

Review

Multilocus Inherited Neoplasia Alleles Syndrome

A Case Series and Review

James Whitworth, MRCP; Anne-Bine Skytte, MD; Lone Sunde, PhD, MD; Derek H. Lim, MRCPCH; Mark J. Arends, PhD, FRCP; Lisa Happerfield, FIBMS, MSc; Ian M. Frayling, PhD, FRCP; Rick van Minkelen, PhD; Emma R. Woodward, PhD, MRCP; Marc D. Tischkowitz, PhD, FRCP; Eamonn R. Maher, FRCP, FMedSci

Mendelian causes of inherited cancer susceptibility are mostly rare and characterized by variable expression and incomplete penetrance. Phenotypic variability may result from a range of causes including locus heterogeneity, allelic heterogeneity, genetic and environmental modifier effects, or chance. Another potential cause is the presence of 2 or more inherited cancer predisposition alleles in the same individual. Although the frequency of such occurrences might be predicted to be low, such cases have probably been underascertained because standard clinical practice has been to test candidate inherited cancer genes sequentially until a pathogenic mutation is detected. However, recent advances in next-generation sequencing technologies now provide the opportunity to perform simultaneous parallel testing of large numbers of inherited cancer genes. Herein we provide examples of patients who harbor pathogenic mutations in multiple inherited cancer genes and review previously published examples to illustrate the complex genotype-phenotype relationships in these cases. We suggest that clinicians should proactively consider the likelihood of this phenomenon (referred to herein as multilocus inherited neoplasia alleles syndrome [MINAS]) in patients with unusual inherited cancer syndrome phenotypes. To facilitate the clinical management of novel cases of MINAS, we have established a database to collect information on what is likely to be an increasingly recognized cohort of such individuals.

JAMA Oncol. doi:10.1001/jamaoncol.2015.4771
Published online December 10, 2015.

 Supplemental content at jamaoncol.com

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Eamonn R. Maher, FRCP, FMedSci, Department of Medical Genetics, University of Cambridge, Cambridge Biomedical Campus, Addenbrooke's Treatment Centre, Cambridge CB2 2QQ, England (erm1000@medschl.cam.ac.uk).

In clinical practice the maxim of Occam's razor is often adopted,¹ such that, whenever possible, a single diagnosis is favored over multiple diagnoses. Rare diseases have a frequency of less than 1 in 2000,² and statistically the chances of an individual being affected by 2 or more rare diseases would seem to be remote. However, with more than 6000 rare diseases and up to 6% to 8% of the European population estimated to experience a rare disease at some time in their lifetime,² there is potential for 2 or more rare disorders to occur by chance. This scenario has been reported in various constitutional disorders with both distinct and overlapping phenotypes, including familial neoplasia and/or patients with multiple primary tumors. If Occam's razor is applied, then the detection of a mutation in a specific inherited cancer gene might lead the clinician to attribute any tumors that are not typical features of the relevant inherited cancer syndrome to examples of variable phenotypic expression or coincidence. In such circumstances, the patient may receive suboptimal treatment and the estimated cancer risks to relatives could be erroneous. In addition, studies of patients harboring multiple mutations in different familial cancer syndrome genes could provide insights into how the function of the relevant gene products may be related, for example, if a particular combination resulted in a more pronounced or novel phenotype (analogous to the differences in phenotype between patients with monoallelic and biallelic mismatch repair gene mutations³). The best-known

examples of patients with multiple inherited cancer gene mutations are reports of patients with mutations in *BRCA1* and *BRCA2*.⁴⁻²² Interestingly, the phenotype in these patients has generally not been shown to be more severe than when a single mutation is present.

Herein we report 5 new cases with multiple pathogenic germline mutations in rare inherited cancer syndrome genes. Three involve the combination of mutations in *FLCN* with *NF1*, *TP53*, and *MSH2*, respectively, 1 in *MLH1* and *XPA*, and the fifth in *NF1* and *BRCA2*. In addition, we reviewed the published literature to identify other cases and provide a summary of the published experience to date. We suggest that this phenomenon will be increasingly recognized and careful descriptions of such cases will inform the treatment of similar patients.

Reports of Cases

Written informed consent was obtained for all case reports described herein.

Case 1

A 39-year-old man presented with testicular seminoma, and a routine abdominal scan 4 years later revealed a pheochromocytoma. Following his seminoma diagnosis, he also developed a pneumo-

thorax and went on to have 6 further occurrences. At age 55 years he complained of abdominal/back pain, and a computed tomographic scan revealed bilateral renal masses that were demonstrated to be renal cell carcinomas (RCCs) following removal. Reinvestigation following further episodes of abdominal pain identified 2 gastrointestinal stromal tumors (GISTs). At age 56 years, a computed tomographic lung scan (to investigate a pneumothorax) revealed a malignant peripheral nerve sheath tumor (MPNT). Skin examination revealed multiple skin neurofibromas, 2 café-au-lait patches, and axillary freckling but no fibrofolliculomas. A clinical diagnosis of neurofibromatosis type 1 (NF1) was made, and although this was considered to be the cause of his MPNT and possibly pheochromocytoma and GIST, the history of renal cancers and recurrent pneumothorax were considered unrelated.

