritable malformations.

Syndromes can be genetic in origin but not necessarily heritable. Happle postulated that such disorders arise as the result of somatic rather than germline mutations, i.e., because complete heterozygosity for a causative mutation would either be lethal to the affected individual or be incapable of transmission through egg or sperm.[1] Happle's hypothesis was confirmed by the discovery of somatic mosaic mutations in several diseases, including McCune-Albright syndrome (MIM 174800) and Proteus syndrome (MIM 176920).[2,3]

Isolated malformations and nonhereditary syndromes with malformation as a component feature could also be caused by a somatic mutation. One such candidate is the recently described disorder CLOVES (*c*ongenital *l*ipomatous asymmetric *o*vergrowth of the trunk with lymphatic, capillary, venous, and combined-type *v*ascular malformations, *e*pidermal nevi, and *s*keletal anomalies [MIM 612918]) (Figure 1).[4–8] Therefore, we sought to identify postzygotic mutations in individuals with CLOVES by employing massively parallel sequencing of DNA and RNA from affected tissue to look for mutations that are present at low frequencies. The study was approved by the institutional review board at Boston Children's Hospital. Study participants provided written informed consent to participate in the study and to authorize the publication of clinical images.

We used fresh or frozen affected tissue from four CLOVES-affected individuals, each of whom had undergone resection of a lipomatous overgrowth or a vascular malformation with lipomatous overgrowth (Tables 1 and 2), and we produced barcoded DNA sequencing libraries as described previously.[9] We assumed that lipomatous tissue would contain mutant cells given that incompletely resected lesions often regrow.[5] We also recovered mRNA and generated barcoded cDNA sequencing libraries[10] from these same specimens. For two of these individuals, we prepared DNA sequencing libraries from white blood cell (blood or saliva) DNA. We assumed that this unaffected tissue would not contain a CLOVES causative mutation. Formalin-fixed paraffin-embedded tissue blocks were available from two other individuals (Table 2) from whom we recovered DNA by using thin sections to produce additional barcoded sequencing libraries (see Figure S1, available online, for the experimental design).[9] Saliva DNA was available from one of these individuals and was used for the preparation of a library.

We enriched each DNA library generated from the fresh or frozen samples for exonic sequences by using the SureSelect human exome kit (Agilent Technologies, Santa Clara, CA, USA). For DNA libraries generated from the paraffin tissue blocks, we employed a custom-designed enrichment array that contained exonic sequences from 77 genes involved in signaling pathways for several growth factors. We had previously employed this targeted array to screen individuals with metachondromatosis.[9] Several genes included in this array, e.g., *PTEN* (MIM 601728), *AKT1* (MIM 164730), *AKT2* (MIM 164731), and *AKT3* (MIM 611223), have been implicated in other overgrowth syndromes.[3,11–14] We performed RNA sequencing in case the gene responsible for CLOVES was abundantly

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DOI 10.1016/j.ajhg.2012.05.006.

nature genetics

CLOVES variant

Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in *PIK3CA*

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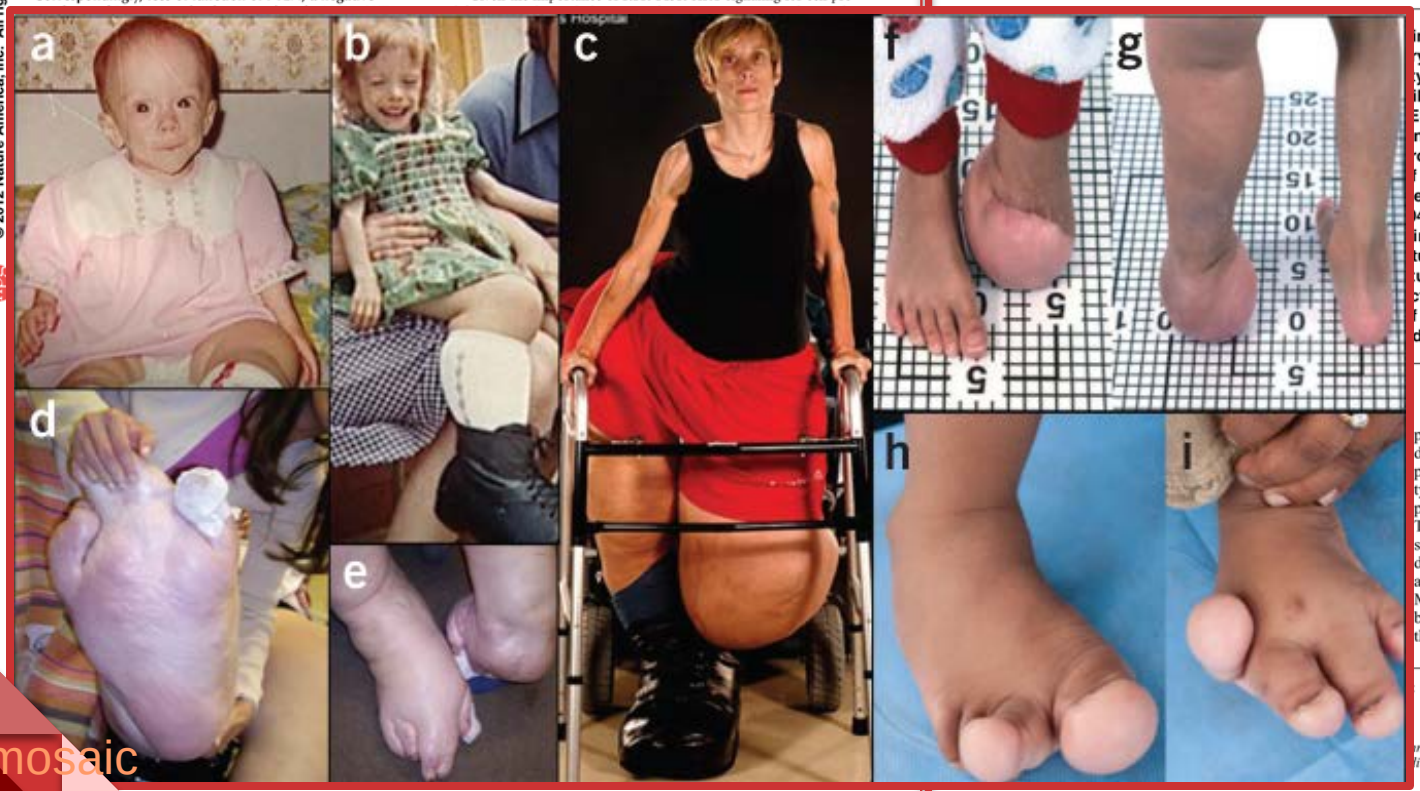
The phosphatidylinositol 3-kinase (PI3K)-AKT signaling pathway is critical for cellular growth and metabolism. Correspondingly, loss of function of PTEN, a negative

pathway is the receptor tyrosine kinase (RTK)-PI3K-AKT pathway, which harbors activating mutations in most solid tumors[1].

Given the importance of RTK-PI3K-AKT signaling for cell pro-



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Somatic gain-of-function mutations in *PIK3CA* in patients with macrodactyly

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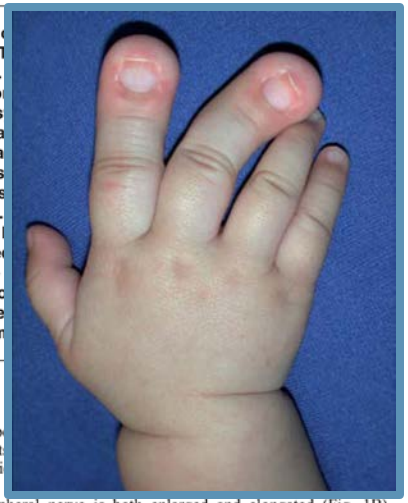

peripheral nerve is both enlarged and elongated (Fig. 1B). The treatment of macrodactyly to date has been ablative, consisting of debulking by excision of a portion of the involved digit, epiphysiodesis to stop longitudinal skeletal growth and amputation when the enlarged parts are no longer functional. Macrodactyly has been described as an unsolved condition by the International Federation of Societies for Surgery of the Hand.

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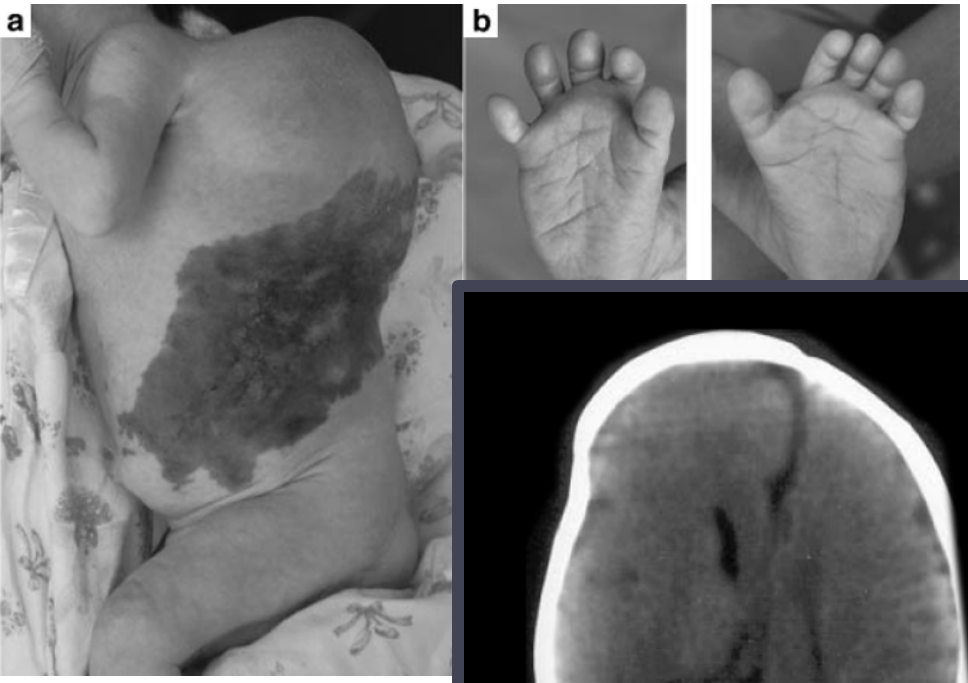
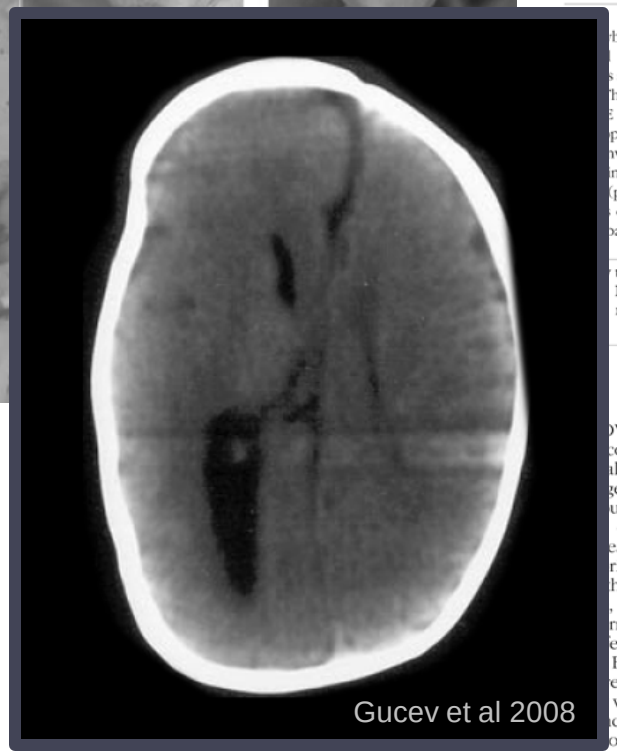
CLOVES | DMEG




Gucev et al 2008

American Journal of Medical Genetics Part A 146A:2688–2690 (2008)

Clinical Report

Congenital Lipomatous Overgrowth, Vascular Malformations, and Epidermal Nevi (CLOVE) Syndrome: CNS Malformations and Seizures may be a Component of This Disorder

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born girl was found to have a massive lymphatic vascular malformation with overlying cutaneous anomaly associated with overgrown feet and splayed These manifestations comprise the recently described syndrome. She also had cranial asymmetry and ped generalized seizures, which were treated with nvulsants. Cranial CT showed encephalomalacia, ng of the ventricles and the sulci, hemimegalence- (predominantly white matter) and partial agenesis of callosum. Review of the literature identified several patients with CLOVE syndrome, some of whom were misdiagnosed as having Proteus syndrome, with strikingly similar manifestations. We conclude that CNS manifestations including hemimegalencephaly, dysgenesis of the corpus callosum, neuronal migration defects, and the consequent seizures, may be an rarely recognized manifestation of CLOVE syndrome. © 2008 Wiley-Liss, Inc.

Key words: CLOVE syndrome; lymphatic vascular malformation; agenesis of corpus callosum; hemimegalencephaly; overgrowth; cataracts

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INTRODUCTION

VE syndrome is a recently delineated disorder comprises vascular malformations (typically l), dysregulated adipose tissue, scoliosis, ged bony structures (typically of the legs) ut progressive or distorting bony overgrowth et al., 2007]. This recent delineation distin- es it from Proteus syndrome, a disorder that rises localized, progressive, postnatal over- h with bony distortion, dysregulated adipose , cerebriform connective tissue and linear rmal nevus, hemimegalencephaly, and other estations [Biesecker et al., 1999; Turner et al., Biesecker, 2006]. We describe a neonate with es of CLOVE syndrome and hemimegalence- with agenesis of the corpus callosum, features cluded in the previous description of CLOVE ome.

CLINICAL REPORT

A 15-day-old Caucasian girl was the first child of nonconsanguineous and otherwise healthy and young parents. At the end of the third trimester an abdominal wall mass was noted on ultrasound examination. She was delivered by cesarean at 31 weeks gestation, with an Apgar score of 7/8. Her birth weight was 3,660 g (≫97th centile), her length was 50 cm (~90th centile) and her OFC was 36 cm (≫97th centile). She had downslanting palpebral fissures, flattening of the malar bones, relative lengthening of the face, and a persistently open

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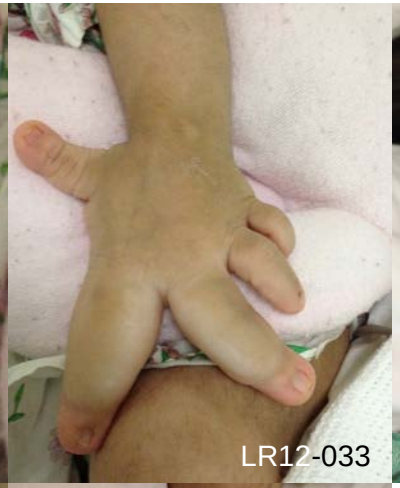
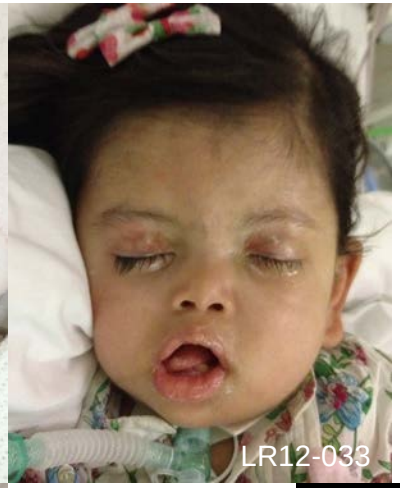
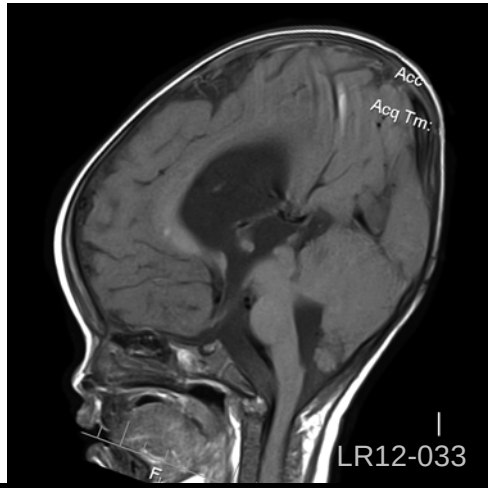
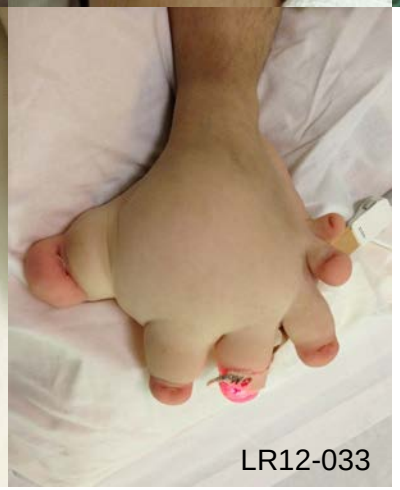
CLOVES | DMEG

