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Copy Number Variation in the Human Genome and Its Implications for Cardiovascular Disease

Rebecca L. Pollex, MSc; Robert A. Hegele, MD, FRCPC

Unlocking the information contained within the human genome will likely advance our understanding of cardiovascular (CV) health and disease by leading to discovery of new molecules, pathways, and networks. A central strategy in genetic studies of CV disease has been to correlate human genomic DNA variation with clinical phenotypes, such as myocardial infarction, heart failure, stroke, and their risk factors, with a range of experimental designs and analytical procedures. The ability to detect genomic differences between individuals is the foundation of this research. Human genomic variation exists in many forms, each of which has unique qualitative and quantitative features. Each form of human genomic variation is composed of many individual variants that occur across the genome. The population frequency of individual variants can range from rare to common. The effect of a specific genomic variant can range from beneficial to neutral to deleterious. To rapidly translate genomic knowledge into diagnosis and treatment of CV disease, it is logical to search for common genomic variants that have a non-neutral impact. In the recent past, one form of genomic variation, the single-nucleotide variant, has dominated the experimental landscape: It is the currency of present genetic CV disease studies. However, recent developments indicate that the focus on single-nucleotide polymorphisms (SNPs) alone will not capture the full range of meaningful human genomic variation, such as a newly characterized and annotated form called copy number variation (CNV).

Main Varieties of Human Genomic Variation

The main forms of human genomic variation are shown in Figure 1. These include SNPs, which are qualitative in nature and involve only a single nucleotide, and a family of genomic changes collectively called structural variations, which are quantitative in nature because they affect the dosage or copy number of a particular genomic region. Structural variant types include deletions, duplications, inversions, and rearrangements of "chunks" of the genome, which range from small insertions and deletions that involve 1 to 50 base pairs through to very large cytogenetic changes that involve entire chromosomes. It is estimated that roughly 5% of the human genome is structurally variable.[1] The recently characterized CNVs comprise structural variants of intermediate size that range from 1000 to 5×10^6 bases of DNA. Furthermore, the types of CNV changes (ie, deletions, duplications, and inversions) are analogous to large cytogenetic changes that were previously visualized through microscopy.

Other variable regions include short repetitive sequences, 1 to 5 base pairs in length, termed short tandem repeat sequences or microsatellite repeats, such as (A)n, (CA)n, or (AAG)n, where n is variable. These repeat units, being neutral and widely dispersed, have been used as markers to "tag" segments of the genome that can then be tracked through families in linkage studies. A few short tandem repeats are functional and give rise to human diseases [eg, neurological disorders resulting from expansion of (CAG)n repeats].[2] Minisatellites or "variable number tandem repeats," which are 5 to 64 base pairs in length and extend over several thousand base pairs, are less evenly distributed but highly informative.[2] Transposons and transposon-like repetitive elements, such as the ubiquitous ≈300–base pair *Alu* repeat sequence, also contribute to human genomic variation.[3] Whereas this review will focus on the relationship of SNPs and CNVs to the CV system, a more detailed description of all forms of human genomic variation, such as tandem repeats and various interspersed genomic elements, can be found in a recent comprehensive review.[1]

Mutations Versus Polymorphisms

When genomic variants are discussed, both "mutation" and "polymorphism" are used, sometimes interchangeably. By convention in human genetic research, any genomic variant with population frequency <1% is termed a "mutation," whereas a variant with population frequency >1% is termed a "polymorphism,"[4] a convention that will be followed in this review. The distinction is based on population frequency of the variant rather than the type of variant or its possible functional impact. However, as a rule, rare mutations tend to have a functional impact that deviates from the "wild type" (or most common form), yielding a higher signal-to-noise ratio for detection of a genetic influence on a trait. In contrast, polymorphisms tend to connote less functionally deviant genomic variants. However, some rare genomic mutations have been found to be functionally neutral, whereas some common polymorphisms also have a major functional impact.

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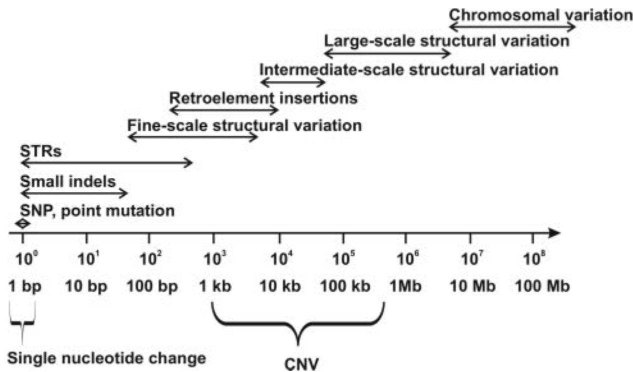

Figure 1. The spectrum of variation in the human genome. A logarithmic *x*-axis measures the number of nucleotides, from 1 bp to ≥100 Mb. Above the axis, types of genetic variation are shown, with their size range depicted below by a double-headed arrow. Size ranges are not definitive. SNP indicates single-nucleotide polymorphism; indels, insertions and deletions; STR, short tandem repeat; bp, base pair; kb, kilobase; Mb, megabase; and CNV, copy number variation.

