Multi-locus Inherited Neoplasia Alleles Syndrome (MINAS) with Birt-Hogg-Dube Syndrome (BHG) and Li-Fraumeni Syndrome (LFS): Case

Kashyap Patel; Emma Gillespie; Hirangi Mukhi; Maharshi Patel; Ashima Kodali; Powers Thaddeus Norrell; Resha Kodali; Seeley Spaulding; Ola Jane Gulledge; Dhwani Mehta; Asutosh Gor; Niyati Nathwani; Viral Rabara; Taylor Lavender; Megan Morava; Christian McKenzie Saunders; Chelsey Miller; Naina Patel; Karina Husband; Angela Feasel; Gabriel Hansen; Sandra Nixon; Olivia Wolfe; Adara Hubbert

Background: Multi-locus inherited neoplasia alleles syndrome (MINAS) is a rare genetic syn- drome resulting from pathogenic variants in two or more cancer susceptibility genes' (CSG) loci associated with uncommon phenotypic expression of cancers. It is an extremely rare inherited condition. MINAS is being reported increasingly as the availability of whole exome sequencing (WES) has grown. So far, 385 cases of MINAS have been reported in the literature, and most of these cases (287/385) involved at least one pathogenic variant in either BRCA1 or BRCA2.
108/385 MINAS cases had multiple primary tumors and unusual multiple tumor phenotypes at presentation. Our extensive literature review revealed very few cases of Birt-Hogg-Dube Syn- drome (BHG) and Li Fraumeni syndrome (LFS). To the best of our knowledge, only one case of MINAS involving LFG and BHG has been reported in the literature. We report a patient who de- veloped renal cell carcinoma at a late age and has had multiple recurrences and lesions over the adrenocortical region, as well as in the thyroid and pancreas. She also has had melanoma. We describe (perhaps the second ever reported case) of MINAS with CSGs involving LFS and BHG.

Methods: A 77-year-old woman presented almost 11 years back in November of 2011 (she was 66 then) to the ER with massive hematuria. Her imaging studies revealed arteriovenous malfor- mation in her left kidney leading to profuse bleeding. She ended up requiring left nephrectomy in November of 2011 that revealed renal cell carcinoma (clear cell carcinoma). She underwent routine follow up and surveillance scans. In April of 2014, her CT scan revealed a lesion on her right lung. Her oncologist and urologists decided to place her on an observation schedule. She continued to have slow growth of the lesion in October of 2014 and was advised to undergo sur- gery for removal of the lesion. However, a second opinion recommended the watch and wait ap- proach, and her surgery was canceled. Her follow up scan in January of 2015 revealed continued growth in the lesion. She was recommended surgery to remove the lesion. She sought a third opinion in April of 2015 in Texas, where she underwent CT-guided biopsy that revealed metasta- ses from clear cell carcinoma consistent with the original renal cell carcinoma.

She was started on combination Nivolumab and Ipilimumab. After the second cycle of treatment, she developed severe colitis and an adverse reaction which required hospitalization. She also had immunotherapy- associated pneumonitis and missed four doses of treatment due to a con- tinued adverse reaction. Finally, her oncologist in Texas decided to switch her over to Pazopinib in November of 2015. She continued Pazopinib until December of 2017, when she developed a lesion on her thyroid. Her ultrasound-guided biopsy revealed a neoplastic lesion; it was unknown whether this lesion represented metastasis or a primary thyroid neoplasm. Her oncologist decid- ed to stop Pazopinib and place her on Nivolumab beginning January of 2018. Her follow up imag- ing studies revealed slight progression and hence Axitinb was added to her regimen. She contin- ued on this combination until the summer of 2019, at which point all treatment was stopped due to side effects. She continued to have imaging studies until 2021, when she developed a le- sion over her right adrenal gland (adrenocortical region). She also developed lesions over her liv- er and right kidney. She has had multiple recurrences including lung metastases, liver metasta- ses, a lesion over her thyroid, right adrenal gland, and melanoma. She had brought up issue of suggested the possibility of genetic susceptibility to her oncologists at three different institutions prior to her consultation with Dr. Kashyap Patel. Since her cancer journey did not fit any stereo- typical pattern of familial cancer, she was told that she did not meet the requirements for germline testing. Dr. Patel chose to test her for germline testing for hereditary cancer.

Results: To Dr. Patel's surprise, he discovered that she harbors MINAS in two separate loci: a germline TP53 mutation with possible mosaic zygosity and autosomal dominant inheritance leading to LFS, and a heterozygous germline FLCN mutation with autosomal dominant inher- itance leading to BHG syndrome (Figure 1). We believe that she is the second case reported with MINAS carrying CSG TP53 and FLCN.

