Arch Public Health
2003, 61, 297-312

Comparing the performance of family characteristics and predictive models for germline *BRCA1/2* mutations in breast cancer families

by

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Abstract

Aim: *The probability of finding a* BRCA1/2 *mutation is an important factor in the decision to initiate a mutation screen in individual families. This may be based on family characteristics or the probability that a mutation will be identified that may be calculated by various models. The current study compares the relative usefulness of these indicators.*

Methods: *All 155 Belgian families that had been counselled and accepted for screening for* BRCA1/2 *germline mutations at the Vrije Universiteit Brussel Family Cancer Clinic and that had enough clinical information on file for at least two of the Couch (1997), Shattuck-Eidens (1997), Frank (1998),* BRCAPRO *(1998), and Vahteristo (2001) models to be applied post hoc were included in the study sample. The sensitivity*

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and specificity of relevant family characteristics were plotted on the receiver operating characteristic curves of the probability models.

Results: *The presence of ovarian cancer in the family is associated with a mutation identification rate of nearly 60%. Breast cancer families without ovarian cancer are a clinically relevant but difficult subgroup for predicting the identification of a* BRCA1/2 *mutation.* BRCAPRO *was the only informative predictive model in this subgroup but did not perform better than the number of breast cancers in the family used as the sole criterion for mutation screening.*

Conclusion: *Two family characteristics, the presence of ovarian cancer and the number of breast cancers in the families without ovarian cancer, are as useful for estimating the probability of finding a* BRCA1/2 *gene mutation as the available probability models. Such models, such as* BRCAPRO*, may, however, merit further validation.*

Keywords: *breast cancer; ovarian cancer; BRCA genes; predictive models; ROC-curve*

Introduction

The *BRCA1* and *BRCA2* genes may be screened for germline mutations in families with a number of breast and/or ovarian cancers occurring in the same lineage. The identification of a *BRCA1* or *BRCA2* gene mutation allows family members to opt for testing their individual carrier status. The probabilities of finding a linkage to or a mutation in the *BRCA1* and *BRCA2* genes have been defined in large families. A *BRCA1/2* gene mutation can only be found in a fraction of all families with a linkage to these genes. Moreover, in the majority of families seeking genetic counseling no linkage analysis can be performed. In these families the decision whether to initiate a *BRCA1/2* mutation screen may be based directly on the familial cancer phenotype. Alternatively, the decision may be based on the estimates of the probability that a mutation will be identified (1). Various models have been proposed to estimate this probability. The available models require the input of specific elements of the personal and family history of breast and ovarian cancer.

The clinical characteristics that are considered predictive for finding a mutation are the presence in the family of breast and ovarian cancer in a single woman, the presence of ovarian cancer, bilateral breast cancer, male breast cancer, early age at diagnosis of cancer and the number of breast cancers, c.q. ovarian cancers, in the family (2-6). The number

of first-degree relatives with breast cancer is an important parameter for estimating a woman's composite risk for the disease (7). There are, however, no reports available on the importance of the number of affected first-degree relatives for the probability of identifying a *BRCA1/2* gene mutation. This parameter has been added to the clinical characteristics to be considered in the current study.

All but one of the published models, that estimate the probability of the identification of a germline mutation in a family from an admixed population, have been developed from a logistic regression. Such logistic regression entails modeling the logarithm of the odds of identifying a germline *BRCA1/2* mutation in the respective study population as a linear function of covarying phenotype characteristics, such as the presence of ovarian cancer in the family and the age at onset of breast cancer. The coefficients from the regression analysis can then be used to calculate the logarithm of the odds of identifying a mutation in a new family (L). For instance, the Vahteristo 2001 model, which is the simplest of these models, estimates L=2.87+ (-0.14) x (age of the youngest breast cancer patient)+2.11x(number of ovarian cancer cases). The probability of identification of a mutation in the family (p) can then be written as p = exp(L)/[1+exp(L)]. Four of such logistic regression based models are considered in the current study: the Couch (1997), Shattuck-Eidens (1997), Frank (1998), and Vahteristo (2001) models (2-4, 8).

