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ARTICLE

Copy number variants and infantile spasms: evidence for abnormalities in ventral forebrain development and pathways of synaptic function

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Infantile spasms (ISS) are an epilepsy disorder frequently associated with severe developmental outcome and have diverse genetic etiologies. We ascertained 11 subjects with ISS and novel copy number variants (CNVs) and combined these with a new cohort with deletion 1p36 and ISS, and additional published patients with ISS and other chromosomal abnormalities. Using bioinformatics tools, we analyzed the gene content of these CNVs for enrichment in pathways of pathogenesis. Several important findings emerged. First, the gene content was enriched for the gene regulatory network involved in ventral forebrain development. Second, genes in pathways of synaptic function were overrepresented, significantly those involved in synaptic vesicle transport. Evidence also suggested roles for GABAergic synapses and the postsynaptic density. Third, we confirm the association of ISS with duplication of 14q12 and maternally inherited duplication of 15q11q13, and report the association with duplication of 21q21. We also present a patient with ISS and deletion 7q11.3 not involving *MAGI2*. Finally, we provide evidence that ISS in deletion 1p36 may be associated with deletion of *KLHL17* and expand the epilepsy phenotype in that syndrome to include early infantile epileptic encephalopathy. Several of the identified pathways share functional links, and abnormalities of forebrain synaptic growth and function may form a common biologic mechanism underlying both ISS and autism. This study demonstrates a novel approach to the study of gene content in subjects with ISS and copy number variation, and contributes further evidence to support specific pathways of pathogenesis.
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INTRODUCTION

Infantile spasms (ISS) are characterized by clusters of epileptic spasms usually before the age of 1 year and are often associated with an electrodecrement and hypsarrhythmia on electroencephalogram (EEG).[1] Children with ISS tend to have poor developmental outcome, with an increased prevalence of autism.[2] The increasing number of new single genes associated with ISS, including *ARX, CDKL5, FOXG1, GRIN1, GRIN2A, MAGI2, MEF2C, SLC25A22, SPTAN1,* and *STXBP1*, suggests diverse genetic causes.[3–14] Many of these genes were identified from copy number variants (CNVs) or constitutional chromosomal rearrangements, suggesting that further study will lead to discovery of new ISS-associated genes.

Our initial review shows many of the ISS-associated genes cluster in two specific biological pathways: ventral forebrain development and forebrain synapse function. Abnormalities in ventral inhibitory GABAergic interneurons are implicated by the involvement of *ARX* and *FOXG1*, as both are transcription factors important in ventral forebrain development.[15,16] *MEF2C* may be a downstream transcriptional target of *ARX* and is differentially expressed in the forebrain in a mouse model of ISS with loss of *Arx*.[17] Disturbance of the gene regulatory network involved in the development of the ventral forebrain, including GABAergic interneurons, is therefore our first hypothesis for ISS pathogenesis.

Second, several ISS-associated genes – *GRIN1, GRIN2A, MAGI2, SPTAN1,* and *STXBP1* – are expressed in the pre- and/or postsynaptic membrane.[18–21] At the synapse a biologic relationship between ISS and autism is apparent, as several proteins associated with ISS interact with proteins implicated in autism pathogenesis.[22–26] Forebrain synaptic dysfunction is therefore our second hypothesis for ISS pathogenesis.

Here, we present novel CNV data in 18 children with ISS, including 7 with deletion 1p36, and combine these with data from the literature

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to provide a more detailed bioinformatics analysis of gene content. We developed bioinformatics tools that integrate publicly available databases and network analysis algorithms to look for evidence of pathways of pathogenesis in ISS, and found enrichment for genes associated with ventral forebrain development and forebrain synaptic function, in addition to several other pathways.

SUBJECTS AND METHODS

CNVs associated with ISS

Novel ISS Cohort. Subjects with ISS were referred to the Infantile Spasms Registry and Genetic Studies (ARP) or to WBD for research analysis after abnormal findings on clinical chromosomal microarray (CMA). Our inclusion criteria consisted of diagnosis of ISS based on epileptic spasms with electrodecrement or diagnosis of other seizure type in the presence of hypsarrhythmia. Clinical CMA was performed on a variety of platforms according to the manufacturer's specifications as part of routine care. The inheritance of CNVs was established using fluorescence *in situ* hybridization (FISH) on parental samples, except for LR09-044 where confirmation studies on parental samples were performed using the BlueGnome 180K array (Bouty Technogenetics, Milan, Italy). Subject LR11-055 was validated by D7S2476 and D7S3196 in parents, and subject LR11-056 was validated on a higher resolution 244K Agilent array. Inheritance of the duplication of 15q11q13 in subject IS09-007 from the maternal allele was demonstrated by genotyping of 21376915. The research protocols were approved by the IRB committees of the University of Chicago, the University of Washington, and Washington University.

