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Citation: Pérez-Palma, Eduardo, Elmo Saarentaus, Marie Ravoet, Giancarlo V. De Ferrari, Peter Nürnberg, Bertrand Isidor, Bernd A. Neubauer, and Dennis Lal. 2018. “Duplications at 19q13.33 in patients with neurodevelopmental disorders.” Neurology: Genetics 4 (1): e210. doi:10.1212/NXG.0000000000000210. http://dx.doi.org/10.1212/NXG.0000000000000210.

Published Version: doi:10.1212/NXG.0000000000000210

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Duplications at 19q13.33 in patients with neurodevelopmental disorders

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Neurol Genet 2018;4:e210. doi:10.1212/NXG.0000000000000210

Abstract

Objective

After the recent publication of the first patients with disease-associated missense variants in the GRIN2D gene, we evaluate the effect of copy number variants (CNVs) overlapping this gene toward the presentation of neurodevelopmental disorders (NDDs).

Methods

We explored ClinVar (number of CNVs = 50,794) and DECIPHER (number of CNVs = 28,085) clinical databases of genomic variations for patients with copy number changes overlapping the GRIN2D gene at the 19q13.33 locus and evaluated their respective phenotype alongside their frequency, gene content, and expression, with publicly available reference databases.

Results

We identified 11 patients with microduplications at the 19q13.33 locus. The majority of CNVs arose de novo, and comparable CNVs are not present in control databases. All patients were reported to have NDDs and dysmorphic features as the most common clinical phenotype (N = 8/11), followed by seizures (N = 6/11) and intellectual disability (N = 5/11). All duplications shared a consensus region of 405 kb overlapping 13 genes. After screening for duplication tolerance in control populations, positive gene brain expression, and gene dosage sensitivity analysis, we highlight 4 genes for future evaluation: CARD8, C19orf68, KDELR1, and GRIN2D, which are promising candidates for disease causality. Furthermore, investigation of the literature especially supports GRIN2D as the best candidate gene.

Conclusions

Our study presents dup19q13.33 as a novel duplication syndrome locus associated with NDDs. CARD8, C19orf68, KDELR1, and GRIN2D are promising candidates for functional follow-up.
NMDA receptors are involved in neurodevelopmental processes such as synaptogenesis, learning, and memory. Structurally, NMDA receptors are composed of 2 subunits of GluN1 and GluN2, which are specifically encoded by the GRIN1 and GRIN2A to GRIN2D genes, respectively. While single nucleotide and copy number variants (CNVs) in the NMDA receptor subunits GRIN1, GRIN2A, and GRIN2B have been associated with a range of neurodevelopmental disorders (NDDs), little is known about the association of GRIN2D variants and NDDs. Recently, de novo missense mutations in GRIN2D (p.Val667Ile) have been identified as the cause of severe epileptic encephalopathy in 2 independent patients. However, whether CNVs covering the GRIN2D locus are also associated with disease has not been studied. GRIN2D is encoded at the end of the long arm of chromosome 19 at the 19q13.33 locus. We hypothesize that dosage changes in GRIN2D are highly likely to be disease associated based on the high sequence homology, expression during neurodevelopment, and a functional relationship with the established disease-associated paralogous genes.

Methods

Standard protocol approvals, registrations, and patient consents

We obtained approval from an ethical standards committee on human experimentation (institutional or regional) for any experiments using human subjects. Written informed consent was obtained from all patients (or guardians of patients) participating in the study (consent for research), following the guidelines provided by ClinVar and DECIPHER databases. We obtained authorization for disclosure (consent to disclose) of the photograph that may be published in the journal, in derivative works by the AAN, or on the journal’s website.