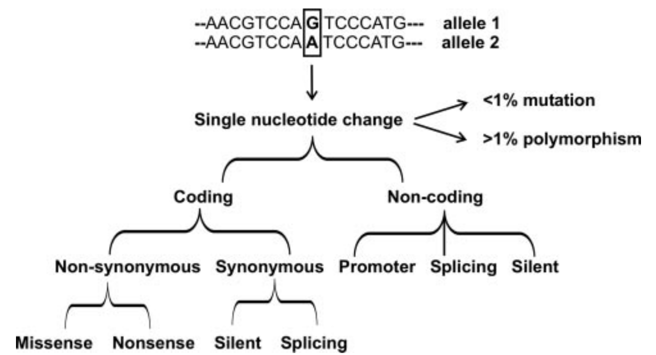

Figure 2. Single-nucleotide genomic changes. The "swap" of a single nucleotide with another, such as the replacement of the wild-type guanine (allele 1) with adenine (allele 2), is referred to either as a mutation, if present in <1% of the general population, or as SNP, if present at a frequency >1%. SNPs are common and span the human genome. Most single-nucleotide changes are found outside coding regions (noncoding) and have no impact on the biological function of a protein (silent), though they may affect gene expression or splicing. However, variants found within the coding region may code for functional changes in amino acid structure (missense) or predict premature protein truncation (nonsense) and thus may have a possible direct association with disease.

Technologies to Visualize Human Genomic Mutations

Technology is an important determinant of the type of genomic mutation detected. For instance, in the pre–polymerase chain reaction (PCR) era (the 1970s and early 1980s) the most prevalent mutation types in disease databases were large-scale genomic DNA changes, such as large insertions and deletions. One reason for this was that most genomic mutations in this era were detected by the Southern blot method. Southern blots were ideal for detection of large-scale rearrangements that involved >200 bases of target genomic DNA, which was the method's resolution limit. However, detection of small mutations was beyond the Southern blot's resolving capacity, unless a mutation altered a recognition site for a restriction endonuclease. Another key methodology to pinpoint variants involved cloning of human genomic DNA coupled with manual DNA sequence analysis, a combination of technologies that were comparatively inefficient and required high levels of skill, labor, and exposure to radioactivity.

These now almost forgotten methods were superseded in the 1980s by PCR and automated DNA sequence analysis. With the invention of PCR, it became possible to rapidly amplify discrete regions of genomic DNA (up to 2000 base pairs) in sufficient quantity and with sufficient quality to allow for high-capacity, high-resolution automated DNA sequence analysis, which in turn enabled rapid ordering of the 4 letters of the genomic alphabet (A, C, G, T) into a continuous data string. Since the late 1980s, the complementary fundamental technologies of gene amplification by PCR and automated genomic DNA sequencing, together with advances in DNA cloning, drove genomic research and ultimately enabled the determination of the entire sequence of the human genome.[5,6] These methods were ideally suited to detect small qualitative changes in the genomic sequence of an individual: 1 or a few genomic DNA nucleotide bases.

SNPs in the Human Genome

The result of the domination of these methods in human genetic research has been that most genomic variation detected over the past 20 years has been small and qualitative—mainly single-nucleotide changes. Their potential pathogenicity is summarized in Figure 2. The 2001 draft of the human genome sequence provided, in effect, an initial but imperfect reference standard for subsequent annotation of genomic variation. Every examination of partial or complete genomic DNA sequence since then has essentially been a "resequencing" experiment that has built on the first draft. The cumulative results of automated DNA sequence analysis over the past 6 years have helped identify millions of common differences between people at the level of single letters of DNA code. These differences, called SNPs, occur with a frequency of ≈1 of every 400 bases of DNA sequence. The extent of SNP variation in populations was exquisitely defined by the International HapMap project[7]: If one considers only SNPs that are present in >5% of specific population samples, SNPs may involve up to 10 million nucleotide bases of DNA, or ≈0.3% of the total genome. Once SNPs have been defined, various dedicated technologies to assay individual nucleotides can be used to screen human samples. A popular current platform is the high-density SNP microarray, which permits simultaneous assay of >500 000 and soon >1 000 000 SNPs from a human genomic DNA sample.

Until recently, SNPs were considered to be the major source of genomic variation in the phenotypic differences between people, which include physiologically neutral features like eye color as well as medically relevant phenotypes such as disease susceptibility and differential response to medications. However, individuals with a hammer seem to see nails everywhere; by analogy, investigators with technologies designed to assess single nucleotides soon regard these as the principal form of genetic variability. The publication of SNP-based studies that attempt to identify the genetic basis of disease traits has become commonplace in the CV field. As important as current SNP-based methods are, however, it is

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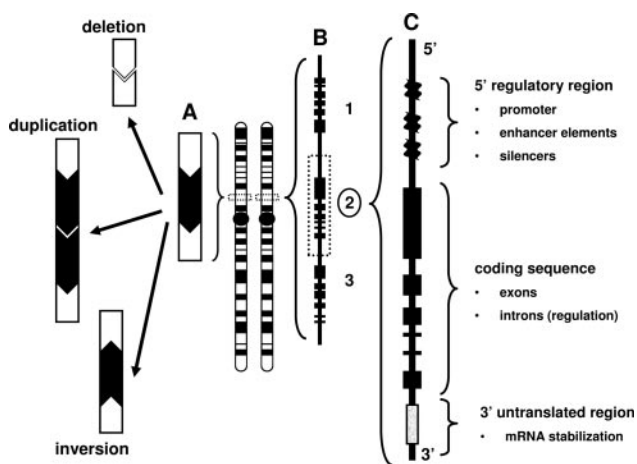

