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SNP array mapping of 20p deletions: Genotypes, Phenotypes and Copy Number Variation

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Abstract

The use of array technology to define chromosome deletions and duplications is bringing us closer to establishing a genotype/phenotype map of genomic copy number alterations. We studied 21 patients and 5 relatives with deletions of the short arm of chromosome 20 using the Illumina HumanHap550 SNP array to 1) more accurately determine the deletion sizes, 2) identify and compare breakpoints, 3) establish genotype/phenotype correlations and 4) investigate the use of the HumanHap550 platform for analysis of chromosome deletions. Deletions ranged from 95kb to 14.62Mb, and all of the breakpoints were unique. Eleven patients had deletions between 95kb and 4Mb and these individuals had normal development, with no anomalies outside of those associated with Alagille syndrome. The proximal and distal boundaries of these eleven deletions constitute a 5.4MB region, and we propose that haploinsufficiency for only 1 of the 12 genes in this region causes phenotypic abnormalities. This defines the *JAG1* associated critical region, in which deletions do not confer findings other than those associated with Alagille syndrome. The other 10 patients had deletions between 3.28Mb and 14.62Mb, which extended outside the critical region, and notably, all of these patients, had developmental delay. This group had other findings such as autism, scoliosis and bifid uvula. We identified 47 additional polymorphic genome-wide copy number variants (>20 SNPs), with 0–5 variants called per patient. Deletions of the short arm of chromosome 20 are associated with relatively mild and limited clinical anomalies. The use of SNP arrays provides accurate high-resolution definition of genomic abnormalities.

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Keywords

SNP array analysis; 20p deletion; copy number variants; Alagille syndrome; haploinsufficiency; JAG1

Introduction

Chromosomal aberrations that are detectable by cytogenetic methods cause a wide variety of developmental abnormalities, which vary with the size and gene content of the aberration (Brewer, et al., 1998). Recent studies using array based platforms allow genomic analysis at much higher resolution, and are now confirming, refining and extending the abnormalities detectable by cytogenetic techniques (Bejjani, et al., 2005a; Bejjani, et al., 2005b; de Vries et al., 2005; Ming, et al., 2006; Shaffer, et al., 2006; Sharp, et al., 2006; Vissers, et al., 2003). Array based studies have also demonstrated that smaller deletions and duplications are present as polymorphisms in the general population (Iafrate, et al., 2004; Redon, et al., 2006; Sebat, et al., 2004; Sharp, et al., 2005; Tuzun, et al., 2005) and a current challenge in the field of molecular cytogenetics is to sort the disease causing abnormalities from the benign.

Deletions of the short arm of chromosome 20 are a relatively rare chromosome abnormality, with less than 35 such patients reported in the literature (Anad, et al., 1990; Byrne, et al., 1986; Dutta, et al., 1991; Garcia-Cruz, et al., 1985; Garcia-Heras, et al., 2005; Kiss and Osztovics, 1988; Krantz, et al., 1997; Laufer-Cahana, et al., 2002; Legius, et al., 1990; Li, et al., 1996; Michaelis, et al., 1997; Oda, et al., 2000; Rand, et al., 1995; Rovet, et al., 1995; Sauter, et al., 2003; Schnittger, et al., 1989; Shohat, et al., 1991; Silengo, et al., 1988; Spinner, et al., 1994; Teebi, et al., 1992; Zhang, et al., 1990). The observation that multiple patients with deletions of 20p12 had the autosomal dominant disorder Alagille syndrome (AGS; MIM# 118450), led to the positional cloning of the AGS disease gene, Jagged1 (*JAG1*; MIM# 601920) (Li, et al., 1997; Oda, et al., 1997). *JAG1* mutations can be identified in about 94% of patients with AGS and deletions of the entire *JAG1* gene are found in 3–6% of patients, while the remainder have intragenic *JAG1* mutations (Crosnier, et al., 1999; Krantz, et al., 1998; Warthen, et al., 2006). Deletions can either be cytogenetically visible or sub-microscopic, diagnosable only by molecular cytogenetic or other molecular level studies (Oda, et al., 2000; Oda, et al., 1997; Pilia, et al., 1999; Rand, et al., 1995). In addition to the clinical features of AGS (cholestatic liver disease, congenital heart defects, ocular and skeletal anomalies and characteristic facial features), individuals with deletions of chromosome 20p have also been reported to manifest other abnormalities. These include developmental delay, hearing loss, autism, brain malformations, cleft lip or palate and spina bifida (Anad, et al., 1990; Krantz, et al., 1997; Michaelis, et al., 1997; Rovet, et al., 1995). Precise characterization of the extent of deletion is a crucial step toward addressing genotype-phenotype correlation and investigating whether common deletion breakpoints are found in multiple patients. A subgroup of chromosomal deletion syndromes have been shown to be associated with genomic architecture that pre-disposes to recurrent deletions (Lupski, 2007), but these have not previously been investigated for deletions of 20p. The studies reported here allow us to define a critical region surrounding the *JAG1* gene on chromosome 20p, deletion of which is associated with AGS, but no additional abnormalities.

