Swedish University of Agricultural Sciences
The Faculty of Natural Resources and Agricultural Sciences
Uppsala BioCenter
Department of Microbiology

Identification of somatic mosaicism in the blood and uninvolved margin in breast cancer patients

Kancherla Reddy Prakash

Independent project • 30 hec • Second cycle, A1E
Biotechnology • ISRN: SLU-MIKRO-EX - 11/15-SE: 2011:15 • ISSN 1101-8151
Uppsala 2011

Identification of somatic mosaicism in the blood and uninvolved margin in breast cancer patients

Kancherla Reddy Prakash

Supervisor: Tomas Linder, Swedish University of Agricultural Sciences, Department of Microbiology

Assistant Supervisor: Jan P. Dumanski, Uppsala University, Department of Immunology, Genetics and Pathology, Rudbeck Laboratory

Hamid Reza Razzaghian, Uppsala University, Department of Immunology, Genetics and Pathology, Rudbeck Laboratory

Lars Forsberg, Uppsala University, Department of Immunology, Genetics and Pathology, Rudbeck Laboratory

Examiner: Volkmar Passoth, Swedish University of Agricultural Sciences, Department of Microbiology

Credits: 30 hec
Level: Second cycle, A1E
Course title: Independent project in Biology D
Course code: EX0564
Programme/education: Biotechnology

Place of publication: Uppsala
Year of publication: 2011
Title of series: no: ISRN: SLU-MIKRO-EX - 11/15-SE: 2011:15
ISSN: 1101-8151
Online publication: http://stud.epsilon.slu.se

Key Words: Breast cancer, uninvolved margin, somatic mosaicism and illumina genotyping

Swedish University of Agricultural Sciences
The Faculty of Natural Resources and Agricultural Sciences
Uppsala BioCenter
Department of Microbiology

ABSTRACT

Somatic mosaicism is the presence of genetically diverse lineage of somatic cells within an organism even though deriving from a single zygote. Mosaicism is known to occur in many diseases. Breast cancer is the second most cause of deaths in woman caused by malignant tumor. Breast cancer was a common malignancy and main cause of morbidity and disease related mortality among women. By studying the genetic profiles of the cells surrounding the primary tumor and blood of same patient may reveal the cause of tumor cells. The purpose of this study was to identify the presence of somatic mosaicism in healthy uninvolved margin of breast tissue surrounding the primary tumor and blood of breast cancer patient by using Illumina 1M BeadChip and Nimblegen 720K array.

So far, all long and very rigorous research explains only 10% of the breast cancer. This indicates the complexity of cancer and the factors responsible for the development of tumor in healthy tissues. Results from whole genome genotyping by Illumina 1M beadchip of blood and cells surrounding the primary tumor of same patient indicate the presence of somatic mosaicism in chromosome 6, 8 and 18 of ML36B (uninvolved margin), ML36A2 and ML36A3 (primary tumor) which was confirmed by genotyping with Nimblegen 720K microarrays. Genotyping also showed the presence of genomic imbalance in genetic makeup of the healthy tissue surrounding the primary tumor.

It is to be likely that rate of discovery of somatic mosaicism in the small proportion of studies cells might continue to accelerate the studies on genetic heterogeneity of breast malignancies. As well as studies by comparing the genetic profiles of tumours and tissue surrounding the primary tumour may lead to identify the genomic aberrations in context to cancer progression.

ABBREVIATIONS

BRCA	Breast cancer susceptibility gene
cDNA	complementary Deoxyribonucleic acid
CGH	Comparative Genomic Hybridization
DNA	Deoxyribo Nucleic acid
EDTA	Ethylenediaminetetra acetic acid
EGF	Epidermal growth factor
ERBB	Avian erythroblastosis oncogene B
FAH	Fumarylaceto acetate hydrolase
gDNA	Genomic Deoxyribonucleic acid
LOH	Loss of Heterozygosity
NaAc	Sodium acetate
NaCl	Sodium chloride
PBS	Sodium perborate
PTEN	Phosphatase and tensin homolog
rpm	Revolutions per minute
SNP	Single nucleotide polymorphism
TGFβ	Transforming Growth Gactor beta
WGG	Whole Genome Genotyping

CONTENTS **PAGE NO**

INTRODUCTION

Cancer

Cancer is a class of genetic disease that evolves through genetic alterations. During last decades the extensive research of molecular mechanism of tumour formation has broadened our knowledge about tumour formation[1]. The generation of cancer is due to the alteration in three types of gene families: proto oncogenes, tumour suppressor genes and stability genes [2]. The normal function of proto oncogenes is to promote cell growth or survival, when mutated or expressed at high levels can cause tumour formation. The functional absence of tumour suppressor genes can lead to cancer. Carcinogenic mutation in these genes may result in inactive gene or gene products which are then unable to inhibit cell growth. The stability genes also known as caretaker genes are types of genes responsible for DNA repair. When stability genes are inactivated, mutation occurs at higher rates in other genes and genetic rates can't be controlled [2].

A single mutation cannot transform normal cell to a cancer cell as there are safeguard mechanisms existing. For example two genetic alterations is required for cultured rodent cells where as human cells require at least three genetic alterations [3].

All the cells have multiple control systems. When these control systems are disrupted it always leads to tumour development. These genetic changes include evasion of apoptosis, tissue invasion and metastasis, self-sufficiency in growth signals, block of differentiation, limitless replicative potential, sustained angiogenesis and genomic instability (Figure 1) [1].

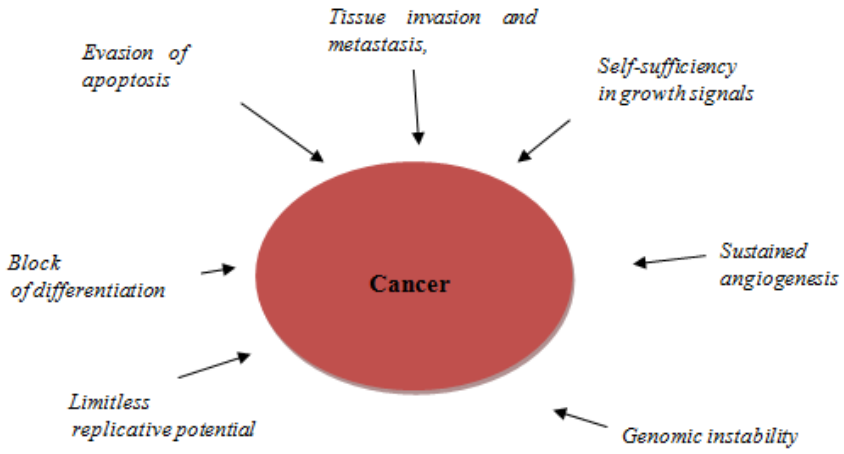


Figure 1. Schematic diagram of cellular process affected by genetic alterations in cancer cells.