Figure 3. Large-scale genomic DNA CNV. The left side of the figure shows some of the types of CNVs; the right side of the figure shows some of the consequences of CNVs at the level of the gene. Segment A represents the normal structure of a region of one of a homologous pair of chromosomes. The type of genomic alterations of the normal structure that lead to deletion, duplication, and inversion are shown at the top. Segment B represents the normal structure of a chromosomal locus that contains a cluster of 3 genes (1, 2, and 3). Segment C shows the detailed structure of gene 2 and includes key functional elements, such as the 5′ regulatory region, with promoter, enhancer, and silencer elements; the 3′ untranslated regions that can regulate message stability; and the intron–exon structure of the gene. Any of these structural and functional elements can become involved in a CNV, with a range of functional consequences that depends on the size and nature of the variant and the affected functional domains. Adapted from Hegele[8] with permission from NRC Research Press. Copyright 2006.

important to recall that large-scale, cytogenetic, chromosomal changes rather than single-nucleotide variants have long been recognized to cause certain CV disorders, even before recent developments that culminated in publication of the CNV map of the human genome.

Historical Snapshot: CV Disorders Caused by Cytogenetic Chromosomal Changes

Since the 1960s, traditional microscopy-based karyotype analysis and, more recently, higher-resolution fluorescent dye–based visualization with microscopy would occasionally detect patients whose nuclei harbored large-scale rearrangements that affected whole chromosomes or sizable fragments of chromosomes. The changes that were cytogenetically visible included deletion (loss of one or both copies), duplication (gain of one or more copies), inversion (flipped orientation of a chromosomal segment), and translocation (transfer of a piece of one chromosome to another). A single instance of such a dramatic chromosomal rearrangement would encompass millions to hundreds of millions of DNA nucleotides. The major types of chromosomal alterations are shown in Figure 3.

These major rearrangements were considered to be rare events and were frequently associated with clinical syndromes: The most familiar example would be the duplication of one copy of chromosome 21 entirely or in part, which is known as Down syndrome. Several disorders that result from large cytogenetic changes involved the CV system and some of these are listed in Table 1.[9–18] At the same time, some dramatic, large-scale, cytogenetic variants were detected incidentally without apparent clinical impact.[19] The standard method for detection of large-scale chromosomal variants has been GTG-banded karyotyping, which has a resolution of 3 to 5 Mb. Finer-resolution methods, collectively called fluorescent in situ hybridization (FISH), are based on hybridization of fluorescent probes onto chromosomes that have been captured in metaphase or interphase. Interestingly, improvements in cytogenetic technology have allowed for detection of smaller structural variants (or CNVs) approached from the low-resolution side of the methodology spectrum, whereas microarray-based platforms have permitted the detection of structural changes from the higher-resolution side of the spectrum. The net result has been that a growing number of common, smaller, quantitative genomic variants are being independently discovered by various technologies. In contrast to the large, uncommon, and frequently pathogenic cytogenetic changes, the much smaller-sized CNVs appeared to be prevalent in the healthy control population. Some genetics researchers clearly foresaw that these genomic structural variants would be a ubiquitous source of variation and likely

TABLE 1. CV Disorders Caused by Large Chromosomal Changes—Detected by Cytogenetics

	CV Component	Chromosomal Abnormality
Alagille syndrome (MIM 118450)[9]	>95% have defects, including peripheral pulmonic stenosis, valvular pulmonic stenosis, tetralogy of Fallot, coarctation of the aorta, and atrial-septal defects	Deletion of 20p11.2-p12
DiGeorge syndrome (MIM 188400)[10] and velocardiofacial syndrome (MIM 192430)[11]	Conotruncal defects, including tetralogy of Fallot, truncus arteriosus, interrupted aortic arch type B	Deletion of 22q11.2; small number of cases defects in 10p13
Down syndrome (MIM 190685)[12]	Major cardiac malformations, primarily atrioventricular septal defects	Trisomy 21
4q- Syndrome[13]	CV malformations	Terminal deletion of 4q
5q- Syndrome[14]	Ventricular myocardial noncompaction, atrial septal defects, cardiomyopathy	Distal 5q deletions
8p- Syndrome[15]	Wide spectrum of heart defects	Distal 8p deletions
Trisomy 18 syndrome[16,17]	Congenital heart disease, particularly ventricular septal defect and patent ductus arteriosus; also congenital polyvalvular disease	Trisomy 18
45,X/46,XY gonadal dysgenesis[18]	CV anomalies	45,X/46,XY mosaicism

MIM indicates Mendelian Inheritance in Man.

