Title Page

Multiple skin hamartomata: A possible novel clinical presentation of *SUFU* neoplasia syndrome

Kirsty Mann¹, Jill Magee², Marine Guillaud-Bataille³, Christophe Blondel³, Brigitte Bressac-de Paillerets³, Josie Yeatman⁴, Ingrid Winship^{1,5}

1. Genetic Medicine and Familial Cancer Centre, Royal Melbourne Hospital, Grattan St, Parkville 3050, Victoria, Australia
2. Dorevitch Pathology, Heidelberg 3084, Victoria, Australia
3. Gustave Roussy, Service de Génétique, Département de Biopathology, Villejuif, F-94805, France
4. Department of Dermatology, Austin Hospital, Heidelberg 3084, Victoria, Australia
5. Department of Medicine, University of Melbourne, Parkville 3052, Victoria, Australia

Corresponding Author

Ms Kirsty Mann

Associate Genetic Counsellor

Genetic Medicine and Familial Cancer Centre

Royal Melbourne Hospital

Grattan Street

Parkville VIC 3050

Phone: (03) 9342 7151

Fax: (03) 9342 4267

Email: kirsty.mann@mh.org.au

Abstract

Medulloblastoma tumours may arise sporadically or as part of an inherited syndrome. A subset of children with medulloblastoma carry germline and somatic mutations in the *SUFU* tumour suppressor gene located at 10q24. We report a 55 year old woman referred for investigation on the basis of skin lesions and a family history of two children from different unions with medulloblastoma. Examination of our patient revealed facial papules (classified as benign folliculosebaceous hamartomatous lesions) and dysmorphology (macrocephaly, hypertelorism and prognathism). She reported her father and her son share the same dermatological features; photographs of the son display hypertelorism. Sequencing in our patient revealed a splice-site mutation in intron 6 of *SUFU* (c. 756+1G>A), predicted to lead to skipping of exon 6. We suggest that the emerging phenotype in *SUFU* associated with familial medulloblastoma may include hamartomatous skin lesions. Consideration of these features, along with macrocephaly will alert clinicians to the likely genetic basis of the syndrome, affording the opportunity for genetic counselling, prenatal or pre-implantation genetic diagnosis in at-risk families.

Keywords

SUFU; Medulloblastoma; Germline Mutation; Genodermatosis

Introduction

Medulloblastoma is a common childhood malignancy where one of five histological subtypes of tumour arise in the cerebellum [1]. These tumours may arise sporadically or as part of an inherited syndrome. Medulloblastoma is seen in five per cent of cases of the Gorlin Syndrome (GS), also known as the Basal Cell Naevus Syndrome, as a result of heterozygous mutations in the *PTCH1* gene [2-4]. In addition, a subset of children with medulloblastoma was shown to carry germline and somatic mutations in *SUFU* tumour suppressor gene located at 10q24 [5]. *SUFU* loss of function predisposes individuals to medulloblastoma by modulating the sonic hedgehog (HH) signalling pathway which plays an important role in the early human embryonic development. In a larger study of 131 children with medulloblastoma, germline mutations in *SUFU* were found in eight probands [6]. All of the affected children were diagnosed with medulloblastoma under the age of three years – macrocrania and hypertelorism were noted as part of the phenotype.

Case report

A 55 year old woman was referred for investigation on the basis of skin lesions and a family history of brain tumours. She had a partial thyroidectomy for a benign cystic lesion and a cholecystectomy, aged in her forties. She had been otherwise well. She presented to the dermatologist for cosmetic advice regarding multiple skin coloured papules on her face, which had first developed in her thirties and had slowly increased in size and number. The lesions were asymptomatic, with neither scaling nor ulceration. Coincidentally, a basal cell carcinoma (BCC) was noted on her upper lip. She had two previous BCCs removed in her fifties from her right forearm and back.

Her family history was highly significant (**Fig. 1**). She had seven children to two unrelated partners. In each union she had a child with medulloblastoma. Her son, born in 1976, was diagnosed aged six months and died at the age of 26 months. A daughter born in 1994 was diagnosed at the age of seven months with a desmoplastic medulloblastoma, and died aged 25 months. Both infants were noted to have large heads at birth. Photographs show similar facial features, with bossed foreheads and hypertelorism.

