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Critical regions for human brain development include *SON* and *ITSN1* defined by a panel with chromosome 21 interstitial deletions.

Julie R. Korenberg

Julie.Korenberg@hsc.utah.edu

University of Utah

Harvey B. Sarnat

University of Calgary

Stefan Pulst

UNIVERSITY OF UTAH

Masahira Hattori

Waseda University

Xiao-Ning Chen

retired

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Abstract

Aneuploidies of chromosome 21 (CHR21), including Down syndrome and partial monosomy 21, are major causes of mental retardation and provide unique opportunities to identify candidate genes regulating human brain development. We identified 10 individuals with partial CHR21 deletions and defined molecular breakpoints with high resolution genomic arrays, FISH (fluorescence in situ hybridization) and quantitative Southern blots. Patient records and neuroimaging generated by the authors or by referring physicians were reviewed. We defined regions from proximal to distal on CHR21, a region consistent with a normal range of cognition, a region associated with microcephaly, and a third region associated with polymicrogyria and partial agenesis of the corpus callosum. Three individuals with severe cortical dysgenesis including polymicrogyria (PMG) with callosal hypoplasia or agenesis shared a ~649 kb minimal region at 21q22.11-22.2 whose distal border lies within *ITSN1* and contains SON. Seven individuals with large proximal deletions outside this interval, had mild cognitive deficits and did not share this phenotype. Comparison of the human phenotypes with those seen with loss-of function mutations in *SON* or *ITSN1* indicate that the 649kb deleted region disturbs vertebrate brain development with components likely attributable to ITSN1 and SON.

Introduction

Down syndrome is caused by trisomy of human chromosome 21 (CHR21) and is the most common human aneuploidy at birth. Rather than changing the essential structures of gene products, aneuploidies change only the relative expression of genes. The large number of genes implicated in whole chromosome trisomies or monosomies, can be refined by the study of smaller interstitial deletions or duplications [1]. These analyses provide an important model for identifying and understanding functions of the genes involved – including those important for cognitive and neuroanatomical development.

Aneuploidies thus provide a “model” for investigating the regulatory interactions important for human brain development and function that may mirror the mechanisms underlying evolution and horizontal phenotypic variation. Moreover, human aneuploidies with brain anomalies can help focusing animal models on those genetic pathways that are important in human developmental disabilities.

The study of interstitial deletions is highly important because it permits pinpointing defined disease-causing genes or clusters, diagnosing rare developmental disorders, and understanding basic cell survival mechanisms. Unlike a whole-chromosome loss, which is usually lethal to human cells, studying these smaller, localized losses provides a unique window into human biology. We have used this approach to define critical regions in CHR21 duplications [2, 3].

To extend the study of duplications of single genes or contiguous genes on brain structure we have now identified a panel of 10 individuals with nine chromosome 21 aneuploidies that represent partial deletions of human CHR21. We defined individual molecular breakpoints, reviewed clinical data, and analyzed brain abnormalities with MRI (magnetic resonance imaging) or CT (computed axial tomography).

MATERIALS AND METHODS

Patient Examination.

The corresponding author examined all patients personally or their medical records in conjunction with their primary physicians or medical geneticists. All data were taken from the original medical records and confirmed by follow-up examinations by the corresponding author and discussions with the family and patient. Neuroimaging was obtained as part of the initial work-up in the respective home institution either by computed tomography (CT) or magnetic resonance imaging (MRI). Procedures for human subjects and confidentiality were followed as approved by the Institutional Review Board of Cedars–Sinai Medical Center and the University of Utah.

Mapping of interstitial deletions

CHR21 deletions abnormalities were mapped with several approaches by using DNA and chromosomes generated from blood cells and cell lines. These methods are described in detail in Korbel et al [5]. In brief, karyotypes were first ascertained through standard cytogenetic analyses. To determine deletion breakpoints, genomes were analyzed by FISH with subsets of a panel of 350 BAC/PACs, each of which was validated for copy-number normals [6], followed by a high-resolution mapping using quantitative Southern blot analysis of single copy fragments [7]. Sources and references for DNA probe are available in Korbel et al (Table S6; NCBI Build 35). To define breakpoints, we performed an initial screen followed by more specific testing with BACs progressively closer to breakpoints. In each case, 10–30 copy-number determinations were performed. 20–50 cells were analyzed with 2–4 BAC DNAs simultaneously. Multicolor images were captured with a Zeiss Axioplan 2 microscope equipped with an AxiocamMRMcamera and conjugated to Metasystem software (Microsoft).