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TABLE 2. Methods for Detection of Structural Variants in the Human Genome

Method	Translocation/Inversion	CNV (>1 kb)	Small Sequence Variants (<1 kb)
Genome-wide scans			
Karyotyping	Yes (>3 Mb)	Yes (>3 Mb)	No
Large-insert clone array-CGH	No	Yes (>50 kb)	No
Oligonucleotide-based array-CGH/ROMA	No	Yes (>35 kb)	No
SNP oligonucleotide array	No	Yes	Yes
Sequence-assembly comparison	Yes	Yes	Yes
Clone paired-end sequencing (fosmid)	Yes (breakpoints)	Yes (>8 kb deletions; <40 kb insertions)	No
Targeted scans			
Microsatellite genotyping	No	Yes (deletions)	Yes
MAPH, MLPA, QMPSF, real-time qPCR	No	Yes	Yes
FISH	Yes	Yes	No
Southern blotting	Yes	Yes	Yes

CGH indicates comparative genomic hybridization; CNV, copy number variant; FISH, fluorescence in situ hybridization; MAPH, multiplex amplifiable probe hybridization; MLPA, multiplex ligation-dependent probe amplification; QMPSF, quantitative multiplex PCR of short fluorescent fragments; and qPCR, quantitative PCR.

disease mechanism,[20,21] but most geneticists and nongeneticists have only recently begun to appreciate the potential relevance of structural variants. Selected technologies to detect specific forms and sizes of genomic variants are shown in Table 2.

Brief Overview of New Genomic Technologies

With variation characterized at the single-nucleotide level and also the microscopic level, present-day strategies and tools now examine the variation between these 2 extremes: examination of submicroscopic structural variants on the scale of ≈1 kb to 3 Mb (Table 2). When screening is performed on a genome-wide scale, the main approach is array-based analysis, which was used in the first studies that described the global presence and distribution of CNVs in the human genome.[22,23] The driving force behind this breakthrough technology was the development of microarrays.[24] Composed of thousands of microscopic DNA probes spotted onto a solid surface such as a glass slide, microarrays allow for much greater resolution. In array-based comparative genome hybridization, the whole genome is fragmented, labeled, and then competitively hybridized to arrays spotted with one of several DNA sources, such as BACs (clone based) or PCR fragments.[25,26] Representational oligonucleotide microarray analysis, is a variation of array-based comparative genome hybridization. It includes an additional preparative step to reduce the complexity of the input DNA.[27] High-density SNP arrays can also be used, such as those used in the development of the most recent CNV map of the human genome.[28]

In the future, it is likely that with the possible development of inexpensive and reliable whole-genome sequencing, computational approaches (eg, sequence-assembly comparison) will become the most popular choice for identification of structural variants. Here, the advantage is that all types of variants, such as balanced inversions, can be easily detected, and the resolution will be down to the nucleotide level. A recent in silico strategy mapped over 1.1 million paired-end sequences from a high-density fosmid library against a reference assembly and discovered numerous CNVs that had not been identified previously, the majority below the expected resolution of other array platforms.[29] Also, SNP genotypes that are obtained from high-density microarrays will need to be further assessed for the possibility that they overlap with CNVs. This could be resolved bioinformatically or perhaps will require a complete overhaul of high-density SNP microarray design.

Newer methods have also been developed to detect structural changes in targeted regions in a more cost-effective and higher-throughput fashion than the traditional fluorescent in situ hybridization (FISH) and Southern blot methods. Such alternative methods include quantitative multiplex PCR of short fluorescent fragments,[30] multiplex amplifiable probe hybridization,[31,32] and multiplex ligation-dependent probe amplification.[33,34] Such methods allow for the scoring of up to 50 independent regions in one experiment and can detect deletions or insertions that involve whole exons that would otherwise be overlooked by traditional exon-by-exon sequence analysis.

CNVs in the Human Genome

The meteoric ascent of CNVs into contemporary genetic discourse began with 2 seminal 2004 publications.[22,23] Each research effort used distinct but complementary technologies designed to detect dosage differences of genes and chromosomal regions compared with the standard 2 copies (maternal and paternal) that are expected in each genome. Both teams saw numerous submicroscopic chromosomal alterations in the genomes of small samples of healthy control subjects. These quantitative genomic variants, eventually called CNVs, were analogous to the chromosomal changes detected by classical cytogenetic methods described above. Initially, larger variants were found, which ranged between 10 kb and 500 kb in size and numbered in the tens to hundreds per

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genome.[22,23] Recently, much smaller variants, mainly deletions that ranged from 500 bp to 10 kb in size have been found; they number in the hundreds and perhaps the thousands in the genome.[35,36] CNVs can also include variants that primarily affect qualitative genomic attributes of large chromosomal segments, such as through inversion of genomic regions with no change in copy number per se. The potential mechanisms that underlie the generation of CNVs may have broader implications as a source of variation between species and a means to generate new genes with new functions.[37]

Surprising features of CNVs included their ubiquity in the genome, high population frequency, and frequent lack of association with disease phenotypes. The genomes of any 2 individuals might differ from each other by hundreds to thousands of CNV events. This high prevalence in the genomes of apparently healthy individuals motivated efforts to create a unified CNV map in control samples and to integrate these with SNP maps by study of samples that had already been mapped for SNPs. In late 2006, Redon and colleagues published the CNV map of the genome,[28] followed by a similar map published by Wong and colleagues in early 2007.[38]

Redon and colleagues defined a CNV to encompass any submicroscopic chromosomal change that affected >1000 (and up to half a million or more) nucleotides of genomic DNA. These authors used both SNP microarrays and comparative hybridization to identify a total of 1447 CNVs in the genomes of 270 healthy individuals from 4 different geographical ancestries.[28] The extent of the variation was breathtaking: These relatively common CNVs cumulatively affected 360 million nucleotides, or ≈12% of the human genome (one of a homologous pair of chromosomes was often 1 million nucleotides and 20 genes shorter than the other). The map subsequently generated by Wong and colleagues[38] was based on study of the genomes of 105 individuals with the use of a whole-genome comparative hybridization assay and reported ≈800 CNVs that had a frequency of >3%; about two thirds of these CNVs overlapped with known genes.