Next-generation sequencing of 94 inherited cancer genes was performed using the Illumina TruSight cancer panel.²³ A previously reported splice site mutation in *FLCN* (c.1062 + 2T>G)^{24,25} and a nonsense mutation in *NF1* (c.1381C>T p.[Arg461*]) were detected and confirmed by means of Sanger sequencing. *FLCN* mutations cause Birt-Hogg-Dube (BHD) syndrome, a rare condition in which affected individuals are predisposed to RCC, pulmonary cysts and pneumothoraces, and fibrofolliculomas. A first-degree relative had also received a diagnosis of bilateral chromophobe RCCs at age 45 years and was found to have facial fibrofolliculomas but did not undergo genetic testing. Presence of the *FLCN* mutation was demonstrated in a further first- and second-degree relative, but both were asymptomatic with normal renal scan results. It was also identified in a second-degree relative with numerous fibrofolliculomas and a history of recurrent pneumothorax (2 occurrences). An obligate carrier in the family had pancreatic adenocarcinoma but was not known to have features of BHD syndrome during life, although autopsy revealed bilateral renal oncocytomas. There was no known family history of NF1.

Neurofibromatosis type 1 has a population frequency of 23 per 100 000²⁶ and might be expected to exist in combination with another inherited cancer syndrome only rarely, although phenotypic variability and use of clinical diagnostic criteria (rather than genetic testing) may underestimate this. It is associated with predisposition to a variety of neoplasms including pheochromocytoma, GIST, carcinoid tumor, cutaneous/plexiform neurofibromas, and MPNT. Thus, in this case associated with 2 pathogenic inherited cancer syndrome gene mutations, the occurrence of the MPNT, pheochromocytoma, GIST, and RCC can be explained but testicular seminoma has not been associated with mutations in either gene.^{27,28} This suggests that the seminoma might be a consequence of the combination of *FLCN* and *NF1* mutations (seminoma has been linked to aberrations in the c-kit, RAS/mitogen-activated protein kinase [MAPK], and phosphatidylinositol 3-kinase [PI3K]/Akt pathways,²⁹ and the *NF1* and *FLCN* gene products regulate RAS/MAPK and mammalian target of rapamycin [mTOR]/PI3K/Akt signaling, respectively^{29,30}) or be coincidental, testicular being the most common solid tumor among men aged 15 to 34 years.³¹

Case 2

A 32-year-old man presented with dysphagia. He had a history of ulcerative colitis for which he had undergone a panproctocolectomy at age 27 years; pathological examination of the colectomy specimen had revealed an incidental rectal adenocarcinoma. Endos-

At a Glance

- The identification of a constitutional deleterious variant at more than 1 locus associated with inherited neoplasia has substantial potential implications for cancer risks, but these are not readily predictable.
- We propose the term *multilocus inherited neoplasia alleles syndrome* (MINAS) to describe such cases, which are expected to become more frequently reported with increased clinical application of next-generation sequencing.
- Five new MINAS cases are described, and a systematic literature review was undertaken, revealing 82 reported cases involving 17 genes.
- Deleterious variants appeared to act independently in many cases, although no consistent effect was observed.
- To facilitate accurate information as to the effect of particular deleterious variant combinations, cases have been uploaded to a public database (phenotypic term "MINAS") and further cases are invited.

copy revealed a gastroesophageal junction adenocarcinoma, and staging imaging demonstrated a 6-cm left kidney tumor. Biopsy of the latter suggested a primary renal neoplasm, prompting nephrectomy. Histologic analysis of the resected kidney confirmed a chromophobe RCC. Examination of the skin showed facial fibrofolliculomas. There was no history of cancer in first-degree relatives, but tumors in second-degree relatives included esophageal squamous cell carcinoma at age 54 years, a brain tumor of uncertain histologic subtype at 50 years, and an oropharyngeal carcinoma at 49 years.

Genetic investigations revealed 2 pathogenic mutations in *FLCN* (c.715C>T p.[Arg239Cys])³² and *TP53* (c.526T>C p.[Cys176Arg]). The latter has been reported as a somatic mutation on multiple occasions,^{33,34} including in colorectal adenocarcinoma,³³ but not previously in germline samples.³⁴ It is rare and does not appear in the ExAC data set.³⁵ In silico tools predict a damaging or function-altering effect.³⁶⁻³⁸ No other family members were available for genetic testing.

Kidney tumors, typically with a hybrid chromophobe/oncocytic RCC histopathologic profile, are a major feature of BHD syndrome. Renal cell carcinoma has been reported in *TP53* mutation carriers, although no firm association been made.³⁹ The relationship between colorectal cancer and BHD syndrome is controversial,^{24,40} but an increased risk of colorectal cancer has been reported with ulcerative colitis (although typically in those with disease for >10 years⁴¹) and also in *TP53* mutation carriers. To our knowledge, esophageal cancers have not been reported in *FLCN* mutation carriers but have occurred in Li-Fraumeni syndrome families, although again the association with this condition is not clear.^{39,42} We note that the median age at diagnosis of renal tumors in *FLCN* mutation carriers (48 years)²⁵ is older than the age at onset of these tumors in this case.