We have analyzed the 20p deletions of a group of 21 patients to estimate the extent and the location of their boundaries, and compared these findings with the clinical features manifested by these individuals. We used the Illumina HumanHap550 SNP array platform (Peiffer, et al., 2006) which allowed fine mapping of the breakpoints and subsequent identification of the specific genes deleted in each patient, comparison of the breakpoints

from patient to patient, and correlation of the genomic alterations with clinical features. Our analysis also identified various copy number alterations elsewhere in the genome that appear to be polymorphisms. These studies demonstrate the high resolution and specificity of the HumanHap550 array in identification of clinically significant copy number alterations.

Materials and Methods

Twenty-one patients and 5 relatives with deletions of the short arm of chromosome 20 were recruited into the study, under a protocol of informed consent, which was approved by the IRB of the Joseph H. Stokes Research Institute at The Children's Hospital of Philadelphia. The chromosomal and clinical features of the patients are listed in Table 1. Facial features of 11 probands and the affected father and brother of patient 4 are shown in Figure 1. Although each of these patients has a deletion of at least 850kb (up to 14.62Mb in patient 21), facial features are relatively mild and dysmorphic features are related to the features of AGS in all patients shown (Kamath, et al., 2002). Six patients were identified following chromosome studies requested because of multiple anomalies. These included 5 patients with cytogenetically visible deletions of 20p (patients 17–21), and one patient with a translocation between chromosomes 2 and 20 (patient 5, 46,XX, t(2;20)(q21.3;p12)). The remaining 19 patients came to medical attention due to a clinical diagnosis of AGS, which led to studies to identify mutations or deletion of the *JAG1* gene. This included fluorescence in situ hybridization (FISH) using a BAC containing *JAG1* (Warthen, et al., 2006) or multiplex ligation-dependent probe amplification using a commercially available kit (MRC-Holland, Inc.). The method of deletion detection for each patient is included in Table 1 along with the clinical features of the patients. DNA for SNP array analysis was isolated from peripheral blood lymphoblastoid cell lines, or fixed pellets using the Puregene kit (Gentra Systems, Inc.) according to the manufacturer's instructions. SNP array genotyping was carried out by the Center for Applied Genomics using the Illumina Infinium SNP genotyping platform (HumanHap550 chips and BeadStation Scanner and BeadStudio analysis software). The Human Hap550 chip contains 555,352 SNP probes, distributed with an average interSNP distance of 6 kb. However, the SNPs are not evenly distributed across the genome. On the short arm of chromosome 20, there are 6,846 SNP probes, with an average distance of 4 kb between probes. DNA samples were diluted to 50ng/ul and 750ng of each sample was isothermally amplified to generate sufficient DNA for hybridization. The amplified product was enzymatically fragmented, precipitated and resuspended, and the resulting product hybridized to the HumanHap550 chip overnight. After hybridization and enzymatic extension, the products were fluorescently stained and visualized using the Beadstation scanner and data collection software. The genotyping yield was greater than 99%. Analysis was carried out using the BeadStudio Genotyping module (Illumina, Inc.), and DNA copy number changes were visualized using B-allele frequency and Log_2R ratio. In this study, we report the base pair of the first and last SNP deleted for each patient. Therefore, it is possible that the actual breakpoint lies somewhere between the first or last deleted SNP and the adjacent non-deleted SNP.

