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, FRCPath; Lisa Happenfield, FIBMS, MSc; Ian M. Frayling, PhD, FRCPath; 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.

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,3 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 mutations5). 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
and phosphatidylinositol 3-kinase [PI3K]/Akt pathways,29 and the among men aged 15 to 34 years.31

even 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 sug-

gests that the seminoma might be a consequence of the combina-

Next-generation sequencing of 94 inherited cancer genes was performed using the Illumina TruSight cancer panel.23 A previously reportedsplice 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 affec-
ted 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 un-
dergo genetic testing. Presence of the FLCN mutation was demon-

Neurofibromatosis type 1 has a population frequency of 23 per 100 00026 and might be expected to exist in combination with an-
other 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 syn-
drome gene mutations, the occurrence of the MPNT and possibly pheochromocytoma and GIST, the history of renal can-

cancers and recurrent pneumothorax were considered unrelated.

Case 2

A 32-year-old man presented with dysphagia. He had a history of ul-
cerative colitis for which he had undergone a panproctocolectomy at age 27 years; pathological examination of the colectomy spec-

Case 3

A 53-year-old woman presented with a rectal adenocarcinoma and had a history of spontaneous pneumothorax at age 46 years. Immuno-

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 nephrec-
tomy. Histologic analysis of the resected kidney confirmed a chro-

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 muta-
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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 a pathogenic 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. Fibrofolliculomas, RCC, and pneumothoraces are not associated with Lynch syndrome, but these are characteristically sebaceous in origin, which was not observed in this case.

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 (see eTable 2 in the Supplement). The combination of coexisting mutations in BRCA1/BRCA2, APC/MLH1, and MMR genes 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. 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 al described 12 cases of combined BRCA1/BRCA2 mutation and suggested that there was no evidence of increased severity, whereas Heidemann et al 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 cancer (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. 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. Although the mouse model is not directly comparable to the human status, more than 2 mutations occurred, as well as recurrent pneumothoraces and facial papules. The features are consistent with an independent mechanism, although the authors suggested that the TP53 mutation might have enhanced the tumorigenic process given the observation that somatic TP53 mutations frequently occur in (microsatellite unstable) colorectal cancers.

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). 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.

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 presence, 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 multigene 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 TPS3 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 severe 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/04296). 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.

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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).

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