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DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
282

PRIIT PALTA

Computational methods for DNA copy number detection

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Computational methods for DNA copy number detection

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Institute of Molecular and Cell Biology, University of Tartu, Estonia

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Supervisor:
Prof. Maido Remm, Ph.D.
University of Tartu
Tartu, Estonia

Opponent:
Assoc. Prof. Lars Feuk, Ph.D.
University of Uppsala
Uppsala, Sweden

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which will be referred to in the text by their Roman numerals:

I Patsalis PC, Kousoulidou L, Männik K, Sismani C, Zilina O, Parkel S, Puusepp H, Tõnisson N, Palta P, Remm M, Kurg A. **Detection of small genomic imbalances using microarray-based multiplex amplifiable probe hybridization.** *Eur J Hum Genet.* 2007 Feb;15(2):162-72.

II Männik K, Parkel S, Palta P, Zilina O, Puusepp H, Esko T, Mägi R, Nõukas M, Veidenberg A, Nelis M, Metspalu A, Remm M, Ounap K, Kurg A. **A parallel SNP array study of genomic aberrations associated with mental retardation in patients and general population in Estonia.** *Eur J Med Genet.* 2011 Mar-Apr;54(2):136-43.

III Nagirnaja L, Palta P, Kasak L, Rull K, Christiansen OB, Nielsen HS, Steffensen R, Esko T, Remm M, Laan M. **Structural genomic variation as risk factor for idiopathic recurrent miscarriage.** *Hum Mutat.* 2014 Aug;35(8):972-82.

IV Palta P, Kaplinski L, Nagirnaja L, Veidenberg A, Möls M, Nelis M, Esko T, Metspalu A, Laan M, Remm M. **Haplotype phasing and inheritance of copy number variants in nuclear families.** *PLoS One.* 2015;10(4):e0122713.

Author's contributions:

Ref. I: Implemented hybridisation probe design software, performed *in silico* design of the microarray probes. Developed the statistical method for CNV calling and contributed to manuscript preparation.

Ref. II: Performed microarray data QC, CNV calling, filtering and analyses. Developed and implemented software for parent-of-origin analysis, allelic composition determination and performed corresponding analyses in the MR cohort. Contributed to manuscript preparation.

Ref. III: Contributed to microarray data QC, CNV calling and filtering. Performed gene set enrichment and pathway analyses for Estonian population and RM cohort case/control samples. Participated in writing of the first draft of the manuscript.

Ref. IV: Planned and conducted the study, performed Estonian sample selection, microarray data QC, CNV calling and filtering. Developed and partly implemented CNV phasing methodology, phased and analysed Estonian and HapMap family datasets, and wrote the first draft of the manuscript.

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LIST OF ABBREVIATIONS

array-CGH	microarray-based comparative genomic hybridisation
BAF	B allele frequency
BISRS	break-induced serial replication slippage
bp	base pair(s)
CGH	comparative genomic hybridization
CNP	copy number polymorphism
CNV	copy number variant
DNA	deoxyribonucleic acid
FoSTeS	fork stalling and template switching
FSI	fluorescent signal intensity
HMM	Hidden Markov model
k	kilo, thousand (1,000)
kb	1,000 base pairs
LBF	log Bayes Factor
LoF	loss-of-function
LOH	loss-of-heterozygosity
LRR	Log R ratio
MAF	minor allele frequency
MAPH	multiplex amplifiable probe hybridisation
Mb	1,000,000 base pairs
MMBIR	microhomology-mediated break-induced replication
MMRDR	microhomology-mediated replication-dependent recombination
MR	mental retardation
NAHR	non-allelic homologous recombination
NHEJ	non-homologous end joining
QC	quality control
RM	recurrent miscarriage
SNP	single nucleotide polymorphism
SNV	single nucleotide variant
TI	tolerance interval(s)
WES	whole exome sequencing
WGS	whole genome sequencing

INTRODUCTION

Advances in sequencing and microarray technology have enabled the progress of systematic and thorough genome-wide analyses to assess the localisation and frequency of different variants in the human genome. For more than a decade, single nucleotide polymorphisms (SNPs) have been a focus of interest for human geneticists. As a result, a large number of studies have associated these single nucleotide variants with variability in different human phenotypes[1]. In addition to SNPs, short (one to a few tens of base pairs) insertions and deletions of genomic DNA sequences (commonly referred to as indels) are also present in the human genome[2–4]. During the last decade, knowledge concerning even longer unbalanced gains and losses of genomic DNA sequences (conventionally called copy number variants or CNVs) has massively increased and has also excited the interest of human geneticists[5–8]. DNA copy number variation is a type of genetic variation that may increase the copy number of a particular DNA segment from the normal two copies per diploid genome to more than two copies (e.g., duplication or triplication) or decrease it to less than two copies (deletion).

Although the extensive presence of CNVs in the human genome has been studied for the last decade[5,6], the reported extent of copy-number variation in different species and individual genomes differs greatly[7,9]. CNV lengths in the human genome typically range from a few kilo-bases to several mega-bases and are estimated to cumulatively cover at least 5% of the human genome[7,9–11]. In many instances, CNVs have been shown to play an important role in different human phenotypic traits, and their association with disease susceptibility has been increasingly recognised.

Alterations in DNA copy-number are the most likely cause of many genetic disorders (hereditary and *de novo*) and play an important role in tumourigenesis and susceptibility to common diseases. The identification of such disease-associated altered regions in the DNA provides valuable information about the genes involved in the disease and presents one step towards understanding the underlying molecular mechanisms. However, computational methods that can help investigators find and prioritise variants obtained from different experiments are needed.

In the review of literature of the current thesis, I will provide a short overview of copy number variants in the human genome, including how CNVs are discovered with current microarray-based methods, their known effects on human phenotypic traits or disease susceptibility and what is known about their population genetics.

In the results and discussion I will describe and discuss a few methods developed for CNV calling and phasing. I will also describe and discuss the inheritance of copy number variants and their allelic variability.

REVIEW OF LITERATURE

1. Copy number variation in the human genome

DNA copy number variation is a type of genetic variation in which case the number of allelic copies of a particular region of a genome is altered from its normal 'state'. In the non-repetitive portion of the human genome, the normal haploid copy number is one – one copy of each sequence per chromosome. Accordingly, the normal diploid copy number in humans is two – one copy inherited from both parents. A copy number variant (CNV) can result from either a loss of copies (most often called a deletion) or gain of copies (called a duplication or amplification). Different deletion and duplication variants are illustrated in Figure 1.

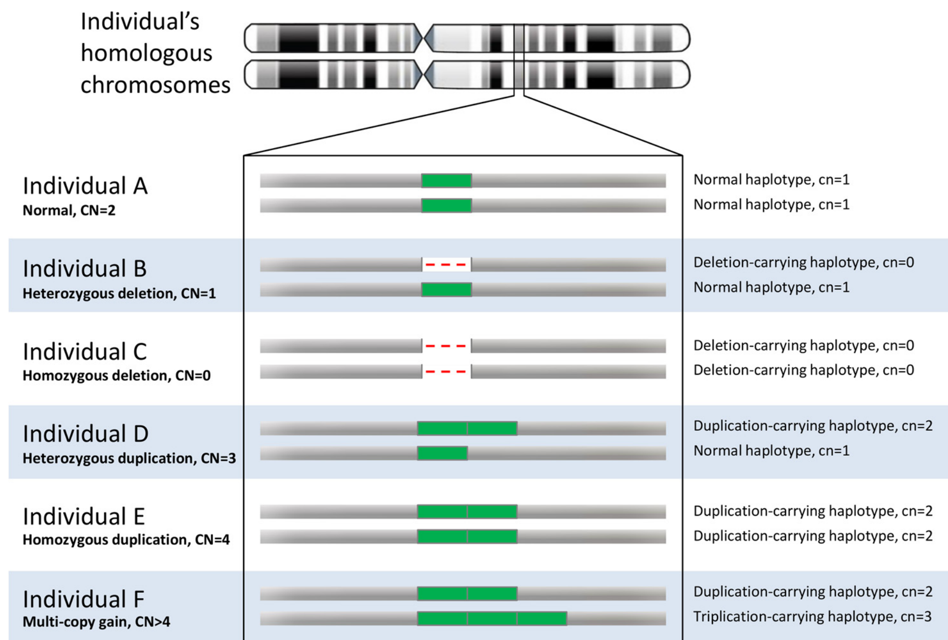

Figure 1. Copy number variants in a diploid genome. Close-up picture of a chromosomal region that contains a CNV (the green region).Whereas the grey regions are present in normal copy numbers (haploid copy number of one: cn=1; diploid copy number of two: CN=2) in individuals A-F, the green region has different haploid and diploid copy numbers in different individuals.