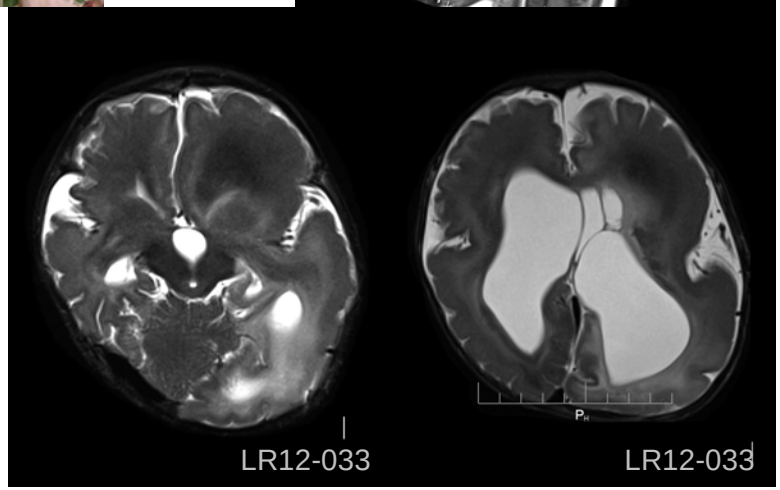
***PIK3CA* p.E545K mosaic**



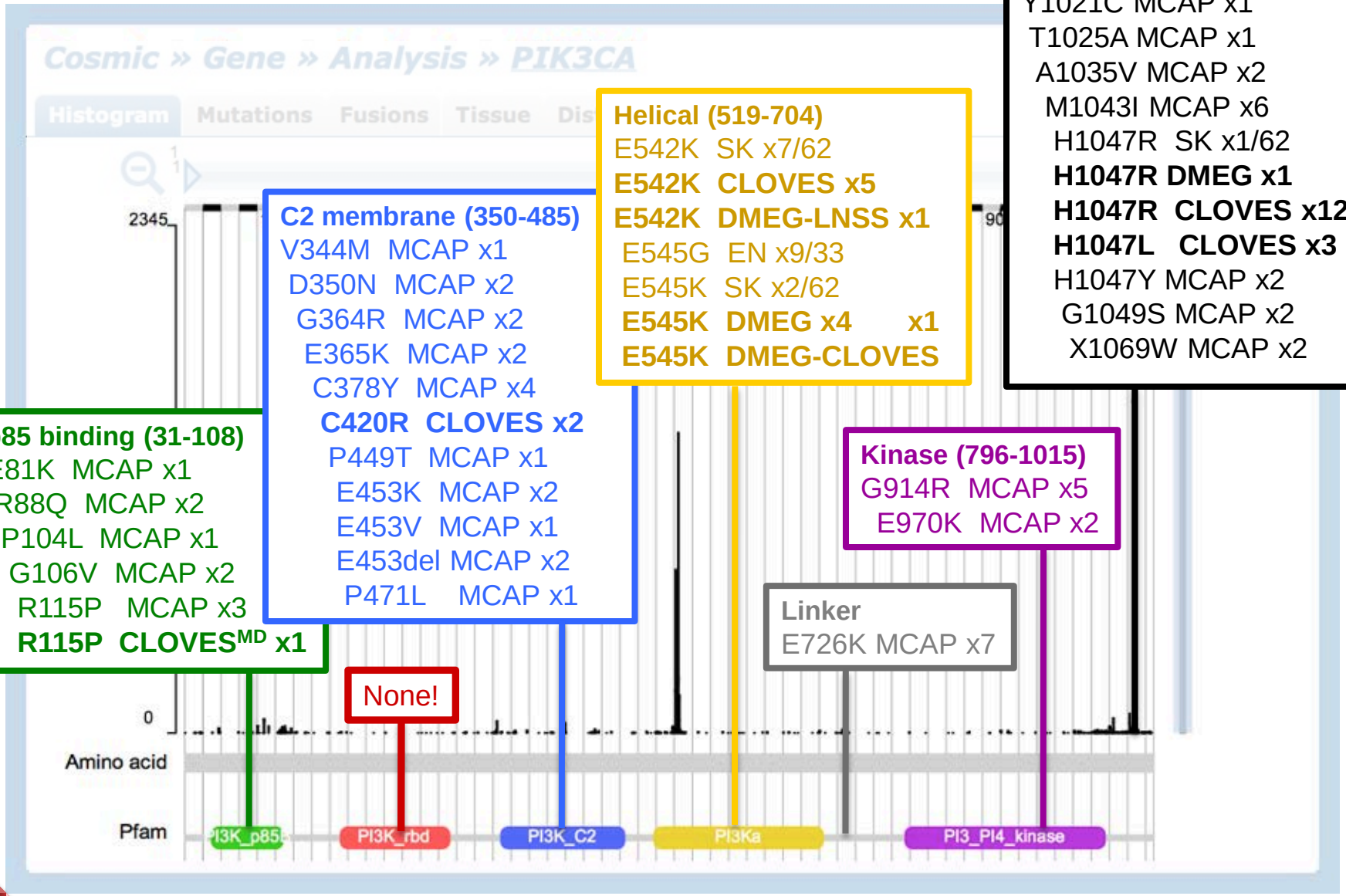




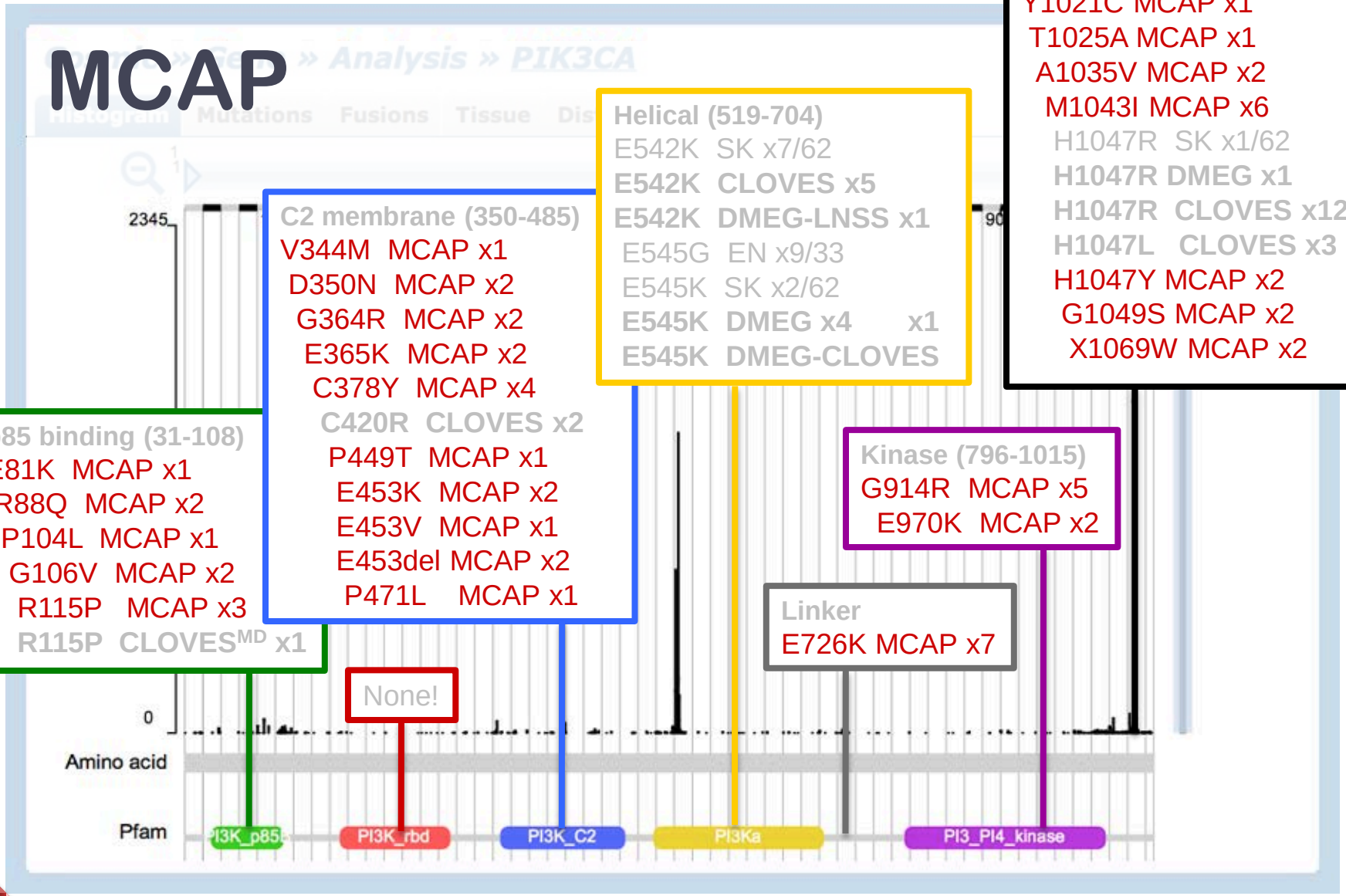




mosaic


Cosmic » Gene » Analysis » PIK3CA
Histogram
Mutations
Fusions
Tissue
2345
0
Amino acid
Pfam
PI3K_p85
PI3K_rbd
PI3K_C2
PI3Ka
PI3_PI4_kinase
p85 binding (31-108)
E81K MCAP x1
R88Q MCAP x2
P104L MCAP x1
G106V MCAP x2
R115P MCAP x3
R115P CLOVES^MD x1
C2 membrane (350-485)
V344M MCAP x1
D350N MCAP x2
G364R MCAP x2
E365K MCAP x2
C378Y MCAP x4
C420R CLOVES x2
P449T MCAP x1
E453K MCAP x2
E453V MCAP x1
E453del MCAP x2
P471L MCAP x1
None!
Helical (519-704)
E542K SK x7/62
E542K CLOVES x5
E542K DMEG-LNSS x1
E545G EN x9/33
E545K SK x2/62
E545K DMEG x4 x1
E545K DMEG-CLOVES
Linker
E726K MCAP x7
Kinase (796-1015)
G914R MCAP x5
E970K MCAP x2
Terminal (1016-1069)
Y1021C MCAP x1
T1025A MCAP x1
A1035V MCAP x2
M1043I MCAP x6
H1047R SK x1/62
H1047R DMEG x1
H1047R CLOVES x12
H1047L CLOVES x3
H1047Y MCAP x2
G1049S MCAP x2
X1069W MCAP x2

MCAP



p85 binding (31-108)
E81K MCAP x1
R88Q MCAP x2
P104L MCAP x1
G106V MCAP x2
R115P MCAP x3
R115P CLOVES^{MD} x1

C2 membrane (350-485)
V344M MCAP x1
D350N MCAP x2
G364R MCAP x2
E365K MCAP x2
C378Y MCAP x4
C420R CLOVES x2
P449T MCAP x1
E453K MCAP x2
E453V MCAP x1
E453del MCAP x2
P471L MCAP x1

None!

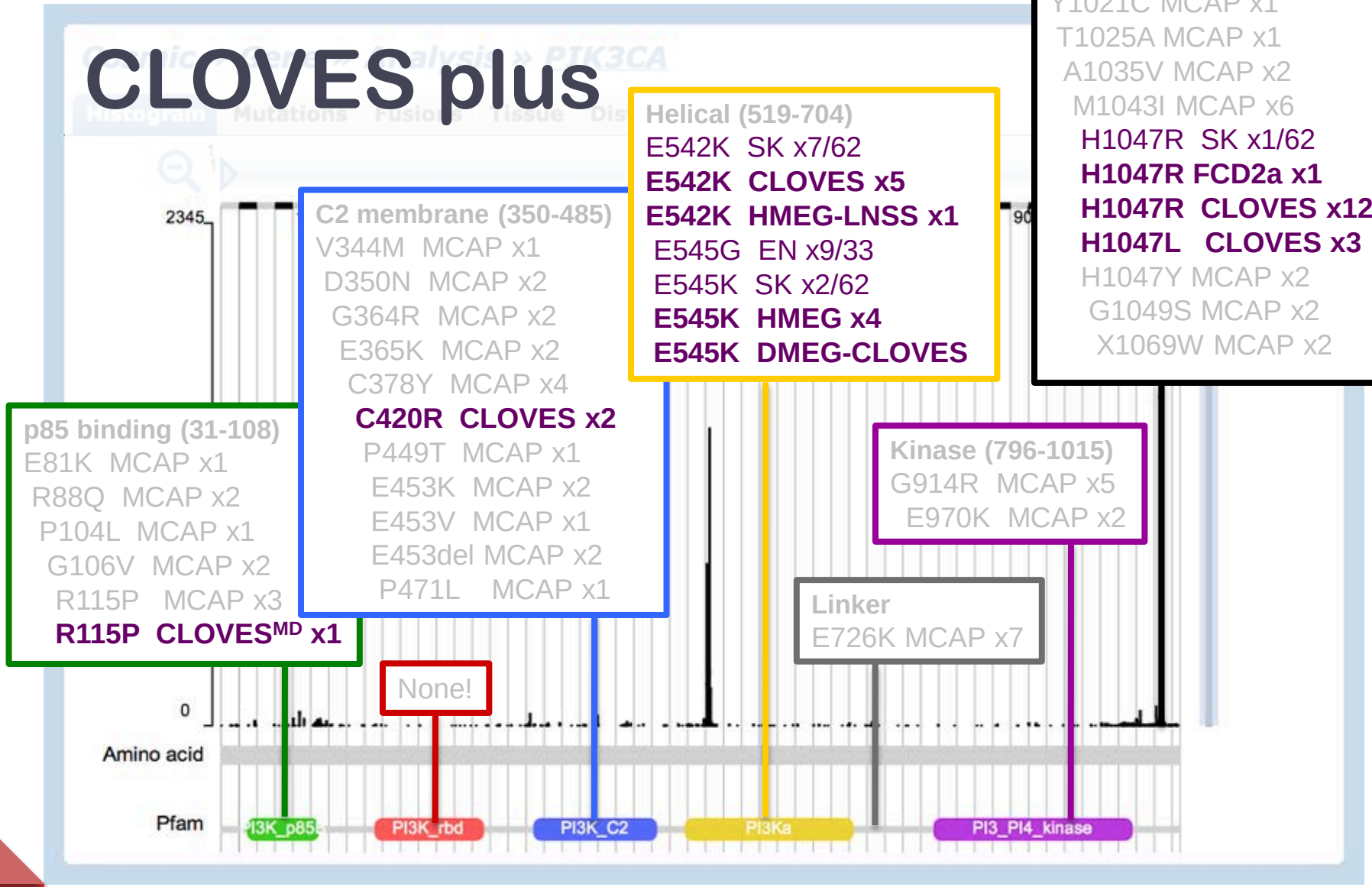
Helical (519-704)
E542K SK x7/62
E542K CLOVES x5
E542K DMEG-LNSS x1
E545G EN x9/33
E545K SK x2/62
E545K DMEG x4 x1
E545K DMEG-CLOVES

Linker
E726K MCAP x7

Kinase (796-1015)
G914R MCAP x5
E970K MCAP x2

Terminal (1016-1069)
Y1021C MCAP x1
T1025A MCAP x1
A1035V MCAP x2
M1043I MCAP x6
H1047R SK x1/62
H1047R DMEG x1
H1047R CLOVES x12
H1047L CLOVES x3
H1047Y MCAP x2
G1049S MCAP x2
X1069W MCAP x2

CLOVES plus



p85 binding (31-108)
E81K MCAP x1
R88Q MCAP x2
P104L MCAP x1
G106V MCAP x2
R115P MCAP x3
R115P CLOVES^{MD} x1

C2 membrane (350-485)
V344M MCAP x1
D350N MCAP x2
G364R MCAP x2
E365K MCAP x2
C378Y MCAP x4
C420R CLOVES x2
P449T MCAP x1
E453K MCAP x2
E453V MCAP x1
E453del MCAP x2
P471L MCAP x1

None!

