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## Phenotypic spectrum of NRXN1 mono- and bi-allelic deficiency: a systematic review

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Key Words:	Autism Spectrum Disorder, Compound heterozygosity, Developmental delay, Neurexin1, Pitt-Hopkins-like syndrome 2, Schizophrenia

**Phenotypic spectrum of *NRXN1* mono- and bi-allelic deficiency:  
a systematic review**

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## ABSTRACT

Neurexins are presynaptic cell adhesion molecules critically involved in synaptogenesis and vesicular neurotransmitter release. They are encoded by three genes (*NRXN1-3*), each yielding a longer alpha ( $\alpha$ ) and a shorter beta ( $\beta$ ) transcript. Deletions spanning the promoter and the initial exons of the *NRXN1* gene, located in chromosome 2p16.3, are associated with a variety of neurodevelopmental, psychiatric, neurological and neuropsychological phenotypes. We have performed a systematic review to define (a) the clinical phenotypes most associated with mono-allelic exonic *NRXN1* deletions, and (b) the phenotypic features of *NRXN1* nullisomy due to compound heterozygous deletions/mutations. Clinically, three major conclusions can be drawn: (a) incomplete penetrance and pleiotropy do not allow reliable predictions of clinical outcome following prenatal detection of mono-allelic exonic *NRXN1* deletions. Newborn carriers should undergo periodic neuro-behavioral observations for the timely detection of warning signs and the prescription of early intervention; (b) the presence of additional independent genetic risk factors should always be sought, as they may influence prognosis; (c) children with exonic *NRXN1* deletions displaying early-onset, severe psychomotor delay in the context of a Pitt-Hopkins-like syndrome 2 phenotype, should undergo DNA sequencing of the spared *NRXN1* allele in search for mutations or very small insertions/deletions.

## KEYWORDS

Autism Spectrum Disorder, compound heterozygosity, developmental delay, neurexin 1, Pitt-Hopkins-like syndrome 2, schizophrenia.

## 1. INTRODUCTION

### 1.1 Neurexin family proteins

Neurexins are presynaptic cell adhesion molecules critically involved in (a) synapse formation and maintenance, by establishing trans-synaptic complexes with postsynaptic ligands including neuroligins, neurexophilin, dystroglycan, LRRTM and cerebellin, among others; and in (b) vesicular neurotransmitter release, by coupling N- and P/Q-type calcium channels with the exocytotic machinery.<sup>1</sup> Three genes (*NRXN1-3*) are transcribed in neurons from two independent promoters, each yielding a longer alpha ( $\alpha$ ) and a shorter beta ( $\beta$ ) isoform, composed of distinct extracellular domains linked to an identical intracellular sequence. Importantly,  $\alpha$ -neurexins show a high degree of evolutionary conservation throughout vertebrates,<sup>2,3</sup> while  $\beta$ -neurexins are not conserved.<sup>2</sup> In line with the evolutionary conservation of  $\alpha$ -neurexins, triple knock-out mice lacking neurexin 1 $\alpha$ , 2 $\alpha$  and 3 $\alpha$ , but not  $\beta$ -neurexins, die on the first day of postnatal life, indicating that  $\alpha$ -, and not  $\beta$ -neurexins, are essential for postnatal survival.<sup>4</sup>

The extracellular portion of  $\alpha$ -neurexins encompasses six LNS (laminin/neurexin/sex hormone binding globulin) domains with three intercalated epidermal growth factor (EGF)-like domains, whereas the shorter  $\beta$ -neurexins have a single LNS domain. Neuroligins bind to the LNS domains of either  $\alpha$ - or  $\beta$ -neurexins.<sup>5</sup> Differential roles in synaptic transmission have been attributed to  $\alpha$ - and  $\beta$ -neurexins, due to isoform-specific patterns of ligand interactions.<sup>6</sup> The extraordinary degree of variation produced through alternative splicing both in neurexins and in neuroligins, profoundly influences intermolecular pairing affinities.<sup>5</sup> All three neurexin genes contain five alternative splice sites (ss1–ss5), yielding nearly 4000 neurexin protein

isoforms.<sup>2</sup> One additional alternative splice site, later described only in the *NRXN1* and *NRXN2* genes, is referred to as ss6 despite residing in the coding region of the fourth LNS domain, between ss2 and ss3.<sup>7</sup>

## 1.2 Neurexin 1 Copy Number Variation

*NRXN1*, located on chromosome 2p16.3, is one of the largest known human genes, with 24 exons spanning 1.1 Mb. The promoter for neurexin-1 $\alpha$  transcripts lies upstream of exon 1, while the promoter for neurexin-1 $\beta$  is located in intron 17.<sup>8</sup> The *NRXN1* locus is particularly susceptible to non-recurrent deletions, resulting from chromosomal rearrangements due to genomic instability.<sup>9</sup> The molecular mechanisms giving rise to these non-recurrent CNVs are very complex and still under investigation. Indeed *NRXN1* deletions often occur in the vicinity of minus self-chains, a novel group of LCRs consisting of short repeats (300-1000 bp) separated by several hundreds to a few thousand nucleotides.<sup>10</sup> These have been shown to cause genomic instability by mediating secondary DNA structures potentially able to stall the replication fork and cause template switching or to act as substrate for non-allelic homologous recombination.<sup>11</sup> However, analyzing the local sequences surrounding the breakpoints of 17 non-recurrent *NRXN1* deletions, Enggaard Hoeffding et al<sup>9</sup> discovered two novel sequence motifs and significantly higher AT nucleotide content at the breakpoints of all 17 deletions, compared to the overall nucleotide content on chromosome 2. Small insertions and duplications giving rise to short microhomologous sequences were also detected at the breakpoints. Hence, the two novel sequence motifs, together with a high AT content, may increase genomic instability and enhance susceptibility to the

formation of single strand structures, potentially fostering repair of double strand breaks by non-homologous end joining or leading to replication errors.<sup>9</sup>

