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ŽIVILĖ ČIULADAITĖ

THE DISTRIBUTION OF UNBALANCED CHROMOSOMAL REARRANGEMENTS IN THE HUMAN GENOME AND ITS ROLE IN THE AETIOPATHOGENESIS OF INTELLECTUAL DISABILITY

Summary of Doctoral Dissertation
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VILNIAUS UNIVERSITETAS

ŽIVILĖ ČIULADAITĖ

NESUBALANSUOTŲ CHROMOSOMINIŲ POKYČIŲ PAPLITIMAS ŽMOGAUS GENOME IR JŲ REIKŠMĖ INTELEKTINĖS NEGALIOS ETIOPATOGENEZEI

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INTRODUCTION

Unbalanced chromosome rearrangements — deletions, insertions and duplications ranging from a kilobase to multiple megabasepairs in length, collectively termed copy number variations (CNVs) (Feuk *et al*., 2006) — are a major component of human genomic variation and are thought to be important contributors to phenotypic diversity and human disease (Stankiewicz&Lupski, 2010). One of the most common diseases associated with CNVs is intellectual disability (ID). The American Association on Intellectual and Developmental Disabilities (AAIDD, 2002) has defined ID as significantly below-average overall intellectual functioning and deficits in adaptive behaviour that start before adulthood (Shevell *et al*., 2003).

Although CNVs have long been implicated in phenotypic diversity and human disease, the mechanisms by which they exert their functional impact have proven elusive. Recently, developments in high-throughput DNA sequencing and microarray-based comparative genome hybridization (array CGH) techniques have greatly facilitated the ability to analyze CNVs in human populations and models organisms and construct maps of CNVs.

Whole human genome analysis has revealed that there are at least 12,000 CNVs overlapping more than 1000 genes, therefore accounting for almost 13 % of the genome, and in any individual there are on average over 1000 CNVs covering some 4 million base-pairs (Conrad *et al*., 2010; Mills *et al*., 2011). Individual CNV events may be benign or pathogenic and can manifest different phenotypes depending on the genomic context of the variant.

With the expansion of knowledge about human genome diversity, particular attention was drawn to genetic causes of ID, which is a big health problem in the world (accounting for approximately 40 % of cases of disability). The prevalence of ID in the general population is reported to be 1 to 3 %, for which a cause is unknown in as many as 60 % of patients (Rauch *et al*., 2006).

In this study, patients with ID and apparently healthy control population were genotyped with a high-resolution array CGH. Use of array technology has resulted in the discovery of widespread CNVs in the human genome, both polymorphic variation in healthy individuals and novel pathogenic copy number imbalances. The deep bioinformatic analysis of rare, probably pathogenic CNVs has revealed the role of CNVs in the aetiopathogenesis of the central nervous system and identified strong candidate genes in ID and/or other clinical features. The information about common and rare CNVs will be helpful in the clinical interpretation of nonreccurent probably pathogenic CNVs.

Aim of the study:

To identify unbalanced chromosomal rearrangements and strong candidate genes, the improper dose of which could be crucial in the aetiopathogenesis of ID.

Main tasks of the research study:

1. To evaluate widespread copy number variants in the human genome according to type of alteration, size, location, and frequency based on molecular karyotyping results obtained from general population of Lithuania.
2. To define criteria for interpretation of rare CNVs according to molecular karyotyping results in a cohort of apparently healthy controls and individuals with ID.
3. To identify possible candidate genes for ID according to bioinformatic analysis: expression pattern and molecular function of genes involved or disrupted by rearrangement, protein-protein interaction, and genotype-phenotype correlation.
4. To investigate possible mechanisms of CNV formation by studying genomic architectural features of the sequence of sites of CNV.
5. To assess the diagnostic yield of 105 K and 400 K microarray.

Relevance and novelty of the research

The prevalence, lifelong socio-economic problems, and poor curability emphasise the impact of ID and the need for research to understand the aetiology and pathogenesis of ID. It is known, that INN is a highly heterogeneous disorder, with the majority of cases having genetic etiology. The establishing of genetic diagnosis in patients with ID is often complicated, mainly because of extensive genetic and phenotypic heterogeneity. The genetic basis of ID may range from large cytogenetic abnormalities to point mutations and epigenetic alterations. The rare monogenic (single gene or Mendelian) disorders are of substantial interest because identification of their genetic basis provides knowledge about disease mechanisms, affected biological pathways, and potential therapeutic targets (Ng *et al*., 2010).

For a long period it has been difficult to elucidate the etiology of ID. Cytogenetic abnormalities are considered to represent a major cause of idiopathic ID (van Karnebeek *et al*., 2005; Rauch *et al*., 2006), but routine karyotype analysis is not sensitive enough to detect subtle chromosome rearrangements (less than 5 Mb). Sequencing of large number of genes was time-consuming and expensive, and most efforts at disease-gene identification involved linkage analysis and positional cloning within large families with a large number of affected individuals. The research was limited by factors such as the availability of only a small number of cases/families, locus heterogeneity, or substantially reduced reproductive fitness.

Recently the rate of identification of novel genes associated with ID has progressed rapidly due to advances in gene discovery by molecular cytogenetic and molecular genetic techniques (array-CGH and NGS, respectively). The introduction of genomic microarray technology to the study of ID has dramatically increased both the diagnostic yield and the biological understanding of the aetiopathogenesis of ID. The ability to recognize dozens of submicroscopic chromosomal rearrangements in all genome array CGH provides a unique possibility to identify novel microdeletion/microduplication syndromes, expand the phenotypes and elucidate the genomic aetiology of

previously well-known conditions, narrow critical regions of known syndromes, reveal the molecular mechanisms of some chromosomal aberrations, and identify candidate genes which changes in expression pattern may be crucial for the pathogenicity of ID.

Theoretically, it is now possible to identify causal mutations in most individuals with ID regardless of frequency, heterogeneity, and inheritance (Topper *et al.*, 2011). Thus every single patient with unrecognized clinical condition becomes the main unit in identifying new candidate genes for Mendelian disorders.

A specific genetic diagnosis facilitates comprehensive medical care and accurate recurrence risk for the family. Establishing the aetiology may also decrease anxiety and allow for proper recurrence risk estimates for future pregnancies when the parents plan to have more children in the future. Although the risk of recurrence for developmental disabilities of unknown aetiology based on empirical datasets is quoted as 3–7% (Van Naarden Braun *et al.*, 2005), a *de novo* microdeletion or microduplication would have a much lower risk of recurrence. In contrast, if autosomal recessive state or unbalanced translocation is identified as a cause of ID, there is a 25–50 % chance of recurrence.

Although high-resolution array CGH is a very useful tool to detect small rare deletions or duplications, the clinical interpretation of rare and small CNVs is still challenging due to genetic heterogeneity and the distinct molecular mechanisms by which they exert their functional impact. To differentiate between CNVs that are likely implicated in the aetiology of ID and CNVs that are not involved in the disease phenotype, it is essential to recognize common variants also identified in normal controls. The analysis of CNVs in patient with ID and apparently healthy individuals in the population of Lithuania is a new area of research. A CNV data set generated from healthy controls has the potential to be very useful in clinical applications as a comparator with CNVs identified in individuals with ID. Detailed analysis of human genome variability will help to better understand the mechanisms of formation of the

CNVs and to verify other scientist's hypotheses about unstable areas of the genome.

