Genome Structural Variation

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Who am I?

- Canadian and American
- 1997 – 2004 – Assistant & Associate Professor Case Western Reserve Univ
- 2004 – present – Professor and HHMI investigator at University of Washington, Seattle

- Recently duplicated genes and dynamic regions of structural variation their role in human disease and evolution
Genetic Variation

Types

- Single base-pair changes – point mutations
- Small insertions/deletions – frameshift, microsatellite, minisatellite
- Mobile elements—retroelement insertions (300bp -10 kb in size)
- Large-scale genomic variation (>1 kb)
  - Large-scale Deletions, Inversion, translocations
  - Segmental Duplications
- Chromosomal variation—translocations, inversions, fusions.

Cytogenetics

Sequence
Genome Structural Variation

Deletion

Duplication

Inversion
Introduction

• **Genome structural variation**: gains and losses of DNA (copy-number variation (CNV)) as well as balanced events such as inversions and translocations—operationally defined >50 bp

• **Objectives**
  1. Genomic architecture and disease impact.
  2. Detection and characterization methods
  3. Primate genome evolution
Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans


The Influence of CCL3L1 Gene–Containing Segmental Duplications on HIV-1/AIDS Susceptibility


Schizophrenia risk from complex variation of complement component 4


Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome

Andrew J Sharp, Sierra Hansen, Rebecca R Selzer, Ze Cheng, Regina Regan, Jane A Hurst, Helen Stewart, Sue M Price, Edward Blair, Raoul C Hennekam, Carrie A Fitzpatrick, Rick Segre, Todd A Richmond, Cheryl Guiver, Donna G Albertson, Daniel Pinkel, Peever S Eis, Stuart Schwartz, Samantha J L Knight & Evan E Eichler

Association between Microdeletion and Microduplication at 16p11.2 and Autism

Lauren A. Weiss, Ph.D., Yiping Shen, Ph.D., Joshua M. Korn, B.S., Dan E. Arking, Ph.D., David T. Miller, M.D., Ph.D., Ragnheidur Fossdal, B.Sc., Evald Saemundsson, B.A., Hreinn Stefansson, Ph.D., Manuel A.R. Ferreira, Ph.D., Todd Green, B.S., Orah S. Platt, M.D., Douglas M. Ruderfer, M.S., Christopher A. Walsh, M.D., Ph.D., David Altshuler, M.D., Ph.D., Aravinda Chakravarti, Ph.D., Rudolph E. Tanzi, Ph.D., Kari Stefansson, M.D., Ph.D., Susan L. Santangelo, Sc.D., James F. Gusella, Ph.D., Pamela Sklar, M.D., Ph.D., Bai-Lin Wu, M.Ed., Ph.D., and Mark J. Daly, Ph.D., for the Autism Consor

Strong Association of De Novo Copy Number Mutations with Autism


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Cite this article.
Perspective: Segmental Duplications (SD)

Definition: Continuous portion of genomic sequence represented more than once in the genome ( >90% and > 1kb in length)—historical copy number variation
Importance:
SDs promote genome structural variation

Non Allelic Homologous Recombination (NAHR)

Human Disease
Triplosensitive, Haploinsufficient and Imprinted Genes
Importance: Evolution of New Gene Function

GeneA → Duplication → GeneA’

Acquire New/Modified Function

Mutation

Loss of Function

Mutation

Maintain old Function

Mutation
Human Genome Segmental Duplication Pattern

- ~4% duplication (125 Mb)
- >20 kb, >95%
- 59.5% interspersed
- gene/transcript rich
- Associated with Alu repeats

Mouse Segmental Duplication Pattern

- 118 Mb or ~4% dup
- >20 kb, >95%
- 89% are tandem
- Gene/transcript poor
- Associated with LINEs

She, X et al., (2008) Nature Genetics
Human Segmental Duplications Properties

- Large (>10 kb)
- Recent (>95% identity)
- **Interspersed** (60% are separated by more than 1 Mb)
- Modular in organization
- Difficult to resolve
Rare Structural Variation & Disease