Case 3

A 53-year-old woman presented with a rectal adenocarcinoma and had a history of spontaneous pneumothorax at age 46 years. Immunohistochemical analysis of the proband's rectal tumor showed no abnormality, but the colon cancer of a relative (who also had multiple pneumothoraces) demonstrated loss of staining of MSH2 and MSH6 proteins. Germline genetic testing in the proband did not detect a

Table. Molecular Analysis of Tumors From Case 4

Tumor	MLH1 IHC	PMS2 IHC	MSI Assessment
Mucinous cecal adenocarcinoma	Loss	Loss	High
Sigmoid colon adenocarcinoma	Loss	Loss	High
Squamous carcinoma (No. 1)	Present	Present	Stable
Squamous carcinoma (No. 2)	Present	Present	Stable
Lentigo maligna	Present	Present	High
Actinic keratosis	Present	Present	High
Squamous carcinoma in actinic keratosis	Present	Present	High

Abbreviations:
IHC, immunohistochemistry;
MSI, microsatellite instability.

pathogenic mismatch repair gene mutation, but a pathogenic *FLCN* mutation (c.1285delC p.[His429Thrfs*39]) was identified. Three siblings had phenotypic similarities to the proband. A sister developed a pneumothorax at age 37 years and had facial fibrofolliculomas. She developed endometrial cancer at 52 years. Genetic testing demonstrated the familial *FLCN* mutation and an *MSH2* truncating mutation (c.892C>T p.[Gln298*]). The twin sister of this individual had pneumothoraces, RCC, and colorectal polyps. She also carried both mutations, as did a brother with facial fibrofolliculomas.

Colorectal and endometrial cancers are characteristic of Lynch syndrome caused by *MSH2* mutations, and the ages at diagnosis seen in this family are typical.⁴³ However, the proband did not carry the pathogenic *MSH2* mutation detected in her siblings and may represent a phenocopy. Also, a role of the *FLCN* mutation in the development of colorectal tumors in the family cannot be excluded.^{24,40} Fibrofolliculomas, RCC, and pneumothoraces are not associated with Lynch syndrome.⁴⁴

Case 4

A male proband presented with a mucinous cecal cancer at age 65 years and a metachronous sigmoid colon cancer in his remaining large bowel at 67 years. One first-degree relative had developed colon cancer at 42 years, but there was no other family history of Lynch syndrome-related tumors. His parents were not knowingly consanguineous but were both from the same small community in India. The proband had received a clinical diagnosis in early childhood of xeroderma pigmentosum (XP). At least 1 other first-degree relative was known to have a similar pattern of skin tumors, but that individual had no internal malignant neoplasms. Neither parent had any reported skin abnormalities. On examination, his sun-exposed skin showed considerable signs of UV damage (eg, severe freckling and loss of pigment), but he had no other features of XP such as neurological or intellectual deficits. His skin tumors over the previous 20 years had included a squamous carcinoma in an actinic keratosis, several seborrheic keratoses, 2 keratoacanthomas/squamous carcinomas, junctional nevi, a squamous carcinoma, and 2 lentigo malignae (pre-malignant melanoma). Immunohistochemical analysis demonstrated loss of MLH1 and PMS2 expression in both colon cancers. Constitutional genetic testing revealed *MLH1* c.306G>T p.(Glu102Asp) (classed as likely pathogenic⁴⁵). Fibroblasts from a skin biopsy were tested for XP, which showed reduced levels of nucleotide excision repair. He therefore did not have mild XP variant as might be expected, but rather had mild variant XP-A, consistent with survival into his 60s. Constitutional genetics analysis revealed a homozygous *XPA* intron 4 splice mutation (c.555 + 8A>G). Molecular analysis of his various tumors is summarized in the Table.

The prevalence of microsatellite instability in skin tumors in XP is unknown. A contribution of the *MLH1* mutation to the dermatological phenotype may be suggested by the presence of microsatellite instability in some of the skin tumors, but the presence of normal MLH1 and PMS2 expression argues against this. Skin tumors are associated with Lynch syndrome but these are characteristically sebaceous in origin, which was not observed in this case.

Case 5

A woman with NF1, having 1 café-au-lait patch, numerous cutaneous neurofibromas, possible Lisch nodules, and an MPNT, received a diagnosis of a ductal breast carcinoma at age 48 years and subsequently went on to develop a cutaneous melanoma at 57 years. Constitutional genetic testing revealed both *NF1* c.6792C>G p.(Tyr2264*) and *BRCA2* c.5213_5216del p.(Thr1738Ilefs*2).⁴⁶ Mutations in both genes can be associated with breast cancer,⁴⁷ but the risk is much higher for *BRCA2*. The breast cancer could be consistent with either syndrome, and no tumor analysis was reported that could help determine which gene was more significant in its initiation.

Having identified 5 cases harboring multilocus inherited neoplasia gene mutations, we proceeded to review the published literature to determine the nature and frequency of similar cases in a systematic fashion. We propose the term *multilocus inherited neoplasia alleles syndrome* (MINAS) to describe this phenomenon.

Literature Survey of Multilocus Inherited Neoplasia Alleles Syndrome

Identification of Cases

To review published cases with MINAS, we undertook a systematic review of the published literature (see eMethods in the Supplement) based on a list of inherited cancer genes (n = 94) (eTable 1 in the Supplement).