How Do CNVs Cause Disease or Influence Phenotypes?

CNVs can affect phenotypes by alteration of levels of genes and gene products both at the level of transcription of genomic DNA to the RNA message and presumably at translation of the RNA message to the protein product.[4] For instance, deletion of one copy of a dosage-sensitive gene results in deficient function that cannot be rescued. Also, genomic deletions in apparently normal individuals might not directly cause a simple monogenic disease, but in the presence of additional genetic or environmental factors may contribute to development of complex polygenic CV diseases with late onset. Similarly, gene-dosage increases are already known to cause a few diseases in humans, but the ubiquity of CNVs means this could be a much more important and general disease mechanism. Furthermore, CNVs may have a role in common diseases if only for the simple reason that certain CNVs span regions that contain many genes. Therefore, the study of SNPs alone when genomic variation is correlated with disease is now inadequate in the context of knowledge of CNVs. A focus on SNPs will literally "miss the forest for the trees." For instance, we recently showed that testing for both SNPs and CNVs expands the molecular diagnosis of familial hypercholesterolemia.[39]

Like SNPs, smaller CNVs will affect only single genes and thus contribute, together with SNPs, to single-gene disorders. However, unlike SNPs, larger CNVs can affect 2 or more contiguous genes and thus contribute to syndromic or complex disorders caused by defects in multiple genes. Finally, some CNVs involve gene-poor regions and may either be functionally neutral or may still have an impact on disease susceptibility through their effects on nontranscribed domains that regulate gene expression at a distance.[4] Like any genetic variant, any deleterious effect must be considered in the context of redundancy of other related genes and gene products that might rescue a deficiency that results from the CNV.

An estimation of the relative impact of SNPs and CNVs on gene expression phenotypes was recently reported by Stranger and collegues.[40] With the use of lymphoblastoid cell lines of all 210 unrelated individuals from the International HapMap project, association analyses compared the expression levels of ≈14 000 genes with SNPs and CNVs. Of the 1061 genes found to be associated, 83.6% were associated with SNPs, 17.7% were associated with comparative genome hybridization clones, and only 1.3% were associated with both types of genetic variation, which clearly indicated that exploration of only one source of variation may not be enough to explore the genetic causes of disease.

CNVs Associated With Known Genetic Cardiovascular Diseases

Table 3 shows a list of selected CV diseases, each of which has an established and strong genetic component, often single-gene or "monogenic" mendelian disorders, for which CNVs overlap the chromosomal region that harbors the disease gene.[28] These represent ≈7% of the total of ≈300 disease genes that are contained within CNVs from the map by Redon and colleagues. Most CNVs that overlap with CV monogenic disorders are present at a frequency between 1% and 5%, which seems reasonable when the fact that these disorders are rare in the general population is considered. One exception that shows a high degree of common variability is *LPA*: The region that harbors *LPA* on chromosome 6 was remarkably variable and commonly polymorphic in both the Redon and Wong CNV maps.[28,38] The findings are consistent with the known biology of *LPA*, which encodes the atherogenic apolipoprotein(a). This protein has long been known to have marked size heterogeneity as a result of variability in the number of tandem repeats of genomic DNA sequence that encodes a critical expressed functional domain. With regard to other genes in Table 3, the original study samples were relatively small, so it remains important to replicate these findings in larger independent samples to demonstrate that these diseases are associated with CNVs. If replicated, it may be worthwhile to consider these CNVs in studies of association or linkage with specific CV disease traits. If these variants actually occur with such frequency in the general

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TABLE 3. CV Disease Genes Affected by CNVs