Results

Results of the array analysis are provided in Table 2, with examples of Illumina BeadStudio output presented in Figure 2. Overall, the deletions in these patients lay within the region extending from the distal SNP at base pair 3,987,627 through the proximal SNP at base pair 26,257,255 (Build 35) a 22.3Mb region extending from 20p12.3 to 20p11.21 that includes approximately 75 known or predicted genes. The deletions were *de novo* in 12 of the 21 patients, maternally inherited in 2 and paternally inherited in 2, and one or both parents were unavailable for study in 5 cases. Deletion sizes in the inherited cases were 0.85, 1.55, 2.31 and 5.66Mb. The parent with the largest deletion also had mild learning disabilities. The

clinical findings of the 21 patients and 5 affected relatives are summarized in Table 1 and individuals are listed in order of increasing deletion size.

Eleven probands (probands 1–8 and 10–12) had AGS only, and examination of their breakpoints revealed a genomic region that is associated with AGS and no other clinical features. These deletions ranged from 95kb to 4.0Mb in size, and their overlap defined a 5.4Mb genomic region (Figure 3). These 11 individuals all met classic criteria for AGS, namely cholestatic liver disease in association with cardiac, skeletal, and facial characteristics. Two of the probands had renal disease, which is not described as a classic criterion for AGS but which occurs in 30–50% of AGS individuals (Alagille, et al., 1987;Emerick, et al., 1999). Three of these deletions were inherited, and of these parents, one met classic criteria, one was mildly affected and one is a mosaic and clinically unaffected. The mildly affected parent of proband 4 had mild features of AGS (facial features and a history of a heart murmur during childhood, which was diagnosed as aortic and mitral valve anomalies in adulthood, see Figure 1) and interestingly he also had sensorineural hearing loss. The region defined by the most proximal to the most distal deleted nucleotide in these 11 patients (7,383,615–12,746,054) encompasses a 5.4Mb region that includes 12 Reference Sequence genes (RefSeq) as listed in the UCSC Genome Browser (Figure 3). This defines the *JAG1* associated deletion size that does not confer additional clinical findings, outside of those associated with AGS. Of the 12 genes that are located within this region only *JAG1* and the *MKKS* gene have been previously associated with a human disorder. The *MKKS* gene codes for a chaperonin-like protein, and mutations in this gene have been associated with two recessive disorders (McKusick-Kaufman syndrome and Bardet Biedl syndrome). These 2 disorders share hydrometrocolpos and postaxial polydactyly, although the more severe Bardet Biedl syndrome patients go on to develop additional features including retinitis pigmentosum, obesity and learning disabilities (Slavotinek and Biesecker, 2000). Our data is consistent with the hypothesis that the 11 genes in this region (not including *JAG1*) do not cause phenotypic abnormalities when hemizygous (Table 3). We looked at each of these genes to determine if it was covered by a CNV in either the Database of Genomic Variants, or the CHOP database. Five genes were covered by CNVs (TXNDC13, PLCB1, PLCB4, PAK7 and BTBD3), while 6 were not covered (HAO1, C20orf103, ANKRD5, SNAP25, MKKS, C20orf94) (Table 3).

Three probands (15,17 and 18) had deletions that extended distally from the AGS-only region. Patient 15's deletion began 329kb distal to the AGS-only region border (at base pair 7,054,353), and while there were no genes in this region, only 32% of this region was covered by copy number polymorphisms as reported in the Database of Genomic Variants (DGV) (http://projects.tcag.ca/variation/). From a clinical standpoint, this individual had liver, cardiac and facial features consistent with AGS and cognitive developmental delay. Patient 17's deletion extended 3.39Mb distal to the AGS boundary (from base pair 3,987,627) and this is a region with 16 known genes and 2 open reading frames, including *PRNP* (Huntington disease-like-1), a gene associated with human disease. Patient 17 died at 18 months of AGS related complications, so it therefore impossible to assess additional abnormalities. Patient 18's deletion is 1.67Mb distal to the AGS-only region, and this region contains 7 genes and 1 open reading frame, one of which (*FERMT1*) has been associated with autosomal recessive Kindler syndrome; a condition characterized by skin blistering and related dermatologic abnormalities. Patient 18 has AGS with prominent cardiac disease, a bifid uvula and relatively mild developmental delay. The varied phenotypes of these 3 patients do not allow us to identify any specific candidate regions.

