known to be required for this process, such as *TFAPA2* and *ZEB2*. In effect, *BAZ1B* is a master regulator of gene expression for genes required for the bones of the face and acts in a dose-sensitive fashion.

The second part supplies the possible connection to human self-domestication. Comparing the DNA sequences in and around the *BAZ1B* gene from the genomes of contemporary anatomically modern humans (AMHs), with those of archaic hominins, Neanderthals, and Denisovians, the authors found fixed mutations in the regulatory regions of the gene from AMHs, but not the archaics. These mutations are almost certainly mild loss-of-function mutations, which would cause slight reductions in the amounts of the gene's expression and, ultimately, in the bones of the face. This is significant because AMHs have slightly reduced facial bones compared with the archaic hominins; this parallels the domestication syndrome in other mammals. Thus, this work ties neural crest cell molecular biology to the expected phenotype one expects for the human developmental biology that might go along with domestication.

Altogether, Zanella *et al.* have produced an elegant and convincing piece of work that strengthens both the NCDS as a mechanism underlying domestication and the case that humans are a domesticated, indeed a self-domesticated, species. Many questions about these issues remain, of course, in particular concerning the links between the molecular biology of the genes involved and the neurobiological foundations of the social behaviors in Williams syndrome. Nevertheless, the work described in Zanella *et al.* is a model for exploring the possible connections between complex behavioral states and their developmental origins and evolutionary correlates. Not least, such studies should deepen our understanding of what domestication is.

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<https://doi.org/10.1016/j.tig.2020.01.002>

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Forum

Genetic Variation across Phenotypic Severity of Autism

Claudio Toma^{1,2,3,*}

It is still unclear how genetic factors of autism spectrum disorder (ASD) are implicated in the significant clinical heterogeneity ranging from intellectual disability (ID) to high-functioning profiles. Here, evidence from recent genetic studies encompassing common and rare variants are combined to suggest

a genetic model that may explain the broad gradient of phenotypic severity observed in ASD.

ASD represents one of the most prevalent disorders in childhood [1]. Family and twin studies that started more than 20 years ago have pointed clearly to a strong genetic component underlying this disorder [1].

Hundreds of studies have thus far substantially increased our knowledge of the genetics of ASD implicating several genes in the disorder. Only a decade ago we were debating which genetic hypothesis – ‘common disease, common variants’ or ‘common disease, rare variant’ – was better at modelling the genetics of autism. Currently, we know that both hypotheses are partially correct.

Many different classes of variants shape ASD genetic liability [1,2], including: (i) common risk alleles from SNPs; (ii) mutations that are rare in the general population or single nucleotide variants (SNVs); (iii) loss/gain of genetic material defined as copy number variants (CNVs); (iv) and *de novo* variants (SNVs or CNVs). Genetic studies conducted to date converge on a genetic model in which both multiple common variants of small effect size and rare variants with moderate or higher penetrance are implicated in the genetic landscape of ASD (Figure 1). Besides the genetic changes identified thus far, DNA methylation and other epigenetic alterations are also likely to contribute to autism. However, DNA methylation studies have generally been performed in small samples [1] and currently no conclusions can be drawn about the extent of epigenetic heritability in ASD.

Notwithstanding the recent advances in unravelling the genetic causes of autism, we acknowledge that the real genetic architecture is far more complex than previously thought. We have journeyed

Figure 1. The Autism Spectrum Disorder (ASD) Phenotype Is the Result of Additive Risk Loci of Both Common and Rare Variants. Each dot represents a risk allele: in blue common variants or SNPs (minor allele frequency higher than 0.01 in general population), which are detected through genome-wide association studies (GWASs); in red, rare variants or single nucleotide variants (SNVs) (minor allele frequency lower than 0.01 in general population) identified through whole-exome sequencing (WES)/whole-genome sequencing (WGS); in black, structural variants (deletions or duplications) detected by copy number variant (CNV) arrays; and in green *de novo* mutations that can involve a nucleotide change or structural variants. The model follows a distribution with rarer risk alleles characterised by higher penetrance effect compared with the lower effect size of common variants.

from the first estimates of approximately 15 genes implicated in ASD to the current estimate of around 1000 genes [2]. However, it is still largely unclear how these genes or sets of genes contribute to the extremely heterogeneous ASD phenotype, which is characterised by impairments in social interactions and communication and repetitive behaviours, as well as accompanied by a large range of comorbidities including epilepsy, language impairment, anxiety, sleep disorders, and attention-deficit hyperactive disorder (ADHD) [1]. Nevertheless, one of the most remarkable clinical features in ASD is represented by ID, which is currently estimated to be associated with approximately 30% of patients [3], although depending on clinical criteria and study design this figure can reach up

to 70% of the total autistic patients in sample collections, as recently discussed by Thurm *et al.* [4]. The subgroup of ASD with comorbid ID is associated with the most severe phenotypic outcomes across the spectrum. ASD individuals with an intelligence quotient (IQ) of 70 or more are generally defined as high-functioning individuals, although ‘high-functioning autism’ is not considered a diagnostic category. High-functioning individuals may have an average intelligence, but often they may also achieve higher scores (>100) in terms of full-scale IQ. Nevertheless, they meet diagnostic criteria for the core symptoms of autism.

The question is, then, how the same genetic factors are involved in such distinct

phenotypes under the same diagnostic category, ranging from ASD patients with comorbid ID to those that are high-functioning individuals.

Sequencing Studies and ASD

Results from next-generation sequencing studies are converging on a preliminary genetic mechanism that can explain the phenotypic differences observed across the autism spectrum. The aim of this commentary is to summarise the recent evidence and propose a potential genetic model.

