INTRODUCTION

Base excision repair (BER) is the main mechanism for repairing endogenous DNA damage that results from oxidation, deamination, depurination, and alkylation, thereby protecting the genome from mutations (Krokan & Bjørås, 2013). Biallelic germline loss-of-function variants in the BER pathway initiating DNA glycosylase (Limpose et al., 2018), NTHL1 (OMIM *602656), were recently reported to underlie a novel recessive adenomatous polyposis and colorectal cancer predisposition syndrome (OMIM #616415) (Kuiper & Hoogerbrugge, 2013).
The most prevalent NTHL1 variant in these polyposis families was the nonsense variant p.Q90* (c.268C>T, rs150766139), which to date has been reported in 18 families (Grolleman et al., 2019; Kuiper & Hoogerbrugge, 2015; Weren et al., 2015). Studies on the polyposis families suggested that besides polyposis colorectal cancer, also the risk for various other cancer types, including breast cancer, is increased. Grolleman et al. (2019) provided additional support for this by reporting women with biallelic deleterious NTHL1 variants having an unexpectedly high breast cancer incidence (60%, 9 out of 15 of the studied cases). While the increased cancer risk is established for individuals with biallelic NTHL1 pathogenic variants, the risk estimates for heterozygous carriers are unclear (Grolleman et al., 2019).

According to public databases, the NTHL1 p.Q90* allele is enriched in the Finnish population (Finnish minor allele frequency [MAF] 0.0038 versus global MAF 0.0014) with a carrier frequency of 19/1324 (1.4%, MAF 0.007) in North Ostrobothnia (gnomAD, https://gnomad.broad institute.org/; SISu, http://www.sisuproject.fi/). This geographical enrichment provides an excellent opportunity to test the association of NTHL1 p.Q90* with breast cancer susceptibility at the population level, along with the potential to establish risk estimates for the allele at heterozygous state. For this purpose, here, we have tested the prevalence of NTHL1 p.Q90* in breast cancer patients with an indication of hereditary disease susceptibility and those unselected for the family history of cancer and age at disease onset, all collected from the North Ostrobothnia area.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This study included informed consent from all participating individuals, and it was approved by the Ethical Board of the North Ostrobothnia Health Care District.

2.2 | Breast cancer cohorts

The hereditary cohort (n = 234), collected from the North Ostrobothnia area (Oulu University Hospital), included BRCA1/BRCA2/PALB2 mutation-negative breast cancer cases with the indication of an inherited predisposition to the disease. Cases were selected using the following criteria: 1) index cases from families with three or more breast and/or ovarian cancer cases in first- or second-degree relatives (n = 125), and 2) index cases from families with two cases of breast, or breast and ovarian cancer in first- or second-degree relatives, of which at least one with early disease onset (<35 years), bilateral disease or multiple primary tumors (n = 29), and 3) breast cancer cases diagnosed at or below the age of 40 (n = 80). The young breast cancer cases were included based on the assumption that when a woman below the age of 40 years develops breast cancer, a hereditary predisposition can be suspected regardless of the family history (Brunet, 2010). The unselected breast cancer cohort consisted of 1099 consecutive breast cancer cases diagnosed at the Oulu University Hospital during the years 2000–2016 (with a mean age of 58 years at diagnosis) and were unselected for the family history of cancer and age at disease onset.

2.3 | Variant detection

Genotyping was performed for DNA samples extracted from peripheral blood by using high-resolution melt analysis (CFX96, Bio-Rad) with Type-It HRM reagents (Qiagen). All assays included heterozygous and homozygous NTHL1 p.Q90* (NM_002528.5) genomic DNA samples as positive controls. Verification of all detected p.Q90* variants were confirmed with Sanger sequencing (ABI3130xl, Applied Biosystems).

2.4 | Statistical analyses

Fisher’s exact test was used to compare the carrier frequency between cases and controls, and Mann–Whitney
U test to compare the mean age at diagnosis between carrier and noncarrier cases in unselected cohort (IBM SPSS Statistics 24.0 for Windows, IBM Corp.). All p values were two-sided and values <0.05 were considered statistically significant.