Clinical Aspects

We identified 82 cases involving 17 inherited cancer genes^{4-7,20,48-69} (see eTable 2 in the Supplement). The combination of coexisting mutations in *BRCA1/BRCA2*, *BRCA2/TP53*, *BRCA1/MLH1*, and *APC/MLH1* were the only combinations that occurred in more than 1 family. This may reflect ascertainment bias (certain genes are commonly screened for simultaneously), common founder mutations present in specific populations, and the fact that hereditary breast cancer, followed by colorectal cancer, is the most common indication for cancer genetic assessment.⁷⁰ Indeed, 13 patients had a combination of 2 of the 3 Ashkenazi founder *BRCA1* and *BRCA2* mutations.

An interesting aspect of patients with MINAS is whether mutations in particular combinations of genes are associated with a more (or less) severe phenotype (eg, earlier onset of cancer or cancer types not usually seen in individuals with a single mutation). The wide variety of combinations of individual germline mutations means that, with the exception of *BRCA1/BRCA2*, for mutation combinations the information on phenotypic effects is limited.

Leege et al⁸ described 12 cases of combined *BRCA1/BRCA2* mutation and suggested that there was no evidence of increased severity, whereas Heidemann et al⁷ reported 8 cases and suggested that a more severe phenotype was observed in 2. Other combinations of inherited breast cancer genes have been described. For example, a combination of mutations in *BRCA1* and *PALB2* was described in a patient with multifocal breast cancer⁶⁷ (case 25, eTable 2 in the Supplement). Uterine leiomyomas and a meningioma were also diagnosed, but it is impossible to know whether these were related to a specific mutation or were coincidental.

Two reports of germline *BRCA2* and *TP53* mutations were identified.^{53,54} In a mouse model in which the homologues of both of these genes are conditionally knocked out in epithelial tissues (to avoid embryonic lethality), a greater incidence and earlier onset of mammary and skin carcinomas was observed in comparison with mice in which only *Trp53* or *Brca2* was conditionally knocked out (with conditional knockout/wild-type heterozygosity in the other gene), suggesting a synergistic effect in these tissues.⁷¹ Although the mouse model is not directly comparable to the human status, more than 2 cancers had occurred in both cases of *BRCA2/TP53* MINAS, though 1 tumor was within the radiotherapy field and the ages of diagnosis in these cases are not atypical for mutations in either gene.^{39,72}

The second most frequently reported examples of specific MINAS were combinations of genes predisposing to inherited colorectal cancers⁶²⁻⁶⁶ (cases 20-24, eTable 2 in the Supplement). Interestingly, severe phenotypes were noted in 2 patients with *APC/MLH1* mutation combinations, with jejunal cancer seen in 1 case⁶² and accelerated polyp progression in the other.⁶⁵

The phenotypic consequences of MINAS may be easier to interpret when the 2 genes involved are associated with dissimilar and narrow phenotypes. Thus, there are various reports of a *BRCA1/BRCA2* mutation in combination with a mismatch repair gene mutation (see cases 1-4, eTable 2 in the Supplement). In general, these have not demonstrated clear evidence of a synergistic effect of these types of mutations on the severity of the phenotype, although 1 reported case with *BRCA1* and *MLH1* mutations had a severe phenotype involving early-onset bilateral breast cancer, and endometrial, ovarian, and clear-cell renal cancers diagnosed at age 39 years. Both breast tumors showed loss of the wild-type *BRCA1* allele but also showed absent staining of *MLH1* on immunohistochemical analysis and loss of the wild-type *MLH1* allele. This suggests that both germline mutations were significant in breast tumorigenesis in this patient. The high number of tumors and the development of early-onset RCC (not usually associated with *BRCA1* or *MLH1* mutations) suggests a possible synergistic effect.⁴⁹

Reports of other MINAS cases with specific gene combinations are rare. For example, mutations in the phosphatase and tensin homolog gene (*PTEN*), which affect the PI3K/Akt signaling pathway,^{73,74} are reported in combination with mutations in *TP53*,⁵⁹ *APC*,⁶⁰ and *SDHC*,⁶¹ with tumors characteristic of each mutation being observed in all 3 cases. A number of the tumors in the *PTEN/*

TP53 case were not typical of a mutation in either gene, and early onset of colonic polyps and paraganglioma were noted in the other patients. *PTEN* normally acts via Akt to downregulate MDM2 (and therefore increase p53 levels) in addition to its other roles,^{73,74} so this interaction may lead to a more severe phenotype.

A further case of *BRCA1* and *NF1* mutations in a patient with cutaneous features of NF1 and early-onset (35 years) breast cancer has also been described.⁶⁹ Of note is the fact that *NF1* and *BRCA1* are both located on the long arm of chromosome 17. The presence of early-onset breast cancer and NF1 in the patient's mother, along with both mutations being found in the proband, may suggest that the 2 altered genes were *in cis*. Such information has important implications for genetic counseling of families in which multiple mutations are identified although interestingly, the proband's brother, who also had NF1, did not carry the *BRCA1* mutation, suggesting a recombination event in the mother.