The model for calculating the probability that a particular individual carries a *BRCA1* or a *BRCA2* mutation published by Berry (1997) and Parmigiani (1998), also known as BRCAPRO, starts from the a priori probability for anyone to carry such a mutation, which is based on published mutation prevalence estimates (9, 10). This probability is then adjusted by applying Bayes' theorem to the individual's first- and second-degree relatives' history of breast and ovarian cancer or lack of such history, the age specific penetrance of the mutations as well as the age specific incidence of sporadic breast and ovarian cancer.

The current study assesses the predictive value of specific family characteristics and various probability models' estimates in 155 Belgian families with breast cancer, ovarian cancer, or both, that were counselled and screened for the presence of *BRCA1/2* germline mutations at the Vrije Universiteit Brussel Family Cancer Clinic. Particular attention is paid to breast cancer-only families, as the known association of the presence of ovarian cancer in the family with a high mutation identification rate relegates most of the clinically relevant uncertainty about deciding on a *BRCA* screen to these families (11-13).

Material and methods

Study Sample

All families that had been counselled and screened for *BRCA1* and *BRCA2* germline mutations at the Family Cancer Clinic of the Vrije Universiteit Brussel on January 1st 2002, with enough clinical information on file for at least two of the available probability models to be applied post hoc, i.e. the Couch, Schattuck-Eidens, Frank, Vahteristo, and BRCAPRO models, were included in the study sample (2-4, 8-10). A total of 155 families could thus be recruited. The families had been referred for the most part by oncologists, gynaecologists and general practitioners, a minority was self-referred. Nearly all individuals who attended the clinic, resided in Belgium, a few resided in one of the neighbouring Western-European countries. Families were accepted for screening for *BRCA1/2* germline mutations on a case by case basis. Preference was given to families that had at least two affected family members, of which one was either affected with ovarian cancer or diagnosed with breast cancer before the age of 50. There were, however, 11 breast cancer only families screened with only one affected individual. Tables 1 and 2 detail the characteristics of the included families.

Mutation Analysis and DNA Sequencing

The *BRCA1/2* mutation screen was performed on genomic DNA extracted from white blood cells of one individual diagnosed with breast and/or ovarian cancer per family. Exon 11 of *BRCA1* was screened by the Protein Truncated Test (PTT) as described by F.B.L. Hogevorst et al (14). The exons 2, 5 and 20 of *BRCA1* were submitted to a combined Single Strand Conformation Polymorphism/Heteroduplex analysis according to P.A. Futreal et al. for the first 70 screens performed and to a high throughput fluorescence – based conformation – sensitive gel electrophoresis as described by Ganguly et al., and Markoff et al. for the remainder of the screens (15-17). Exons 10 and 11 of *BRCA2* were assessed by PTT.

When putative mutations were detected, the corresponding fragments were reamplified from the original genomic DNA, the amplified fragments were purified with the High Pure PCR Product Purification Kit (Boeringer Mannheim) and the mutations confirmed by sequence analysis (Sequenase Version 2,0 DNA Sequencing Kit from USB). These analyses were performed at the Laboratory of Molecular Oncology, Oncology Center and Genetics Center, Vrije Universiteit Brussel, Brussels, Belgium, between October 1st 1994 and October 1st 2001.

TABLE 1
BRCA1/2 gene mutation predictive family characteristics in families with breast cancer, ovarian cancer, or both*

	Nr. of families N=155	Nr. of BRCA1/2 mutations N=37	Mutation identification rate (%)	Statistical significance
Breast and ovarian cancer in a single individual				
present in family	8	7	88	
not present in family	147	30	20	p=0.0002
Ovarian cancer				
present in family	37	22	59	
not present in family	118	15	13	p<0.00005
Nr. of breast and ovarian cancers in the family *				
1	13	0	0	
2	51	9	18	
3	45	9	20	p=0.001
4 or more	46	19	41	
Nr. of breast and ovarian cancers per 100 women *				
less than 20	45	7	16	
20-39	75	18	24	
40-59	18	5	28	p=0.06
60 or more	17	7	41	
Nr. of affected first-degree relatives				
< 2 affected	23	2	9	
2	61	11	18	
3	39	10	26	p=0.002
4 or more	32	14	44	
Average age at breast cancer# (y)				
lower than 30	3	2	67	
30-39	19	5	26	
40-49	68	20	29	p=0.05
50 or over	61	9	15	

* A case of breast and ovarian cancer in a single individual adds one to the count of breast cancers and one to the count of ovarian cancers in the family. A case of bilateral breast cancer adds one to the count of breast cancers in the family.
# Four families with no known age at diagnosis of breast cancer were excluded from this part of the analysis.

