Review

Multilocus Inherited Neoplasia Alleles Syndrome
A Case Series and Review

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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.

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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 mutations[3]). 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

pneumothorax 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 100 000[26] 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 panprotocolectomy at age 27 years; pathological examination of the colectomy specimen had revealed an incidental rectal adenocarcinoma. Endoscopy 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 years[41]) 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

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.

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 keratoacanthomata/squamous carcinomas, junctional nevi, a squamous carcinoma, and 2 lentigo malignae (premalignant 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 pathogenic[45]). 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.

Leegte et al[8] described 12 cases of combined *BRCA1/BRCA2* mutation and suggested that there was no evidence of increased severity, whereas Heidemann et al[7] 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[67] (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 cancers[62-66] (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 case[62] 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 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 *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 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 heath care professionals and researchers will contribute their cases in order to increase knowledge of this emerging phenomenon.

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