10.1515/chilat-2016-0002

ORIGINAL ARTICLE

Chromosomal Aberration in Colorectal Cancer Family

Edvins Miklasevics, Mikko Kupila, Dagnija Kalniete, Inese Eglite, Dace Berzina, Monta Ustinova, Gunta Purkalne, Zanda Daneberga
Institute of Oncology, Riga Stradins University, Latvia

SUMMARY
Introduction. Lynch syndrome, previously more commonly known as hereditary nonpolyposis colorectal cancer, is a hereditary cancer syndrome with an autosomal dominant inheritance pattern. Usually it is caused by mutations the MMR genes. In 20 – 25% of cases patients are not found to have mutations in any of these genes. Chromosomal aberrations as a cause of the Lynch syndrome were examined in this study.
Aim of the study. To identify chromosomal aberrations which may lead to colorectal cancer.
Material and methods. Twelve patients, corresponding to either Amsterdam I/II criteria or Bethesda guidelines, which have been tested negative for mutations in Lynch genes have been karyotyped were karyotyped with SNP array chips, in order to determine if they had potentially heritable chromosomal aberrations which could be responsible for increased risk of malignancy.
Results. One patient with a 14.7Mbp duplication framed by small deletions was chosen to be the most likely patient to suffer from an inherited carcinogenic chromosomal aberration. The preceding deletion was found to contain the coding region of *BRE*, encoding a component of the BRCA1-A complex; we believe that this deletion is the most carcinogenic component of the aberration and likely responsible for Lynch syndrome in this case. The larger duplication furthermore contained the coding regions for 83 genes, some of which have been shown to promote malignant disease when overexpressed.
Conclusion. Because of the clinically grossly tolerable nature of the aberration it is possible that it was vertically transmitted and contributed to the onset of colorectal cancer in the patient and his mother and maternal aunt.
Key words: Lynch syndrome, karyotyping, BRCA1-A complex, *BRE gene*

INTRODUCTION

Colorectal cancer (CRC), which collectively refers to cancers of the colon and rectum, is one of the most significant contributors to overall cancer morbidity. In Latvia CRC has the third highest incidence of all malignancies in both genders; in women it is superseded only by breast and skin malignancies, whilst in men by cancers of the prostate and lungs (6). The overall incidence of CRC is increasing. There is a causal relationship between urbanisation, with its associated lifestyle risk factors, and increased risk of CRC (5).

Lynch syndrome, previously more commonly known as hereditary nonpolyposis colorectal cancer (HNPCC), is a hereditary cancer syndrome with an autosomal dominant inheritance pattern; it predisposes affected individuals to several types of malignancies, especially CRC. Lynch syndrome is the most common hereditary cancer syndrome associated with CRC and is accountable for 2-5% of all cases. Patients with Lynch syndrome have a 70-80% lifetime risk of developing CRC. A patient can be diagnosed with Lynch syndrome if she satisfies Amsterdam I/II criteria or Bethesda guidelines, with increasing sensitivity respectively. Mutations in several DNA mismatch repair (MMR) genes are known to cause Lynch syndrome. MMR genes code for proteins which identify and correct errors inevitably occurring during DNA replication; failure of these genes leads to a massive accumulation of these errors, predisposing to malignancy. Listed in order of decreasing contribution to Lynch syndrome cases are mutations in: *MSH2* (45-50%), *MLH1* (20%), *MSH6* (10%), *PMS2* (1%), *PMS1* (<1%), *MSH3* (<1%), *EXO1* (<1%). In 20 – 25% of cases patients are not found to have mutations in any of the known Lynch genes (8).

Unbalanced chromosomal aberrations are characterised by changes in the genome that involve copy number changes in the form of deletions or duplications. Genes have varying sensitivities to changes in their copy number, depending on the task of the coded protein and the sensitivity of associated pathways to changes in protein concentrations (3). Especially aberrations which have only a mild, or no, phenotype can be vertically transmitted. In a study investigating 130 families with vertically transmitted unbalanced chromosomal aberrations, the size of duplications was a poor indicator as to the presence or absence of a clinical phenotype. The same study showed that the majority of families were affected phenotypically. However it was noted that, as with other subclinical diseases, chromosomal aberrations without, or with only mild, phenotypes may suffer from negative acquisition bias (1). In a study of 10,000 healthy sperm donors in France, 0.17% had clinically silent chromosomal aberrations (10).

AIM OF THE STUDY

To identify chromosomal aberrations which may lead to colorectal cancer.

