Comprehensive analysis of NuMA variation in breast cancer

Outi Kilpivaara*1, Matias Rantanen1, Anitta Tamminen1, Kristiina Aittomäki2, Carl Blomqvist3,4 and Heli Nevanlinna1

Address: 1Department of Obstetrics and Gynecology, Helsinki University Central Hospital (HUCH), Helsinki, Finland, 2Department of Clinical Genetics, Helsinki University Central Hospital (HUCH), Helsinki, Finland, 3Department of Oncology, Helsinki University Central Hospital (HUCH), Helsinki, Finland and 4Dept. Oncology, Uppsala University Hospital, Sweden

Email: Outi Kilpivaara* - outi.kilpivaara@helsinki.fi; Matias Rantanen - matias.rantanen@helsinki.fi; Anitta Tamminen - anitta.tamminen@hus.fi; Kristiina Aittomäki - kristiina.aittomaki@hus.fi; Carl Blomqvist - carl.blomqvist@hus.fi; Heli Nevanlinna - heli.nevanlinna@hus.fi

* Corresponding author

Abstract

Background: A recent genome wide case-control association study identified NuMA region on 11q13 as a candidate locus for breast cancer susceptibility. Specifically, the variant Ala794Gly was suggested to be associated with increased risk of breast cancer.

Methods: In order to evaluate the NuMa gene for breast cancer susceptibility, we have here screened the entire coding region and exon-intron boundaries of NuMA in 92 familial breast cancer patients and constructed haplotypes of the identified variants. Five missense variants were further screened in 341 breast cancer cases with a positive family history and 368 controls. We examined the frequency of Ala794Gly in an extensive series of familial (n = 910) and unselected (n = 884) breast cancer cases and controls (n = 906), with a high power to detect the suggested breast cancer risk. We also tested if the variant is associated with histopathologic features of breast tumors.

Results: Screening of NuMA resulted in identification of 11 exonic variants and 12 variants in introns or untranslated regions. Five missense variants that were further screened in breast cancer cases with a positive family history and controls, were each carried on a unique haplotype. None of the variants, or the haplotypes represented by them, was associated with breast cancer risk although due to low power in this analysis, very low risk alleles may go unrecognized. The NuMA Ala794Gly showed no difference in frequency in the unselected breast cancer case series or familial case series compared to control cases. Furthermore, Ala794Gly did not show any significant association with histopathologic characteristics of the tumors, though Ala794Gly was slightly more frequent among unselected cases with lymph node involvement.

Conclusion: Our results do not support the role of NuMA variants as breast cancer susceptibility alleles.

Background

Recently, a genome-wide association study with over 25 000 single-nucleotide polymorphisms (SNP) was conducted to discover variants associated with increased breast cancer risk [1]. The initial sample set comprised of 254 German breast cancer cases and 268 controls [1].
Fifty-two SNPs were selected for replication genotyping in two independent sample series, one German (188 cases, 150 controls) and one Australian (180 cases, 180 controls) and, among others; a putative breast cancer susceptibility locus was identified at 11q13. The variant with strongest association in the 300 kb region was SNP rs673478 (p = 0.001 and OR = 4.45 [1]). A number of identified variants were selected, based on bioinformatic analyses, for further exploratory screening in familial breast cancer patients (n = 341) and healthy population controls (n = 368). The previously identified possible risk variant Ala794Gly was screened in larger series of familial (n = 910) and unselected breast cancer cases (n = 884), and compared in frequency with population controls (n = 906). The unselected breast cancer case series included consecutive newly diagnosed breast cancer patient samples collected at the Department of Oncology, Helsinki University Central Hospital at two separate periods in 1997–1998 and 2000 covering 79% of all newly diagnosed breast cancer cases at respective study periods combined (for more detailed description, see [11,12]). The series of familial breast cancer cases in this study have been collected at the Helsinki University Central Hospital as described in [13]. The successfully genotyped series included 378 patients with strong family history, defined as three or more breast cancer cases in first degree family members (including the index case).