- **Genomic Disorders:** A group of diseases that results from genome rearrangement mediated mostly by non-allelic homologous recombination. *(Inoue & Lupski, 2002).*

**Human Disease**
Triplosensitive, Haploinsufficient and Imprinted Genes
DiGeorge/VCFS/22q11 Syndrome

1/2000 live births
180 phenotypes
75-80% are sporadic (not inherited)
• 130 candidate regions (298 Mb)
• 23 associated with genetic disease
• Target patients array CGH
~14.2% of genetic cause of developmental delay explained by large CNVs (>500 kbp)

Cooper et al., Nat. Genet, 2011
Common and rare structural variation are linked to 17q21.31 deletion syndrome.
17q21.31 inversion

• Region of recurrent deletion is a site of common inversion polymorphism in the human population
• Inversion is largely restricted to Caucasian populations
  – 20% frequency in European and Mediterranean populations
• Inversion is associated with increase in global recombination and increased fecundity

Stefansson, K et al., (2005) Nature Genetics
Direct Orientation allele (H1)
Inverted orientation allele (H2)

- Tested 17 parents of children with microdeletion and found that every parent within whose germline the deletion occurred carried an inversion
- Inversion polymorphism is a risk factor for the microdeletion event
Duplication Architecture of 17q21.31 Inversion (H2) vs. Direct (H1) Haplotype

- Inversion occurred 2.3 million years ago and was mediated by the LRRC37A core duplicon.
- H2 haplotype acquired human-specific duplications in direct orientation that mediate rearrangement and disrupts KANSL1 gene.

Summary

- Human genome is enriched for segmental duplications which predisposes to recurrent large CNVs during germ-cell production
- 15% of neurocognitive disease in intellectual disabled children is “caused” by CNVs—8% of normals carry large events
- Segmental duplications enriched 10-25 fold for structural variation.
- Increased complexity is beneficial and deleterious: Ancestral duplication predisposes to inversion polymorphism, inversion polymorphisms acquires duplication, haplotype becomes positively selected and now predisposes to microdeletion
II. Genome-wide SV Discovery Approaches

Hybridization-based
- Iafrate et al., 2004, Sebat et al., 2004
- SNP microarrays: McCarroll et al., 2008, Cooper et al., 2008, Itsara et al., 2009
- Array CGH: Redon et al. 2006, Conrad et al., 2010, Park et al., 2010, WTCCC, 2010

Single molecule mapping
- Optical mapping: Teague et al., 2010 e.g. Bionano Genomics: Levy-Sakin et al, 2019

Sequencing-based
- Read-depth: Bailey et al, 2002
- Next-gen sequencing: Korbel et al. 2007, Yoon et al., 2009, Alkan et al., 2009, Chen et al. 2009; Mills 1000 Genomes Project, 2011, Sudmant et al. 2015a,
- Long-read Sequencing: Chaisson et al., 2015, 2019, Pendleton et al., 2015, Sedlazeck et al., 2018 Audano et al, 2019, Ebert et al., 2021

Hybridization-based Sequencing-based
Array Comparative Genomic Hybridization

One copy gain = \( \log_2(3/2) = 0.57 \) (3 copies vs. 2 copies in reference)

One-copy loss = \( \log_2(1/2) = -1 \)
SNP Microarray detection of Deletion (Illumina)

Human chromosome 3 position

~55 kbp

LogR and B-AIlele Frequency

AB

A- or B-

AB
Using sequence read pairs to resolve structural variation

Dataset: 1,122,408 fosmid pairs preprocessed (15.5X genome coverage)
639,204 fosmid pairs BEST pairs (8.8 X genome coverage)
Genome-wide Detection of Structural Variation (>8kb) by End-Sequence Pairs

Next-Generation Sequencing Methods

- **Read pair analysis**
  - Deletions, small novel insertions, inversions, transposons
  - Size and breakpoint resolution dependent to insert size

- **Read depth analysis**
  - Deletions and duplications only
  - Relatively poor breakpoint resolution