Deletion 1p36-ISS cohort. Subjects with deletions of 1p36 and ISS were identified by chromosome analysis or subtelomere FISH. Informed consent was obtained to collect further clinical information or had previously been obtained as part of a long standing research project under one investigator, with informed consent in each case (LGS). Array CGH was performed using various versions of the SignatureChip (Signature Genomics, Spokane, WA, USA; see Table 2). Inheritance was established using FISH or genotyping with microsatellite markers on parental samples. To verify *KLHL17* deletion in subject 1p09-C, STS (sequence-tagged site) markers were designed and polymerase chain reaction (PCR) analyses were performed as described previously,[27] using genomic DNA from the subject, her parents, hybrids containing the chromosome 1 with deletion from 1p09-C, and control DNA samples. These research studies were approved at Poznan University of Medical Sciences.

Published ISS loci. PubMed (http://www.ncbi.nlm.nih.gov/pubmed) was searched from 1975 to 2010 for all abstracts in English containing patient reports of ISS associated with a CNV or chromosomal translocation. Publications were included if the clinical information was sufficient to confirm a diagnosis of ISS by criteria stated above, the CNV or chromosomal translocation was *de novo*, and breakpoints could be obtained. The Decipher database (https://decipher.sanger.ac.uk/) was searched using the term 'infantile spasms', and the clinical and genomic data of identified subjects were examined.

Bioinformatics analysis of gene content

Evaluation for evidence of brain expression. The gene content from the loci of interest was extracted based on the hg18 version of the UCSC genome browser (http://genome.ucsc.edu), using tools available through GEDI (http://gedi.ci.uchicago.edu/), and then handled with customized Perl and PHP scripts that interfaced with a MySQL database. Genes were evaluated for evidence of expression in the brain using the publicly available mouse databases in Supplementary Methods Table 1. Each gene was ranked for brain expression as yes, no, or unknown.

Evaluation for evidence of role in forebrain development/function. Genes with evidence of expression in the brain were then evaluated for a role in any of the eight steps of forebrain development and function, as detailed in the Supplementary Methods Table 2. This evidence was collected assisted by Pubmatrix (http://pubmatrix.grc.nia.nih.gov./) to carry out parallel searches of PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), followed by manual review of the abstracts generated. Information about Gene Ontology (GO; http://geneontology.org) molecular function and biological process was also extracted with GEDI. A list of preliminary candidate genes for ISS was then compiled that had evidence for (1) expression in the brain followed by (2) role in one or more stages of forebrain development/function. These genes were termed *preliminary candidate genes*.

Evaluation for pathways of ISS pathogenesis. The list of preliminary candidate genes was then combined with the list of known ISS-associated genes and subjected to network analysis using String 8.3 (http://string-db.org/), an algorithm that mines publicly available protein–protein interaction data for direct and indirect associations.[28] In order to minimize spurious associations, String analysis was performed with a confidence level of 0.9 (most stringent), and the Markov Clustering algorithm was used to identify the most relevant interactions.[29] Additionally, GEDI functionality that integrates multiple sources of clinical, genomic, and biologic data were used to identify relationships between the preliminary candidate genes and potential pathways of ISS pathogenesis. To specifically evaluate the gene regulatory network of ventral forebrain development, the list of preliminary candidate genes was compared with the list of 84 genes differentially expressed in the forebrain of the *Arx* conditional knockout mouse.[17] Genes shared in both lists were identified as potential genes interacting with this gene regulatory network. To specifically evaluate pathways of synaptic function, the list of preliminary candidate genes was compared with a list of 2912 genes extracted from GO that are involved in synaptic function (http://geneontology.org search on 'synapse') as well as the more specific child GO terms for synaptic transmission (GO:0007268), synaptic vesicle transport (GO:0048489), presynaptic membrane (GO:0042734), and postsynaptic density (GO:0014069). Genes present on overlapping lists were considered potential interactants in a specific synaptic biological process.

Statistical analysis. A Fisher's *F*-test was performed on the variance of the number of genes with role(s) in forebrain development that overlapped with pathways identified, compared with the variance in 200 genes with role(s) in forebrain development randomly selected from the BGEM data set (http://www.stjudebgem.org/web/mainPage/mainPage.php; Supplementary Table 4). A value of $P<0.05$ was used as an indicator of statistically significant enrichment for a pathway. Statistical analysis was performed using R version 2.9.2 (obtained from http://www.r-project.org/).