TABLE 2
BRCA1/2 gene mutation predictive family characteristics in subset of families with breast cancer but no ovarian cancer

	Nr. of families N=118	Nr. of BRCA1/2 mutations N=15	Mutation identification rate (%)	Statistical significance
Bilateral breast cancer				
present in family	30	6	20	
not present in family	88	9	10	p=1
Male breast cancer				
present in family	5	0	0	
not present in family	113	15	13	p=0.5
Nr. of breast cancers in the family *				
1	11	0	0	
2	40	3	8	
3	35	4	11	p=0.02
4 or more	32	8	25	
Nr. of breast cancers per 100 women *				
less than 20	34	5	15	
20-39	57	6	11	
40-59	13	0	0	p=0.8
60 or more	14	4	29	
Nr. of affected first-degree relatives				
< 2 affected	20	1	5	
2	46	4	9	
3	29	4	14	p=0.03
4 or more	23	6	26	
Average age at breast cancer (y)				
lower than 30	1	0	0	
30-39	13	1	8	
40-49	55	10	18	p=0.5
50 or over	49	4	8	

* A case of bilateral breast cancer adds one to the count of breast cancers in the family, as does a case of breast and ovarian cancer in the same individual.

The *BRCA* screen that was used in the current study may differ from the screens that were applied to the samples that provided the data for the development of the tested probability models. However, any differences in the number of identified mutations, caused by differences in screening techniques, are assumed not to be linked to particular clinical characteristics, leaving the relative validity of various models and clinical characteristics unaffected. The entire coding sequence of both genes was investigated in most families but only the mutations found by the above methods, which have been applied to all the families in the study sample, have been included in the analysis.

Deleterious mutations could be identified in 37 of the 155 families (24%), twenty-four of these mutations were at *BRCA1* (65%) and 13 at *BRCA2* (35%). Six mutations occurred in more than one family but overall 26 different deleterious mutations could be identified, 14 of which had not been previously entered in the Breast cancer Information Core database at the time of identification.

Data Collection and Analysis

The family histories representing lineages suggestive of an autosomal dominant predisposition to breast cancer, ovarian cancer, or both, were based on the information provided by the patients of the Family Cancer Clinic, supplemented with pathology reports pertaining to consenting family members' neoplasms, when available.

The individuals in the lineage of interest who had been diagnosed with breast cancer, ovarian cancer, or both, were considered affected family members. Lineage members related through an unaffected male were added to the number of first-degree relatives for the purposes of the analysis. Affected family members add one to the count of affected first-degree relatives if they belong to the largest string of affected first-degree relatives in the family. The average age at breast cancer diagnosis is the arithmetic mean of the known ages at diagnosis of breast cancer in the family.

Statistical significance was assessed with two-tailed Fisher's exact and Mann-Whitney tests calculated with the IDAMS software programme (18).

The Couch, Shattuck-Eidens, Frank, and Vahteristo models were applied to the study data in the exact same manner as described in the original publication of reference (2-4, 8). The BRCAPRO model was applied by means of a dedicated computer programme obtained

from M. Euhus at the University of Texas Southwestern Medical Center. Probability estimates were calculated for the individual that provided the sample for the *BRCA1/2* screen except for the Couch and Vahteristo models that provide estimates for families as a whole. The probability for the screened affected family member to carry the mutation was taken to be equal to the probability for the identification of a mutation in the family in these cases.

Predicted probabilities were compared with the outcome in terms of identified mutations by means of receiver operating characteristic (ROC) curves that plot the sensitivity against the false-positive rate, calculated as 1 - specificity. The sensitivity of a genotype family characteristic, or a probability model threshold, is calculated for the purposes of the analysis as either the probability that the family characteristic will be present, or either that the threshold will be reached, in the subset of families with an identified mutation. The specificity is the probability that either the family characteristic is absent, or either the calculated probability score does not reach the threshold, in the families with a negative screen. The various models' ROC curves are constructed by plotting 0.1 probability interval thresholds' sensitivities against their false positive rates, starting with the probability 1 threshold entailing a 0 or near 0 sensitivity and false positive rate, the probability 0 threshold entailing a sensitivity and false positive rate of 1, other thresholds entailing values in between. The area under the ROC curve represents the probability that a set of two families, one with and one without an identified mutation, will be scored in the correct order by the model, i.e. the family with an identified mutation scoring higher than the family without.