However cancer in fundamental nature is a disease in which environmental factors and causal genetic abnormalities act together [4]. Most of mutations in cancer occur in somatic cells and are not inherited to next generation. On the other hand inherited mutations that are carried in germ line may influence cells to tumorigenesis specially when combined with somatic mutation. Somatic mutation include several classes of DNA changes such as insertion or deletion of DNA fragments, copy number increases and reductions, base substitution and chromosomal rearrangements [5]. Projects that resequence tumour genomes have analytically discovered thousands of genes which are not linked to tumorigenesis but in a small fraction they are somatically mutated and may be vital for tumour progression and initiation [6-11]. Several of these somatic changes are expected to be passengers which have no functional effects but are already present in cell that rise to tumour or acquire during tumour growth [6].

The most pressing challenge in cancer genetics is determining mutations which are drivers and passengers. Driver genes are mutated very frequently and genes which are discovered thus far are mutated in a relatively small fraction of tumours [6]. The examination of large number of tumour can give information for classification of drivers versus passengers, but capability of sequencing alone to give definite results is limited due to variation in mutation frequency in individual tumours and individual genes [6].

Breast Cancer

Breast cancer constitutes 10.4% of all cancer prevalence worldwide among women and it is the second common type of non-skin cancer and fifth common causes of cancer deaths [12]. The cancer that originates from epithelium of glandular tissue, most commonly from lobules which are milk-producing glands or ducts which produce milk to nipple is referred as breast cancer (Figure 2). The carcinomas that originate from ducts are called ductal carcinomas and carcinomas that originate from lobules are called lobular carcinomas.

Breast tumours can be classified histologically into invasive or non-invasive (*in situ*) carcinoma. Invasive ductal carcinoma is the most common of all cases and it accounts for 80% [13]. Cancer cells can attack nearby healthy breast tissue and metastasize to lymph nodes. Through spreading via lymph nodes or blood they can spread to other distal parts of the body [14]. The fatal outcome of the disease is caused by the metastatic spread of tumour cells.

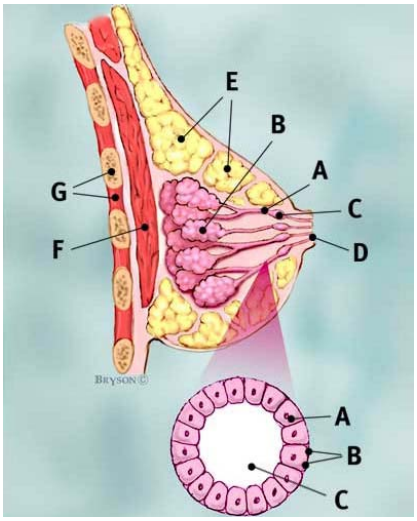


Figure 2. Breast anatomy. **A** milk ducts, **B** milk lobules, **C** dilated section of duct to hold milk, **D** nipple and areola, **E** fatty tissue, **F** pectoralis major muscle, **G** chest wall/rib cage; **Enlargement**: **A** normal duct cells, **B** basement membrane, **C** lumen (center of duct). Adapted and modified from http://www.breastcancer.org/illustrations/i0013.html.

Like many other malignancies, breast cancer also results from stepwise genetic alterations of host cells and from other epigenetic changes in the behavior of not only cancer cells but also the host cells which interact with the tumor, like vascular, immune and stromal cells [15]. However, only a small proportion of breast cancer can be attributed to inherited mutation. Around 5-10% of mutations causing breast cancer are due to the inactivation of autosomal dominant genes: *BRCA1* and *BRAC2* the breast cancer susceptibility genes. *BRCA1* and *BRAC2* genes operate as tumor-suppressor genes have been identified as main contributors to hereditary breast cancer [16, 17]. Mutations in either of these genes account for majority of families in multiple cases. Up to 85% confer a lifetime risk of breast cancer in female *BRCA1*

and *BRAC2* mutation carriers [17]. In addition, other germ line mutations in tumor-suppressor genes like *PTEN* or *TP53* genes has been implicated to increase susceptibility to breast cancer [16].

Of all the cases, the most widespread is sporadic breast cancer which is non-hereditary. Sporadic cases occur due to gradual accumulation of uncorrected and acquired mutations in somatic mutations during lifetime [14, 18].

One of the most important oncogenes essential in development of breast cancer is tyrosine kinase *ERBB2,* which belongs to *ERBB* family. Approximately 60% of *in situ* carcinomas are excessively amplified in *ERBB2* [19]. It has been found that over expression of *ERBB2* can lead to tumorigenesis, transformation and metastasis. Growth factors like *EGF* and *TGFβ* can also be involved in mammary growth and differentiation at different stages of development [14].

Breast cancer in perspective of somatic mosaicism

Somatic mosaicism implies the presence of genetically distinct lineages of somatic cells in a single organism that are resulting from same zygote. The term mosaicism can be used to all types of aberrations from point mutations to aneuploidies. Humans develop from a single cell. From this single cell an individual grows up to 10^{13} to 10^{14} cells. During the growth cells undergo many cell divisions and as a result much somatic mutation occurs. These mutations occurring during the development likely have major consequences for genetic mosaicism in the body and for the risk of cancer that occur from those mutations [20, 21]. The remarkable evolutionary history has been hidden by the difficulty of measuring the genetic changes in the cell. Recently new high-throughput technologies are just opening up the possibility of measuring somatic mutations and evolution [22]. To understand the evolutionary history of an individual and cause of disease, somatic genomics must be considered within the context of rate and pattern evolutionary changes in cell lineages.

