KD Gonzalez[1], L Shahmirzadi[1] , E Palmaer[1], S Tang[1], X Li[1], EC Chao[1], J Neidich[1], JJ Wei[1], W Zeng[1]
1) Ambry Genetics, Aliso Viejo, CA

Family-based exome sequencing reveals that *de novo* alterations make up a significant portion of previously undiagnosed patients

BACKGROUND

- The last two years has demonstrated the diagnostic utility of exome sequencing in establishing a molecular diagnosis, enhancing genetic counseling, and aiding in clinical management.
- It has been widely suggested that although *de novo* alterations may be individually rare in a given disease, collectively they are likely to account for a significant portion of patients with complex genetic disease.
- Exome sequencing has been particularly effective in the identification of *de novo* alterations, of which traditional molecular methods have been particularly feeble.
- It is established that exome sequencing has been powerful in the identification of de novo mutations among patients with autism and schizophrenia (Girard, 2011; Iossifov, 2012; Neale, 2012; O'Roak, 2012; O'Roak, 2012; Sanders, 2012; and Xu, 2011).

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from patient samples provided to the laboratory for diagnostic exome sequencing (DES). Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) and sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).
- Data is annotated with the Ambry Variant Analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, predicted functional impact, and clinical disease associations (Human Gene Mutation Database (HGMD; Stenson, 2009)), OMIM, and several other databases). A molecular geneticist performed interpretive filtering based on the deleterious nature of the candidate alterations, literature search, and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype.
- Each candidate variant was analyzed by Sanger sequencing for mutation confirmation and co-segregation analysis.
- Positive findings were curated into a database along with patient referral indications and clinical history. Each positive gene finding was categorized by inheritance pattern and inherited vs *de novo* mutation occurrence.

Figure 1. Overall Detection Rates Among Patients Referred for DES

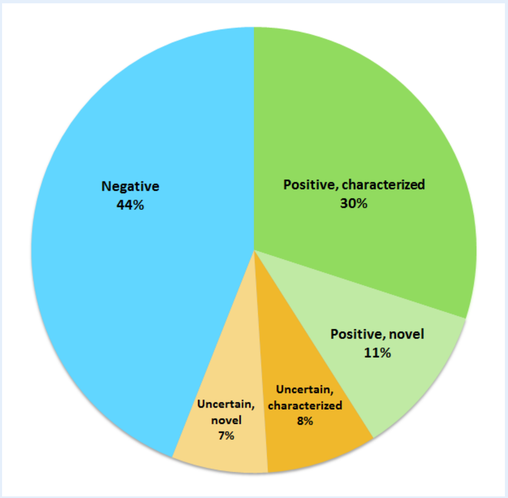
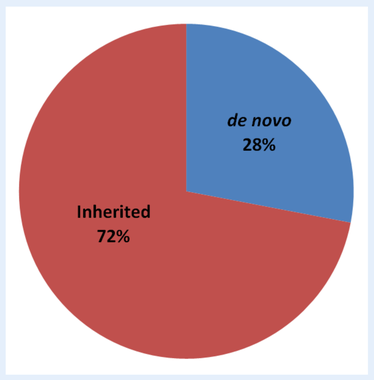


Figure 2. Family-based exome sequencing reveals that *de novo* alterations make up a significant portion of previously undiagnosed patients.



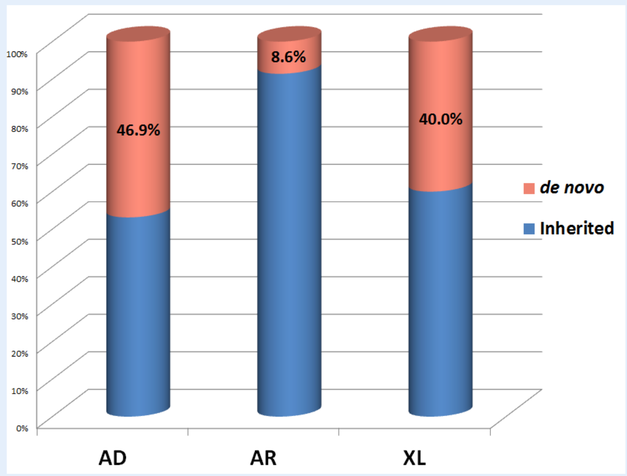


Table 1. Truncating alterations account for half of identified *de novo* alterations