RESULTS

CNVs associated with ISS

Novel ISS cohort. Eleven subjects had CNVs identified on clinical CMA. The clinical and laboratory data are summarized in Table 1, and the genomic data are illustrated in Supplementary Figure 1. All CNVs were confirmed to be *de novo*, with the exception of the large 21.5 Mb duplication of 2q24.3q32.1 in subject IS10-021, which was presumed to be *de novo* although confirmatory samples were not available from the phenotypically normal parents. We also note the association of ISS in subject LR11-055 with deletion of the Williams syndrome region 7q11.23, subject IS09-007 with duplication of maternal 15q11q13, and duplication of 21q21 in subject LR10-282. The entire gene content of each CNV was included for bioinformatics analysis, with the exception of subject IS10-028, as the ISS critical region for duplication 14q12 has been narrowed down to *FOXG1*.[11] Clinical details are presented in the Supplementary Clinical Data.

Deletion 1p36-ISS cohort. Seven subjects with deletions of 1p36 and ISS were studied. The clinical and laboratory data are summarized in Table 2, and the genomic data are illustrated in Figure 1. We compared deleted regions in this cohort and concluded that *KCNAB2*, previously suggested as a candidate for epilepsy[30] was not likely critical for ISS pathogenesis, as this gene was not deleted in subjects 1p73-C and 1p93-C. The commonly deleted region in all seven subjects includes another epilepsy candidate, *GABRD*. However, ISS were not present in five subjects with interstitial deletions including *GABRD,* and epilepsy was only present in two of those patients.[31] In subject 1p09-C, STS

Table 1 Data on 11 subjects with ISS and CNVs

Subject	*ISS/HYPS*	*Genomic locus/coordinates (hg18)*	*Genomic event/size*	*De novo*	*Array platform*	*Best candidate gene(s)*
LR10-050	–/+	2p16.1p15 60 525 759–62 998 199	Del 4.0 Mb	+	Affymetrix 6.0	*BCL11A, REL*
IS10-021	+/+	2q24.3q32.1 164 579 191–186 069 420	Dup 21.5 Mb	NK[a]	Nimblegen 135K	*DLX1, DLX2, GAD1, NEUROD, PDK1*
LR10-281	+/+	3p26.3 35 000–1 540 000	Dup 1.6 Mb	+	Affymetrix 5.0	*CHL1*
LR11-055	+/+	7q11.23 72 161 000–73 583 000	Del 1.4 Mb	+	Agilent 44K	*FZD9, STX1A*
IS10-028	+/+	14q12 27 165 797–30 192 375	Dup 3.3 Mb	+	OligoHDScan 99K	*FOXG1*
LR09-044	+/+	14q32.33 104 763 000–105 080 000	Dup 317 kb	+	Agilent 44K	*CRIP2*
IS09-007	+/+	15q11.2q13.1 21 192 943–26 213 571	Dup 5.3 Mb	+	Affymetrix 6.0	*GABRA5, GABRB3, GABRG3*
LR10-283	+/+	19q12 34 845 623–35 853 961	Del 1.3 Mb	+	Illumina 660 Quad	*ZNF536*
IS09-013	+/+	20p13 9 536–1 813 622	Del 1.9 Mb	+	Affymetrix 6.0	*SNPH*
LR10-282	+/–	21q21.1q21.2 21 750 000–22 950 000	Dup 1.3 Mb	+	Affymetrix 5.0	*NCAM2*
LR11-056	+/+	Xp22.2 9 290 000–9 725 000	Dup 435 kb	+	Agilent 244K	*SHROOM2, TBL1X*

Abbreviations: CNV, copy number variant; Del, deletion; Dup, duplication; HYPS, hypsarrhythmia; ISS, infantile spasms; NK, not known.
[a]The large 21.5-Mb duplication 2q24.3q32.1 in subject IS10-021 was presumed *de novo*, as the parents were phenotypically normal.
Maximum CNV size is reported for all subjects.