CNVs in the human genome range from tens of bases to several mega-bases[12,13]. One of the largest sequencing-based studies estimated that the median CNV size in the human genome was 729 bp, with mean of 8 kb[11]. Although the abundant presence of CNVs in healthy individuals has been known for many years[5,7,14], the reported number and extent of CNVs has been wide-ranging and has varied from a few to up to a few thousand per diploid genome[9,15]. CNVs are cumulatively estimated to cover 1–18% of the DNA sequence in the human

diploid genome[7,10,15–17]. Often, these discrepancies are due to differences in the technology and the experimental or computational methods used; the broad consensus is that CNVs cover approximately 5–10% of human genomic DNA sequences[9,10,18,19]. Thus, CNVs cover approximately 0.3–0.8% of the genome in every individual[9,20], corresponding to 9–24 Mb of DNA sequence.

Although copy number variants occur virtually everywhere in the human genome, there are many CNV hotspots where CNVs occur more frequently. This is well illustrated by an autosomal map of long (>100 kb) CNVs from approximately 2500 individuals from ethnically diverse populations (Figure 2).

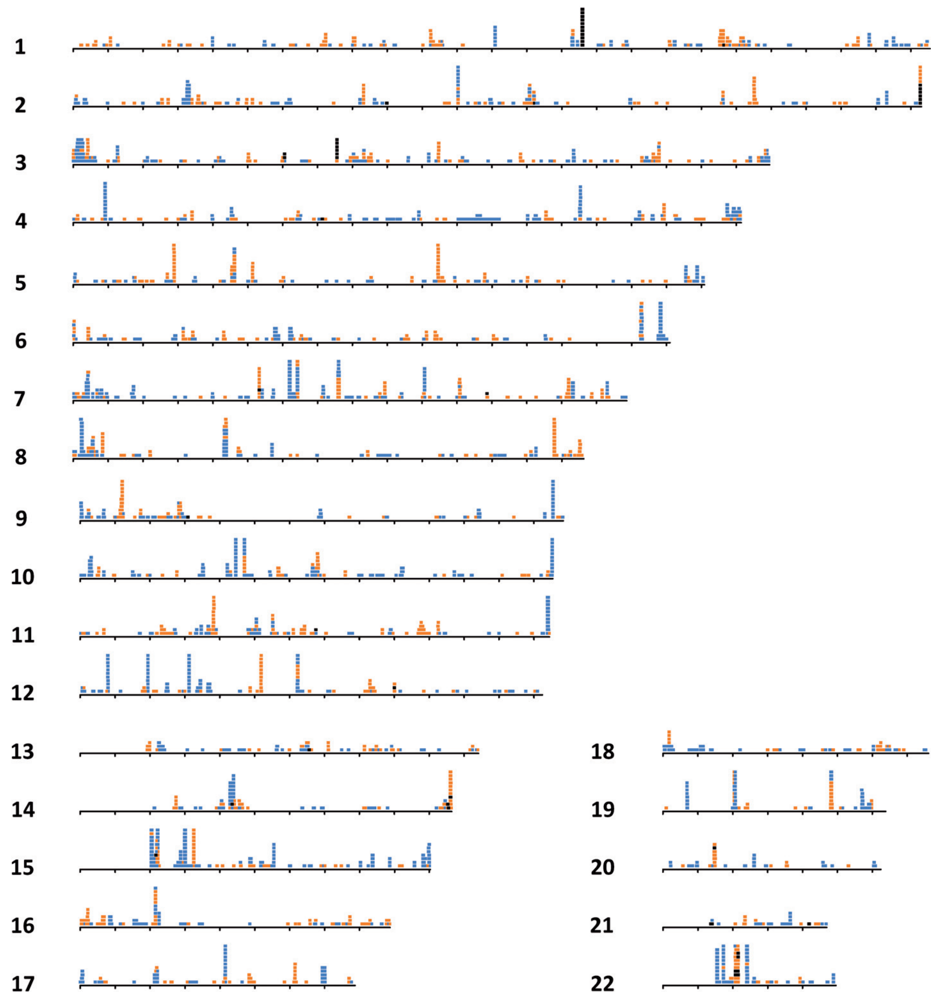

Figure 2. Autosomal landscape of large CNVs. Large (>100 kbp) copy number variants in the human genome based on analysis of 2493 individuals. Observed duplications are depicted in blue, heterozygous deletions in orange and homozygous deletions with black rectangles. Chromosomes are drawn to their size scale (outside tick marks indicate 10 Mb), and CNVs observed more frequently than ten times for a given locus are cropped (based on Itsara *et al.*, 2009).

Many CNVs in different individuals have identical recurrent breakpoints. However, some CNVs have slightly different breakpoints, often depending on the mechanism responsible for the introduction of the CNV[20].

1.1 Mechanisms of CNV formation

There are three primary classes of known mechanisms that create CNVs. A large number of copy number variants that often have recurrent breakpoints in the same locus arise by non-allelic homologous recombination (NAHR) between segmental duplication or low-copy repeats[16,21]. NAHR can result in deletions and duplications as well as inversions and translocations depending on the location and orientation of the interacting homologous sequences[22,23]. Inter-chromosomal and intra-chromosomal (inter-chromatidal) NAHR events can facilitate both deletions and duplications, whereas intra-chromatidal events result in only deletions[24,25]. Non-homologous end joining (NHEJ) is the most common DNA repair mechanism in mammalian cells; this mechanism involves ligating together any two broken DNA ends and does not require homologous sequences, although it has been shown that the presence of terminal micro-homologies also facilitates NHEJ[26]. NHEJ can result in small scale non-recurrent deletions and translocation rearrangements with minimal to no junction homology[21]. Microhomology-mediated mechanisms (collectively referred to as MMRDR) include microhomology-mediated break-induced replication (MMBIR), break-induced serial replication slippage (BISRS), and fork stalling and template switching (FoSTeS)[22,27–29]. These mechanisms are consistent with a common hypothesis that the leading/lagging strand primer/ polymerase disengages from its template upon replication fork stalling, translocates and then re-associates (probably using short microhomology at the 3′ end) to another available template/replication fork in physical proximity and resumes replication[27,30]. This phenomenon can result in non-recurrent deletions, duplications or complex rearrangements, including triplications and inversions[28].

Considering these most prevalent mutational mechanisms, it would be intuitive to propose that deletions and duplications are not generated and do not occur at equal rate in the human genome because some mechanisms give rise to only deletions. It has been estimated that deletions and duplications are likely to be generated in proportions of approximately 2:1 to 3:1, with the ratio varying widely from region to region[17]. Based on empirical genome-wide studies we know that large deletions and duplications occur with an approximately 2:1 ratio[23].

2. Microarray-based CNV detection

Despite the decreasing costs of whole-genome sequencing (WGS) and whole-exome sequencing (WES), microarrays are still extensively used in genetic studies of disease susceptibility and clinical diagnostics. Although sequencing-based applications have great deal of potential and enable investigators to interrogate almost every base pair in the studied genomes, both experimental and computational methods to infer copy number variants from sequencing data are still being developed and standardised[31,32]. Microarrays still represent the gold standard for both genotyping and CNV calling, especially in case of rare variants. Furthermore, increased sample sizes and improved analytical tools have stimulated what could be called a 'microarray-based genotyping renaissance'.

DNA copy number detection using microarray techniques is an indirect method to estimate diploid copy numbers of the studied DNA at pre-defined genomic loci by interrogating the sample with specifically designed or selected microarray probes. Briefly, the genomic DNA of interest is amplified and hybridised to a solid chip matrix carrying locus-specific capture probes. These probes most often are 25–60 bp in length and include *in situ* synthesised or spotted oligonucleotide molecules that are complementary to a specific unique sequence in the human genome assembly. In the case of array-CGH (microarray-based comparative genomic hybridisation), an additional reference DNA (often a cytogenetically controlled DNA or pool of DNAs) is co-hybridised to the same array. After the hybridisation process in which amplified and fluorescent dye-labelled genomic sequences are specifically bound to capture probes, the arrays are washed to remove any non-specifically bound material and the quantity of the specifically hybridised DNA is estimated by measuring the fluorescent signal intensity (FSI) for each capture probe by scanning the microarray chip.

FSIs from microarray probes exhibit dosage-dependent responses to alterations in the DNA copy number up to a certain level. Thus, the more copies of a certain sequences there are in the studied sample, the higher the corresponding probe's FSI level will be. In the case of array-CGH, two different fluorescent dyes are used for labelling – one to label the studied DNA and one to label the reference DNA. In the case of SNP genotyping microarrays, two different dyes are used to label different alleles of the bi-allelic SNP variants interrogated[33,34]. The most commonly used dyes for labelling are fluorescent cyanine-3 and cyanine-5 (abbreviated Cy3 and Cy5, respectively). These two fluorescent dyes are excited at different wavelengths and are quantified by a two-channel microarray scanner[33].