Data analysis

Using the gene-oriented query “GRIN2D,” we accessed 2 publicly available repositories of clinical genetic variation: (1) The Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources, DECIPHER (URL: https://decipher.sanger.ac.uk, accessed on July 2016) and (2) The public archive of interpretations of clinically relevant variants, ClinVar (URL: http://www.ncbi.nlm.nih.gov/clinvar, accessed on July 2016). For DECIPHER patients, the individual scientists were contacted to acquire further phenotype information including the presence of intellectual disability (ID), developmental delay (DD), seizures, hypotonia, dysmorphism (Dysm), learning difficulties, behavioral problems as well as social communication, and behavioral disorders of the autism spectrum disorder. We considered only DECIPHER entries with positive subverter contact. All phenotypes evaluated were considered as binary denominators (i.e., Yes/No). Gene annotations of the extracted CNVs refer to the genome build GRCh37/hg19. A consensus region was determined with an in-house Python script (available on request). Genes inside the consensus region were further evaluated as disease candidate genes with additional publicly available resources for (1) brain expression, strongly brain-expressed genes (n = 4,756), specified by a log (RPKM) >4.5 of the BrainSpan RNA-Seq transcriptome data set; (2) overlapping CNVs reported in the curated control inclusive map of the Database of Genomic Variants; (3) loss-of-function (LoF) intolerance reported in the Exome Aggregation Consortium, given by a probability of being LoF intolerant (pLI score) equal to or greater than 0.9 based on the observed genetic variation of 60,706 healthy individuals; and (4) overlapping CNVs reported in 20,227 controls. Genome-wide brain-specific noncoding functional elements were extracted from the GenoSkyline; project (http://genocanyon.med.yale.edu/GenoSkyline), which implements a statistical framework based on high-throughput genetic and epigenetic data to predict tissue-specific functional noncoding elements.

Results

We detected 11 patients with CNVs overlapping the 19q13.33 locus (table 1). Of interest, all of them were duplications. Three were annotated in ClinVar (patients 1 through 3) and 8 in DECIPHER (patients 4 through 11). Although a ninth individual did fulfill the inclusion criteria (DECIPHER entry 275388), given the actual size of the reported variant in comparison with the entire chromosome 19 (CNV = 58.83 Mb vs Chr19 = 59.12 Mb), it was considered a chromosome trisomy and therefore was excluded. Detailed clinical phenotypes are provided in table 1. Notably, all patients were reported to have mild to severe forms of NDDs. Of all the phenotypes evaluated, mild but distinct dysmorphic features were the most frequent (n = 8), followed by seizures (n = 6, including generalized tonic and febrile seizures), ID (n = 5), and DD (n = 4). In particular, dysmorphisms were present in patients carrying CNVs larger than 3 Mb (pathogenic size according to the American College of Human Genetics). The image of 1 of such patients is shown in figure, A, showing a child with signs of macrostomia, midface hypoplasia, and progenia.

For DECIPHER entries with available parental information, 85.7% (n = 6) of observed microduplications were de novo, and only 1 was inherited from an affected family member. The majority of (87.5%, n = 7) patients did not carry additional CNVs, and none of the additional CNVs found in 3 patients covered a known disease locus or known disease

Glossary

DD = developmental delay; ID = intellectual disability; LoF = loss of function; NDD = neurodevelopmental disorder.
Table 1 Clinical phenotypes of the 11 retrieved patients with GRIN2D variants at the 19q13.33 locus

| Patient | Resource | Database entry ID | Size (Mb) | Sex | Type of variant | De novo | No CNVs | ID | DD | Seizures | Hypotonia | ASD | Dysm | Learning difficulties | Behavioral problems |
|---------|----------|-------------------|-----------|-----|-----------------|---------|---------|----|----|----------|-----------|-----|------|----------------------|----------------------|
| 1       | ClinVar  | 59117             | 10.65     | —   | Gain            | —       | —       | —  | Yes| Yes      | —         | —   | —    | —                    | —                    |
| 2       | ClinVar  | 59116             | 10.64     | —   | Gain            | —       | —       | —  | Yes*| —        | —         | —   | ?    | —                    | —                    |
| 3       | ClinVar  | 59115             | 2.39      | —   | Gain            | —       | —       | —  | Yes| —        | —         | —   | —    | —                    | —                    |
| 4       | DECIPHER | 257554            | 0.91      | M   | Gain            | No**    | 1       | Yes| —  | Yes      | —         | —   | —    | —                    | —                    |
| 5       | DECIPHER | 262506            | 2.51      | F   | Gain            | Yes     | 1       | Yes| Yes| Yes      | —         | Yes | Yes  | —                    | —                    |
| 6       | DECIPHER | 269407            | 3.57      | M   | Gain            | Yes     | 1       | Yes| —  | —        | —         | —   | —    | Yes                   | —                    |
| 7       | DECIPHER | 274058            | 10.64     | F   | Gain            | Yes     | 1       | —  | Yes| Yes      | —         | —   | —    | —                    | Yes                  |
| 8       | DECIPHER | 275426            | 7.78      | F   | Gain            | Yes     | 1       | —  | Yes| Yes      | —         | Yes | Yes  | —                    | —                    |
| 9       | DECIPHER | 282304            | 2         | F   | Gain            | —       | 2       | Yes| —  | Yes      | —         | —   | —    | Yes                   | —                    |
| 10      | DECIPHER | 282364            | 2.72      | F   | Gain            | Yes     | 1       | —  | Yes| Yes      | —         | Yes | Yes  | —                    | —                    |
| 11      | DECIPHER | 328426            | 1.16      | F   | Gain            | Yes     | 1       | Yes| —  | —        | —         | —   | —    | Yes                   | —                    |