- **Split read analysis**
  - Small novel insertions/deletions, and mobile element insertions
  - 1bp breakpoint resolution

- **Local and de novo assembly**
  - SV in unique segments
  - 1bp breakpoint resolution

Alkan et al., *Nat Rev Genet*, 2011
Using Sequence Read Depth

- Map whole genome sequence to reference genome
  - Variation in copy number correlates linearly with read-depth

Illumina Sequence

Random Genome Sample

Bailey et al., Science, 2002
Personalized Duplication or Copy-Number Variation Maps

Venter (Sanger)

Watson (454)

NA12878 (Solexa)

NA12891 (Solexa)

NA12892 (Solexa)

• Two known ~70 kbp CNPs, CNP#1 duplication absent in Venter but predicted in Watson and NA12878, CNP#2 present mother but neither father or child

Alkan, Nat. Genet, 2009
Read-Depth CNV Heat Maps vs. FISH

Interphase FISH

GM19240

GM12878
Indirect sequence-based approaches are incomplete 159 genomes (2-4X) (deletions only)

Read-Pair

6855 (63%)

3250

1772 (33%)

Read-Depth

3223 (80%)

486

Mills et al., Nature 2011
Challenges

- Size spectrum—>5 kbp discovery limit for most experimental platforms; NGS can detect much smaller but misses events mediated by repeats.
- Class bias: deletions>>>duplications>>>>>balanced events (inversions)
- Multiallelic copy number states—incomplete references and the complexity of repetitive DNA
- False negatives.
Long read Genome Sequencing Revolution

Pacific Biosciences (PacBio)—single-molecule real-time sequence (SMRT) data (15-50) kbp sequence reads
ONT (Oxford Nanopore Technology)—higher error rate but, portable, scalable native DNA sequencing of long-reads
Advances in long-read sequencing

HiFi Pac Bio Sequencing

- Double-stranded DNA
- Ligate Adapters
- Anneal Primer and Bind DNA Polymerase
- Sequence
- Generate Consensus Read

99.9% accurate 18-23 kbp reads

Ultra-long reads ONT

- Ultra-long reads ONT > 100 kbp in length

19.5X coverage

- Median = 36.7
- Mean = 67.3
- N50 = 139.9
- N1 = 631.9
- Max = 1538.3

Total Gb = 62.56
Advantages of long read sequencing

Ultra-long Oxford Nanopore Technology (ONT)  
\(~ 139 \text{ kbp}\)

HiFi PacBio  
\(~ 18-20 \text{ kbp}\)

Illumina  
\(150-300 \text{ bp}\)
More uniform coverage and sequencing of native DNA

**SHANK3**

- PacBio Sequence Coverage
- Illumina Sequence Coverage
Increased sensitivity for structural variation (SV)

- ~25,000 PacBio SVs vs. 11,000 Illumina SVs >50 bp
- Eleven Illumina callers combined detect 49% of deletions and 11% of insertions in a human genome--NGS misses 75% of SVs

Chaisson et al, Nature, 2015; Chaisson et al., Nat Comm, 2019
LRS has transformed how we characterize copy number and structural variation

Sudmant et al, Science, 2015,
Porubsky et al, Cell, in press
Complete sequence of human genome

2021 (T2T-CHM13)

Different contigs □ Absent sequences □ Centromeres

So how did we do it?

We used an **effectively haploid** human cell line known as CHM13

CHM13 is a complete **hydatidiform mole**

This greatly simplifies this problem because it allows us to assemble each chromosome without interference from a second set of chromosomes

We used two long-read sequencing technologies with complementary strengths

1. Pacific Biosciences (PacBio) high-fidelity (HiFi)
   
   • 15-25 kbp long
   • >99% accurate (similar to Illumina)
   • Strength: Extremely accurate