Table 2 Data on seven subjects with ISS and deletions of 1p36

Subject	*ISS*	*Genomic coordinates (chr1; hg18)*	*Deletion size*	*De novo*	*Array platform*	*Best candidate gene(s)*
1p105-C	+	1–8 609 755	8.6 Mb	+	SignatureChipOS V 2.0	*GABRD, KCNAB2, KLHL17*
1p09-C[a]	EIEE	Complex rearrangement with three interstitial deletions and an inversion	2.7 Mb, 2.5 Mb, and 19 kb	+	SignatureChip V 2.0 BAC	*GABRD, KCNAB2, KLHL17*
1p97-C	+	1–7 303 017	7.2 Mb	+	SignatureChipOS V 2.0	*GABRD, KCNAB2, KLHL17*
1p119-C	+	1–7 684 487	7.6 Mb	+	SignatureChipOS V 2.0	*GABRD, KCNAB2, KLHL17*
1p129-C	+	1–7 752 669	7.7 Mb	+	SignatureChipOS V 2.0	*GABRD, KCNAB2, KLHL17*
1p73-C	+	1–4 413 688	4.4 Mb	+	SignatureChipOS V 2.0	*GABRD, KLHL17*
1p93-C	+	1–4 413 688	4.4 Mb	+	SignatureChipOS V 2.0	*GABRD, KLHL17*

Abbreviations: EIEE, early infantile epileptic encephalopathy/Ohtahara syndrome; HYPS, hypsarrhythmia; ISS, infantile spasms.
[a]The genomic data for subject 1p09-C was previously reported.[60]
Maximum deletion size is reported for all subjects.

marker walking confirmed deletion of another candidate, *KLHL17* (data not shown). Subject 1p09-C had electroclinical features consistent with early infantile epileptic encephalopathy (EIEE, Ohtahara syndrome), illustrating the likely biologic similarities between EIEE and ISS. The gene content of the largest deletion in this cohort (1p105-C) was included in bioinformatics analysis. Clinical details for the deletion 1p36-ISS cohort are presented in the Supplementary Clinical Data.

Published ISS loci. Our search of PubMed found 189 publications, of which 13 had data of sufficient detail to derive breakpoints, and in which the diagnosis was clearly ISS. Reports of Pallister–Killian syndrome and Down's syndrome were excluded because of the large number of genes. Three additional reports of balanced translocations in patients with ISS were identified, but in only one was the breakpoint mapped and the translocation disrupted a gene. Our search of Decipher (accessed January 2011) found 8 subjects reported with ISS, but only in subject 1229 was the CNV *de novo*. This subject with a 3.69-Mb deletion of 1p36 was previously reported,[32] and did not have ISS but rather partial epilepsy, so this patient was not included (personal communication, K Devriendt). Altogether, the gene content from five published loci (Table 3) underwent bioinformatics analysis.

Bioinformatics analysis of gene content

Evaluation for evidence of brain expression. The 11 novel CNVs, the largest region from our deletion 1p36-ISS cohort, and the 5 published loci analyzed contained 521 genes (Supplementary Table 1). Evidence for brain expression was found for 230 genes (Supplementary Table 2). Evidence that a gene was not expressed in the brain was found for 94 genes, and no data were found for 197 genes. Therefore, 291 genes were excluded from further analysis.

Evaluation for evidence of role in forebrain development/function. Of the 230 genes with evidence for brain expression, 82 had evidence of a role in at least one of the eight steps of forebrain development or function (Supplementary Table 3). These 82 genes were termed *preliminary candidate genes*. On the basis of the information presented below, the candidate genes were further refined to those that were the best candidates (Table 4).

Evaluation for pathways of ISS pathogenesis. Network analysis on the list of preliminary candidate genes identified several pathways likely to be significant in neurodevelopment. These included gap junction signaling, WNT signaling, ERBB-family signaling, GDNF-family signaling, neuronal cell adhesion, semaphorin signaling, including, as expected, ventral forebrain development, and several pathways of forebrain synapse function. Network analysis on the larger list of brain-expressed genes was not informative, because of the incompleteness of data regarding detailed expression patterns for many of the genes (data not shown).

Ventral forebrain development. We explored the connection between the preliminary candidate genes and the gene regulatory network of

ventral forebrain development in more detail because of the association of *ARX, FOXG1,* and *MEF2C* with ISS. An additional subject with duplication of *FOXG1* at 14q12 is reported here. *DLX1* and *DLX2* were present within the large duplication of 2q24.3q32.1; these are transcription factors directly upstream of *ARX*. Additionally, two other genes within 2q24.3q32.1, *GAD1* and *PDK1* are important in GABAergic interneuron development.[33] Finally, one of the preliminary candidate genes, *MAGEL2*, was differentially expressed in the *Arx* conditional knockout mouse and on this basis was considered a candidate for involvement in ventral forebrain development. Figure 2a illustrates this expanded gene regulatory network with these genes as proposed members. Our gene content was significantly enriched for members of this pathway ($P<0.001$).