Statements to be defended:

1. Copy number variants are widespread in the human genome; most of them are common and benign.
2. Rare CNVs in patient with ID are larger and have a significant excess of genes when compared with controls analyzed on the same microarray platform.
3. The gene-encoded transcriptional factors or proteins that are involved in synapse formation and maintenance, chromatin remodeling, or neurotransmission play a crucial role in normal brain development and function and are potential candidate genes in the pathogenesis of ID.
4. Unique chromosomal rearrangements occur via replication-based mechanism and common chromosomal rearrangements occur via recombination.
5. 400 K microarray is a high-resolution tool for molecular karyotyping, appropriate for scientific study of population CNVs as well as clinical diagnostic test.

PATIENTS, MATERIALS AND METHODS

In this study, 175 patients with idiopathic ID and 150 apparently healthy individuals were recruited after the appropriate approval and informed consent of the human subjects.

Array CGH experiments were carried out according to the recommendations of the manufacturer and performed at the cytogenetic laboratory of the Cyprus Institute of Neurology and Genetics. We used a consensus microarray design, focusing on unique genomic regions and avoiding repetitive sequences. The arrays were either 105 K or 400 K custom-designed 60-mer oligonucleotide arrays (Agilent Technologies, Santa Clara, CA) with a whole-genome backbone, plus targeted, higher density coverage of known disease-causing regions. The backbone coverage included probes spaced approximately every 7-35 kb, allowing for CNVs of approximately 23 kb and greater to be detected. For the targeted regions, we could identify imbalances of approximately 600 bp –20 kb.

Arrays were scanned using an Agilent SureScan Scanner (Agilent Technologies, Santa Clara, CA). Results were extracted using the Feature Extraction program (Agilent Technologies, Santa Clara, CA) and analyzed with Nexus Copy Number software (Biodiscovery, CA). Data include only those imbalances that contained at least three consecutive probes with abnormal log2 ratios. Data were presented as minimum coordinates (sequence positions of the first and last probes within the CNV) in the NCBI36 genome assembly.

CNVs were categorized as pathogenic, VOUS or benign based on overlapping with CNVs in the healthy control population, known clinically relevant regions, gene content, and inheritance pattern as described previously. CNVs overlapping with CNVs reported in at least two studies in the Database of Genomic Variants (DGV) or in our internal controls were considered common CNVs; those that overlapped partially (<50 %) or did not overlap with CNVs reported in the DGV or our internal controls were called rare. All

rare CNVs were confirmed by RT-PCR or FISH methods. For both deletions and duplications, the genes located within the CNVs and neighboring genes were assessed. Imbalances that involved large genomic segments from the chromosomal backbone coverage were considered likely to be pathogenic if they contained multiple known genes and did not overlap a confirmed benign CNV region. CNVs were classified as pathogenic if the CNV included an autosomal dominant gene known to cause a disease phenotype.

RESULTS AND DISCUSSION

4.1. CNV distribution among apparently healthy individuals

To understand the role of CNVs in human disease, we first need insight into the prevalence of structural variants in the general population. Uncovering the nature and patterns of CNVs in the general population can help to uncover the biological significance of *de novo* and infrequent hereditary CNVs in rare genomic disorders and Mendelian diseases (Lupsky, 2007) and facilitate the identification of variants that may confer increased risk for common diseases or act as modifiers of a given phenotype (McCarroll et al., 2007; Beckmann *et al*., 2007).

In this study, we investigated genome-wide characteristics of CNV in apparently healthy individuals. The result is the first comprehensive map of copy number variation in the Lithuanian genome, which provides an important resource for studies of genome structure and human disease. We predict that this map of CNVs and potentially dosage-sensitive genes will be invaluable for both clinical and research purposes in the future.

A total 32,463 CNV calls were detected among 150 apparently healthy individuals. The frequency of copy-number losses was 1.1-fold greater than the occurrence of gains, with a total of 17,231 losses (average size 117 kb, median 57 kb) versus 15,232 gains (average 141 kb, median 82 kb).

When we classified all CNVs by ≤50 kb , 51–100 kb, 101–200 kb, 201–500 kb, 501 kb – 1 Mb and >1 Mb lengths, we found that a longer length was associated with a less frequent distribution (Fig. 1). We found an excess of deletions versus duplications for all events, but this trend was reversed for events >500 kb. The relative enrichment of deletions at smaller sizes may reflect higher *de novo* rates of occurrence of deletions (Turner *et al*., 2008), whereas their depletion at larger sizes is consistent with large deletions being more deleterious with stronger prenatal selection than duplications. The most common CNVs (with a frequency of >75 %) are smaller than 100 kb and include less than 10 genes (Fig.2).

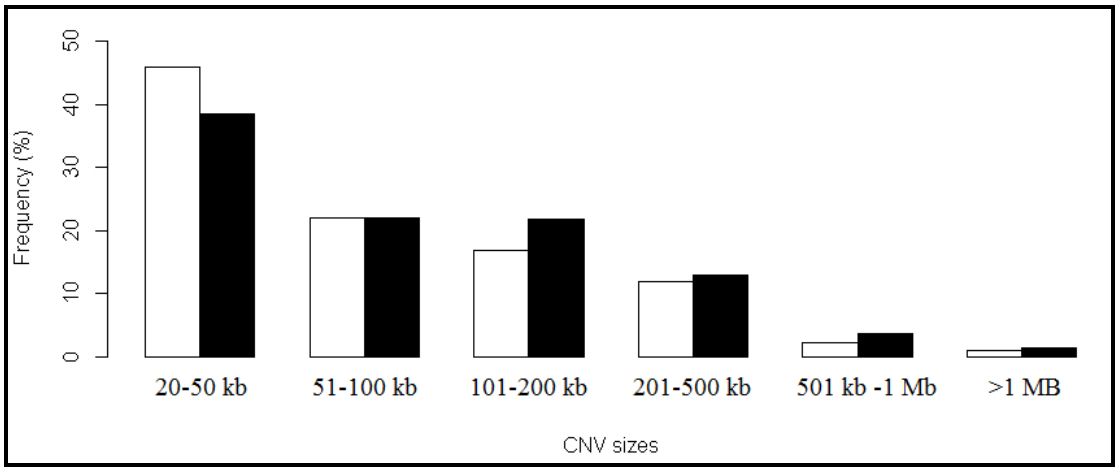

Figure 1. The distribution of CNVs length.