Gene Name	Gene Symbol	Band	Size, Mb	Disease	OMIM	Mode	Replicated	Region of Gene	Gain/Loss	Frequency	Mouse Models
Calsequestrin 2	*CASQ2*	1p13.2-1p13.1	3.5	Ventricular tachycardia	114251	AR	Yes	100%	Both	<5%	None
Ryanodine receptor 2	*RYR2*	1q43	0.73	Arrhythmogenic right ventricular dysplasia type 2; ventricular tachycardia	180902	AD	No	3′ end 12%	Gain	<1%	KO: less MI-induced CHF
Titin	*TTN*	2q31.2	0.21	Cardiomyopathy; Edstrom myopathy	188840	AD	No	Middle 60%	Loss	<1%	KO: heart defects; embryonic lethal
Myosin, light polypeptide 3, alkali	*MYL3*	3p21.31	0.29	Hypertrophic cardiomyopathy	160790	AD;AR	No	100%	Loss	>5%	None
Natriuretic peptide receptor C/guanylate cyclase C	*NPR3*	5p13.3	0.49	Overgrowth syndromes	108962	CD	No	5′ end 25%	Loss	<1%	TG: skeletal overgrowth
ADAM metallopeptidase with thrombospondin type 1 motif, 2	*ADAMTS2*	5q35.3	0.38	Ehlers-Danlos syndrome type VIIC	604539	AR	Yes	5′ end 15%	Gain	<1%	KO: multiple abnormalities
Major histocompatibility complex, class II, DR β1	*HLA-DRB1*	6p21.32	0.33	Immune disease; cardiomyopathy	142857	CD	No	100%	Both	>5%	None
Lipoprotein, Lp(a)	*LPA*	6q25.3-6q26	0.86	CHD; stroke; thrombosis	152200	CD	Yes	3′ end 3%	Both	>20%	TG: increased atherosclerosis
Neutrophil cytosolic factor 1	*NCF1*	7q11.23	3.1	Chronic granulomatous disease; Williams-Beuren syndrome with hypertension	608512	AR	No	100%	Both	1-5%	TG: collagen-induced diseases
K+ voltage-gated channel, subfamily H, member 2	*KCNH2*	7q36.1	0.13	Long-QT syndrome 2; short-QT syndrome 1; bradycardia	152427	AD	No	100%	Loss	<1%	KO: sinus brachycardia
Storkhead box 1	*STOX1*	10q21.3-10q22.1	0.17	Preeclampsia	609397	AD	No	5′ end 25%	Loss	<1%	None
K+ voltage-gated channel, KQT-like subfamily, member 1	*KCNQ1*	11p15.5-11p15.4	0.18	Long-QT syndrome 1	607542	AD;AR	No	3′ end 33%	Gain	<1%	KO: deaf, shaker-waltzer phenotype
CD44 molecule	*CD44*	11p13	0.21	Abnormal arteriogenesis	107269	CD	Yes	5′ end 50%	Loss	<1%	KO: impaired arteriogenesis
Solute carrier family 6, member 2	*SLC6A2*	16q12.2	0.31	Orthostatic intolerance	163970	AD	No	100%	Gain	1-5%	KO: abnormal nor-adrenaline response
Nitric oxide synthase 2A	*NOS2A*	17q11.2	0.30	Hypertension susceptibility	163730	CD	No	100%	Gain	1-5%	None
Thrombomodulin	*THBD*	20p11.21	0.15	Thrombophilia; atherosclerosis	188040	AD	Yes	5′ end 10%	Loss	<1%	KO: embryonic lethal
SCO cytochrome oxidase deficient homolog 2	*SCO2*	22q13.33	0.09	Cytochrome c oxidase deficiency	604272	AR	No	100%	Loss	<1%	None
Lysosomal-associated membrane protein 2	*LAMP2*	Xq24	0.18	Danon disease	309060	XLR	No	3′ end 33%	Loss	<1%	KO: cardiomyopathy

Replicated refers to a positive finding from both methods used in Redon et al.[28] CHD indicates coronary heart disease; AR, autosomal recessive; AD, autosomal dominant; CD, complex disease; XLR, X-linked recessive; KO, knockout; MI, myocardial infarction; CHF, congestive heart failure; TG, transgenic; and OMIM, reference number in Online Mendelian Inheritance in Man database.

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population, then perhaps subtle or later-onset forms of the monogenic phenotypes might be more prevalent than has been generally recognized.

Curbing Enthusiasm: Time Needed to Characterize, Validate, and Associate

Although completion of genome-wide CNV maps has been a great accomplishment, it is important to recognize certain limitations. For instance, genomic DNA from cell cultures was used almost exclusively, which, although it provided numerous practical advantages, has the potential limitation that cells in culture might more readily acquire genomic CNVs that were not present in the starting material. Expansion of the databases to contain CNV information from multiple platforms on thousands of individuals analyzed according to established standards is essential if CNV mapping is to become a more routinely used technology in research and diagnosis.

On the assumption that technical issues are dealt with, assay performance is optimized, and consensus standards are applied to CNV mapping, the next logical step would be to determine the potential role of CNVs in rare or common diseases. Large-scale genome-wide association and case–control studies will have to incorporate CNV analyses into their designs. Future comprehensive studies of CV disease will require reliable, complementary, and harmonized technologies that account for several forms of genomic variation simultaneously. For any particular CNV, increased confidence of its validity would derive from confirmation of the same CNV in the same individual by different technologies and from observation of the same CNV in multiple individuals by multiple methodologies.

Because CNVs are so prevalent and because certain chromosomal regions that harbor CNVs recur across multiple normal samples, it is important to curb the inclination to provide clinical advice on the basis of the presence of CNVs until CNVs have been even more completely mapped in a much wider range of normal healthy subjects, and their association with phenotypes, particularly congenital syndromes, single-gene disorders, and complex diseases of later life, has been more fully characterized.

Implications of CNV Knowledge to Cardiovascular Health and Disease

There is considerable excitement in the recognition of the research opportunities created by awareness of CNVs as an entire new family of genomic DNA variants. In the clinical arena, will the benefits of new technologies with improved resolution be offset by problems that arise from the detection of large numbers of heretofore-unseen genomic abnormalities? The probability of finding normal genomic variants in screened samples is now markedly increased, given the sensitivity of the new methods and the ubiquity of CNVs >1 kb in the human genome. In a clinical research setting, how will the management and counseling of a patient and his/her family unfold with and without taking CNVs into account? Will ethical issues that arise from analysis of CNVs simply mirror past issues encountered when cytogenetic methods are used? Could past medical genetic diagnosis be revised in light of new knowledge afforded by CNVs? Should archived specimens be reevaluated in light of the CNV map? These questions and others[41] will require attention soon.