For High-Density Oligonucleotide Tiling Array Analysis, DNA from cell lines or blood was used to probe a custom tiling array platform (Nimblegen Technology) containing 45–85-bp oligonucleotide probes with a median probe distance of 90 bp. Array normalization was performed with the Qspline algorithm and c Copy-number changes called with the *BreakPtr* algorithm as previously described [8].

RESULTS

We identified a panel of 10 individuals that had come to medical attention in a number of academic medical centers for developmental disabilities, short stature, microcephaly, cardiac or pulmonary abnormalities or an abnormal neurological examination or imaging. Two individuals were siblings with identical deletions and are listed as case 6 EH/AH. After initial chromosomal analysis suggested interstitial deletions of human chromosome 21, they were referred for a detailed molecular definition of the deletion extent.

Table 1 shows a summary of the clinical and neuroimaging findings associated with specific interstitial deletions and table 2, their precise molecular definition. Figure 1 summarizes deletions from left to right

beginning with the most proximal breakpoints and groups of characteristic phenotypes. Examples of neuroimaging findings are shown in Fig. 2.

The deletions on CHR21 cause changes in a large number of genes and organ systems Table 1). Although all individuals show partially overlapping presentations, there are distinctive presentations based on presence or absence of specific features. Changes in ear position or morphology were seen in all cases. Short stature, broad nasal bridge or short philtrum, and clinodactyly were seen in at least 6 of the 9 cases. Seizures were surprisingly rare and observed in only 2 cases. Severe intellectual disability was present in 3, but normal range IQ was present in 3 cases as well.

To allow for a more detailed correlation of genotype and phenotype, we mapped the location and extent of interstitial deletions combining a number of techniques (Table 2). We defined their molecular breakpoints with high resolution genomic arrays, FISH (fluorescence in situ hybridization) and quantitative Southern blots.

The deletions observed in RB, JC, EO, GM6918, AH, EH and FB (14,261 – 33,449 Mb), comprise the most centromeric 13,966 Mb region including LIP1 through ADAMTS 1/5. These deletions are associated with normal range structural neuroimaging with or without white matter hypoplasia and low normal range intellectual function (Cases EO with the 14.0 Mb deletion and Case RB with the 10.3 Mb deletion), with normal cognitive function, (Case JC with the 13.8 Mb deletion), or with mild microcephaly (Case FB with the 11.7 Mb deletion). In contrast, cases KS, HJ and LS whose deletions include the region from 33,449 – 34,098 Mb, have severe cortical malformations including polymicrogyria, microcephaly and partial agenesis of the corpus callosum.

Based on the presence of severe cortical dysgenesis characterized by polymicrogyria (PMG) and hypoplasia or agenesis of the corpus callosum (ACC) in cases LS, HJ and KS, the human deletion data reported here suggest that the chromosome 21 region deleted in common among these 3 cases is likely to contain dose-sensitive genes involved in development of human cerebral cortex and hippocampal formation. In contrast, the seven deletion cases FB, GM6918, AH, EH, EO, JC and RB, which do not include this region (Table 2), do not show profound cognitive or structural abnormalities, but rather normal (case EO) or mild neuroimaging defects (cases FB and RB), normal range cognition (cases AH and JC) or reduced cognitive function without microcephaly (case GM6918).

We thereby narrow a chromosomal region likely to contain genes primarily responsible for PMG and callosal dysgenesis to a region of ~ 650 kb including a dozen known genes at 21q22.11-22.2. The proximal border of the candidate region is defined by the 6 cases without severe cognitive deficits or PMG, whereas the distal border of the candidate region is defined by three cases (KS, HJ, LS) with severe polymicrogyria (PMG) at 34.098 Mb (NCBI Build 36/hg18 genome assembly) and occurs within the gene for *ITSN1*.

DISCUSSION

We examined a panel of rare CHR21 interstitial deletions with a wide range of physical and brain features (Table 1). Using a number of genetic mapping tools, we fine-mapped the deleted regions and defined the regions of overlap (Table 2). The association of specific overlapping deleted regions and their associated brain abnormalities are summarized in Fig. 1. With regard to central nervous system abnormalities, interstitial deletions can be grouped into three large regions.