Six patients (9, 13, 14, 16, 18 and 21) had deletions that included the *JAG1* gene, but extended proximally beyond the AGS-only region. The distances between the proximal AGS boundary at basepair 12,746,054 and the respective deletions were 914kb for patient 9

(3 known genes and 1 open reading frame), 1.37Mb for patient 13 (5 genes and 2 open reading frames), 449Kb for patient 14 (1 gene and 1 open reading frame) 2.3Mb for patient 16 (6 genes and 2 open reading frames), 1.3Mb for patient 18 (5 genes and 2 open reading frames) and 9.18Mb for patient 21 (29 known genes and 8 open reading frames). Patients 9 and 13 had AGS, cognitive delay and patient 9 had hearing loss. Patient 21 has the largest deletion and not surprisingly has the most extensive abnormalities, which include cleft lip and palate, visual loss, seizures, tethered spinal cord, pseudotumor cerebri, obesity and foot deformities. Again, none of the genes mapping to this region have been associated with a known genetic disorder. Two patients (19 and 20) had large (8.68 and 11.95Mb) deletions that started proximal to the *JAG1* gene, and as expected these patients did not have AGS, although they did have significant other abnormalities. These included significant cognitive developmental delay, autistic features, hearing loss, Hirschprung disease, growth hormone deficiency, and central nervous system anomalies.

In addition to confirming and precisely defining the size and boundaries of the 20p deletions, analysis with the Illumina HumanHap550 array also identified other loci, elsewhere in the genome that varied in copy number. This was expected based on the recent demonstration by multiple groups, that there are a large number of copy number variable regions, which have been shown to include known genes, disease loci, functional elements and segmental duplications (Iafrate, et al., 2004; Sebat, et al., 2004; Sharp, et al., 2005). In this paper, we report copy number variations that contain a minimum of 20 consecutive SNPs, as experimental validation using qPCR and FISH have been 100% successful for all such CNVs that we tested. We have found that copy number variants identified by 20–25 SNPs ranged from 28 to 298 kb, with an average of 90.35 kb. Among the 21 patients we studied, there were from 0–5 CNVs outside of the known 20p deletions (Supplementary Table S1). These variants ranged from 28kb to 2.35Mb, and many of them included known genes, with 0 to 13 genes per variant. There were a total of 47 CNVs identified. The most frequent CNV occurred at 6q14, and was seen as a duplication or a deletion in 10 of the 21 patients (47.6%). Comparison with all copy number variations compiled in the DGV from 44 different studies (http://projects.tcag.ca/variation/) reveals that 5 of the 47 CNVs do not overlap with any known CNVs, 6 CNVs share between 15%–80% overlap with a known copy number variation region, and the remaining 36 CNVs are fully contained within a known copy number variation region. We have conducted a large-scale study to map the location of copy number variants in a cohort of 2,026 healthy individuals genotyped with the Illumina HumanHap550K SNP arrays (Gai et al, submitted 2008). Comparison of the CHOP control database with the DGV, revealed that 68.3% of all CHOP CNVs were reported in the DGV, and conversely, 53.5% of variants in the DGV were found in the CHOP series. Comparison of the 47 CNVs identified in our 20p deletion patients with over 54,000 CNVs (defined by 2 or more SNPs) identified in the 2,206 controls was carried out. Thirty of the 47 CNVs identified in our 20p deletion patients, were fully contained within the CNV regions identified in our control group. Fifteen of the CNVs in our 20p deletion patients had between 2% and 96% overlap with the control CNVs. Two of the 47 CNVs do not overlap at all with any of the controls CNVs in our cohort. These were a 23 SNP duplication on chromosome 17, from basepair 1,658,228 through 1,747,939 (90Kb), which included 2 genes (SMYD4 and RPA1). This region is reported as a copy number variable region in the DGV, with 7/50 individuals studied by de Smith et al. 2007 having a loss of this region (de Smith, et al., 2007). The second region is a 24 SNP deletion on chromosome 4, from basepair 48,027,080 to 48,098,543 (71kb). This region includes the SLAIN2 gene, and no variants including this gene are included in the DGV.

