events at the same loci, Cole, Baudat and colleagues demonstrated that crossovers involved reciprocal exchange, as expected from the resolution of a double Holliday junction. Further, crossover-associated gene conversion events, at approximately 500 bp in length, were five times longer than noncrossover gene conversion events. Importantly, they recovered 143 instances of noncrossover gene conversion and found no evidence for double–Holliday junction resolution during the DSB repair process, providing strong evidence that noncrossovers derive from a nonreciprocal mechanism such as SDSA.

Analyzing recombination hotspots

Recombination hotspots are small regions of the genome, usually a few kilobases in length, where crossovers occur at a higher than expected rate[8]. The location of hotspots in humans and mice is determined by PRDM9, a meiosis-specific histone H3 methyltransferase that binds sequence motifs to direct the formation of SPO11-dependent DSBs[9]. Cole, Baudat and colleagues focused their attention on two mouse recombination hotspots, *Psmb9* and *A3*, to increase their chances of observing both crossover-associated and noncrossover-associated gene conversions. The analysis of these two hotspots indicated that the majority of noncrossover gene conversions did not alter the PRDM9 binding site, providing a plausible answer to the paradoxical question of how PRDM9 binding sites can promote the formation of a DSB yet be maintained in the genome over many generations[10]. In theory, a specific sequence that promotes the formation of a DSB should be lost over time as the DSB is repaired and replaced by allelic, non-hotspot-specifying sequences. However, the authors found that 80% of noncrossover gene conversions converted polymorphisms that flanked the *A3* PRDM9 binding site but did not include it. This property allows the maintenance of the PRDM9 binding site and, therefore, of the hotspot for longer than expected.

The finding that gene conversions consistently associated with crossovers had longer tract lengths than those associated with noncrossovers in the mouse is different from what has been observed in yeast, where both classes of gene conversion events were found to average ~2 kb in length[11,12]. This difference, along with the presence in the mouse of the hotspot-determining protein PRDM9, which yeast lack, causes us to speculate that, in mammals, shorter conversion tracts might exist to protect the integrity of the PRDM9 binding sites. It is also possible that shorter conversion tracts serve to lessen the severity of meiotic errors, such as those seen with nonallelic homologous recombination[13].

It is possible to imagine the approach described by Cole, Baudat and colleagues being applied in whole or part to other model systems. The power of tetrad analysis is that it gives a complete picture of all four meiotic products, providing important clues about the machinery involved in the break repair process. These clues may prove valuable in understanding not only meiotic DSB repair but also mitotic DSB repair. Indeed, tetrad analysis is conspicuously absent from the toolkit of worm and fly geneticists, among others. The elegant solution provided by Cole, Baudat and colleagues should be both encouraging and a springboard to promote the development of this technique in other model systems.

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Reaching a CNV milestone

Arthur L Beaudet

A new study compares the copy number variants (CNVs) in 29,085 children with developmental delay to those in 19,584 healthy controls, providing a valuable compilation of such data. The phenotypic variability and wide range of penetrance for these variants present societal challenges regarding how these findings might be incorporated into newborn screening, early intervention and, perhaps, carrier testing and prenatal diagnosis.

About a decade ago, chromosomal microarray analysis in the form of array comparative genomic hybridization (CGH) and SNP arrays came into routine clinical use for the evaluation of children with various disabilities and congenital malformations. This technology was a major advance in clinical genetics, increasing the proportion of children with severe intellectual disability and developmental delay for whom an underlying causative cytogenetic abnormality was identified from about 5% using Giemsa banded karyotyping to 20–25% using chromosomal microarrays, depending on the details of the phenotype[1,2]. This increased ability to identify an underlying genetic cause was perhaps the most substantial clinical benefit to come directly from the Human Genome Project in the first decade of the twenty-first century, although sequencing-based diagnosis is having an even greater impact in the second decade. On page 1063 of this issue, Evan Eichler and colleagues report the analysis of CNVs in 29,085 children with developmental delay in comparison to those identified in 19,584 healthy controls[3]. They combined this herculean effort with the resequencing of 26 genes in a smaller subset to combine CNV and point mutation analyses. Although there are databases and tracks on genome browsers with data for CNVs in healthy controls and in symptomatic individuals (Database of Genomic Variants, DGV; International Collaboration for Clinical Genomics, ICCG; Decipher; and dbVar, a database of genomic structural variation), it is difficult even for those immersed in CNV biology and clinical practice to determine