Because only pre-defined regions of the genome can be investigated with this method, CNV detection relies on the array design (i.e. how many probes are used and how these probes are distributed across the chromosomes). Therefore, as a rule of thumb (and quite intuitively), the use of more probes to cover all chromosomes uniformly results in a more accurate and higher resolution CNV

profile. It has been shown that the differences between platforms are more pronounced for short CNVs, whereas the differences resulting from genotyping platform largely disappear for larger (>500kb) CNVs[16]. In their high resolution study, Conrad *et al.* used custom-designed array-CGH arrays carrying 42 million oligonucleotide probes that covered the assayable portion of the genome with median spacing of 56 bp[9]. Currently (*Anno Domini* 2015), top-notch genotyping microarrays have been produced with as many as 4.3 million probes (Illumina HumanOmni5Exome, www.illumina.com) and often additionally carry non-polymorphic probes to uniformly interrogate CNVs in the human genome. For example, the Affymetrix Genome-Wide Human SNP Array 6.0 includes approximately 946 thousand CNV probes, with 200 thousand of such probes targeting common CNP loci and an additional 744 thousand probes evenly spaced along all of the chromosomes (www.affymetrix.com).

In addition to high density and coverage, one obvious advantage of SNP genotyping arrays over array-CGH is the identification of allele-specific information[34,35]. In addition to SNP genotype calling, the simultaneous measurement of total and allele-specific FSI makes it possible to detect both copy number changes and copy number neutral events, such as loss-of-heterozygosity (LOH).

Known problems with genotyping arrays are their higher intrinsic variability caused by the PCR-based approach used to amplify the studied genomic material and the optimisation of these platforms for SNP variant calling (i.e., oligonucleotide design and the hybridisation chemistry/conditions used) rather than for CNV detection[33,36]. Consequently, these confounding factors can increase the proportion of false positive (region incorrectly called a variant) and false negative (undetected true variant) CNV calls if not properly taken into account. A relatively high rate of false positive and false negative findings has raised important concerns regarding the suitability of microarray-based copy number detection for clinical diagnostics applications. Although microarrays are suitable for CNV screening, reliable assays that provide clear and high quality results of measurable significance are required in a clinical diagnostics set-up. Moreover, false findings should be minimal and their rate should be accurately predictable[37,38].

2.1 Nature and principles of SNP genotyping microarray data

Aside from the experimental microarray assay set-up, copy number detection for the studied sample can be quite complicated and cannot be regarded as an easy task. The reason for this is that even though the underlying biological nature of DNA copy numbers is always discrete (i.e., in one cell, genomic DNA copy number in a certain genomic region always corresponds to a certain non-negative integer), the measured FSIs of the studied and reference DNA material from the microarray experiment most often come from a mixture of cells. Thus, these FSI values are continuous variables with a considerable amount of non-biological variance and comprehensive and precise computational and statistical

methods are required to estimate the DNA copy number for different loci and to confidently detect CNVs[34].

As the first step in *in silico* copy number analysis, FSIs for all probes are organised by their genomic coordinates and normalised. For arrayCGH, logarithmic FSI ratios of the studied and reference DNA are used to infer CNVs[9,20,39]. For SNP genotyping arrays, FSIs from both channels (denoted X and Y values and representing different SNP alleles usually indicated as alleles A and B) for the studied DNA are normalised against a panel of reference samples[34,40,41]. For the ith probe on the microarray, the total normalised logarithmic FSI (aka the Log R ratio or LRR) is calculated as follows:

$$LRR_i = \log_2\left(\frac{R_i observed}{R_i expected}\right)$$

where $R_i observed = X_i + Y_i$ measures the total combined signal intensity of two FSI channels and $R_i expected$ is measured from a set of 'normal' reference samples.

Several adjacent positive or negative LRR values that significantly deviate from the expected value of zero corresponding to the most frequent diploid copy number of two observed in most loci for most individuals can be indicative of copy number gain (i.e., duplication and triplication) or loss (i.e., hetero- or homozygous deletion).

Another relevant measure – the B allele frequency (BAF) is the normalised measurement of the relative FSI ratio corresponding to the B and A alleles:

$$BAF_i = \begin{cases} 0, \text{if } \theta_i < \theta_{i\,\mathrm{AA}} \\ 0.5(\theta_i - \theta_{i\,\mathrm{AA}})/(\theta_{i\,\mathrm{AB}} - \theta_{i\,\mathrm{AA}}), \text{if } \theta_{i\,\mathrm{AA}} \le \theta_i < \theta_{i\,\mathrm{AB}} \\ 0.5 + 0.5(\theta_i - \theta_{i\,\mathrm{AB}})/(\theta_{i\,\mathrm{BB}} - \theta_{i\,\mathrm{AB}}), \text{if } \theta_{i\,\mathrm{AB}} \le \theta_i < \theta_{i\,\mathrm{BB}} \\ 1, \text{if } \theta_i \ge \theta_{i\,\mathrm{BB}} \end{cases}$$

where the ith SNP-specific theta-value $\theta_i = \arctan(X_i/Y_i)/(\pi/2)$ refers to the relative allelic signal intensity ratio and where $\theta_{i\,\mathrm{AA}}$, $\theta_{i\,\mathrm{AB}}$, and $\theta_{i\,\mathrm{BB}}$ are the theta values for three canonical two-letter genotype clusters generated from a large set of reference samples[42]. This transformation from theta to BAF values adjusts for different characteristics of each probe so that the BAF values for different SNPs are comparable to each other[41]. A few examples of LRR and BAF values for normal, deletion and duplication CNVs are depicted in Figure 3.

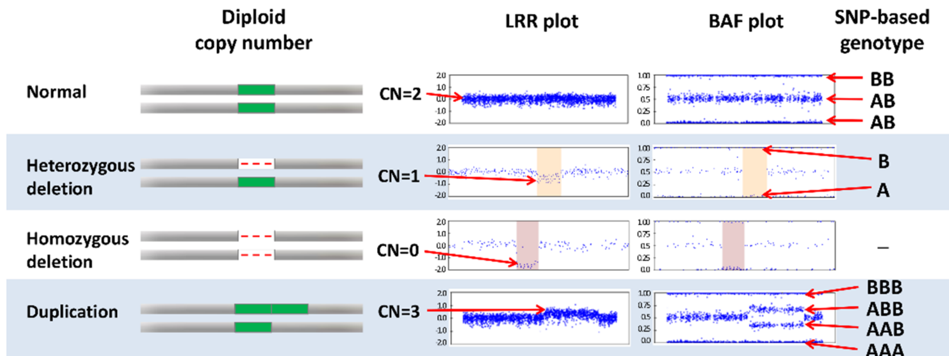

Figure 3. Examples of plots with different LRR and BAF values (and corresponding SNP genotypes) for normal (diploid copy number of two – CN=2), heterozygous deletion (CN=1), homozygous deletion (CN=0 with 'null' alleles) and duplication (CN=3) copy number variants.

2.2 Computational methods for CNV detection

A naïve method for copy number variant detection is to identify genomic regions where the consecutive microarray probes demonstrate LRR values that significantly deviate from the expected LRR value (corresponding to a diploid copy number of two). This approach is often called the 'fixed threshold method' in the pertinent literature[43–46]. The problem with this simple method is that threshold-based methods have limited specificity (i.e., many false CNV calls are made) and sensitivity (i.e., many true CNVs are missed) because microarray FSIs exhibit considerable variance due to technical reasons that are often specific for each assay and probe, resulting in a high rate of false positive and negative findings[47].

More advanced methods were developed to overcome these problems by considering the intrinsic variance of microarray assays. Some methods consider the variance across all microarray probes ('information-lending' methods)[48,49] or take advantage of a permutation procedure to find a better balance between the false positive and false negative findings within each assay[47,50,51]. If reference data are available (e.g., healthy individuals typed on the same platform), it is possible to consider the estimated FSI distribution for each microarray probe and to separately calculate a specific threshold for each probe[33]. These methods are one step forward compared to the naïve method and allow for the estimation of the proportion of false positive and negative CNV regions to decrease their proportion in the called CNVs.

Additionally, several more sophisticated methods for CNV calling have been developed to date[41]. Hidden Markov model (HMM) and Bayesian methods are frequently used for CNV calling from high-density microarray data. Most of these methods use LRR values for CNV detection. A few algorithms use LRR and BAF values together and some also include user-specified or automatically

estimated experiment-related parameters. Hidden states in HMM represent the actual underlying copy number that is estimated by considering FSI and often also reflect other relevant measures. For example QuantiSNP – one of the first tools developed for CNV calling from SNP genotyping microarray data – uses an Objective-Bayes-HMM approach and simultaneously incorporates the LRR and BAF as well as a (fixed) value for the expected frequency of the B allele for all microarray probes in a HMM framework[52]. Another popular algorithm (PennCNV) uses the total diploid copy number specific and probe distance-dependent transition probabilities in its HMM state-transition matrix, which is a more realistic model for the state transition between different diploid copy numbers in genomic DNA. PennCNV incorporates additional sources of relevant data to achieve finer breakpoint mapping and improve a posteriori CNV validation, including optionally population-specific values for the expected frequency of the B allele for each SNP and the distance between adjacent probes[42].

