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SHORT COMMUNICATION

Germline mutation p.N363K in *POLE* is associated with an increased risk of colorectal cancer and giant cell glioblastoma

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Abstract

Germline mutations of the *POLE* gene are responsible for polymerase proofreading-associated polyposis syndrome (PPAP). These mutations were hypothesised to predispose to extra-gastrointestinal tumours (ovary, endometrium, brain), but this association has not been confirmed so far. We report a family with an autosomal dominant inheritance of PPAP due to a c.1089C>A; p.Asn363Lys mutation in the proofreading exonuclease domain of *POLE*. Ten patients presenting a history of colorectal tumours and three patients with polyposis are indexed in this family. Three carriers (including siblings and a distant cousin at 30, 45 and 52 respectively) and another member (at 37 not tested) presented glioblastoma. This is the second family reported to carry this mutation. Among the four glioblastomas in the family that we report, both show similar pathology: giant cell glioblastoma. These cases suggest that the c.1089C>A germline *POLE* mutation may confer an increased risk of brain cancer [incidence 17.4% (4/23) in mutation carriers combining the two families]. More observations are needed to support this hypothesis. It seems that not all mutations of *POLE* are equally associated with extra-gastrointestinal tumours. Although carriers of a mutation responsible for PPAP should benefit from screening for colorectal and uterine cancer, due to the rapid evolution of glioblastoma the value of neurological follow-up and brain imaging screening remains questionable. Nevertheless, considering the limitations of standard therapy for glioblastoma, mutation status could be useful for targeting therapy. The biological mechanism linking *POLE* mutation to glioblastoma remains to be determined.

Keywords *POLE* · Polymerase epsilon · PPAP · Proofreading · Glioblastoma · Giant cells

Introduction

Germline mutations in the proofreading exonuclease domain of the polymerase ε gene (*POLE*) were described in association with a familial predisposition to colorectal cancer (CRC) [1, 2]. Polymerase proofreading-associated polyposis syndrome (PPAP), caused by autosomal dominant mutations of polymerases genes *POLD1* and *POLE* [3], predispose to colorectal cancer, with or without polyposis. Rohlin et al. described a family carrying the mutation of *POLE* c.1089C>A, p.Asn363Lys and suggested that *POLE* mutations can predispose carriers to a broad spectrum of tumours [4]. We report a family with the same mutation displaying a high incidence of CRC and glioblastoma. We support the hypothesis that this mutation is associated with brain tumours.

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Subjects and methods

Family report

The family we report (Fig. 1) has been followed up in the Medical Oncogenetics Department of the Institut Universitaire du Cancer de Toulouse-Oncopole (IUCT-O). Four members, patient III:3, III:4, IV:15 and IV:19, developed a brain cancer (glioblastoma) at age 45, 52, 30 and 37 respectively. Ten members have developed at least one colorectal (I:1, II:8, II.10, III:5, III:3, III:4, III:8, III:18, III:16, and IV:15). The main phenotypic information relating to the family members is summarized in Table 1. Informed written consent for genetics germline analysis was collected from all participating subjects in the context of medical care.

Analysis of tumour tissue

Somatic MMR status was determined by assessing microsatellite instability (MSI) and by immunohistochemistry (IHC) on colorectal cancer tissues of patient III:3 and III:4 and the glioblastoma of patient III:3. MSI status was established in all cases on the DNA from paired tumour and normal tissue samples as recommended by the National Cancer Institute workshop [5]. Expression of MLH1, MSH2 and MSH6 was assessed by IHC as previously described [6].

Germline mutation analysis

Analysis of *MLH1* (NM_000249.3), *MSH2* (NM_000251.2), MS*H*6 (NM_000179.2) and *APC* (NM_000038.4) genes was performed in the Laboratoire d'Oncogénétique of the IUCT-O using Sanger sequencing. The screening method of these two genes is described in Supplementary Material.

Mutation screening of the exonuclease coding region of *POLD1* (NM_001308632.1) codons 268–471 and *POLE* (NM_006231) codons 304–517 was performed in the Laboratoire d'Oncogénétique Biochimie Génétique et Moléculaire of the Hôpital Cochin, Paris using custom made Ion Torrent Ion AmpliSeq panel (Life Technologies) on DNA extracted from peripheral blood samples. Mutations were confirmed using Sanger sequencing.