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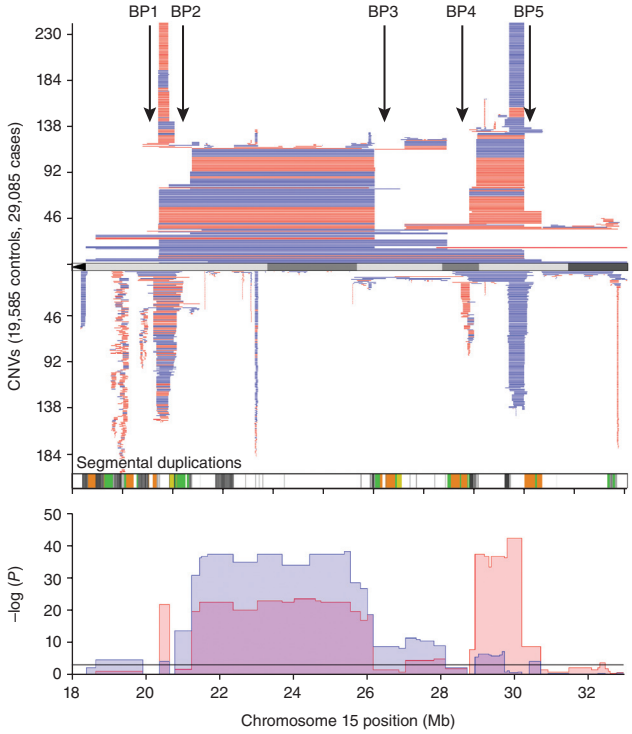

Figure 1 CNVs at chromosome 15q11-q13.3, with the critical region for Prader-Willi and Angelman syndromes between breakpoints 2 and 3 (BP2–BP3). Breakpoints are regions of segmental duplication leading to recurrent CNVs primarily through non-allelic homologous recombination. Data were obtained from ref. 3. Red indicates deletions, and blue indicates duplications.

the likely pathogenicity of a particular variant. The new findings reported by Coe *et al.*[3] are thus enormously helpful in associating CNVs with phenotypes, including cognitive disability (in particular, see Supplementary Fig. 3 and Supplementary Tables 2 and 3 in this report).

The extent of variation in copy number in the human genome took biomedical science by surprise with the recognition that it greatly exceeds heterogeneity in sequence variation[4]. It rapidly became obvious that many human phenotypes could be caused by CNVs and that prominent among these abnormalities were cognitive impairments (developmental delay and intellectual disability), behavioral abnormalities (schizophrenia, bipolar disorder and autism), epilepsy and many congenital malformations (for example, congenital heart disease).

Many reviews of the genomic architecture, clinical significance and mechanisms of CNV occurrence are available[5,6]. Noteworthy observations include the occurrence of single CNVs with many phenotypes and many CNVs causing the same phenotype, complicating the assignment of genotype-phenotype correlations. CNVs currently represent a major component of what is known about the etiology of intellectual disability, autism, schizophrenia, bipolar disorder and epilepsy[7–9]. CNVs account for 20–25% of severe intellectual disability, and their analysis in prenatal diagnosis has become widespread[10]. Other observations suggest that variability in the other allele at heterozygous CNV regions can modify phenotypes, and there is evidence of interaction between CNVs and point mutations with compound heterozygous genotypes, as in thrombocytopenia and absent radius[11]. CNVs might also interact in a two-locus mode, such that two CNVs in an individual might give a phenotype different from either CNV alone[12].

An attempt at classification

It has become clear that severity of phenotype and penetrance vary widely and that many regions are prone to both deletion and duplication. At the risk of oversimplification, CNVs might be divided into five groups (**Supplementary Table 1**). There would be many subgroups and exceptions to this nonstandard classification, but it might be of some help in discussions to follow. Individual CNVs might belong in one group in terms of severity and in a different group in terms of penetrance (for example, colorblindness has high penetrance in males but a mild phenotype). Group 1 would include CNVs resulting in conditions with severe phenotypes, very high or complete penetrance, uniform *de novo* inheritance and little to no reproduction (for example, Angelman syndrome or Smith-Magenis syndrome). Group 2 would include CNVs resulting in conditions with moderate severity, 50–90% penetrance, both *de novo* and inherited cases, and reduced but not absent transmission (for example, deletion of 15q13.3 with breakpoints BP4–BP5; **Fig. 1**). Although the CNV comprising deletion of 15q13.3 is not shown in controls in **Figure 1**, there are some parents of probands who are at least superficially normal, particularly parents of probands with homozygous deletion[13]. Group 3 would include CNVs resulting in conditions with mild severity, penetrance ranging from 5 to 100%, 70–90% inherited cases, and normal or near-normal reproduction (for example, duplication *CMT1A* and reciprocal deletion hereditary neuropathy with liability to pressure palsies showing high and low penetrance, respectively). Group 4 would include CNVs for which the majority of carriers do not meet the criteria for any medical diagnosis or disability but for which there is undeniable evidence that a small percentage of carriers are diagnosed with mild intellectual disability, autism or even schizophrenia. Deletion of 15q11.2 (BP1–BP2; **Fig. 1**) is an excellent example of this phenomenon. In probands with developmental delay and deletion of 15q11.2, there is almost always a parent with the same CNV who is either entirely healthy or has 'subclinical penetrance'. Subclinical penetrance is a concept that has not been well documented but is thought by many clinicians to be a real phenomenon, whereby an individual has an IQ that is 10–15 points lower than would be the case without the CNV but does not meet the criteria for any medical or disability diagnosis and has normal reproduction[14]. Group 5 would be reserved for totally benign CNVs with no effect on phenotype whatsoever. Duplication of 15q11.2 (BP1–BP2) appears to belong in this group.