Gene & Transcript	Variant Nomenclature	Zygosity	Classification	Disease	Inheritance
TP53 NM_ooo546.5	c.659A>G p.Y22oc	'Possible Mosaic	Pathogenic	Li-Fraumeni Syndrome	Autosomal Dominant
FLCN NM_144997,5	c.1274A>G p.Q425R	Heterozygous (one copy)	Variant Of Uncertain Significance	Birt-Hogg-Dube Syndrome	Autosomal Dominant

Fig 1: Results of Next Generation Germline Sequencing

Discussion: Inherited cancer predisposition is supposedly rare, as a very small number of pa- tients undergo germline testing. There is large phenotypic variability in expression of inherited cancer syndrome. This variability can be explained by factors such as allelic heterogeneity, envi- ronmental effects, or the presence of mutations on two or more inherited cancer genes in the same individual (defined as MINAS). While past literature reports that inherited mutations are thought to play a role in about 5-10 % of all cancers, recent publications, including a large study carried out at Mayo (INTERCEPT), reported the prevalence of pathogenic variants in germline mutations as high as 28% in certain cancers. Combining these reports in literature and additional factors like environmental influences (including the impact of the social determinants of health) there can be can lead to the detection of more than 50hereditary cancer syndromes with differ- ent phenotypic expressions. The standard clinical practice for guidelines in concordant testing has it's its own limitations. After detecting a mutation in a specific gene, the clinician may attrib- ute any tumors that are not typical of the detected syndrome to phenotypic variability. Thus, the patient may receive suboptimal treatment and any risks to relatives might be incorrectly esti- mated go undetected. Next-generation sequencing (NGS) techniques particularly using whole exome sequencing (WES) and whole genome sequencing (WGS) now offer the opportunity of simultaneous testing of large numbers of inherited cancer genes. Studying patients with multi- ple mutations in different cancer syndrome genes could provide insights into how the functions of the relevant genes products may be related and result in an enhanced or novel phenotype.

Reviews of MINAS: A recent study by Whitworth, Skytte, Sunde, et al. (2016)[1] reported five new cases of multiple germline mutations in inherited cancer syndrome genes, three of them involve involving the combination of mutations in FLCN with NF1, TP53, and MSH2, respectively. Whit- worth, et al.l identified 82 cases involving 17 inherited cancer genes [2-5]. The combination of coex- isting mutations in BRCA1/BRCA2, BRCA2/TP53, BRCA1/MLH1, and APC/MLH1 were the only combinations that were reported in multiple family members in their observations and review of literature.

Phenotypic Consequences of MINAS: Whitworth, et al.l proposed that the adverse phenotypic consequences of MINAS could be additive (i.e., the observed cancer risks reflect those of each of the relevant CSGs independent of the presence of the other) or synergistic (i.e., some CSG com- binations could result in notably more severe phenotypes such as earlier age of onset or the oc- currence of tumor types that are atypical for the relevant CSGs. Theoretically MINAS might also be associated with protective effects (e.g. through synthetic lethality) but this would be likely re- quire analysis of healthy control cohorts rather than patients tested through diagnostic laborato- ries. To review the evidence for additive/synergistic effects, McGuigan, Whithworth, Andreou, et al.,[13] subdivided the 385 MINAS cases in the current cohort into those with a BRCA1/BRCA2 MI- NAS combination (n = 206) and those with other combinations of CSGs and then examined pos- sible evidence for additive/synergistic interactions. However, assessing the occurrence of tumor types that are atypical for the relevant CSGs, for non-BRCA1/BRCA2 MINAS combinations, the number of instances of specific CSG combinations was generally very small. 108/385 (28%) MI- NAS cases had multiple primary tumors at presentation. Among the 108 cases, 2 (1.9%) had an unknown number of multiple primaries, 75 (69%) had two primary tumors, 18 (17%) had three and 13 (12%) had four or more. The most common multiple primary tumor combinations were breast-ovarian, with 33 cases; breast-breast, with 24 cases; and colon-colon, with 6 cases.

Phenotypic Associations of Non-BRCA1/BRCA2 MINAS: In the MINAS historical subgroup, it was estimated that 14.6% of patients (13/89) had at least one tumor type that was not typical of the relevant CSGs (e.g. renal clear cell carcinoma in a patient with variants in both BRCA1 and MLH1). However, in the recent cohort an atypical tumor phenotype was present in 15.8% of non-BRCA1/BRCA2 MINAS cases.