To diminish the rate of false positive findings, additional filtering steps are often applied to raw CNV calls. For example, very short predicted CNVs that are especially enriched in false positives or CNVs with low confidence metrics are often excluded[33,34]. Additionally, considering only CNVs detected by more than one algorithm can effectively minimise the proportion of false positive calls[53–55].

3. CNVs in health and disease

The widespread distribution of CNVs in the human genome suggests that they play an important role in different human phenotypic traits, including disease susceptibility[8,56]. As many as 13% of the RefSeq genes cumulatively overlap with CNVs[9], and CNVs have been demonstrated to have a direct impact on the gene expression of the encompassed or nearby genes[57,58]. CNVs can have functional and phenotypic consequences by changing gene expression patterns or altering the coding sequences of these genes.

3.1 Genomic impact of CNVs

Both deletion and duplication variants can act as loss-of-function (LoF) variants by simply disrupting coding sequences[9]. Heterozygous deletion variants (i.e., if one haplotype has been deleted, but the other haplotype is still present) can unmask other functional variants or even recessive deleterious mutations in the remaining haplotype[59]. Shorter intragenic CNVs can also generate novel genes and transcripts by fusing coding regions from different genes or by deleting exons of existing genes[11,29].

Another obvious mechanism through which CNVs can effect on phenotypic traits is gene dosage[60]. Copy number variants encompassing dosage-sensitive genes or their regulatory regions can directly or through positional effects result in over- or under-expressed genes and consequent disparity in the corresponding phenotypes[41]. CNVs can also induce changes in DNA methylation with functional and phenotypic consequences[61].

Copy number variants can cumulatively ('genomic burden') or in combination with other variants ('two-hit' model) alter human phenotypes and cause sporadic or Mendelian and complex disease[62]. In contrast, it has been shown that there are approximately 100 genes in humans that can be completely or partially deleted without producing any apparent trait or disease consequences[2,13].

3.2 Associated trait and disease phenotypes

The following three categories of genes are often enriched for copy number variants in healthy individuals: a) genes involved in extracellular biological processes and immunity (e.g., cell adhesion, cell recognition, signalling and immune response); b) genes involved in environmental response functions (e.g., sensory perception) and c) retrovirus- and transposition-related protein coding genes[9,18,62]. Additional known examples of normal phenotypical variability include CNVs associated with salivary amylase production[5,7,63], red-green colour vision[64–66], testosterone excretion level[67] and rhesus blood group sensitivity[68].

CNVs have also been associated with several Mendelian or common disease phenotypes and infectious diseases[69–74]. Furthermore, it has been shown that newly arisen *de novo* copy number variants (germline mutations present in the offspring but not in the parents) can cause a variety of different diseases[19,75]. Examples of known CNV-driven disease associations include HIV infection susceptibility[76,77], haemophilia A[78], Parkinson's disease[79,80], Alzheimer's disease[81–83], obesity[84–86], schizophrenia[87–90], susceptibility to autism spectrum disorders[91–95], systemic lupus erythaematosus[96], psoriasis[97,98] and other immune system-related diseases[99,100]. As with dominant *de novo* and recessive disease-causing SNP variants, *de novo* copy number alterations alone and in combination with inherited CNVs can be responsible for more severe clinical phenotypes[101] or cause sporadic disease[75,102].

The penetrance is nearly complete for some copy number variants[84,89,95,103–106]; however, this is not the case for most CNV loci (especially for duplications)[89,107–110]. Possible reasons include epigenetic modifications, other genetic variants in the vicinity, modifier genes, regulatory elements (reviewed by Cooper *et al.*)[111] and potentially alternative allelic copies present within the CNV regions.

4. Population genetics of CNVs

Deleterious CNVs that predispose individuals to severe phenotypes are to some extent most likely to occur under purifying selective pressure[18,112]. This reasoning is supported by the observation that CNVs overlap with genes and other functional elements (e.g., enhancer regions and ultra-conserved elements such as exons in genes involved in RNA processing and introns or nearby genes involved in the regulation of transcription and development) less frequently in the human genome than could be expected to occur by chance[7,113,114]. In their high resolution study of HapMap individuals, Conrad *et al.* reported that the strongest negative selection was observed for exonic CNVs, followed by intronic and then intergenic CNVs[9].

Considering the frequency distribution of copy number variants in outbred populations, CNVs have clear tendency towards a lower frequency of variant-carrying haplotypes[20]. Approximately 50% of all observed CNVs are singletons or are observed at very low frequency[9,16,115]. Indeed, CNV frequency has been shown to be strongly negatively correlated with CNV gene density and size (i.e., CNVs larger than 100 kb are rare, whereas common CNVs tend to be relatively small (<10 kb))[10,16]. Similarly, common CNVs usually harbour fewer genes than rare CNVs which can be relatively gene-rich. On the population level approximately 65–80% of individuals carry at least one CNV that is at least 100 kb in size and variants larger than 500 kb are present in 5–10% of individuals. In contrast, very large copy number variants (>1 Mb) are relatively rare and observed in only 1–2% of individuals on the population level[16,62].

Most CNV loci in the human genome can be explained by normal and simple deletion- and duplication-carrying haplotypes. As expected, deletion-carrying haplotypes have been observed 1.3–2.2 -fold more frequently than duplication-carrying haplotypes in both microarray- and sequencing-based CNV studies[11,20]. An analysis of 849 whole genomes sequenced by the 1000 Genomes Project showed that there were simple deletion-carrying haplotypes in 55% of all detected CNVs; simple duplication-carrying haplotypes were found in 29% of CNVs and multi-allelic CNVs with three or more different segregating haplotypes (i.e., normal, deletion-carrying, and duplication-carrying haplotypes) were found in 16% of CNVs, thereby demonstrating that a considerable proportion of such multi-allelic loci exist in the human genome[116]. It should be noted that the term ‘multi-allelic’ in the context of CNVs can be confusing because it is commonly used to note the fact that different types of copy number variants (i.e., deletion, duplication, triplication, etc.) segregate in the same region at the population level.

Compared to frequency-matched SNP variants, ‘bi-allelic’ CNVs with normal and deletion- or normal and duplication-carrying haplotypes exhibit greater population stratification[39]. Additionally, bi-allelic copy number polymorphisms (CNPs – CNVs with a frequency ≥1%) show a strong correlation with flanking bi-allelic SNP genotypes. Furthermore, deletion variants can be

tagged more efficiently than duplications. In a study by Handsaker *et al.*, duplication and multi-copy CNPs with a MAF >10% showed an almost uniform distribution of imputation correlation (r^2) values between 0–1, whereas in another study, only 40% of duplication CNVs showed a high correlation (r^2>0.8) with neighbouring SNPs[39,116]. This discrepancy could be due to allelic heterogeneity in proximity to the duplicated CNV or the fact that the transposed duplications are actually located farther away from the SNPs being tested for LD[9,39,116].

5. Haplotype and allelic variability in CNV regions

It has been somewhat disregarded that in addition to variable copy numbers, the allelic composition (i.e., the actual DNA sequence) can differ slightly from copy to copy (Figure 4). In CNV studies published to date, only dichotomous conditions (i.e., gain or loss[7], estimated total number of copies on two homologous chromosomes (0, 1, 2, 3, 4, etc.)[73,74,108,117] or the continuous normalised microarray intensity data[57,115,118]) have been considered.

A few studies have demonstrated that in addition to CNV detection it is also possible to infer and output the total allelic composition for each SNP marker (called the 'CNV genotype' or 'CNV-based SNP genotype call') within individuals' CNV regions using SNP genotyping arrays[52,119,120]. Similar to two-letter genotypes (e.g., 'AA' or 'AB' or 'BB'), these null, mono-, tri- and tetraploid genotypes (e.g., '–', 'A', 'AAB' and 'AABB', respectively) are inferred from the B allele frequency (BAF) data for each SNP variant and represent the total allelic composition from both homologous chromosomes in the studied individual[52,121,122]. When these SNP-based CNV genotypes (i.e., the exact allelic composition within the CNV region) are considered, it is possible to infer the haplotypic phase of the allelic copies within each copy number variable region – a problem that was well defined for trisomic chromosomes by Clark and colleagues[123].

The correct phase information has been shown to be important for SNP variants[124–126]. Therefore, it is important to know, how exactly the allelic copies of a DNA sequence are distributed on different haplotypes on two sets of chromosomes within a CNV region[8,127]. The ability to phase and differentiate between normal haplotypes and CNV-carrying haplotypes with different allelic composition and to determine their parental origin would enable new, possibly more powerful association analyses with human disease traits[128,129].