Past clinical experience with cytogenetic abnormalities may provide important direction for new CNV information. For instance, in postnatal diagnosis of a child with a CV developmental or morphological abnormality, it has been generally accepted that any genomic CNV inherited from a phenotypically normal parent is probably less clinically significant than a variant that has arisen de novo.[42] When a CNV has been detected in the genome of a child with a clinical abnormality, it would thus be essential to exclude a de novo chromosomal change in the parents, only after nonpaternity is excluded. The same CNV in a healthy parent suggests it might be a normal variant. If the CNV is not present in either parent, it could then be searched against the database of known CNVs; potential pathogenicity could be inferred through homology of the CNV with known non–disease-related CNVs. However, creation of an extensive and authoritative archive that relates CNVs to disease will require time, resources, and cooperation between research and clinical communities. A current Web site that contains integrated data and includes CNVs is found at http://projects.tcag.ca/variation.

Conclusions

Thus, the CNV maps in healthy "normal" individuals add a new dimension to the study of the human genome. These new comprehensive genomic maps and analyses have initiated a paradigm shift that might profoundly affect CV biology and medicine. They have altered the notion that SNPs are the main source of interindividual genomic variation. The size and ubiquity of CNVs suggest a potential role for susceptibility to common complex, polygenic CV diseases. Although it is promising as a hypothesis for human genetic research, the ultimate proof of the involvement of CNVs in CV disease phenotypes will require large-scale studies that comprise well-phenotyped cohorts and comprehensive, robust methods to classify individuals according to their CNV status. In any event, future genomic mapping experiments and genome-wide association analyses, and their respective detection technologies, will need to account for the presence of CNVs. Current platforms to study the genome may need to be redesigned either to optimize detection of CNVs or minimize their interference with detection of other forms of genomic variation. As the "personal genome" moves closer to reality, it will be important to interpret the biological meaning of all forms of genomic variation (SNPs and CNVs) for any individual. Finally, CNVs are now part of the contemporary discourse on genomic variation studies and their biological, health, and societal implications. More research is required to fully understand the implications and potential applications of human genomic CNVs.

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References

1. Sharp AJ, Cheng Z, Eichler EE. Structural variation of the human genome. *Annu Rev Genomics Hum Genet*. 2006;7:407–442.
2. Wright AF. Genetic variation: polymorphisms and mutations. In: *Nature Encyclopedia of the Human Genome.* London: Nature Publishing Group; 2003:959–968.
3. Bennett EA, Coleman LE, Tsui C, Pittard WS, Devine SE. Natural genetic variation caused by transposable elements in humans. *Genetics*. 2004;168:933–951.
4. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. *Nat Rev Genet*. 2006;7:85–97.
5. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ. Initial sequencing and analysis of the human genome. *Nature*. 2001;409:860–921.
6. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. *Science*. 2001;291:1304–1351.
7. The International HapMap Project. *Nature.* 2003;426:789–796.
8. Hegele RA. Genetic susceptibility to heart disease in Canada: lessons from patients with familial hypercholesterolemia. *Genome*. 2006;49: 1343–1350.
9. Byrne JL, Harrod MJ, Friedman JM, Howard-Peebles PN. del(20p) with manifestations of arteriohepatic dysplasia. *Am J Med Genet*. 1986;24: 673–678.
10. Greenberg F, Elder FF, Haffner P, Northrup H, Ledbetter DH. Cytogenetic findings in a prospective series of patients with DiGeorge anomaly. *Am J Hum Genet*. 1988;43:605–611.
11. Lindsay EA, Goldberg R, Jurecic V, Morrow B, Carlson C, Kucherlapati RS, Shprintzen RJ, Baldini A. Velo-cardio-facial syndrome: frequency and extent of 22q11 deletions. *Am J Med Genet*. 1995;57:514–522.
12. Catovic A, Kendic S. Cytogenetic findings at Down syndrome and their correlation with clinical findings. *Bosn J Basic Med Sci*. 2005;5:61–67.
13. Huang T, Lin AE, Cox GF, Golden WL, Feldman GL, Ute M, Schrander-Stumpel C, Kamisago M, Vermeulen SJ. Cardiac phenotypes in chromosome 4q-syndrome with and without a deletion of the dHAND gene. *Genet Med*. 2002;4:464–467.
14. Pauli RM, Scheib-Wixted S, Cripe L, Izumo S, Sekhon GS. Ventricular noncompaction and distal chromosome 5q deletion. *Am J Med Genet*. 1999;85:419–423.
15. Hutchinson R, Wilson M, Voullaire L. Distal 8p deletion (8p23.1–8pter): a common deletion? *J Med Genet*. 1992;29:407–411.
16. Matsuoka R, Misugi K, Goto A, Gilbert EF, Ando M. Congenital heart anomalies in the trisomy 18 syndrome, with reference to congenital polyvalvular disease. *Am J Med Genet*. 1983;14:657–668.
17. Van Praagh S, Truman T, Firpo A, Bano-Rodrigo A, Fried R, McManus B, Engle MA, Van Praagh R. Cardiac malformations in trisomy-18: a study of 41 postmortem cases. *J Am Coll Cardiol*. 1989;13:1586–1597.
18. Gantt PA, Byrd JR, Greenblatt RB, McDonough PG. A clinical and cytogenetic study of fifteen patients with 45,X/46XY gonadal dysgenesis. *Fertil Steril*. 1980;34:216–221.
19. de la Chapelle A, Schroder J, Stenstrand K, Fellman J, Herva R, Saarni M, Anttolainen I, Tallila I, Tervila L, Husa L, Tallqvist G, Robson EB, Cook PJ, Sanger R. Pericentric inversions of human chromosomes 9 and 10. *Am J Hum Genet*. 1974;26:746–766.