2. Oxford Nanopore Technologies (ONT)
   
   • No limit in read length!
   • 93-99% accurate
   • Strength: Extremely long
- 8% of missing genome sequence added (>200 Mbp)
- Complete sequence of centromeres, acrocentric and segmental duplications
- Adds 1956 gene predictions of which 130-190 are protein coding
- Framework for understanding the genetically most complex regions of our genome.
A 6 Gbp Human Genome Assembly
(contig N50=25-28 Mbp)

Porubsky et al, Nat. Biotech, 2020
Combining HiFi & UL-ONT improves contiguity with maternal and paternal complements nearly resolved

- Verkko assembly with >30X HiFi + >30UL-ONT
- Generates phased human genome assemblies with <50 gaps
- Bandage representation: maternal (red) and paternal (blue)
- 99% of the human genome can now be phased & assembled

Rautiainen et al., bioRxiv, 2022, Nat. Biotech, 2023
Primate phased genome assembly efforts

4.5-6 mya
6-8 mya
12-16 mya
18-20 mya
25-33 mya

Human (87 genomes)
Chimpanzee (2)
Gorilla (2)
Orangutan (2)
Gibbon (1)
Macaque (1)

Complete sequencing of ape chromosomes

Chromosome 12

Chimp h1

Bonobo h2

Human T2T

Gorilla

Orang (Bor.)

Orang (Sum.)

DongAhn Yoo & the T2T Primates Consortium
Complete sequencing of ape chromosomes (SVbyEye)

Chromosome 16

Chimp h1

Bonobo h2

Human T2T

Gorilla

Orang (Bor.)

Orang (Sum.)

DongAhn Yoo unpublished
A graph can capture such variation e.g. Minigraph

1. Generate phase genome assemblies
2. Iteratively introduce assembly sequence to a graph.
3. Distinguish query sequence already present in graph from novel sequence
4. Include novel sequence as new segments or edges between segments in graph.
5. Repeat with next assembly

Guitart, X
A graph-based representation of structural variation

Liao et al., bioRxiv, 2022, Nature, 2023
A graph-based representation of the entire human genome as a conceptual new reference.
Access to previously inaccessible regions: Centromeres

1. Mirror symmetry
2. Layered nature

“StainedGlass” Visualization, Vollger et al, Biorxiv, 2021

Understanding centromere structure and function

Chromosome 8 position (Mbp)

Repeat elements

- Monomeric α-satellite
- 2.08 Mbp α-satellite HOR
- Monomeric α-satellite

Fold coverage of ONT reads

- methylated
- unmethylated

Location of the kinetochore?

~76 kbp dip in methylation!
Understanding centromere structure and function

Copy number and structural variation of \textit{TBC1D3}
Summary

• Short read NGS approaches
  – Multiple methods are needed—readpair+read-depth+splitread often with orthogonal validation such as SNP microarray
  – ~75% of SVs are missed because SVs are non-randomly distributed to repetitive regions where mapping quality is low
  – Read-depth approaches allow CNV prediction but not structure

• Long-read sequencing methods provide complete SV but currently limited throughput
  – Read-based versus assembly-based approaches
  – Telomere-to-telomere assemblies of human genomes now possible or nearly so for diploid—complete genetic information where all variants are phased.
  – First human pangenome now available—a new concept to eventually replace a singular reference.
III. Why?

• Ohno—Duplication is the primary force by which new gene functions are created.

• There are 990 annotated genes completely contained within segmental duplications.
Dynamic Genetic Variation

- Genomic copy number changes contributes more genetic difference between apes and humans than SNVs
- 468 Mbp CNV vs. 167 Mbp SNVs (ration: 2.8)

Rate of Duplication

Sudmant PH et al., *Genome Res.* 2013

\[ p = 9.786 \times 10^{-12} \]
Mosaic Architecture

- A mosaic of recently transposed duplications
- Duplications within duplications.
- Potentiates “exon shuffling”, regulatory innovation
• The burst of segmental duplications 8-12 mya corresponds to core-associated duplications which have occurred on six human chromosomes (chromosomes 1, 2, 7, 15, 16, 17).

• Most of the recurrent genomic disorders associated with developmental delay, epilepsy, intellectual disability, etc. are mediated by duplication blocks centered on a core.