In biology, during the past several years, germ-line and somatic mosaicism have emerged as important factors that put in phenotypic variability. More than 30 monogenic disorders show variable expressivity due to somatic mosaicism [23]. Mosaicism also leads to certain aspects like embryo twinning and mitochondrial disorders which is a principle cause of antigenic diversity [24, 25]. It was proposed that neoplasm results from a single cell which undergoes genetic changes and later studies on nucleotides and chromosomes proved the existence of somatic mutation in most cancers [26]. Unregulated cell divisions occur due to the accumulation of somatic mutation in certain pathways [27]. The difference between somatic mosaicism and germline mosaicism (gonadal mosaicism) is based upon the findings of population of cells which are genetically different in somatic and germline tissue [28].

Chimerism is a closely related but different term, and should not be confused with mosaicism. Chimerism is defined as the presence of two or more cell lines in an organism that are derived from different zygotes. During embryogenesis cells from two different embryos might mix. Although this is thought to be rare event, but quite common in dizygotic twins. This has been shown by transplantation experiments that such chimeric individuals do not reject the graft from their twins as foreign [25, 29]. The rate of mosaicism may depend on the particular disorder, tissue of origin or selective pressure. More than 83% of patients with inherited mutations in fumarylacetoacetate hydrolase (FAH) gene which causes hereditary tyrosinaemia type 1 appear to have mosaic liver that have mutant and reverted population of hepatocytes [30]. The two monogenic disorders – Blooms syndrome and Fanconi anaemia are used to illustrate the cellular basis, clinical importance and mechanistic range of somatic mosaicism [31, 32]. Structural abnormalities of chromosomes also cause somatic mosaicism. Cytogenetic

studies in more than one-third of patients with Fanconi anaemia have detected clonal variations which arise due to failure of cellular mechanisms that prevent non-disjunction or abnormal chromosome segregation [33].

In hemizygous males, certain X-linked disorders like incontinentia pigment and Rett syndrome are usually fatal. In rare cases males who survive with these conditions are informative about somatic mosaicism. Moreover, in these cases mosaicism for a DNA mutation or chromosome can result in survival of male patients [34, 35].

Monozygotic twins also called identical twins signify an extraordinary source for understanding many disorders, phenotypes and complex genetic diseases. Approximately 3% of deliveries are twin births, in which ~10% are monozygotic [36, 37]. While most of the studies to date have observed that somatic mutations are connected with various forms of cancer, but there is mounting proof that other diseases are also caused due to somatic mutation. In recent studies multiple somatic mutations was reported in two brothers with Wiskott-Aldrich syndrome an immunodeficiency disease [38].

Another example of somatic mutation and mosaicism is Heteroplasmy, a phenomenon found in diseases associated with alterations in human mitochondrial DNA. There are different percentages of affected mitochondria in different tissues or cells in within the tissue which was found in maternally inherited diabetes and deafness [39].

METHODOLOGICAL BACKGROUND

Genotyping Methods

Development of new genotyping methods led to advanced studies on genetic variations. The process of determining the genetic variants of an individual by means of molecular techniques such as SNP microarray technology is called genotyping. The process of microarrays is based up on hybridization between two strands, one DNA oligonucleotide and a complementary sequence. Standard DNA microarrays are made up of glass or plastic slide which is coated with a frame of approximately 200,000 microscopic spots, each subsequent to a gene or DNA segment. By using microarrays the expression levels of thousands of genes in a cell can be determined by directly measuring the amount of mRNA attached to each probes on the array. The fluorescent tags are excited by laser and with microscope and camera the digital image of the array is made together. The florescent signal intensity of each spot on the array is calculated by computer program and creates a profile of gene expression in the cell.

The completion of human genome sequence has unraveled the genetic causes of human diseases. In recent times, the international HapMap consortium refined collecting SNP haplotype information from three major populations [40]. This haplotype and associated tag SNP information may help in studying the genetics of may complex diseases. Recent development in genotyping technology have allowed in analysis of 100,000 of SNPs across hundreds to thousands of samples. Furthermore, whole genome SNP arrays has been used for high-resolution analysis of loss of heterozygosity (LOH), DNA copy number and other chromosomal aberrations in cancer and inherited diseases [41].

During the past, based upon the comparative genomic hybridization (CGH) several technologies have been used to study gain and loss of genomic DNA in cancers and developmental disorders. It is well known that physical changes in DNA copy number and/or in allelic ratios of particular genomic regions are associated with tumor development [42]. In specific, LOH has been widely used in discovery of tumor suppressor genes like RB1, TP53 and WT1 which are involved in formation of retinoblastoma, Li-Fraumeni syndrome and Wilm's tumour respectively [43]. Compared to earlier techniques, the introduction of whole genome genotyping (WGG) array technology allows combined DNA copy number and LOH analysis at high resolutions.

Nowadays there are different types of commercial SNP arrays available in the market. One of them is Illumina Human1M BeadChip. Illumina Human1M BeadChip uses the established Infinium assay to profile human genome at more than one million loci, used for detection of structural variations (chromosomal aberration) found across the genome. SNP genotyping can indicate duplications, deletions and amplifications which can be associated with cancer. Illumina Human1M BeadChip detects chromosomal aberrations by comparing the normalized intensity of signal (R) of a subject sample and a pool of reference samples. The genomic plots of the log2 (R subject / R reference) called log R ratio, and B allele frequency (BAF) value are the basis for detection of chromosomal aberrations. By measuring the intensities of signals in test DNA versus reference DNA the loss and gain of chromosomal aberrations can be identified.

Nimblegen

High-resolution array CGH has quickly emerged as a method for detection of chromosomal abnormalities related with cancer and other complex phenotypes. Nimblegen Human CGH 3x720K Whole-Genome Tiling array contains empirically tested 720,000 probes per array, which provides analysis of whole human genome. The test and reference genomic DNA were separately labelled with different fluorescent dyes and co-hybridized to a 720K whole genome

tiling array. By using Nimblescan software, the log2-ratio values of the probe signal intensities were calculated. The advantages of Nimblegen 720K array are detection of copy number variations, segmental duplications and chromosomal aberrations like duplication, deletions and amplifications across the genome.

AIM

The main objective of the study was to identify the presence of somatic mosaicism in blood and healthy uninvolved margin of breast tissue surrounding the primary tumor in breast cancer patients. The validation of the presence of somatic mosaicism was carried out by Nimblegen Human CGH 3x720K Whole-Genome Tiling array.