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20. Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet*. 1998; 14:417–422.
21. Stankiewicz P, Lupski JR. Genome architecture, rearrangements and genomic disorders. *Trends Genet*. 2002;18:74–82.
22. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. Detection of large-scale variation in the human genome. *Nat Genet*. 2004;36:949–951.
23. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lucito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A, Wigler M. Large-scale copy number polymorphism in the human genome. *Science*. 2004;305:525–528.
24. Pinkel D, Albertson DG. Comparative genomic hybridization. *Annu Rev Genomics Hum Genet*. 2005;6:331–354.
25. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. High-resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet*. 1998;20: 207–211.
26. Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Dohner H, Cremer T, Lichter P. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromosomes Cancer*. 1997;20:399–407.
27. Lucito R, Healy J, Alexander J, Reiner A, Esposito D, Chi M, Rodgers L, Brady A, Sebat J, Troge J, West JA, Rostan S, Nguyen KC, Powers S, Ye KQ, Olshen A, Venkatraman E, Norton L, Wigler M. Representational oligonucleotide microarray analysis: a high-resolution method to detect genome copy number variation. *Genome Res*. 2003;13:2291–2305.
28. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. *Nature*. 2006;444:444–454.
29. Tuzun E, Sharp AJ, Bailey JA, Kaul R, Morrison VA, Pertz LM, Haugen E, Hayden H, Albertson D, Pinkel D, Olson MV, Eichler EE. Fine-scale structural variation of the human genome. *Nat Genet*. 2005;37:727–732.
30. Charbonnier F, Raux G, Wang Q, Drouot N, Cordier F, Limacher JM, Saurin JC, Puisieux A, Olschwang S, Frebourg T. Detection of exon deletions and duplications of the mismatch repair genes in hereditary nonpolyposis colorectal cancer families using multiplex polymerase chain reaction of short fluorescent fragments. *Cancer Res*. 2000;60:2760–2763.
31. Armour JA, Sismani C, Patsalis PC, Cross G. Measurement of locus copy number by hybridisation with amplifiable probes. *Nucleic Acids Res*. 2000;28:605–609.
32. Hollox EJ, Akrami SM, Armour JA. DNA copy number analysis by MAPH: molecular diagnostic applications. *Expert Rev Mol Diagn*. 2002; 2:370–378.
33. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 2002;30:e57.
34. Sellner LN, Taylor GR. MLPA and MAPH: new techniques for detection of gene deletions. *Hum Mutat*. 2004;23:413–419.
35. Hinds DA, Kloek AP, Jen M, Chen X, Frazer KA. Common deletions and SNPs are in linkage disequilibrium in the human genome. *Nat Genet*. 2006;38:82–85.
36. McCarroll SA, Hadnott TN, Perry GH, Sabeti PC, Zody MC, Barrett JC, Dallaire S, Gabriel SB, Lee C, Daly MJ, Altshuler DM. Common deletion polymorphisms in the human genome. *Nat Genet*. 2006;38:86–92.
37. Freeman JL, Perry GH, Feuk L, Redon R, McCarroll SA, Altshuler DM, Aburatani H, Jones KW, Tyler-Smith C, Hurles ME, Carter NP, Scherer SW, Lee C. Copy number variation: new insights in genome diversity. *Genome Res*. 2006;16:949–961.
38. Wong KK, deLeeuw RJ, Dosanjh NS, Kimm LR, Cheng Z, Horsman DE, MacAulay C, Ng RT, Brown CJ, Eichler EE, Lam WL. A comprehensive analysis of common copy-number variations in the human genome. *Am J Hum Genet*. 2007;80:91–104.
39. Wang J, Ban MR, Hegele RA. Multiplex ligation-dependent probe amplification of LDLR enhances molecular diagnosis of familial hypercholesterolemia. *J Lipid Res*. 2005;46:366–372.
40. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, Redon R, Bird CP, de Grassi A, Lee C, Tyler-Smith C, Carter N, Scherer SW, Tavare S, Deloukas P, Hurles ME, Dermitzakis ET. Relative impact of nucleotide and copy number variation on gene expression phenotypes. *Science*. 2007;315:848–853.
41. Daar AS, Scherer SW, Hegele RA. Implications of copy number variation in the human genome: a time for questions. *Nat Rev Genet*. 2006;7:414.
42. Feuk L, Marshall CR, Wintle RF, Scherer SW. Structural variants: changing the landscape of chromosomes and design of disease studies. *Hum Mol Genet.* 2006;15:R57–R66.

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