Her father died aged 57 of non Hodgkins lymphoma. He had multiple facial papules. He had a cousin who died aged 52 of a brain tumour; no further details are available.

A son from the first union is now 30 years of age. He is described as having skin lesions similar to his mother.

Photographs reveal a broad forehead, hypertelorism, and a ridge of skin coloured papules below each eye.

Examination of the proband revealed facial dysmorphology (**Fig. 2**). She had macrocephaly with a head circumference of 58.5cm, relative to a height of 159cm. She had hypertelorism and prognathism.

Her skin examination revealed profuse pearly white and skin coloured papules on her face in a centropacial distribution. These were especially dense around her eyes and nose, and were evident behind her ears and on her scalp. She also had multiple achrochordons on her neck and trunk. Her skin showed evidence of significant sun damage. She had small amounts of plaque psoriasis.

Histological assessment of a lesion excised from her nose showed several small aggregates of bland basaloid cells extending downward from the epidermis into the papillary dermis (**Fig. 3**). These aggregates exhibited folliculosebaceous differentiation and were classified as benign folliculosebaceous hamartomatous lesions with some features of tumour of the follicular infundibulum.

She had no other features of GS and a skull radiograph did not show evidence of calcification of the falx cerebri.

Other family members were not available for examination or genetic testing.

Method

Sequence of testing

Based on the hamartomatous lesions and macrocephaly, Cowden syndrome was queried as a potential diagnosis and genetic testing was performed to exclude mutations in the *PTEN* genes. Similarly, *PTCH1* mutation analysis was performed since Gorlin syndrome was considered based on the family history of medulloblastoma, and personal history of macrocephaly and BCCs. Cancer predispositions as a result of microdeletions and microduplications have been identified through SNP array and so a SNP microarray was undertaken as part of her initial clinical investigation. *SUFU* mutation analysis was then offered.

SUFU mutation analysis

The twelve exons of the *SUFU* gene were amplified by quantitative PCR using Quantitect SYBR Green (Qiagen), 12ng of DNA, and 375nM of each primer. Each quantitative PCR primer contains a universal sequence component, which has been used for further Sanger sequencing of the PCR products using Big Dye with an ABI 3730 sequencer. Sequences of quantitative PCR and sequencing primers are available upon request. Sequences were analysed using Seqscape Software (Life Technologies).

Results

No mutations were identified in *PTEN* or *PTCH1*. SNP array revealed a long continuous stretch of homozygosity on chromosome 5q; no genes of interest were evident in this region. Sequencing revealed a splice-site mutation in intron 6 of *SUFU* (c. 756+1G>A) (**Fig. 4**). In silico bioinformatics tools (SpliceSiteFinder-like, NNSplice, MaxEntScan, Gene splicer, and Human Splicing Finder) available in Alamut (Interactive Biosoftware) all predicted a complete loss of the donor splice site. This probably leads to skipping of exon 6, frameshift, and a premature STOP codon, probably associated with degradation of the mRNA by the mRNA decay system. Viable lymphocytes were not available for RNA extraction and subsequent RT-PCR analysis to experimentally test the consequences of the mutation on mRNA splicing. There were no large deletions nor duplications in *SUFU* using quantitative PCR.

Discussion

This family supports autosomal dominant inheritance of a predisposition to medulloblastoma conferred by a germline mutation in *SUFU*, with a mutation carrier having children with medulloblastoma to two separate and unrelated partners. A phenotype has emerged, with macrocephaly and hypertelorism. This family suggests that hamartomatous skin lesions are likely to be an additional syndromic feature.

In 2002, Taylor discerned a subset of children with desmoplastic medulloblastoma on the basis of germline heterozygous mutations in *SUFU* [5]. The role of *SUFU* in tumorigenesis has been investigated, illustrating that the thresholds of sensitivity of the malignant transformation differ between the skin and the cerebellum [7]. While the role of *SUFU* has been demonstrated in tumour suppression in the cerebellum [8], inactivation of *SUFU* does not transform embryonic or adult keratinocytes to BCC [9]. Interestingly activation of the HH pathway by *SUFU* mutation does appear to cause basaloid follicular hamartomas.