NuMA (Nuclear Mitotic Apparatus protein) gene is located on chromosome 11q13 and it encodes a 236 kDa nuclear protein essential for normal mitotic spindle organization. NuMA protein consists of globular head and tail domains and a separating long coiled-coil domain [2] which mediates dimerization and oligomerization of NuMA [3]. The tail domain itself is bipartite including a region critical for interaction with the mitotic spindle and another region needed for accurate nuclear reformation including a nuclear localization signal [4-6]. Expression of NuMA with deletion in either head or tail domains results in dominant defects in mitosis [7].

NuMA has been associated with acute promyelocytic leukemia (APL). In very rare cases of APL translocation NuMA-RARα (retinoic acid receptor α) t(11;17)(q13;q21) has been observed instead of the common translocation involving a gene fusion of promyelocytic leukemia protein (PML) and RARα [8]. The fusion protein consists of 1883 amino acids of NuMA protein in the aminoterminal end the rest of the total 2285 amino acid protein being derived from RARα – the structure similar to all RARα fusion proteins seen in APL. The essential feature of the fusion partner NuMA is to be capable of establishing protein-protein interactions that may result in formation of defective heterodimers interfering with retinoid signaling [8]. It has been reported that NuMA is an interaction partner of GAS41 (glioma-amplified sequence 41) [9], which is a highly conserved protein and a putative transcription factor amplified in even at early stages of human glioma [10]. This interaction links the structural protein NuMA to the regulation of gene expression. In addition to the previously mentioned studies the role of NuMA has not been under extensive research in association with cancer.

In this study, we screened the NuMA gene for genetic variants in breast cancer cases and studied their relevance for breast cancer risk. In particular, we studied the association of the reported breast cancer susceptibility allele NuMA Ala794Gly in larger series of unselected and familial breast cancer cases and controls, and examined the association of this variant with clinicopathologic characteristics of breast tumors.

**Methods**

**Breast cancer patients**

The 28 exons and exon-intron boundaries as well as 5’ and 3’ untranslated regions (ENST00000358965) were screened for sequence variants in a total of 92 familial (non-BRCA1/2) breast cancer cases. Familial breast cancer case was here, in this initial screening, defined as three or more breast cancer cases in first degree family members (including the index case).

Histopathologic data were collected from pathology reports for all the primary breast tumors available from
the unselected case series (889 tumors from 842 successfully genotyped cases). The data set in this study includes information on tumor histology, grade, estrogen and progesterone receptor status, p53 immunohistochemistry, tumor diameter, lymph node status, distant metastases, and the age at the time of diagnosis.

The study was performed with informed consent from the patients and permissions from the Ethics Committee of the Department Obstetrics and Gynecology, as well as from the Ministry of Social Affairs and Health in Finland.

**Variant screening**

All NuMA exons were screened for sequence variants using single strand conformation polymorphism (SSCP) or conformation sensitive gel electrophoresis (CSGE) method. For further screenings restriction fragment polymorphism (RFLP) method was used when applicable. Variant Arg972Gln (R972Q) was screened by RFLP using enzyme BsrBI, Arg1471Trp (R1471W) using enzyme MspI, and Arg1665Cys (R1665C) using enzyme HhaI. For the screening of intronic variant IVS2+34G > C a mutagenesis primer was designed to create a restriction site for enzyme NdeI. Primer sequences are available on request. All endonucleases are products of New England Biolabs, Beverly, MA. All variants found in the screening have been confirmed by either DNA sequencing or repeating the screening by respective method (SSCP, CSGE, RFLP) for the genomic DNA sample. The genotyping Ala794Gly has been performed using Amplifluor™ fluorescent genotyping (K-Biosciences, Cambridge, UK). The quality of the Ala794Gly genotyping was ascertained by analyzing duplicate samples (92 samples were genotyped with 100% concordance). Genotyping success rate for unselected cases was 95% (842/884), for familial cases 98% (893/910), and 100% for population controls (906/906). The unsuccessful genotypes were due to poor quality or lack of DNA in the analysis.