Discussion

We have used genome-wide SNP analysis to study 25 individuals from 21 families with known deletions of the short arm of chromosome 20, in order to precisely map the deletion sizes, establish genotype-phenotype correlations for this region of the genome, and test this SNP platform for delineation of chromosomal abnormalities. Nineteen of the 21 deletions were identified in patients with the autosomal dominant disorder AGS, which is known to be caused by haploinsufficiency for the *JAG1* gene, which maps to 20p12. We were able to size each of the deletions, which ranged from 95kb to 14.62Mb and all of the deletions were interstitial. The breakpoints were variable, indicating there is no rearrangement hotspot as has been seen with some other, more common deletion syndromes. Furthermore, there are no segmental duplications associated with any of these deletions. This lack of common breakpoints speaks against a mechanism of non-allelic homologous recombination between repeated sequences that flank commonly deleted regions, which is implicated in some chromosome deletion or microdeletion syndromes such as the 22q11.2 deletion (Edelmann, et al., 1999; Shaikh, et al., 2000), Angelman syndrome and Prader-Willi syndrome (Amos-Landgraf, et al., 1999), William syndrome (Osborne, 1999) and the Smith-Magenis syndrome (Potocki, et al., 2000). A chromosomal fragile site such as that seen in Jacobson syndrome (deletion 11q23.3) (Jones, et al., 2000) is also unlikely.

The uniqueness of each deletion and the small number of patients deleted for each region makes assignment of phenotypes to specific regions challenging. However, several points that are highly relevant to genetic counseling can be made. Eleven patients had deletions of 4.00 Mb or smaller that included the *JAG1* gene. Each of these patients met the clinical criteria for AGS, but they had no additional features and four of these deletions were inherited. These deletions described a 5.4Mb region taken from the most proximal of the breakpoints to the most distal. Mental and physical development was normal in these patients, indicating that loss of one copy of the 11 genes (besides *JAG1*) in this region is not correlated with clinical abnormalities. It should be noted, of course, that since this is a cross-sectional study, it is possible that some later onset complication may arise as a result of haploinsufficiency of one or more of these genes. Five of these genes were covered by purportedly benign copy number variation, as reported in either the Database of Genomic Variants, or our CHOP in-house database, and six of these genes were not covered by CNVs. The significance of CNV coverage as an indicator for a dosage-associated phenotype will be clarified as the number of samples reported in these databases increases. However, if indeed this lack of phenotype with haploinsufficiency is correct, this provides some estimate of the number of genes whose loss causes a dominant phenotype. It is of interest that estimates from other organisms such as Drosophila, has suggested that the majority of mutations are recessive and therefore do not cause disease when a single copy is deleted (Wilkie, 1994). The finding of a lack of haploinsufficient genes is not surprising given that very few known genetic syndromes have been mapped to 20p besides AGS. Autosomal recessive disorders mapped to this region include McKusick-Kaufman, Bardet Biedl, and Kindler syndrome; and as these are recessive, deletion would not be anticipated to cause these disorders in the absence of a mutant allele on the non-deleted chromosome. Some dominant disorders are mapped to 20p, but these are more likely to be caused by a dominant negative mechanism. These include Creutzfeld-Jakob, Gerstmann-Straussler, Huntington-disease like-1 and fatal familial insomnia, all associated with mutations in the prion protein (PRNP) gene. Additional disorders include thrombophilia, late fetal loss and possible myocardial infarction, which are associated with a defect in thromodulin (*THBD*). Keratoconus is a dominant, variably expressed disorder of the cornea, associated with *VSX1* mutations (although again, there is no evidence that haploinsufficiency would cause disease). Deletions that are proximal or distal to the 5.4Mb AGS-only region can cause additional clinical features including developmental delay. There are 20 RefSeq genes

reported in the distal region, and 39 in the proximal region and at this time there is insufficient information regarding the clinical consequences of loss of these. A more complete understanding will await analysis of additional patients with deletions of 20p, as well as further information on which of these genes is associated with benign copy number variation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Facial features of 11 probands (P) (2P, 4P, 5P, 7P, 9P, 10P, 11P, 12P, 13P, 18P and 21P) and the affected father (F) (4F) and sibling (S) (4S) (brother) of proband 4.