Unlike recurrent deletions mediated by flanking terminal repeats, *NRXN1* deletions vary in size and breakpoint location. Exonic *NRXN1* deletions are significantly associated with neurodevelopmental and neuropsychiatric disorders, displaying an 8-fold increase in prevalence among 19263 cases compared to 15624 controls (0.213% vs 0.026%, O.R. = 8.14).<sup>12</sup> Furthermore, 34% of these deletions were *de novo*,<sup>12</sup> supporting relatively high, albeit not full penetrance, due to controls, parents and unaffected siblings occasionally carrying the same CNV as the affected proband. Clinically disruptive exonic *NRXN1* deletions usually span the promoter region and the first few exons encoding the *NRXN1-α* transcript, which indeed represent the most critical portion of the *NRXN1* gene.<sup>12</sup> In fact, in these individuals the *NRXN1-β* transcript is often spared and individuals with deletions limited to the 3' end of the gene display a 7-fold increased likelihood of carrying a second clinically relevant CNV, compared to subjects with a *NRXN1* deletion affecting the 5' end.<sup>12</sup> Also genomic instability or increased recombination rates could affect more frequently the 5' end of the gene, enhancing the probability of deletions in the promoter region.<sup>13</sup> Interestingly, these 5' deletions encompass or indirectly influence the long noncoding RNA *AK127244* adjacent to the promoter of *NRXN1-α*, increasingly involved in the etiology of several neuropsychiatric disorders.<sup>14-17</sup>

Contrary to exonic *NRXN1* deletions, cases and population-based controls do not differ in prevalence of intronic *NRXN1* deletions.<sup>12,18,19</sup> Furthermore, intronic *NRXN1* deletion carriers are two-fold more likely to harbor a second clinically relevant CNV compared to exonic *NRXN1* deletion cases.<sup>12</sup> Therefore, *NRXN1* intronic deletions do

not appear to substantially increase the risk for neurodevelopmental or neuropsychiatric disorders. Also the effects of duplications and point mutations can greatly vary and cannot be reliably predicted without specific functional studies.

Based on the evidence summarized above, this systematic review is focused on mono- and bi-allelic exonic *NRXN1* deletions and their relationship with neurodevelopmental/neuropsychiatric disorders or transdiagnostic neuropsychological phenotypes.

## 2. METHODS

### 2.1 Research strategy

This systematic review has been conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines employing the PubMed and Scopus databases (last update: November 30, 2018).<sup>20</sup> Both were searched for full-length articles that discussed CNVs and *NRXN1* using the following search terms:

(*NRXN1* OR Neurexin1) AND Deletion.

### 2.2 Study selection

Articles were included in our review according to the following criteria: (a) English, French, Italian, Spanish or German languages, (b) publication in peer reviewed journals, (c) case-control studies or screenings for *NRXN1* exonic deletions in large clinically-referred cohorts, and (d) case reports about *NRXN1* nullisomy. Articles were excluded by title, abstract, or full text for irrelevance to the investigated issue. Finally, in order to identify additional studies that could fulfill inclusion criteria, we also reviewed the



references listed in Béna et al<sup>21</sup> and Dabell et al,<sup>13</sup> the two major reviews published to date on the clinical phenotypes associated with exonic *NRXN1* deletions.

**2.3 Data extraction and quality assessment**

Data were independently extracted by 2 reviewers (P.C., M.B.) according to the Cochrane guidelines. A third reviewer (A.R.) then performed quality control on all data extracted from each article, including: (a) total number and localization of mono-allelic exonic *NRXN1* deletions; (b) total number of cases and controls analyzed; (c) type, genomic coordinates and familial transmission of compound heterozygous *NRXN1* deletions/mutations present in nullisomic patients, as well as (d) other molecular findings, (e) family history and (f) clinical phenotype.

**2.4 Recruitment of a new case with *NRXN-1* nullisomy**

The new case of *NRXN-1* nullisomy hereby reported was recruited and clinically characterized within the framework of an ongoing study on the genetics of Autism Spectrum Disorder and neurodevelopmental delay, as previously described.<sup>22</sup> This study was approved by the Institutional Review Boards of University of Messina (Messina, Italy) and University “Campus Bio-Medico” (Rome, Italy). Written informed consent was provided by parents for themselves and for their children. A separate written consent was provided for the publication of the clinical summary presented below and of this patient’s photographs.

**3. RESULTS AND DISCUSSION**

The multi-step process and the number of articles retrieved at each step for this systematic review is depicted in Figure 1. A total of 57 published studies was included in this review. All 51 studies evaluating the frequency of monoallelic *NRXN1* exonic deletions in clinically referred patient/control cohorts are listed in Table 1. Two of these same studies, as well as 6 additional case reports describing *NRXN1* nullisomic patients are listed in Table 2.

### 3.1 Phenotypic spectrum of heterozygous *NRXN1* exonic deletions

Heterozygous exonic microdeletions in *NRXN1* have been reported in association with susceptibility to neurodevelopmental and neurocognitive disabilities, including developmental delay (DD) and/or intellectual disability (ID), autism spectrum disorder (ASD), schizophrenia and others (epilepsy, ADHD, Tourette syndrome, obsessive-compulsive disorder, etc) (Table 1). Heterozygous deletions have also been found in normal parents, siblings and healthy controls (Table 1), suggesting reduced penetrance with gene-gene and gene-environment interactions influencing clinical features and severity.

Several case-control studies have been published and thirty of them provide control data for *NRXN1* (Table 1). Studies describing large numbers of cases and controls have found statistically significant enrichment of exonic *NRXN1* deletions in cases vs controls [0.18% vs 0.02%, respectively,  $P < 0.0001$ ; OR=10.3 (7.03-15.06, 95% confidence interval)], supporting a role for these deletions in the development of abnormal phenotypes (Table 1). However, the possibility to reliably meta-analyze these case-controls studies is undermined by the same control samples and data sets being shared across more than one study.

In clinically ascertained populations, moderate-to-severe ID represents the clinical phenotype most frequently associated with *NRXNI* exonic deletions (77–92%).<sup>13,21,28</sup> Other commonly co-occurring behavioral conditions include ASD (43–70%),<sup>13,21,28,69,70</sup> ADHD (9–41%),<sup>12,28</sup> anxiety (6–7%)<sup>12,13</sup> and schizophrenia (5%).<sup>12</sup> Among neurological phenotypes, epilepsy has been reported in 14–53% of *NRXNI* deletion carriers<sup>13,21,28,69</sup> and muscle hypotonia in 38–47%.<sup>21,28</sup> As expected for many of the above-mentioned neurodevelopmental phenotypes, delays in receptive and/or expressive language development have been consistently reported in children with *NRXNI* deletions.<sup>13,21,25,27,60</sup> Brignell et al<sup>71</sup> made a comprehensive evaluation of speech and language phenotypes, reporting speech difficulties in 69% of the patients with *NRXNI* deletions.