**Statistical and Bioinformatic Methods**

Standard chi squared or Fisher exact tests were used to assess the differences in genotype frequencies between groups. Per allele odds ratios for each SNP, together with 95% confidence intervals, were estimated using logistic regression. Differences in survival by genotype were assessed using the log-rank test. Analyses were performed using SPSS (SPSS Inc., Chicago, IL). All \( p \)-values are two-sided. SIFT [17,18] and PolyPhen [19,20] were used for predicting the impact of observed amino acid substitutions on the structure and function of NuMA protein. The most probable haplotypes were reconstructed using the program PHASE [21-23].

**Results**

A total of 11 exonic and 12 intronic or untranslated region (UTR) variants in NuMA were identified (Table 1). We selected 5 of these variants, in addition to Ala794Gly, for further analysis. These five variants had a SIFT score lower than or similar to the score for Ala794Gly (SIFT score 0.29). In particular, two variants (Arg218Trp and

---

**Table 1: Variants identified in NuMA and their predicted effect on protein level by SIFT and PolyPhen**

| Location | Variant | Frequency | SIFT | PolyPhen |
|----------|---------|-----------|------|----------|
| IVS2     | IVS2+34 G > C | 4/92 | 0.00 | unknown |
| IVS4     | IVS4+46 delCA | 19/92 | 0.92 | - |
| IVS5     | IVS5+23 T > A | 3/92 | 0.03 | benign |
| IVS6     | IVS6-53 A > G | 19/92 | 0.00 | possibly damaging |
| IVS8     | IVS8-52 T > A | 19/92 | 0.92 | benign |
| ex 10    | 652 C > T, Arg218Trp | 1/92 | 1.00 | benign |
| IVS12    | IVS12-37 insA | 19/92 | 0.00 | unknown |
| IVS14    | IVS14+32 G > T | 9/92 | 0.92 | benign |
| ex 15    | 2381 C > G, Ala794Gly | 9/92 | 0.29 | benign |
| ex 15    | 2484C > T, Gly828Gly | 1/92 | 0.92 | - |
| ex 15    | 2915 G > A, Arg972Gln | 3/92 | 0.30 | benign |
| ex 15    | 4012 G > A, Glu1338Lys | 1/92 | 0.28 | benign |
| ex 15    | 4411 C > T, Arg1471Trp | 2/92 | 0.00 | possibly damaging |
| ex 17    | 4785 G > A, Lys1595Lys | 1/92 | 0.28 | benign |
| ex 18    | 4996 C > T, Arg1665Cys | 1/92 | 0.03 | possibly damaging |
| IVS18    | IVS18+15 C > G | 1/92 | 0.92 | benign |
| ex 20    | 5335 G > A, Asp1779Asn | 3/92 | 1.00 | benign |
| ex 21    | 5619 C > T, Ala1873Ala | 9/92 | 0.92 | benign |
| ex 25    | 6083 G > A, Arg2028Gln | 1/92 | 0.94 | benign |
| 3' UTR   | 6517 A > C | 10/92 | 0.00 | unknown |
| 3' UTR   | 6519 C > G | 10/92 | 0.00 | unknown |
| 3' UTR   | 6580 T > C | 1/92 | 0.00 | unknown |
Arg1471Trp) had a SIFT score was 0.00, which may indicate that the amino acid substitution is harmful to the protein. We genotyped these variants in 341 familial breast cancer patients and 368 population controls. All were present at a similar frequency in cases and controls. (Table 2).

The Ala794Gly variant was present in 5.8% of population controls (53/906), 5.7% of unselected breast cancer cases (48/842) and 4.8% of familial breast cancer cases (43/893) (Table 3). Thus there was no evidence for an association between Ala794Gly genotype and breast cancer risk.