The first whole-exome sequencing (WES) studies focused on identifying the role of *de novo* variants in ASD [5] and suggested that this class of variants explained approximately 5% of the genetic liability [5,6]. Several of these WES studies showed a correlation between the higher burden of *de novo* ‘gene-truncating variants’ (nonsense, in-dels leading to frameshift, canonical splice site variants) and lower IQ in patients with ASD [6–8]. In previous studies a similar association has also been described for CNVs, which have been found in excess in ASD patients with ID (IQ<70) [9].

However, despite the fact that *de novo* highly damaging mutations are considerably higher in ASD patients with comorbid ID [8], they cannot fully explain this tail of the spectrum, considering the low rate of less than 0.2 *de novo* truncating variants per ASD proband [8] and the fact that it is unlikely that they would be fully penetrant. *De novo* variants, especially those that truncate genes are considered exceptional candidates for explaining severe phenotypes, but they are responsible for only a small fraction of ASD patients with ID.

Subsequent WES studies were performed to explore the impact of inherited rare variants, suggesting that this class of

Key Figure

Impact of Different Classes of Genetic Variants on Phenotypic Severity in Autism Spectrum Disorder (ASD).

Figure 2. Phenotypic severity is shaded in different tones of blue, from ASD patients with intellectual disability (30% of individuals) in dark blue to high-functioning individuals in light blue, including approximately 25% of ASD patients with borderline-range intelligence quotient (IQ) scores ($70 < \text{IQ} < 85$) and approximately 45% of ASD patients with average or above-average scores ($\text{IQ} > 85$). In the upper part of the figure, dark tones of green correspond to higher numbers of disrupting gene variants [*de novo* or inherited truncating variants and copy number variants (CNVs)] potentially highly penetrant (lightning bolt). These variants have a higher impact on patients with intellectual disability. Yellow tones represent the cumulative impact of hundreds of risk alleles from common variations (both SNPs and copy number polymorphisms). These variants with lower penetrance effects (four-point stars) are observed to have a higher impact in the high-functioning range of autism.

variants may explain a considerable portion of the unaccounted heritability in ASD. These early studies suggested that genome-wide inherited truncating alleles play a significant role in disease pathogenesis [10], although this impact is restricted to severe ASD patients with ID [10–12]. In the first reports, transmitted truncating alleles of ASD patients were compared with those that were not transmitted from parents [10,11], whereas in a successive larger sample truncating alleles in ASD patients were compared

with their unaffected siblings [12]. Both studies concluded that gene-disruptive alleles may play a major role when ASD is associated with ID. However, gene-truncating alleles may impact cognitive functions more broadly in humans. Recent studies have shown that they may impact symptom severity across various psychiatric phenotypes, including schizophrenia (SCZ) with ID [13], and in bipolar disorder (BD) with earlier age of onset [14]. A recent large study also reported a significant association between

higher burden of gene-truncating alleles and measures of symptom severity across several psychiatric disorders (ASD, ADHD, SCZ, and BD) [15].

While it is still unclear how the pool of gene-truncating alleles may modulate the phenotype in ASD or other psychiatric diseases, one possibility is that they may directly occur in genes associated with ASD. Thus, higher numbers of disrupted ASD genes may result in increased symptom severity. Alternatively, they may

act additively on a pre-existing genetic makeup of ASD risk alleles (common variants, structural variants, and rare variants of moderate effect), impacting general cognitive functions and shifting symptoms toward more severe manifestations. However, the extremes of a likely model may include, on the one side, ASD patients with ID characterised by a higher burden of severe mutations and lower polygenic risk of combined risk alleles of SNPs, and on the other side high-functioning ASD patients who accumulate more common risk variants of small effect sizes plus a discrete number of gene-truncating variants and other variants (Figure 2, Key Figure). This potential model is supported by the results of the latest and largest genome-wide association study (GWAS) performed thus far, in 18 381 ASD patients, which reported that the polygenic contribution of common variants may be more prominent in high-functioning ASD patients than in ASD patients with comorbid ID [16].

Concluding Remarks

The GWAS and WES studies completed to date have probably described two sides of the same coin: a genetic mechanism where severe mutations and common alleles of small effect act inversely at the two tails of the phenotypic symptom severity spectrum of ASD. Whole-genome sequencing (WGS) represents the new technological frontier that allows researchers to uncover most classes of genetic variants in coding and noncoding

regions (common variants, structural variants, and rare variants) and can be used to further elucidate the model provided here. Large WGS studies are currently being performed in ASD exploring the impact of *de novo* and inherited rare variants in coding and non-coding genomic regions, but generally they are not focused to comprehensively address also the role of common variants. Future WGS studies may explain the diverse phenotypic outcomes considering all classes of genomic variants simultaneously and suggest future strategies for clinical testing based on phenotypic profiles. Therefore, the next step in ASD genetic research is to clarify the relationship between ASD phenotypic severity and the relative impact of different classes of genetic variation in this highly heterogeneous condition.

Acknowledgments

Support was received from the (Australian National Medical and Health Research Council (NHMRC) Project Grants 1063960 and 1066177 and Program Grant 1037196. C.T. is a recipient of a 'Ramón y Cajal' fellowship (RyC2018-024106-I) from the Spanish MINECO. C.T. acknowledges Dr Janice Fullerton and Bronwyn Overs from Neuroscience Research Australia for providing their suggestions and constructive comments.

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<https://doi.org/10.1016/j.tig.2020.01.005>

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