A case of MINAS involving *FLCN* and *APC* mutations has been reported.⁵⁶ Typical colonic polyps and a colorectal cancer at age 28 years occurred, as well as recurrent pneumothoraces and facial papules. The features are consistent with an independent mechanism, although the authors suggested that the *FLCN* mutation might have enhanced the tumorigenic process given the observation that somatic *FLCN* mutations frequently occur in (microsatellite unstable) colorectal cancers.⁴⁰

Molecular Genetics Aspects

In theory, insights into the role of individual inherited cancer gene mutations in the pathogenesis of tumor types that are rarely associated with either of the relevant genes (or tumor types associated with both genes) might be derived from loss-of-heterozygosity (LOH) studies (assuming the relevant inherited cancer genes are tumor suppressor genes [TSGs]). Loss-of-heterozygosity analysis, however, can be uninformative if the somatic mutation ("second hit") is a point mutation or promoter methylation of the wild-type allele (ie, no LOH).⁵ For example, LOH analysis of 3 primary breast cancers from a woman with *BRCA1/BRCA2* MINAS demonstrated LOH at *BRCA1* in 1 tumor and at *BRCA2* in the other 2—suggesting that there was no direct interaction between the 2 loci in the tumors. However, in another case report of *BRCA1/BRCA2* MINAS, LOH at both loci was demonstrated in an ovarian cancer from the same patient.¹⁹

Future Perspectives

There are inherent ascertainment biases influencing which MINAS cases are present in the literature, including more frequent analysis of combinations of particular genes, the range of phenotypes referred for testing, and the restriction of analyzed genes to only those most strongly suggested by the tumor history. Availability, or lack thereof, of analysis of certain genes in some centers may also be a factor and is likely to have led to recognition of 3 *FLCN* MINAS cases at our center where this gene is tested frequently. Clinical features such as the skin manifestations of BHD syndrome and NF1 can indicate the need for analysis of specific genes, but increasing use of cancer gene panels or whole-exome/genome sequencing provides the opportunity for a more comprehensive genetic testing strategy and is likely to result in increased recognition of cases of MINAS. Increasing detection will lead to increased demand for accurate information on

whether particular combinations of mutations are likely to result in a particularly severe phenotype (ie, a synergistic interaction) or whether the resulting phenotype is typical of each mutation having an independent effect. Review of previous reports of MINAS reveals that although a more severe phenotype seems likely in some cases, this cannot be concluded in the majority. In utero death from more severe manifestations of mutation combinations may account for some milder phenotypes, but where survival occurs, MINAS may be more likely suspected in severe cases or those with an atypical phenotype. We suggest that as further cases are uncovered by routine multi-gene testing strategies, those cases with a less severe phenotype will be recognized more easily. However, in certain circumstances it may be prudent to expect that a particular combination of mutations might result in a more severe phenotype. Thus, if an individual has mutations in TSGs that map to the same chromosome region, loss of a chromosome (or part of it) harboring the wild-type alleles will result in a tumor homozygous null for both TSGs (this may have occurred in case 2 because *FLCN* and *TP53* map to 17p11.2 and 17p13.1, respectively). Also, if there is a direct relationship between the mechanisms of tumorigenesis of the 2 mutations (eg, *APC* and mismatch repair gene mutations), a more severe phenotype may occur. In addition, 2 gain-of-function mutations in proto-oncogenes might predict a more se-

vere phenotype (although we have not found reports of such cases) because, in contrast to TSGs, an additional event (somatic inactivation of a wild-type allele) is not required to initiate tumorigenesis. As mutation-dependent targeted therapies for the treatment of cancers become a more common option in oncology, the recognition of MINAS and application of tumor analysis to define the most likely driver mutation will become more important.

Conclusions

The optimum resource with which to discern the effects of individually rare mutation combinations and improve future management of patients with MINAS is a reference database containing clinical, genetic, and tumor information. Such information could guide the clinician as to what the effect of each combination of mutations might be. To facilitate sharing of such information, cases can be uploaded to the Leiden Open Variant Database and identified by "MINAS" phenotype (<http://databases.lovd.nl/shared/diseases/O4296>). We hope that other oncology and genetics health care professionals and researchers will contribute their cases in order to increase knowledge of this emerging phenomenon.

ARTICLE INFORMATION

Accepted for Publication: September 21, 2015.

Published Online: December 10, 2015.
doi:10.1001/jamaoncol.2015.4771.

Open Access: This article is published under JAMA Oncology's open access model and is free to read on the day of publication.

Author Affiliations: Department of Medical Genetics, University of Cambridge and Cambridge NIHR Biomedical Research Centre, Cambridge, England (Whitworth, Tischkowitz, Maher); Department of Clinical Genetics, Aarhus University Hospital, Denmark (Skytte, Sunde); West Midlands Regional Genetics Service, Birmingham Women's Hospital NHS Trust, Birmingham, England (Lim); Division of Pathology, University of Edinburgh and Institute for Genetics and Molecular Medicine, Western General Hospital, Edinburgh, Scotland (Arends); Department of Pathology, Addenbrooke's Hospital, Hills Road, Cambridge, England (Happerfield); Institute of Cancer and Genetics, Cardiff University School of Medicine, Wales (Frayling); Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands (van Minkelen); Department of Genomic Medicine, St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, England (Woodward).

Author Contributions: Dr Whitworth had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Whitworth, Skytte, Frayling, Woodward, Tischkowitz, Maher.

Acquisition, analysis, or interpretation of data: Whitworth, Sunde, Lim, Arends, Happerfield, Frayling, van Minkelen, Woodward, Tischkowitz, Maher.