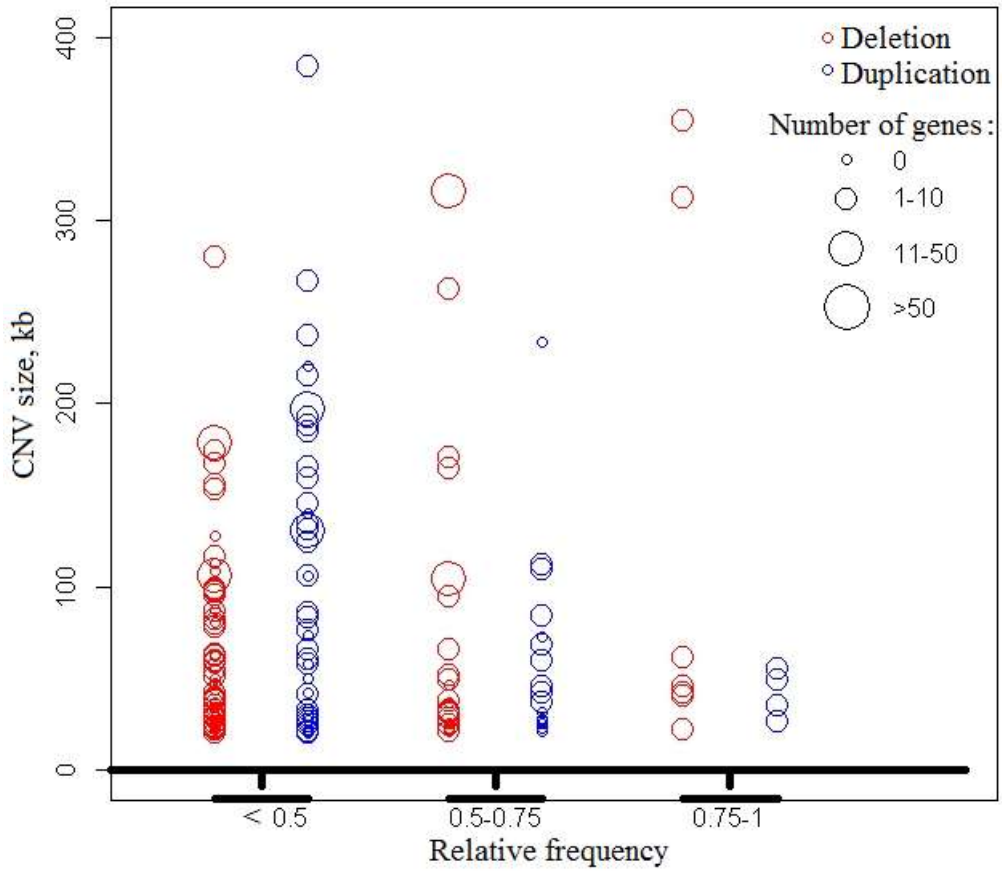

Figure 2. The distribution of common CNVs among apparently healthy individuals in association with length and number of genes.

By merging overlapping CNVs identified in each individual, we delineated a minimal set of 1072 discrete CNV regions (CNVRs) spanning 166 Mb (5.1 %) of the autosomal genome. These were similar to the previous

1081 regions reported by Redon *et al.* (2006) and 1083 regions reported by Pinto *et al*. (2007), but less then Kato *et al*. (2010) (699 for 500KEA; 669 for WGTP). However, the genomic coverage was less than the previous 253 Mb (Redon *et al*., 2006) or 240 Mb for WGTP (Kato *et al*., 2010) and more than 72 Mb for 500KEA (Kato *et al*., 2010). The length of the median CNV region was 65 kb, which was longer than the previous 13 kb (Kato et al., 2010) and shorter than the previous 163 kb (31 kb for 500KEA; 229 kb for WGTP) in the Redon *et al*. (2006) study or 185 kb in the Pinto *et al*. (2007) study. These differences presumably result from differences in the platforms.

87% (969 out of 1072) of these CNVRs were common. Among them we found four common CNVRs that were not reported CNVs in the Database of Genomic Variants (DGV). Interestingly, in the population of Lithuania 53 % (72 out of 137) of rare CNVRs are well known from previously studies. 47% (65 out of 137) of rare CNVRs were novel and not present in the DGV.

For a whole-genome view of CNVs, we drew CNV lengths and the number of individuals with CNVR on a chromosome map (Fig. 3). The number of CNVRs was roughly proportional to chromosome length (Fig. 4), but chromosome coverage by CNVs was not proportional to chromosome length (Fig. 5). In particular, chromosomes 9, 16 and 22 were highly covered by CNVs (Fig. 6), because of the presence of many large CNVs. The association clearly increased according to the increase in the population frequencies of CNVs. These results indicate that segmental duplications are involved in the recurrent generation of CNVs.