MATERIALS AND METHODS

Assortment of Samples

A total of 60 pairs of samples blood and uninvolved margin of same breast cancer patient from Gdansk were included in this study. Prior to DNA extraction the samples was stored in -70ºC.

DNA extraction from tissue

A small amount of tissue was taken and chopped with a sterile scalpel. The tissue was transferred into a labeled 15 ml falcon tube. To this 4 ml of lysis buffer (10 mM EDTA, pH 8.0. 10 mM Triscl pH 7.5-8.0 and 50 mM NaCl) was added. The tissue was disrupted with Qiagen Tissue Ruptor at full speed until tissue was ruptured thoroughly. Disposable probes for each sample were used to prevent cross-contamination. A pinch of proteinase K was added and mixed well. Later 500 µl of 10% Sarcosyl was added and mixed gently until the solution became viscous. The tubes were incubated overnight at 50ºC. To this equal amount of phenol was added and mixed well for 20 min on a suitable mixer. The contents were centrifuged for 10 min at 4000 rpm in room temperature. Maximum volume of upper phase was transferred to a fresh tube; to this equal volume of chloroform+isoamyl alcohol (24:1) was added. The content was mixed on suitable mixer for 20 min and centrifuged for 20 min at 4000 rpm in room temperature. The upper phase was transferred to a new tube and 1/10 volume of 3 M NaAc was added and mixed well. Later twice the volume of 95% ethanol was added and mixed gently by inverting tubes for few minutes until precipitate was formed and later incubated in -70ºC for 15 min. The contents were allowed to thaw and centrifuged at 4000 rpm for 15 min. The supernatant was discarded and to this 80% ethanol was added and pellet was washed by disrupting and centrifuged at 4000 rpm for 15 min. The wash solution was discarded and the pellet was allowed to air dry. As soon as the pellet was dried, based on size of pellet, DNA was resuspended in 100-200 µl sterile distilled water.

DNA extraction from blood

DNA was extracted from blood by using QIAamp DNA Blood Maxi Kit (Qiagen), according to manufactures instructions.

To the empty 50 ml falcon tubes 500 µl protease K was added provided along with kit. The blood sample was added to the tubes and volume was brought up to 10 ml by adding PBS. To this 12 ml of AL buffer (Lysis buffer) was added and the contents was mixed thoroughly by inverting tubes 15 times and vortexing for 1min. The tubes were incubated at 70ºC for 10 min. 10 ml of 96 % ethanol was added and mixed by inverting the tubes several times and by vortexing for 1 min. The obtained volume was transferred to QIAamp Maxi column and centrifuged for 3 min at 1850xg (3000rpm). The filtrate was discarded and to the QIAamp Maxi column 5 ml of AW1 buffer (wash buffer) was added. The tubes were centrifuged for 1 min at 4500xg. To this 5ml of AW2 buffer was added and centrifuged for 15 min at 4500xg. QIAamp Maxi column was placed into a new 50 ml tubes provided along with kit and to this 500 µl sterile distilled water was added directly on to the QIAamp Maxi column membrane and incubated for 5 min at room temperature and centrifuged for 4500xg for 3min. The eluate was transferred into fresh tubes.

Measurement of DNA quality and concentration

The quality and concentration of DNA extracted from tissue and blood was measured by using spectrophotometer- NanoDrop® ND-1000 and Quant-iT™ PicoGreen® dsDNA Assay Kit.

Picogreen

A standard curve was plotted by diluting λ DNA 100 µg/mL provided along with the Quant-iT™ PicoGreen® Kits (Molecular Probes™, Invitrogen) with TE buffer (1 mM EDTA and 10 mM Tris). A series of dilutions from 0.0 to 2000 ng/ml was made (Table:1)

To the 96 well spectroflourimeter plate 100 µl of the prepared standard dilutions was added.

1 µl of each DNA sample was added in duplicates and to this 99µl of 1x TE buffer was added. The plate was incubated on a shaker for 1 hr at 200rpm.

Tube	Final concentration [ng/ml]
1	2000
2	1000
3	500
4	250
5	125
6	62,5
7	31,25
8	0

Table 1: Serial dilutions of λ DNA with their final concentrations used to obtain a standard curve for measurement of DNA concentration with picogreen.

A working solution of picogreen reagent was made by a 200 fold dilution of the concentrated DMSO stock with TE-buffer. To achieve 2000ng/ml final concentration of Lambda DNA 40µl of stock □ DNA was mixed with 1960 µl of TE buffer. 1000 µl from this solution was transferred to a tube with 1000 µl of TE buffer. The next step was performed in the same way and last solution was left blank. After incubation 100 µl of standard working picogreen solution was added to each well containing DNA and to the wells containing standard dilutions. Fluorescence was determined by using Wallac 1420 Victor 2 plate reader machine.

Fluorescence of standard curve was determinate by using Wallac 1420 Victor 2 plates reader and plotted in excel (Figure 3).

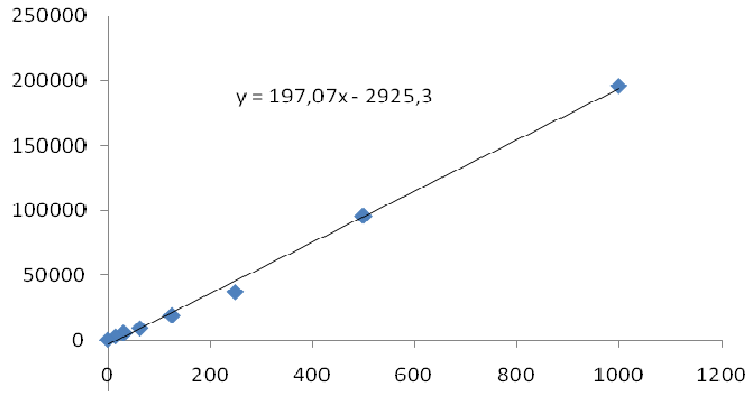


Figure 3. Lambda DNA standard curve was used to determine the concentration of DNA. The X-axis shows the concentration (ng/ml) and Y-axis depicts florescence signal. The standard curve was made by 10 times serial dilutions of the Lambda DNA

Gel electrophoresis

The quality of the extracted genomic DNA was verified before genotyping. The gDNA was analyzed on 0.8% agarose gel with 1 X TAE. Approximately 5 µl gDNA and 2 µl of loading dye was added to the wells. The gel electrophoresis was allowed to run for 1 hr at 120 voltes. The band size was verified using 1 kb ladder.