Correlation of the various probability models' estimates was assessed by Bland-Altman diagrams that plot the difference between the probability estimates of two models against their arithmetic mean for each of the families in the study sample that both models could be applied to (19). But 9 pairs of models could thus be compared in individual Bland-Altman diagrams, the Couch and Shattuck-Eidens models being limited to estimating the probability that a mutation will be identified at *BRCA1* and the Vahteristo model being limited to estimating the aggregated probability that a mutation will be identified at either the *BRCA1* or the *BRCA2* gene.

Results

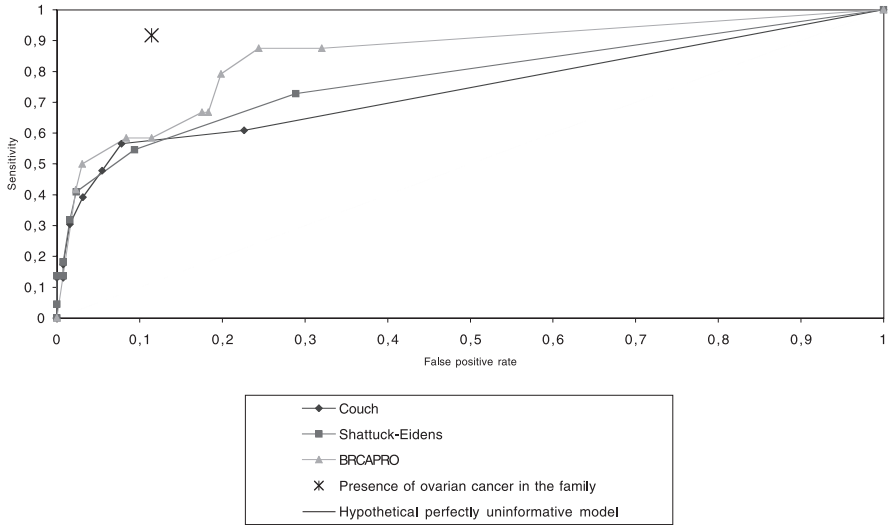
The presence of ovarian cancer is, as confirmed by table 1, a strong predictor of the identification of a germline *BRCA1/2* mutation in a hereditary breast and ovarian cancer family. The presence of breast

and ovarian cancer in a single individual, which occurs in fewer families, is even a stronger predictor. However, this characteristic may be of limited use in the context of the current study since the mutation identification rate that is associated with ovarian cancer has been high enough in itself to warrant the initiation of a *BRCA* screen in most clinical situations. Other characteristics illustrated in table 1 that appear to be weaker, but statistically significant predictors of a mutation, may be of limited practical significance for the same reason. The data on the clinically relevant subset of breast cancer-only families have been analyzed separately as is shown in table 2. There is no significant association of identified mutations with a family history of bilateral breast cancer in the study sample as a whole nor in the subset of breast cancer-only families. No mutations were identified in the five families that had male breast cancer. There is a statistically significant correlation of the number of affected individuals, and the number of affected first-degree relatives in the family, with identified mutations in the study sample as a whole, and in the subset of families without ovarian cancer. The association of identified mutations with the number of affected individuals per 100 women in the family was at the border of significance in the study sample as a whole but not at all statistically significant in the subset of families without ovarian cancer. The same can be said for the average age at which breast cancer was diagnosed in the family. The association of the lowest age at which diagnosis of breast cancer occurred with identified mutations, which is taken into account in the Vahteristo model, scored even lower than the average age at diagnosis.

The Couch, Shattuck-Eidens, Frank, Vahteristo, and BRCAPRO models could be applied to respectively 151, 150, 73, 150, and all 155 of the study sample families and the Frank and Vahteristo models respectively to 50 and all 117 of the subset of breast cancer-only families. The Frank model could be applied to less than half of the families because it is limited, when applied exactly as published, to predicting the probability that a *BRCA1/2* mutation will be identified in a woman with breast cancer under 50 years of age.