Despite *NRXNI*'s widespread systemic expression and possible roles outside of the nervous system,<sup>72</sup> congenital anomalies are relatively rare, with the most prevalent affecting skeletal muscle and heart.<sup>13,25,28,73</sup> Mild dysmorphic features have been described in 45–71% of the patients.<sup>13,21</sup>

As discussed above, the majority of exonic *NRXNI* deletions identified in clinical cases involves the promoter and the first few exons of the *NRXNI-α* transcript. However, one published report supports an association between *NRXNI-β* deletions and more severe phenotypes, possibly including enhanced prenatal lethality.<sup>74</sup> In particular, a deletion of *NRXNI-β* exons 2–4, corresponding to *NRXNI-α* exons 19–22, carried by a woman with a history of five first-trimester spontaneous abortions and diagnosed with high-functioning autism, anxiety and depression, was transmitted to all four of her children affected with autism, anxiety, developmental and speech delay, but not to an unaffected child.<sup>74</sup> Deletions involving also *NRXNI-β* exons or *NRXNI-α* exons 6–17

may be associated with macrocephaly and seizures, according to some,<sup>13,28</sup> though not all studies.<sup>21</sup> Apart from the involvement of *NRXN1-β*, increased phenotypic severity may be due to *NRXN1-α* exon 6-17 deletions being in-frame and possibly yielding an abnormal *NRXN1-α* protein, devoid of one or several of its extracellular domains, responsible for a peculiar dominant negative effect.

The availability of knock-out mice carrying deletions of the promoter and first exon of the *NRXN1-α* gene provides direct access to the biological effects of neurexin-1α disruption on neurodevelopmental phenotypes. These rodent models recapitulate some of the behavioral deficits observed in schizophrenia and ASD: mice lacking *NRXN1-α* show impaired sensorimotor gating, increased grooming behavior, impaired nest building and parenting abilities.<sup>75</sup> Parallel electrophysiological studies performed in these same animals show weaker spontaneous and evoked excitatory synaptic transmission, due to presynaptic glutamatergic deficits.<sup>75</sup> Neurexins' role in synapse formation, functioning and alignment may thus well explain the reduced connectivity between distant brain regions believed to underlie social cognition deficits pathognomonic of ASD and the disorganization of thought processes typical of schizophrenia. This conclusion is further reinforced by behavioral deficits, especially in social memory, recorded even in heterozygous mice,<sup>76</sup> which usually do not display behavioral phenotypes in rodent models of human neurodevelopmental disorders.

The putative effects of dysfunctional synaptic adhesion molecules, such as *NRXN1*, on neural circuitry support pathophysiological theories postulating “two hit” events as pivotal to neurodevelopmental disorders. In particular, the highly variable clinical outcome associated with *NRXN1* deletions, ranging from unaffected carrier status to severe neurodevelopmental phenotypes, suggests they may confer disease risk

rather than play a causal role *per se*. Incomplete penetrance may be explained by: (a) secondary and independently segregating genetic risk factors, as supported by enhanced frequencies of additional pathogenic CNVs in cohorts of patients carrying *NRXNI* deletions;<sup>21,40</sup> (b) exposure to environmental factors, especially active prenatally;<sup>77,78</sup> and/or (c) unfavorable stochastic events, able to synergistically foster the development of clinical signs and of full-blown psychiatric disorders.<sup>27</sup> However, *NRXNI* deletion carriers, even when apparently unaffected, seemingly share a common phenotype characterized by high levels of anxiety, a dysexecutive syndrome with prominent impulsivity, and borderline intelligence in the absence of aphasia, amnesia, agnosia, and apraxia.<sup>79,80</sup> These clinical features do not immediately fit into any single nosological entity, but rather predispose to a variety of behavioral disorders. Additional evidence comes from anedotal studies describing two mothers with a *NRXNI* deletion apparently devoid of psychiatric disorder requiring treatment, but showing subclinical depressive and anxiety symptoms as well as a dependent personality disorder.<sup>42,53</sup> Interestingly, high levels of anxiety are also characteristic of *NRXNI* knock-out mice,<sup>81</sup> although they are not recorded in hz mice.<sup>76</sup> Collectively, these data highlight the need for a thorough neuropsychological and psychiatric evaluation of apparently unaffected *NRXNI* deletion carriers before providing clinical advice and genetic counseling (see below).

Most studies performed to date present several limitations: (a) phenotypic information on deletion-transmitting parents and on deletion-carrying siblings is frequently lacking. This seriously limits the reliability of penetrance estimations, currently ranging between 46% and 78%;<sup>25,27,61,70</sup> (b) the majority of index cases are children, not allowing accurate prevalence or penetrance estimations for adult-onset

neuropsychiatric conditions, e.g psychotic or mood disorders; (c) sample sizes are usually small. These methodological limitations, paired with our insufficient understanding of factors and processes influencing the clinical trajectory of *NRXN1* deletions, pose a major challenge to genetic counseling over both prognosis and recurrence risk.

### 3.2 Phenotypic spectrum of *NRXN1* nullisomy

*NRXN1* nullisomy, also referred to as Pitt-Hopkins-like syndrome 2 (OMIM n. 614325), represents the “extreme” phenotype associated with *NRXN1* defects. In this condition, compound heterozygous inherited *NRXN1* deletions/mutations produce a recessive and extremely severe phenotype, including moderate to severe DD/ID, absence of expressive language, muscle hypotonia, motor stereotypes, chronic constipation and abnormal sleep/wake cycle. Only ten patients with *NRXN1* biallelic loss have been reported to date (Figure 2).<sup>21,29,57,82-86</sup> We shall now describe the clinical presentation of a new case carrying compound heterozygous *NRXN1* deletions. The clinical phenotype associated with biallelic *NRXN1* loss-of-function will be then reviewed, highlighting the clinical features most consistently shared by the overall set of 11 known *NRXN1*-null patients (Table 2).