Societal implications

All CNVs, benign and severely disabling, could potentially be detected through newborn screening. It is possible that noninvasive prenatal diagnosis capable of detecting all of these CNVs will become a routine option for all pregnancies. For CNVs in group 1, the circumstances are unfortunate, and there is often irreversible damage before birth and little opportunity for cure or dramatic correction. All variants would be *de novo* and detectable by universal prenatal diagnosis or newborn screening. Groups 2, 3 and 4 cover a wide range of severity and penetrance, including severe, moderate, mild and

borderline intellectual disability. Particularly when under- or overexpression of a single gene in a CNV mediates the phenotype, pharmacological treatments or even cures are imaginable. Group 4 CNVs, such as the BP1–BP2 deletion shown in **Figure 1**, represent the greatest challenges to society. On the one hand, huge numbers of people might be stigmatized, and one could argue that diagnosing these CNVs would do far more societal harm than good. On the other hand, these CNVs might be contributing substantially to societal disability and disparity, and affected individuals might be precisely the group that could benefit from early supportive intervention as described in the Marmot Review[15] or pharmacological cure. There is general acceptance that early intervention is desirable and beneficial in autism and mild intellectual disability. Could such intervention start even before the onset of symptoms in infants at twofold or greater relative risk for mild intellectual disability? The social and ethical issues surrounding the detection and reporting of milder and incompletely penetrant CNVs are complex and present serious challenges for discussion.

Note: Supplementary information is available in the online version of the paper (doi:10.1038/ng.3106).

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NLRC4 gets out of control

Hanif Javanmard Khameneh & Alessandra Mortellaro

The NLRC4 inflammasome mediates the rapid release of proinflammatory cytokines in response to various microbial stimuli, but its role in the pathology of human diseases remains unknown. Two new studies now report gain-of-function mutations in the *NLRC4* gene that cosegregate with distinct autoinflammatory syndromes in affected families.

NLRC4 is a member of the NOD-like receptor (NLR) family of intracellular sensors that was first identified and characterized more than a decade ago[1,2]. The NLRC4 protein interacts with the enzyme caspase-1 and has a pivotal role in antimicrobial defense via intracellular sensing of a wide range of pathogens, including *Pseudomonas aeruginosa*[3] and *Salmonella enterica* serovar *typhimurium*[4]. NLRC4 is activated by NAIP-mediated recognition of flagellin or components of bacterial type 3 secretion systems (TTSS) in the host cell cytosol, leading to the assembly of a multimolecular complex known as the inflammasome that mediates proteolytic cleavage of immature pro-IL-1β and pro-IL-18 and rapid release of the bioactive forms of these proinflammatory cytokines into the extracellular space (**Fig. 1**)[5–8]. Current knowledge of NLRC4 functions comes primarily from *in vitro* studies and *in vivo* transgenic models[9], and until now there had been no reports of disease associations with *NLRC4* genetic variants. Now, Barbara Kazmierczak, Richard Lifton and colleagues[10] and Scott Canna, Raphaela Goldbach-Mansky and colleagues[11] report that two *de novo* gain-of-function mutations in human *NLRC4*, encoding p.Val341Ala and p.Thr337Ser substitutions in close proximity to each other in the nucleotide-binding domain (NBD) of the protein, are associated with distinct autoinflammatory syndromes in humans.

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New faces of autoinflammation

On page 1135 of this issue, Romberg *et al.*[10] report on several members of the same family who presented with a syndrome of enterocolitis and autoinflammation associated with mutation in *NLRC4* (SCAN4) due to a newly identified shared variant in the HD1 domain of the protein. Whereas the father of the index patient and one child experienced relatively mild symptoms, the same mutation in these individuals was associated with severe gastrointestinal complications, fever and systemic inflammation in a newborn that resulted in death by just four weeks of age. The genetic defect described by Romberg *et al.*[10] resulted in constitutive activation of the NLRC4 inflammasome in patient-derived macrophages, as well as elevated secretion of IL-1β and IL-18. Upon infection with *S. typhimurium*, patient-derived macrophages exhibited an unusually high rate of pyroptosis, a form of cell death triggered by caspase-1 activation and overproduction of IL-1 family cytokines. Unlike cells from healthy control donors, macrophages derived from patients with hyperactive NLRC4 exhibited the formation of multiple ASC (apoptosis-associated speck-like protein containing a CARD) foci that rarely colocalized with activated caspase-1. Expression of the mutant NLRC4 variant *in vitro* recapitulated the phenotype of patient-derived macrophages, indicating that the mutation alone was sufficient to critically alter cellular function.

In a separate report on page 1140, Canna *et al.*[11] describe a second autoinflammatory syndrome associated with *NLRC4* mutation in a seven-year-old patient who presented with symptoms comparable to those for a secondary form of hemophagocytic lymphohistocytosis (HLH) known as macrophage activation syndrome (MAS). The new disorder (termed