Analysis of *BMPR1A* and *MUTYH* was performed in the Laboratoire de Génétique Moléculaire of the CHU de Rouen by Sanger sequencing.

Results

All tumours tested (Table 1) were micro-satellite stable (MSS) with maintained expression of the MMR proteins MLH1, MSH2, MSH6 (patients III:3, III:5, III:4) and PMS2 (patient IV:15) except the sigmoid polyp of patient IV:15 which presented an isolated loss of MSH6 expression (checked on two occasions).

Germline DNA sequencing of *MLH1, MSH2* (patients III:3, III:4, III:5) and *MSH6* (patients III:5, III:3, IV:15) did not reveal any mutation or large rearrangement of these genes. *APC* (patients II:10, III:5, III:4), *MUTYH* (patients II:10, III:5, III:4, IV:5, IV:15), *BMPR1A* (patient IV:15) and *POLD1* (patient IV:5) were also sequenced and no mutation was detected.

Finally, a c.1089C>A, p.Asn363Lys mutation of *POLE* was detected in patient III:4. She developed a rectal adenocarcinoma (MSS) diagnosed at age 37 years and a right temporal glioblastoma at 52 years of age. Her brother, patient III:3, was a carrier of the familial mutation and developed an adenocarcinoma of the caecum, a synchronous cancerous sigmoid polyp and a glioblastoma at 42 years of age. Patient IV:15 (distant cousin of patient III:4 and patient III.3), carrying the familial mutation, developed a colonic adenocarcinoma at 23 years of age, associated with multiple polyposis, and a glioblastoma at 30 years of age.

The mutation status of another distant cousin (IV:19) was not tested, but this patient also developed a glioblastoma

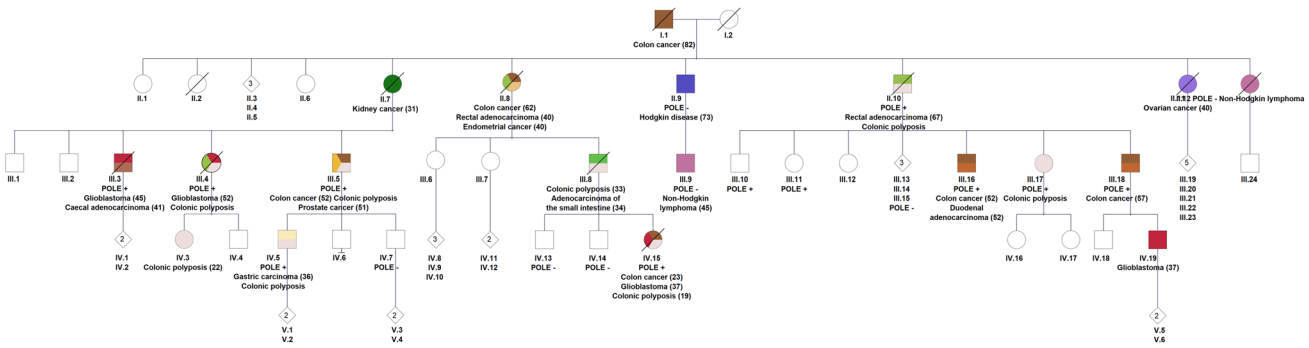

Fig. 1 Pedigree of the family carrying *POLE* c.1089C>A mutation reported in the current study. Age at diagnosis is bracketed when this information is available. POLE+: *POLE* c.1089C>A mutation carriers, POLE−: wild type *POLE*

Table 1 Summary of phenotypic characteristics of *POLE* c.1089C>A, p.Asn363Lys pathogenic mutation carriers and their siblings presenting symptoms