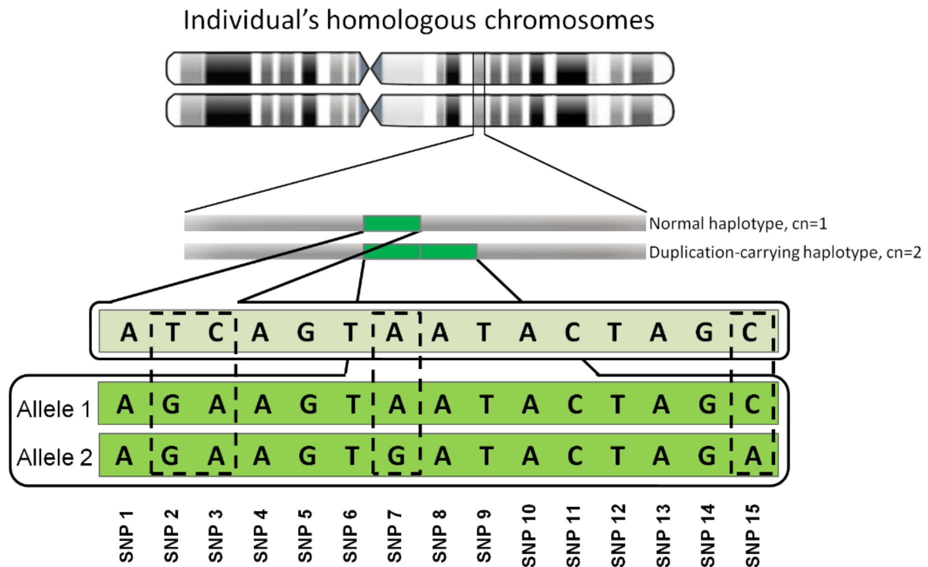

Figure 4. Haplotypes with slightly different allelic copies in a duplication CNV region. Haplotype-informative SNP genotypes in a duplicated region that are polymorphic between normal or duplication-carrying haplotypes and that can be used for phasing of given normal and CNV-carrying haplotypes are indicated with dashed rectangles.

AIMS OF THE PRESENT STUDY

In the era of high-throughput genetics and genomics, bioinformatics and statistical tools are required for the critical interpretation of thousands or even millions of parallel experiments and to help draw the correct conclusion(s) from these experiments. The present thesis aimed to find and develop statistical and computational tools and methods for DNA copy number variant calling and analysis from DNA microarray data.

The main research aims of the thesis were as follows:

1. To develop a statistical framework for CNV detection from human chromosome X-specific microarrays (Ref. I)

2. To develop a computational pipeline for CNV calling from whole-genome SNP genotyping microarray data (Ref. II–IV)

3. To develop a computational method for phasing of normal and variant-carrying haplotypes within CNV regions in families (Ref. II and IV)

4. To study and describe the transmission and allelic variability of CNV haplotypes (Ref. II and IV)

RESULTS AND DISCUSSION

1. Statistical method for detecting copy number variants from a chromosome X-specific microarray (Ref. I)

Prior to any analysis of interesting copy number variants (i.e., association analysis, experimental CNV validation, breakpoint refinement, and functional consequences), the variants have to be detected from the raw data. For this, special statistical tools and methods are required.

For the first practical application, we developed a computational workflow to detect copy number variants from a chromosome X-specific microarray containing 558 probes spanning almost the entire chromosome (with median spacing of 238 kb) and 111 control probes representing all autosomal chromosomes and chromosome Y. This single-channel array platform was developed in-house to screen patients with idiopathic mental retardation (MR). For the first step in CNV calling, microarray signals were transformed to the logarithmic scale and normalised between the arrays with respect to the median of: a) all fluorescent signal intensity (FSI) values or b) autosomal control probe-specific FSIs from microarrays included in the analysis. This was necessary because the absolute FSI values from different microarrays often varied several fold, and between-array normalisation enabled comparisons between FSI values from different microarrays. Next, the average and 90% tolerance interval values (TI90%) were calculated for each microarray probe using the data from the control panel containing FSIs from 5–15 cytogenetically controlled and phenotypically normal individuals. This step was performed separately for the male and female control panels.

To discover possible CNVs, FSI values for the studied individuals were compared to the calculated 90% upper and lower tolerance interval values. Regions with two or more adjacent probes deviating in the same direction were marked as putative copy number variants and visually inspected from the FSI plots (Figure 5c and d). Theoretically, such criteria would have resulted in a false positive proportion of 0.5%. Empirically, we calculated the false positive rate of 1% on average and 3% in the worst case scenario when comparing normal females against the panel of normal females (and male to males).

In practice, if no more than 10–20 probes deviated from the expected FSI values in a particular array-MAPH experiment, single probe alterations that might otherwise be interpreted as false positives were often further investigated, allowing smaller deletions or duplications to be detected or rejected.

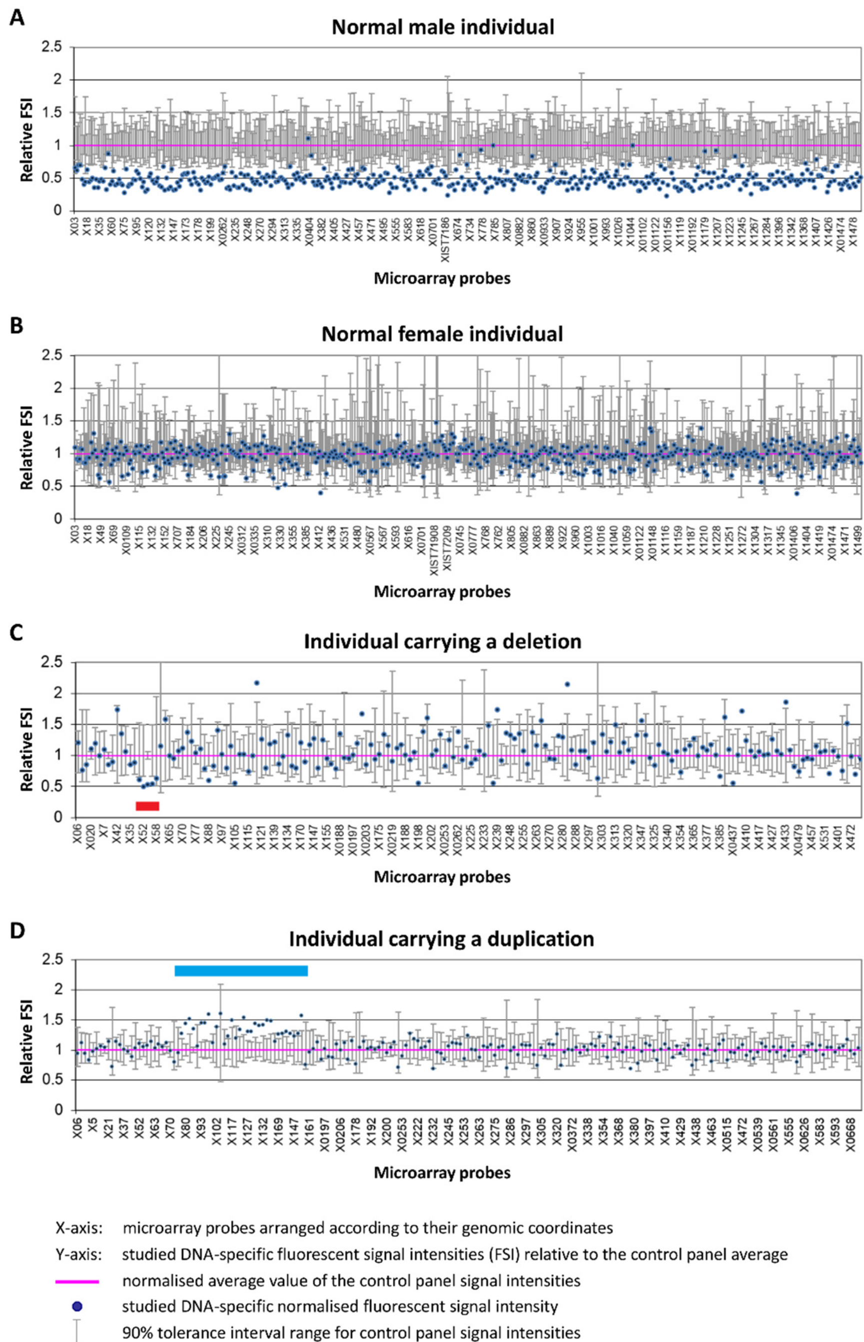

Figure 5. Different samples analysed on a chromosome X-specific single-channel microarray. Normal male individual (A) and normal female individual (B) compared to a panel of healthy female controls illustrate how one copy of chromosome X (male, XY) compares to two copies of the chromosome (female, XX). The examples show individuals carrying a deletion (C) and duplication (D) CNV detected by the array-MAPH method.