MATERIAL AND METHODS

The study group consisted of 12 Lynch syndrome patients who matched Amsterdam I/II criteria or Bethesda guidelines. All patients had been hospitalised during

2002 and 2012 in Pauls Stradiņš Clinical University Hospital, Oncology Centre of Latvia or Oncology Centre of Daugavpils. Samples were retrieved from the bio bank of Riga Stradiņš University Institute of Oncology. All patients signed informed consent forms. For this case report one colorectal cancer patient was selected.

DNA samples were extracted from 2ml of venous blood by FlexiGene DNA Kit (Qiagen) according to the instructions of the manufacturer. The presence of point mutations in *MLH1*, *MSH2* and *MSH6* genes were excluded by Sanger sequencing. The presence of the chromosomal aberrations was assessed by microarray technology using HumanExome-12 v.1.1 (>240 000 SNP markers) and HumanOmniExpress-24 v.1.1 (>713 014 SNP markers) BeadChip Kits (Illumina) according to the instructions of the manufacturer. Data analysis was performed by GenomeStudio v1.8 software (Illumina). B allele frequency and Log R ratio were used to visualize and interpret chromosomal aberrations.

RESULTS

Of the sampled patients, one male was selected for this case report due to our findings. The selected patient was diagnosed with CRC at age 67; his mother and aunt were also diagnosed with CRC at 64 and 62 years of age respectively. The family did not meet Amsterdam I/II, due to relatively high age of members at cancer diagnosis, but did meet Bethesda guidelines. Both relatives died at the age of 65 (Figure 1). In our first run with the lower SNP density chip (>240,000 SNP markers), we found an alteration consistent of a large duplication in chromosome 2 (Figure 2). The aberration spans bands 2p21 – 2p23.2, with a total length of approximately 15.7Mbp.

During the second run with the higher SNP density chip (>713 014 SNP markers), the alteration was more clearly visualised. A scattering of the B allele frequency plot together with a deviation of the Log R Ratio is indicative of a copy number aberration, with duplications and deletions showing as increases and decreases of the log R ratio respectively when compared to the baseline. With this increased resolution, we were able to determine that the anomaly is in fact a duplication framed by two smaller deletions (Figure 3). The following SNPs illustrate the approximate thresholds of the different components of the aberration: rs466601 at base position 28,019,175 marks the beginning of the preceding deletion, rs6715256 at 28,723,942 marks the transition from deletion to duplication, rs10203499 at 43,466,619 marks the ending of the duplication and beginning of the trailing deletion with rs17030798 at 43,670,183 marking the end of the aberration. Thus the base pair lengths of the preceding deletion, trailing deletion, and duplication are 705.767Kbp, 203.564Kbp and 14,742.677Kbp respectively.

DISCUSSION

A significant number of people have clinically silent chromosomal aberrations. On the outset of this study, we were expecting to find a deletion of chromosome segments containing MMR genes, which in turn would have resulted in the classical mechanism of Lynch syndrome. Instead we found one patient to have a large, 14.7Mbp, duplication of genomic DNA within chromosome two, framed by small deletions. Deletions and duplications in this area of the genome have been documented in the Database of Genomic Variants, but no duplications of this size (11).

The duplication contains coding regions of 76 genes, according to RefSeq; among these are three: *PRKD3*, *MTA3* and *SOS1* which we would like to highlight because their overexpression has been associated with malignancies. Expression of the *PRKD3* (protein kinase D3) gene has been shown to be elevated in triple negative breast cancer (TNBC); accordingly knockout of *PRKD3* has been shown to strongly inhibit proliferation of TNBC (4). Overexpression of the *MTA3* (metastasis associated 1 family member 3) gene has been shown to promote progression of non-small cell lung cancer (7). Enhanced expression of the *SOS1* (son of sevenless) gene has been shown in prostatic carcinoma; down regulated expression on the other hand decreases metastatic potential of prostatic carcinoma cells (10). We do not know, however, if a duplication of these genes would lead to a sufficient overexpression in order to predispose to malignancy.