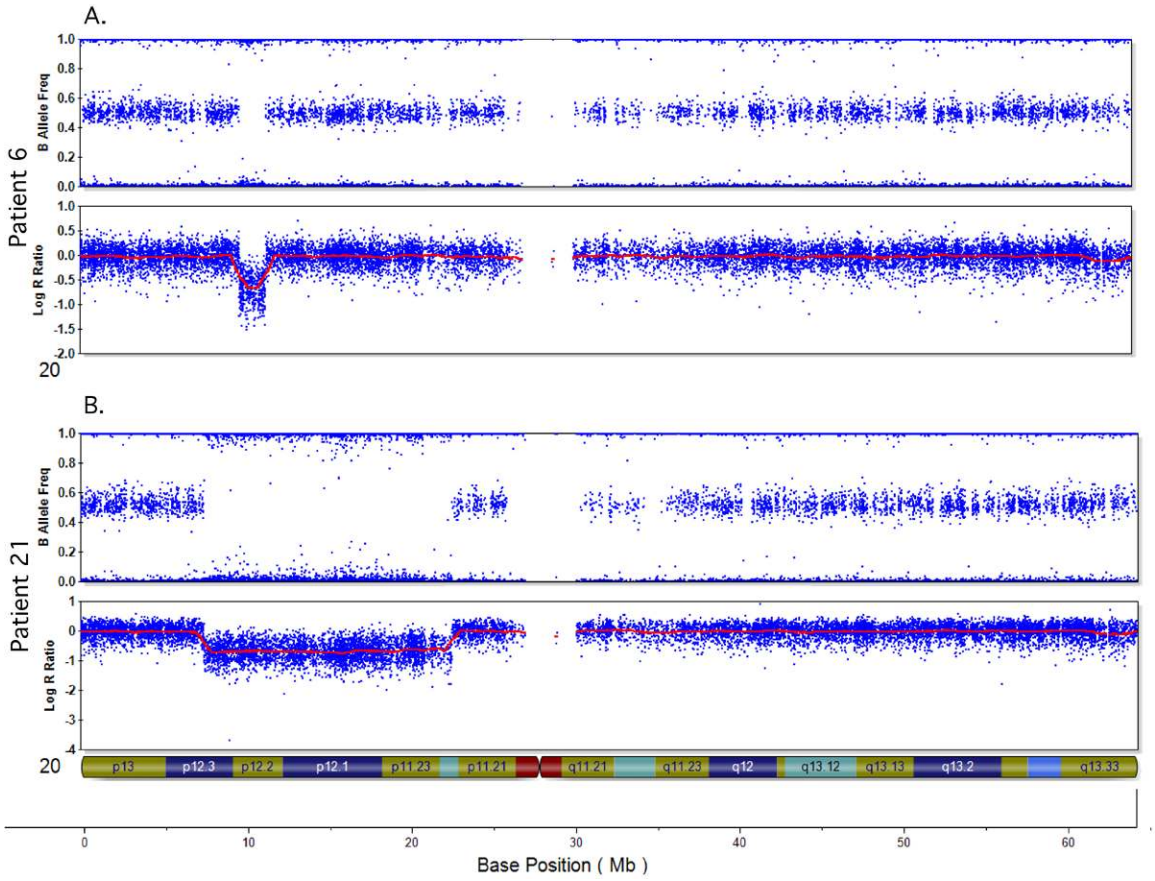

Figure 2.

Representative Illumina BeadStudio output from 2 patients with 20p deletions. Panel 2A shows patient 6, who demonstrates a 576 SNP, 2.4 Mb deletion, which can be seen as a loss of heterozygosity in the B allele frequency track, and a decreased Log_2R ratio in the lower track. Panel 2B shows a larger deletion in patient 21 (3,987 SNP, 14.6Mb), which can also be seen in both the B allele frequency and Log_2R ratios.