| Literature | Patient | Size (Mb) | Sex | Type of variant | De novo | No CNVs | ID | DD | Seizures | Hypotonia | ASD | Dysm | Learning difficulties | Behavioral problems |
|------------|---------|-----------|-----|-----------------|---------|---------|----|----|----------|-----------|-----|------|----------------------|----------------------|
| 12         | Dorn et al.13 | [1]       | 15.7 | M              | Gain     | —       | 1  | Yes| Yes      | —         | —   | —    | Yes                  | —                    |
| 13         | Dorn et al.13 | [2]       | 15.7 | F              | Gain     | —       | 1  | Yes| Yes      | —         | —   | —    | Yes                  | —                    |
| 14         | Carvalheira et al.11 | [1] | 10.6 | F              | Gain     | Yes     | 1  | Yes| Yes      | —         | —   | —    | Yes                  | —                    |
| 15         | Wang et al.12 | [1]       | 1.22 | —              | Gain     | —       | 1  | —  | Yes      | —         | —   | —    | —                    | —                    |
| 16         | Wang et al.12 | [5]       | 1.22 | —              | Gain     | —       | 1  | —  | Yes      | —         | —   | —    | —                    | —                    |
| 17         | Wang et al.12 | [7]       | 1.22 | —              | Gain     | —       | 1  | —  | Yes      | —         | —   | —    | —                    | —                    |

| GRIN2D pathogenic SNV | Patient | Mutation    | Sex | Effect | De novo | No CNVs | ID | DD | Seizures | Hypotonia | ASD | Dysm | Learning difficulties | Behavioral problems |
|----------------------|---------|-------------|-----|--------|---------|---------|----|----|----------|-----------|-----|------|----------------------|----------------------|
| 18                   | Li et al.2 | p. Val667Ile | —   | Gain of function | Yes     | —       | —  | Yes| Yes      | —         | —   | —    | Yes                  | —                    |
| 19                   | Li et al.2 | p. Val667Ile | —   | Gain of function | Yes     | —       | —  | Yes| Yes      | —         | —   | —    | Yes                  | —                    |

Abbreviations: ? = not clear; * = developmental delay and/or other significant developmental or morphological phenotypes; ** = from affected parent; ASD = autism spectrum disorder; DD = developmental delay; Dysm = dysmorphism; ID = intellectual disability; No CNVs = number of copy number variants annotated in patient; P = patient; SNV = single nucleotide variant.
All 11 CNVs were highly heterogeneous in their size (average = 4.99 Mb; SD = 4.05 Mb) and breakpoint distribution (encompassing from Chr19: 45.38 Mb–59.09 Mb, Hg19) (table 1).

To identify additional CNVs absent in ClinVar and/or DECIPHER databases, we screened the literature and retrieved 3 additional studies, including 6 patients with duplications at the 19q13.33 locus. All of these patients had seizures. Three patients carried CNVs of 1.22 Mb size, whereas the remaining 3 duplications were >10 Mb. Patients affected by the large CNVs were, in addition to seizures, also affected by other NDDs including ID and dysmorphism.
Of interest, the 2 independent patients with the p.Val667Ile mutation on GRIN2D featured similar NDDs including DD, dysmorphism, seizures, and muscular hypotonia (table 1).

Overall, the consensus duplicated region was determined to be located within the coordinates 48,520,809 bp–48,926,006 bp, with a final size of 405 kb. This is consistent with previous reports. The consensus region overlapped 13 RefSeq genes (figure, B) that were further examined for brain expression, the presence of CNVs overlapping these genes in control cohorts, and variation intolerance (table 2). Four genes persisted above all available filters, namely, the caspase recruitment domain family member 8 (CARD8), the chromosome 19 open reading frame 68 (C19orf68), the KDEL endoplasmic reticulum protein retention receptor 1 (KDELR1), and the glutamate ionotropic receptor NMDA type subunit 2D (GRIN2D). In our view, these 4 genes represent the most promising candidates.