NuMA Ala794Gly did not show any significant association with histopathologic parameters of the tumors (Table 4). There was some evidence of an association between the Ala794Gly variant and positive lymph node status in unselected breast cancer case series \( (p = 0.008) \), but this association was not seen among familial patient series (data not shown). Furthermore, if Bonferroni correction for multiple testing was applied, only \( p \)-value of 0.006 or smaller would be considered significant. There was no difference in either overall or disease-free survival by Ala794Gly genotype (data not shown).

Observed variants in NuMA were used for reconstructing haplotypes using PHASE program (Table 5). Altogether 15 different haplotypes defined by 23 sequence variants in NuMA were observed in the screening of 92 familial breast cancer patients (Table 5). The most common haplotype (without any observed DNA sequence variants) accounts for 80% of observed haplotypes, and together the three most common haplotypes account for 90% of haplotypes. Each of the missense variants that were further studied represent a unique haplotype except the Ala794Gly which is present in two haplotypes (numbers 12 and 14 in Table 5) defined by an intronic variant IVS2+34 G > C. In order to study the possibility that either of haplotypes 12 or 14 would associate with breast cancer risk we screened the haplotype-defining intronic variant IVS2+34G > C in 337 familial breast cancer cases and 359 controls. Variant IVS2+34G > C was present in cases and controls in similar proportions (cases 49/337, 14.5% and controls 57/359, 16.0%; \( p = 0.6 \)). Furthermore, we did not observe any haplotype 12 carriers in this further screening (variant IVS2+34G > C was always present in Ala794Gly carriers).

### Table 2: Further screening of selected variants in familial breast cancer cases and controls.

| Exon | Variant | Familial Cases | Controls |
|------|---------|----------------|----------|
| 10   | ex10 652 C > T, Arg218Trp | 1/341 | 1/368 |
| 15   | ex15 2915 G > A, Arg972Gln | 6/333 | 12/364 |
| 15   | ex15 4012 G > A, Glu1338Lys | 1/323 | 1/364 |
| 15   | ex15 4411 C > T, Arg1471Trp | 2/339 | 0/368 |
| 18   | ex 18 4996 C > T, Arg1665Cys | 7/337 | 6/366 |

### Table 3: Results from NuMA A794G screening

|                      | total | C:C | C:G | \( p^2 \) | OR | 95%CI |
|----------------------|-------|-----|-----|---------|----|-------|
| **Population controls** |       |     |     |         |    |       |
| Unselected cases      | 906   | 853(94.2%) | 53(5.8%) | 0.89  | 0.97 | 0.65–1.45 |
| bilateral breast cancer | 842  | 794(94.3%) | 48(5.7%) | 0.541 | 1.35 | 0.47–3.92 |
| unilateral breast cancer | 788  | 744(94.4%) | 44(5.6%) | 0.617 | 0.59 | 0.18–1.93 |
| multiple cancers\(^1\) | 84    | 81(96.4%) | 3(3.6%)  |       |     |       |
| no multiple cancers   | 758   | 713(94.1%) | 45(5.9%) |       |     |       |
| **Familial cases**    | 893   | 850(95.2%) | 43(4.8%) | 0.33  | 0.81 | 0.54–1.23 |
| 3+ families           | 378   | 359(95.0%) | 19(5.0%) | 0.56  | 0.85 | 0.50–1.46 |
| breast cancer only    | 298   | 283(95.0%) | 15(5.0%) |       |     |       |
| breast and ovarian cancer | 80  | 76(95.0%) | 4(5.0%)  |       |     |       |
| Small families        | 515   | 491(95.3%) | 24(4.7%) | 0.34  | 0.79 | 0.48–1.29 |

\(^1\)case with at least one other cancer than breast cancer

\( ^2 \)compared to population controls, except comparisons between unilateral vs. bilateral and multiple cancers vs. one cancer

\( ^3 \)Fisher’s exact test

No G:G genotypes were observed.