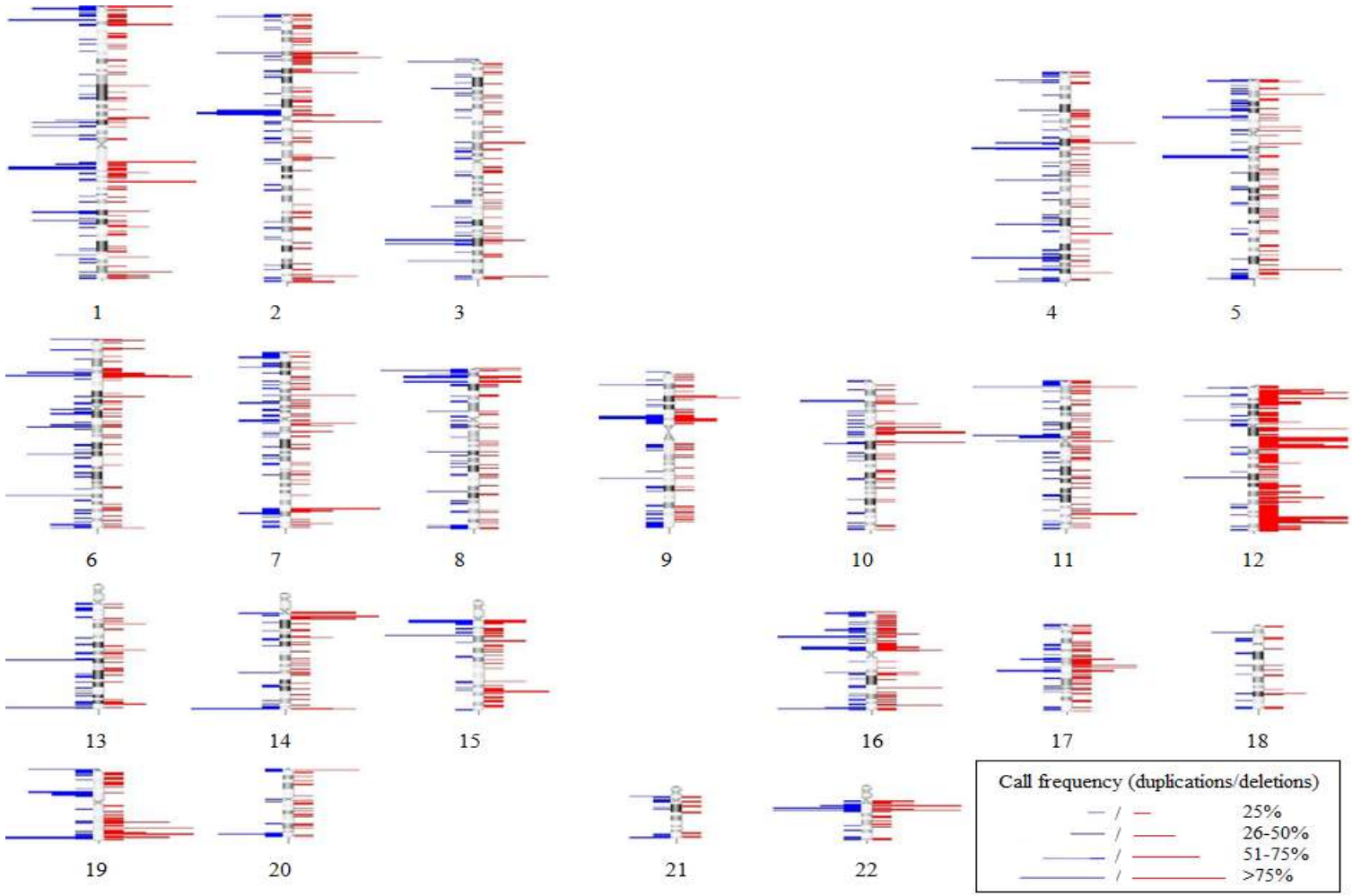

Figure 3. Genomic distribution of CNVRs. The chromosomal locations of 1072 CNVRs are indicated by lines to either side of ideograms. Blue lines denote – duplications; red lines denoted deletions. The length of lines represents the frequency of each CNVR. The most frequent CNVRs are in pericentromeric regions on chromosome 1, chromosome 2, chromosome 9, chromosome 11, and chromosome 22.

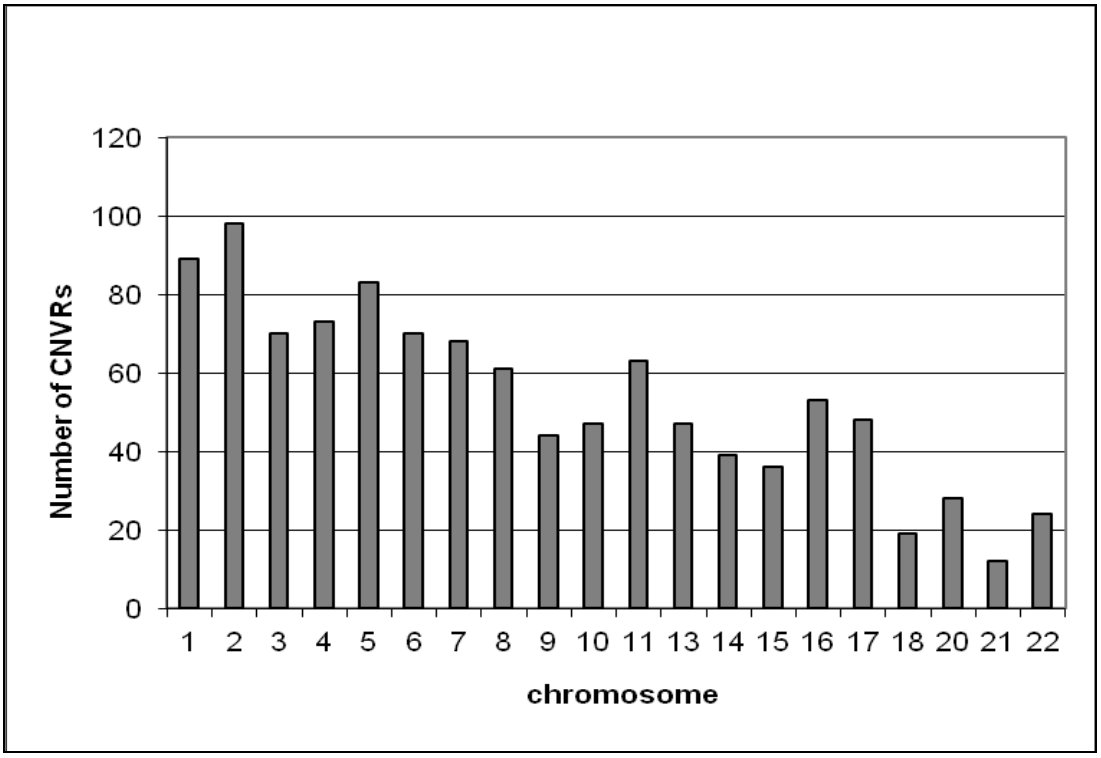

Figure 4. Number of CNVRs per autosome (excluding chromosome 12 and 19). The number of CNVRs is roughly proportional to chromosome length.

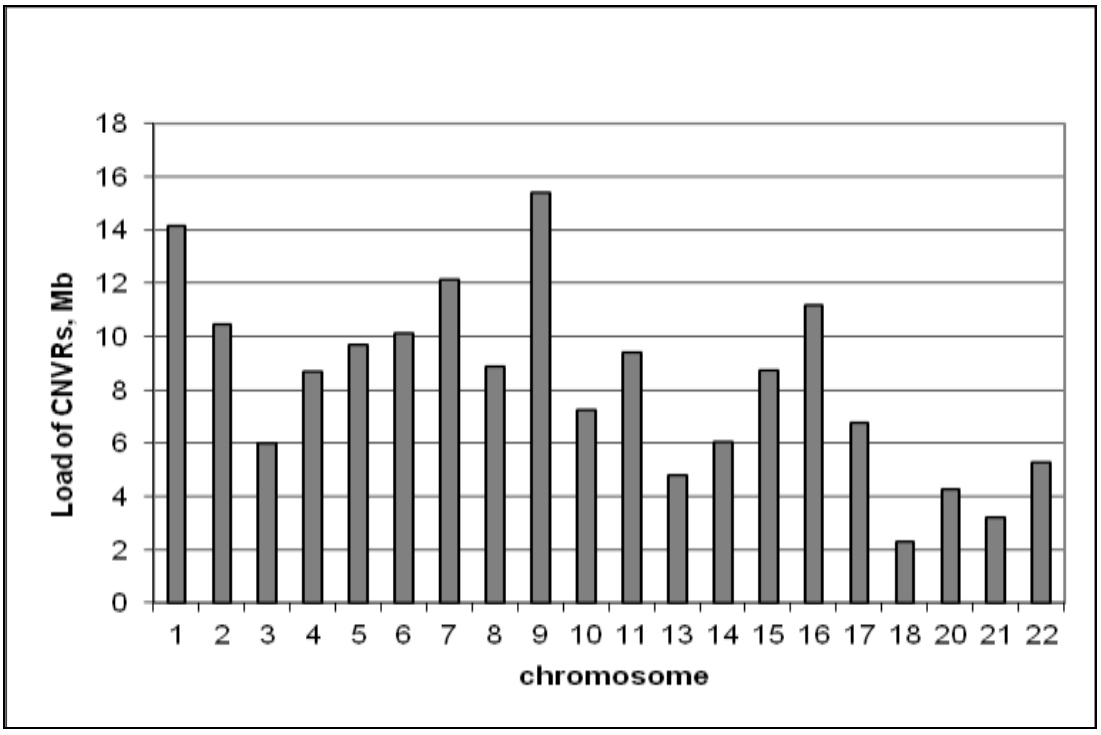

Figure 5. Merged CNVR coverage separately by chromosome (excluding chromosome 12 and 19).