The most proximal region encompassing several individuals with large interstitial deletions, surprisingly showed normal cognitive abilities. Despite several physical features such as short stature and bilateral epicanthal folds with a ~ 14Mbp deletion, individual JC had normal cognitive function, although possibly decreased with respect to familial expectation. This individual was previously reported but given fewer molecular probes, his deletion was reported as being somewhat larger [4]. Similar to JC, individual EO with a nearly identical deletion, has normal cognitive abilities and shares with JC, the presence of eczema and seizures. Case RB has a slightly smaller deletion and normal cognitive function, but lacks seizures. Of note, all three are deleted for APP, the amyloid precursor protein. These findings are important for gene-targeted therapies reducing APP abundance and support results in model systems. [12, 13]The mild effects on cognition are consistent with feasibility of reducing overall abundance of APP in humans with AD or Down syndrome-AD. However, in contrast with these proximal deletions, deletions distal to and including *ZNF294 (LTN1*), were not associated with normal cognitive abilities and associated with cortical abnormalities. The human subjects with the most severely abnormal cortical features (Cases HJ, LS, and KS), had deletions that encompassed a small region of 650 kb that includes fewer than a dozen genes. The proximal border of this region is defined by case FB that retains these genes and lacks the cortical features of poly- and pachymicrogyria or lissencephaly. We note that the human candidate region for polymicrogyria found here does not overlap previously reported individuals with deletion 21 and cortical dysplasia suggesting the existence of at least two regions on chromosome 21 that affect brain development [9].

Of the genes in the 650kb region only few are known to be expressed in brain and to be associated individually with brain abnormalities by heterozygous single loss-of-function mutations. *DONSON* variants lead to severe neurodevelopmental and growth disorders (Meier-Gorlin syndrome), but those *DONSON* LOF mutations associated pathologic phenotypes including cognitive dysfunction, are all biallelic [14]. GART and GARS proteins continue to be continuously expressed during postnatal development in the cerebellum of individuals with Down syndrome, unlike in neurotypical brains where expression decreases rapidly after birth. This prolonged overexpression is hypothesized to disrupt normal purine metabolic balance and contribute to the neurological and cognitive phenotypes of DS [15] but single gene variants have not been described. TMEM50B (C21orf4) is expressed in brain and has been nominally associated with human hydrocephalus, but no single gene variants have been reported [16].

The two genes that stand out as candidates for haploinsufficiency effects are *SON* and *ITSN1*. SON is a large Ser/Arg (SR)-related protein and functions as a splicing co-factor and nuclear speckle scaffold protein [17]. ITSN1 is an endocytic scaffold protein that participates in exocytosis and coordinates

endocytic membrane traffic with the actin assembly machinery [19]. In addition, it may regulate the formation of clathrin-coated vesicles and synaptic vesicle recycling. Heterozygous loss-of-function *SON* mutations cause Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome. Individuals with ZTTK syndrome have profound developmental delays, variable dysmorphic features and congenital anomalies, intellectual disabilities, brain malformations including microcephaly, and seizures [18].

Point mutations and truncating mutations of ITSN1 [19, 20] have been shown to cause neurodevelopmental disorders ranging from autism spectrum disorders to intellectual disability. Truncating variants were found in the N-terminal half, whereas missense variants predominated in the C-terminal half. Interestingly, ITSN1 loss-of-function variants are also associated with an increased risk of Parkinson disease and may even appear as mendelian PD gene in some families [21]. Therefore, we speculate that *ITSN1* and SON deletion may contribute to most of the defects. As found in deletions of chromosome 17p, in which codeletion of 14-3-3 increases the severity of *LIS1* cortical dysplasia, the more severe human gyrification anomalies observed in larger deletions of chromosome 21 may reflect combined deleterious effects from loss of ITSN1 and *SON* as well as of neighboring genes including *SYNJ1*, *SON*, and possibly *TIAM1*, all of which are also involved in endocytosis[22, 23]. It is interesting to note that neuronal migration defects and dendritic spine abnormalities are observed in knockout or knockdown of either *Itsn1* or *Son* in the mouse, which may contribute to the significant intellectual disability observed in our cases deleted for both genes [24].

Infants and children with Down syndrome have a low incidence of epilepsy despite cortical dyslaminations and anomalies in gyration/sulcation that, in non-Down patients, would likely be epileptogenic. One explanation for the paucity or absence of seizures in Down syndrome is that beginning at birth a postnatal deletion of terminal dendrites and dendritic spines from the molecular layer results in loss of excitatory glutamatergic synapses of pyramidal neurons without concomitant loss of inhibitory GABAergic axosomatic synapses [28, 29], This progressive change shifts the excitatory/inhibitory ratio in individual pyramidal neurons toward greater inhibition, a genetically programmed neurodegenerative process at the cellular level. Later, in adolescence and early adult life, patients with Down syndrome also suffer another neurogenerative process of Alzheimer disease with neurofibrillary tangles and plaques and deposition of tau protein. Alzheimer disease causes dementia but not epilepsy.