Drafting of the manuscript: Whitworth, Skytte, Frayling, Woodward, Maher.

Critical revision of the manuscript for important intellectual content: Whitworth, Skytte, Sunde, Lim, Arends, Happerfield, Frayling, van Minkelen, Woodward, Tischkowitz.

Obtained funding: Maher.

Administrative, technical, or material support: Skytte, van Minkelen, Tischkowitz.

Study supervision: Skytte, Woodward, Maher.

Conflict of Interest Disclosures: None reported.

Funding/Support: Dr Whitworth is supported by the Cambridge Biomedical Research Campus. Dr Tischkowitz is supported by a European Research Council Researcher Award. Dr Maher is supported by the Cambridge Biomedical Research Campus and a European Research Council researcher award.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank the patients for granting permission to publish this information.

REFERENCES

- Mani N, Slevin N, Hudson A. What three wise men have to say about diagnosis. *BMJ*. 2011;343:d7769.
- EURORDIS. What is a rare disease? 2015. <http://www.eurordis.org/content/what-rare-disease>. Accessed May 14, 2015.
- Bakry D, Aronson M, Durno C, et al. Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. *Eur J Cancer*. 2014;50(5):987-996.
- Augustyn AM, Agostino NM, Namey TL, Nair S, Martino MA. Two patients with germline mutations in both BRCA1 and BRCA2 discovered unintentionally: a case series and discussion of BRCA testing modalities. *Breast Cancer Res Treat*. 2011;129(2):629-634.
- Caldes T, de la Hoya M, Tosar A, et al. A breast cancer family from Spain with germline mutations in both the BRCA1 and BRCA2 genes. *J Med Genet*. 2002;39(8):e44.
- Choi DH, Lee MH, Haffty BG. Double heterozygotes for non-Caucasian families with mutations in BRCA-1 and BRCA-2 genes. *Breast J*. 2006;12(3):216-220.
- Heidemann S, Fischer C, Engel C, et al. Double heterozygosity for mutations in BRCA1 and BRCA2 in German breast cancer patients: implications on test strategies and clinical management. *Breast Cancer Res Treat*. 2012;134(3):1229-1239.
- Leegte B, van der Hout AH, Deffenbaugh AM, et al. Phenotypic expression of double heterozygosity for BRCA1 and BRCA2 germline mutations. *J Med Genet*. 2005;42(3):e20.
- Liede A, Rehal P, Vesprini D, Jack E, Abrahamson J, Narod SA. A breast cancer patient of Scottish descent with germ-line mutations in BRCA1 and BRCA2. *Am J Hum Genet*. 1998;62(6):1543-1544.
- Loubser F, de Villiers JNP, van der Merwe NC. Two double heterozygotes in a South African Afrikaner family: implications for BRCA1 and BRCA2 predictive testing. *Clin Genet*. 2012;82(6):599-600.
- Moslehi R, Russo D, Phelan C, Jack E, Antman K, Narod S. An unaffected individual from a breast/ovarian cancer family with germline mutations in both BRCA1 and BRCA2. *Clin Genet*. 2000;57(1):70-73.
- Musolino A, Naldi N, Michiara M, et al. A breast cancer patient from Italy with germline mutations in both the BRCA1 and BRCA2 genes. *Breast Cancer Res Treat*. 2005;91(2):203-205.
- Noh JM, Choi DH, Nam SJ, et al; Korea Breast Cancer Study Group. Characteristics of double heterozygosity for BRCA1 and BRCA2 germline mutations in Korean breast cancer patients. *Breast Cancer Res Treat*. 2012;131(1):217-222.