We also searched for noncoding brain-specific functional elements within the consensus region. A total of 291 were found overlapping 9.87% of the consensus region (40,019 bp). Within the consensus regions of the duplications, the density of noncoding elements was not significantly higher than that outside of chromosome 19.

### Table 2 Consensus region gene annotation and candidate gene filtering

| Transcript ID | chrom | cdsStart | cdsEnd | Gene | Size (bp) | Brain expressed? | DGV [clean] CNVs not present in controls | ExAC LoF Intolerance | PCG Browser CNV not present in controls | Total |
|---------------|-------|----------|--------|------|-----------|------------------|----------------------------------------|----------------------|--------------------------------------|-------|
| NM_022142     | chr19 | 48,511,924 | 48,525,584 | ELSPBP1 | 13,660 | No               | Yes                                    | No                   | Yes                                 | 2/4   |
| NM_019855     | chr19 | 48,533,813 | 48,547,179 | C4BP5 | 13,366 | No               | Yes                                    | No                   | Yes                                 | 2/4   |
| NM_001159322  | chr19 | 48,551,599 | 48,613,772 | PLA2G4C | 62,173 | Yes              | Yes                                    | Yes                  | Yes                                 | 3/4   |
| NM_001320971  | chr19 | 48,618,905 | 48,668,823 | LIG1  | 49,918 | Yes              | Yes                                    | Yes                  | No                                  | 3/4   |
| NM_199341     | chr19 | 48,675,059 | 48,700,084 | C19orf68 | 25,025 | Yes              | Yes                                    | NA                   | Yes                                 | 3/3   |
| NM_014959     | chr19 | 48,714,966 | 48,744,277 | CARD8 | 29,311 | Yes              | Yes                                    | Yes                  | Yes                                 | 4/4   |
| NM_153608     | chr19 | 48,783,056 | 48,790,135 | ZNF114 | 7,079  | No               | Yes                                    | No                   | Yes                                 | 2/4   |
| NM_144577     | chr19 | 48,800,232 | 48,822,028 | CCDC114 | 21,796 | Yes              | Yes                                    | No                   | Yes                                 | 3/4   |
| NM_001313905  | chr19 | 48,830,101 | 48,833,727 | EMP3  | 3,626  | Yes              | Yes                                    | No                   | Yes                                 | 3/4   |
| NM_018273     | chr19 | 48,836,475 | 48,867,177 | TMEM143 | 30,702 | Yes              | No                                     | Yes                  | Yes                                 | 3/4   |
| NM_012451     | chr19 | 48,869,099 | 48,879,575 | SYNGR4 | 10,476 | No               | Yes                                    | Yes                  | Yes                                 | 3/4   |
| NM_006801     | chr19 | 48,886,549 | 48,894,615 | KDELR1 | 8,066  | Yes              | Yes                                    | Yes                  | Yes                                 | 4/4   |
| NM_000836     | chr19 | 48,901,649 | 48,947,194 | GRIN2D | 45,545 | Yes              | Yes                                    | Yes                  | Yes                                 | 4/4   |

aBrain-expressed genes from Uddin et al.6
bDGV curated CNV control map from Zarrei et al.7
ExAC LoF Intolerant genes from Lek et al.8
PCG Control CNVs from Marshall et al.9
Bold genes are positive in all applicable filters and are highlighted as “candidates genes” for future evaluation.

### Discussion

Here, we report on 11 patients with duplications at a potential novel disease locus within 19q13.33. Several lines of evidence support the hypothesis that duplications at this locus are associated with NDDs: (1) duplications at this locus are virtually absent in healthy individuals from the general population; (2) all of the identified duplications with parental information arose de novo with the exception of patient 4, which according to DECIPHER was inherited from an affected parent with a similar phenotype (DECIPHER entry 257554); (3) none of the patients carried additional likely pathogenic CNVs; and (4) all duplications covered multiple plausible disease candidate genes.