Abnormal activation of the HH signalling leads to BCC. Inactivating mutations in the *PTCH1* gene are found in BCC and cause the phenotype of the GS, where multiple BCC's coexist with a variable dysmorphic phenotype [3].

Medulloblastoma occurs in approximately 5% of affected children with GS [3].

A knockout mouse model with a loss of function mutation in *SUFU* has been generated in which mice *SUFU*^{+/-} (heterozygous for the mutated allele) develop skin lesions consistent with basaloid follicular hamartomas. Simultaneous *TP53* (*Trp53* in mice) loss on a background of *SUFU*^{+/-}, induced medulloblastoma but not BCC in mice [9]. One family with GS that was *PTCH1* negative has been reported with a germline splicing mutation in *SUFU* [10].

In the reported series, penetrance of the predisposition to medulloblastoma is incomplete [11] and the family presented is consistent with these reports. In two families with 25 mutation carriers, seven developed medulloblastoma, all were desmoplastic or nodular, diagnosed under the age of three years the penetrance was estimated at 30%. In a larger study of

131 consecutive patients with medulloblastoma, eight germline mutations in *SUFU* were found [6]. Of these, the seven patients examined had no other feature of GS other than macrocephaly. Six patients had head circumference greater than 97th percentile; three of these were confirmed to have macrocephaly at birth. Eight healthy carriers of the mutation also had relative macrocephaly. Other tumours found in these kindreds [6] included leiomyosarcoma aged 63, breast cancer aged 37, and a meningioma aged 38 years. While the role of the meningioma is plausible, other tumours may be coincidental. Germline *SUFU* mutations have also been detected in familial multiple meningioma [12]. In 2012, Kijma et al [13] reported the finding of meningioma in the context of a germline *SUFU* mutation. The individual had a medulloblastoma at 21 months old, subcutaneous nodules on his face and neck shown to be trichoepithelioma at age 25, BCC at age 28, and meningothelial meningioma at age 35.

In the above group of eight patients with *SUFU* mutations [6], an 18 year old had multiple BCC's in a field of radiation, one patient had perianal skin tag and one had multiple naevi. The family reported here has features similar, with additional dermatologic signs comprising multiple facial hamartomatous papules. In the sixth decade, the proband's BCCs are likely to be coincidence. The proband developed skin hamartomata in early adulthood. Depending on the variability in age of onset, there may be family members carrying the mutation have not yet developed cutaneous signs. Similarly, the triad of skin lesions (fibrofolliculomas, trichodiscomas, and acrochordons) characteristic of Birt Hogg Dube syndrome (OMIM #135150), do not appear until the third or fourth decade of life [14].

There is an increasing number of skin lesions recognised as syndromic features of inherited malignancy. Cowden syndrome (OMIM #158350) and Hereditary Leiomyomatosis and Renal Cell Carcinoma (OMIM #158350) are further examples of conditions that have pathognomonic skin signs, which may precede internal malignancy [15]. Skin lesions may alert clinicians to a range of heritable cancer syndromes [15]. Recognition of these skin signs may raise awareness to an increased risk of malignancy in family members, affording the opportunity to follow optimal strategies to manage the predictable risk of cancer.

We suggest that the emerging phenotype in *SUFU* associated with familial medulloblastoma may include hamartomatous skin lesions. Consideration of these features, along with macrocephaly, will alert clinicians to the likely genetic basis of the syndrome, affording the opportunity for genetic counselling, prenatal or pre-implantation genetic diagnosis in at-risk families.

Acknowledgements

The authors would like to thank Angela Magira, Blake Storey and Iain Gordon for assistance with manuscript preparation and illustrations.