Forebrain synapse function. These pathways were first identified by the association of *MAGI2, SPTAN1,* and *STXBP1* with ISS. During the course of this work, two additional genes expressed in the synapse were associated with ISS – *GRIN1*[12] and *GRIN2A*[13] – providing additional validation for involvement of these pathways in ISS pathogenesis. In all, 15 of the 82 preliminary candidate genes overlapped with synaptic pathways. The presence of the *GABRA5, GABRG3,* and *GABRB3* GABA receptor subunits in the 15q11q13 critical region suggests that abnormalities in GABAergic synapses have a role in ISS pathogenesis when duplicated from the maternal allele (Figure 2b). Additionally, copy number changes in several pre- and postsynaptic expressed genes were identified, including *CRIPT, ERRB4, SNPH,* and *STX1A*. Network analysis identified a functional relationship between SNPH and STX1A, which in turn interacts with the ISS-associated STXBP1 (Figure 3a). ERRB4 interacts with the postsynaptic density protein DLG4, which associates with the ISS-associated GRIN1 and GRIN2A (Figure 3b). KLHL17 is an actin-binding protein that interacts with GRIK2 in the postsynaptic density.[34] Our gene content was significantly enriched for members of the pathway of synaptic vesicle transport (GO:0048489) ($P<0.001$).

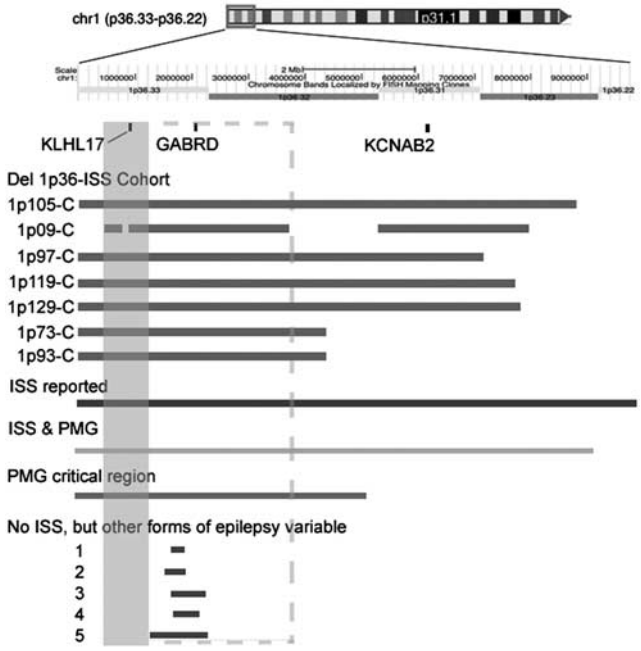

Figure 1 Genomic data on seven subjects with deletion 1p36 and ISS (Del 1p36-ISS Cohort), comparing breakpoints with a previously reported subject (ISS reported),[66] a subject with ISS and PMG,[59] the PMG critical region,[58] and five interstitial deletions without ISS but other forms of epilepsy variable.[31] The region within the dashed line indicates plausible ISS critical region containing *GABRD*, with the solid region representing a smaller hypothesized critical region containing the synaptic-expressed *KLHL17*.

DISCUSSION

ISS are associated with copy number variations that provide evidence for pathogenesis

As these subjects were referred for study based on discovery of a pathogenic CNV, our data cannot be used to determine the frequency of pathogenic CNV in ISS. However, the data generally support the concepts that ISS have a genetic basis and that CNVs are relevant for study in children with this disorder. By analyzing the gene content in these CNVs, we present evidence to support at least two hypotheses of ISS pathogenesis, specifically abnormalities in ventral forebrain development and pathways of pre- and postsynaptic function.

Seven of the novel CNVs reported are microduplications, and assigning pathogenicity remains preliminary until more data exist on the effect of gene dosage on phenotype.[35] More data are available regarding the effects of gene dosage in genomic microdeletions[36] and we addressed these issues by examining DECIPHER for haploinsufficiency scores when compiling our list of ISS candidate genes.

ISS are part of a clinical spectrum of developmental epilepsies

The subjects in this report had a spectrum of developmental epilepsy related to ISS[37] and included 13 children with ISS and hypsarrhythmia on EEG, 2 with other seizure types associated with hypsarrhythmia that we include based on previous studies,[38] and 1 child with Ohtahara syndrome. Some of the subjects responded to standard ACTH therapy, while others developed intractable epilepsy. Several developed severe developmental impairment, autism, self-injurious behavior, or combinations of these problems. This is consistent with the clinical heterogeneity already described in patients with mutations in ISS-associated genes. Phenotype–genotype correlations are not possible with the subject numbers here, but correlations may emerge

Table 3 Data on five published subjects with ISS and CNVs/chromosomal abnormalities