3 | RESULTS

Five cases from the hereditary cohort were identified as heterozygous \textit{NTHL1} p.Q90* carriers (5/234, 2.1%, \(p = 0.39\), odds ratio [OR] = 1.5, 95% confidence interval [CI] = 0.6–4.1, Table 1). The presence of other pathogenic germline \textit{NTHL1} variants in them was ruled out by targeted gene panel sequencing. The carriers were diagnosed with breast cancer at the age of 34, 38, 38, 47, and 49 years, respectively. In these families, two additional breast cancer cases (family members of Her3 and Her4, respectively) were available for testing and one of them (breast cancer at the age of 62 years) was identified to carry \textit{NTHL1} p.Q90* (Table 2). In the family of index Her1, one case with stomach cancer and one with uterus cancer were also identified as carriers, whereas the salivary gland cancer case tested negative. In two other families (index Her3 and Her5, Table 2), DNA samples from altogether four family members diagnosed with other cancer types (basal cell carcinoma, prostate cancer, adenocarcinoma, and renal cancer, respectively) were available for testing, but all turned out as non-carriers. Based on these analyses, the evidence for \textit{NTHL1} p.Q90* segregating with cancer within these families remains uncertain.

In the unselected breast cancer cohort, 11 \textit{NTHL1} p.Q90* carriers were identified (11/1099, 1.0%, \(p = 0.36\), OR = 0.7, 95% CI = 0.3–1.5, Table 1). The mean age at disease onset for the carriers was 64 years (range 58–79 years), which was higher than in the carriers from hereditary cohort and also higher than the mean of the unselected cohort (58 years, range 28–93 years, \(p = 0.032\)). Of these, five had additional breast cancer cases in their first- and/or second-degree relatives (Unsel 1–5, Table 2) and seven had various other cancer types in their family (Unsel 3–9, Table 2). No samples from the relatives were available for testing.

In total, the frequency of \textit{NTHL1} p.Q90* in the studied breast cancer cohorts (16/1333, 1.2%, \(p = 0.61\), OR = 0.8, 95% CI = 0.4–1.6, Table 1) did not significantly differ from the population frequency (19/1324, 1.4%) in this geographical region. No homozygous cases were observed in any of the cohorts.

| Index ID | -cancers/tumors (age at diagnosis) | Breast/ovarian cancer(s) in first- and/or second-degree relatives (age at diagnosis) | Other cancers in first- and/or second-degree relatives (age at diagnosis) |
|----------|-----------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------|
| Her1     | Bil Br (34)                        | Br (u)                                                                          | Stomach (u) [+], Uterus (u) [+], Lung (71), Salivary gland (u) [-], Salivary gland (u), and Lymphoma (u) |
| Her2     | Br (38)                            |                                                                                 | Meningioma (u)                                                  |
| Her3     | Br (38)                            | Br (62) [+], Br (49) [-], Br (u), Br (u)                                        | Basal cell (50) [-], Prostate (67) [-]                          |
| Her4     | Br (47)                            | Br (64), Br (49) [-], Br (u), Br (u)                                            | Pancreatic (50)                                                |
| Her5     | Br (49)                            | Br (65) and Brain (67), Br (42)                                               | Adenocarcinoma\(^b\) (41) [-], Renalb (38) [-]                |
| Unsel 1  | Br (79)                            | Br (65)                                                                         |                                                                  |
| Unsel 2  | Br (71)                            | Br (u)                                                                          |                                                                  |
| Unsel 3  | Br (50) and Thy (u)                | Br (u)                                                                          | Lung (u)                                                       |
| Unsel 4  | Br (66)                            | Bil Br (45, 64)                                                                 | Prostate (70)                                                  |
| Unsel 5  | Br (59)                            | Br (70), Br (u), Br (u)                                                        | Esophagus (u)                                                  |
| Unsel 6  | Br (62)                            |                                                                                 | Renal (71)                                                     |
| Unsel 7  | Br (58)                            |                                                                                 | Hepatic (u)                                                    |
| Unsel 8  | Bil Br (70)                        |                                                                                 | Stomach (46)                                                  |
| Unsel 9  | Br (69)                            |                                                                                 | Throat (u)                                                    |
| Unsel 10 | Br (62)                            |                                                                                 |                                                                  |
| Unsel 11 | Br (64)                            |                                                                                 |                                                                  |

Note: All tested cases marked as [+], if positive and [–], if negative for \textit{NTHL1} p.Q90*.