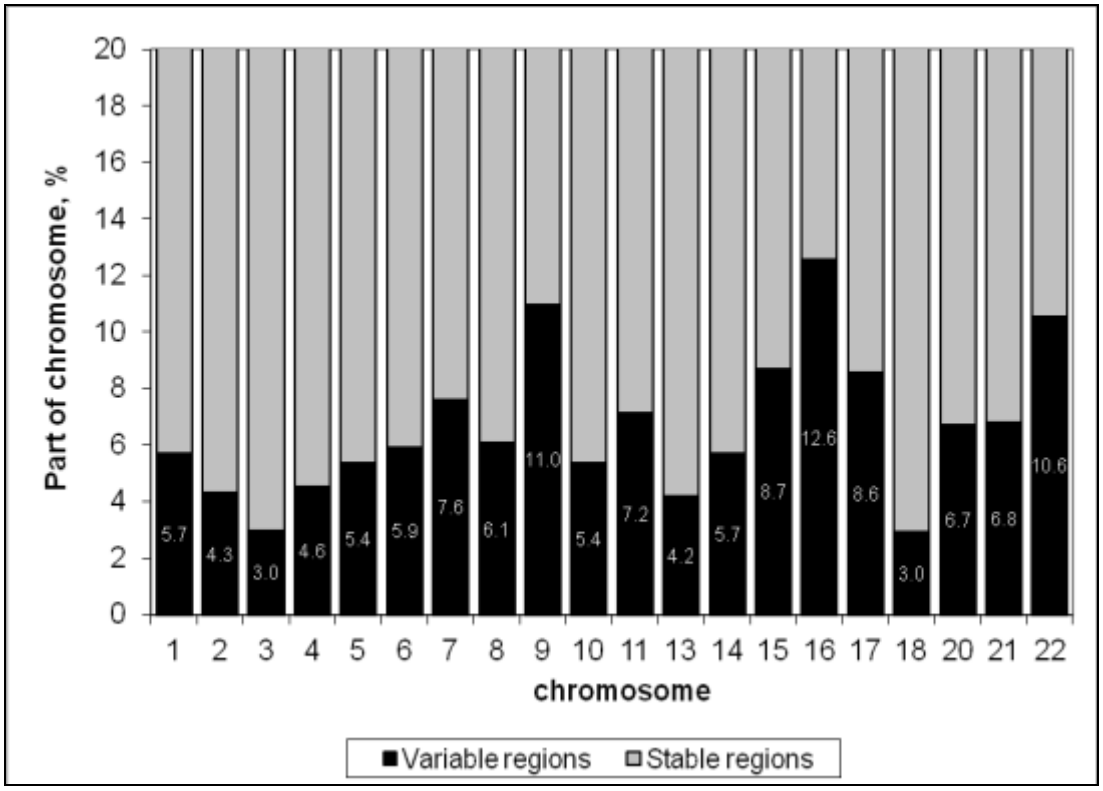

Figure 6. Proportion of variable and stable regions per autosome (excluding chromosome 12 and 19). The most variable in copy number are chromosome 9, 16 and 22.

Analysis of the sequence context of CNVR sites suggested that large deletion and duplication events are much more likely to be mediated by segmental duplications (Fig. 7). This observation is consistent with previous reports that indicated the role of NAHR is more pronounced for larger CNV events than small events (Tuzen *et al*., 2005; Korbel *et al*., 2007; Condrad *et al*., 2009). Also, the loci at which both deletions and duplications occurred were markedly enriched for segmental duplications (Fig. 8). It was not surprising because segmental duplications are substrate for non-alelic homologous recombination that results reciprocal deletions and duplications.

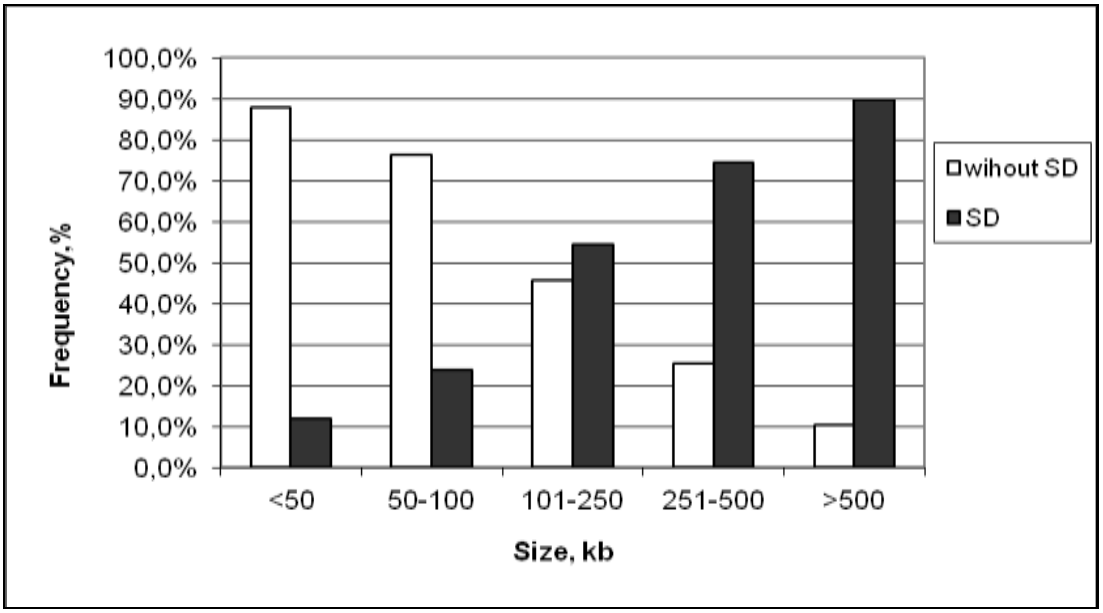

Figure 7. Association between the size of CNVR and the presence of segmental duplication. Most large CNVR events overlap segmental duplications at their ends (up to 89 % for events of more than 500 kb). On the contrary, only 12 % of events of less than 50 kb contain segmental duplications at their end.