#### 3.2.1 Clinical description of a new case of Pitt-Hopkins-like syndrome 2 with compound heterozygous *NRXN1* deletion

The proband is a 6 y.o. girl, second child of non-consanguineous parents with two other children, a 9 y.o. girl with congenital deafness due to a connexin 26 mutation, and a 2½ y.o. girl in good health. Minor bleeding during the I trimester and diminished fetal

movements were reported. Born at full term, her birth weight was 2950 g, length 49 cm, and Apgar score 9 at 1'. Parents observed greyish complexion, weak crying and arm extraflexion during the first 12 hours of postnatal life. Remarkable muscle weakness, hypotonia, reduced spontaneous activity and fatigability were present since birth. Psychomotor development was profoundly delayed: postural control of head and trunk were obtained at 8 and 18 months, respectively; she never crawled and walked only with support until 5 and ½ y.o., when she began walking independently, although to this date she prefers to sit or stand with support, when not prompted to walk; sphincter control has not yet been acquired. Motor coordination is severely impaired. Babbling began at 10 months and never evolved into expressive language. Non-verbal communication, including pointing, is also absent. Stereotypic hand movements for visual self-stimulation are frequent. Additional sensory self-stimulation involves chewing on toys and occasionally fixating rotating objects. Upper limb mannerisms and transient choreic movements are observed. The patient consistently turns when called by name and her visual engagement has improved over time. Imitation skills have begun emerging only after 4 ½ years of age. At kindergarden she appeared quiet, open to interaction with peers but limited by motor and cognitive deficits. No aggressiveness toward self or others was ever observed. Her sleep-wake cycle is disturbed by frequent night awakenings, but her overall daily sleep time is sizable. Adherence to routines and insistence-on-sameness are relevant, especially with regard to her daily schedule. Allergic to milk and egg, she only eats soft foods and has been diagnosed with coeliac disease following tests performed for chronic constipation. No seizure or absence ever occurred, nor recurrent ear/upper airways infections.

Auxometric parameter include head circumference at 3<sup>rd</sup>-5<sup>th</sup> percentile, height at 25<sup>th</sup> percentile and weight at <<3<sup>rd</sup> percentile. Facial dysmorphisms include depressed nasal bridge, telecanthus, frontal bossing with high anterior hairline, low-set ears, slightly prominent glabella, infraorbital creases, prognathism, long filter, thin upper lip, pointed chin (Figure 3a, b); cranial dysmorphisms include brachycephaly with flattened occiput and positional plagiocephaly. No dysmorphisms are present in trunk and limbs. Feet display mallet toes (Figure 3c). Otoacoustic emissions and auditory evoked-potentials, EEG, brain MRI, fragile-X screening and karyotype were all normal.

Parents do not report present or past neuro- or psychopathology. Paternal family history is positive for ID, brain tumor, alcoholism, bulimia and Arnold-Chiari malformation; maternal family history is positive for schizophrenia, mild ID and depression. Spontaneous abortions and infertility were also reported among maternal and paternal family members, respectively.

Array-CGH unveiled a paternally inherited 467 kb deletion (chr2:50,713,464–51,180,620/hg19) and a maternally inherited 269 kb deletion (chr2:50,982,113–51,251,557), both disrupting exclusively the *NRXN1* locus and partly overlapping in bp 50,982,113-51,180,620, yielding nullisomy by compound heterozygosity (Figure 3d).

### 3.2.2 Shared phenotypic features in *NRXN1* nullisomy and genotype-phenotype correlations

Comparison of the phenotypic features present in the eleven patients carrying biallelic *NRXN1* deletions/mutations, as listed in Table 2, allows the identification of the following “core” clinical correlates of *NRXN1* nullisomy: DD/ID, severe muscle hypotonia, absence of expressive language, social interaction deficits, motor



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4 stereotypies, abnormal sleep-wake cycle and chronic constipation. Additional features,  
5  
6 such as failure to thrive, strabismus, seizures, hyperventilation, hearing impairment,  
7  
8 microcephaly, electrophysiological and neuroanatomical abnormalities, are present in a  
9  
10 sizable minority of cases (Table 2).  
11  
12

13  
14 With a single exception,<sup>83</sup> all *NRXN1* defects were inherited from healthy  
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16 parents (Table 2); all are exonic, except for a boy carrying a homozygous intronic  
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18 biallelic deletion, whose pathogenicity is uncertain due to parental consanguinity  
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20 allowing alternative recessive explanations.<sup>85</sup> Two patients are compound heterozygous  
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22 for a deletion and a point mutation, namely a nonsense mutation in exon 15 and a splice  
23  
24 mutation, both leading to a truncated neurexin 1- $\alpha$  protein and to a clinical condition  
25  
26 similar to the one observed in the presence of compound heterozygous deletions.<sup>57,83,87</sup>  
27  
28 The only subject carrying two point mutations has a clinical diagnosis of Pitt-Hopkins-  
29  
30 like syndrome 2, although a missense mutation of uncertain significance and a silent  
31  
32 substitution are unlikely to cause his severe condition,<sup>86</sup> which could well be caused by  
33  
34 the missense mutation on one allele and an unidentified alteration on the second allele.  
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40 As described for heterozygous deletions, most exonic deletions in Pitt-Hopkins-  
41  
42 like syndrome 2 span the promoter region and the first few exons of the *NRXN1- $\alpha$*   
43  
44 transcript, sparing the *NRXN1- $\beta$*  transcript (Figure 2). The only exception is reported by  
45  
46 Harrison et al,<sup>82</sup> who proposed that *NRXN1- $\beta$*  deletions may possibly be associated with  
47  
48 early puberty in girls. The  $\alpha$ - and  $\beta$ - isoforms interact differently with their binding  
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50 partners, so the loss of both isoforms could lead to more severe loss of protein functions  
51  
52 at the synapse. Functional redundancy between the various neurexin  $\alpha$ - and  $\beta$ - isoforms  
53  
54 is certainly not complete, given the abnormal phenotypes in mice and humans with  
55  
56 homozygous loss of *NRXN1- $\alpha$* .  
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Genotype-phenotype correlations are also complicated by the presence of additional CNVs present in the genome of some patients. In particular, three patients listed in Table 2 carry a deletion also spanning *miR-NID1* (*miR-8485*), located in the fifth intron of *NRXN1* (Figure 2). This miRNA directly binds to TDP-43 and this interaction results in repression of *NRXN1* gene expression.<sup>88</sup> There are no apparent phenotypic differences between these three patients and those with biallelic *NRXN1* deletions sparing *miR-8485*, so its phenotypic relevance is still unclear.