Abbreviations: --, none reported; Bil Br, bilateral breast cancer; Br: breast cancer; Her, hereditary cohort; Ov, ovarian cancer; Thy, thyroid cancer; u, unknown; Unsel, unselected cohort.

\(^a\)GenBank reference sequence NM_002528.5.

\(^b\)Third-degree relative, included because sample was available for \textit{NTHL1} p.Q90* genotyping.
Germline loss-of-function variants in genes behind familial colorectal cancer and polyposis syndromes have also been reported to increase the risk for breast cancer. High lifetime breast cancer risk has been established for STK11 and PTEN gene mutations that are causative for dominantly inherited hamartomatous polyposis syndromes, Peutz-Jeghers and Cowden syndrome, respectively (Couch, Nathanson, & Offit, 2014). Also, families with more recently described polyposis predisposition syndrome caused by biallelic mutations in MLH3 gene were reported to have extracolonic tumors, including breast cancer (Okinudowa et al., 2019). The studies from NTHL1 families (Grolleman et al., 2019; Kuiper & Hoogerbrugge, 2015; Weren et al., 2015) add evidence for this: the same causative gene defect(s) in either recessive or dominant mode of inheritance, depending on the colorectal cancer/polyposis syndrome in concern, can also lead to predisposition to cancer in various different tissues. Whereas the evidence for breast cancer being a part of the cancer spectrum of recessively inherited NTHL1 tumor syndrome is strong (Grolleman et al., 2019; Kuiper, Nielsen, De Voer, & Hoogerbrugge, 2020; Rivera, Castellsague, Bah, van Kempen, & Foulkes, 2015), the contribution of NTHL1 p.Q90* heterozygosity, or even homozygosity, to breast cancer incidence in general population requires further investigation.

In the currently analyzed cohorts, heterozygous NTHL1 p.Q90* carriers were identified in 2.1% cases with indication of hereditary predisposition to disease and in 1.0% of the breast cancer cases unselected for family history or age at disease onset. This did not significantly differ from the 1.4% carriers in the healthy population controls. Similar carrier frequencies between studied cases and the general population argue against association of NTHL1 p.Q90* in a heterozygous state with increased breast cancer risk, although we acknowledge that the sample size of hereditary cohort is limited to 234 cases. The absence of homozygotes can also be explained by sample size of the current cohorts, but nevertheless NTHL1 p.Q90* homozygosity appears to be an extremely rare event in cases unselected for the family history of adenomatous polyposis and colorectal cancer. It is noted that current study is limited to investigating only the germline DNA of the patients, and the presence of somatic inactivation of the NTHL1 wild-type allele, and the mutational signature 30 associated with biallelic loss of NTHL1 (Grolleman et al., 2019) in patient tumors, was not addressed.

In conclusion, the current results indicate that NTHL1 p.Q90* allele is unlikely to be a significant contributor to breast cancer risk at the population level and that the risk is not increased in heterozygous carriers, which is in line with results obtained from other cancer types (Belhadj et al., 2019). This result is particularly important for the genetic counseling units in clinical diagnostics, as the use of large gene panels containing a variety of hereditary cancer genes has become routine, even when the patients lack the classical clinical features associated with some of the genes.

ACKNOWLEDGMENTS

We thank all the patients and their family members for volunteering to participate in this study, and Meeri Otsukka, Leena Keskitalo, and Lilian Vreede for technical assistance. Biocenter Oulu sequencing center is acknowledged for providing sequencing services.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Katri Pylkäs, Tuomo Mantere, Anna Tervasmäki, and Timo Kumpula conceived the study. Laura Huilaja, Kaisa Tasanen, Robert Winqvist, Richarda M. de Voer, and Katri Pylkäs provided the study samples. Timo Kumpula, Anna Tervasmäki, Tuomo Mantere, and Susanna Koivuluoma performed the experiments and data analysis, supervised by Katri Pylkäs. Timo Kumpula, Anna Tervasmäki, Tuomo Mantere, and Katri Pylkäs wrote the manuscript, and all the authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data to support the findings of this study is available on request from the corresponding author.