Subject	*ISS/HYPS*	*Genomic locus/coordinates (hg18) or breakpoints*	*Genomic event/size*	*De novo*	*Method*	*Best candidate gene(s)*
Patient 1[61]	+/+	2p22.1p21	Inv dup	+	FISH	*CRIPT*
Patient 2[62]	+/+	4p16.1-4pter	Dup	+	FISH	*CRMP1, JAKMIP1, PPPR2R2C*
Patient 3[63]	+/NK	14q32.32 101 794 000–106 288 000	Del	+	FISH	*RCOR1*
Patient 4[64]	+/+	19p13.13 12 615 927–13 280 259	Del 736 kb	+	Agilent 44B	*SP1*
Patient 5[65]	–[a]/+	t(2;6)(q34;p25.3)	Bal trans	+	Karyotype	*ERBB4*

Abbreviations: Bal trans, balanced translocation; CNV, copy number variant; del, deletion; dup, duplication; FISH, fluorescence *in situ* hybridization; HYPS, hypsarrhythmia; inv dup, inverted duplication; ISS, infantile spasms; NK, not known .
[a]Patient 5 had early myoclonic encephalopathy with hypsarrhythmia.

Table 4 Summary of best candidate genes, loci, and pathway association(s) after bioinformatics analysis

Gene	*Locus*	*Pathway association(s)*
CHL1	3p26.3	Neuronal cell adhesion
		Presynaptic SNARE complex chaperone
CRIPT	2p22.1p21	Postsynaptic membrane
CRMP1	4p16.1-4pter	Semaphorin signaling in neurons
DLX1	2q24q32	GABAergic interneuron development
		Ventral forebrain development
DLX2	2q24q32	GABAergic interneuron development
		Ventral forebrain development
ERBB4	2q34	GABAergic synapse
		Postsynaptic density
		Synapse organization and development
FOXG1	14q12	Ventral forebrain development
FZD9	7q11.23	WNT signaling
GABRA5	15q11q13	GABAergic synapse
GABRB3	15q11q13	GABAergic synapse
GABRD	1p36	GABAergic synapse
GABRG3	15q11q13	GABAergic synapse
GAD1	2q24q32	GABAergic interneuron development
		Ventral forebrain development
JAKMIP1	4p16.1-4pter	GABAergic synapse
KLHL17	1p36	Postsynaptic density
MAGEL2	15q11q13	Ventral forebrain development
NCAM2	21q21.1q21.2	Neuronal cell adhesion
PDK1	2q24q32	GABAergic interneuron development
		Ventral forebrain development
PPP2R2C	4p16.1-4pter	GABAergic synapse
SNPH	20p13	Synaptic vesicle exocytosis
		Synaptic vesicle transport
		Synaptosome
SP1	19q13.13	Gap junction signaling
STX1A	7q11.23	Presynaptic SNARE complex
		Synaptic vesicle exocytosis
RCOR1	14q32.32	Forebrain development
REL	2p16.1p15	ERBB-family signaling
		GDNF-family signaling
SHROOM2	Xp22.2	Actin binding
ZNF536	19q12	Neuronal differentiation

as larger patient numbers are ascertained. We also report ISS with hypsarrhythmia in a subject with duplication of 15q11q13, associating ISS with the most common chromosomal cause of autism.[39] The finding of ISS in a subject with duplication of 21q21 may reduce the critical region for ISS in Down syndrome.

Enrichment of genes involved in ventral forebrain development within CNVs in ISS patients

As *ARX* was the first gene associated with ISS, much information is known about its role in ventral forebrain development. *Arx*-deficient mice have severe deficits in GABAergic interneurons while conditional mutants deleting Arx from ganglionic eminence-derived neurons have seizures similar to those observed in patients with *ARX* mutations.[40,41] Other genes important in ISS pathogenesis may be present in the regulatory network that directs ventral forebrain development, including *FOXG1* upstream and *MEF2C* downstream. Duplications of *FOXG1* at 14q12 have been associated with ISS,[8,11] and we report here an additional subject with this association. This study then identified candidate genes to expand this network further.