14. Pilato B, De Summa S, Danza K, Lambo R, Paradiso A, Tommasi S. Maternal and paternal lineage double heterozygosity alteration in familial breast cancer: a first case report. *Breast Cancer Res Treat*. 2010;124(3):875-878.
15. Smith M, Fawcett S, Sigalas E, et al. Familial breast cancer: double heterozygosity for BRCA1 and BRCA2 mutations with differing phenotypes. *Fam Cancer*. 2008;7(2):119-124.
16. Tesoriero A, Andersen C, Southey M, et al. De novo BRCA1 mutation in a patient with breast cancer and an inherited BRCA2 mutation. *Am J Hum Genet*. 1999;65(2):567-569.
17. Zuradelli M, Peissel B, Manoukian S, et al. Four new cases of double heterozygosity for BRCA1 and BRCA2 gene mutations: clinical, pathological, and family characteristics. *Breast Cancer Res Treat*. 2010;124(1):251-258.
18. Ramus SJ, Friedman LS, Gayther SA, et al. A breast/ovarian cancer patient with germline mutations in both BRCA1 and BRCA2. *Nat Genet*. 1997;15(1):14-15.
19. Randall TC, Bell KA, Rebane BA, Rubin SC, Boyd J. Germline mutations of the BRCA1 and BRCA2 genes in a breast and ovarian cancer patient. *Gynecol Oncol*. 1998;70(3):432-434.
20. Bell DW, Erban J, Sgroi DC, Haber DA. Selective loss of heterozygosity in multiple breast cancers from a carrier of mutations in both BRCA1 and BRCA2. *Cancer Res*. 2002;62(10):2741-2743.
21. Friedman E, Bar-Sade Bruchim R, Kruglikova A, et al. Double heterozygotes for the Ashkenazi founder mutations in BRCA1 and BRCA2 genes. *Am J Hum Genet*. 1998;63(4):1224-1227.
22. Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol*. 2002;20(6):1480-1490.
23. TruSight Cancer Sequencing Panel, Table 1. Illumina website. 2013. http://www.illumina.com/Documents/products/datasheets/datasheet_trusight_cancer.pdf. Accessed November 9, 2015.
24. Toro JR, Wei M-H, Glenn GM, et al. BHD mutations, clinical and molecular genetic investigations of Birt-Hogg-Dubé syndrome: a new series of 50 families and a review of published reports. *J Med Genet*. 2008;45(6):321-331.
25. Schmidt LS, Nickerson ML, Warren MB, et al. Germline BHD-mutation spectrum and phenotype analysis of a large cohort of families with Birt-Hogg-Dubé syndrome. *Am J Hum Genet*. 2005;76(6):1023-1033.
26. Orphanet. Prevalence and incidence of rare diseases: bibliographic data. 2015. http://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf. Accessed November 9, 2015.
27. Friedman J. Neurofibromatosis 1. In: Pagon RA, Adam MP, Bird TD, et al, eds. *GeneReviews*. Seattle, WA: University of Washington; 2012. <http://www.ncbi.nlm.nih.gov/books/NBK1109/>. Accessed July 17, 2014.
28. Toro JR. Birt-Hogg-Dubé syndrome. In: Pagon RA, Adam MP, Bird TD, et al, eds. *GeneReviews*. Seattle, WA: University of Washington; 2008. <http://www.ncbi.nlm.nih.gov/books/NBK1522/>. Accessed July 17, 2014.
29. Duensing A, Medeiros F, McConarty B, et al. Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). *Oncogene*. 2004;23(22):3999-4006.
30. Dowling RJO, Topisirovic I, Fonseca BD, Sonenberg N. Dissecting the role of mTOR: lessons from mTOR inhibitors. *Biochim Biophys Acta*. 2010;1804(3):433-439.
31. Manuel HD, Mitikiri N, Khan M, Hussain A. Testicular germ cell tumors: biology and clinical update. *Curr Opin Oncol*. 2012;24(3):266-271.
32. Woodward ER, Ricketts C, Killick P, et al. Familial non-VHL clear cell (conventional) renal cell carcinoma: clinical features, segregation analysis, and mutation analysis of FLCN. *Clin Cancer Res*. 2008;14(18):5925-5930.
33. cBioPortal for Cancer Genomics. <http://www.cbioportal.org/>. Accessed May 6, 2015.
34. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat*. 2007;28(6):622-629.
35. Exome Aggregation Consortium. ExAC Browser (Beta). <http://exac.broadinstitute.org/>. Accessed June 11, 2015.
36. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4(7):1073-1081.
37. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249.
38. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. *Nucleic Acids Res*. 2011;39(17):e118.
39. Schneider K, Zellek K, Nichols K, Garber J. In: Pagon RA, Adam MP, Bird TD, et al, eds. *Li-Fraumeni Syndrome*. Seattle, WA: University of Washington; 2013. <http://www.ncbi.nlm.nih.gov/books/NBK1311/>. Accessed November 1, 2013.
40. Nahorski MS, Lim DHK, Martin L, et al. Investigation of the Birt-Hogg-Dubé tumour suppressor gene (FLCN) in familial and sporadic colorectal cancer. *J Med Genet*. 2010;47(6):385-390.
41. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut*. 2001;48(4):526-535.
42. University Medical Center Groningen. FaCD online syndrome fact sheet. Name: Li-Fraumeni syndrome. Updated 2010. <http://www.familialcancerdatabase.nl/loggedin/syndromedetails.aspx?SyndromeCode=32>. Accessed May 20, 2015.
43. Stoffel E, Mukherjee B, Raymond VM, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. *Gastroenterology*. 2009;137(5):1621-1627.
44. Kohlmann W, Gruber SB. In: Pagon RA, Adam MP, Bird TD, et al, eds. *Lynch Syndrome*. Seattle, WA: University of Washington; 2014. <http://www.ncbi.nlm.nih.gov/books/NBK1211/>. Accessed July 25, 2014.
45. InSiGHT. Colon cancer gene variant databases: MutL homolog 1 (E.coli) (MLH1). Leiden Open Variation Database website. http://chromium.lodv.nl/LOVD2/colon_cancer/variants.php?select_db=MLH1&action=search_all&search_Variant%2FDNA=c.306G%3ET. Accessed July 25, 2014.
46. Frayling IM, van Minkelen R, Baralle D, et al. Predisposition to breast cancer in NF1 occurs before, but not after, age 50y and is unrelated to NF1 gene mutation type or site (Poster PM12.210). Poster presented at: European Human Genetics Conference; June 2015; Glasgow, Scotland.
47. Sharif S, Moran A, Huson SM, et al. Women with neurofibromatosis 1 are at a moderately increased risk of developing breast cancer and should be considered for early screening. *J Med Genet*. 2007;44(8):481-484.
48. Borg A, Isola J, Chen J, et al. Germline BRCA1 and HMLH1 mutations in a family with male and female breast carcinoma. *Int J Cancer*. 2000;85(6):796-800.
49. Pedroni M, Di Gregorio C, Cortesi L, et al. Double heterozygosity for BRCA1 and hMLH1 gene mutations in a 46-year-old woman with five primary tumors. *Tech Coloproctol*. 2014;18(3):285-289.
50. Kast K, Neuhann TM, Görgens H, et al. Germline truncating-mutations in BRCA1 and MSH6 in a patient with early onset endometrial cancer. *BMC Cancer*. 2012;12:531.
51. Thiffault I, Hamel N, Pal T, et al. Germline truncating mutations in both MSH2 and BRCA2 in a single kindred. *Br J Cancer*. 2004;90(2):483-491.
52. Foppiani L, Forzano F, Ceccherini I, et al. Uncommon association of germline mutations of RET proto-oncogene and CDKN2A gene. *Eur J Endocrinol*. 2008;158(3):417-422.
53. Manoukian S, Peissel B, Pensotti V, et al. Germline mutations of TP53 and BRCA2 genes in breast cancer/sarcoma families. *Eur J Cancer*. 2007;43(3):601-606.
54. Monnerat C, Chompret A, Kannengiesser C, et al. BRCA1, BRCA2, TP53, and CDKN2A germline mutations in patients with breast cancer and cutaneous melanoma. *Fam Cancer*. 2007;6(4):453-461.
55. Ghataorhe P, Kurian AW, Pickart A, et al. A carrier of both MEN1 and BRCA2 mutations: case report and review of the literature. *Cancer Genet Cytogenet*. 2007;179(2):89-92.
56. Kashiwada T, Shimizu H, Tamura K, Seyama K, Horie Y, Mizoo A. Birt-Hogg-Dubé syndrome and familial adenomatous polyposis: an association or a coincidence? *Intern Med*. 2012;51(13):1789-1792.
57. Kilmartin DJ, Mooney DJ, Acheson RW, Payne SJ, Maher ER, Eustace P. von Hippel-Lindau disease and familial polyposis coli in the same family. *Arch Ophthalmol*. 1996;114(10):1294.
58. Mastroianno S, Torlontano M, Scillitani A, et al. Coexistence of multiple endocrine neoplasia type 1 and type 2 in a large Italian family. *Endocrine*. 2011;40(3):481-485.
59. Plon SE, Pirics ML, Nuchtern J, et al. Multiple tumors in a child with germ-line mutations in TP53 and PTEN. *N Engl J Med*. 2008;359(5):537-539.
60. Valle L, Rodríguez-López R, Robledo M, Benítez J, Urioste M. Concurrence of germline mutations in the APC and PTEN genes in a colonic polyposis family member. *J Clin Oncol*. 2004;22(11):2252-2253.
61. Zbuk KM, Patocs A, Shealy A, Sylvester H, Miesfeldt S, Eng C. Germline mutations in PTEN and