Whole genome genotyping using Illumina

The extracted DNA from blood and uninvolved margin from breast cancer patients was genotyped using Illumina Human1M BeadChip at University of Alabama at Birmingham.

RESULTS

Confirmation of quality of gDNA by gel electrophoresis.

The extracted gDNA from 60 pairs of blood and uninvolved margin of breast tissue surrounding the primary tumor was run on 0.8% agarose gel (Figure 4).

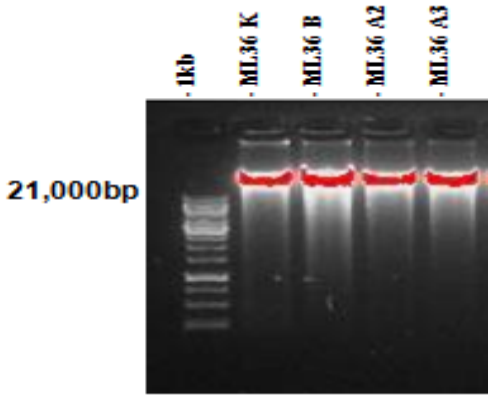
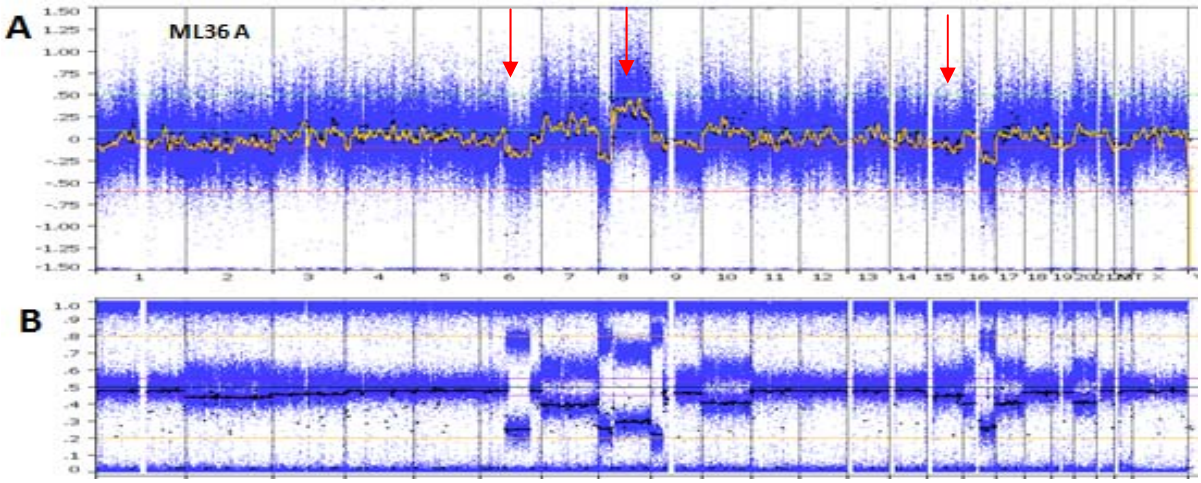


Figure 4. Agarose gel electrophoresis showing high molecular weight of extracted DNA. Lane 1 1kb ladder; lane 2 sample ML36K; lane 3 sample ML36B; lane 4 ML36A2; lane 5 ML36A2.

Illumina genotyping results

The investigation of somatic mosaicism was based upon the results obtained from Illumina 1M DNA analysis BeadChip. The raw data was analyzed using Nexus copy number program, using the graphical interface of Nexus software (version 5, BioDiscovery Inc.). The data analyzed by the Nexus software for uninvolved margin of breast tissue surrounding primary tumour and blood shows the presence of somatic mosaicism in the breast tissue in a given sample (Figure 5).