The presence of *DLX1* and *DLX2* within the large duplication of 2q24.3q32.1 suggests that copy number changes of genes linked in a functional network with *ARX* are involved in ISS pathogenesis. Mice lacking *Dlx1* show loss of specific interneuron subtypes and have epilepsy,[42] although there is not yet a phenotype described with duplication. Other genes interacting with *ARX* were identified to have abnormal copy number in ISS subjects. *MAGEL2* is expressed in the developing hypothalamus,[43] and is a candidate given the hypothesized role for abnormalities in the hypothalamic–pituitary–adrenal axis and ACTH-responsiveness in ISS patients.[44]

Enrichment of genes involved in specific synaptic pathways within CNVs in ISS patients

One specific synaptic functional pathway, synaptic vesicle transport, and was enriched for genes with altered copy number in this study. *SNPH* has a role in presynaptic exocytosis,[45] and *Snph* deletion in the mouse results in abnormal presynaptic function.[46] *CHL1* is a chaperon of the presynaptic SNARE complex.[47] Additional genes expressed in the postsynaptic density/postsynaptic membrane, were of interest as well. *CRIPT* is involved in membrane support in close proximity to SHANK proteins.[48,49] We report the fourth patient with a deletion of 7q11.23 that does not include *MAGI2*,[50,51] raising the possibility that other genes, such as *FZD9* and/or *STX1A* or other factors, may contribute to ISS in patients with Williams syndrome.

Genes were also identified with specific roles in GABAergic synapse function. *ERBB4* is involved in GABAergic signaling in inhibitory interneurons,[52] promotes interneuron migration, and is regulated by ARX.[53] Several genes for GABA receptor subunits – *GABRA5, GABRD, GABRG3,* and *GABRB3* – also had abnormal copy number in ISS subjects. *GABRA5* is notably involved in interneuron function in the hippocampus.[54] *JAKMIP1* regulates GABRB2 mRNA, and can also be added to the list of genes involved in GABAergic synaptic function.[55]

ISS in deletion 1p36 patients associated with loss of KLHL17

As past reports indicated that 25% of deletion 1p36 syndrome patients may develop ISS,[56] we wished to look closely at the gene content of deletion 1p36 subjects with ISS. The critical region(s) for ISS, and for epilepsy in general in deletion 1p36, have included *GABRD* and *KCNAB2*[30] as candidates. Our data suggest that in some subjects overlap exists with *GABRD* and *KCNAB2,* although a recent series of patients with *GABRD* deleted did not have ISS, and epilepsy in general was only variably present.[31] Therefore, a more specific critical region for ISS derived from our data may include *KLHL17*. KLHL17 interacts with the kainate-type glutamate receptor subunit GRIK2, a key member of the postsynaptic density network centered on DLG4.[34] KLHL17 also binds actin in the postsynaptic density,[57] and may be involved in structural support of the postsynaptic membrane in a manner similar to the ISS-associated MAGI2.[19] This candidate region overlaps partially with the critical region for polymicrogyria (PMG)[58] and coincides with a reported deletion 1p36 patient with both PMG and ISS.[59] These findings must be treated with caution, however, as position effect, incomplete penetrance and variable expressivity may have a role in epilepsy type in 1p36 deletion patients.

Abnormalities of forebrain synaptic growth and function suggest common biologic mechanism underlying both ISS and autism

Two themes emerged from these analyses: (1) pathways of GABAergic interneuron development and synaptic function may be linked in ISS pathogenesis; (2) several genes expressed in the presynaptic STX1A and SNARE complexes, as well as others in the postsynaptic density

