ELECTRONIC LETTER

STK11 status and intussusception risk in Peutz-Jeghers syndrome

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Background: Peutz-Jeghers syndrome (PJS) is caused by germline *STK11* mutations and characterised by gastrointestinal polyposis. Although small bowel intussusception is a recognised complication of PJS, risk varies between patients.

Objective: To analyse the time to onset of intussusception in a large series of PJS probands.

Methods: *STK11* mutation status was evaluated in 225 PJS probands and medical histories of the patients reviewed.

Results: 135 (60%) of the probands possessed a germline *STK11* mutation; 109 (48%) probands had a history of intussusception at a median age of 15.0 years but with wide variability (range 3.7 to 45.4 years). Median time to onset of intussusception was not significantly different between those with identified mutations and those with no mutation detected, at 14.7 years and 16.4 years, respectively (log-rank test of difference, $\chi^2=0.58$, with 1df; $p=0.45$). Similarly no differences were observed between patient groups on the basis of the type or site of *STK11* mutation.

Conclusions: The risk of intussusception in PJS is not influenced by *STK11* mutation status.

Peutz-Jeghers syndrome (PJS; MIM 175200[1]) is an autosomal dominantly inherited disorder characterised by mucocutaneous pigmentation and gastrointestinal hamartomas and is associated with considerable morbidity mainly from intussusception of gastrointestinal harmatomas.[2]

Germline mutations in the serine/threonine kinase gene (*STK11*) on chromosome 19p13.3 cause PJS.[3 4] Loss of the wild type allele in harmatomas and adenocarcinomas developing in patients with PJS suggests that STK11 is a tumour suppressor.[5] Depending on the patient population studied and the analytical technique employed, a causative germline mutation in *STK11* is identified in 30% to 80% of PJS patients.[6–12] Most mutations are small deletions/insertions or single base substitutions resulting in an abnormal truncated protein with the consequent loss of kinase activity. Failure to identify mutations in all PJS patients and the report of a possible second disease locus[13] has fuelled debate over whether allelic or genetic heterogeneity is responsible for PJS in which no *STK11* mutation can be detected.

Clinical manifestations differ considerably between PJS patients, motivating researchers to examine the possibility of a relation between genotype and phenotype. Over 90% of patients with PJS develop significant harmartomatous polyps of the small bowel, and polyps commonly arise within the stomach and colorectum.[2] These polyps can cause severe abdominal pain and self limited intussusception, occasionally resulting in bowel obstruction and gastrointestinal haemorrhage, which can be the presenting feature of the disease.[2] While gastrointestinal symptoms typically present in the early part of the second decade of life, there is considerable variation in time to onset.[14] Predicting the risk and onset of intussusception in patients with PJS is therefore important in disease management.

Here we report the relation between *STK11* mutation status and the site, type, and time to onset of intussusception in a series of 225 PJS probands.

METHODS

Clinical data on PJS patients in whom *STK11* had been analysed were collected from participating centres within Europe, Australia, and the USA (Institute of Cancer Research, UK; St Mark's Polyposis Registry, INSERM U343, Marseille, France; Erasmus MC University Medical Centre, Rotterdam, The Netherlands; VU University Medical Centre, Amsterdam, Netherlands; Institute of Human Genetics and Anthropology, University of Düsseldorf, Düsseldorf, Germany; Hunter Family Cancer Service and NSW & ACT Hereditary Cancer Registries, New South Wales, Australia; Mayo Clinic, Minnesota, USA; and The Johns Hopkins University School of Medicine, Baltimore, USA. The study was carried out with ethical review board approval from the relevant authority in each country, in accordance with the tenets of the declaration of Helsinki.

All probands included in the study fulfilled the established criteria for a diagnosis of PJS.[2] Ascertainment and collection of probands was solely systematic, with no selection for medical history. For each patient the following data were obtained: sex, date of birth, diagnosis of PJS, family history of PJS, and diagnosis of intussusception.