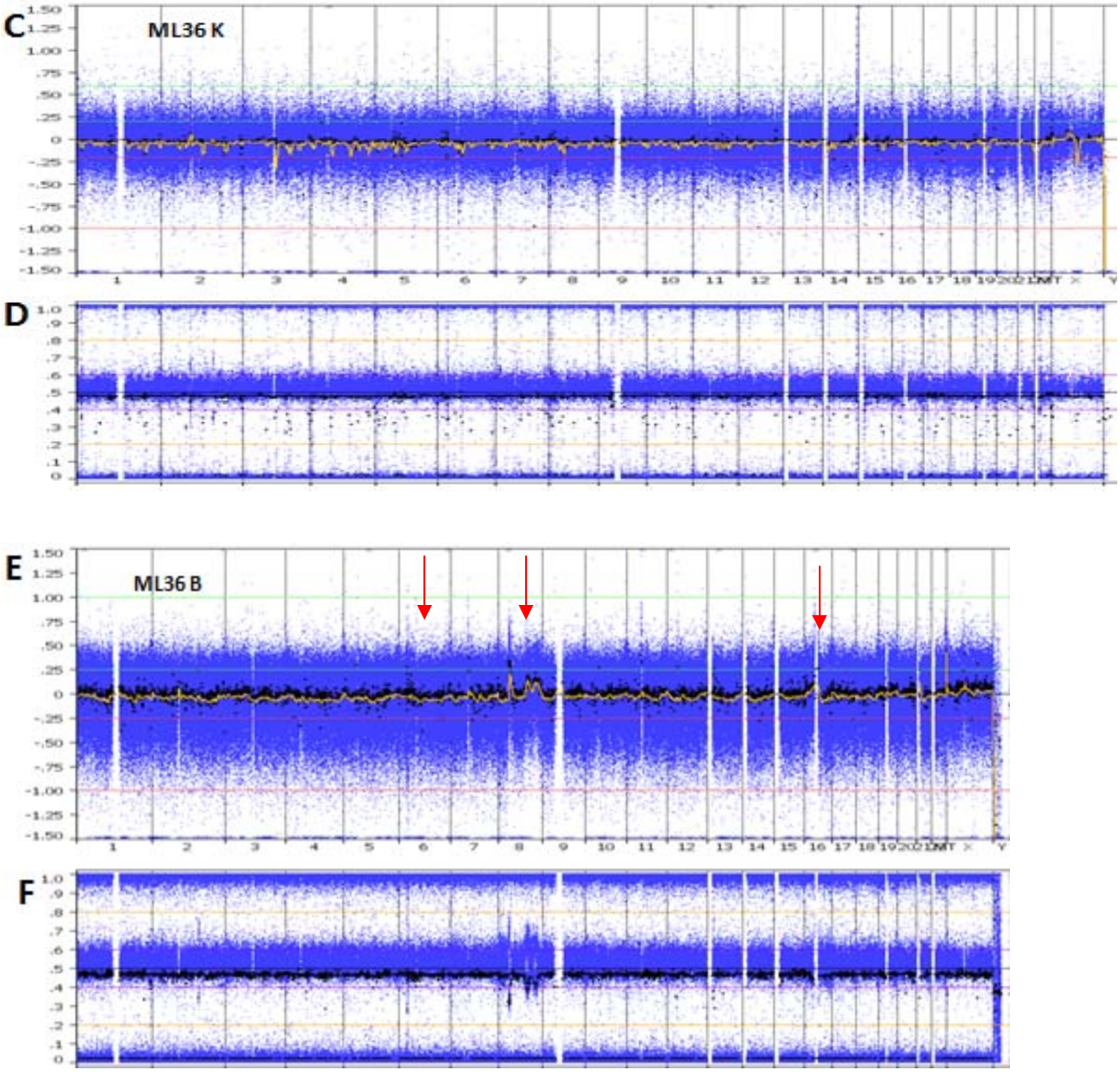


Figure 5. Whole genome results from breast cancer samples ML36 A (Primary tumour), ML36 K (Blood) and ML36 B (Uninvolved margin), profiled with Illumina 1M DNA analysis BeadChip. Panels A,C and E shows Log R ratio, while panels B,D and F represents B-allele frequency (BAF) values. Note the difference between log ratio and altered B allele frequency indicates the presence of mosaicism in the observed sample.

Genotyping results by Nimblegen

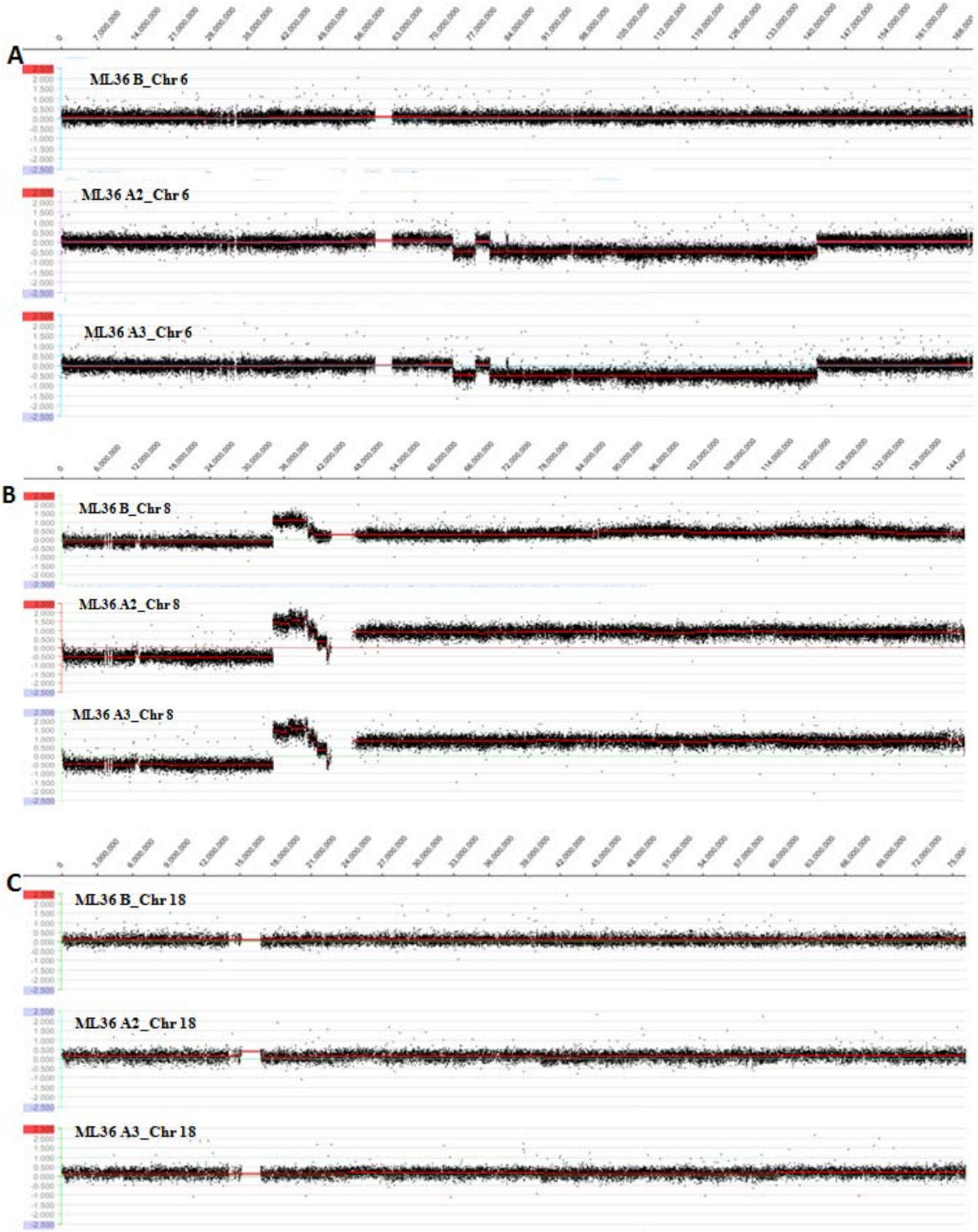


Figure 6. Detection of somatic mosaicism in sample ML36B (uninvolved margin), ML36A2 and ML36A3 using NimbleGen CGH 3x720K Whole-Genome Array. The Y axis represents log2 ratios; the X axis represents genomic positions along chromosome.

The results from the Illumina genotyping and Nimblegen show the presence of somatic mosaicism in the sample ML36B. The chromosomes 6, 8 and 18 as indicated with arrows (Figure 5) show genomic abreactions and this result was compared with the result from Nimblegen. The results from Nimblegen show deletion in the chromosome 6, and gain in chromosome 8 and chromosome 18 of sample ML36B (uninvolved margin), ML36A2 and ML36A3 (primary tumours) (Figure 6).