Figures

Fig. 1 The pedigree of the family

Fig. 2 Facial dysmorphology and multiple skin papules evident in the proband

Fig 3 Histological changes: Superficial benign basaloid proliferation with folliculosebaceous differentiation, consistent with a benign follicular hamartoma (indicated by arrow)

Fig 4 Electrophoregram of the identified SUFU mutation (indicated by arrow) and wildtype sequence

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 114:97-109
2. Ng D, Stavrou T, Liu L, Taylor MD, Gold B, Dean M, Kelley MJ, Dubovsky EC, Vezina G, Nicholson HS, Byrne J, Rutka JT, Hogg D, Reaman GH, Goldstein AM (2005) Retrospective family study of childhood medulloblastoma. *Am J Med Genet A* 134:399-403
3. Evans DG, Farndon PA, Burnell LD, Gattamaneni HR, Birch JM (1991) The incidence of Gorlin syndrome in 173 consecutive cases of medulloblastoma. *Br J Cancer* Nov;64(5):959-61 Review
4. Slade I, Murray A, Hanks S, Kumar A, Walker L, Hargrave D, Douglas J, Stiller C, Izatt L, Rahman N (2011) Heterogeneity of familial medulloblastoma and contribution of germline PTCH1 and SUFU mutations to sporadic medulloblastoma. *Fam Cancer* 10:337-42
5. Taylor MD, Liu L, Raffel C, Hui CC, Mainprize TG, Zhang X, Agatep R, Chiappa S, Gao L, Lowrance A, Hao A, Goldstein AM, Stavrou T, Scherer SW, Dura WT, Wainwright B, Squire JA, Rutka JT, Hogg D (2002) Mutations in SUFU predispose to medulloblastoma. *Nat Genet* 31:306-10
6. Brugières L, Remenieras A, Pierron G, Varlet P, Forget S, Byrde V, Bombled J, Puget S, Caron O, Dufour C, Delattre O, Bressac-de Paillerets B, Grill J (2012) High frequency of germline SUFU mutations in children with desmoplastic/nodular medulloblastoma younger than 3 years of age. *J Clin Oncol* 30:2087-93

7. Li ZJ, Nieuwenhuis E, Nien W, Zhang X, Zhang J, Puviindran V, Wainwright BJ, Kim PC, Hui CC (2012) Kif7 regulates Gli2 through SUFU–dependent and –independent functions during skin development and tumorigenesis. *Development* 139:4152-61
8. Lee Y, Kawagoe R, Sasai K, Li Y, Russell HR, Curran T, McKinnon PJ (2007) Loss of suppressor-of-fused function promotes tumorigenesis. *Oncogene* 26:6442-47
9. Heby-Henricson K, Bergström A, Rozell B, Toftgård R, Teglund S (2012) Loss of Trp53 promotes medulloblastoma development but not skin tumorigenesis in *SUFU* heterozygous mutant mice. *Mol Carcinog* Sep;51(9):754-60
10. Pastorino L, Ghiorzo P, Nasti S, Battistuzzi L, Cusano R, Marzocchi C, Garrè ML, Clementi M, Scarrà GB (2009) Identification of a *SUFU* germline mutation in a family with Gorlin syndrome. *Am J Med Genet A* Jul;149A(7):1539-43
11. Brugières L, Pierron G, Chompret A, Paillerets BB, Di Rocco F, Varlet P, Pierre-Kahn A, Caron O, Grill J, Delattre O (2010) Incomplete penetrance of the predisposition to medulloblastoma associated with germ-line *SUFU* mutations. *J Med Genet* 47:142-44
12. Aavikko M, Li SP, Saarinen S, Alhopuro P, Kaasinen E, Morgunova E, Li Y, Vesanen K, Smith MJ, Evans DG, Pöyhönen M, Kiuru A, Auvinen A, Aaltonen LA, Taipale J, Vahteristo P (2012) Loss of *SUFU* function in familial multiple meningioma. *Am J Hum Genet* Sep 7;91(3):520-6
13. Kijima C, Miyashita T, Suzuki M, Oka M, Fujii K (2012) Two cases of nevoid basal cell carcinoma syndrome associated with meningioma caused by a PTCH1 or *SUFU* germline mutation. *Familial Cancer* 11:565–570.
14. Toro JR, Glenn G, Duray P, Darling T, Weirich G, Zbar B, Linehan M, Turner ML (1999) Birt-Hogg-Dube syndrome: A novel marker of kidney neoplasia. *Arch Dermatol* 135:1195-1202.
15. Winship IM, Dudding TE (2008) Lessons from the skin--cutaneous features of familial cancer. *Lancet Oncol* 9:462-72. Review