Helical (519-704)
E542K SK x7/62
E542K CLOVES x5
E542K HMEG-LNSS x1
E545G EN x9/33
E545K SK x2/62
E545K HMEG x4
E545K DMEG-CLOVES

Linker
E726K MCAP x7

Kinase (796-1015)
G914R MCAP x5
E970K MCAP x2

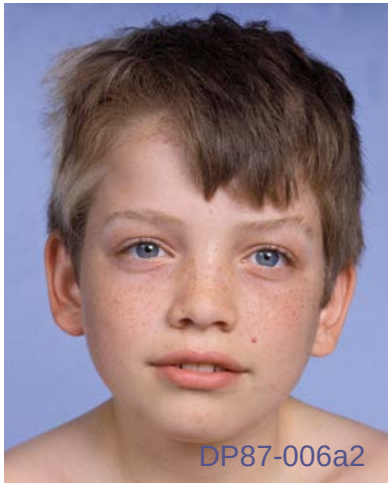
Terminal (1016-1069)
Y1021C MCAP x1
T1025A MCAP x1
A1035V MCAP x2
M1043I MCAP x6
H1047R SK x1/62
H1047R FCD2a x1
H1047R CLOVES x12
H1047L CLOVES x3
H1047Y MCAP x2
G1049S MCAP x2
X1069W MCAP x2

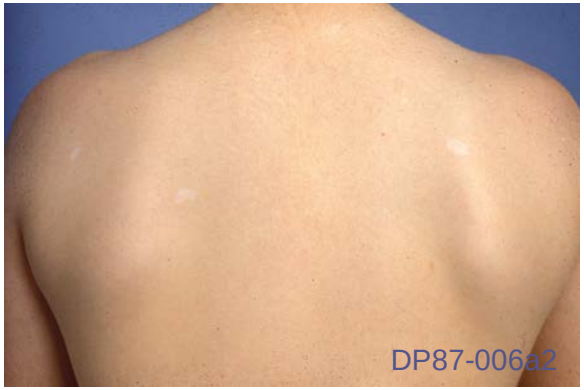
Tuberous sclerosis

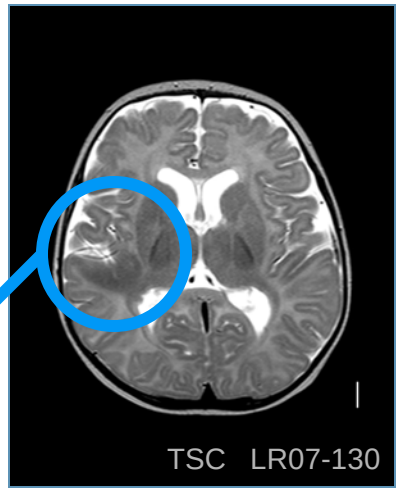
TSC

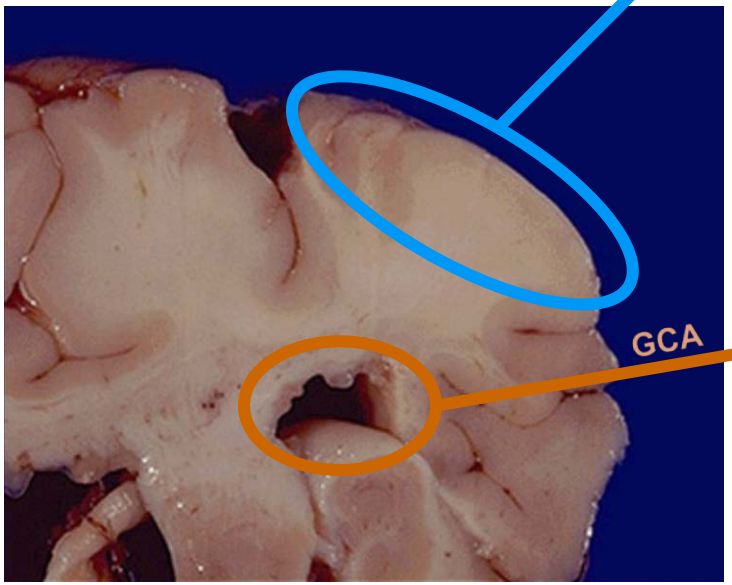
MTOR signaling

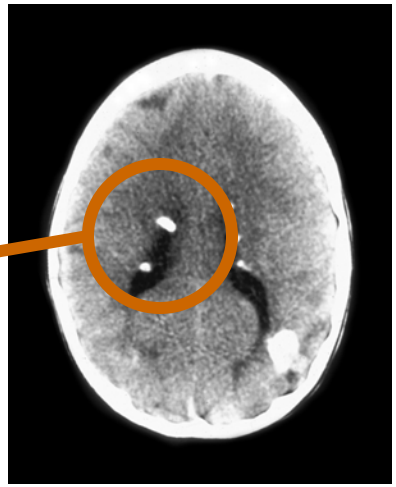
Tuberous sclerosis (TSC)











- Skin
  - Ash leaf spots
  - Fibromas (face, nails)
- Brain
  - Hamartomas
  - Giant cell astroctomas
  - FCD type 2b

Tuberous sclerosis

- *TSC1* and *TSC2* mutations
  - *TSC1* or *TSC2* mutations 75-85%
  - No mutation in 23/157 (15%)
    - Neurological and MRI problems less frequent than *TSC1* or *TSC2*
    - Renal abnormalities intermediate

Annals of human genetics

doi: 10.1111/j.1469-1809.2008.00496.x

Distinct clinical characteristics of Tuberous Sclerosis Complex patients with no mutation identified

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Summary

Tuberous Sclerosis Complex (TSC) is a multi-system disorder that is highly variable in its clinical presentation. Current molecular diagnostic methods permit identification of mutations in either *TSC1* or *TSC2* in 75–85% of TSC patients. Here we examine the clinical characteristics of those TSC patients who have no mutation identified (NMI). A retrospective review of our patient population that had comprehensive testing for mutations in *TSC1/TSC2* identified 23/157 (15%) that were NMI. NMI patients had a lower incidence of brain findings on imaging studies, neurological features, and renal findings than those with *TSC2* mutations. In contrast, NMI patients had a lower incidence of seizures than TSC patients with *TSC1* mutations, but had a higher incidence of both renal angiomyolipomas and pulmonary lymphangioleiomyomatosis. This distinct constellation of findings suggest that NMI patients may have a unique molecular pathogenesis, different from that seen in TSC patients with the usual mutations in *TSC1* and *TSC2*. We suggest that the mechanisms of disease in these patients include both mosaicism for a *TSC2* mutation, and unusual non-coding region

Summary

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mosaic

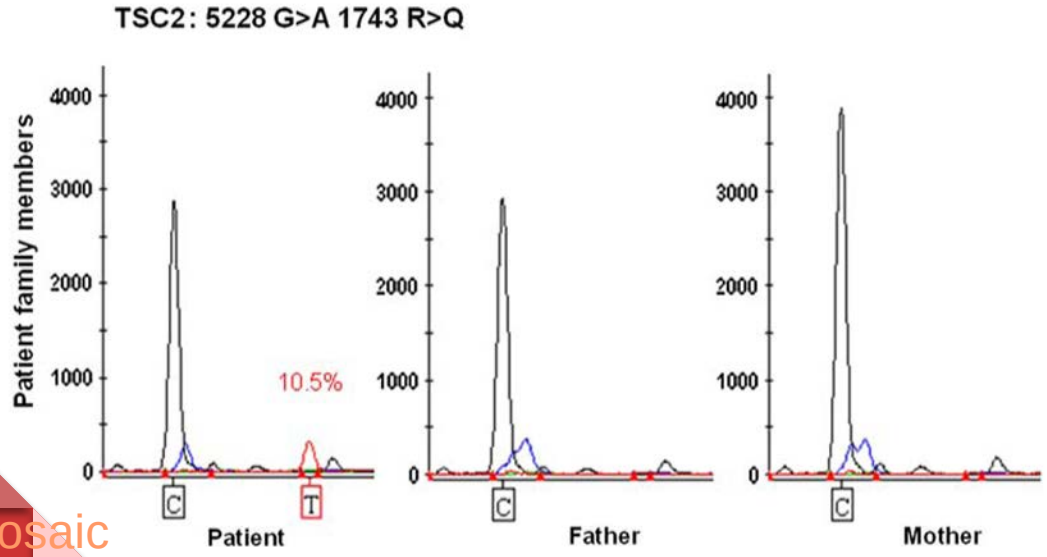
d, mosaicism, lymphangioleiomyomatosis, angiomy-

ions (Janssen et al., 1990; Niida et al., 1999; Dabora et al., 01; Humphrey et al., 2004; Ali et al., 2005; Au et al., 07), many studies show that *TSC2* patients have overall th a higher incidence and more severe features of TSC n patients with *TSC1* mutations, including more signifi- nt neurological, renal and other organ involvement (Jones al., 1999; Dabora et al., 2001; Langkau et al., 2002; Sancak al., 2005; Au et al., 2007). However there are also sev- l missense mutations in *TSC2* which have been associated th distinctly mild clinical features, such that some family embers bearing these *TSC2* missense mutations do not ful- standard diagnostic criteria (Khare et al., 2001; O'Connor al., 2003; Mayer et al., 2004; Jansen et al.,2006). In ad- ion, TSC patients with deletions encompassing the *TSC2* d *PKD1* loci have a severe renal phenotype (Sampson et al., 97).

TSC patients without confirmed disease-causing genetic utations ('no mutation identified', NMI) have been reported having milder phenotypes compared to those with *TSC2* utations and similar phenotypes compared to those with *SC1* mutations (Dabora et al., 2001; Sancak et al., 2005). enal involvement is an exception; it is commonly seen in

Tuberous sclerosis

- *TSC1* and *TSC2* mutations
  - *TSC1* or *TSC2* mutations found in 85-90% patients
  - Type 2 mosaicism (2nd hit) found in 2/33 patients, each in 10.5% of cells (*TSC2*)



mosaic

Hum Genet (2010) 127:573–582
DOI 10.1007/s00439-010-0801-z

ORIGINAL INVESTIGATION

Ultra deep sequencing detects a low rate of mosaic mutations in tuberous sclerosis complex

Wei Qin · Piotr Kozlowski · Bruce E. Taillon · Pascal Bouffard · Alison J. Holmes · Pasi Janne · Susana Camposano · Elizabeth Thiele · David Franz · David J. Kwiatkowski

Received: 22 October 2009 / Accepted: 5 February 2010 / Published online: 18 February 2010

Abstract Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous syndrome caused by mutations in *TSC1* and *TSC2*. However, 10–15% TSC patients have no mutation identified with conventional molecular diagnostic studies. We used the ultra-deep pyrosequencing technique of 454 Sequencing to search for mosaicism in 38 TSC patients who had no *TSC1* or *TSC2* mutation identified nal methods. Two *TSC2* mutations were iden- t 5.3% read frequency in different patients, th mosaicism. Both mosaic mutations were several methods. Five of 38 samples were e heterozygous non-mosaic mutations, which sed in earlier analyses. Several other possible low-frequency mosaic mutations were identified by deep sequencing, but were discarded as artifacts by secondary studies. The low frequency of detection of mosaic mutations, two (6%) of 33, suggests that the majority of TSC patients who have no mutation identified are not due to mosaicism, but rather other causes, which remain to be determined. These findings indicate the ability of deep sequencing, coupled with secondary confirmatory analyses, to detect low-frequency mosaic mutations.

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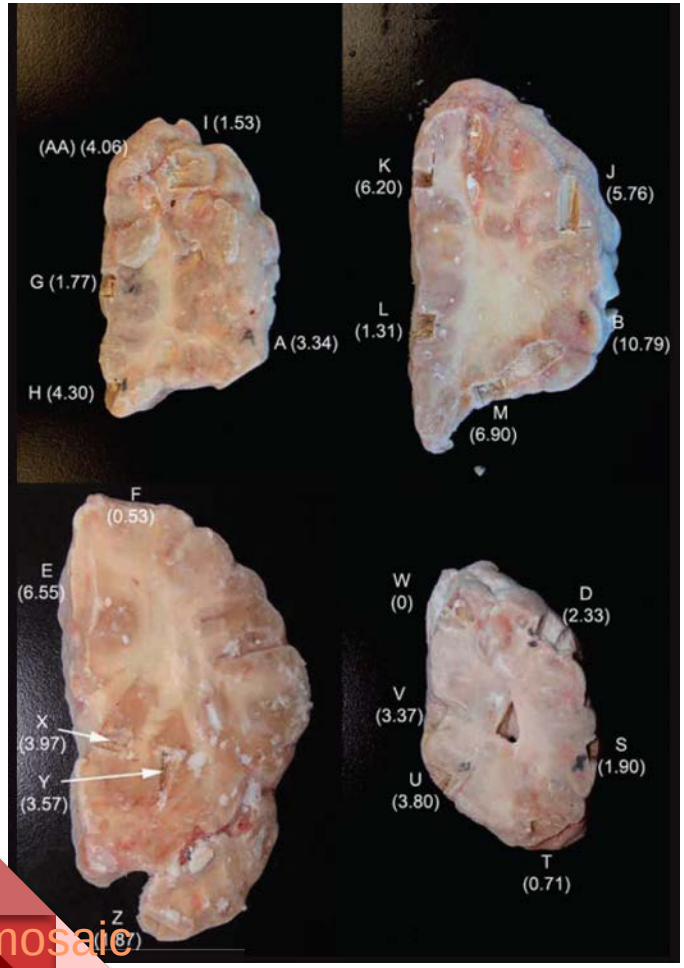
oland

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous syndrome of high penetrance, characterized by a highly variable phenotype and the development of multiple hamartomas at various sites throughout the body (Crino et al. 2006; Gomez et al. 1999). Approximately 60–70% of TSC cases are sporadic, reflecting a high spontaneous mutation rate in the two genes, *TSC1* and *TSC2* (European Tuberous Sclerosis Consortium 1993; Sampson et al. 1989; van Slegtenhorst et al. 1997).

Comprehensive mutation detection studies have led to identification of mutations in 70–90% of TSC patients (Au et al. 2007; Dabora et al. 2001; Jones et al. 1999; Niida et al. 1999; Sancak et al. 2005) (http://chromium.liacs.nl/LOVD2/TSC/home.php). More than 1,500 mutations and more than 800 unique mutations have been identified in *TSC1* and *TSC2* combined. However, 10–15% of TSC patients have no mutation identified (NMI), despite a thorough molecular diagnostic assessment, including analysis for large genomic deletions. These NMI TSC subjects generally have milder clinical features of TSC than patients with identified *TSC1* or *TSC2* mutations (Dabora et al. 2001; Sancak et al. 2005).

Tuberous sclerosis



Brain Pathology ISSN 1015-6305

RESEARCH ARTICLE

Analysis of TSC Cortical Tubers by Deep Sequencing of TSC1, TSC2 and KRAS Demonstrates that Small Second-Hit Mutations in these Genes are Rare Events

Wei Qin[1]*; Jennifer A. Chan[2]*; Harry V. Vinters[3a]; Gary W. Mathern[3b]; David N. Franz[4]; Bruce E. Taillon[5]; Pascal Bouffard[5]; David J. Kwiatkowski[1]

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Keywords
tuber, tuberous sclerosis, TSC1, TSC2.

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Received 5 February 2010; accepted 24 May 2010.

Abstract

Tuberous sclerosis complex (TSC) is an often severe neurocutaneous syndrome. Cortical tubers are the predominant neuropathological finding in TSC, and their number and location has been shown to correlate roughly with the severity of neurologic features in TSC. Past studies have shown that genomic deletion events in TSC1 or TSC2 are very rare in suggested the potential involvement of the MAPK pathway in their pathogen- ed deep sequencing to assess all coding exons of TSC1 and TSC2, and the activating mutation hot spots within KRAS in 46 tubers from TSC patients. Germline heterozygous mutations were identified in 81% of tubers. The same secondary mutation in TSC2 was identified in six tuber samples from one individual. Further study showed that this second hit mutation was widely distributed in the cortex from one cerebral hemisphere of this individual at frequencies up to 10%. No other secondary mutations were found in the other 40 tubers analyzed. These data indicate that small second hit mutations of these three genes are very rare in TSC tubers. However, in one TSC individual, a second hit TSC2 point mutation occurred early during brain development, and likely contributed to tuber formation.

Type 2 mosaicism (2nd hit)

- N = 46 tubers/34 patients
  - 2nd hit in 1/34 patients (3%)
  - 2nd hit = type 2 mosaicism
- TSC patient #1560
  - DNA isolated from 6 tubers
  - TSC2 c.4375C>T and p.R1459X
    - 100% cells: 42-51% reads
  - TSC2 c.1864C>T and p.R622W
    - ~4% cells: 0.7-7.2% reads

for *TSC1* or *TSC2* markers has been demonstrated in 84 of 128 TSC renal AMLs (66%) (1, 15, 34), both TSC-associated and sporadic LAM (5, 33), and in TSC SEGA (6). Point mutation second hit events are also known in TSC lesions (6).

On the other hand, evidence for the two hit model in TSC cortical tubers is much more limited (15, 31), and the true mechanism of disease pathogenesis for these lesions is disputed (18, 23). Recent evidence suggests that the MAPK signaling pathway may contribute to the development of TSC brain lesions, but an underlying genetic lesion causing this has not been found (14, 21). Understanding the pathogenic mechanisms underlying tuber development has considerable importance since neurologic issues (including seizures, intellectual disability and behavioral problems including autism) are the greatest clinical problems in the majority of TSC patients.