Although the chromosome X-specific array platform and related methods functioned quite robustly, the production and usage of this array was discontinued. New emerging computational methods developed for microarray-based genome-wide SNP genotyping platforms provided much higher resolution and more sensitive detection of CNVs.

2. Computational pipeline for CNV calling from SNP genotyping microarray data (Ref. II–IV)

Maintaining a good balance between false positive and false negative findings is nearly always a challenging task. Different scientific questions and analyses might prioritise one or the other, often with a resulting trade-off between specificity and sensitivity.

Because SNP genotyping arrays were first developed and optimised for SNP genotyping rather than genome-wide CNV detection and calling, CNVs detected from the SNP genotyping arrays include a considerable amount of false positive findings. To control the proportion of such false positive CNVs and to avoid possible false associations and/or interpretations in any downstream analyses, we developed a CNV calling pipeline for this application that combined two different CNV calling algorithms and a few additional QC steps in addition to standard quality control and filtering steps. As CNV calls from only one algorithm can include a large number of false positive calls, considering CNVs called by more than one independent algorithm will help decrease the number of false positive CNVs.

For each studied individual, CNV calling was performed in parallel with the QuantiSNP and PennCNV programs. These initial raw CNV calls were further merged as intersections; only CNVs that were called by both algorithms for the same individual in the same genomic loci were considered for most analyses. To achieve unambiguous results in downstream analyses, only CNVs that were similarly called (same type of overlapping copy number change – gain or loss) were considered in Ref. IV. We used population-specific B allele frequency reference files (PFB-file) for different datasets in PennCNV, because this approach seemed to provide higher sensitivity in CNV calling and more accurate CNV start and end coordinates. Separate raw calls in relevant CNV regions from both algorithms were studied either manually (Ref. II and Ref. III) or programmatically (Ref. IV) to avoid false negative findings in individuals without 'called-by-both-programs' CNVs (either in studied cases and healthy controls or family members) resulting from our conservative calling methodology. Additionally, we used breakpoints inferred by one single CNV calling program rather than the QuantiSNP-PennCNV intersection coordinates for experimental validations and other analyses that required CNV breakpoint coordinates because our CNV merging procedure systematically underestimated CNV size and therefore could possibly have provided incorrect starting and

ending positions. We used QuantiSNP coordinates (as described in Ref. II and Ref. III) because, in our hands, this algorithm gave more accurate breakpoint estimates than PennCNV. Discrete CNV regions (CNVRs) were defined by merging overlapping (≥1 bp) CNVs across all individuals in a study group as described previously[7,130].

For additional filtering steps, we filtered out very short putative CNVs and CNVs that contained only a few microarray probes. CNVs that had low log Bayes Factor (LBF) values (representing QuantiSNP confidence estimates for CNV calls) were also filtered out. If raw data were available, putative CNV regions were confirmed or omitted by visually inspecting the LRR and BAF plots for the CNV regions (as described in Ref. II and Ref. IV). We also filtered out CNV regions where any member of the corresponding family had any unclear raw CNV calls ('called-by-one-program') when working with family data (Ref. IV).

3. Computational method for phasing normal and variant-carrying haplotypes within CNV regions (Ref. II and IV)

Considering only CNVs called by two algorithms (and excluding CNV regions with inconclusive raw calls) provided good quality data that we further used to study how exactly new CNVs emerged and how existing CNVs were transmitted from parents to offspring. To accomplish this objective, we developed an algorithm that could computationally differentiate and phase normal and variant-carrying haplotypes within CNV regions in nuclear families based on genotyping microarray data.

Our algorithm works by examining adjacent regular two-letter and null, mono-, tri- or tetraploid CNV genotypes (described earlier) in each family member in a CNV region present in any member of the corresponding family (Figure 6). Our algorithm uses copy number variant calls, user-defined family structure and informative genotypes that are polymorphic between the parents to phase the allelic composition within each copy number variable region in the studied families by deterministically testing all possible molecular haplotypes and their transmission according to the Mendelian inheritance scenarios in the studied CNV locus in a given family. If there is more than one child in the studied family, all children will be considered simultaneously in this step. Finally, our algorithm will select these normal and/or CNV-carrying haplotypes and transmission scenarios that can unambiguously explain the allelic content for every member of the corresponding family for each CNV locus. If it is not possible to explain the allelic composition in the offspring by Mendelian transmission scenarios of parental haplotypes, non-Mendelian scenarios –

A Data collection and removal of uninformative and low-confidence SNP genotype calls

	QuantiSNP CNV genotypes			
Marker ID	**Father**	**Mother**	**Child 1**	**Child 2**
rs589559	AA	BB	B -	AB
rs2867167	AB	BB	B -	BB
rs666536	AB	AA	A -	AB
rs694861	BB	AB	A -	BB
rs12959303	AB	BB	B -	BB
rs608406	AB	AA	A -	AB
rs2124297	AA	AB	B -	AA
rs8083190	AB	AA	A -	AA
rs650464	BB	AB	B -	AB

B Haplotype phasing

	Phased haplotypes							
Marker ID	**Father**		**Mother**		**Child 1**		**Child 2**	
rs589559	A	A	B	B	B	-	B	A
rs2867167	A	B	B	B	B	-	B	B
rs666536	A	B	A	A	A	-	A	B
rs694861	B	B	A	B	A	-	B	B
rs12959303	A	B	B	B	B	-	B	B
rs608406	A	B	A	A	A	-	A	B
rs2124297	A	A	B	A	B	-	A	A
rs8083190	B	A	A	A	A	-	A	A
rs650464	B	B	B	A	B	-	A	B

C Illustrated haplotype segregation

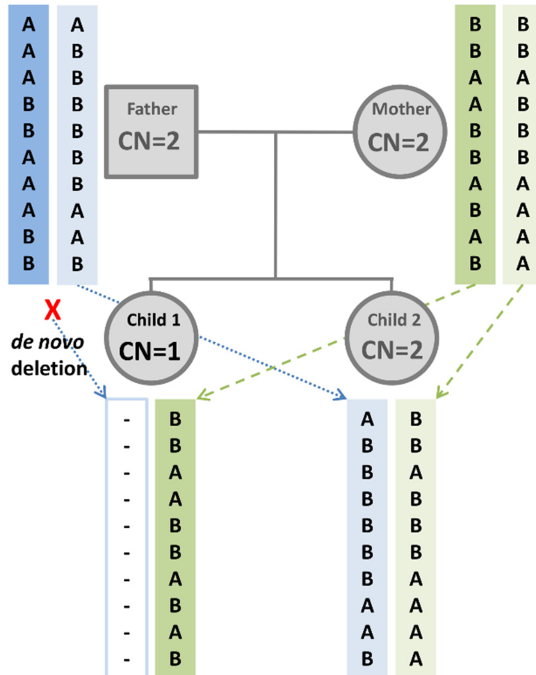

Figure 6. Computational phasing of normal and CNV-carrying haplotypes. A putative *de novo* CNV region where Child 1 has inherited the normal haplotype from the mother and a deletion-carrying haplotype from the father. The other child (Child 2) has inherited normal haplotypes from both parents. In Child 1, both normal and deletion-carrying haplotypes are already inherently 'phased' (A). By considering the haplotypes and genotypes observed in the mother, Child 2 and the father, finding the exact haplotypes transmitted from both parents is intuitively and computationally very straightforward (B and C).

de novo deletion or duplication events and uniparental isodisomy/heterodisomy – are considered. In the presence of informative genotypes between parental haplotypes, our algorithm can also determine on which parental chromosome the mutation occurred (e.g., inter- or intra-chromosomal duplication). If there are no haplotype-informative markers within a CNV region and the simplest haplotypes and Mendelian inheritance scenarios do not explain the allelic composition in all family members, it is not possible to determine a single set of correct haplotypes and inheritance scenarios and several equally possible haplotypes and Mendelian and/or non-Mendelian transmissions are suggested as the result.

4. Transmission and allelic variability of CNV-carrying haplotypes (Ref. II and IV)

It is often important to identify which parent transmitted a certain variant (e.g., a putatively disease-causing CNV). Additionally, it is useful to understand how the variant of interest segregates within the corresponding family and whether the other affected or unaffected siblings have inherited the same variant and alleles (as described in Ref. II). In the case of *de novo* variants that are not directly inherited but have often emerged in parental germline cells, it might be important to elucidate whether the mutation occurred on the maternal or paternal chromosomes.