The ROC curves shown in figure 1 do not indicate the Couch and Shattuck-Eidens models to have a higher validity for predicting the identification of a mutation at *BRCA1* than the BRCAPRO model. It was not considered necessary, for the purposes of the current study, to attempt to adapt the Couch and Shattuck-Eidens models to allow them to predict the identification of a mutation at *BRCA1* or *BRCA2*, since at least one of the models, that are available for this purpose, seemed to perform as well.

Figure 1: Performance of models predicting the identification of a mutation at BRCA1

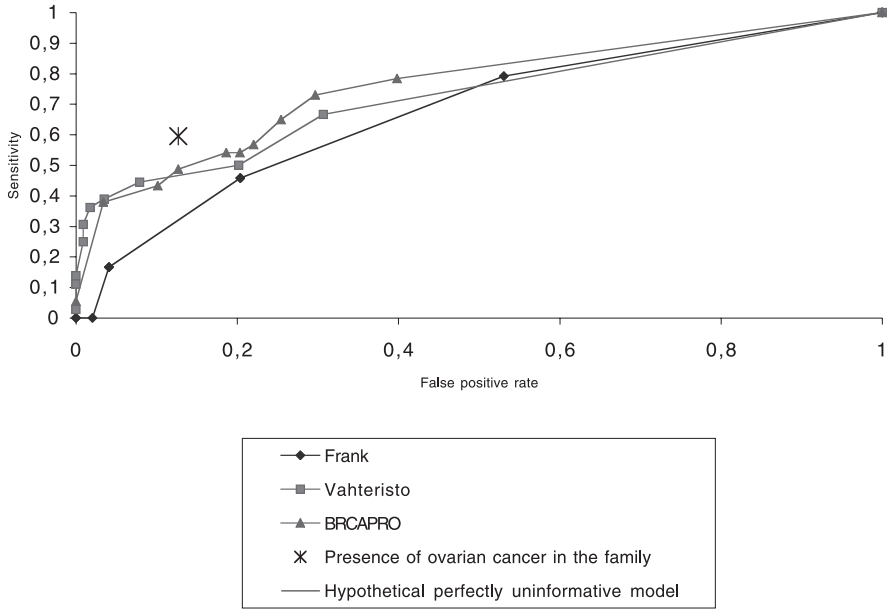


The presence of ovarian cancer in the family is plotted in the same manner as the 0.1 probability interval thresholds of the models.

The assessment of the validity of the estimates of the clinically more relevant probability of identification of a mutation at either *BRCA1* or *BRCA2* was thus limited to the three models that allow this joint probability to be estimated. The Frank, Vahteristo and BRCAPRO models' ROC curves are shown in figure 2 for the entire study sample and in figure 3 for the subset of breast cancer-only families. This subset may be of most interest for the clinician since there are more breast cancer-only families than breast and ovarian cancer families, in few of which there will be doubt about initiating a *BRCA* screen anyway. The Frank and Vahteristo models do not seem to outperform a hypothetical perfectly uninformative model in this subset which leaves the BRCAPRO model to be used in breast cancer-only families.

The two most predictive family characteristics will also be the most readily available: one can always ask patients whether they know of any ovarian cancer in the family and there are bound to be some breast cancers in the families that do not have ovarian cancer but that are still taken in consideration for BRCA screening. These characteristics, i.e. the presence of ovarian cancer and the number of breast cancers in the family, have been selected to be plotted on the graphs that have the relevant ROC curves. The simple family characteristics consistently plot to the area above the ROC curves.

Figure 2: Performance of models predicting the identification of a mutation at either BRCA1 or BRCA2