To explore the hypothesis that tubers develop following the two hit mechanism, we used deep sequencing to search for second hit small mutations in TSC cortical tubers. We recognized that abnormal cell types were a small fraction of all cells seen in cortical

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mosaic

Focal cortical dysplasia

FCD and HMEG

Hemimegalencephaly

FCD1, FCD2 etc

Epilepsia, 52(1):158–174, 2011
doi: 10.1111/j.1528-1167.2010.02777.x

SPECIAL REPORT

The clinicopathologic spectrum of focal cortical dysplasias: A consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission[1]

*[2]Ingmar Blümcke, †Maria Thom, ‡Eleonora Aronica, §Dawna D. Armstrong, ¶Harry V. Vinters, #Andre Palmini, **Thomas S. Jacques, ††Giuliano Avanzini, ‡‡A. James Barkovich, §§Giorgio Battaglia, ¶¶Albert Becker, ##Carlos Cepeda, ***[3]Fernando Cendes, †††Nadia Colombo, ‡‡‡Peter Crino, §§§J. Helen Cross, ¶¶¶Olivier Delalande, ###François Dubeau, ****John Duncan, ††††Renzo Guerrini, ‡‡‡‡Philippe Kahane, §§§§Gary Mathern, ¶¶¶¶Imad Najm, ####Çiğdem Özkara, *****Charles Raybaud, †††††Alfonso Represa, ‡‡‡‡‡Steven N. Roper, §§§§§Noriko Salamon, ¶¶¶¶¶Andreas Schulze-Bonhage, #####Laura Tassi, ******Annamaria Vezzani, and ††Roberto Spreafico

†Department of Neuropathology, Institute of Neuro)Pathology, Academic Medisch Centrum, , The Netherlands; §Formerly Baylor College of nts of Pathology and Laboratory Medicine of Medicine at UCLA, Los Angeles, California,

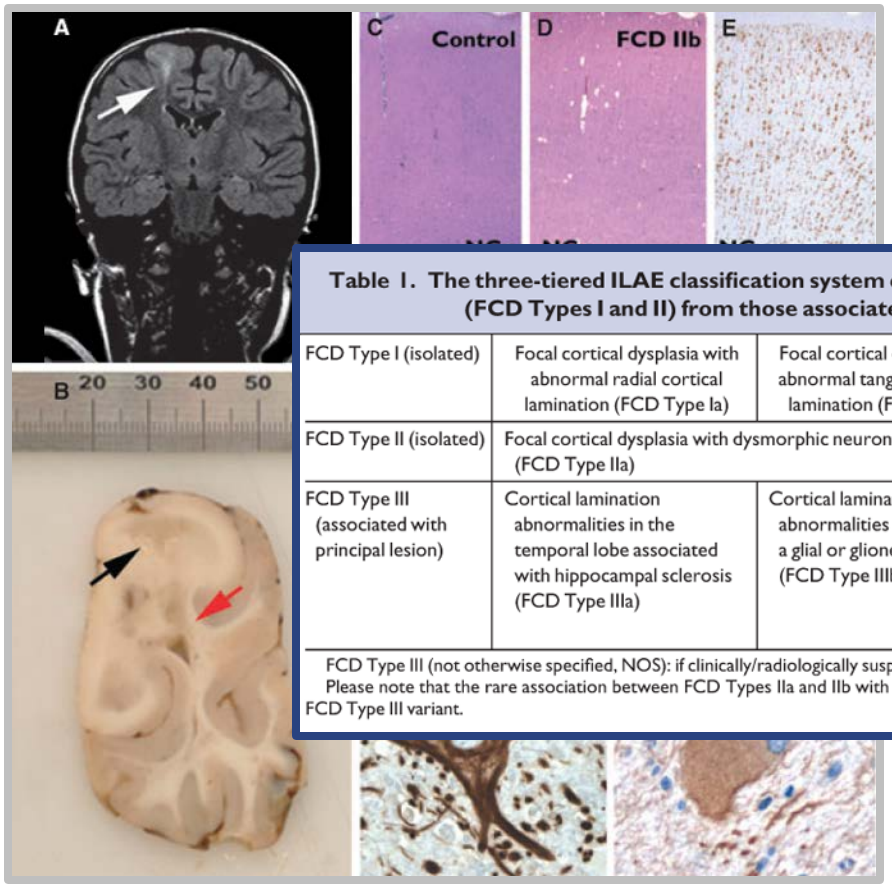
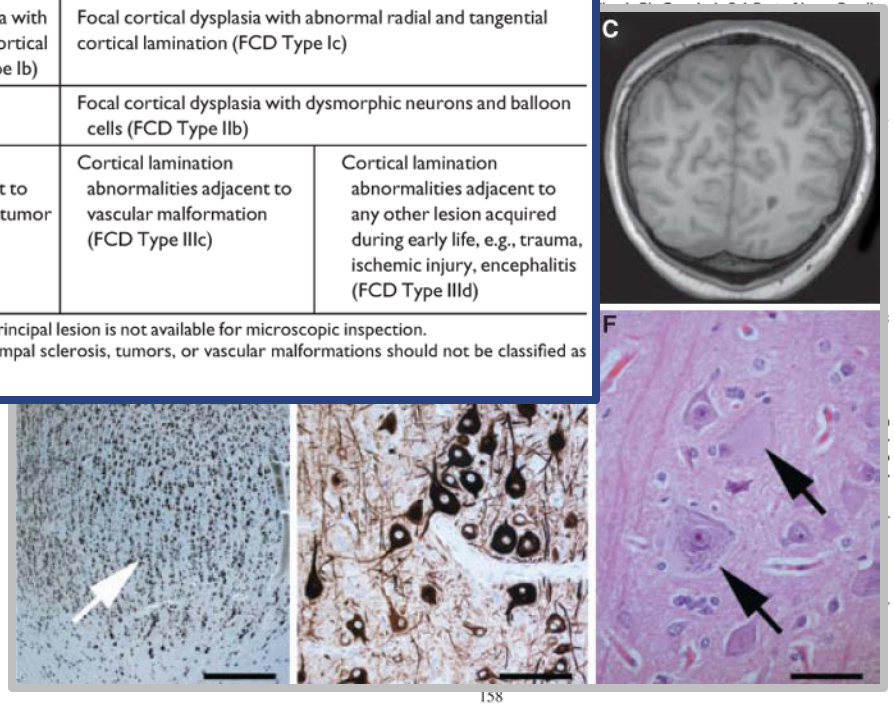


Table 1. The three-tiered ILAE classification system of focal cortical dysplasia (FCD) distinguishes isolated forms (FCD Types I and II) from those associated with another principal lesion (FCD Type III).

FCD Type I (isolated)	Focal cortical dysplasia with abnormal radial cortical lamination (FCD Type Ia)	Focal cortical dysplasia with abnormal tangential cortical lamination (FCD Type Ib)	Focal cortical dysplasia with abnormal radial and tangential cortical lamination (FCD Type Ic)	
FCD Type II (isolated)	Focal cortical dysplasia with dysmorphic neurons (FCD Type IIa)		Focal cortical dysplasia with dysmorphic neurons and balloon cells (FCD Type IIb)	
FCD Type III (associated with principal lesion)	Cortical lamination abnormalities in the temporal lobe associated with hippocampal sclerosis (FCD Type IIIa)	Cortical lamination abnormalities adjacent to a glial or glioneuronal tumor (FCD Type IIIb)	Cortical lamination abnormalities adjacent to vascular malformation (FCD Type IIIc)	Cortical lamination abnormalities adjacent to any other lesion acquired during early life, e.g., trauma, ischemic injury, encephalitis (FCD Type IIId)

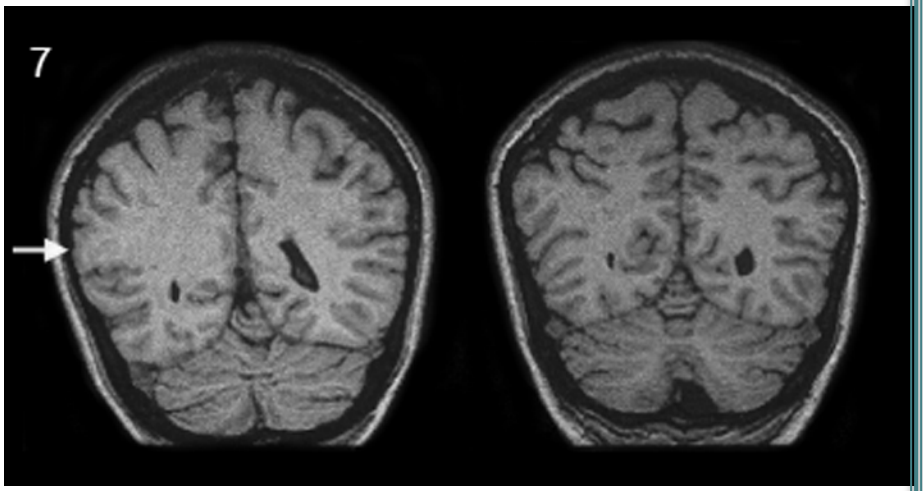
FCD Type III (not otherwise specified, NOS): if clinically/radiologically suspected principal lesion is not available for microscopic inspection.
Please note that the rare association between FCD Types IIa and IIb with hippocampal sclerosis, tumors, or vascular malformations should not be classified as FCD Type III variant.



Transmantle sign: funnel-like hyperintensity that tapers from gyrus to ventricle (FCD2b)

mosaic

Cortical dysplasia



Focal cortical dysplasia (FCD)

MZ twins

Causes of Epilepsies: Insights from Discordant Monozygous Twins

Regula S. Briellmann, MD,[1] Graeme D. Jackson, MD,[1,2] Yvonne Torn-Broers, BA (Hons),[1] and Samuel F. Berkovic, MD[1,2]

Whereas some patients with epilepsy have known acquired or genetic causes, in many the cause is unknown. By analyzing monozygotic twins, discordant for epilepsy, subtle etiological factors may be detected. We analyzed 12 monozygotic, discordant twins for factors explaining discordancy. These factors were presence of major clinical risk factors, presence of possibly epileptogenic lesions on brain magnetic resonance imaging (MRI), and quantitative brain volume abnormalities. Major risk factors, with associated acquired lesions were found in 4 of 12 twins. An MRI lesion without a major risk factor was found in a further 4 of 12 twins. Two of these had unilateral malformations of cortical development, 1 had bilateral periventricular heterotopia, and 1 had focal atrophy. Significant twin–twin differences in MRI volumes without obvious MRI lesions or major risk factors were found in 2 of 12 twins. Both had larger volumes than their co-twins, and idiopathic generalized epilepsy. No clinical or MRI findings accounting for discordance for epilepsy were found in 2 of 12 twins. In 10 of 12 pairs a clinical or MR correlate of epilepsy was found; some of those were subtle and only apparent by twin–twin comparison. They may be due to occult acquired factors, such as prenatal insults, or to genetic abnormalities resulting from postfertilization genetic processes.

Ann Neurol 2001;49:45–52

The epilepsies are a clinically and etiologically heterogeneous group of disorders. Currently they are broadly classified into symptomatic epilepsies where acquired factors are known, cryptogenic epilepsies where undiscovered lesions are believed to be present, and idiopathic epilepsies where the etiology is principally genetic.[1] Disentanglement of the critical etiological factors is difficult because of the great biological variability in the general population, both genetically and for exposure to acquired factors. Variance in clinical research can be greatly reduced by analysis of twin samples.[2–4] Whereas twin studies are best known for identifying genetic components of disease, they are also valuable for estimating effects of environmental factors and for analysis of complex traits.[2,3]

were affected with seizures, there were more concordant pairs among the MZ pairs (48 of 108; concordance rate = 0.62), than among the discordant (DZ) pairs (14 of 145; concordance rate = 0.18). The concordance for generalized epilepsies was higher (MZ = 0.82, DZ = 0.26) than for partial epilepsies (MZ = 0.36, DZ = 0.18). We concluded that genetic factors are important in determination of a specific epileptic syndrome. In accord with other researchers, we observed the puzzling fact of a large proportion of MZ twins who are discordant for epilepsy.

The aim of this study was to analyze such discordant MZ pairs. Noninherited factors are the likely reason for the discordance for epilepsy. Therefore, such twins represent an elegant way to identify such factors. Major noninherited factors such as a brain injury affecting an individual with epilepsy are easily recognized in singleton patients. Subtle developmental abnormalities, however, are difficult to ascertain in population studies. The high degree of similarity in the brain structure between MZ twins allows the detection of such abnormalities. Developmental abnormalities may be the consequence of a brain injury, which may have happened prenatally, or they may be due to genetic factors. Although MZ twins have generally the same genetic

Table 1. Clinical Features, EEG Findings, and Visual MRI Interpretation

Pair	Sex	BO	Age	Onset	Seizure	EEG	FH	Risk Factor	MRI Finding
7	F	1	32	14	Nocturnal TC	Normal	No	No risk factors known	Dysplasia, parietal
	F	2	32			Normal		Face presentation, internal rotation	Normal

first twin of each pair is the affected twin.

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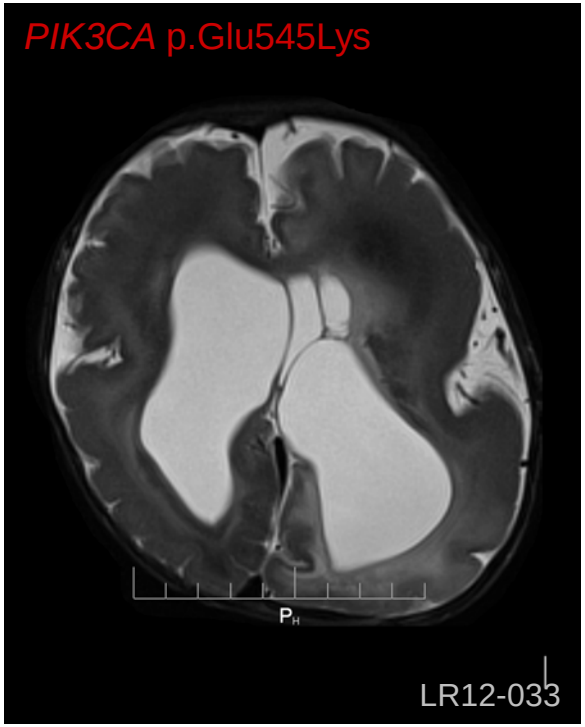
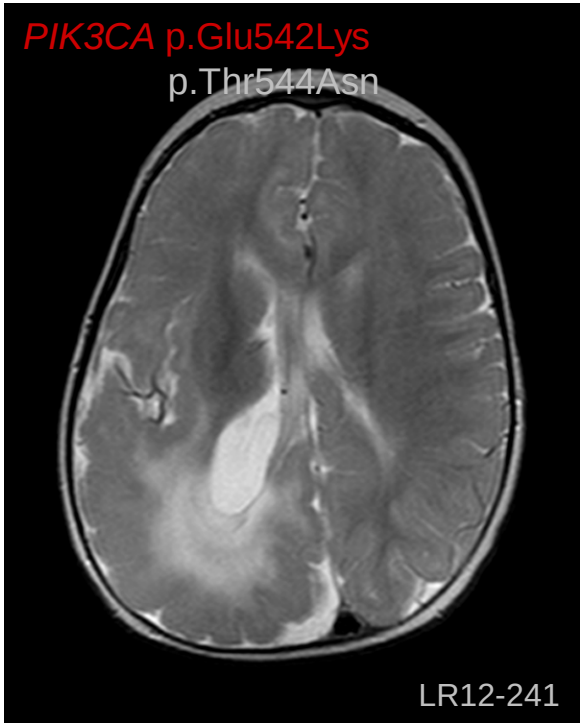
Genetic analysis in DMEG-HMEG-FCD2

N	Imaging	Distribution	NPATH	Gene	Subject
1	DMEG	Bi-hemispheric L=R	FCD2a	***PIK3CA*** p.Glu545Lys (27%)	LR12-033
1	DMEG	Bi-hemispheric L>R	FCD2a	***AKT3*** p.Glu17Lys (10%)	LR11-443
1	HMEG	Hemispheric A>P	FCD2a	***PTEN*** p.Tyr68His (50%)	LR12-123
2	HMEG	Hemispheric A>P	FCD2a		
1	HMEG	Hemispheric P>A	FCD2a	***PIK3CA*** p.Glu542Lys (31%)	LR12-241
1	HMEG	Hemispheric P>A	FCD2a		
6	FCD	Multilobar P>A	FCD2a		
2	FCD	Multilobar A>P	FCD2a		
1	FCD	Frontal	FCD2a		
3	FCD	Temporal	FCD2a		
1	FCD	Occipital	FCD2a	***PIK3CA*** p.His1047Arg (5%)	LR12-251
3	FCD	Frontal	FCD2b		
1	FCD	Temporal	FCD2b		
1	FCD	Parietal	FCD2b		

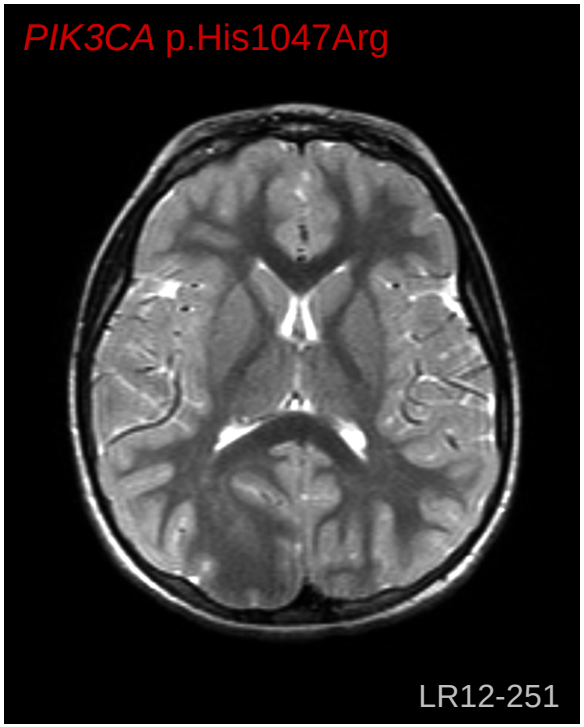
PIK3CA: DMEG-HMEG-FCD2a



DMEG

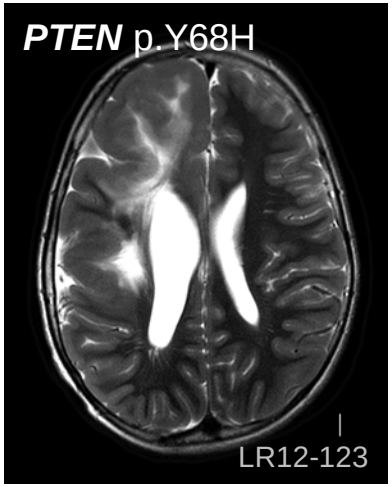


HMEG



FCD2a

More HMEG



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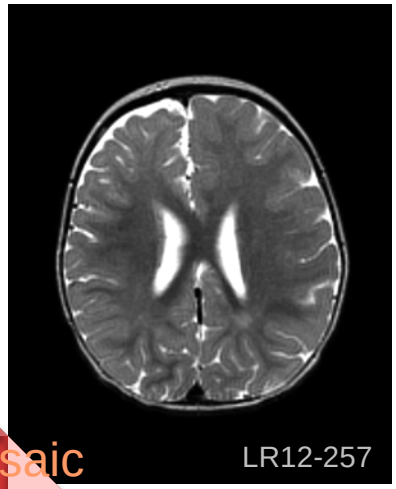