Jiang et al, Nat. Genet., 2007
Human/Great-ape “Core Duplicons” have led to the emergence of new genes

Features: No orthologs in mouse; multiple copies in chimp & human; dramatic changes in expression profile; signatures of positive selection
Core Duplicon Hypothesis

The selective disadvantage of interspersed duplications is offset by the benefit of evolutionary plasticity and the emergence of new genes with new functions associated with core duplicons.

Marques-Bonet and Eichler, CSHL Quant Biol, 2008
Human-specific gene family expansions

Notable human-specific expansion of brain development genes.
Neuronal cell death: $p=5.7 \times 10^{-4}$; Neurological disease: $p=4.6 \times 10^{-2}$

Sudmant et al., Science, 2010
SRGAP2 function

- **SRGAP2** (SLIT-ROBO Rho GTPase activating protein 2) functions to control migration of neurons and dendritic formation in the cortex.
- Gene has been duplicated three times in human and no other mammalian lineage.
- Duplicated loci not in human genome.

Guerrier et al., *Cell*, 2009
SRGAP2 Human Specific Duplication

Dennis, Nuttle et al., Cell, 2012
SRGAP2C is fixed in humans
(n=661 individual genomes)
SRGAP2 duplicates are expressed

RNAseq

In situ
SRGAP2C duplicate antagonizes function

Charrier et al., Cell, 2012
Australopithecus
- A. afarensis
- A. anamensis
- A. aethiopicus
- A. boisei
- A. robustus
- A. garhi
- K. platyops
- Homo

Homo habilis

Sahelanthropus
Orrorin
Ardipithecus

Fixed in human population and expressed in neurons

Dennis, Nuttle et al. Cell (2012)

3.4 mya
2.4 mya

~350 cc ~1000 cc
Example 2: Human-specific Duplication of ARHGAP11B

• Hypothesis: increase in number of basal radial glial cells or prolonged proliferation may lead to enlargement of the subventricular zone in humans

• Search for genes that are dramatically increased in concentration in basal radial glial cells as compared to neurons during development

• Only one gene of 56 not present in mouse ARHGAP11B

**ARHGAP11B induced gyrification of mouse brain**

- E13.5 microinjection of *ARHGAP11B* induced folding in the neocortex by E18.5 in ½ of the cases— a significant increase in cortical area.

*Florea et al., Science 2015*
Transgenic human-specific duplicate *ARHGAP11B*: Marmoset fetal brain with human promoter

WT brain and brain expressing *ARHGAP11B* in neocortex (TG3). Arrowheads indicate cortical folds. R, rostral; C, caudal. Scale bars, 1 mm

- Increased the numbers of basal radial glia progenitors in the marmoset outer subventricular zone, increased the numbers of upper-layer neurons, enlarged the neocortex, and induced its folding.
Duplication of ARHGAP11B and 15q13.3 Syndrome

Duplication from ARHGAP11A to ARHGAP11B estimated to have occurred 5.3 +/- 0.5 million years ago.

Antonacci et al., Nat Genet, 2014,
Human-specific duplicated gene innovations and brain development

- **SRGAP2C**— 3.2 mya—produces a truncated protein that heterodimerizes with the parental product and alters neuronal migration, dendritic morphology and density of synapses ([Dennis et al., Cell, 2012; Charrier et al., Cell, 2012]).

- **ARHGAP11B**— truncated duplicate is expressed in basal radial glial cells appears to expand neuronal count and expand subventricular zone ([Antonacci et al., Nat Genet, 2014; Florio et al., Science, 2015]).

- **BOLA2B**--- (256 kya) duplication of gene family specifically at root of Homo sapiens, rapid fixation and largest difference between Neandertals and human genomes and is important in iron homeostasis ([Nuttle et al., Nature, 2016, Gianuzzi et al., Am J Hum Genet 2019]).