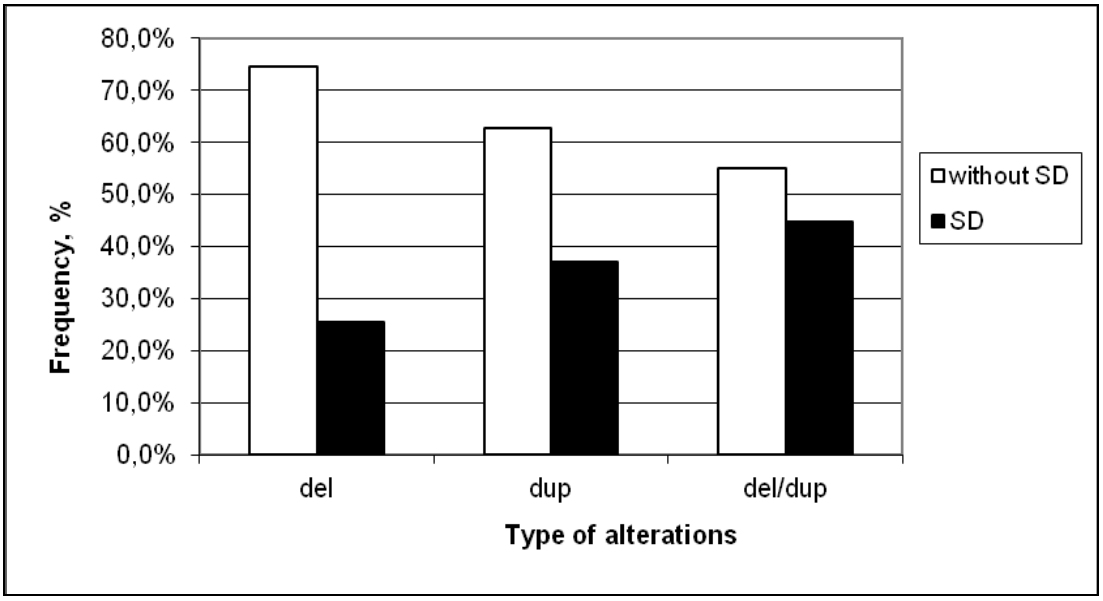

Figure 8. Proportion of CNVRs of different type of changes associated with segmental duplications.

In this study the spectrum and frequencies of the CNVRs identified supplemented the knowledge of the genome peculiarity of the Lithuanian population and will be valuable resources for studying human genome diversity and its association with disease.

4.2. Comparison of CNVs between cases and controls

Since CNVs are common in normal individuals, determining the functional and clinical significance of rare copy number variants in patients remains challenging.

We compared copy number variants (CNVs) in 75 children with ID (cases) to CNVs in 150 unaffected adult (their parents) controls that were tested using the same customized higher density microarray (400 K). A total of 48,437 CNV calls (>20 kb) were detected with an average of 217 events (rare and common) per individual with ID (a median size of 60.9 kb) and 216 events per apparently healthy individual (a median size of 62.2 kb) (p=0.292, CI=95 %).

The global burden of CNVs was examined in terms of type of alteration, length, and number of genic regions. No global burden test results were statistically significant (see Table 1), thus suggesting that there is no enrichment of common CNVs in cases compared with controls.

Table 1. Deletions and duplications between cases and controls.

Characteristics	Rare and common CNVs			Rare CNVs		
	case	control	p-value	case	control	p-value
Number of CNVs/individual	217	216	0,22	0,64	0,39	0,06
Median size of deletions, kb	119	117	0,14	2001	110	0,08
Median size of duplications, kb	147	141	0,75	2203	267	0,017
Average number of deleted genes	3,94	4,09	0,19	34,56	1,92	0,05
Average number of duplicated genes	2,95	2,98	0,74	40,10	5,89	0,012

Then we compared CNV content between the cases and controls, excluding common CNVs (>1 % population frequency). After filtering, a total of 107 rare (<1 % population frequency) autosomal CNV calls were made with an average of 0.64 and 0.39 CNV events for cases (with a median CNV size of 193 kb) and

apparently healthy individuals (with a median CNV size of 124 kb). Consistent with previous studies of neurological disease (Sharp *et al*., 2006; Sebat *et al*., 2007; Walsh *et al*., 2008), we found an excess of rare large CNVs among relative to controls. This excess is evident at 250 kb CNVs and becomes more cases pronounced with increasing CNV size.

4.3. Potential candidate genes

PCDH18 − protocadherin-18

An interstitial deletion at 4q28.3 of maternal origin, 1.53 Mb in size, detected in a boy with severe developmental delay, seizures, microcephaly, hypoplastic corpus callosum, internal hydrocephalus and dysmorphic features, involves only one gene – *PCDH18*, which expression is evident during embryonic development and function is presumably significant in brain development (Aamar *et al*., 2008). Isolation of a full-length zebrafish cDNA that encodes a protein showed high similarity to human and mouse Pcdh18 (Aamar *et al*., 2008). Experiments of cell transplantation revealed that overexpression of *pcdh18a* causes diminished cell migration and reduced cell protrusions, resulting in a tendency of cells to stay more firmly aggregated, probably due to increased cell adhesion (Aamar et al., 2008). In contrast, knockdown of *pcdh18a* by a morpholino oligonucleotide caused defects in epiboly, and led to reduced cell adhesion as shown by cell dissociation, sorting and transplantation experiments (Aamar *et al*., 2008).

In the context of the findings of *PCDH18* gene expression and function, analysis of *DGV*, *DECIPHER* databases, we suggest, that the reduced expressivity or incomplete penetrance in cases of 4q28.3 deletions must be considered and the haploinsufficiency of *PCDH18* gene could be the main factor influencing the manifestation of ID. The complete loss of transcript may be caused by the presence of splicing mutations in the gene's introns, promoter mutations or alterations in regulation sequences of the paternal allele. Gene expression analysis in the patient and his relatives would be useful but is complicated as the expression of the *PCDH18*

gene in the whole blood or skin was evident in very low levels or not detected (Yanai *et al*., 2005). Also altered interaction of gene's product with other compounds in its metabolic pathway may be present, as Pcdh18 interacts with Dab1 on neuronal migration and cell positioning during mammalian brain development (Homayouni *et al*., 2001) and the adequate expression of both products is essential for sufficient function.

We consider this deletion as private inherited copy number variation which may have pathogenic role in manifestation of specific clinical features in the patient and *PCDH18* gene as a possible candidate gene for ID (Kasnauskiene *et al*., 2012).

ERBB4 − v-erb-a erythroblastic leukemia viral oncogene homolog 4

A *de novo* interstitial deletion at 2q34, 958 kb in size, detected in a boy with ID, hyperactivity and severe speech delay, involves the single protein coding gene *ERBB4.* This gene is one of four members of that mammalian ERBB family of transmembrane tyrosine kinases. Among the ERBB proteins, ERBB4 plays a major roll in neurodevelopmental processes. The major ligands of the ERBB4 receptor are neuroregulins (NRG1-4) belonging to a family of growth factors. The *NRG1-ERBB4* signalling pathway is essential for many neurobiological processes. It has a developmental role in the generation, migration and differentiation of interneurons in the neocortex and hippocampus, and by modulating synaptic plasticity and oscillations it is involved in neuronal signal processing in the adult brain (Neddens *et al*., 2010).

Two cases of *ERBB4* gene disruption have been reported previously, one caused by deletion and one caused by the intragenic breakpoint in translocation. In the first report, Walsh *et al.* (2008) has described a patient with schizophrenia and deletion of the distal part of the *ERBB4* gene, starting from exon 19 and expanding to the downstream intergenic sequence. Since the deletion involves the terminal part of the *ERBB4* gene, the clinical consequences might be milder than pure haploinsufficiency, and a specific clinical effect of truncated ERBB4 is possible. Backx *et al.* (2009) reported a patient with *de novo* reciprocal translocation t(2;6)(q34;p25.3) disrupting

the *ERBB4* and *GMDS* genes at chromosome 2 and 6. The phenotypic consequences in this patient were early myoclonic encephalopathy, profound psychomotor delay, microcephaly, axial hypotonia, and peripheral hypertonia with brisk tendon reflexes. The authors suggested that the disruption of the *GMDS* allele most likely had no phenotypic effect, as the patient had no specific clinical features and mutation was not detected in the other *GMDS* allele by sequencing. Thus the phenotype in the patient was attributed to the disruption of the *ERBB4* gene alone. Still as in the previously mentioned patient, the clinical effect of truncated *ERBB4* and additional genetic/epigenetic factors should be considered in this case. The deletion in patient (LT-34-1) involved the proximal part of the *ERBB4* gene (1-3 exons; figure 2). It resulted in pure *ERBB4* haploinsufficiency, which could manifest as hyperactivity and cognitive dysfunction, especially language.