According to RefSeq, the framing deletions contain coding regions of six genes. The larger, preceding deletion, contains *BRE* (Brain And Reproductive Organ-Expressed Protein). *BRE* codes for a component of the BRCA1-A complex, which is the best understood of the BRCA1 complexes and has a significant role in DNA repair (2). Furthermore, levels of *BRE* expression have been shown to correlate with cancer progression in several studies confirming its significance in BRCA1-A complex function (2; 9). With one incapacitated copy of *BRE* at birth, our patient would have had his first hit to a significant DNA repair gene; a scenario, we propose, not dissimilar to inheriting a mutated MMR gene. As second hits are caused by mutations which randomly occur across the genome, and would statistically affect the *BRE* and MMR genes at the same age, we speculate that the damaged BRCA-1A complex would not lead to as high an increase in mutation rate as in the case of lost MMR genes, which in turn would explain the delayed onset of CRC in our patient's family when compared CRC caused by mutations of known Lynch genes. It is thus tempting to infer that the patient and his relatives acquired CRC due to this deletion in particular.

The aberration is grossly tolerated as the patient shows no obvious clinical signs usually associated with chromosomal aberrations such as mental retardation or morphological defects at birth. Thus it is credible that it could have been vertically transmitted, and thereby could have been responsible for an elevated cancer risk in the two affected first degree relatives; furthermore, due to the absence of obvious clinical signs, the mutation would have gone unnoticed in them. Unfortunately, the aforementioned family members are since deceased and thus it is not possible to explore the inheritance of this aberration further.

CONCLUSION

Because of the clinically grossly tolerable nature of the aberration it is possible that it was vertically transmitted and contributed to the onset of colorectal cancer in the patient and his mother and maternal aunt.

Conflict of interest: None

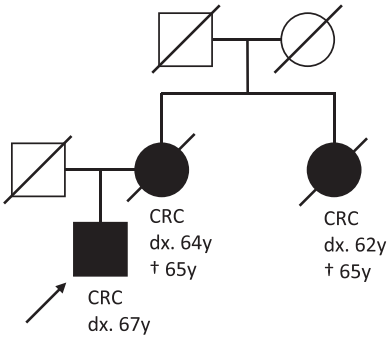

Fig. 1. Pedigree with the sampled patient marked by an arrow. Affected family members annotated with type of cancer, age at diagnosis as well as age on exitus

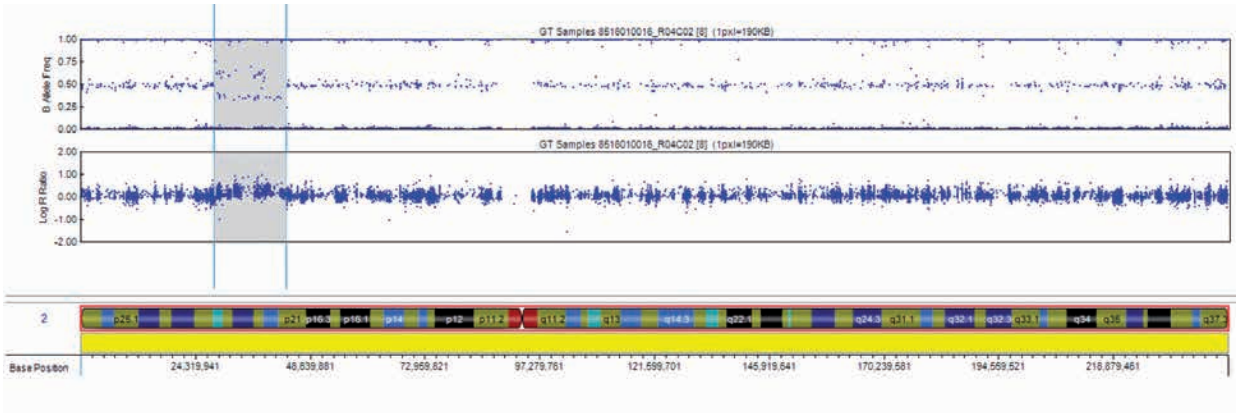

Fig. 2. Low SNP density BeadChip run, 2nd chromosome is shown in its entirety. Blue dots represent individual SNPs. The range of the aberration has been highlighted