Several different techniques were used by each of the centres to identify germline *STK11* mutations—conformational sensitive gel electrophoresis (CSGE), single strand conformational polymorphism (SSCP), denaturing high performance liquid chromatography (DHPLC), denaturing gradient gel electrophoresis (DGGE), and direct sequencing of exons. Two centres (ICR and the Mayo Clinic) additionally made use of long range PCR, and two (ICR and the VU Medical Centre) used multiplex ligation-dependent probe amplification (MPLA) to screen for large scale gene deletions. Probands were classified as carriers if they or a family

Abbreviations: PJS, Peutz-Jeghers syndrome; STK11, serine/threonine protein kinase 11

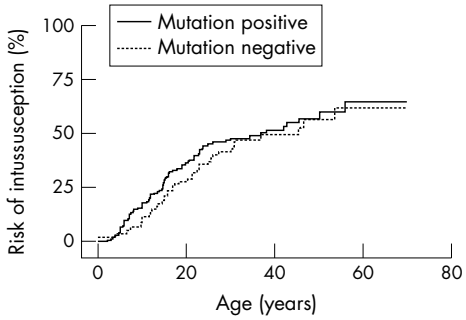

Figure 1 Time to onset of first interssusception in PJS patients stratified by *STK11* mutation status.

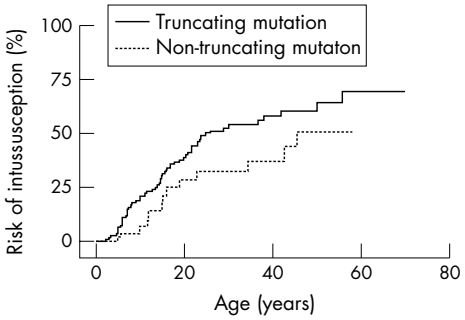

Figure 2 Time to onset of first intussusception in PJS patients stratified by type of *STK11* mutation.

member with PJS had a fully characterised germline *STK11* mutation. In addition, patients in whom no mutation could be demonstrated but who were from a family linked to chromosome 19p13.4 were also considered to be carriers of a germline mutation in *STK11*.

Nucleotide changes identified were coded according to the published sequence of *STK11* (GenBank accession numbers: exon 1, AF032984; exons 2–8, AF032985; exon 9, AF032986), and mutations according to the Human Genome Variation Society (http://www.hgvs.org/mutnomen). STK11 protein sequences of *Homo sapiens* (GenBank accession No NP 000446), *Mus musculus* (NP 035622), and *XEEK1* (Q91604) were obtained from the National Center for Biotechnology Information (NCBI) protein database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = Protein). Alignments were made using the ClustalW (1.82) multiple sequence alignment program (http://www.ebi.ac.uk/clustalw/).

STATA version 8 (Stata Corporation, College Station, Texas, USA; http://www.stata.com) was used to carry out Kaplan–Meier analysis on the data to characterise the time of onset of intussusception. Differences in a time of onset of intussusception between sex, familial, sporadic, and *STK11* mutation status were made using the log-rank test. A probability (p) value less that 0.05 was considered significant.

RESULTS

In all, 225 unrelated PJS probands (101 male and 124 female) were available for analysis; 132 probands had a documented family history of PJS. Germline mutations in *STK11* had been established in 135 patients (60%). The rate of mutation detection varied between different centres (21% to 100%), with detection higher in those centres that had employed multiple analytical methods.

Mutations were scattered throughout the gene, but no mutation in exon 9 was identified in any of the patients. Over 85% of both truncating and missense mutations localised to regions of *STK11* encoding the kinase domain of the expressed protein. Eighty five of the germline *STK11* mutations (83%) identified represent unique sequence changes. Sixty one of the mutations resulted in the truncation of the protein by the creation of premature transcription termination signals, 16 occurred within highly conserved splice sites, four were in-frame deletions predicted to lead to loss of kinase activity, and 15 were missense mutations. All of the missense mutations led to non-conservative amino acid changes that altered amino acids highly conserved in evolution between human, mouse, and *Xenopus* homologues of *STK11* and resided within the kinase domain of the protein encoded by exons 1–8. Other mutations included one large scale genomic deletion and six exonic deletions. Seven families had uncharacterised mutations.