It is supposed that only the gene-implicated deletion has a functional role in neurodevelopmental processes (Kasnauskiene *et al*., 2013). Clinical consequences in the patient are probably determined by haploinsufficiency of the *ERBB4* gene.

ZFHX3 **-** *zinc finger homeobox 3*

An interstitial *de novo* deletion at 16q22.3, 726 kb in size, spanning *ZFHX3* revealed a new potential candidate gene in the aetiopathogenesis of ID. *ZFHX3* encoded protein acts as a transcription repressor. The binding of ZFHX3 to the AT-rich element of the AFP gene suppresses its expression by interfering with the binding of activators. ZFHX3 also regulates the differentiation of myoblasts through the binding to the AT-rich sequence of the MYF6 promoter and promoter repression. Kim et al., 2010 reported that ZFHX3 regulates the expression of adhesion molecules, including procollagen type III and integrin and the platelet-derived growth factor receptor (PDGFRB) (Kim *et al*., 2010).

ZFHX3 is expressed in post-mitotic neurons in the embryonic brain (Jung et al., 2005). Kim *et al*. (2010) showed that the expression of *ZFHX3* is correlated with *PDGFRB* in postitotic neurons. Neurons are highly susceptible to oxidative stress because of their high rate of oxidative metabolism and low level of antioxidant

enzymes (Brooks *et al*., 2000). ZFHX3 might play a key role in inducing both integrin and PDGFRB expression, which would facilitate the associated signal transduction from these membrane receptors. Membrane receptors are required to trigger the activation of ATM in response to oxidative stress. The activation of ATM in the cytoplasm might play a role in autophagy in protection of neurons against oxidative stress (Ishii *et al*., 2006). In addition, variants in *ZFHX3* have been reported to be associated with genetic susceptibility to Kawasaki disease, which induces a high risk of coronary aneurysmal dilatation (Burgner *et al*., 2009) and atrial fibrillation and ischemic stroke (Benjamin *et al*., 2009; Gudbjartsson *et al*., 2009). Thus, *ZFHX3* plays a role in neuron survival, protecting against the formation of aneurysms and healing wounds.

4.4. Diagnostic yield of array CGH

Thirty pathogenic CNVs among twenty-six patients and two potentially pathogenic CNVs were identified. Almost all pathogenic CNVs were larger than 500 kb, significantly larger than the median size of all CNVs detected.

Twenty-six (14.8%) of the samples showed a CNV that was considered clearly pathogenic (Table 2). Of these, thirteen have well established clinical significance — *Angelman*, *DiGeorge*,*Phelan-McDermid*, *Cri du chat*, *Sotos*, *Jakobsen* and 2q37 monosomy syndromes or new microdeletions/duplications that have recently been well described as pathogenic — del5q13.3, del16p11.2, dup16p13.3, del2q24.2q24.3, del4q21.22, del7q35q36.1 and dup7p22.1.

The findings of three patients have not been related to any previously published results. Due to the sizes of dup2p16.1p14.1 (10 Mb), dup2p22.1p16.1 (17 Mb) and dup15q22.21q24.1 (9 Mb). We considered them to be clinically significant.

Seven cases of complex chromosomal rearrangements, including four unrelated unbalanced translocations, a case of complex rearrangements at chromosome 21 and two rearrangements with multiple breakpoints were detected.

Array CGH is very useful when an apparently balanced rearrangement is detected in case with abnormal phenotype and/or intellectual disability because an apparently balanced translocations may in fact be CCRs. We identified two cases of complex chromosomal rearrangements in two out of three patients with *de novo* apperently balanced translocation. In addition, we identified more complex chromosomal rearrangement than conventional cytogenetic methods revealed. Array CGH detected complex chromosomal rearrangement on chromosome 21 in a patient with terminal deletion of chromosome 21 revealed by G banding and FISH methods. These findings further highlight array CGH as first tier test for patients with developmental delay.

Interestingly, array CGH revealed three cases of a single gene deletion: at 4q28.3, 2q34, and 16q22.3. Based on molecular function of the genes and high expression in the brain, these alterations were considered as pathogenic.

Table 2. List of pathogenic CNVs.

Patient ID	Array-CGH result, hg18	Size, Mb	Comment	array design
LT-11-1	arr 22q13.31q13.3(45,834,903-49,529,400)x1 *dn*	3.8	*SHANK3*	400K
LT-43-1	arr 22q11.2(117,018,751-19,795,282)x1 *dn*	2.8	*TBX, COMT, PRODH1*	400K
LT-69-1	arr 15q11.2q13.1(22,293,861-26,890,764)x1 *dn*	4.6	*UBE3A*	400K
LIT-29-1	arr 16p11.2(29,563,985-30,106,254)x1 *mat*	0.6	*MAPK3, TBX6 ect.*	105K
LIT-156-1	arr 16p11.2(29,563,985-30,106,254)x1 dn	0.6	*MAPK3, TBX6 ect.*	105K
LIT-30-1	arr 5p14.3p14.1(23,025,478-26,938,536)x1 *dn*	3.9	*CTNND2*	105K
LIT-227-1	arr2q37.3(239,525,614-242,717,216)x1 *dn*	3.2	*HDAC4*	105K
LIT-19-1	arr 16p13.3(2,589,524-3,911,387)x3 *dn*	1.3	*CREBBP*	105K
LIT-138-1	arr 2q24.2q24.3(162,118,995- 164,461,141)x1 *dn*	2.3	*SLC4A10, KCNH7*	105K
LIT-117-1	arr 4q21.22(83,373,844-84,097.897)x1 *dn*	0.7	*HNRPDL* ir *HNRNP*	105K
LIT-216-1	arr 5q14.3(86,456,211-89,104,733)x1 *dn*	2.7	*MEF2C*	105K
LIT-123-1	arr 7q35q36.1(146,392,196-149,441,454)x1 *dn*	3.1	*CNTNAP2*	105K
LIT-150-1	arr 7p22.1(5,337,072-6,316,915)x3 *dn*	1.0	*ACTB*	105K
LIT-147-1	arr 2p22.1p16.1(40,059,584-57,546,352)x3 *dn*	17.5	Too large	105K
LIT-136-1	arr 2p16.1p14.1(58,656,378- 68,790,747)x3 *dn*	10.2	*CRIP1a, OTX1*	105K
LIT-25-1	arr 4q28.3(137,417,138-138,947,393)x1 *mat*	1.5	*PCDH18*	105K
LT-34-1	arr 2q34(212,505,294-213,463,152)x1 *dn*	1.0	Erbb4	400K
LT-70-1	arr 16q22.3 (70,919,282-71,645,680)x1 *dn*	0.7	*ZFHX3*	400K
LIT-54-1	arr 5q35.3 (176,728,738-176,992,730)x3 *dn*	0.3	*NSD1*	*105 K*
LT-49-1	arr 1p36.11(26,714,062-27,728,654)x1 *dn*	1.0	ARID1A, PIGV, GPR3, FCN3	400K
LT-68-1	arr 5p15.33p15.31(0-8,157,370)x1, 12q24.21 q24.33(113,931,344-132,349,534)x3 *mat*	8.2 18.4	Unbalanced translocation	400K
LT-59-1	arr 8p23.3p23.1 (0-7,036,726)x1, 15q26.1q26.3(90,515,060-100,338,915)x3 *dn*	7.0 9.9	Unbalanced translocation	400K
LT-63-1	arr 11q23.3q25(120,505,418-134,452,384)x1 *mat*	13.9	Unbalanced translocation	400K
LT-67-1	arr 4p16.3(0-4,194,871)x1, 11p15.5p15.4(0-3,357,154)x3 *pat*	4.1 3.4	Unbalanced translocation	400K
LT-36-1	arr 21q22.2q22.3(39,774,417-41,817,957)x3, 21q22.3(41,817,957-46,944,323)x1 *dn*	2.0 5.0	*TRPM2*, *TSPEAR*, *PCNT*, *DIP2A* ir *S100*	400K
LT-38-1	arr 15q22.21q24.1(62,383,648-71,456,752)x3 *dn*	9.0	Too large	400K