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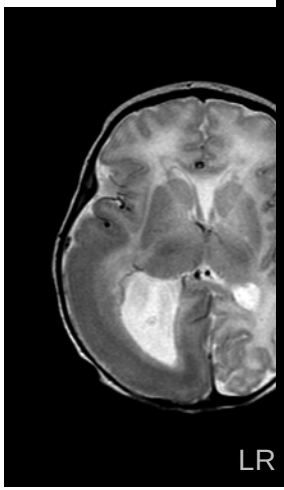
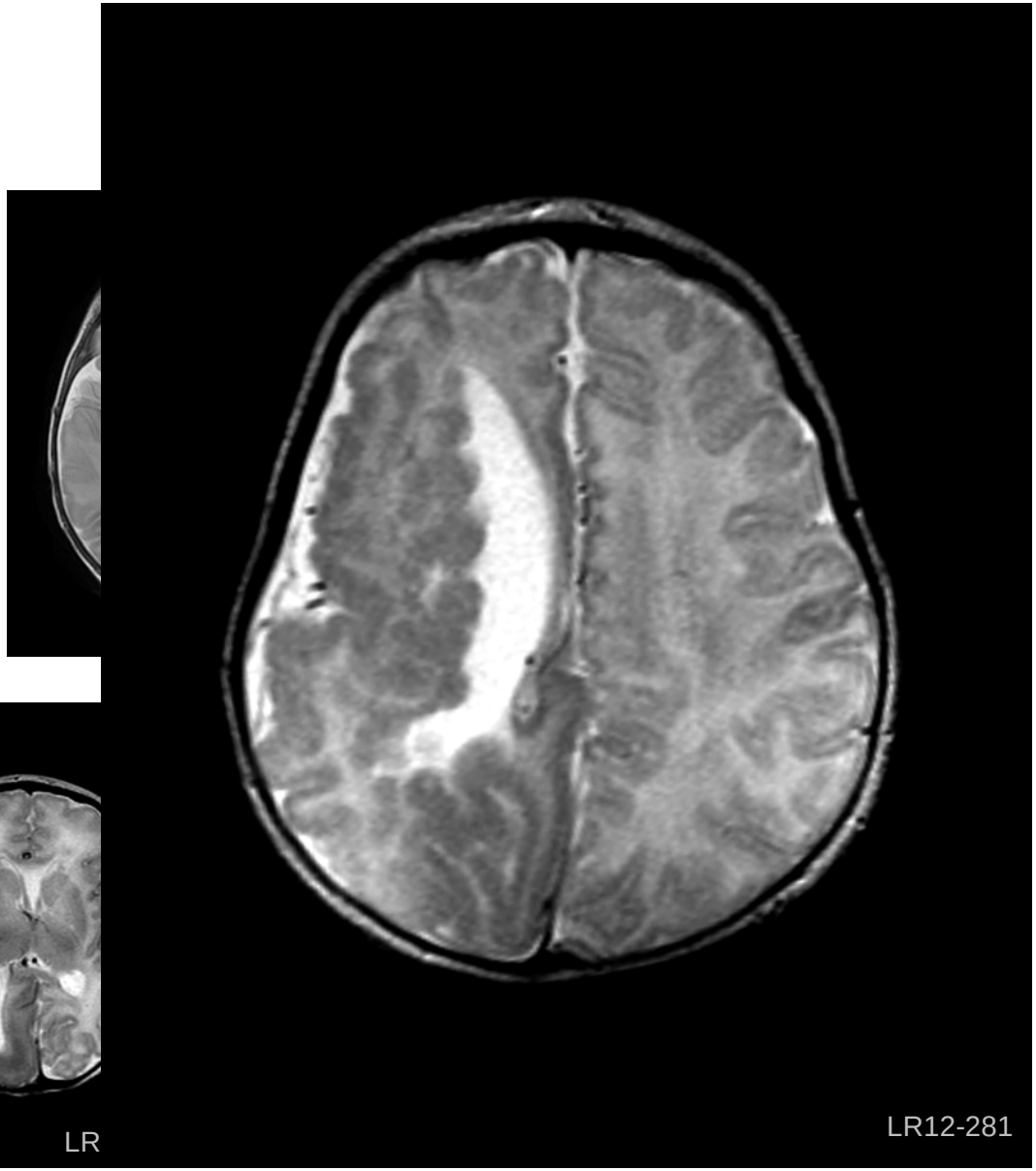
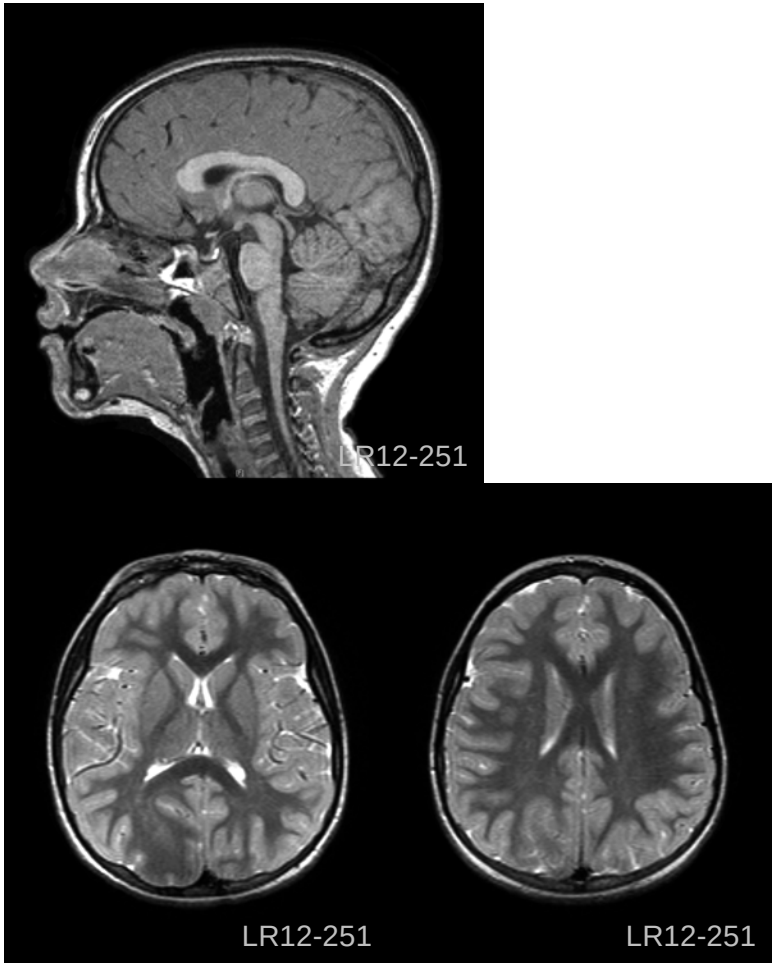




FCD2a and *PIK3CA* p.H1047R

- 6yo girl (LR12-251)
  - Mild DEV delay
  - Intractable focal SZ
  - MCD right medial occipital lobe
  - 5y epilepsy surgery
  - SZ free x 1y
- *PIK3CA*
  - Brain-derived DNA
  - p.His1047Arg (p.H1047R)
  - Mosaic 20/293 reads or 7%



mosaic

Mutations of PTEN in patients with Bannayan-Riley-Ruvalcaba phenotype

Michel Longy, Valérie Coulon, Bernadette Duboué, Albert David, Marc Larrègue, Charis Eng, Patrizia Amati, Jean-Louis Kraimps, Armand Bottani, Didier Lacombe, Dominique Bonneau

Abstract
We report three new mutations in PTEN, the gene responsible for Cowden disease in five patients with Bannayan-Riley-Ruvalcaba syndrome from three unrelated families. This finding confirms that Cowden disease, a dominant cancer predisposing syndrome, and Bannayan-Riley-Ruvalcaba syndrome, which includes macrocephaly, multiple lipomas, intestinal hamartomatous polyps, vascular malformations, and pigmented macules of the penis, are allelic disorders at the PTEN locus on chromosome 10q.
(*J Med Genet* 1998;35:886–889)

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Received 10 February 1998
Revised version accepted for publication 8 April 1998

The term "Bannayan-Riley-Ruvalcaba" syndrome (BRR) (MIM 153480) has been proposed[1] to encompass three autosomal dominant conditions previously described as separate entities : Bannayan-Zonana, Riley-Smith, and Ruvalcaba-Myhre syndromes. Typical clinical features of BRR include macrocephaly, multiple lipomas, intestinal hamartomatous polyps, vascular malformations, and pigmented macules of the penis. At least half of the patients affected with BRR have hypotonia, delayed psychomotor development, mild to severe mental deficiency, and seizures.[2] BRR exhibits a number of clinical similarities to Cowden disease (CD),

cancer in 3-10% of all affected subjects.[3] In addition, about 25% of CD patients have lipoma and macrocephaly.[2] In contrast, hyperpigmented macules of the penis are rare in subjects with CD, having only been reported twice.[4]

Given the clinical similarities between these disorders, the idea of a possible common genetic pathogenesis between BRR and CD was previously put forward.[4] Recently, mutations in the putative tumour suppressor gene PTEN, encoding a protein tyrosine phosphatase, were detected in patients with CD.[5 6] Subsequently, Marsh *et al*[6] discovered germline mutations in P
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Figure 1 Pedigree

Box 2 Revised Cowden syndrome Consortium diagnostic criteria[3]

Pathognomonic criteria
- Lhermitte–Duclos disease (LDD)—adult
- Mucocutaneous lesions:
  - Trichilemmomas, facial
  - Acral keratoses
  - Papillomatous lesions

Major criteria
- Breast cancer
- Thyroid cancer (papillary or follicular)
- Macrocephaly (≥97%ile)
- Endometrial cancer

Minor criteria
- Other structural thyroid lesions (eg, adenoma, multinodular goitre)
- Mental retardation (ie, IQ≤75)
- Gastrointestinal hamartomas
- Fibrocystic disease of the breast
- Lipomas
- Fibromas
- Genitourinary tumours (eg, uterine fibroids, renal cell carcinoma) or
- Genitourinary structural malformations
- Uterine fibroids

Operational diagnosis in an individual (any of the following):
1. Mucocutaneous lesions alone if:
   a) there are six or more facial papules, of which three or more must be trichilemmoma, or
   b) cutaneous facial papules and oral mucosal papillomatosis, or
   c) oral mucosal papillomatosis and acral keratoses, or
   d) palmoplantar keratoses, six or more
2. Two or more major criteria, but one must include macrocephaly or LDD; or
3. One major and three minor criteria; or
4. Four minor criteria

ORIGINAL ARTICLE

Predicting *PTEN* mutations: an evaluation of Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome clinical features

Robert Pilarski,[1,2] Julie A Stephens,[3] Ryan Noss,[1,2] James L Fisher,[2] Thomas W Prior[4]

ABSTRACT
Background Cowden syndrome (CS) is associated with benign hamartomatous lesions and risks for thyroid, breast and endometrial cancers. Bannayan–Riley–Ruvalcaba syndrome is an allelic disorder characterised by macrocephaly, intestinal polyps, lipomas, and pigmented penile macules. Diagnostic criteria for CS are based on the presence of a range of clinical features. However, prior data on the component clinical features have been based primarily on compilations of cases reported before development of consensus diagnostic criteria.
Objective This study sought to determine the clinical features most predictive of a mutation in the largest single cohort of patients with clinical testing for *PTEN* mutations reported to date.
Methods Molecular and clinical data were reviewed on 802 patients referred for *PTEN* analysis by a single laboratory.
Results Deleterious mutations were found in 172 (21.4%) subjects. Among mutation carriers significant differences from previous reports were found for the frequencies of several clinical features, including macrocephaly, uterine fibroids, benign breast disease, and endometrial cancer. Logistic regression analyses indicated that female mutation carriers were best identified by the presence of macrocephaly, endometrial cancer, trichilemmomas, papillomatous papules, breast cancer, benign thyroid disease, and benign gastrointestinal (GI) lesions. For males, the most discriminating features were macrocephaly, lipomas, papillomatous papules, penile freckling, benign GI lesions, and benign thyroid disease. Age related differences were also identified.
Conclusion The mutation frequency in patients meeting CS diagnostic criteria (34%) was significantly lower than previously reported, suggesting a need for reevaluation of these criteria. A mutation prediction model has been developed which can help identify patients appropriate for *PTEN* testing in clinical practice.

INTRODUCTION
The *PTEN* (*p*hosphatase and *ten*sin homologue on chromosome 10') tumour suppressor gene on chromosome 10q23 is a dual specificity phosphatase with multiple and as yet incompletely understood roles in cellular regulation. Germline mutations in *PTEN* cause a spectrum of clinical syndromes, including Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome (BRRS), and autism spectrum disorders with macrocephaly.

CS is a multisystem disorder involving increased risks for malignancy and hamartomatous tissue overgrowth. Diagnostic criteria for CS were initially established in 1996[1] and subsequent modifications have been proposed, including the addition of endometrial cancer[2] and renal cell carcinoma[3] and moving adult Lhermitte–Duclos disease (LDD) into the pathognomonic category.[4]

PTEN mutations were first reported in individuals with CS in 1997.[5 6] It was subsequently suggested that approximately 80% (30 of 37) of individuals with a clinical diagnosis of CS have a detectable germline coding sequence mutation in *PTEN*.[7] However, variable mutation detection rates have been found in other studies, including three of 27 (11%),[8] nine of 19 (47%),[9] and eight of 13 (61%) patients.[9] A small but as yet undefined proportion of CS patients have deletions or large rearrangements of the *PTEN* gene. A recent report found such changes in three of 15 patients who were negative for mutations using denaturing gel gradient electrophoresis (DGGE).[10] Another study found no deletions, but approximately 10% of DGGE mutation negative patients had variants in the *PTEN* promoter.[11]

BRRS is a congenital disorder whose hallmark features are macrocephaly, hamartomatous intestinal polyps, lipomas, and pigmented macules on the penis. Additional features include developmental delay, large birth weight, and joint hyperextensibility.[12] Consensus diagnostic criteria have not been established but diagnosis is based upon presence of the hallmark features. Initially thought to be a separate disorder, BRRS was subsequently shown to be allelic to CS with approximately 60% of patients with BRRS having detectable coding sequence mutations in *PTEN*.[13]

Most previous reports on the prevalence of the malignant and non-malignant features of CS/BRRS have been based upon data compiled from individual case reports and studies of small cohorts. More importantly, the majority of these reports were published before the adoption of the Consortium diagnostic criteria for CS in 1996. Thus, the true frequencies of the clinical features in CS and BRRS are not clearly known (see Pilarski[14] for review). The commonly reported frequencies for CS are shown in Box 1.

In an effort to obtain better data on these features and their correlation with *PTEN* mutations, we report here our experience with 172 patients with *PTEN* mutations, the largest single

doi:10.1136/jmg.2011.088807

PTEN c.507delC

LETTER TO JMG

Germline mutation of the tumour suppressor *PTEN* in Proteus syndrome

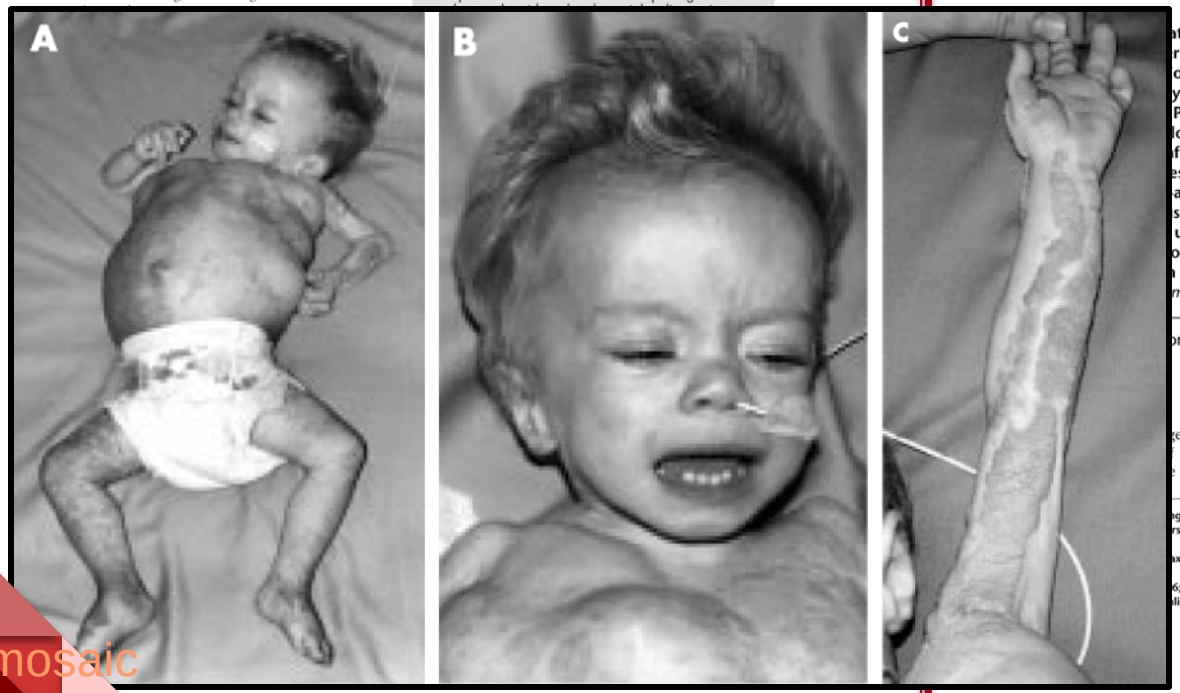
J M Smith, E P E Kirk, G Theodosopoulos, G M Marshall, J Walker, M Rogers, M Field, J J Brereton, D J Marsh

