

EDITORIAL ARTICLE

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A new era in the discovery of *de novo* mutations underlying human genetic disease

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Editorial text

Germline mutations arise anew during meiosis in every generation. Such spontaneously occurring genetic alterations are termed *de novo* mutations and serve to describe those heritable mutations that neither parent possessed or transmitted. Thus, *de novo* mutations denote mutations that arose in the gametes of the parents as distinct from post-zygotic somatic mutations that arise during embryonic development. Studies of *de novo* mutations in the human genome have been very challenging owing to past technological limitations. However, the advent of high-throughput next-generation sequencing (NGS) technologies has ushered in a new era in the study of *de novo* mutations. Whole-genome sequencing (WGS) and whole-exome sequencing (WES) can now be performed on parent-offspring trios to identify *de novo* point mutations in the entire genome or within protein-coding regions, respectively [1-3]. Although genome-wide *de novo* copy number variants (CNVs) were previously studied using microarrays [4-7], the study of *de novo* point mutations or single nucleotide variants (SNVs) required the advent of large-scale sequencing, which was not feasible using Sanger sequencing [1-3]. As with inherited mutations, *de novo* mutations range in size from point mutations and small indels of multiple bases to much larger CNVs and structural rearrangements [8]. In this article, we focus on *de novo* point mutations/SNVs and their implications for our understanding of the etiology of common complex diseases as well as rare Mendelian diseases.

The availability of NGS has allowed first estimations of the human genome mutation rate as well as a glimpse of

the spatial distribution of *de novo* point mutations in the human genome and their association with heritable diseases [1-3,9-13]. Recent studies have estimated the per-generation mutation rate in humans as approximately 10^{-8} . For example, sequencing the complete genomes from two parent-offspring trios (from the HapMap CEU and YRI, to >22-fold mapped depth) identified a total of 49 and 35 germline *de novo* point mutations in the two trios, respectively [2]. Similarly, an average *de novo* mutation rate of 1.20×10^{-8} per nucleotide per generation was also reported by sequencing the entire genomes of 78 Icelandic parent-offspring trios [3]. Interestingly, the diversity in the mutation rate of SNVs was found to be strongly influenced by the age of the father when the child was conceived (the impact of paternal age corresponds to about two mutations per year). These findings have highlighted the likely importance of the father's age for the risk of those diseases which appear to be strongly affected by *de novo* mutations, e.g., schizophrenia and autism (see below) [3]. However, the mutation rate is critically dependent upon the type of mutation being considered, as the CNV mutation rate is somewhat higher than the single base-pair substitution mutation rate [14].

Owing to technical limitations, the contribution of *de novo* point mutations to human genetic diseases (both common complex disease and rare Mendelian disorder) has remained largely unexplored. *De novo* point mutations are nevertheless likely to have severe biological or phenotypic consequences when they impact functionally important nucleotides in the genome (for example, nonsense mutations or missense mutations in evolutionarily conserved regions). However, only a relatively small proportion of the *de novo* point mutations occurring at each meiosis will alter functionally important nucleotides [1-3]. Although *de novo* CNVs have been shown, using whole-genome genotyping arrays, to be associated with

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several common neurodevelopmental diseases such as schizophrenia and autism [4-7], it was not until very recently that *de novo* point mutations were also implicated in the etiology of schizophrenia, autism, and mental retardation through the WES of affected trios [9-12,15-17]. In similar vein, *de novo* point mutations have also been found to be responsible for sporadic cases of various rare dominant Mendelian disorders such as Kabuki syndrome, Schinzel-Giedion syndrome, and Bohring-Opitz syndrome [18-20]. These Mendelian disorders are also characterized by multiple neurodevelopmental defects; thus, for example, Bohring-Opitz syndrome is a clinically recognizable syndrome characterized by severe intellectual disability, distinctive facial features, and multiple congenital malformations [20].

It has been postulated that the occurrence of *de novo* mutations might explain why diseases characterized by dramatically reduced fecundity such as mental retardation and schizophrenia nevertheless remain frequent in the population. If this were indeed to be the case, the *de novo* mutations occurring in sporadic cases of these diseases would serve to replenish the number of highly penetrant disease mutations in every generation despite the continual negative selection. Indeed, *de novo* CNVs are a known cause of schizophrenia, autism, and mental retardation [21,22]. In addition, *de novo* point mutations have also been identified in schizophrenia through the WES of affected case-parent trios. In the exomes of 53 sporadic schizophrenia cases (with no history of the disease in a first- or second-degree relative), 22 unaffected controls and their parents were sequenced which led to the identification of a total of 34 *de novo* point mutations/SNVs and 4 *de novo* indels in the affected trios. However, it is noteworthy that some *de novo* SNVs (four non-synonymous and three synonymous) were also identified in the control subjects. This suggests that identifying the pathogenic and/or deleterious *de novo* mutations from a non-disease-associated mutational background may be a tricky task [11]. In a parallel development, *de novo* mutations were also found to be enriched in autism spectrum disorder (ASD)/autism [15-17]. A large WES study of 238 families from a comprehensively phenotyped ASD cohort comprising pedigrees with two unaffected parents and an affected proband (and in 200 families, an unaffected sibling) found that highly disruptive (nonsense and splice-site) *de novo* point mutations in brain-expressed genes were associated with ASDs and that these mutations carried large effects. *De novo* point mutations have also been identified in individuals with unexplained mental retardation [9]. These findings provide further support for the likely importance of *de novo* mutations in the etiology of these complex neurodevelopmental disorders.