To solve these questions, we used our phasing algorithm to resolve the normal and CNV-carrying haplotypes in trios or larger nuclear families based on genotype and copy number estimates from SNP microarray data. By studying 34 Estonian families (22 mother-father-child trios and 12 families with multiple siblings) and 30 HapMap Yoruban (YRI) trios, we discovered that it was computationally possible to unambiguously determine and phase the underlying normal and CNV-carrying parental haplotypes in vast majority (94%) of CNV regions and follow their transmission in offspring (Ref. IV). We observed that haplotype phasing was more efficient in CNV regions where only one CNV-carrying haplotype was present in one of the parents, resulting in a single or very few equally possible Mendelian and/or non-Mendelian transmission scenarios. In comparison, when *de novo* CNVs or multiple CNV-carrying haplotypes were present in the parents (especially duplication-carrying haplotypes), two or more equally possible haplotype combinations/transmission scenarios were often plausible (Ref. IV).

4.1 Transmission of normal and CNV-carrying haplotypes within CNV regions in nuclear families (Ref. IV)

We further analysed CNV regions that could be unambiguously phased in Estonian and HapMap YRI families and counted the number of transmission

events of normal or CNV-carrying haplotypes from the parents carrying a CNV. CNV-carrying haplotypes were observed in the offspring slightly less frequently than expected by random Mendelian inheritance. To further investigate this phenomenon, we analysed the transmission of deletion and duplication-carrying haplotypes in the combined dataset while also considering CNV length. Although this analysis revealed small deviations from the expected Mendelian transmission rate of 50% for both deletions and duplication in nearly all CNV length intervals, the previously observed differences were mainly driven by the under-transmission of short (<10 kb) deletion-carrying haplotypes in the HapMap dataset.

Although these CNVs in the HapMap dataset were validated by other independent means, it is possible that these short under-transmitted CNVs contained proportionally more false positive CNV calls (either cell-line artefacts of actually incorrect CNV calls) that could not be transmitted and therefore appeared to be under-transmitted. However, a similar tendency was observed for longer (>10 kb) deletion-carrying haplotypes in both studied cohorts. Such bias might be expected in the case of some high penetrance CNVs associated with severe disease phenotypes. Moreover, it has been suggested that this effect could be more pronounced for larger deletion variants interrupting genes of vital importance; consequently this effect is more likely under stronger (prenatal) selection. The opposite effect that was observed for a slightly increased transmission rate for longer (>100 kb) duplications (although this did not reach statistical significance) could possibly be explained by the contribution of duplications in providing the means for functional redundancy and facilitating exon shuffling, gene fusion and gene duplication. By generating new functional genes, duplication events may be an important mechanism for long-term evolutionary changes in humans and thus occur under positive selection. Although similar results were also observed in several earlier studies, only a very cautious interpretation of both findings should be considered. Larger studies with high-resolution platforms and validation techniques are merited to confirm and further investigate these interesting phenomena.

4.2 Putative *de novo* copy number variants (Ref. IV)

The process of directly deleterious variants being under negative selection and thus systematically under-transmitted from generation to generation is balanced by germline mutation events that generate *de novo* variants that are observable in new generations.

In our combined dataset we detected 27 CNVs that were identified as putative *de novo* copy number alterations in the offspring. Out of these 27 putative *de novo* CNVs, 20 were deletions and 7 were duplication events. As determined by our algorithm and confirmed by manual inspection, five out of nine unambiguously phased putative *de novo* CNVs appeared on maternally inherited chromosomes and four on a paternally inherited chromosome. We use

the term 'putative' *de novo* CNVs because such variants might not be true *de novo* mutations even if these CNVs are unambiguously phased and validated by other experimental methods. Alternatively, these can be somatically derived cloned mutations or artefacts often observed when DNA from cell-lines is analysed. *De novo* CNVs might also appear due to complex haplotype compositions of a studied family in a given locus (e.g., in CNP loci where haplotypes with 0 and 2 copies are combined in one parent, leading to incorrect calling of *de novo* variants from unphased CNV data, thereby highlighting the relevance of phasing of the exact parental haplotypes).

4.2 Allelic variability in duplication CNV regions (Ref. IV)

Deterministic phasing within CNV regions allowed us to differentiate between normal and CNV-carrying haplotypes with different allelic compositions and to more extensively study the allelic variability within the discovered copy number gain-carrying haplotypes. Because alternative allelic copies within CNV regions may modify the severity of a phenotype (including disease susceptibility), we aimed to determine the occurrence of alternative allelic copies within these multi-copy haplotypes.

We studied these CNV regions in a combined dataset where one or both parents of the same family had duplication or triplication-carrying haplotypes in the same regions. We determined that informative polymorphic genotypes were present within the multi-copy haplotypes in 93% of such CNV regions. Therefore, it was possible to phase and differentiate between normal and multi-copy haplotypes (as shown in Figure 3). Furthermore, heterozygous genotypes were also present within the multi-copy haplotypes in 67% of these CNV regions (Figure 7), thereby allowing us to define alternative allelic copies within these copy number gain-carrying haplotypes and demonstrating extensive and to-date unmeasured allelic variability in multi-copy CNV regions in the human genome.

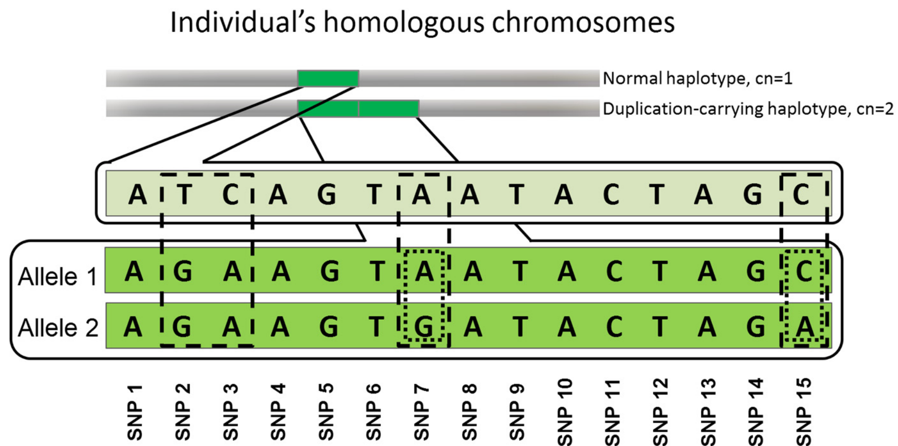

Figure 7. Allelic variability in CNV regions and within duplication CNVs. SNP-based genotypes within duplicated region that are polymorphic between normal (haploid copy number cn=1) and duplication-carrying haplotypes (cn=2) are indicated with dashed rectangles. The duplication-carrying haplotype is composed of two allelic copies distinguished by single nucleotide variants (indicated as SNP7 and SNP15) that are polymorphic within the duplication-carrying haplotype (depicted by a dotted rectangle).

SUMMARY AND CONCLUSIONS

Using statistical and computational methods, we studied copy number variants (CNVs) and different CNV-related aspects of the human genome. The most important results of this thesis are as follows:

1. We developed computational methods and software for phasing haplotypes within CNV regions in nuclear families based on SNP genotyping microarray data. Using two different datasets, we determined that it was possible to unambiguously phase all parental haplotypes and follow their transmission in offspring in the vast majority of CNV regions.

2. By counting the number of transmission events of normal or CNV-carrying haplotypes in two family datasets, we found that CNV-carrying haplotypes were observed in offspring less frequently than expected by Mendelian inheritance. This results suggests the systematic under-transmission of CNVs. This trend was stronger for deletion-carrying haplotypes.

3. We determined that the copies represented different alleles in two-thirds of the studied duplication-carrying CNV regions. This result demonstrates the extensive allelic variability in multi-copy CNV regions of the human genome.

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SUMMARY IN ESTONIAN

Arvutuslikud meetodid DNA koopiaarvu määramiseks

Arusaamine, et inimesed on oma geneetiliste materjali poolest erinevad, on suhteliselt vana. Ometigi sai võimalikuks ja algas selliste erinevuste ulatuslik ja süstemaatiline uurimine inimese genoomis alles suhteliselt hiljuti, uue aastatuhande alguses. Pärast inimese genoomi täieliku primaarjärjestuse esimese versiooni avaldamist[131] algas ulatuslik indiviidide ja erinevate populatsioonide vaheliste geneetiliste erinevuste (variantide) otsimine ja kaardistamine. See andis uut hoogu ka inimese haigusseoseliste geneetiliste variantide otsimisele ja kirjeldamisele. Paljude aastate jooksul keskenduti enamasti ühenukleotiidsete variantide ehk SNP-de otsimisele, sest olemasolevad tehnoloogilised vahendid ja metoodikad võimaldasid neid kõige lihtsamini suures mahus leida ja kirjeldada.