POLE protein change	Case (gender)	Tumours (age at diagnosis)	Polyps (localisation)	MSI status and somatic IHC	Publication
p.N363K	II.2 (♀)	CRC (43 years old), OC (50 years old), EC (50 years old)			Rohlin et al.
p.N363K	II.4 (♂)	CRC (56 years old)			Rohlin et al.
p.N363K	II.7 (♂)	CRC (28 years old)			Rohlin et al.
p.N363K	II.9 (♀)	2xCRC (52, 78 years old) OC (45 years old), pancreatic cancer (78 years old)			Rohlin et al.
p.N363K	II.11 (♀)	OC and EC (45 years old)			Rohlin et al.
p.N363K	III.2 (♂)	CRC (35 years old)			Rohlin et al.
p.N363K	III.4 (♀)	2xCRC (47, 53 years old)	Prophylactic colectomy (polyposis)		Rohlin et al.
p.N363K	III.6 (♂)	CRC (55 years old)			Rohlin et al.
p.N363K	III.9 (♂)	CRC (40 years old)			Rohlin et al.
p.N363K	III.10 (♂)	giant cell glioblastoma (28 years old)			Rohlin et al.
p.N363K	IV.2 (♂)	CRC (38 years old)			Rohlin et al.
p.N363K	IV.3 (♂)	–	AP (28 years old)		Rohlin et al.
	I.1 (♂)	CRC (82 years old)			Current study
	II.7 (♀)	Kidney cancer (31 years old)			Current study
	II.8 (♀)	Colonic (62 years old) and Rectal (40 years old) cancer, EC (40 years old)			Current study
WT	II.9 (♂)	Hodgkin disease (73 years old)			Current study
p.N363K	II.10 (♂)	Rectal cancer (67)	Colonic polyposis		Current study
	II.11 (♀)	OC (died at 42 years old)			Current study
WT	II.12 (♀)	Mesenteric and retroperitoneal non-Hodgkin Lymphoma B type with giant cells			Current study
p.N363K	III.3 (♂)	CRC (41 years old) Glioblastoma (45 years old)	No	MSS and MLH1+/MSH2+ (for both glioblastoma and caecal adenocarcinoma)	Current study
p.N363K	III.4 (♀)	Rectal adenocarcinoma (37 years old), glioblastoma (52 years old)	5 AP, 1 gastric glandulocystic adenoma	MSS MLH1+/MSH2+/MSH6+ (rectal cancer)	Current study
p.N363K	III.5 (♂)	CRC (52 years old) prostatic adenocarcinoma (51 years old)	5 Colonic polyps (tubular)	MSS and MLH1+/MSH2+/MSH6+	Current study
	III.8 (♂)	Small intestine adenocarcinoma with multiple brain metastases (34 years old)	19 Polyps (33 years old)		Current study
WT	III.9 (♂)	Rectal non-Hodgkin lymphoma B type with giant cells (45 years old)	No		Current study
p.N363K	III.10 (♂)	– (52 years old)	Colonic polyps		Current study
p.N363K	III.11 (♀)	– (50 years old)	Colonic polyps		Current study
p.N363K	III.16 (♂)	Synchronous duodenal adenocarcinoma and CRC (52 years old)	Colonic polyps (19 years old)	MSS	Current study
p.N363K	III.17 (♀)	– (> 50 years old)	6 Colonic polyps—tubulovillous with low grade dysplasia		Current study

Table 1 (continued)

POLE protein change	Case (gender)	Tumours (age at diagnosis)	Polyps (localisation)	MSI status and somatic IHC	Publication
p.N363K	III.18 (♂)	Duodenal adenocarcinoma (48 years old) and CRC (57 years old)		MLH1+/MSH2+ (colon)	Current study
	IV.3 (♀)	–	Colonic polyposis (22 years old) with > 20polyps (tubular with low grade dysplasia)		Current study
p.N363K	IV.5 (♂)	Gastric carcinoma (36 years old)	Mixed polyposis (> 50 colonic polyps), rectal intraepithelial adenocarcinoma (26 years old)		Current study
p.N363K	IV.15 (♀)	CRC (23 years old) Glioblastoma (30 years old)	3 Colonic polyps, a cancerous sigmoid polyp pT1sm1	MSS MLH1+/MSH2+/MSH6- / PMS2+ (checked twice)	Current study
	IV.19 (♂)	Glioblastoma (37 years old)			Current study

AP adenomatous polyp; *CRC* colorectal cancer; *EC* endometrial cancer; *OC* ovarian cancer; *MSI* microsatellite instability; *MSS* microsatellite stability; *WT* not carrier; ♀: female ; ♂: male; *IHC* immune-histochemical profile (+: conserved/− extinction of protein expression)

diagnosed at 37 years of age. His father (III:18) is a carrier of the familial mutation.

Glioblastoma pathology

Glioblastoma biopsies were performed, before initiating any treatment, for patient III:3, patient III:4, patient IV:19 and patient IV:15. Pathological characteristics of these glioblastoma biopsies are summarized in Table 2 and their histological appearances are illustrated in Fig. 2. All were giant cell glioblastomas.