The NDDs observed in the 11 patients were characterized by dysmorphism as the most prominent feature, followed by ID and seizures (table 1). Our observations are in agreement with previous reports. Although, 1 example focused exclusively on seizures, we cannot rule out that other NDDs were actually present in those patients. Similarly, we acknowledge that DD, behavioral problems, and learning difficulties may be subject to interobserver variability to some extent. In this regard, future clinical studies of 19q13.33 duplication carriers need to be conducted to draw detailed and robust genotype-phenotype conclusions. Since previous reports from the
literature were based on low-resolution cytogenetic methods, identification of the underlying disease gene was not possible. Here, we show that by integration of multiple CNVs data sets from public repositories, we are able to narrow down the disease-associated genomic sequence to a few candidate genes at the 19q13.33 locus (figure).

Our included data sets do not allow estimation of 19q13.33 duplication frequency. However, the absence of 19q13.33 duplications in CNVs databases of the general population and the presence of only a few variant carrying patients in diagnostic CNVs databases with heterogeneous breakpoints indicate that 19q13.33 duplications are extremely rare (table 2).

All 11 of the identified patient CNVs shared a genomic interval of 405 kb, which includes 4 genes with genetic, population and biological support of disease association. These included CARD8, C19orf68, KDELRI, and GRIN2D. For CARD8, C19orf68, and KDELRI, no association with NDDs has been reported in the literature to date. Although we cannot rule out that brain-specific noncoding elements at 19q13.33 could be involved in the development of NDDs, GRIN2D represents a plausible candidate gene for association with NDDs. GRIN2D, encoding the NMDA receptor subunit GluN2D, is highly expressed prenatally and after birth before progressively declining through adulthood.14 It is possible that GRIN2D microduplications may predispose to disease susceptibility in a dose-dependent manner by enhancing GluN2D expression during development, thereby influencing the NMDA receptor composition, which might provoke changes in neuronal networks, thus contributing to hyperexcitability and neurologic diseases.15 Besides CNVs, the GRIN2D gene is also depleted due to negative selection in the general population, supporting the GRIN2D association with disease.8 In agreement, 2 recently identified GRIN2D single nucleotide variants also lead functionally to a gain-of-function mutation in 2 patients with similar outcomes2 (table 1). Beyond the potential diagnostic relevance, our identification of GRIN2D as a possible new NDD gene has a potential clinical application, since memantine, a low-affinity therapeutic NMDA channel blocker, selectively blocks extrasynaptic NMDA receptors that are likely to contain GluN2C/2D subunits.16 This might especially be relevant for patients with gain-of-function mutations or microduplications.2

Author contributions
Eduardo Pérez-Palma: analysis and interpretation of data and wrote the manuscript. Elmo Saarentaus: analysis and interpretation of clinical data. Giancarlo V. De Ferrari: critical revision of the manuscript for intellectual content. Marie Ravoet, Giancarlo V. De Ferrari, Peter Nürnberg, and Bertrand Isidor: clinical and critical revision of the manuscript for intellectual content. Bernd A. Neubauer: drafting of the manuscript and critical revision of the manuscript for intellectual content. Dennis Lal: study concept and design, analysis and interpretation of data, and wrote the manuscript.

Acknowledgment
The authors thank all the clinicians, patients, and their families. This study makes use of data generated by the DECIPHER community. A full list of centers who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust.

Study funding
Eduardo Pérez-Palma was supported by the Chilean Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) regular grant number 1140353 to Giancarlo V. De Ferrari. Dennis Lal received funds from the German Academic Exchange Service (DAAD), grant number 57073880. Bernd A. Neubauer received funding from the Deutsche Forschungsgemeinschaft (Ne 416/5-1).

Disclosure
E. Pérez-Palma has received research support from Fondo Nacional de Ciencia y Tecnología (FONDECYT), Chile. E. Saarentaus has received research support from the Institute for Molecular Medicine Finland (FIMM) and Svenska Studiefond. M. Ravoet has been involved in clinical procedures/imaging studies at the Center of Human Genetics, Cliniques Universitaires St-Luc (10%). 2016. G.V. De Ferrari has received research support from Fondo Nacional de Ciencia y Tecnología (FONDECYT), Chile, and CONICYT. Chile. P. Nürnberg is a founder, CEO, and shareholder of ATLAS Biolabs GmbH (ATLAS Biolabs GmbH is a service provider for genomic analyses). B. Isidor reports no disclosures. B. Neubauer has served on the scientific advisory boards of and has received travel funding/speaker honoraria from Eisai, Bial, and UCB Pharma; has served on the editorial board of Neuropediatrics; and has received research support from DFG 416/5-1 (German national funding agency). D. Lal reports no disclosures. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

Received August 17, 2017. Accepted in final form September 11, 2017.

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