De novo heterozygous point mutations are also believed to be a common cause of sporadic cases of

those rare Mendelian diseases that are characterized by multiple congenital malformations or anomalies, developmental delay, and mental retardation or intellectual disability such as Schinzel-Giedion syndrome and Bohring-Opitz syndrome, the genetic basis of which had previously remained elusive [19,20]. These disease-causing *de novo* mutations were undetected by linkage analysis because they were not transmitted between the generations. Although the conventional gene-centric approach of limited Sanger sequencing is capable of identifying *de novo* point mutations, this method cannot realistically be scaled up to encompass the entire genome, exome, or even all genes on a chromosomal arm. Further, it is a hypothesis-driven approach whereby the selection of candidate genes is highly dependent upon an *a priori* hypothesis which is in turn constrained by our still limited knowledge of the biological pathways that characterize the diseases in question. By contrast, WES constitutes a powerful and robust tool which can be used to unravel *de novo* point mutations by screening the entire coding region of the genome (i.e., all the exons) of the affected parent-offspring trios. The first success in identifying novel *de novo* mutations for rare diseases came with the identification of the *MLL2* gene underlying Kabuki syndrome, even although this study did not adopt a trio design [18]. Instead, the exomes of unrelated probands from different families were sequenced in order to identify the causal genes, and in families where parental DNA was available, the mutations were confirmed to be *de novo* in some of them [18]. However, similar success was also obtained in studies that applied WES to affected trios for various rare diseases. Thus, *de novo* mutations in the *EZH2* gene underlying Weaver syndrome [23] and in the *ACTB* and *ACTG1* genes underlying Baraitser-Winter syndrome were identified by means of WES of trios [24].

The advantage of applying WES to parent-case trios is that it narrows down the list of potential candidate disease mutations very considerably because of the limited number of *de novo point* mutations to be expected within protein-coding sequences. This is of course especially useful for disorders in which *de novo* mutations are strongly suspected, e.g., Baraitser-Winter syndrome (a rare but now well-defined developmental disorder). No familial recurrence or consanguinity has ever been observed in families affected with this syndrome; hence, the genetic basis of Baraitser-Winter syndrome was always likely to be due to the occurrence of *de novo* point mutations (as no pathogenic CNVs had been detected using microarrays) [24]. WES would be sufficient to identify the mutations underlying most rare Mendelian disorders as long as the causative mutations reside within the coding regions. In contrast to WGS, WES is more cost-effective and analytically less challenging.

However, some studies have also applied the WGS approach, for example, to a family quartet affected by a sporadic case of severe epileptic encephalopathy. WGS of the family, containing an affected proband and her unaffected parents and sibling, revealed a *de novo* heterozygous missense mutation in *SCN8A* [25].

Although numerous *de novo* point mutations have now been identified which are responsible for rare disorders, it remains unclear what constitutes evidence for causality. It is both difficult and challenging to establish the causative role of newly identified mutations, even for *de novo* events. Co-segregation of the putative causal mutation with a disease phenotype in large pedigrees might provide strong genetic evidence of causality, but this would not be applicable in the case of *de novo* mutations which are, by definition, not transmitted through the generations. Therefore, for a newly identified putative pathological mutation which appears to have occurred *de novo*, further screening of additional cases is invariably required. Detection of recurrent mutation (or identical mutations) or different mutations in the same gene in additional cases and their absence in healthy individuals constitutes strong evidence of causality. However, it might be quite difficult, in the context of extremely rare disorders, to find additional cases with which to validate the newly identified *de novo* mutation. Although challenging, further validation in additional samples has generally been successful in recent WES studies of rare disorders. Most notably, after the discovery of *de novo* point mutations in *ACTG1* and *ACTB* by WES of three parent-case trios with Baraitser-Winter syndrome, the study used Sanger sequencing to screen the coding sequence of both genes in 15 additional affected individuals and was successful in detecting pathogenic mutations in one or other of these genes in all subjects studied [24]. These mutations were then shown to have occurred *de novo* in all 11 subjects from whom parental DNA was available. Moreover, further validation in normal controls showed that none of the mutations identified in Baraitser-Winter syndrome were present in several large control datasets [24]. As an alternative, molecular functional studies to confirm the functional significance of newly identified *de novo* mutations could be considered confirmatory if and when the mutated gene had a clear role in conferring a well-defined molecular pathology.

The studies of *de novo* mutations that have been performed so far have adopted the WES approach. However, the current shortcoming of WES lies in its necessary focus on coding regions. Hence, the clinicobiological importance of *de novo* mutations in non-coding regions remains unclear. Although very challenging, WGS eventually promises to permit the comprehensive analysis of *de novo* mutations (and other inherited variants) in

the human genome in collections of case-parent trios affected by various heritable diseases. Since a considerable number of inherited mutations for various diseases have now been found in non-coding regions [26], it is reasonable to suppose that this could also pertain in the context of *de novo* mutations within non-coding regions. This in turn supports the likely efficacy of a strategy of adopting WGS to ascertain the full impact of *de novo* mutations in human genetic disease and to study the molecular characteristics of such lesions. The collection of a large number of such trios would be important in this context. The discovery to date of *de novo* mutations underlying common complex diseases as well as rare Mendelian disorders is likely to represent only the tip of the iceberg; more discoveries would be anticipated in the near future.

Competing interests

The authors declared that they have no competing interests.

Authors' contributions

CSK and WV contributed to the conceptualization of this article. CSK and DNC contributed to the writing of this article. WV contributed to proofreading. CSK and DNC approved the final manuscript. All authors read and approved the final manuscript.

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