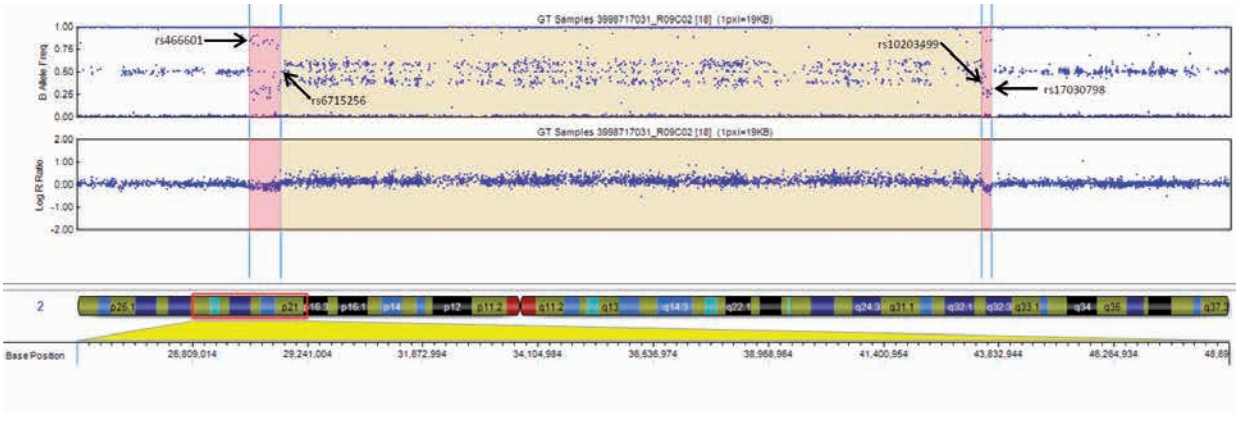

Fig. 3. High SNP density BeadChip run, zoomed into the affected bands. Deletions highlighted in red, duplication in yellow. SNPs selected to illustrate thresholds have been annotated

REFERENCES

1. Barber J. C. K. Directly transmitted unbalanced chromosomal abnormalities and euchromatic variants // Journal of Medical Genetics, 2005; 42: 609-629. doi: 10.1136/jmg.2004.026955
2. Bian C., Wu R., Cho K., Yu X. Loss of BRCA1-A Complex Function in RAP80 Null Tumor Cells // PLoS ONE, 2012; 7 (7): e40406. doi: 10.1371/journal.pone.0040406
3. Clancy S., Shaw K. DNA deletion and duplication and the associated genetic disorders // Nature Education, 2008; 1 (1): 23
4. Huck B., Duss S., Hausser A., et al. Elevated Protein Kinase D3 (PKD3) Expression Supports Proliferation of Triple-negative Breast Cancer Cells and Contributes to mTORC1-S6K1 Pathway Activation // The Journal of Biological Chemistry, 2013; 289 (6): 3138-3147. doi: 10.1074/jbc.M113.502633
5. Järvinen H. J., Kouri M., Österlund P. Paksusuolen ja peräsuolen syövän yleisyys // Syöpätaudit / Ed. by Joensuu H., Roberts P. J., Kellokumpu-Lehtinen P., et al. – 5th ed. – Helsinki: Duodecim, 2013. – Pp. 483
6. Lepiksone J., Mauriņa A., Misiņš J., et al. STATISTICAL YEARBOOK OF HEALTH CARE IN LATVIA. – 15th ed. – Riga: Centre for Disease Prevention and Control of Latvia, 2014. – Pp. 94-100
7. Li H., Sun L., Xu Y., et al. Overexpression of MTA3 Correlates with Tumor Progression in Non-Small Cell Lung Cancer // PLoS ONE, 2013; 8 (6): e66679. doi: 10.1371/journal.pone.0066679
8. Munoz J. C., Lambiase L. R. Hereditary Colorectal Cancer // http://emedicine.medscape.com/article/188613 (accessed 13.06.2015.)
9. Noordermeer S. M., Wennemers M., Bergevoet S. M., et al. Expression of the BRCA1 complex member BRE predicts disease free survival in breast cancer // Breast Cancer Research and Treatment, 2012; 135: 125-133. doi: 10.1007/s10549-012-2122-5
10. Racel C., Berthaut I., Bresson J. L., et al. Prevalence of chromosomal abnormalities in phenotypically normal and fertile adult males: large-scale survey of over 10 000 sperm donor karyotypes // Human Reproduction, 2006; 21 (6): 1484-1489. doi: 10.1093/humrep/del024
11. The Center for Applied Genomics. Database of Genomic Variants // dvg.tcga.ca (accessed 15.06.2015.)
12. Timofeeva O. A., Zhang X., Ressom H. W., et al. Enhanced expression of SOS1 is detected in prostate cancer epithelial cells from African American men // International Journal of Oncology, 2009; 35 (1): 751-760. doi: 10.3892/ijo_00000388

ACKNOWLEDGEMENT

This study was supported by ESF grant Agreement No. 2013/0047/1DP/1.1.1.2.0/13/APIA/VIAA/017

Address:
Edvins Miklasevics
Institute of Oncology
Riga Stradins University
Dzirciema iela 16, Riga LV1007
e-mail: Edvins.Miklasevics@rsu.lv