Discussion

Kammerer et al. utilized a genome-wide association analysis to identify breast cancer susceptibility regions and identified, among other regions, a high-linkage disequilibrium region on chromosome 11q13 [1]. This region contains several genes and NuMA was chosen as a most
likely candidate for breast cancer susceptibility gene, and variant Ala794Gly was hypothesized to be functionally impaired and suggested to be associated with breast cancer risk [1].

Our thorough screening of NuMA gene in breast cancer cases resulted in identification of several variants of which eight were missense changes and the rest were synonymous variants or not located in coding regions. Missense variants that warranted further screening after bioinformatic analyses were present in the breast cancer cases in similar frequencies as in controls. Missense variant Arg1471Trp was not detected in controls, however, but being present in only 0.6% of the cases would have only marginal effect on breast cancer even if having any effect on breast cancer risk for the carriers. These variants represented also unique haplotypes, not suggesting presence of other risk alleles in linkage disequilibrium with these variants either, although due to low power, very low risk alleles may go unrecognized.

We also specifically evaluated the variant NuMA Ala794Gly for breast cancer risk in an extensive patient series (884 unselected cases, 910 familial cases and 906 population controls) as compared to those used by Kammerer et al, (2005) [1]. For this analysis, our material has 98% power to detect a difference in frequency of that magnitude.

Variant Ala794Gly was detected in almost equal frequencies in all our study series as well as in population controls, which is consistent with the results from a large

Table 4: Tumor characteristics among 842 unselected breast cancer cases analyzed for NuMA A794G.

| NuMA A794G (2381C > G) | p |
|------------------------|---|
| Total                  |   |
| n = 889                |   |
| Total                  |   |
| CC                     |   |
| 837(94.2%)             |   |
| CG                     |   |
| 52(5.8%)               |   |
| Histology              |   |
| n = 889                |   |
| Ductal                 |   |
| 599(71.6%)             |   |
| Lobular                |   |
| 132(15.8%)             |   |
| Medullary              |   |
| 11(1.3%)               |   |
| other                  |   |
| 95(11.4%)              |   |
| Gradus                 |   |
| n = 793                |   |
| 1                      |   |
| 208(27.9%)             |   |
| 2                      |   |
| 316(42.4%)             |   |
| 3                      |   |
| 222(29.8%)             |   |
| pT Stage               |   |
| n = 801                |   |
| pT1-pT2                |   |
| 699(92.6%)             |   |
| 44(95.7%)              |   |
| pT3-pT4                |   |
| 56(7.4%)               |   |
| 2(4.3%)                |   |
| Lymph Nodes            |   |
| n = 858                |   |
| pN0                    |   |
| 446(55.3%)             |   |
| 360(44.7%)             |   |
| pN1-2                  |   |
| 19(36.5%)              |   |
| 33(63.4%)              |   |
| Estrogen Receptor Status |   |
| n = 845                |   |
| Positive               |   |
| 622(78.4%)             |   |
| 42(80.8%)              |   |
| Negative               |   |
| 171(21.6%)             |   |
| 10(19.2%)              |   |
| Progesterone Receptor Status |   |
| n = 846                |   |
| Positive               |   |
| 538(67.8%)             |   |
| 38(73.1%)              |   |
| Negative               |   |
| 256(32.2%)             |   |
| 14(26.9%)              |   |
| p53 Status             |   |
| n = 653                |   |
| Positive               |   |
| 132(21.3%)             |   |
| 2(6.1%)                |   |
| Negative               |   |
| 488(78.7%)             |   |
| 31(93.9%)              |   |
| Distant metastasis     |   |
| n = 853                |   |
| Positive               |   |
| 41(5.1%)               |   |
| 0(0.0%)                |   |
| Negative               |   |
| 765(94.9%)             |   |
| 47(100.0%)             |   |

The mean age of diagnosis was 57 years for C:C, and 56 for C:G genotypes.
breast cancer patient and control series recently studied by the Breast Cancer Association Consortium [24] and does not support the previously proposed association with breast cancer risk. However, as the variant Ala794Gly was initially found here to be present in two distinct haplotypes defined by an intronic variant it is possible that differences in the relative haplotype frequencies could have masked any associated risk. Further evaluation of these two haplotypes, however, did not support this possibility.