#### 4. RECOMMENDATIONS FOR CLINICAL PRACTICE

The association of *NRXN1* deletions with several human disease phenotypes underscores their clinical relevance. Indeed converging lines of evidence firmly establish *NRXN1* deletions as risk factors for a variety of neurological, psychiatric and neurodevelopmental disorders, like other recurrent CNVs endowed with incomplete penetrance. Nonetheless, genetic counseling remains a challenge, especially when the phenotype is atypical or includes congenital malformations. *It is not possible at this time to reliably predict the clinical outcome of a heterozygous exonic NRXN1 deletion detected through prenatal testing.* In these cases, it is advisable to set up a periodic neuro-behavioral follow-up program for the timely detection of warning signs and the prescription of early interventions possibly effective on neurodevelopmental deficits.<sup>89,90</sup> It is also important to consider that *NRXN1* deletion carriers reported in the Literature likely represent the severe end of a phenotypic spectrum and, as discussed above, *NRXN1* deletions likely require “second-hit” genetic contributors, able to synergistically impair neurodevelopment and push phenotypic severity above clinical threshold. Hence, *the presence of additional independent genetic risk factors should be*

sought in *NRXN1* deletion carriers. Large population-based and clinical cohort studies, as well as longitudinal follow-up studies able to catch late-onset phenotypes, such as schizophrenia and depression, will indeed represent a key area of future research, aimed at enhancing the reliability of genetic counselling and the efficacy of targeted therapeutic interventions in the presence of mono-allelic *NRXN1* deletions.

Our phenotypic review of Pitt-Hopkins-like syndrome 2 bears another important consequence in clinical practice: *the diagnostic work-up of heterozygous exonic NRXN1 deletion carriers must include DNA sequencing of the spared NRXN1 allele in search for mutations or very small insertions/deletions, when children display early-onset, severe psychomotor delay in the context of a Pitt-Hopkins-like syndrome 2 phenotype.* Genetic and genomic defects affecting the *NRXN1* locus seemingly produce this extreme degree of clinical severity and full penetrance only in the presence of nullisomy, which may not be entirely explained by CGH-array alone.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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**FIGURE AND TABLE LEGENDS**

**Figure 1.** Flowchart of the Literature search.

**Figure 2.** Location of compound heterozygous deletions described so far, in reference to *NRXNI*  $\alpha$  and  $\beta$  isoforms (top). Horizontal bars represent deletions, vertical bars represent point mutations.

**Figure 3.** Phenotypic and molecular findings in our new case of *NRXNI* nullisomy. (a, b) Facial and cranial dysmorphisms; (c) Mallet toes; (d) Array-CGH profile of the proband shown in parallel with the 467 kb paternal deletion at 2p16.3 (chr2:50,713,464–51,180,620) and the 269 kb maternal deletion (chr2:50,982,113–51,251,557).

**Table 1.** List of case/control studies describing the frequency and characteristics of monoallelic *NRXNI* exonic deletions ordered by disease phenotype.

**Table 2.** Phenotypic and molecular characteristics of individuals with biallelic *NRXNI* deletions/mutations. “Core” phenotypic features shared by most or all patients are highlighted in gray.

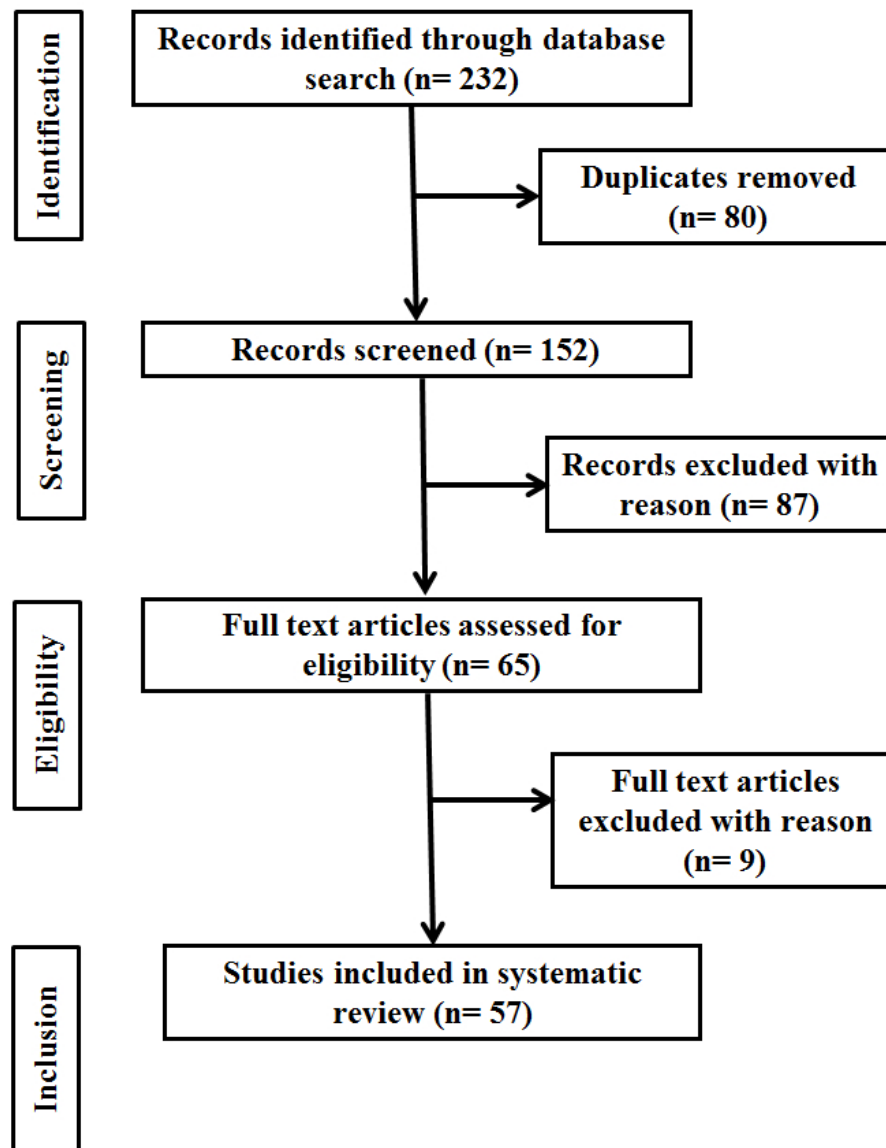


Figure 1. Flowchart of the Literature search.