J Med Genet 2002;**39**:937–940

Proteus syndrome (PS, OMIM 176920) is a hamartomatous disorder characterised by overgrowth of multiple tissues, connective tissue and epidermal naevi, and vascular malformations.[1] These presentations are usually apparent at birth or soon after and continue to develop as the patient ages. It is named after the Greek god Proteus who, legend has it, could change his shape at will to avoid capture. It is probably the disease suffered by the Elephant Man.[2] Tumours, mostly benign but some malignant, have also been reported in PS, generally presenting by the age of 20 years and including papillary adenocarcinoma of the testis, meningioma, and cystadenoma of the ovaries.[3] Given the predominantly sporadic nature of this syndrome and the mosaic distribution of lesions, it has been suggested that PS may be caused by somatic mosaicism for a genetic change that is lethal in the

Key points

- Proteus syndrome (PS) is a hamartomatous disorder characterised by overgrowth of multiple tissues that usually manifests early and continues to develop as the patient ages. Clinical overlap exists between PS and another hamartoma syndrome, Bannayan-Riley-Ruvalcaba (BRR) syndrome.
- Up to 60% of subjects affected by BRR carry a germline mutation of the tumour suppressor gene *PTEN*, which encodes a dual specificity phosphatase. *PTEN* mutations can also be identified in the germline of up to 80% of subjects with Cowden syndrome (CS), characterised by the presence of hamartomas in multiple organs as well



mosaic

PTEN c.491delA; p.Lys164Arg_fsX2 (pt2)

European Journal of Human Genetics (2007) 15, 767–773

www.nature.com/ejhg

ARTICLE

Segmental overgrowth, lipomatosis, arteriovenous malformation and epidermal nevus (SOLAMEN) syndrome is related to mosaic *PTEN* nullizygosity

Frédéric Caux[1], Henri Plauchu[2], Frédéric Chibon[3], Laurence Faivre[4], Olivier Fain[5], Pierre Vabres[6], Françoise Bonnet[3], Zied Ben Selma[1], Liliane Laroche[1], Marion Gérard[7] and Michel Longy*[,3]

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atients from distinct Cowden disease families with specific germline *PTEN* mutations
rs from the usual appearance of Cowden disease. Their phenotype associates classical
owden disease and congenital dysmorphisms including segmental overgrowth,
ymphatic vascular malformations, lipomatosis and linear epidermal nevus reminiscent
Proteus syndrome. We
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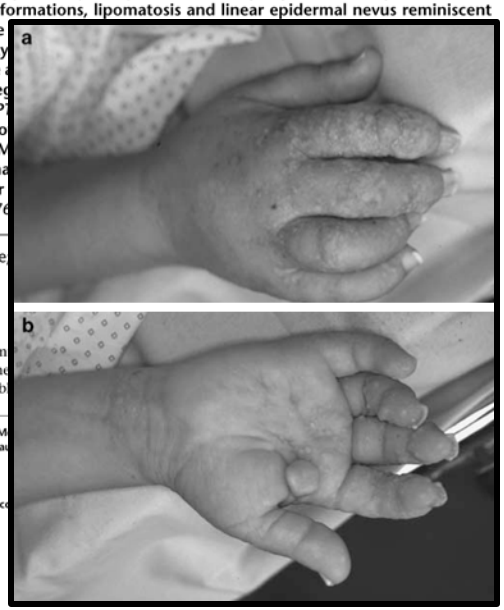

PTEN p.Tyr68Asp
PTEN LOH in skin

CASE REPORT DOI 10.1111/j.1365-2133.2006.07196.x

Epidermal naevus in Proteus syndrome showing loss of heterozygosity for an inherited

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Accepted for publication
27 November 2005

Key words
loss of heterozygosity, Proteus syndrome, PTEN

Conflicts of interest
None declared.

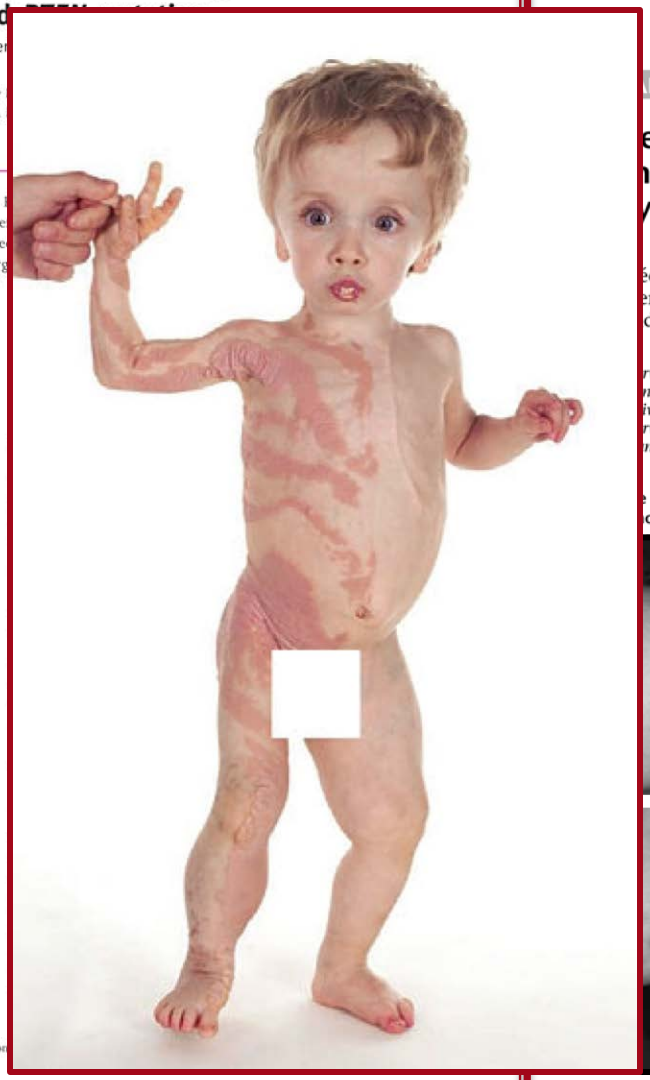
Summary

A 3-year-old boy with
of the PTEN gene inhe
addition, DNA extracte
shows loss of heterozyg
been described before.

Proteus syndrome (PS; OMIM 17690) is a rare sporadic hamartomatous syndrome with postnatal asymmetrical overgrowth of different tissues including the skeletal system, the skin and the vascular system. It was first described in 1979 by Cohen and Hayden.[1] The term *Proteus syndrome* was suggested by Wiedemann et al. in 1983 after the Greek god Proteus who was able to change his shape at will.[2] Some patients with PS, like those with other hamartomatous syndromes such as Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome (BRRS) and Proteus-like syndrome (PLS), have germline PTEN (OMIM 601728, GenBank NM_000314) mutations.[3] We therefore investigated PTEN in our patient with PS.

Case report

This boy of 3 years was born at 35 weeks gestation to unrelated healthy white parents. At birth he appeared healthy but had a very extensive epidermal naevus affecting most of the right side of his body below the neck following Blaschko's lines (Fig. 1a,b). A 'bruise-like' appearance of his right leg, groin, scrotum and less so of the left groin became apparent over the first year, and the right thigh was clearly thinner than the left. On review at 7 months his head circumference had risen from 34 cm (centile 91) at 1 month to 48·3 cm (above 97th centile) and there was downwards deviation of the eyes ('sun setting') suggesting raised intracranial pressure. Urgent computed tomographic scan of the head showed dilated 3rd and lateral ventricles. The 4th ventricle was small and the cerebellar tonsils were lying in the foramen magnum. A mag-
tic resonance imaging (MRI) scan showed no structural



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PTEN c.405A>G; p.Ile135Val
PTEN LOH in lesions (pt1)

European Journal of Human Genetics (2007) 15, 767–773

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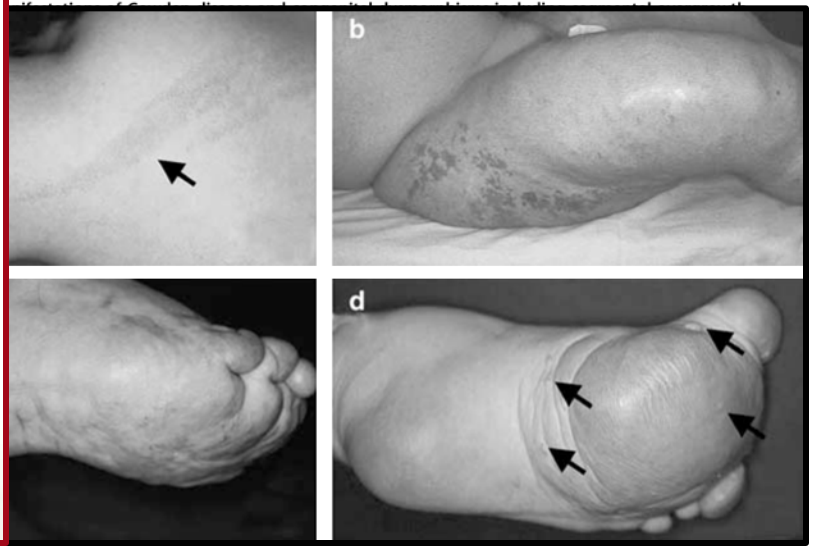
RTICLE

egmental overgrowth, lipomatosis, arteriovenous alformation and epidermal nevus (SOLAMEN) yndrome is related to mosaic *PTEN* nullizygosity

édéric Caux[1], Henri Plauchu[2], Frédéric Chibon[3], Laurence Faivre[4], Olivier Fain[5], erre Vabres[6], Françoise Bonnet[3], Zied Ben Selma[1], Liliane Laroche[1], Marion Gérard[7] d Michel Longy*[,3]

*rvice de Dermatologie, Hôpital Avicenne & ERI 18, Université Paris 13, Bobigny, France; [2]Service de Génétique nique, Hôpital Hôtel-Dieu, Lyon, France; [3]Laboratoire de Génétique Moléculaire, Institut Bergonié & EA 3669, iversité Victor Ségalen- Bordeaux 2, Bordeaux, France; [4]Centre de Génétique, Hôpital d'Enfants, Dijon, France; rvice de Médecine Interne, Hôpital Jean-Verdier, Bondy, France; [6]Service de Dermatologie, Hôpital du Bocage, Dijon, nce; [7]Service de Génétique Médicale, Hôpital Robert Debré, Paris, France

e describe two patients from distinct Cowden disease families with specific germline *PTEN* mutations ose disease differs from the usual appearance of Cowden disease. Their phenotype associates classical



PTEN hamartoma tumor syndrome

PHTS with germline mutations

- Pathognomic criteria
  - Lhermitte-Duclos disease
  - Mucocutaneous lesions
- Major criteria (2 or more)
  - Macrocephaly (>97%ile)
  - Breast or endometrial cancer
  - Thyroid cancer (papillary, follicular)
- Minor criteria
  - Intellectual disability, mild to severe
  - Hamartomas
    - Lipomas
    - Pigmented macules on penis
    - Lots of them
  - Vascular malformations
  - Genitourinary malformations
- PTEN
  - Germline heterozygous mutations

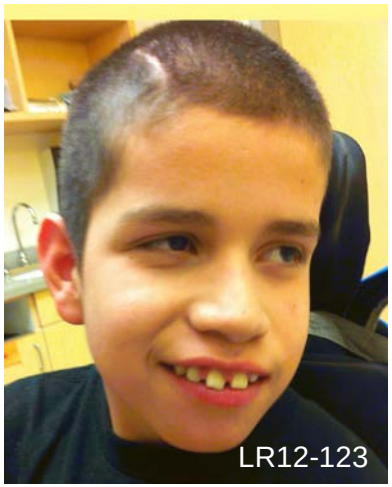
mosaic

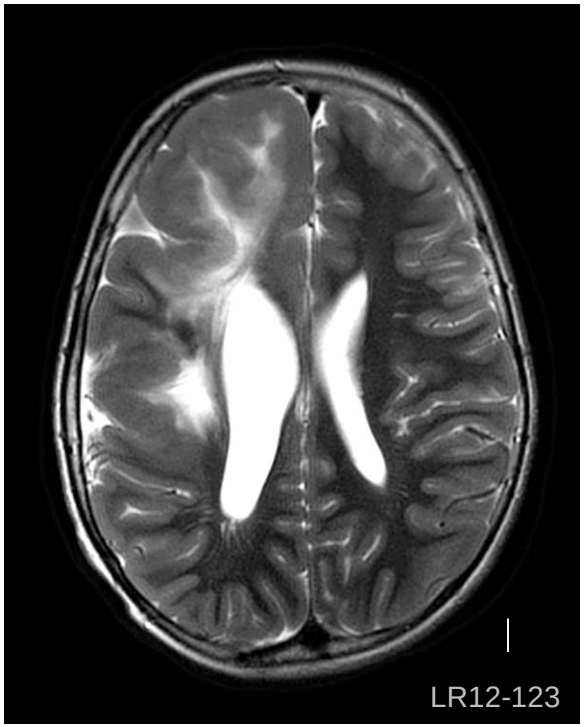
PHTS with type 2 mosaicism

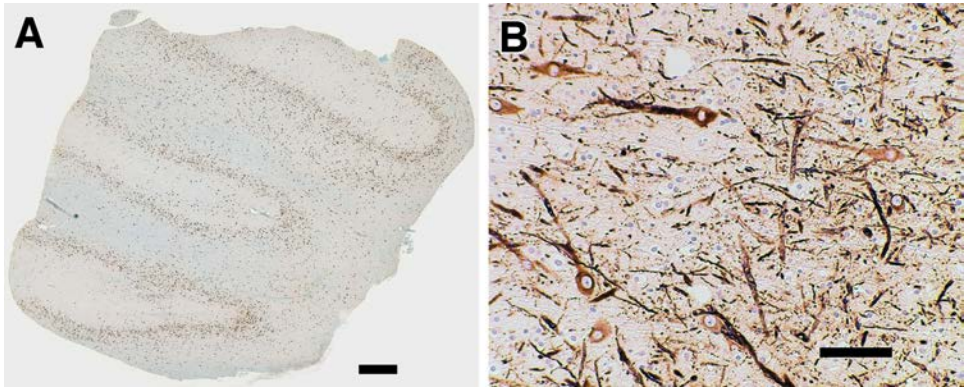
- PHTS
- Segmental dysplasias
  - Asymmetric overgrowth
  - Lipomatosis
  - Vascular malformations
  - Epidermal nevi
- PTEN
  - Germline heterozygous mutations
  - Mosaic LOH or 2nd mutation
- Nomenclature is a mess
  - Bannayan-Riley-Ruvalcaba (?)
  - Proteus (wrong) or Proteus-like
  - SOLAMEN syndrome
  - PHTS with type 2 mosaicism

PTEN and HMEG

- PHTS – Cowden disease (?)
  - OFC
  - Mild ID
  - SZ intractable
- ***PTEN*** p.Tyr68His
  - *PTEN* LOH negative

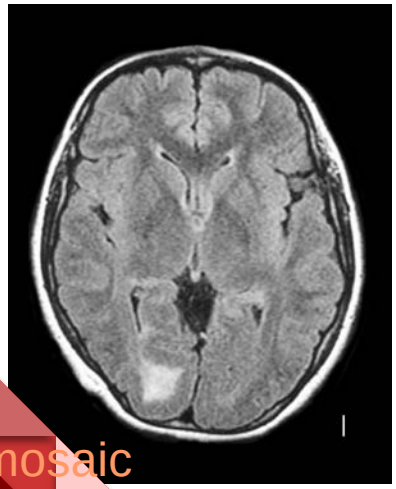






PTEN and FCD

- PHTS – Cowden disease
  - OFC 61 cm, IQ and exam normal at 10 years
  - Goiter and later other hamartomas from 16 years
- ***PTEN*** c.1023delT and p.Phe341Leu_fs*3
  - PTEN LOH not tested



mosaic

CASE REPORT

Atypical focal cortical dysplasia in a patient with Cowden syndrome

KM Cheung *, CW Lam, YK Chan, WK Siu, L Yong

ABSTRACT

A macrocephalic girl presented with generalised epilepsy due to focal cortical dysplasia. She later developed multiple hamartomatous lesions and was diagnosed to have Cowden syndrome. The diagnosis was confirmed by identification of a novel frameshift mutation in the *PTEN* gene of the patient.