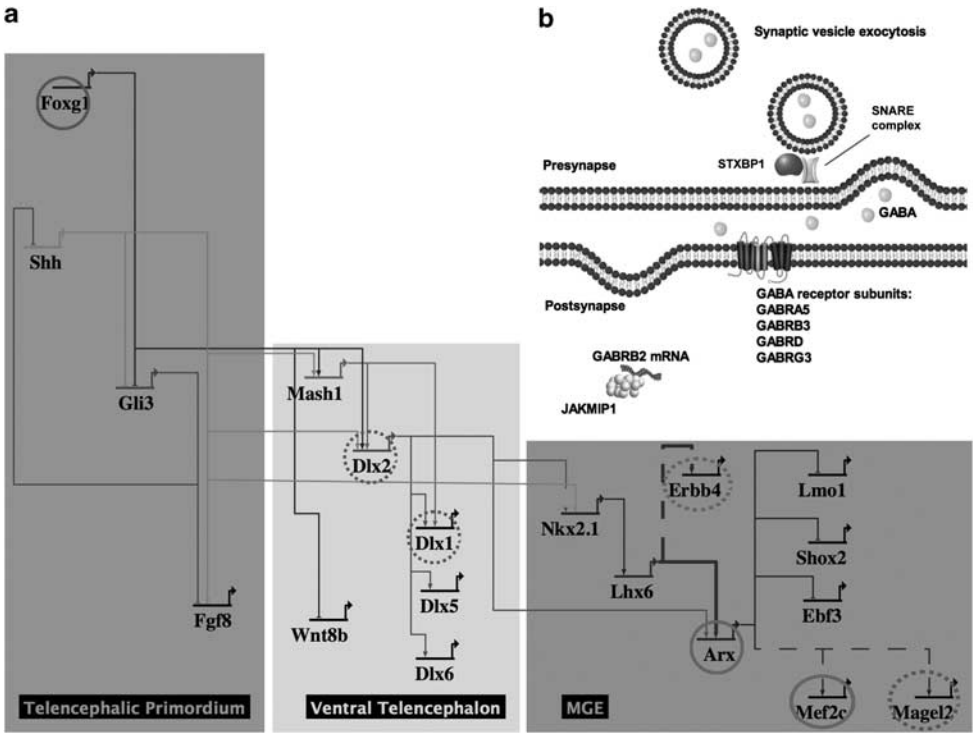

Figure 2 (**a**) The expanded gene regulatory network for ventral forebrain development. ISS-associated genes are indicated by closed circles, candidate members of the network identified in this study are indicated by dashed circles. In the color version, red indicates gene deletion or intragenic mutation and green indicates gene duplication. Dashed lines represent putative gene regulatory relationships that require further validation. Figure generated using BioTapestry (http://www.biotapestry.org/). (**b**) Illustration of the GABA-receptor subunit genes expressed in the post-synapse with abnormal copy number in subjects with ISS identified in this study. The involvement of STXBP1 in synaptic vesicle exocytosis is also shown. Figure generated using ProteinLounge (http://www.proteinlounge.com/). GABA, gamma-aminobutyric acid; MGE, medial ganglionic eminence. The color reproduction of this figure is available at the *European Journal of Human Genetics* journal online.

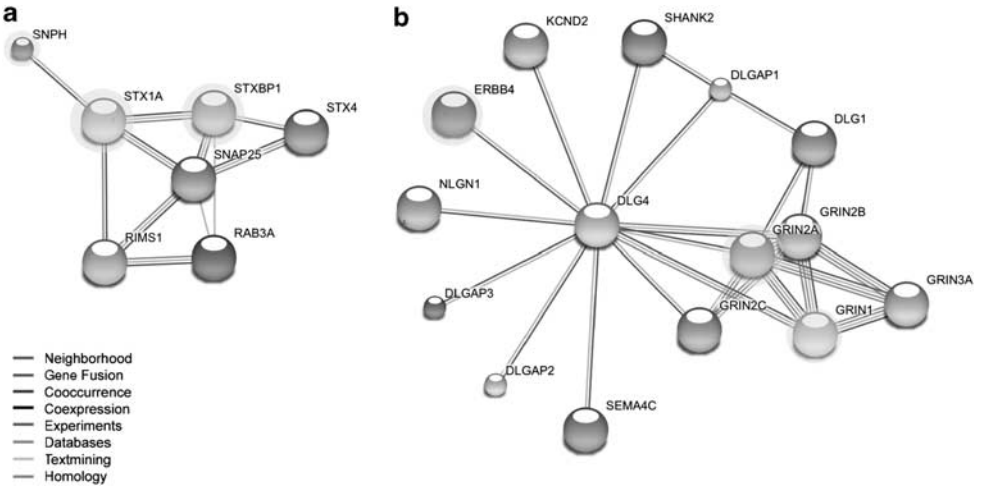

Figure 3 (**a**) Protein–protein interaction network of synaptic vesicle exocytosis (GO:0016079), illustrating a relationship between ISS candidate SNPH with STX1A, which in turn interacts with the ISS-associated STXBP1. (**b**) Protein–protein interaction network of the postsynaptic density (GO:0014069), illustrating a relationship between ERBB4 with DLG4, which in turn interacts with the ISS-associated GRIN1 and GRIN2A.

DLG4 complex, were identified with altered copy number in ISS subjects. These gene products are in close proximity to several autism-associated proteins. This leads to the hypothesis that abnormalities in proteins involved in forebrain GABAergic synaptic growth and function may form a common biologic mechanism underlying both autism and ISS, and that the increased prevalence of autism in children with a history of ISS may not be simply a result of epileptic encephalopathy.

In conclusion, we report 8 novel CNVs and additional children with 4 known CNVs from a group of 23 children with ISS and abnormal

clinical CMA. We then used a novel bioinformatics approach to evaluate these CNVs to study several hypotheses of pathogenesis. We identified candidate genes and biologic pathways that will serve as targets for further validation studies. Finally, we argue that several of these biologic pathways of ISS pathogenesis are linked and involve forebrain GABAergic interneuron development, as well as GABAergic and other pathways of pre- and postsynaptic function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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