180x232mm (96 x 96 DPI)



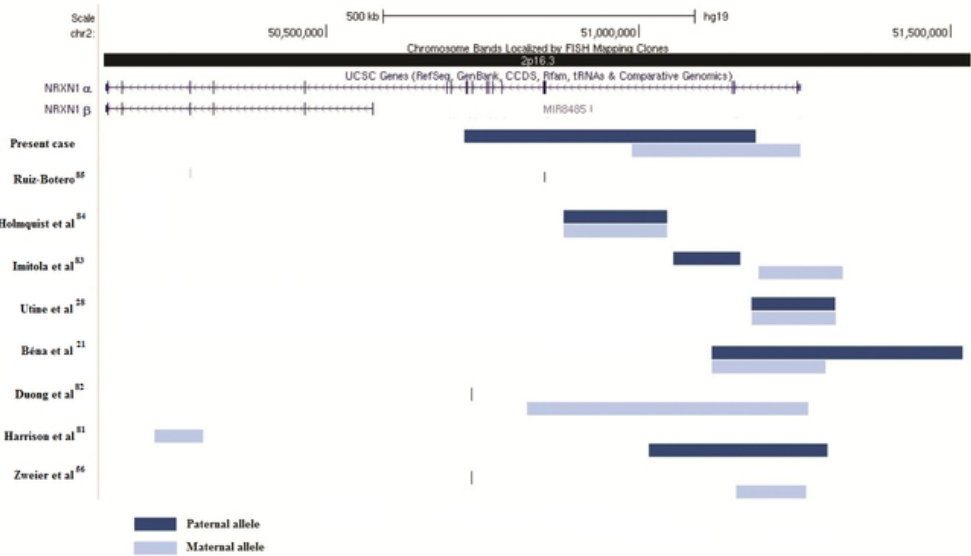


Figure 2. Location of compound heterozygous deletions described so far, in reference to NRXN1  $\alpha$  and  $\beta$  isoforms (top). Horizontal bars represent deletions, vertical bars represent point mutations.

55x31mm (300 x 300 DPI)

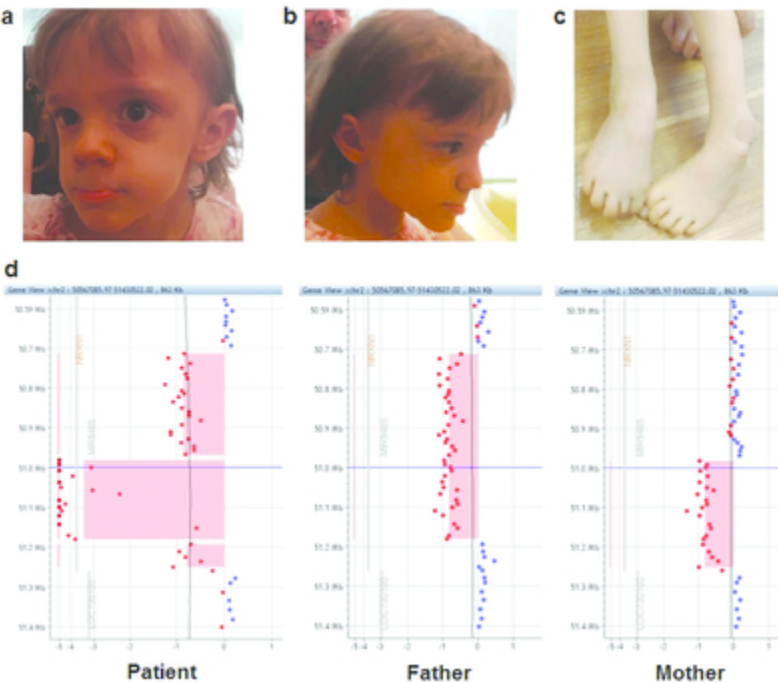


Figure 3. Phenotypic and molecular findings in our new case of NRXN1 nullisomy. (a, b) Facial and cranial dysmorphisms; (c) Mallet toes; (d) Array-CGH profile of the proband shown in parallel with the 467 kb paternal deletion at 2p16.3 (chr2:50,713,464–51,180,620) and the 269 kb maternal deletion (chr2:50,982,113–51,251,557).

33x30mm (300 x 300 DPI)

Reference	only $\alpha$ exons	$\alpha$ and $\beta$ exons	Unspecified location	Cases tested	Frequency of exonic deletions in cases (%)	Deletions in controls	Controls tested	Frequency of exonic deletions in controls (%)
<b>DD/ID cohorts</b>								
Friedman et al (2006) <sup>22</sup>	1	0	0	100	1.00	NA	NA	NA
Guilmatre et al (2009) <sup>23</sup>	1	0	0	247	0.40	0	236	0
Ching et al (2010) <sup>24</sup>	7	2	0	3540	0.25	10	51939	0.02
Sahoo et al (2011) <sup>25</sup>	12	3	0	1150	1.30	NA	NA	NA
Gregor et al (2011) <sup>26</sup>	1	0	0	45	2.22	NA	NA	NA
Schaaf et al (2012) <sup>27</sup>	8	3	0	8051	0.14	NA	NA	NA
Chen et al (2013) <sup>10</sup>	12	0	0	6623	0.18	NA	NA	NA
Dabell et al (2013) <sup>13</sup>	27	2	0	25610	0.11	NA	NA	NA
Uttine et al (2014) <sup>28</sup>	1	0	0	100	1.00	NA	NA	NA
Roberts et al (2014) <sup>29</sup>	0	0	0	150	0.00	NA	NA	NA
Wolfe et al (2017) <sup>30</sup>	1	0	0	202	0.50	NA	NA	NA
<b>Sum of DD/ID cohorts</b>	<b>71</b>	<b>10</b>	<b>0</b>	<b>45818</b>	<b>0.18</b>	<b>10</b>	<b>52175</b>	<b>0.02</b>
<b>ASD cohorts</b>								
Szatmari et al (2007) <sup>31</sup>	0	1	0	173	0.58	NA	NA	NA
Marshall et al (2008) <sup>32</sup>	1	0	0	427	0.23	0	1652	0
Bucan et al (2009) <sup>33</sup>	4	0	5	1771	0.51	0	2539	0
Guilmatre et al (2009) <sup>23</sup>	2	0	0	260	0.77	0	236	0
Glessner et al (2009) <sup>34</sup>	3	0	3	1637	0.37	0	2519	0
Sanders et al (2011) <sup>35</sup>	3	0	0	1124	0.27	NA	NA	NA
Prasad et al (2012) <sup>36</sup>	0	0	2	676	0.30	NA	1000	NA
Gai et al (2012) <sup>37</sup>	2	0	0	689	0.29	NA	NA	NA
Hedges et al (2012) <sup>38</sup>	1	0	0	168	0.60	1	149	0.67
Chen et al (2013) <sup>10</sup>	2	0	0	751	0.27	1	13991	0.01
Girirajan et al (2013) <sup>39</sup>	7	0	0	2588	0.27	1	2770	0.04
Roberts et al (2014) <sup>29</sup>	0	0	0	65	0.00	NA	NA	NA
Görker et al (2018) <sup>40</sup>	1	0	0	53	1.89	NA	NA	NA
<b>Sum of ASD cohorts</b>	<b>26</b>	<b>1</b>	<b>10</b>	<b>10382</b>	<b>0.36</b>	<b>3</b>	<b>23856</b>	<b>0.01</b>