- **NOTCH2NL**--- (<3 mya) partial duplication expressed in radial glial where interacts with NOTCH2 receptors and delays neuronal progenitor differentiation([Fiddes et al., Cell, 2018])

- Properties: Nearly fixed for copy number in the human population, predispose to disease instability and the duplications are incomplete with respect to gene structure. **NONE present in original human genome.**
Summary

• Interspersed duplication architecture sensitized our genome to copy-number variation increasing our species predisposition to disease—children with autism and intellectual disability.

• Duplication architecture has evolved recently in a punctuated fashion around core duplicons which encode human great-ape specific gene innovations (eg. *NPIP*, *NBPF*, *LRRC37*, etc.).

• Cores have propagated in a stepwise fashion “transducing” flanking sequences---human-specific acquisitions flanks are associated with brain developmental genes.

• **Core Duplicon Hypothesis:** Selective disadvantage of these interspersed duplications offset by newly minted genes and new locations within our species. Eg. *SRGAP2C*
Overall Summary

• **I. Disease:** Role of CNVs in human disease—relationship of common and rare variants—a genomic bias in location and gene type

• **II. Methods:** NGS Read-pair and read-depth methods to characterize SVs within genomes—long-read genomes can now fully phase and assemble achieving complete telomere-to-telomere assembly

• **III: Evolution:** Rapid evolution of complex human architecture that predisposes to disease also coupled to gene innovation that makes us human
Disease

Evolution
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<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>SV</td>
<td>structural variation</td>
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<tr>
<td>CNV</td>
<td>copy number variation</td>
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<tr>
<td>CNP</td>
<td>copy number polymorphism</td>
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<tr>
<td>NGS</td>
<td>next generation sequencing</td>
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<tr>
<td>(eg. Illumina short read)</td>
<td></td>
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<tr>
<td>Indel</td>
<td>insertion/deletion event</td>
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<tr>
<td>SD</td>
<td>segmental duplication</td>
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<tr>
<td>SMRT</td>
<td>single-molecule real-time sequencing</td>
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<tr>
<td>CCS</td>
<td>circular consensus</td>
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<tr>
<td>sequencing</td>
<td>HiFi-high fidelity long-read sequencing</td>
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SV Software

- **PennCNV** (Kai Wang) and **CNVPartition**—calling CNVs from SNP microarray
- **Genomestrip**—Handsaker/McCarroll—combines read-depth and readpair data to identify potential sites of SV data from population genomic data; **dCGH**—Sudmant/Eichler—measure Illumina read-depth using multi-read sequence mapper (mrsFAST/mrFAST); **Delly**—EMBL Rausch/Korbel—uses split-read and readpair signatures to increase sensitivity and specificity; **Lumpy**—Quinlan/Hall—uses probabilistic framework to integrate multiple SV such as discordant paired-end alignments and split-read alignments; **GATK-SV**—Talkowski—integrates multiple short reads signatures
- **Conifer /XHMM**—Krumm/Eichler & Frommer/Purcell-exome CNV calling
- **PBSV**—Aaron Wenger (PacificBiosciences software) signatures from pbmm2 alignments; **SNIFFLES2**—Sedlaczeck/Schatz—NGLMR mapping of PacBio or ONT data using split-read alignments, high-mismatch regions, and coverage
- **PAV**—Audano/Eichler & **SVIM-asm**—Heller/Vingron--assembly-to-assembly based discovery of SVs using minimap and LR assembled genomes
- **Verkko**—Koren/Philippy & **HiFiasm-UL**—Heng Li—graph based approaches to generate near T2T assemblies using UL-ONT and HiFi sequencing data
- **Saffire-SV, StainedGlass & SVbyEye**—(Vollger/ Porubsky/Eichler)—visualization tools to characterize chromosomal level SV and centromeric satellite DNA
SD-Mediated Rearrangements

(a) Interchromosomal Direct
(b) Inverted Direct
(c) Complex Direct
(d) Intrachromosomal Direct
(e) Intrachromosomal Inverted
(f) Intrachromosomal Complex
(g) Intrachromatid Direct
(h) Intrachromatid Inverted
(i) Intrachromatid Complex