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Case report

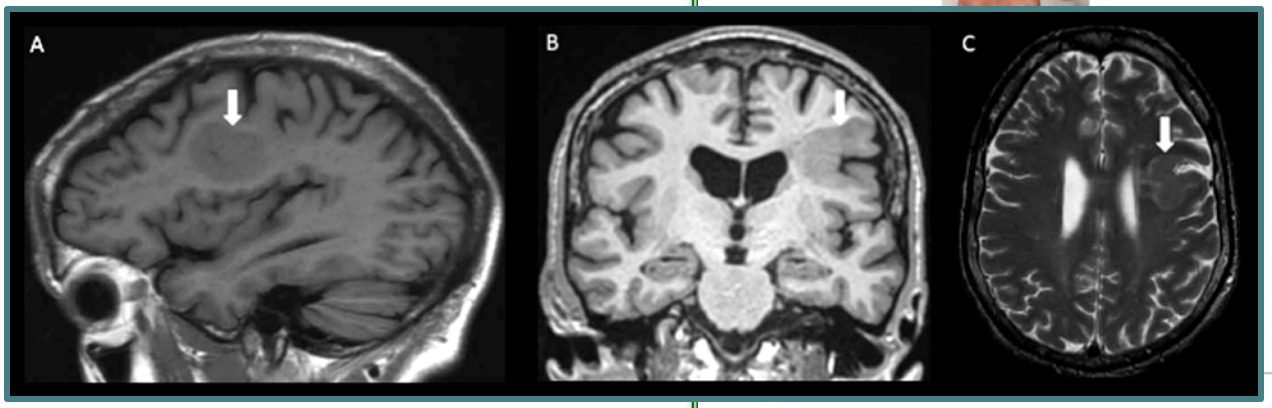
A 10-year-old Chinese girl presented to a paediatric clinic with epilepsy. She had normal intelligence and social interaction ability, and no relevant family history. She had been having recurrent sleep seizures, with generalised twitching of all four limbs, cyanosis, up-rolling of eyeballs, drooling, and urinary incontinence. Her head circumference was 61 cm (5 cm > the 97th percentile). Physical examination was otherwise normal. The electroencephalogram showed an abnormal focus at the right temporo-occipital region. Goldmann perimetry revealed no abnormality. Magnetic resonance imaging (MRI) with contrast showed pachygyria (total loss of sulcation) with hyperplastic white matter and disorganisation of the grey and white matters in the right occipital lobe. A 2.5 cm x 1.8 cm T2 hyperintense area around the right occipital horn was consistent with gliosis. The right occipital lobe was mildly enlarged. Repeated MRI 5 and 7 years after presentation showed no interval changes. Her findings were compatible with focal cortical dysplasia involving the right occipital lobe (Fig 1). The epilepsy was under good control with carbamazepine treatment. She developed a goitre at the age of 16 years, but was euthyroid and ultrasonography showed a multinodular goitre. Hypertrichosis was noted when she was 17 years old. Multiple tiny papules were noted at the perinasal region, of which the patient regarded as coarse skin. At the age of 19 years, she was incidentally found to have iron deficiency anaemia (haemoglobin 96 g/L [reference range, 117-149 g/L], mean corpuscular volume 68.5 fL [82-97 fL], serum iron 2 μmol/L [5.0-30.4 μmol/L], total iron binding capacity 77.1 μmol/L [44.8-76.0 μmol/L]). She had no gastro-intestinal symptoms, but did receive iron supplements.

She had a left hemithyroidectomy at the age of 22 years for increasing size of a dominant left-sided thyroid nodule that expanded from 1.7 × 1.4 × 0.8 cm to 3.6 × 2.9 × 4.2 cm over 10 months. Fine-needle aspiration showed lymphocytic thyroiditis, and excisional biopsy revealed adenomatous hyperplasia. At the age of 23 years, enlargement of a thyroid nodule in the right thyroid lobe (3 cm in diameter) was noted; fine-needle aspiration suggested it was an adenomatous nodule. Total thyroidectomy was performed, and excisional biopsy revealed an atypical nodule with atypical enlarged vesicular nuclei and small distinct nucleoli. The patient also developed a 3-cm scalp papilloma, shown by excisional biopsy to be fibrous papule. At the age of 24 years, she also experienced coffee ground vomiting; oesophagogastroduodenoscopy showed diffuse glycogen deposits at the lower oesophagus and multiple gastric polyps. Polypectomy yielded lymphoid hyperplasia, and oesophageal mucosa biopsy was reported as showing glycogenic acanthosis.

In view of her multiple benign tumours, the possibility of a hamartomatous polyposis syndrome was suspected. Subsequent clinical examination yielded multiple papillomas over the face and tongue, but there was no pigmentation of the lips. These mucocutaneous features were pathognomonic criteria of Cowden syndrome. Notably, macrocephaly, thyroid adenoma, gastro-intestinal hamartomas (oesophageal glycogenic acanthosis and gastric polyps), and skin fibromas

PTEN and FCD

- PHTS – Cowden disease
  - 10 years OFC 61 cm, IQ and exam normal at 10 years
  - Goiter and other hamartomas from 16 years
- ***PTEN*** mutation not specified
  - PTEN LOH not tested



RESIDENT & FELLOW SECTION

Section Editor
Mitchell S.V. Elkind, MD, MS

Mystery Case: Cowden syndrome presenting with partial epilepsy related to focal cortical dysplasia

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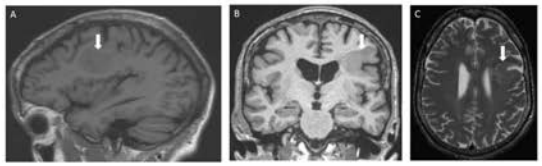
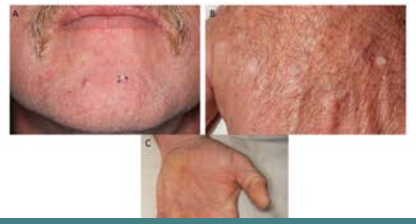
Figure 1 MRI brain



Sagittal T1-weighted MRI (A), oblique-coronal T1-weighted MRI (B), and axial T2-weighted MRI (C) show focal cortical dysplasia with abnormal sulcation involving the middle and inferior frontal gyrus posteriorly on the left with T2 signal extending to the ventricular surface thought to represent neuronal migration lines.

Figure 2 Skin changes found in Cowden syndrome



CS is an autosomal dominant condition of hamartomas and tumors.[1] MRI is abnormal in 35%, commonly showing dysplastic gangliocytomas of cerebellum, meningiomas, and vascular malformations.[2] CS has not been reported presenting with partial epilepsy and focal cortical dysplasia.

AUTHOR CONTRIBUTIONS
N.D. Child and Dr. Cascino contributed to drafting and revising the manuscript. Dr. Cascino was responsible for the study concept.

STUDY FUNDING
No targeted funding reported.

DISCLOSURE
N. Child reports no disclosures. G. Cascino serves as an Associate Editor for *Neurology*®. Go to Neurology.org for full disclosures.

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2. Lok C, Viseux V, Avril MF, et al. Brain magnetic resonance imaging in patients with Cowden syndrome. Medicine 2005;84:129–136.

MYSTERY CASE RESPONSES The Mystery Case series was initiated by the *Neurology*® Resident & Fellow Section to develop the clinical reasoning skills of trainees. Residency programs, medical student preceptors, and individuals were invited to use this Mystery Case as an educational tool. Responses were solicited through a group e-mail sent to the American Academy

PTEN and FCD

- PHTS – BRRS
  - OFC 38.2 cm (+2 SD) at birth at 61 cm (+4 SD) at 8 years
  - Epidermal nevi over scalp and limbs, lipoma over right flank
  - FH: father with Cowden disease
- MRI
  - Bilateral frontal parasagital FCD and L temporal arachnoid cyst
- ***PTEN*** c.389G>A, p.Arg130Gln
  - *PTEN* LOH not tested

Cortical dysplasia associated with the *PTEN* mutation in Bannayan Riley Ruvalcaba syndrome: a rare finding

Declan John O'Rourke[a], Eilish Twomey[b], Sally-Ann Lynch[c] and Mary D. King[a]

Clinical Dysmorphology 2012, **21**:91–92

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List of key features

Bannayan Riley Ruvalcaba
PTEN mutation
Cortical dysplasia
Macrocephaly

Summary

A male, born at term without complications, presented with infantile spasms at 6 weeks of age. Remission occurred with sodium valproate and nitrazepam. At 2 years, partial seizures emerged that responded to carbamazepine. Thereafter, seizures were infrequent. Migraine was diagnosed at 8 years.

Neurodevelopment was delayed and a diagnosis of autistic spectrum disorder with moderate learning disability and motor dyspraxia was made.

Head circumference at birth was 38.2 cm (98th centile) and at 8 years was 61 cm, 4 SD above the mean. There

Discussion

The *PTEN* mutation has only rarely been associated with cortical dysplasia. Hemimegalencephaly, being part of the spectrum of focal cortical dysplasia, was described in an infant with a germline *PTEN* mutation by Merks *et al.* (2003). In a case report of a girl with a germline *PTEN* mutation associated with multiple vertebral hemangiomas, Jenny *et al.* (2007) comment on the sister of the index case who had macrocephaly, cortical dysplasia, an intraorbital tumor and the same mutation. To our knowledge, no other cases have reported this association.

Mutations in the *PTEN* gene (phosphatase and tensin homologue) may present as BRRS, Cowden syndrome, Proteus syndrome, or Lhermitte–Duclos disease. *PTEN* is a dual-specificity phosphatase and tumor suppressor that negatively regulates the cell-survival-signaling pathway initiated by phosphatidylinositol 3-kinase and the mitogen-activated protein kinase (Sansal and Sellers, 2004). Mutations in the *PTEN* gene confer cancer predisposition, and sporadic loss of heterozygosity mutations results in a variety

nd endometrium.

acrocephaly, lipomas, delay, autism, scolio-s (Longy *et al.*, 1998).

TEN mutations in-plastic gangliocytoma nd venous angiomas 005), and dural arte-an *et al.*, 2007).

etween cortical dys-r. Studies in resected at a misregulation of OR-signaling pathway growth of dysplastic y is usually controlled amartin) and TSC2 *et al.*, 1999). Neuronal cortical dysplasia have ut lack morphological ss of apical dendrites

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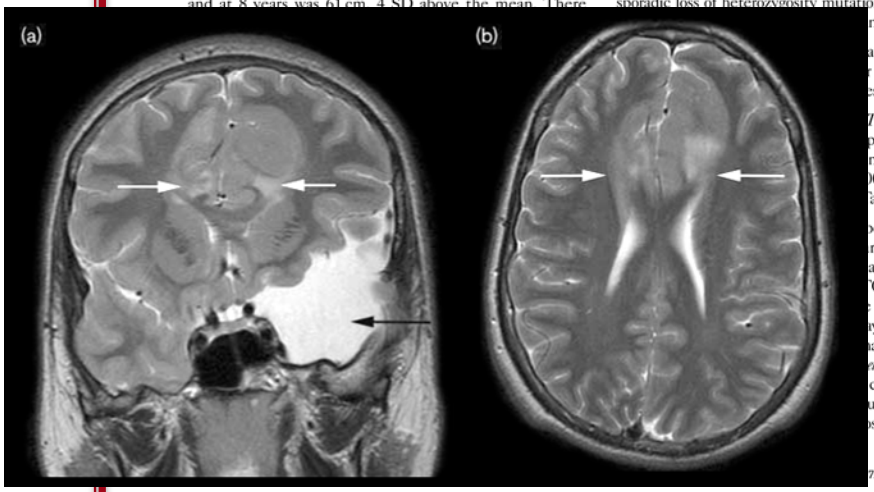


PTEN and FCD

- PHTS – Cowden disease
  - OFC 42 cm at 1 month, 57 cm at 6y7mo (both +4.5 SD)
  - Congenital L hemiparesis
  - 4 months onset SZ intractable
  - DEV delay, moderate ID
  - 7y HYD Rx shunt, CBL surgery
  - 12y intestinal polyps
- MRI
  - R FCD (more than PMG)
  - L Lhermitte-Duclos hamartoma
- Neuropathology
  - CBL gangliocytoma

- ***PTEN*** c.385G>A, p.G129Arg
  - *PTEN* LOH not tested

ELSEVIER

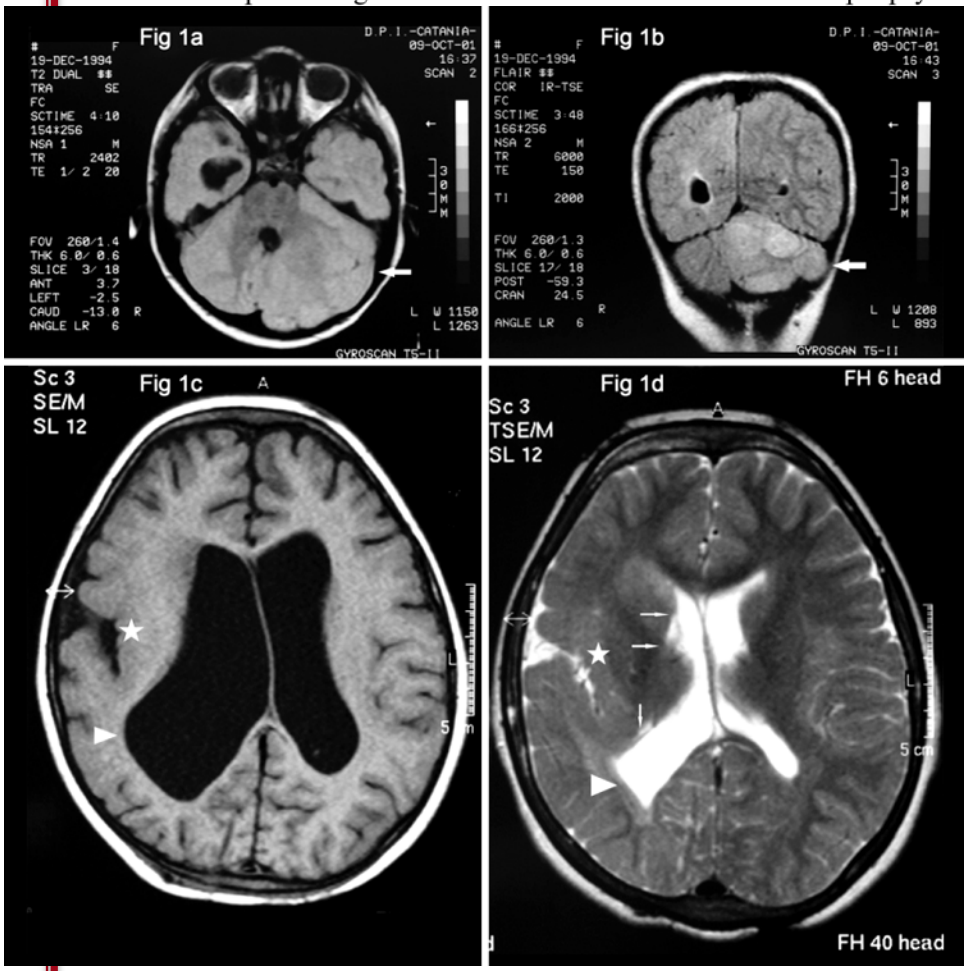
Brain & Development 34 (2012) 873–876

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case report

An atypical patient with Cowden syndrome and PTEN gene mutation presenting with cortical malformation and focal epilepsy



PTEN and HMEG

- PHTS – BRRS
  - OFC 38 cm at 32wkg (+5 SD)
  - Asymmetric skull bulging on the L
  - Linear nevus sebaceous over L face
  - Onset lateralized seizures day 1
  - Seizures intractable, died on day 3
  - Brain weight 510 g (NL 217±49 g)
- ***PTEN*** c.IVS5+1delG
  - *PTEN* LOH negative

ELECTRONIC LETTER

PTEN hamartoma tumour syndrome: variability of an entity

J H M Merks, L S de Vries, X-P Zhou, P Nikkels, P G Barth, C Eng, R C M Hennekam

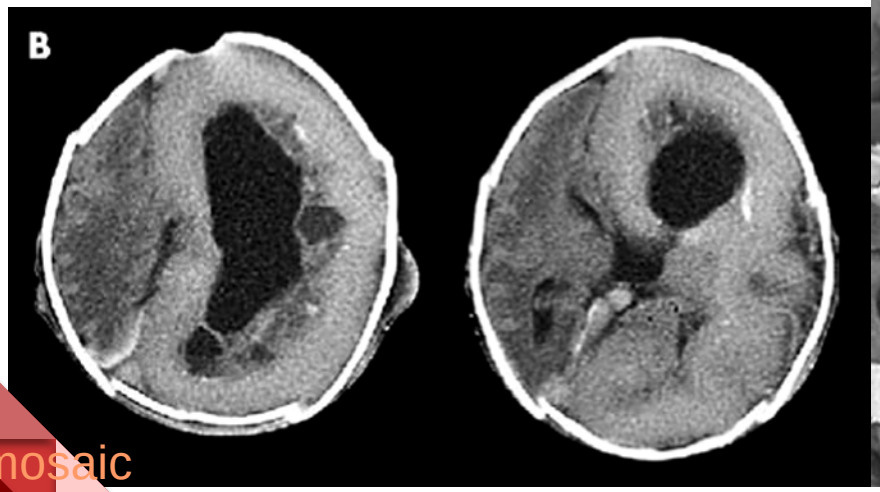
J Med Genet 2003;**40**:e111 (http://www.jmedgenet.com/cgi/content/full/40/10/e111)