- SDHC in a woman with epithelial thyroid cancer and carotid paraganglioma. *Nat Clin Pract Oncol*. 2007;4(10):608-612.
62. Lindor NM, Smyrk TC, Buehler S, et al. Multiple jejunal cancers resulting from combination of germline APC and MLH1 mutations. *Fam Cancer*. 2012;11(4):667-669.
63. Soravia C, DeLozier CD, Dobbie Z, et al. Double frameshift mutations in APC and MSH2 in the same individual. *Int J Colorectal Dis*. 2005;20(5):466-470.
64. Uhrhammer N, Bignon Y-J. Report of a family segregating mutations in both the APC and MSH2 genes: juvenile onset of colorectal cancer in a double heterozygote. *Int J Colorectal Dis*. 2008;23(11):1131-1135.
65. Scheenstra R, Rijcken FEM, Koornstra JJ, et al. Rapidly progressive adenomatous polyposis in a patient with germline mutations in both the APC and MLH1 genes: the worst of two worlds. *Gut*. 2003;52(6):898-899.
66. van Puijbroek M, Nielsen M, Reinards THCM, et al. The natural history of a combined defect in MSH6 and MUTYH in a HNPCC family. *Fam Cancer*. 2007;6(1):43-51.
67. Pern F, Bogdanova N, Schürmann P, et al. Mutation analysis of BRCA1, BRCA2, PALB2 and BRD7 in a hospital-based series of German patients with triple-negative breast cancer. *PLoS One*. 2012;7(10):e47993.
68. Ercolino T, Lai R, Giachè V, et al. Patient affected by neurofibromatosis type 1 and thyroid C-cell hyperplasia harboring pathogenic germ-line mutations in both NF1 and RET genes. *Gene*. 2014;536(2):332-335.
69. Campos B, Balmaña J, Gardenyes J, et al. Germline mutations in NF1 and BRCA1 in a family with neurofibromatosis type 1 and early-onset breast cancer. *Breast Cancer Res Treat*. 2013;139(2):597-602.
70. Wonderling D, Hopwood P, Cull A, et al. A descriptive study of UK cancer genetics services: an emerging clinical response to the new genetics. *Br J Cancer*. 2001;85(2):166-170.
71. Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet*. 2001;29(4):418-425.
72. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*. 2003;72(5):1117-1130.
73. Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44-57.
74. Huang W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. 2009;37(1):1-13.