Our cases provide a unique opportunity to observe the effects of combined haploinsufficiency of genes in this critical CHR21 region. Just as with other genetic variants, penetrance and expressivity apply to the phenotypes observed with large interstitial deletions syndromes that reflect differing genetic backgrounds as well as intrauterine and environmental factors. Thus, more cases, especially individuals from varied ancestries, will be needed to confirm our results. (Fig. 1). With these caveats in mind, the results of our study suggest that the relatively minor effects of large proximal deletions of CHR21 including more than a dozen protein-coding genes contrast with more severe phenotypic effects associated with more distal deletions, especially those involving *ITSN1* and *SON*.

Declarations

All patients or their legal guardians consented to participate in the study.

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Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

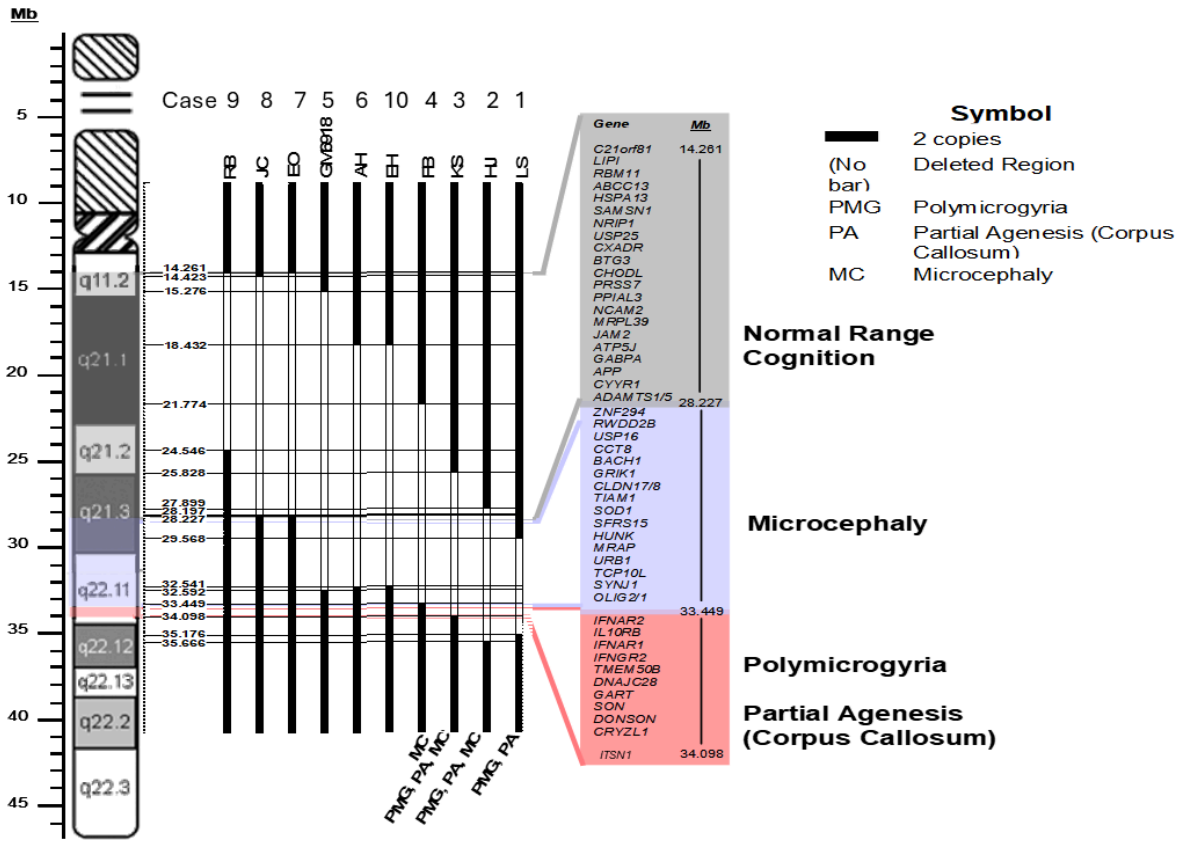


Figure 1

Schematic representation of CHR21 interstitial deletions and CNS phenotypes

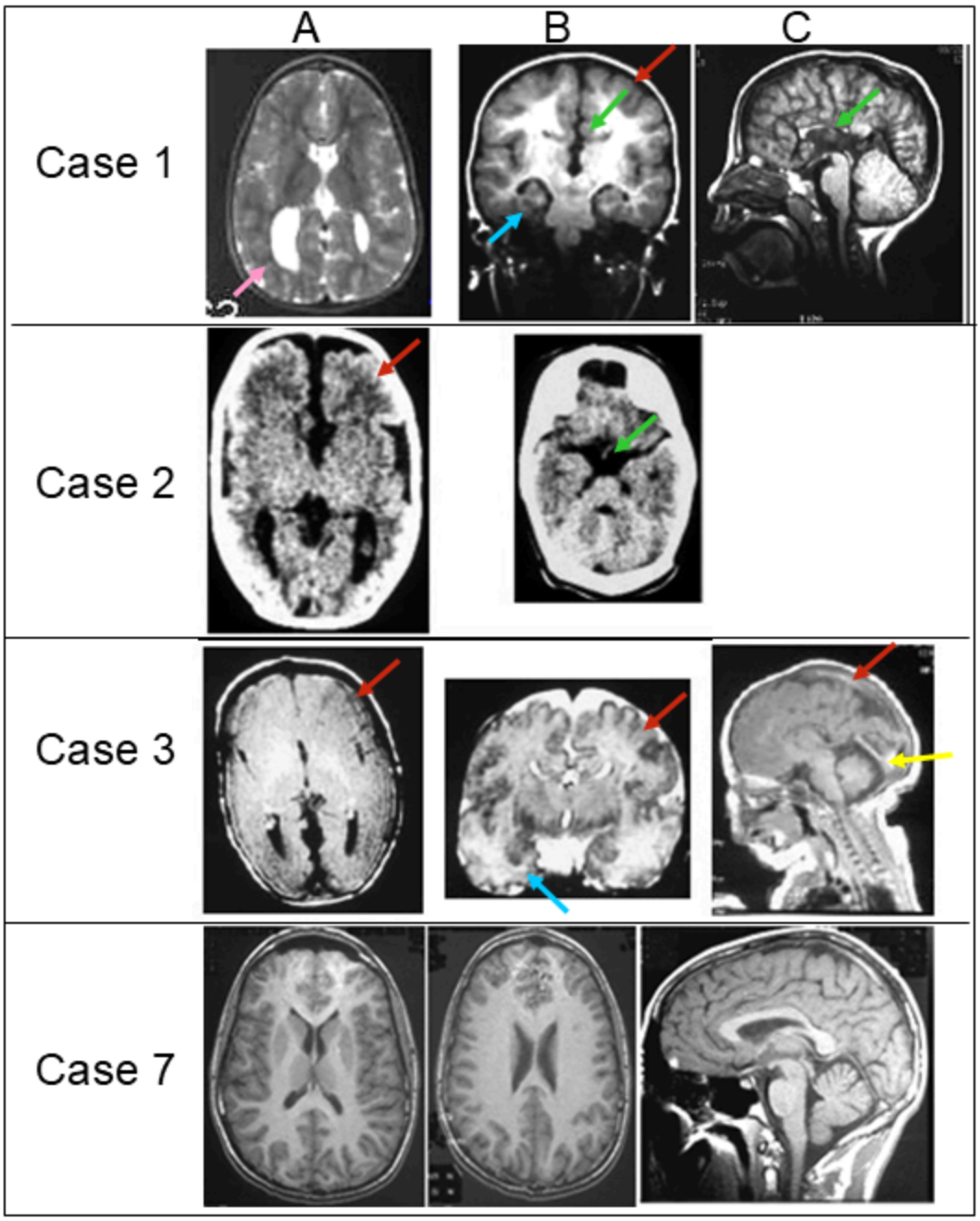


Figure 2

MRI/CT scans from subjects with deletion of chromosome 21. Case 7 (EO) is not deleted for *ITSN1* presents normal T_2-weighted MRI as shown on the bottom panel. Case 1 (T_2-weighted MRI), Case 2 (CT scan), and Case 3 (T_2-weighted MRI) who are deleted for *ITSN1* present brain malformation as shown in Figure 1b, including cortical dysplasia (red arrows); small cerebellum or vermis (yellow arrows), agenesis or dysgenesis of corpus callosum (green arrows), dysgenesis of hippocampus (blue arrows), colpocephaly shown in Case 3, and asymmetric colpocephaly shown in Case 1 (pink arrows).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1PhenotypesCorrected.docx
- Table2.docx
- SupplementaryTable1.xlsx