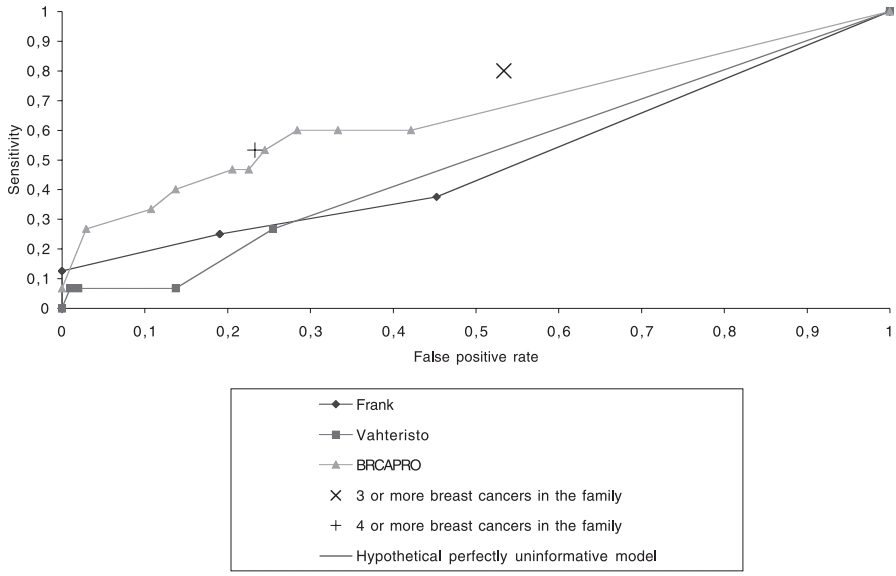
The presence of ovarian cancer in the family is plotted in the same manner as the 0.1 probability interval thresholds of the models.

The correlation between the estimates of the available probability models was revealed to be poor in the Bland-Altman diagrams. Interested readers can obtain copies of the diagrams by contact with the corresponding author.

Discussion

The presence of ovarian cancer in families that are suspected to be hereditary breast and ovarian cancer families is associated with a mutation identification rate of nearly 60% in this study sample, which is consistent with published data (3, 5, 6, 11-13). The presence of ovarian cancer in the family may thus be used as the sole criterion for initiating a *BRCA1/2* gene mutation screen because it would seem to entail a probability of identifying a mutation that is much higher than, for instance, the 10% threshold proposed in the relevant American Society of Clinical Oncology statement (20). The same can be said of breast and ovarian cancer in a single individual which appears to be of even higher significance in the limited number of families that have this characteristic.

Figure 3: Performance of models predicting the identification of a mutation at BRCA1



The presence of ovarian cancer in the family is plotted in the same manner as the 0.1 probability interval thresholds of the models.

The mutation identification rate is much lower in breast cancer-only families and the available means to distinguish the families harbouring a mutation show limited promise in this subset of families. The number of breast cancer cases in the family is slightly more significant than the number of first-degree relatives with breast cancer. The clinician may thus tend first to take account of the number of breast cancers, which is also the easier of the two characteristics to determine. The lack of association of identified mutations with the number of breast cancers per 100 women in the family may be due to incomplete information gathered on unaffected women. The fact that other associations of clinical characteristics with family mutation status could not be shown to be statistically significant in the study sample needs to be interpreted in view of the sample size and the clinical significance of bilateral breast cancer, male breast cancer, and age at diagnosis of breast cancer is in no way disproven.

The available probability models perform better in predicting a mutation in the *BRCA1* gene than in predicting the identification of a mutation at either *BRCA1* or *BRCA2*. The three available models for predicting the identification of a mutation at either *BRCA1* or *BRCA2* show less validity in the subset of breast cancer-only families than in the

study sample as a whole. It is precisely the breast cancer-only families who form the clinically more relevant group, since a *BRCA* screen may be initiated in the other families on the strength of the presence of ovarian cancer alone. The poor correlation between the probability models, demonstrated in the Bland-Altman diagrams, indicates the possibility for the clinician to be confronted with a number of mathematically precise, but widely divergent, probability estimates. One may prefer to use just one probability model, in which case BRCAPRO may be indicated in view of the Vahteristo and Frank model being close to the uninformativity threshold in the breast cancer-only families. The BRCAPRO model may benefit from further validation (21).

The current study's findings may also allow clinicians justifiably to decide whether or not to screen for a *BRCA1* or *BRCA2* gene germline mutation on the basis of a direct assessment of the familial cancer phenotype. Families that have ovarian cancer may be screened if there are at least two affected lineage members. Breast cancer only families may be screened if there are at least three affected lineage members. These criteria may be applied to individual families on a case by case basis, in which case they may be modified in view of the family members' wishes, the extent of the known family history and additional clinical characteristics, such as bilateral breast cancer, male breast cancer, and the age at diagnosis of cancer.