Discussion

Glioblastoma is the most frequent primary brain tumour. In European and North American countries, glioblastoma incidence is approximately 3 cases per 100,000 people per year [7, 8].

Table 2 Summary of pathological characteristics glioblastoma tissue of patient III:3, III:4, IV:15 and IV:19

Cases	III:3	III:4	IV:19	IV:15
Age at diagnosis	45 years old	52 years old	37 years old	30 years old
Giant cells	Multinucleated cells are angulated and large. They can contain from a few nuclei up to10. These nuclei can be prominent with cytoplasmic inclusion			
% of giant cells	60	70	50	20
Histological patterns	Giants cells Small poorly differentiated cells with nuclear atypia and atypical mitoses			
Neovascularization	Microvascular proliferation with multi-layered mitotically active endothelial cells			
Lymphocytic-style infiltration	Few perivascular lymphocytic cuffing			No perivascular cuffing
Necrosis	Ischemic and palisading necrosis			Necrosis
IHC				
IDH1(R132H)	–	–	–	–
P53	70%	90%	80%	70%
OLIG2	55%	60%	50%	60%
Hypermutated phenotype	Not available—not performed			
Anatomical location	Right frontal lobe	Right temporal lobe	Left temporal lobe	Right pedunculo thalamo-pituitary location, including caudate nucleus
Maximum Ki-67 index	30%	30%	30%	80%

IHC immuno-histo-chemistry; −: loss of protein expression

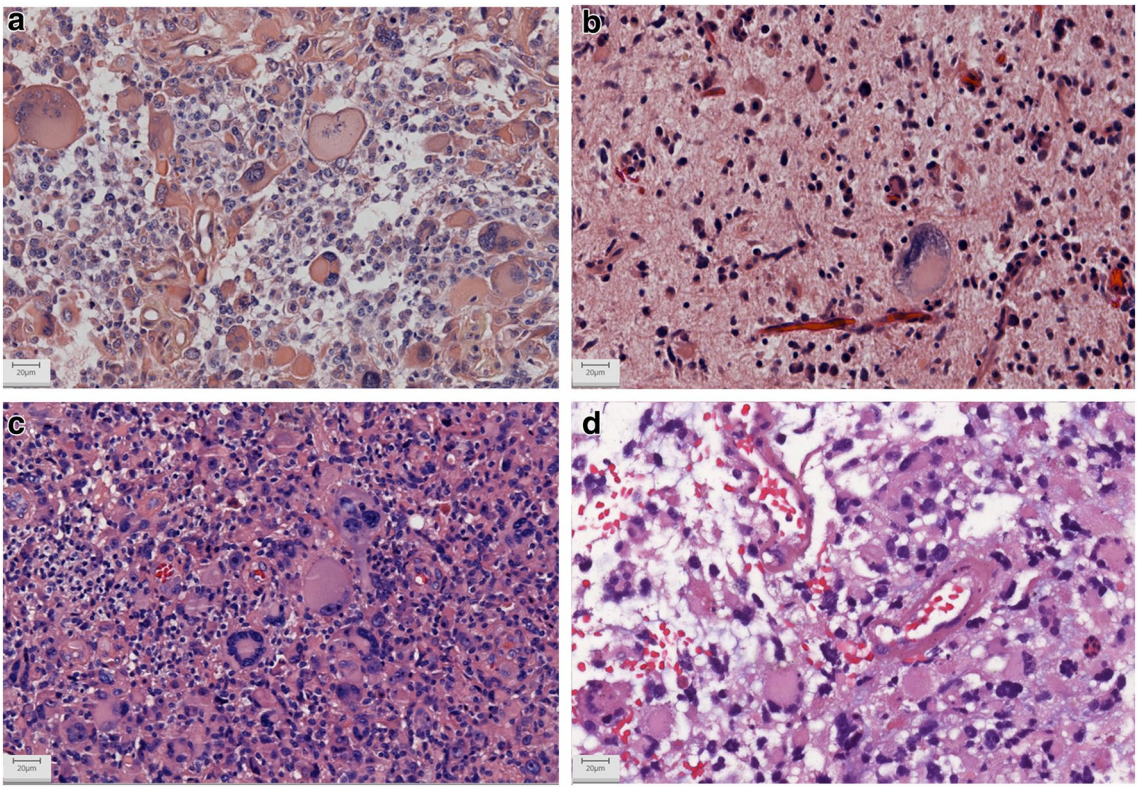

Fig. 2 Resected giant cell glioblastomas of patient III:3 (1.c), III:4 (1.b), IV:19(1.a) and IV:15 (1.d) stained with haematoxylin and eosin (H&E). These reveal numerous multinucleated giant cells with atypical mitoses. Histological descriptions are summarized in Table 2