Kuigi juba aastakümneid oli tegelikult olnud teada, et erinevatel inimestel võivad olla erinevused ka pikemates DNA primaarjärjestuse lõikudes, sai DNA pikemate erinevuste, sealhulgas ka DNA koopiaarvu muutuste (ingl. *copy number variant* ehk CNV) süstemaatiline ja ulatuslik uurimine hoo sisse alles 2003. aastal. Ja kui esialgu uuriti ja seostati selliseid suuremaid muutusi DNA primaarjärjestuses tihtipeale erinevate tõsiste geneetiliste haigustega ja kasvajatega[132,133], sai peagi ühemõtteliselt selgeks, et sellised pikemad DNA ümberkorralduste variandid ei esine ainult seoses tõsiste haigustega, vaid on suhteliselt sagedased ka tervete inimeste geneetilises materjalis ja moodustavad suure osa indiviidide vahelistest erinevustest DNA tasemel[5–7].

Sellised muutused võivad olla väga erineva suurusega, alates tervete kromosoomide kordistumisest/kaotsiminekust kuni geenide ja üksikute eksonite duplitseerumiseni (koopiate kordistumine) või deleteerumiseni (koopiate kaotsiminek). Et eristada haigustpõhjustavaid CNV-sid normaalsetest ”tervete” inimeste vahelisest koopiaarvu variantidest, on tähtis aru saada sellest, kui suur on inimeste vaheline normaalne varieeruvus DNA koopiaarvu variantide tasemel. Seetõttu on oluline töötada välja uusi ja arendada edasi olemasolevaid ekperimentaalseid ja arvutuslikke meetodeid, millega detekteerida ja uurida DNA koopiaarvu muutusi inimese genoomis.

Käesoleva doktoritöö kirjanduse ülevaade keskendub DNA koopiaarvu variantide ja tekkemehhanismide kirjeldamisele. Samuti antakse ülevaade koopiaarvu variantidega senini seostatud fenotüüpidest, sealhulgas haigustest. Kirjeldatakse DNA koopiaarvu leidmiseks kasutatavate mikrokiipide tööpõhimõtteid ja olemasolevad arvutuslikke vahendeid.

Antud doktoritöö raames töötati välja erinevaid arvutuslikke meetodeid ja lahendusi DNA koopiaarvu muutuste leidmiseks ja nendega töötamiseks. Kõigepealt töötati välja statistiline raamistik ja arvutuslik metoodika CNV-de detekteerimiseks X-kromosoomi spetsiifiliselt mikrokiibilt. Kui hakkasime kasutama algselt SNP-de genotüpiseerimiseks mõeldud kogu genoomi katvaid mikrokiipe, tekkis vajadus leida ja automatiseerida meie andmetele ja kiipidele

sobivad CNV-de detekteerimise ja filtreerimise arvutuslikud lahendused tark-vara töövoogudena.

Uurides CNV-sid perekondade andmetest oli vaja täpselt määrata, milline (oletatavalt haigus-seoseline) koopiaarvu muutus milliselt vanemalt on lapsele/lastele pärandunud. Selle tarvis sai välja töötatud uudne arvutuslik metoodika, mis kasutades SNP mikrokiibi andmeid võimaldab faasida ja „järgida“ CNV-sid kandvate haplotüüpide pärandumist vanematelt lastele.

Käesoleva töö viimases osas uurisime me lisaks haigusseoseliste variantidele ka Tartu Ülikooli Eesti Geenivaramu ja rahvusvahelise HapMap projekti poolt kogutud tervetel inimestel esinevaid DNA koopiaarvu muutusi ja nende pärandumist perekondades. Selle uuringu üheks huvitavamaks tulemuseks oli deletsioonide „alapärandumine“ vanematelt lastele, st. deletsioone kandvaid haplotüüpe esines laste genoomides oluliselt vähem, kui Mendeliaalse pärandumise korral oodata oleks võinud. Teiseks huvitavaks tulemuseks oli *de novo* ehk uute DNA koopiaarvu muutuste leidmine – nimelt esines paljude laste genoomides selliseid DNA koopiaarvu variante, mida kummagi vanema genoomis ei olnud. Kolmandaks, uurides duplikatsioone perekondades faasitud CNV-de regioonides, leidsime me, et kaks kolmandikku (67%) duplikatsioonides esinevatest alleelsetest koopiatest ei olnud identsed, vaid mõnevõrra erinevad, demonstreerides seni teadmata olnud alleelse varieeruvuse määra DNA koopiaarvu korduste regioonides.

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Dear Lord, do not give me light;
Let it be yours and yours alone;
But grant me a sincere desire
To strive towards it.

Desiderius Erasmus

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PUBLICATIONS

CURRICULUM VITAE

Name: Priit Palta
Date of birth: April 25, 1981
Address: Institute of Molecular and Cell Biology, 23 Riia St., 51010 Tartu
E-mail: priit.palta@ut.ee
Current position: Research scientist, Institute for Molecular Medicine Finland (FIMM), University of Helsinki

Education:

2007–2011 Ph.D. student in bioinformatics, Institute of Molecular and Cell Biology, University of Tartu
2005–2007 M.Sc. student in bioinformatics, Institute of Molecular and Cell Biology, University of Tartu
2000–2005 B.Sc. student in bioinformatics, Institute of Molecular and Cell Biology, University of Tartu
1994–2000 Tartu Mart Reinik's Gymnasium
1988–1994 12th High School of Tartu

Working experience:

Since 2013 Research scientist, Institute for Molecular Medicine Finland (FIMM), University of Helsinki
2011–2013 Visiting scientist, Wellcome Trust Sanger Institute
2007–2011 Research scientist, Estonian Biocentre
2005–2007 Bioinformatics specialist, Estonian Biocentre
2004–2008 Bioinformatics specialist, Institute of Molecular and Cell Biology, University of Tartu

Main fields of research:

DNA copy number variants in health and disease; rare genetic variants in migraine and schizophrenia; enriched (loss-of-function) variants in isolated populations.

Publications:

Palta P, Kaplinski L, Nagirnaja L, Veidenberg A, Möls M, Esko T, Nelis M, Metspalu A, Laan M, Remm M. **Haplotype phasing and inheritance of copy number variants in nuclear families.** *PLoS One.* 2015;10(4):e0122713. doi: 10.1371/journal.pone.0122713. PubMed PMID:25853576.

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Scholarships and awards:

2013	Stipend of Finnish Cultural Foundation
2007	Artur Lind’s stipend, Estonian Genome Foundation
2007	Kristjan Jaak’s stipend, Archimedes Foundation

Other scientific activities:

Since 2012	Member of International Headache Society
Since 2007	Member of the Estonian Society of Human Genetics

Supervised dissertations:

2008–2009	Andres Veidenberg, M.Sc., Department of Bioinformatics, Institute of Molecular and Cell Biology, University of Tartu
2007–2008	Taavi Võsumaa (co-supervisor), M.Sc., Department of Bioinformatics, Institute of Molecular and Cell Biology, University of Tartu
2007–2008	Ksenia George, B.Sc., Department of Bioinformatics, Institute of Molecular and Cell Biology, University of Tartu

ELULOOKIRJELDUS

Nimi: Priit Palta
Sünniaeg: 25. aprill, 1981
Aadress: TÜ Molekulaar- ja Rakubioloogia Instituut, Riia 23, Tartu 51010
E-post: priit.palta@ut.ee
Praegune töökoht: Teadur, Soome Molekulaarse Meditsiini Instituut (FIMM), Helsingi Ülikool

Haridus:

2007–2011 Ph.D. õpingud bioinformaatikas, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2005–2007 M.Sc. õpingud bioinformaatikas, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2000–2005 B.Sc. õpingud bioinformaatikas, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
1994–2000 Tartu Mart Reiniku Gümnaasium
1988–1994 Tartu 12. Keskkool

Töökogemus:

Alates 2013 Teadur, Soome Molekulaarse Meditsiini Instituut (FIMM), Helsingi Ülikool
2011–2013 Külalisteadur, Wellcome Trust Sanger'i Instituut
2007–2011 Teadur, Eesti Biokeskus
2005–2007 Bioinformaatika spetsialist, Eesti Biokeskus
2004–2008 Bioinformaatika spetsialist, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool

Peamised uurimisvaldkonnad:

DNA koopiaarvu variandid ja nende seos haigustega, migreeni ja skisofreeniaga seotud mittesagedased geneetilised variandid ja kõrgenenud alleelisagedusega geneetilised variandid isoleeritud populatsioonides.

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Saadud stipendiumid:

2013	Soome Kultuuri Sihtasutuse stipendium
2007	Artur Linnu nimeline stipendium, Sihtasutus Geenikeskus
2007	Kristjan Jaagu nimeline stipendium, Sihtasutus Archimedes

Muu teaduslik organisatsiooniline ja erialane tegevus:

Alates 2012	International Headache Society liige
Alates 2007	Eesti Inimesegeneetika Ühingu liige

Juhendatud väitekirjad:

2008–2009	Andres Veidenberg, M.Sc., bioinformaatika õppetool, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2007–2008	Taavi Võsumaa (co-supervisor), M.Sc., bioinformaatika õppetool, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2007–2008	Ksenia George, B.Sc., bioinformaatika õppetool, Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool

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