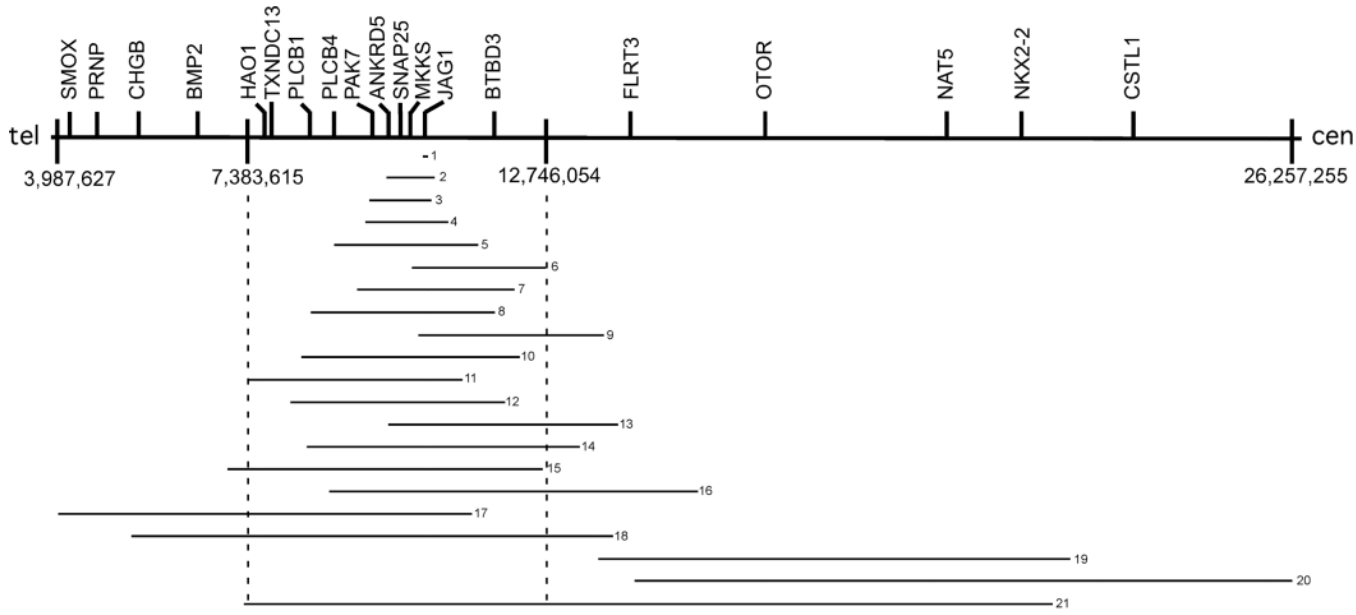

Figure 3.

Map of the 20p deletions from each of the patients studied. A map of selected genes from 20p is presented along the top, and the extent of the deletions is represented by a line. The dotted lines indicate the boundaries of the "Alagille only" critical region. Deletions that are fully within these boundaries do not appear to be associated with clinical features outside of those seen in Alagille syndrome.

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TABLE 1

Ascertainment of deletions and overview of clinical features

Patient	Deletion detection	AGS features	Cognitive Delay	Autistic Features	Hearing Loss	Other
1	MLPA	L, H, V, F	-	-	-	-
2	FISH	L, H, E, F	-	-	-	-
2-Mother	FISH (mosaic)	-	-	-	-	-
3	FISH	L, H, V, F, R	-	-	-	-
4	FISH	L, H, F	-	-	-	-
4-Father	FISH	H, F	-	-	YES	-
4-Sibling	FISH	L, H, F	-	-	-	-
5	46,XX,t(2;20)	L, H, V, E, F, R	-	-	-	-
5-Father	46,XY, t(2;20)	L, H, F	-	-	-	-
6	FISH	L, H, V, E, F	-	-	-	-
7	MLPA	L, H, V, E, F	-	-	-	-
8	FISH	H, E, F	-	-	-	-
9	FISH	L, H, E, F	YES	-	YES	-
10	FISH	L, H, V, E, F, R	-	-	-	-
11	FISH	L, H, E, F	-	-	-	-
12	MLPA	L, H, V, E, F	-	-	-	-
13	FISH	L, H, V, F	YES	-	-	-
14	MLPA	L, H, E, F	YES	-	-	-
15	FISH	L, H, E, F, R	YES	-	-	Scoliosis
15-Mother	FISH	H	YES	-	-	Scoliosis, pes planus, pectus excavatum
16	FISH	L, H, V, F, R	YES	-	YES	-
17	46, XX, del(20)(p11.23p12)	L, H, V, E, F	YES	-	-	-
18	46, XX, del(20)(p11.23p12)	L, H, V, E, F	YES	-	-	Bifid uvula
19	46, XY, del(20)(p11.22p11.23)	Not AGS	YES	YES	YES	Short segment Hirschprung disease, 4th cranial nerve palsy