In addition to identifying new potentially pathogenic loci high-resolution aCGH provided the opportunity to identify atypical deletions or duplications and suggested the new molecular mechanism of pathogenicity. For example, we identified an atypical deletion within the 1p36 microdeletion syndrome region. The breakpoints in this case contrast with previously reported cases of 1p36 deletion and are likely not generated by non-allelic homologous recombination. Detailed clinical information of the case with atypical deletion showed strong phenotypic similarity with the known syndrome, including developmental delay, absent speech, sensorineural hearing loss, heart defect, plagiocehaly, flat nasal bridge, pointed chin, and low-set, abnormal ears. Although the main clinical features of our patient are common for 1p36 monosomy syndrome, however, critical region for 1p36 monosomy syndrome located at 1p36.33 is 25 Mb proximally from interstitial deletion at 1p36.11 in our patient. We propose the common phenotype of nonoverlapping deletions within 1p36 region could be the cause by haplinsufficiency of one or more deleted genes, disruption of an essential regulatory element or by position effect as the juxtaposition of a euchromatic gene with a region of heterochromatin that might effect expression of genes outside the deletion (non deleted genes).

An unexpected rearrangement was detected in a patient LIT-54-1. This girl was with developmental delay and a *de novo* 264 kb interstitial duplication in the region of Sotos syndrome at 5q35.3 in the immediate vicinity of critical *NSD1* gene, but manifesting the phenotype, of overgrowth both prenatal stage and postnatal, macrocephaly, developmental delay, and resembling that of Sotos syndrome, rather than the recently reported syndrome of reciprocal duplication. The duplication is located right downstream from the *NSD1* gene, a region which appears critical for the expression of the gene as regulatory elements might be disrupted or the expression of a not amplified critical gene might be otherwise affected by the duplicated region. Thus, in the process of evaluating identified CNVs attention should be drawn to the possible influence of chromosomal rearrangement on distant genes, which could add additional diversity to genomic disorders. Our case demonstrates that evaluation of

the size of chromosomal alteration and gene content are not sufficient for assessment of CNV's pathogenicity and the context of adjacent genes should be considered.

CONCLUSIONS

1. This study confirmed that copy number variants are widespread structural variants. Copy-number variation analysis in a control group of people in Lithuania reveals that CNV regions compose 5.1 % of the human genome. Of the detected CNVs detected, 99.8 % are common and without clinical significance. The most common CNVs (with a frequency of >75 %) are smaller than 100 kb and include less than 10 genes.
2. From a cytogenetic point of view, most individuals are unique, having rare deletions/duplications. Only 35.7% (30/84) of rare CNVs have clinical significance. The clinical significance of CNVs was evaluated using several criteria, including occurrence and frequency in healthy controls; size, type and origin of alteration; and most importantly expression and function of the affected genes and neighbouring genes.
3. In reduced dosage, *PCDH18*, *ErbB4* and *ZFHX3* are strong candidates for causing ID. As normal cognition and behaviour depend on tight neuronal homeostatic control mechanisms, the altered dosage of genes having products crucial to neuron homeostasis may cause neurological or psychiatric phenotypes, because changes in neuronal excitability demand compensation.
4. Genomic architectural features have an influence on the occurrence of chromosomal rearrangements. Most CNVs bigger than 500 kb (89.7 %) are associated with segmental duplications resulting in nonallelic homologous recombination. It is thought that CNVs that are not associated with segmental duplication occur due to replication errors.
5. The diagnostic yield of array-CGH in this study was 14,8 %. 400 K microarray is an efficient tool for detecting subtle unbalanced chromosomal rearrangements and evaluating variability in the human genome.

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SANTRAUKA

Intelektinė negalia (INN) yra viena iš dažniausių šių dienų neįgalumo problemų, kurios dažnumas populiacijoje yra 1 – 3%. INN priežastys labai heterogeninės, daugeliu atvejų – genetinės. Specifinės diagnozės nustatymas prevencijos ir gydymo galimybės priklauso nuo genų kandidatų, siejamų su INN, identifikavimo.

Ilgą laiką buvo sunku išaiškinti genetinius INN etiologijos aspektus. Daugiausia tyrimų siekiant nustatyti ligą lemiančius genus buvo atliekami sankibos analizės ir pozicinio klonavimo metodais, į tyrimus įtraukiant dideles šeimas su daug ta pačia liga sergančių individų. Šiuos tyrimus ribojo nedidelis tinkamų analizei šeimų skaičius, su liga siejamų genetinių sričių heterogeniškumas arba sumažėjęs reprodukcinis tinkamumas. Genominių mikrolustų technologijos (vLGH) pritaikymas INN tyrimuose paspartino naujų citogenetinių INN priežasčių nustatymą, padėjo geriau suprasti INN etiopatogenezės mechanizmus ir atskleidė naujus genus kandidatus.

Šio mokslinio darbo metu buvo atlikti vLGH tyrimai asmenims, turintiems INN, ir kontrolinės grupės asmenims. Išsami bioinformacinė retų galimai kliniškai reikšmingų variantų analizė suteikė galimybę nustatyti galimus genus kandidatus INN bei kitiems klinikiniams požymiams bei įvertinti KSP įtaką centrinės nervų sistemos funkcionavimui. Gauta informacija apie plačiai žmogaus genome paplitusius kopijų skaičiaus pokyčius (KSP) ir retuosius Lietuvos populiacijai būdingus KSP bus pagrindas galimai patogeninių, su tiriamu klinikiniu požymiu susijusių, KSP klinikiniam įvertinimui.

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