DISCUSSION

DNA sequences in every nucleated cell of an organism are identical. An adult human is made up of ~10^{14} cells and it is not surprising that errors can occur during DNA replication and cell division needed to form a complete individual. In this study in a few set of breast cancer samples a remarkable variation was found by using Illumina 1M DNA analysis BeadChip. The genotyping data from Illumina indicates that there are variations in the genomic profiles of breast tissue surrounding the primary tumour that is health uninvolved margin and blood of same patient. The results from NimbleGen 720K microarray also show the presence of genetic variations in primary tumour and healthy uninvolved margin of the breast tissue.

While investigating the whole genome of the breast cancer patients by Illumina genotyping numerous genetic variations were observed in few numbers of samples. Over the past few years there has been a considerable increase in number of reported cases of somatic mosaicism in genetic disease and disorders. In cases where recognition of direct genetic evidence was not possible, indirect evidence for possible somatic mosaicism was found in discordant phenotypes in monozygotic twins (44, 45). A mutation can also have influence on the phenotype of an individual (46). This depends on the particular DNA affected, stage of development and type of cells involved. Most of cancers occur due to somatic mutations, and mutations in many other diseases like Hemophilia A, Tuberous Sclerosis, Rubinstein-Taybi Syndrome and Chronic Granulomatous Disease have shown in some cases to be mosaic either in the patient of one of the parents.

In this study however there is genomic imbalance and presence of somatic variations which seem to be more frequent in numerous cohorts of cancer patients. These mutations occur in many forms; from point mutation to large structural alterations affecting millions of base pairs in the genome. CNVs are subclass of structural variants, commonly defined as region of DNA segment more than 1kb that is present a variable number of times in a genome when compared to reference genome. These CNVs occur in a proportion of cells where its analysis is more demanding. The studies on genetic abnormalities between different populations of somatic cells are challenging because the cells might have variant genotypes, probably causing phenotypes which can be mixed with other types of normal cells in any tissue. Therefore it is necessary to enrich the target cells that have methodological obstacles which requires sorting of cells or micro-dissections.

The application of Illumina beadchips via BAF value as a tool for detection of somatic mosaicism on the genome wide scale, introduced an extensive improvement in the detection of mosaicism in a range of 5% of affected cells [47]. The rate of discovery of somatic mosaicism in the small proportion of studied cells might continue to accelerate the studies on genetic heterogeneity of breast malignancies.

FUTURE PERSPECTIVES

The results from this study show the presence of somatic mosaicism. To assess the cause of phenotypic expression, somatic mosaicism must be considered. To measure the influence of specific gene in particular tissue, the genome sequence of different cells types in large number has to be analyzed. Cancer tissues are known to be heterogeneous, it is important to sequence all grades and diverse types of tissue surrounding the primary tumour. In specific, polymorphisms like CNVs and trinucleotide repeats which are associated with number of cancer require a careful genetic analysis of all types of cells that make up tumours.

Even though techniques such as microarray analysis and high-throughput sequencing analysis are readily available, the capability to sequence very specific and possibly a very little number of cells will be needed (isolated by e.g. laser capture microdissection) to analyse the phenotype expression completely. However, the major outcome of the discovery of somatic mosaicism is that we will cease to regard an individual's genome as fundamentally homogeneous and stable throughout individual's life time. We can view it, to a certain extent, as a structure that undergoes considerable changes, such that the genome one is borne with will not remain the same when one dies. Research related to metastasis in breast cancer is not well developed. Studies that might compare genetic profiles of primary tumour and uninvolved margin may lead to identify genomic aberrations in context to cancer development.

ACKNOWLEDGEMENTS

I am heartily thankful to my supervisor, Prof. Jan P. Dumanski, whose encouragement, supervision and support from the preliminary to the concluding level enabled me to develop an understanding of the subject.

Lastly, I offer my regards to Hamid, Lars, Chiara and Geeta who supported me in any respect during the completion of the project.

Last but not the least; I would like to thank my parents, for giving birth to me at the second place and supporting me spiritually throughout my life.

REFERENCES

1. Hanahan, D.a.W., R. A, (2000). The hallmarks of cancer Cell, 100, 57- and 70.
2. Vogelstein, B. and Kinzler, K. W, (2004). Cancer genes and the pathways they control *Nat Med,* 10, 789-99.
3. Hahn, W. C., Counter, C. M., Lundberg, A. S., Beijersbergen, R. L., Brooks, M. W. and Weinberg, R. A, (1999). Creation of human tumour cells with defined genetic elements Nature, 400, 464-8.
4. Nagasaki, K. and Y. Miki, (2006). Gene expression profiling of breast cancer. Breast Cancer, 13(1): p. 2-7.
5. Stratton, M.R., P.J. Campbell, and P.A. Futreal, (2009). The cancer genome. Nature, 458(7239): p. 719-24.
6. Greenman C, Stephens P, Smith R, et al, (2007). Patterns of somatic mutation in human cancer genomes. Nature; 446: 153–8. CrossRefMedline
7. Jones S, Zhang Z, Parsons DW, et al, (2008). Core signaling pathways in human pancreatic cancer revealed by tumor genome analysis. Science; 321: 1801–6.
8. Kaminker JS, Zhang Y, Waugh A, et al, (2007). Distinguishing cancer-associated missensemutations from common polymorphisms. Cancer Res; 67: 465–73.
9. Parsons DW, Jones S, Zhang X, et al, (2008). An integrated genomic analysis of glioblastoma multiforme. Science; 321: 1807–12.
10. Sjoblom T, Jones S, Wood LD, et al, (2006). The consensus coding sequences of human breast and colorectal cancers. Science; 314: 268–74.
11. Wood LD, Parsons DW, Jones S, et al, (2007). The genomic landscapes of human breast and colorectal cancers. Science; 318: 1108–13.
12. "World Cancer Report", (June 2003). International Agency for Research on Cancer.
13. Benetkiewicz, M., et al, (2006). Chromosome 22 array-CGH profiling of breast cancer delimited minimal common regions of genomic imbalances and revealed frequent intra-tumoral genetic heterogeneity. Int J Oncol, 29(4): p. 935-45.
14. Kenemans, P., R.A. Verstraeten, and R.H. Verheijen, (2004). Oncogenic pathways in hereditary and sporadic breast cancer. Maturitas, 49(1): p. 34-43.
15. Balmain A, Gray J, Ponder B., (2003). The genetics and genomics of cancer. Nat Genet;33(suppl):238
16. Baynes, J.W. and M.H. Dominiczak, 2(005). Medical Biochemistry. Second Edition ed. Elsevier Mosby.
17. Benetkiewicz, M., et al., (2006). Chromosome 22 array-CGH profiling of breast cancer delimited minimal common regions of genomic imbalances and revealed frequent intra-tumoral genetic heterogeneity. Int J Oncol, 29(4): p. 935-45.
18. Plo, I., et al., (2008). AKT1 inhibits homologous recombination by inducing cytoplasmic retention of BRCA1 and RAD51. Cancer Res, 68(22): p. 9404-12.
19. Stern, D.F., (2000). Tyrosine kinase signalling in breast cancer: ErbB family receptor tyrosine kinases. Breast Cancer Res, 2(3): p. 176-83.
20. Frank SA, Nowak MA, (2003). Developmental predisposition to cancer. Nature 422:494.
21. Frank SA, (2007). Dynamics of Cancer: Incidence, Inheritance, and Evolution (Princeton Univ Press, Princeton).
22. Stratton MR, Campbell PJ, Futreal PA, (2009). The cancer genome. Nature 458:719–724.
23. Gottlieb B, Beitel LK, Trifiro MA, (2001). Somatic mosaicism and variable expressivity. Trends genet. 17. 79-82.
24. Wallace, D. C. & Lott, M, (2002). in Principles and Practice of Medical Genetics 4th Edn (eds Rimoin, D. L., Connor, J. M., Pyeritz, R. E. & Korf, B. R.) 299–409 (Churchill Livingstone, Edinburgh,).