One hundred and nine of the PJS probands (48%) had a history of one or more episodes of intussusception. The median time to first reported intussusception was 15.0 years (mean 17.7; SD 12.0). There was no significant difference in time to onset of intussusception according to the sex of the proband or whether the case had a family history of the disease (log-rank test of difference $\chi^2 = 0.28$ with 1df, $p = 0.59$; and $\chi^2 = 2.14$ with 1df, $p = 0.14$, respectively).

The time to onset of intussusception in probands with *STK11* mutations and those in whom no mutation was detected was similar (fig 1). Median times to onset were 14.7 years (mean 16.9, SD 11.9) and 16.4 years (mean 19.1, SD 12.0), respectively (log-rank test of difference $\chi^2 = 0.58$ with 1df, $p = 0.45$).

Probands with truncating mutations tended to have an earlier age of onset than those harbouring non-truncating mutations (fig 2), but the difference was not statistically significant. Median age to onset 14.5 years (mean 16.4; SD 11.7) and 15.6 years (mean 20.8; SD 13.1) in the two groups, respectively (log-rank test of difference $\chi^2 = 2.63$ with 1df; $p = 0.11$).

To examine whether the site of germline mutation influenced time to onset of intussusception, the different mutation types were analysed separately. We grouped mutations according to their site within the functional domains of the expressed protein; specifically, the N-terminal domain (amino acids 1–48), the kinase domains I-IVA responsible for ATP binding and the site of catalysis (amino acids 49–171), the kinase domains VIB-VIII responsible for substrate recognition (amino acids 172–225), the kinase domains IX-XI (amino acids 226–309), and the C-terminal domain (amino acids 310–433). Stratifying truncating and non-truncating mutations by site within these functional domains of the expressed protein did not influence age of onset of intussusception (log rank tests $\chi^2 = 5.83$ with 4df, $p = 0.21$; and $\chi^2 = 4.37$ with 4df, $p = 0.36$, respectively).

DISCUSSION

Data were not available to fully characterise the pattern and timing of intussusception in patients with more than one episode, but this is an important area of investigation for improving the management of those individuals with early onset intussusception. The current investigation, however, did not show any relation between *STK11* mutational status and risk or time of onset of intussusception. Furthermore, mutation site and type of *STK11* mutation also had no significant influence on presentation. Our results do not therefore confirm a previous report based on a small number of PJS patients (42 probands and a historical cohort of 51 affected individuals)[14] that found that individuals with missense mutations in *STK11* typically had a later time to onset of first polypectomy and other symptoms compared with patients with truncating mutations or no detectable

mutation. Importantly several of the clinical end points evaluated by Amos *et al*[14] are difficult to evaluate. For example, unless all patients had endoscopic surveillance, then data on the development and detection of polyps would be inaccurate. Hence, we chose to only examine intussusception as a marker of disease severity, as this phenotype requires medical intervention and therefore is well documented in the medical records.

The notion of genetic heterogeneity in PJS is founded on the observation that mutations in *STK11* cannot always be identified in PJS patients and on evidence of genetic linkage of a PJS phenotype to 19q14 in a single family.[13] Detection of *STK11* mutations in PJS has been primarily through conventional polymerase chain reaction (PCR) based systems, which have sensitivities of approximately 70%.[15] If the number of patients having PCR detectable mutations is adjusted accordingly and the relatively high prevalence of exonic *STK11* deletions in PJS[16–18] is taken into account, almost all cases of the syndrome can be reconciled by *STK11* mutations, thereby strongly questioning the existence of locus heterogeneity. Identifying a mutation for PJS is clearly highly desirable for the management of families with the syndrome and for predictive testing. Our data, however, suggest that since *STK11* status does not appear to significantly affect the risk of intussusception, such information does not provide a rationale for the differential management of bona fide PJS patients to avoid this clinical event.

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