None of the identified NuMA variants was associated with breast cancer risk in our study. Furthermore, Ala794Gly variant was not significantly associated with any of the tumor characteristics.

Conclusion
In conclusion, our results do not support the role of NuMA variants as breast cancer risk alleles.

Abbreviations
NuMA: Nuclear Mitotic Apparatus protein; SNP: Single nucleotide polymorphism; APL: Acute promyelocytic leukemia; RARα: Retinoic acid receptor α; PML: promyelocytic leukemia protein; GAS41: Glioma-amplified sequence 41; SSCP: Single strand conformation polymorphism; CSGE: Conformation sensitive gel electrophoresis; RFLP: Restriction fragment length polymorphism; UTP: Untranslated region.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
OK did the statistical analyses and drafted the manuscript and did some of the molecular genetic studies. MR and AT were responsible for the majority of molecular genetic studies involving screening of NuMA. KA and CB collected the patient samples and clinical data in the study. HN participated in the study design and helped in drafting the manuscript.

Acknowledgements
Professor Douglas Easton is gratefully acknowledged for his constructive comments on the manuscript and Rainer Fagerholm for his help with haplotype construction. This study has been financially supported by the Helsinki University Central Hospital Research Fund, Academy of Finland, Finnish Cancer Society, the Sigrid Juselius Foundation, Maud Kuistila Memorial Foundation, and Ida Montin Foundation.