Patient	Deletion detection	AGS features	Cognitive Delay	Autistic Features	Hearing Loss	Other
20	46,XY, del(20)(p11.1p12.2)	Not AGS	YES	YES	YES	Empty sella on CT scan, Growth hormone deficiency
21	46,XY, del(20)(p11.1p12.3)	H, V, E, F	YES	-	YES	Pierre Robin sequence, Cleft lip and palate, visual loss, seizure disorder, tethered spinal cord, pseudotumor cerebrii, obesity, obstructive sleep apnea, vertical talus deformity

Key: AGS Features: L=liver involvement, H=heart manifestations, V=vertebral anomalies, E=eye features, R=renal involvement, F=characteristic facies

MLPA=Multiplex Ligation-dependent Probe Amplification; FISH = Fluorescence *in situ* Hybridization

TABLE 2

20p deletion size by SNP array analysis

ID#	Origin	Distal end*	Proximal end*	SNPs	Size (Mb)
1	Unknown	10,508,458	10,604,087	23	0.095
2	Maternal	9,867,383	10,717,877	224	0.85
3	Unknown	9,510,922	10,691,024	355	1.18
4	Paternal	9,473,601	11,027,205	473	1.55
5	Paternal	9,246,889	11,558,454	659	2.31
6	de Novo	10,308,051	12,746,054	576	2.44
7	de Novo	9,452,468	12,280,526	743	2.83
8	de Novo	8,813,006	11,844,206	809	3.03
9	Unknown	10,380,766	13,660,393	807	3.28
10	de Novo	8,521,437	12,366,350	1,024	3.84
11	de Novo	7,383,615	11,268,829	1,125	3.89
12	de Novo	8,044,130	12,044,897	1,129	4.00
13	Unknown	9,952,874	14,120,443	1,015	4.17
14	Unknown	8,756,310	13,195,568	1,206	4.44
15	Maternal	7,054,353	12,717,237	1,516	5.66
16	de Novo	9,039,235	15,011,679	1,518	5.97
17	de Novo	3,987,627	11,390,419	2,120	7.40
18	de Novo	5,709,024	14,014,276	2,221	8.31
19	de Novo	13,566,382	22,251,261	2,338	8.68
20	de Novo	14,300,641	26,257,255	2,904	11.96
21	de Novo	7,318,428	21,933,498	3,987	14.62

*all position coordinates are stated according to Human Genome Build 35.

Table 3

Genes in 5.4 Mb AGS only region

Genes in AGS-Only Region	Associated Phenotype	Inheritance of Phenotype if Known	Covered by CNVs in CHOP Database	Covered by CNVs in DGV*
HAO1	None		No	No
TXNDC13	None		Yes	No
PLCB1	None		Yes	Yes
PLCB4	None		Yes	Yes
C20orf103	None		No	No
PAK7	None		No	Yes
ANKRD5	None		No	No
SNAP25	None (questionable association with ADHD**) (Thapar et al. 2005)		No	No
MKKS	McKusick Kaufman syndrome, Bardet Biedl Syndrome	Recessive	No	No
C20orf94	None reported		No	No
JAG1	Alagille syndrome	Dominant	No	No
BTBD3	None		Yes	No

*DGV = Database of Genomic Variants (http://projects.tcag.ca/variation/)

**ADHD=attention deficit hyperactivity disorder