ORCID

Katri Pylkäs https://orcid.org/0000-0002-2449-0521

REFERENCES

Belhadj, S., Quintana, I., Mur, P., Munoz-Torres, P. M., Alonso, M. H., Navarro, M., … Valle, L. (2019). NTHL1 biallelic mutations seldom cause colorectal cancer, serrated polyposis or a multi-tumor phenotype, in absence of colorectal adenomas. Scientific Reports, 9(1), 9020. https://doi.org/10.1038/s41598-019-45281-1
Brunet, J. (2010). Hereditary breast cancer and genetic counseling in young women. Breast Cancer Research and Treatment, 123, 7–9. https://doi.org/10.1007/s10549-010-1050-5
Couch, F. J., Nathanson, K. L., & Offit, K. (2014). Two decades after BRCA: Setting paradigms in personalized cancer care and prevention. Science, 343(6178), 1466–1470. https://doi.org/10.1126/science.1251827
Grolleman, J. E., de Voer, R. M., Elsayed, F. A., Nielsen, M., Weren, R. D. A., Palles, C., … Kuiper, R. P. (2019). Mutational signature analysis reveals NTHL1 deficiency to cause a multi-tumor phenotype. Cancer Cell, 35(2), 256–266.e5. https://doi.org/10.1016/j.ccell.2018.12.011
Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., … MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. Nature, 581(7809), 434–443. https://doi.org/10.1038/s41586-020-2308-7
Krokan, H. E., & Bjørås, M. (2013). Base excision repair. Cold Spring Harbor Perspectives in Biology, 5(4), 1–22. https://doi.org/10.1101/cshperspect.a012583

Kuiper, R. P., & Hoogerbrugge, N. (2015). NTHL1 defines novel cancer syndrome. Oncotarget, 6(33), 34069–34070. https://doi.org/10.18632/oncotarget.5864

Kuiper, R. P., Nielsen, M., De Voer, R. M., & Hoogerbrugge, N. (2020). NTHL1 tumor syndrome. In: M. P. Adam, H. H. Ardinger … R. A. Pagon (Eds.), GeneReviews® [Internet] (pp. 1993–2020). Seattle, WA: University of Washington.

Limpose, K. L., Trego, K. S., Li, Z., Leung, S. W., Sarker, A. H., Shah, J. A., … Doetsch, P. W. (2018). Overexpression of the base excision repair NTHL1 glycosylase causes genomic instability and early cellular hallmarks of cancer. Nucleic Acids Research, 46(9), 4515–4532. https://doi.org/10.1093/nar/gky162

Olkinuora, A., Nieminen, T. T., Mårtensson, E., Rohlin, A., Ristimäki, A., Koskenvuo, L., … Peltomäki, P. (2019). Biallelic germline nonsense variant of MLH3 underlies polyposis predisposition. Genetics in Medicine, 21(8), 1868–1873. https://doi.org/10.1038/s41436-018-0405-x

Rivera, B., Castellsague, E., Bah, I., van Kempen, L. C., & Foulkes, W. D. (2015). Biallelic NTHL1 mutations in a woman with multiple primary tumors. New England Journal of Medicine, 373(20), e33. https://doi.org/10.1056/NEJMc15068780

Weren, R. D. A., Ligtenberg, M. J. L., Kets, C. M., de Voer, R. M., Verwiel, E. T. P., Spruijt, L., … Hoogerbrugge, N. (2015). A germ-line homozygous mutation in the base-excision repair gene NTHL1 causes adenomatous polyposis and colorectal cancer. Nature Genetics, 47(6), 668–671. https://doi.org/10.1038/ng.3287

How to cite this article: Kumpula T, Tervasmäki A, Mantere T, et al. Evaluating the role of NTHL1 p.Q90* allele in inherited breast cancer predisposition. Molecular Genetics & Genomic Medicine. 2020;8:e1493. https://doi.org/10.1002/mgg3.1493