References
1. Kammerer S, Roth RB, Hoyal CR, Reneland R, Marnellos G, Kiechle M, Schwarz-Boeger U, Griffiths LR, Ebner F, Rehbock J, Cantor CR, Nelson MR, Braun A. Association of the NuMA region on chromosome 11q13 with breast cancer susceptibility. Proc Natl Acad Sci USA 2005, 102:2004-2009.
2. Harborth J, Weber K, Osborn M. Epitope mapping and direct visualization of the parallel, in-register arrangement of the double-stranded coiled-coil in the NuMA protein. EMBO J 1995, 14(11):2447-60.
3. Merdes A, Ramyar K, Vechio JD, Cleveland DW: A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly in 1996, 87(3):447-58.
4. Compton DA, Luo C: Mutation of the predicted p34cdc2 phosphorylation sites in NuMA impair the assembly of the mitotic spindle and block mitosis. J Cell Sci 1995, 108(Pt 2):621-633.
5. Gueth-Hallonet C, Weber K, Osborn M: NuMA: A bipartite nuclear location signal and other functional properties of the tail domain. Exp Cell Res 1996, 225:207-218.
6. Tang TK, Tang CJ, Chao YJ, Wu CW: Nuclear mitotic apparatus protein (NuMA): Spindle association, nuclear targeting and differential subcellular localization of various NuMA isoforms. J Cell Sci 1994, 107(Pt 6):1389-1402.
7. Compton DA, Cleveland DW: NuMA is required for the proper completion of mitosis. J Cell Biol 1993, 120:947-957.
8. Wells RA, Cazavuelos C, Kamel-Reid S: Fusion of retinoic acid receptor alpha to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukemia. Nat Genet 1997, 17:109-113.
9. Harborth J, Weber K, Osborn M: GAS41, a highly conserved protein in eukaryotic nuclei, binds to NuMA. J Biol Chem 2000, 275(31):29779-29785.
10. Fischer U, Heckel D, Michel A, Janka M, Hulsebos T, Meese E: Cloning of a novel transcription factor-like gene amplified in human glioma including astrocytoma grade I. Hum Mol Genet 1997, 6:1817-1822.
11. Syrjakoski K, Vahteristo P, Eerola H, Tamminen A, Kivinummi K, Saharainen A, Kononen J, Aittomaki K, Heikkila P, Holli K, Blomqvist C, Nevanlinna H: Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. J Nat Cancer Inst 2000, 92:1529-1531.
12. Kilpivaara O, Bartkova J, Eerola H, Syrjakoski K, Vahteristo P, Lukas J, Blomqvist C, Holli K, Heikkila P, Sauter G, Kallioniemi OP, Bartek J, Nevanlinna H: Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. Int J Cancer 2005, 113:275-280.
13. Eerola H, Blomqvist C, Pukkala E, Pyrhonen S, Nevanlinna H: Familial breast cancer in southern Finland: How prevalent are breast cancer families and can we trust the family history reported by patients? Eur J Cancer 2000, 36:1143-1148.
14. Vehmanen P, Friedman LS, Eerola H, McClure M, Ward B, Sarantaus L, Kainu T, Syrjakoski K, Tamminen A, Kononen J, Aittomaki K, Heikkila P, Blomqvist C, Kallioniemi OP, Lucente F, Frank TS, Nevanlinna H: Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: Evidence for additional susceptibility genes. Hum Mol Genet 1997, 6:2309-2315.
15. Vahteristo P, Eerola H, Tamminen A, Blomqvist C, Nevanlinna H: A probability model for predicting BRCA1 and BRCA2 mutations in breast and breast-ovarian cancer families. Br J Cancer 2001, 84:704-708.
16. Vahteristo P, Bartkova J, Eerola H, Syrjakoski K, Ojala S, Kilpivaara O, Tamminen A, Kononen J, Attomaki K, Heikkila P, Holli K, Blomqvist C, Bartek J, Kallioniemi OP, Nevanlinna H: A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. Am J Hum Genet 2002, 71:432-438.
17. SIFT [http://blocks.fhcrc.org/sift/SIFT.html]
18. Ng PC, Henikoff S: Accounting for human polymorphisms predicted to affect protein function. Genome Res 2002, 12:436-446.
19. PolyPhen [http://www.bork.embl-heidelberg.de/PolyPhen/] PolyPhen: A tool for predicting damaging missense mutations.
20. Polsensky V, Bork P, Sunyea S: Human non-synonymous SNPs: Server and survey. Nucleic Acids Res 2002, 30:3894-3900.
21. PHASE Software for Haplotype Estimation [http://www.stat.washington.edu/stephens/software.html]
22. Stephens M, Smith NJ, Donnelly P: A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001, 68:978-989.
23. Stephens M, Donnelly P: A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003, 73:1162-1169.
24. Cox A, Dunning AM, Garcia-Closas M, Balasubramanian S, Reed MW, Pooley KA, Scollan S, Baynes C, Ponder BA, Chanock S, Lissowska J, Brinton L, Peplonska B, Southey MC, Hopper JL, McCredie MR, Giles GG, Fletcher O, Johnson N, dos Santos Silva I, Gilson L, Bojesen SE, Nordenskjold BG, Axelson CK, Torres D, Hamann U, Justenhoven C, Brauch H, Chang-Claude J, Kropp S, Risch A, Wang-Gohrke S, Schurmann P, Bogdanova N, Dork T, Fagerholm R, Aaltonen K, Blomqvist C, Nevanlinna H, Seal S, Renwick A, Stratton MR, Rahman N, Sargaziyad S, Hughes D, Odofrey F, Brennan P, Spurdle AB, Chenevix-Trench G, Kathleen Cunningham Foundation Consortium for Research into Familial Breast Cancer, Beesley J, Mannervik A, Hartikainen J, Kataja V, Kesma VM, Couch FJ, Olson JE, Goode EL, Brooks A, Schmidt MK, Hogervorst FB, Vant Veer LJ, Kang D, Yoo KY, Noh DY, Ahn SH, Wedren S, Hall P, Low YL, Liu J, Mline RL, Ribas G, Gonzalez-Neira A, Benitez J, Sgurdom AJ, Stredick DL, Alexander BH, Struwing JP, Pharaoh PD, Easton DF, Breast Cancer Association Consortium: A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet 2007, 39(3):352-358.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/8/71/prepub