25. Hall, J. G, (1996). Twinning: mechanisms and genetic implications. Curr. Opin. Genet. Dev. 6, 343–347.
26. Boveri, T, (1929). The Origin of Malignant Tumors (Williams & Wilkins, Baltimore, Maryland).
27. Jackson, A. L. & Loeb, L. A, (1998). The mutation rate and cancer. Genetics 148, 1483–1490.
28. Hall, J. G, (1988). Review and hypotheses: somatic mosaicism: observations related to clinical genetics. Am. J. Hum. Genet. 43, 355–363. An excellent synthesis of observations from clinical genetics that relate to germinal and somatic mosaicism.
29. Van Dijk, B. A., Boomsma, D. I. & De Man, A. J. M, (1996). Blood group chimerism in human multiple births is not rare. Am. J. Med. Genet. 61, 264–268.
30. Kvittingen, E. A., Rootwelt, H., Berger, R. & Brandtzaeg, P, (1994). Self-induced correction of the genetic defect in tyrosinemia type I. J. Clin. Invest. 94, 1657–1661.
31. German, J., Ellis, N. A. & Proytcheva, M, (1996). Bloom's syndrome. XIX. Cytogenetic and population evidence for genetic heterogeneity. Clin. Genet. 49, 223–231.
32. Gregory, J. J. et al., (2001). Somatic mosaicism in Fanconi anemia: evidence of genotypic reversion in lymphohematopoietic stem cells. Proc. Natl Acad. Sci. USA 98, 2532–2537.
33. Berger, R. & Jonveaux, P, (1996). Clonal chromosome abnormalities in Fanconi anemia. Hematol. Cell. Ther. 38, 291–296.
34. The International IP Consortium. Survival of male patients with incontinentia pigmenti carrying a lethal mutation can be explained by somatic mosaicism or Klinefelter syndrome. Am. J. Hum. Genet. 69, 1210–1217 (2001). This study illustrates the unmasking of lethal genetic mutations in the somatic mosaic state.
35. Armstrong, J., Pineda, M., Aibar, E., Gean, E. & Monros, E, (2001). Classic Rett syndrome in a boy as a result of somatic mosaicism for a MECP2 mutation. Ann. Neurol. 50, 692.
36. Hall JG, (2003). Twinning. Lancet; 362:735-43.
37. Gringras P, Chen W, (2001). Mechanisms for differences in monozygous twins. Early Hum Dev; 64: 105-17.
38. Lutskiy MI, Park JY, Renold SK, Remold-O'Donnell E, (2008). Evolution of highly polymorphic T cell populations in siblings with the Wiskott-Aldrich syndrome. PLoS One 3;e3444.
39. Wong, L.-J. C., Wong, H. & Liu, A, (2002). Intergenerational transmission of pathogenic heteroplasmic mitochondrial DNA. Genet. Med. 4, 78–83.
40. Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P, (2005). A haplotype map of the human genome. Nature; 437 (7063): 1299-320.
41. Slater HR, Bailey DK, Ren H, Cao M, Bell K,Nasioulas S,Henke R, Choo KH, Kennedy GC, (2005). High-resolution identification of chromosomal abnormalities using oligonucleotide arrays containing 116,204 SNPs. Am J Hum Genet; 77: 709-26.
42. Albertson DG, Collins C, McCormick F, Gray JW, (2003). Chromosome aberrations in solidtumors. Nat Genet; 34 : 369-76.
43. Greenwald BD, Harpaz N, Yin J, Huang Y, Tong Y, Brown VL,McDaniel T, Newkirk C, Resau JH, Meltzer SJ, (1992). Loss of heterozygosity affecting the p53, Rb, and mcc/apc tumor suppressor gene loci in dysplastic and cancerous ulcerative colitis. Cancer Res 52: 741-5.
44. Shotelesruk, V. et al., (1999). Discordance of oralfacial-digital syndrome type 1 in monozygotic twin girls. Am. J. Med. Genet. 86, 269–273
45. Yamagishi, H. et al., (1998). Phenotypic discordance in monozygotic twins with 22q11.2 deletion. Am. J. Med. Genet. 78, 319–321
46. Hall JG, (1988). Review and hypotheses: somatic mosaicism: observations related to clinical genetics. Am J Hum Genet 43: 355–363.
47. Conlin L, Theil B, Bonnemann C, et al. 2010. Mechanisms of mosaicism, Chimerism and uniparental disomy identified by SNP array analysis. Human Molecular Genetics, published on line human Molecular Genetics Advance Access, (January 6, 2010).