<b>SCZ cohorts</b>								
Kirov et al (2008) <sup>41</sup>	1	0	0	93	1.08	0	372	0
Walsh et al (2008) <sup>42</sup>	0	1	0	233	0.43	0	268	0
Vrijenhoek et al (2008) <sup>43</sup>	3	0	0	806	0.37	0	706	0
ISC (2008) <sup>44</sup>	3	0	0	3391	0.09	1	3181	0.03
Need et al (2009) <sup>45</sup>	0	0	3	1073	0.28	NA	NA	NA
Rujescu et al (2009) <sup>18</sup>	5	0	0	2977	0.17	5	33746	0.01
Kirov et al (2009) <sup>46</sup>	1	0	0	471	0.21	2	2792	0.07
Guilmatre et al (2009) <sup>23</sup>	1	1	0	236	0.85	NA	NA	NA
Bradley et al (2010) <sup>47</sup>	0	0	2	480	0.42	NA	NA	NA
Magri et al (2010) <sup>48</sup>	1	0	0	172	0.58	NA	NA	NA
Ikeda et al (2010) <sup>49</sup>	0	0	0	519	0.00	0	513	0
Levinson et al (2011) <sup>50</sup>	9	1	0	3945	0.25	1	3611	0.03
Levinson et al (2012) <sup>51</sup>	1	0	0	2461	0.04	NA	NA	NA
Van Den Bossche et al (2013) <sup>52</sup>	1	0	0	8	12.50	NA	NA	NA
Rees et al (2014) <sup>53</sup>	11	0	0	6882	0.16	0	6316	0
Todarello et al (2014) <sup>54</sup>	3	0	0	635	0.47	0	635	0
Li et al (2016) <sup>55</sup>	3	0	0	6588	0.05	0	11904	0
<b>Sum of SCZ cohorts</b>	<b>43</b>	<b>3</b>	<b>5</b>	<b>30970</b>	<b>0.16</b>	<b>9</b>	<b>52140</b>	<b>0.02</b>
<b>Mixed cohorts</b>								
Zweier et al (2009) (PTHS like) <sup>56</sup>	1	0	0	179	0.56	0	667	0
Bradley et al (2010) (ADHD) <sup>47</sup>	0	0	1	440	0.23	NA	NA	NA
Sundaram S (2010) (TS) <sup>57</sup>	2	0	0	111	1.80	0	73	0
Stewart et al (2011) (E + SCZ) <sup>58</sup>	0	0	0	235	0.00	0	191	0
(E + BD) <sup>58</sup>	0	0	0	80	0.00	NA	NA	NA
Curran et al (2013) (DD/ID/ASD/MCA) <sup>59</sup>	15	3	0	10397	0.17	NA	NA	NA
Møller et al (2013) (IGE) <sup>60</sup>	0	0	5	1569	0.32	2	6201	0.03
Nicholl et al (2013) (E + ID/DD/ASD/MCA) <sup>61</sup>	0	1	0	247	0.40	NA	NA	NA
Noor et al (2013) (BD) <sup>62</sup>	3	0	0	936	0.32	0	940	0
Nag 2013 (TS) <sup>63</sup>	2	0	0	179	1.12	0	234	0
Wang et al (2016) (ID/DD/ASD/MCA) <sup>64</sup>	0	0	35	38000	0.09	NA	NA	NA
Lowther et al (2017) (DD/ID/ASD/MCA) <sup>12</sup>	0	0	41	19263	0.21	4	15264	0.03

Pérez-Palma et al (2017) (GGE/RE/AFE) <sup>65</sup>	0	0	3	2454	0.12	0	6476	0
Grünblatt et al (2017) (OCD) <sup>66</sup>	1	0	0	121	0.83	0	124	0
Huang 2017 (TS) <sup>67</sup>	0	0	12	2434	0.49	1	4093	0.02
Rizzo et al (2018) (NCD) <sup>17</sup>	9	2	0	2470	0.45	NA	NA	NA
Sum of mixed cohorts	33	6	97	79115	0.17	7	34263	0.02
Sum of all cohorts	173	20	112	166285	0.18	29	162434	0.02

**Abbreviations:** DD/ID: Developmental Delay/Intellectual Disability; ASD: Autism Spectrum Disorder; SCZ: schizophrenia; PTHS: Pitt-Hopkins Syndrome; ADHD: Attention Deficit Hyperactivity Disorder; TS: Tourette Syndrome; E: Epilepsy; IGE: Idiopathic Generalised Epilepsy; MCA: Multiple Congenital Anomalies; BD: Bipolar Disorder; GGE: Genetic Generalized Epilepsy; RE:Rolandic Epilepsy; AFE: Adult Focal Epilepsy; OCD: Obsessive compulsive Disorder; NCD: Neurocognitive Disorders