In *POLE*, only the germline mutation p.Leu424Val was associated with glioblastoma with an incidence ranging from 4% [2] to 6.4% [9].

The current family presents an unusually high frequency of early onset glioblastoma, which occurred in three mutation carriers (III:4, III:3 and IV:15) and a suspected carrier (IV:19) who had a high probability (50%) of carrying the familial mutation via paternal transmission.

Rohlin et al. reported, in a Norwegian family, a patient displaying a glioblastoma of early onset (at age 28 years) who carried the same mutation [4]. His grandfather developed a CRC and a glioma at 65 and 68 years of age respectively. Of his seven children, five were carriers of a *POLE* familial mutation. Even though the mutation status was not determined for this person, we can therefore hypothesise that he is most likely the transmitter of the familial mutation [4].

Compiling information from the two families, *POLE* mutation p.Asn363Lys appears to be associated with a high rate of primary grade IV glioma with a relative risk of glioblastoma of at least 17.4% (4/23) in the carrier population. Glioblastoma incidence in carriers of the p.Asn363Lys mutation seems to be more than twice as high as in families with a p.Leu424Val mutation, or any other mutation of the proofreading exonuclease domain of *POLE*.

We can put forward three hypotheses to explain these findings. Firstly, the incidence of glioblastoma associated with mutations of the exonuclease domain of the polymerase ε may have been overlooked or underestimated in previous studies. Secondly, the p.Asn363Lys *POLE* mutation may be associated with a higher relative risk (20%) of developing glioblastoma than the other *POLE* mutations. Thirdly, the increased incidence of glioblastoma could be explained, in our family, by synergistic mutations located in unexplored genes, completely segregating with the familial *POLE* mutation.

Tumour tissue from patients (III:3, III:4, IV:19, IV:15) revealed giant cell glioblastoma histology with a high proportion of multinucleated giant cells, a rare variant which account for only 2–5% of glioblastomas and which was previously associated with somatic mutation of *POLE* [10]. The occurrence of this rare histological type in all four of our patients' glioblastomas in the same family allows us to propose a link between germline *POLE* mutation and the occurrence of glioblastoma with giant cell morphology. A hypermutated tumour phenotype has been described in glioblastoma tissue from a *POLE* germline mutation carrier [11] and in a case of glioblastoma bearing a somatic *POLE* mutation (a patient without a germline *POLE* defect) [10]. Unfortunately, the rate of glioblastoma mutations in patients

III:3, III:6, IV:15, IV:19 has not been tested in the context of medical care, so we cannot provide this information.

Among the *POLE* mutated population of the two families the incidence of colorectal cancer was particularly high, 69.5% (16/23) with a mean age at diagnosis of 45 (ranging from 23 to 67). These findings are consistent with previous observations [2]. There are colorectal follow-up recommendations for carriers of the *POLE* germline mutation [9], but no recommendation relating to the increased susceptibility to brain tumours has been proposed so far. The clinical history of glioblastoma is usually short (< 3 months in > 50% of patients), so the benefit of neurological follow up with MRI imaging for *POLE* mutation carriers should be carefully assessed. Given the short clinical history of such tumours, enhancing patient vigilance to early symptoms (unusual headaches, sensory or motor problems) would not help to improve their early care and would be stressful for patients and their families.

We report the second family in the literature bearing the *POLE* c.1089C>A, p.Asn363Lys mutation. Carriers have an increased risk of brain cancer, with an estimated incidence of at least 17.4% and a colorectal cancer risk of 69.5%. Even though these results could be improved with more patients, with functional testing and glioblastoma sequencing (to assess mutational burden and second hit of POLE), practitioners treating patients bearing such a mutation should be informed about this increased relative risk of high grade glioma so that they can adapt patient management and follow up accordingly.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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