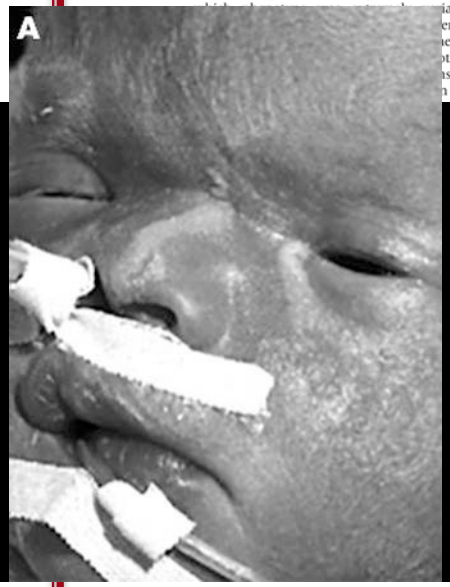
Cowden syndrome (CS; OMIM 158350) is an autosomal dominant disorder with age related penetrance characterised by mucocutaneous lesions, macrocephaly and an increased risk of cancer, especially of the breast, thyroid and endometrium.[1 2] The phenotype in CS has proven to be highly variable, which became especially evident after identification of the susceptibility gene *PTEN*.[3 4] This is also shown in the change in incidence figures, which were found to be at least five times higher after *PTEN* was identified (estimated incidence before PTEN identification 1:1 000 000,[5] and after >1:200 000.[6 7]). Bannayan-Riley-Ruvalcaba syndrome (BRRS; OMIM 153480) is allelic to CS and is characterised by the triad of macrocephaly, lipomas, and pigmented macules of the glans penis.[8] Proteus syndrome (PS; OMIM 176920) is a disorder characterised by overgrowth of hands and/or feet, asymmetry of limbs, connective tissue, and epidermal naevi, vascular and lymphatic malformations, and cranial hyperostosis.[8] Proteus-like syndrome (PLS) is another closely related disorder, where individuals are characterised by the presence of macrocephaly, lipomas and overgrowth not meeting the criteria for CS, BRRS, or PS.[8] Germline *PTEN* mutations have been found in 80% of individuals with CS, 60% of individuals with BRRS, up to 20% with PS, and 50% with PLS.[9 10]

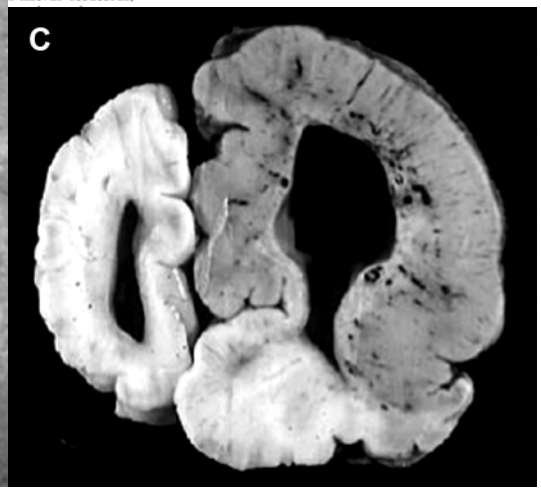
Here, we present a family (a mother and three sons) in [illegible], one member [illegible]ence, and minimal [illegible]ember with macro- [illegible]other with macroce- [illegible]st member having [illegible] naevus sebaceous,

Key points

- Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), proteus and proteus-like syndrome are entities that can show remarkable clinical overlap, and are all shown to be caused by germline *PTEN* mutations (80% of CS cases, 60% of BRR cases, up to 20% of Proteus syndrome cases, and 50% of Proteus-like cases).
- We describe four members of a single family with a heterogeneous phenotype that at present most closely fits BRRS, although further development of symptoms with time may eventually lead to the diagnosis of CS.
- All four cases were shown to harbour the same *PTEN* mutation (IVS5+1delG).
- One of the cases was first suspected of having Jadassohn naevus sebaceous syndrome, a diagnosis that was refuted only after the birth of the other family members and *PTEN* mutation analysis. This patient also had a hemimegalencephaly, not reported before in a case with BRRS or CS. No loss of heterozygosity was found in the megalencephalic part of the brain.
- The family can best be classified by the molecular cause as having *PTEN* hamartoma tumour syndrome. Hemimegalencephaly as part of Jadassohn naevus sebaceous syndrome can be added as further manifestations of germline *PTEN* mutations.







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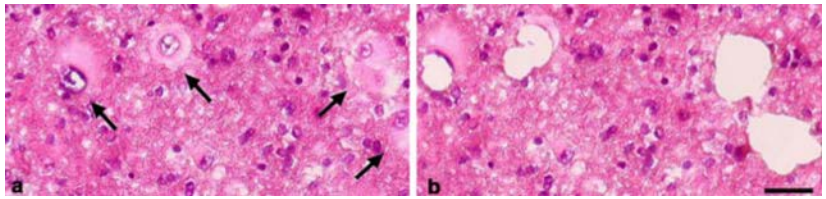
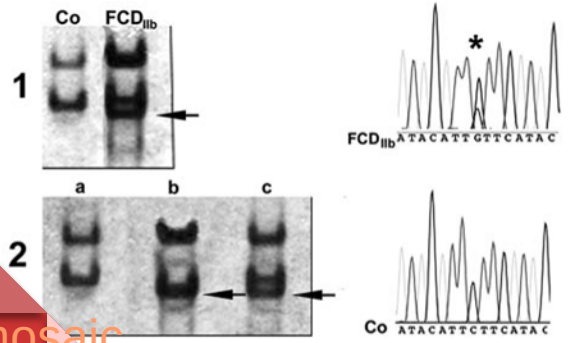
PTEN and FCD2b



- FCD2b
  - Balloon cells and dysplastic neurons taken with LCM and sequenced for PTEN
- ***PTEN*** c.834C>G, p.Phe278Leu
  - Not present in EVS or HGMD, not seen at GeneDX
  - Polyphen-2 score 0.948 (very high)
  - *PTEN* LOH negative



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Acta Neuropathol (2006) 112:715–725
DOI 10.1007/s00401-006-0128-y

ORIGINAL PAPER

Activation of Akt independent of *PTEN* and *CTMP* tumor-suppressor gene mutations in epilepsy-associated Taylor-type focal cortical dysplasias

Volker Schick · Michael Majores · Gudrun Engels · Sylvia Spitoni · Arend Koch · Christian E. Elger · Matthias Simon · Christiane Knobbe · Ingmar Blümcke · Albert J. Becker

Received: 31 January 2006 / Revised: 28 July 2006 / Accepted: 28 July 2006 / Published online: 30 September 2006

Abstract Focal cortical dysplasias (FCD) with Taylor-type balloon cells (FCD_{IIb}) are frequently observed in biopsy specimens of patients with pharmacoresistant focal epilepsies. The molecular pathogenesis of FCD_{IIb}, which lack familial inheritance, is only poorly understood. Due to their highly differentiated, malformative nature and glioneuronal phenotype, FCD_{IIb} share neuropathological characteristics with lesions observed in familial disorders such as cortical tubers present in patients with autosomal dominant tuberous sclerosis complex (TSC), related to mutations in the *TSC1* or *TSC2* genes, and dysplastic gangliocytomas of the cerebellum found in Cowden disease. Current data have indicated distinct allelic variants of *TSC1* to accumulate in FCD_{IIb}. TSC1 represents a tumor suppressor operating in the phosphatidylinositol 3-kinase (PI3K)/insulin pathway. The tumor-suppressor gene *PTEN* is mutated in Cowden disease. Like PTEN, also carboxyl-terminal modulator protein (CTMP) modulates PI3K-pathway signaling, both via inhibition of Akt/PKB, a kinase inactivating the TSC1/TSC2 complex. Here, we have analyzed alterations of Akt, *PTEN* and *CTMP* relevant for insulin signaling upstream of TSC1/TSC2 in FCD_{IIb}. Immunohistochemistry with antibodies against phosphorylated Akt (*phospho*-Akt; Ser 473) in FCD_{IIb} ($n = 23$) showed strong *phospho*-Akt expression in dysplastic FCD_{IIb} components. We have further studied sequence alterations of *PTEN* ($n = 34$ FCD_{IIb}) and *CTMP* ($n = 20$ FCD_{IIb}) by laser microdissection/single-strand conformation polymorphism analysis. We observed a somatic mutation in an FCD_{IIb}, i.e., amino-acid exchange at nucleotide position 834 (*PTEN* cDNA, GenBank AH007803.1) in exon 8 with replacement of phenylalanine by leucine (F278L). We also found several silent polymorphisms of *PTEN* in exon 2 and exon 8 as well as silent and coding polymorphisms but no mutations in *CTMP*. No loss of heterozygosity in FCD_{IIb} ($n = 6$) at *10q23* was observed. To our knowledge, we here report on the first somatic mutation of a tumor-suppressor gene, i.e., *PTEN*, in FCD_{IIb}. However, our study also demonstrates that mutational alterations of *PTEN* and *CTMP* do not play major pathogenetic roles for activation of Akt in FCD_{IIb}. Future studies need to determine the origin of insulin pathway activation upstream of TSC1/TSC2 in FCD_{IIb}.

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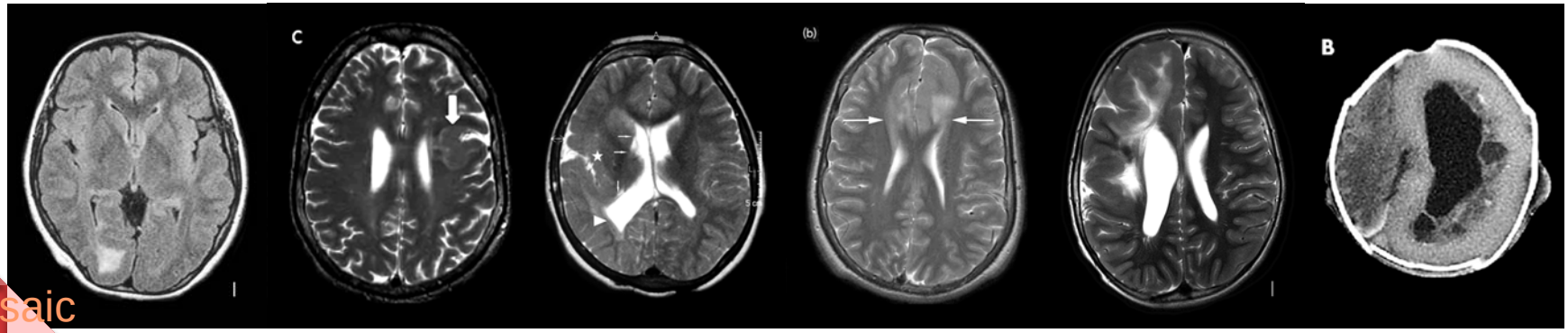
Keywords PTEN · Akt · PI3K-pathway · CTMP · Glioneuronal lesion · FCD

PTEN and segmental cortical dysplasia

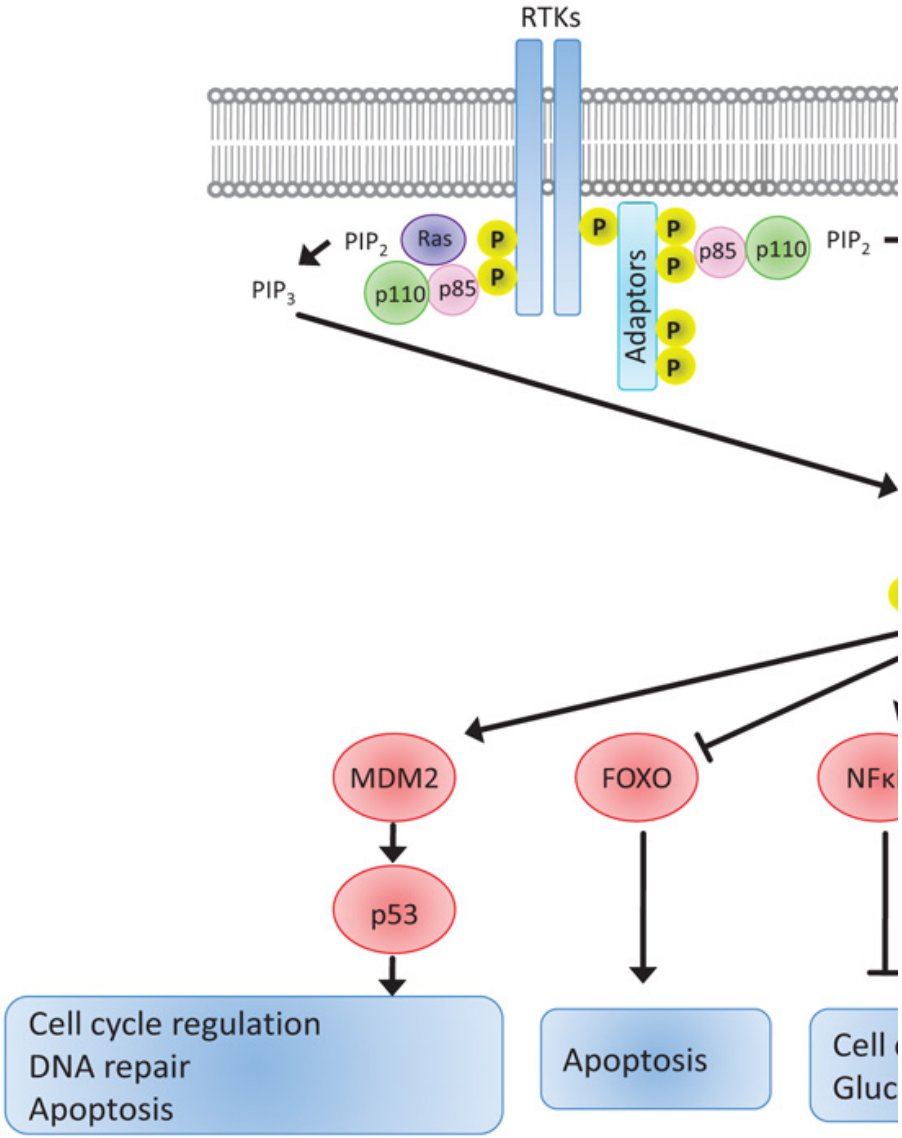
Diagnosis	Subtype	Brain	PTEN	LOH	Reference
PHTS	BRRS	HMEG	c.IVS5+1delG	NEG	Merks et al., 2003
PHTS	No data	**FCD2b**	p.Phe278Leu	NEG	Schick et al., 2006
PHTS	Cowden	FCD, LDD	p.Gly129Arg	•••	Elia et al., 2012
PHTS	BRRS	FCD	p.Arg130Gln	•••	O'Rourke et al., 2012
PHTS	Cowden	FCD-NOS	Not provided	•••	Child et al., 2013
PHTS	Cowden	FCD-NOS	p.Phe341Leu_fs*3	•••	Cheung et al., 2014
PHTS	TBD	**HMEG-FCD2a**	p.Tyr68His	NEG	LR12-123



Need expanded nomenclature

- Segmental cortical dysgenesis (dysplasia)
  - Dysplastic megalencephaly (bilateral)
  - Hemimegalencephaly (but not really)
  - Multilobar cortical dysplasia (dysgenesis)
    - Anterior quadrantic dysplasia (dysgenesis)
    - Posterior quadrantic dysplasia (dysgenesis)
    - Multilobar dysplasia (dysgenesis) NOS
  - Focal cortical dysplasia type 2a, type 2b a.k.a. “lobar cortical dysplasia (dysgenesis)”

PI3K-AKT



nature genetics

De novo CCND2 mutations leading to stabilization of cyclin D2 cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome

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Activating mutations in genes encoding phosphatidylinositol 3-kinase (PI3K)-AKT pathway components cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH, OMIM 603387)[1–3]. Here we report that individuals with MPPH lacking upstream PI3K-AKT pathway mutations carry *de novo* mutations in *CCND2* (encoding cyclin D2) that are clustered around a residue that can be phosphorylated by glycogen synthase kinase 3β (GSK-3β)[4]. Mutant CCND2 was resistant to proteasomal degradation *in vitro* compared to wild-type CCND2. The PI3K-AKT pathway modulates GSK-3β activity[4], and cells from individuals with *PIK3CA*, *PIK3R2* or *AKT3* mutations showed similar CCND2 accumulation. CCND2 was expressed at higher levels in brains of mouse embryos expressing activated AKT3. *In utero* electroporation of mutant *CCND2* into embryonic mouse brains produced more proliferating transfected progenitors and a smaller fraction of progenitors exiting the cell cycle compared to cells electroporated with wild-type *CCND2*. These observations suggest that cyclin D2 stabilization, caused by *CCND2* mutation or PI3K-AKT activation, is a unifying mechanism in PI3K-AKT–related megalencephaly syndromes.

Germline mutations of *PIK3R2* and *AKT3* result in MPPH[3], whereas postzygotic mutations of *PIK3CA* cause megalencephaly-capillary malformation syndrome[3], and postzygotic mutations of *PIK3CA* and *AKT3* cause isolated hemimegalencephaly[1,5]. We previously failed to detect mutations in *PIK3R2* or *AKT3* in 35% of probands with MPPH[3]. We hypothesized that mutation-negative patients have *de novo* mutations in one or more previously unreported genes, and thus we performed whole-exome sequencing in two child-parent trios (JT-144 and LR11-424) and one child-parent pair (LR02-064).

We identified germline mutations in *CCND2* in all three affected individuals (resulting in p.Thr280Ala in one subject and p.Lys270* in two unrelated subjects), and we confirmed these mutations as *de novo* by Sanger sequencing. None of the identified variants was present in dbSNP build 132, the 1000 Genomes Project, the National Heart, Lung, and Blood Institute (NHLBI) exome variant server of 6,500 exomes or in-house exome databases (Online Methods). We identified *de novo* heterozygous *CCND2* mutations in nine additional patients with MPPH by conventional Sanger sequencing. The clinical and molecular findings of this cohort are shown in **Figure 1** and are summarized in **Table 1**, **Supplementary Table 1** and **Supplementary Note**.

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- 1 Nil recurrence risk in families
- 2 Asymmetry or focal/multifocal anomalies
- 3 Dysplastic growth especially overgrowth
- 4 Predisposition for tumors
- 5 Discordant monozygotic twins