Abstract

Doel: *De waarschijnlijkheid een* BRCA1/2 *mutatie te vinden, is een belangrijke factor bij de beslissing om al dan niet een mutatiescreening te beginnen in bepaalde families. Hierbij kan men zich baseren op de karakteristieken van de familie of op de kans dat er een mutatie zal gevonden worden zoals die kan berekend worden aan de hand van verschillende modellen. Deze studie vergelijkt de relatieve bruikbaarheid van deze indicatoren.*

Methode: *Alle 155 Belgische families die werden gecounseld en aanvaard voor screening voor* BRCA1/2 *kiemcelmutaties bij de Raadpleging Familiale Kanker van de Vrije Universiteit Brussel en waarvan het dossier genoeg klinische informatie bevat om ten minste twee van de Couch (1997), Shattuck-Eidens (1997), Frank (1998), BRCAPRO (1998), of Vahteristo (2001) modellen post hoc te kunnen toepassen, werden in de studie opgenomen. De sensitiviteit en specificiteit van de relevante karakteristieken van de families werden uitgezet op de receiver operating characteristic-grafieken van de waarschijnlijkheidsmodellen.*

Resultaten: *Aanwezigheid van ovariumkanker in de familie gaat gepaard met bijna 60% geïdentificeerde mutaties. Borstkankerfamilies zonder ovariumkanker zijn een klinisch relevante maar moeilijke subgroep voor het voorspellen van de identificatie van een* BRCA1/2 *mutatie. BRCAPRO was het enige informatieve waarschijnlijkheidsmodel*

in deze subgroup maar deed het niet beter dan het aantal borstkankers in de familie als enige criterium om over te gaan tot mutatiescreening.

Conclusie:*Twee familiale karakteristieken, de aanwezigheid van ovariumkanker en het aantal borstkankers in de families waar geen ovariumkanker voorkomt, zijn even bruikbaar voor het inschatten van de kans dat er een* BRCA1/2 *genmutatie wordt gevonden als de beschikbare waarschijnlijkheidsmodellen. Verdere validatie van dergelijke modellen, zoals het BRCAPRO model, lijkt aangewezen.*

Résumé

But: *La probabilité de trouver une mutation constitutive dans les gènes* BRCA1/2 *au sein d'une famille est un facteur important lorsqu'il faut décider d'entamer une analyse génétique. Cette décision peut être prise sur base des caractéristiques de la famille, mais la probabilité de trouver une mutation peut également être calculée à l'aide de différents modèles. La présente étude compare l'utilité relative des ces différents indicateurs.*

Méthodes: *Toutes les 155 familles Belges entrevues lors d'un counseling génétique à la Clinique du Cancer Familial de la Vrije Universiteit van Brussel qui furent admises pour une analyse moléculaire des gènes* BRCA1/2*, et pour lesquelles le dossier médical contenait suffisamment de données cliniques pour pouvoir appliquer post hoc au minimum deux modèles parmi les modèles de Couch (1997), Shattuck-Eidens (1997), Frank (1998), BRCAPRO (1998) ou Vahtersto (2001) furent incluses dans la présente étude. La sensibilité et la spécificité des caractéristiques relevantes des familles ont été mises en graphique sur les receiver operating characteristic graphiques de modèles prédictifs.*

Résultats: *La présence d'un cancer de l'ovaire dans la famille est associée avec une probabilité de presque 60% de trouver une mutation. Le sous-groupe des familles avec plusieurs cas de cancers du sein mais sans cancer de l'ovaire est cliniquement relevant mais difficile pour prédire la probabilité d'identifier une mutation. BRCAPRO était le seul modèle prédictif informatif dans ce sous-groupe, mais sa performance ne dépasse pas l'utilisation du nombre de cas de cancer du sein comme seul critère pour initier une analyse génétique.*

Conclusion: *L'utilisation de deux caractéristiques familiales, la présence de cancer de l'ovaire et le nombre de cas de cancer du sein, est tout aussi utile pour estimer la probabilité de trouver une mutation dans les gènes* BRCA1/2 *que l'utilisation des modèles prédictifs disponibles. Certains modèles, comme BRCAPRO, méritent toutefois d'être plus amplement validés.*

Acknowledgements

The authors gratefully acknowledge the statistical advice of L. Kaufman and the continuing contributions of W. Lissens, R. Sacré, Ph. De Sutter, S. Bauwens, P. Wylock and A. Rigo to the Familial Cancer Team of the Oncology Center of the Vrije Universiteit Brussel, as well as the administrative assistance of I. Pauwels.

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