Patients	Present case	Ruiz-Botero <sup>85</sup>	Holmquist et al <sup>84</sup>	Imitola et al <sup>83</sup> Case 1
<b><i>NRXN1</i> deletions/mutations</b>	(p) 467-kb del [chr2:50,713,464-51,180,620]	(p) c.1405C>T p.Pro469Ser [chr2:50,847,195]	(p) 170-kb del [chr2:50,871,825-51,036,925]	(ns) 107-kb del [chr2:51,047,060-51,154,995]
(p) paternal allele				
(m) maternal allele	(m) 269-kb del [chr2:50,982,113-51,251,557]	(m) c.4053A>G p.Ala1351Ala [chr2:50,280,606]	(m) 170-kb del [chr2:50,871,825-51,036,925]	(ns) 135-kb del [chr2:51,184,729-51,319,450]
(ns) not specified				
<b><i>NRXN1</i> isoform</b>	$\alpha$	$\alpha+\beta$	$\alpha$	$\alpha$
<b>Other molecular findings</b>	489-kb dup [chr15:32,021,733-32,510,863]	NR	NR	NR
<b>Parental consanguinity</b>	No	No	Yes	No
<b>Family history</b>	Depression, schizophrenia, brain tumor, Arnold-Chiari malformation, alcoholism, cognitive disability and bulimia	None	None	None
<b>Age (years)</b>	6	7	4	7
<b>Sex</b>	Female	Male	Male	Male
<b>Height (centile)</b>	25th	90,3th	<3rd	5th
<b>Weight (centile)</b>	<<3rd	34,5th	<3rd	10th
<b>Head circumference</b>	3-5th	60,8th	<<3rd (-4 sd)	25-50th
<b>Developmental delay</b>	Severe	Severe	Severe	Severe
<b>Seizures</b>	No	No	No	Yes, starting at 5 y.o.
<b>EEG</b>	Normal	NR	NR	Generalized paroxysmal spike wave followed by 2Hz delta waves
<b>Age at walking</b>	5.5 y.o.	2 y.o.	Unable to walk	2 y.o.

Abbreviations: NR, not reported; G-E, gastroesophageal reflux; y.o., years old; EEG, electroencephalography; MRI, magnetic resonance imaging; TSH, thyroid-stimulating

<b>Imitola et al<sup>83</sup></b>	<b>Utine et al<sup>28</sup></b>	<b>Béna et al<sup>21</sup></b>	<b>Duong et al<sup>82</sup></b>	<b>Harrison et al<sup>81</sup></b>
<b>Case 2</b>				<b>Case 1</b>
(ns) 107-kb del [chr2:51,047,060- 51,154,995]	(p) 130-kb del [NRXN1 ex 1-2]	(p) 400-kb del [chr2:51,109,690- 51,510,961]	(p) c.IVS14-1G>A [chr2:50,723,234]	(p) 287-kb del [chr2:51,008,023-51,294,599]
(ns) 135-kb del [chr2:51,184,720- 51,362,512]	(m) 130-kb del [NRXN1 ex 1-2]	(m) 180-kb del [chr2:51,100,600- 51,362,512]	(m) 451kb-del [chr2:50,812,891- 51,362,512]	(m) 79-kb del [chr2:50,214,717-50,293,739]
$\alpha$	$\alpha$	$\alpha$	$\alpha$	$\alpha+\beta$
NR	NR	NR	NR	742-kb dup [chr5:168,866,009-169,607,847]
No	Yes	NR	No	No
None	NR	None	Paranoid schizophrenia, schizotypal personality disorder and bipolar disorder	None
7	10	10	33	16
Female	Male	Female	Male	Female
5th	$\leq 3$ rd	NR	NR	<0.4th
10th	$\leq 3$ rd	NR	NR	<0.4th
<5th	$\leq 3$ rd	70th (+1 sd)	NR	0.4-9th
Severe	Severe	Moderate	Severe	Severe
No	Yes (intractable seizures)	Yes	Yes (West syndrome)	Yes (severe epileptic encephalopathy, starting at 6 months)
Normal	NR	NR	Hypsarhythmia	Diffusely slow for age in wake; disorganized in sleep, with posterior epileptiform discharges limited to early childhood.
2 y.o.	> 6 y.o.	NR	3 y.o.	5 y.o.



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2	NR	Normal	NR	Moderate cortical atrophy, cyst	Normal
3				deflecting the right hippocampus	
4					
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8					
9	Hyperventilation	No	NR	No	Hyperventilation
10	Yes	NR	Yes	Yes	Yes
11	2-5 words	2 words	No	No	20-25 words
12	Severe	Severe	Severe	Severe	Severe
13	Severe	Severe	Severe	Severe	Severe
14	Yes	No	NR	Yes	Yes
15	Yes	NR	NR	Yes	No
16	NR	NR	NR	Yes	No
17	NR	Yes	No	No	Yes
18	NR	No	No	Yes	No
19	Yes	NR	NR	NR	Yes
20	Yes	No	NR	NR	Yes
21	Aggressive behavior.	NR.	Hyperactivity.	NR.	G-E- reflux requiring gastrostomy and fundoplication; scoliosis, pulmonary stenosis, early-onset puberty (age 9). Bruxism. Self-harm behavior.
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31	hormone; WM, white matter				
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**Harrison et al<sup>81</sup>****Zweier et al<sup>56</sup>****Case 2**

(p) 287-kb del  
[chr2:51,008,023-51,294,599]  
(m) 79-kb del  
[chr2:50,214,717-50,293,739]

$\alpha+\beta$   
742-kb dup  
[chr5:168,866,009-169,607,847]  
No  
None

11  
Female  
25th  
25th  
9th  
Severe  
Yes (severe myoclonic epilepsy  
with occasional atypical absences,  
starting at 4 months)  
NR

Unable to walk

(p) c.2936C>G, p.Ser979X  
[chr2:50,723,289]  
(m) 113-kb del  
[chr2:51,147,499-  
51,260,172]

$\alpha$   
NR  
NR  
None

18  
Female  
50-75th  
50-75th  
25th  
Severe  
No  
NR

2 y.o.

For Review Only

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Normal

Normal

Breath holding

Hyperventilation

Yes

NR

No

No

Severe

Severe

Severe

Severe

Yes

Yes

No

Yes

No

No

Yes

No

No

Yes

Yes

Yes

Yes

Yes

G-E reflux, scoliosis,  
pulmonary stenosis, early-  
onset puberty (age 6).

Hyperactivity and  
excessive drooling.

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