Tumor Studies and Mechanisms of Tumorigenesis in MINAS: Multiple primaries occur in asso- ciation with MINAS. However, relevant CSGs have very different tumor associations, and this may be due to an impact of environmental and acquired factors leading to different phenotyp- ic expressions. In cases whereCSGs' overlapping tumor associations may not explain phenotyp- ic variability,the application of tumor studies might provide insights into whether a single CSG or multiple CSGs are implicated in the occurrence of a tumor. As most CSGs follow a tumor suppressor 'two hit model' of tumorigenesis(,) the most readily available strategy for investi- gating mechanisms of tumorigenesis is tumor loss of heterozygosity (LOH) studies (though LOH may not be observed if the somatic inactivating event is a point mutation or promoter methyl- ation of the wild-type allele) [8,9]. Rebbeck, et al.[6], described LOH analysis from 14 informative cancers from BRCA1/BRCA2 MINAS cases and found LOH at a single locus in four tumors, sug- gesting that in most cases, the tumor develops from a second hit at a single CSG and the effects of MINAS are generally additive rather than synergistic. Nevertheless, with increasing awareness of acquired factors triggering oncogenic drivers and mutations, we will need a lot more information from combining whole exome and whole genome sequencing as well as massive amounts of bioinformatics and SDoH data to reach to some type of consensus.

Conclusion: Since the term MINAS was coined 5 years ago, this phenomenon has received in- creasing awareness reflected in the increasing numbers of publications on this topic. There are some limitations to our analysis that would cause us to underestimate the frequency of MI- NAS. Firstly, only a fraction of MINAS cases are likely to be included in the published literature. Secondly, to reliably compare the frequency of MINAS, it is important to include recently iden- tified cancer susceptibility genes, which is not the case in the majority of the publications.
Thirdly, the cases reported to date are predominantly based on diagnostic gene-panel data. Studies based on WES and WGS data would likely yield more cases of MINAS. There is also the MINAS database (https://databases.lovd.nl/shared/diseases/04296) that can be accessed to identify dynamic changes in newer additional data on published and unpublished cases of can- cer combinations. Though in most cases the evidence would appear to suggest that the effects are likely to be additive, reports of some MINAS cases with atypical tumors are a concern (though these cases may be overrepresented because of ascertainment bias). To provide the best prognostic information for individuals with MINAS, long-term follow up and molecular ge- netic analysis of any MINAS-related cancers (e.g. for LOH, cancer signature etc.) should be un- dertaken. In summary, germline testing outside of current guidelines should be offered to all patients with solid tumors, in particular,those patients with abnormal phenotypic expres- sion,to detect true prevalence of MINAS. In another study (in the process of submission), we observed that nearly 59% of patients tested outside of conventional guidelines for germline testing revealed findings with significant implications either for disease or offspring.

References:

1)James Whitworth, MRCP[1]; Anne-Bine Skytte, MD[2]; Lone Sunde, PhD, MD[2]; et al Multilocus Inherited Neoplasia Alleles Syndrome. A Case Series and Review *JAMA Oncol. 2016;2(3):373-379. doi:10.1001/jamaoncol.2015.4771*
2)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.
3)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.*
4)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.PubMedGoogle ScholarCrossref*
5)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.*
6)Rebbeck TR, Friebel TM, Mitra N, Wan F, Chen S, Andrulis IL, et al. Inheritance of deleterious mutations at both BRCA1 and BRCA2 in an international sam- ple of 32,295 women. Breast Cancer Res. 2016;18:112.
7)Breast Cancer Association Consortium, Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, Wahlström C, et al. Breast Cancer Risk Genes — associa- tion analysis in more than 113,000 women. N Engl J Med. 2021;384:428–39.
8)Baylin SB, Jones PA. Epigenetic determinants of cancer. Cold Spring Harbor Perspect Biol. 2016;8:a019505.
9)Thiagalingam S, Laken S, Willson JKV, Markowitz SD, Kinzler KW, Vogelstein B, et al. Mechanisms underlying losses of heterozygosity in human colorectal cancers. Proc Natl Acad Sci U.S.A. 2001;98:2698–702.
10)Kaliyappan K, Palanisamy M, Duraivan J, Govindarajan R. Applications of immunohistochemistry. J Pharm Bioallied Sci. 2012;4:S307–9.
11)Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen H-Z, et al. Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol. 2017;1:PO.17.00073.
12)Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, et al. The repertoire of mutational signatures in human cancer. Nature. 2020;578:94– 101.
13)McGuigan, A., Whitworth, J., Andreou, A. *et al. Multilocus Inherited Neoplasia Allele Syndrome (MINAS): an update. Eur J Hum Genet 30, 265–270 (2022). https://doi.org/10.1038/s41431-021-01013-6*