de novo mutation types		
Missense	**52%**	
Truncating	**48%**	
	Frameshift	22%
	Nonsense	19%
	Splice	7%

Figure 3. The clinical spectrum of these patients with *de novo* alterations

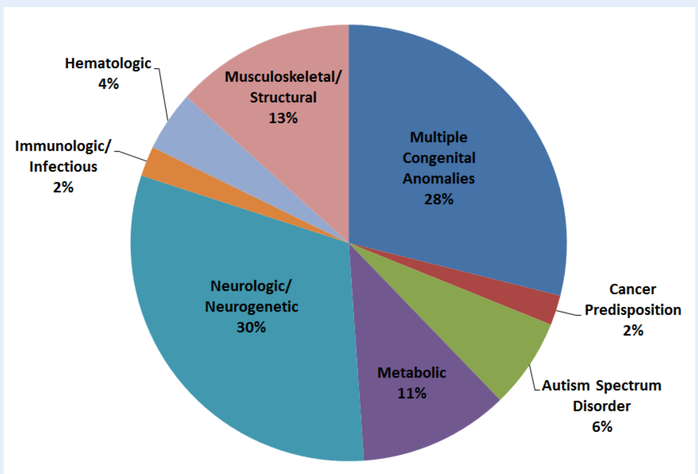


Table 2. Most patients with *de novo* alterations did not have a diagnosis prior to diagnostic exome sequencing

Prior diagnoses among patients with *de novo* alterations	
Patients with a prior diagnosis	4%
Patients with a prior differential diagnosis	15%
Total with prior diagnosis or differential	19%

RESULTS/ DISCUSSION

- A pathogenic alteration(s) within a characterized gene (genes reported in HGMD, OMIM-Morbid, or the medical literature in association with a disease phenotype) in about **one third** of the patients (**Figure 1**).
- Among these patients with positive findings in characterized gene, one-third of the alterations were *de novo* (**Figure 2)**.
- *de novo* alterations were identified within every major inheritance pattern, including recessive inheritance in a minority of cases (~9% of patients with autosomal recessive gene findings) (**Figure 2)**.
- Not surprisingly, truncating alterations make up a significant portion of identified *de novo* alterations (48%); consisting of 22% frameshift, 19% nonsense, and 7% splice alterations (**Table 1**).
- The clinical spectrum of these patients with *de novo* alterations was wide with the majority falling within the categories (neurologic/ neurogenetic (30%), multiple congenital anomalies (28%), and musculoskeletal (13%). A minority of patients also presented with metabolic (11%), autism spectrum (6%), hematologic (4%), immunologic (2%), and cancer predisposition (2%) (**Figure 3**).
- Virtually all patients had not previously received a clinical diagnosis consistent with the gene finding or had been provided with a consistent differential diagnosis (**Table 2**).

CONCLUSIONS

- A family-based approach to clinical diagnostic exome sequencing (CDE) has enabled us to overcome the limitations in detection of *de novo* mutations posed by traditional molecular strategies for mapping traits such as linkage analysis and positional cloning.
- The findings offer a unique look at a mixed cohort of patients with de novo alterations whose diagnoses, without diagnostic exome sequencing (DES), would have been exceedingly difficult to make.
- Overall, these results highlight the value of family-based exome sequencing and suggest an important role for new mutations among a wide spectrum of disease categories. Further, these results reveal that *de novo* alterations make up a significant portion of previously undiagnosed patients.

References

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