

ARTICLE

PIAS4 is associated with macro/microcephaly in the novel interstitial 19p13.3 microdeletion/microduplication syndrome

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Array comparative genomic hybridization (aCGH) is a powerful genetic tool that has enabled the identification of novel imbalances in individuals with intellectual disability (ID), autistic disorders and congenital malformations. Here we report a 'genotype first' approach using aCGH on 13 unrelated patients with 19p13.3 submicroscopic rearrangement (11 deletions and 2 duplications) and review cases in the literature and in public databases. Shared phenotypic features suggest that these patients represent an interstitial microdeletion/microduplication syndrome at 19p13.3. Common features consist of abnormal head circumference in most patients (macrocephaly with the deletions and microcephaly with the duplications), ID with developmental delay (DD), hypotonia, speech delay and common dysmorphic features. The phenotype is associated with at least a ~0.113 Mb critical region harboring three strong candidate genes probably associated with DD, ID, speech delay and other dysmorphic features: *MAP2K2*, *ZBTB7A* and *PIAS4*, an E3 ubiquitin ligase involved in the ubiquitin signaling pathways, which we hypothesize for the first time to be associated with head size in humans.

European Journal of Human Genetics (2015) 23, 1615–1626; doi:10.1038/ejhg.2015.51; published online 8 April 2015

INTRODUCTION

Chromosome 19 has the highest gene density of the human chromosomes.¹ However, only a few disease-associated microdeletion/duplication regions have been described.^{2–11} On the short arm, microdeletion/duplication syndromes have been proposed for 19p13.13,² 19p13.12³ and terminal 19p13.3 microdeletions.⁴ Pure terminal 19p13.3 duplications⁷ have also been described as syndromic entities.

High-resolution microarray comparative genomic hybridization (aCGH) is a powerful genetic tool implemented as a first-tier test for diagnosis of genomic imbalances.^{12,13} A 'genotype first' approach, in which patients are characterized by a similar genomic rearrangement before a common clinical presentation is observed, has proven to be successful in characterizing the growing list of microdeletion/

duplication syndromes. In fact, using this experimental approach, we and others recently described new microdeletion or microduplication syndromes.^{14–17}

Here we report 13 new patients with proximal 19p13.3 submicroscopic rearrangements (11 deletions and 2 duplications) and review patients from the literature (14 cases; 13 deletions and 1 duplication)^{18–22} and public genomic databases such as DECIPHER and ISCA Consortium (10 cases; 6 deletions and 4 duplications) for a total of 37 cases. We describe the phenotypic findings and suggest that these patients represent a new microdeletion/duplication syndrome at 19p13.3, with a 113.5 Kb critical region harboring three genes: *ZBTB7A*, *MAP2K2* and *PIAS4*, the latter being a candidate gene for abnormal head size.

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Received 12 October 2014; revised 23 December 2014; accepted 17 February 2015; published online 8 April 2015

MATERIALS AND METHODS

Individuals

Patients 1–4 were referred for genetic assessment and patients 5–9 and 12–13 for a customized aCGH analysis to the Institute of Medical and Molecular Genetics at the University Hospital La Paz in Madrid (Madrid, Spain). Patients 5, 7 and 9–13 were originally ascertained following referral for clinical aCGH testing to Signature Genomics, Spokane, WA, USA. The clinical investigations were performed according to the guidelines in the Declaration of Helsinki. Informed consent was obtained from all family members and specific permission to publish photographs was obtained. The studies were approved by the IRB of both institutions. All data reporting variants/phenotypes linked to all rearrangements described herein have been submitted to DECIPHER public data base.

aCGH, fluorescence *in situ* hybridization and parent-of-origin analysis with short tandem repeats

The aCGH experiments were performed using previously described methods.^{23–26} Details on the used arrays and specific techniques are given in Supplementary Materials. FISH studies²⁷ and microsatellites analyses were performed following standard procedures.

Detection of breakpoints and junction fragment analysis with long-range PCR and Sanger sequencing

To determine the sequence at the breakpoints for some 19p13.3 deletions (patients 1–7, 9, 12 and 13), we designed a different set of primers (available upon request); according to our custom 19p13.3 aCGH results and followed previously described methods.¹⁶

RESULTS

Clinical data

Clinical and phenotypic findings of all 13 individuals are listed in Table 1, and the facial phenotypes of some patients are shown in Figure 1 (patients 1, 3–6 and 13). Table 1 also analyzes the frequency of several phenotypic features, and compares with previously reported cases (reviewed in Table 2 and references^{18–22}). Among them, developmental delay (DD), abnormal head size, speech delay, intellectual disability (ID), feeding problems, hypotonia and other dysmorphic features were the most prevalent, present in most deletion cases. Individuals with duplications share many of these main phenotypic features. Unique traits only reported with microdeletion include ophthalmologic alterations, gastroesophageal reflux, sleep disorders, congenital heart disease, and in a lower incidence, behavioral disorders such as autism or aggressive behavior. Distinct recognizable facial features, including a short philtrum, thin upper lip, abnormal ears, wide nasal bridge, depressed nose and root, downslanting palpebral fissures and hypertelorism were apparent in many patients (see Figure 1 and Tables 1 and 2). Ocular anomalies included strabismus, amblyopia, astigmatism and myopia. Congenital heart disease, such as atrial septal defect (the most frequent), dilated aorta, tetralogy of Fallot, bicuspid aortic valve, prominent aortic root and patent ductus arteriosus were observed. In addition, six out of nine patients, including both individuals with duplication, showed proportionate short stature, and three others had a postnatal growth pattern above the mean. In total, 9/13 showed some height alteration. Interestingly, head circumference was abnormal in all but two patients (patients 6 and 10). Head circumference had an almost complete phenotype–genotype correlation among our and previously reported patients: 21/24 patients with deletion have macrocephaly and 2/3 patients with duplication have microcephaly. In fact, macrocephaly (OFC > 97th centile) or microcephaly (OFC < 3rd centile) was observed at birth in patients with 19p13.3 genomic rearrangements and remained a medical concern throughout their lives.

Additional cases (without full clinical information available) with genomic rearrangements at this region and published in public databases, such as DECIPHER and ISCA consortium, were summarized in Supplementary data (Supplementary Table S1).

aCGH studies

Genomic rearrangements within 19p13.3 were demonstrated by aCGH in 13 patients (see Supplementary Figure S1 and Table 3 for genomic coordinates in hg19; NCBI build 37). Eleven had deletions, ranging in size from 151 kb (patient 11) to 1.70 Mb (patient 7), and two had duplications, 1.48 Mb (patient 10) and 2.39 Mb (patient 8). In all but three (patients 6, 9 and 13), the 19p13.3 deletion was the sole significant aberration (Table 3). In patient 6 we also observed a *de novo* 0.226 Mb deletion at 19q13.2, and patients 9 and 13 showed two genomic rearrangements within 19p13.3 separated by a normal region (see supplementary data Supplementary Figure S3). FISH and/or other aCGH assays confirmed the initial aCGH results (data not shown). Most of the deletions/duplications have different breakpoints, although the breakpoints in some cases are in close proximity (Figure 2 and Supplementary Figure S1 and Table 3). When parental samples were available, the rearrangements were found to be *de novo* (by means of either FISH or aCGH, see supplementary data).

Among individuals with interstitial 19p13.3 rearrangements (sharing characteristic features of abnormal head size, DD, speech delay, hypotonia and dysmorphic features) the shortest region of overlap (SRO) in our series is delineated distally and proximally by patient 11 with estimated breakpoints at genomic positions chr19:3979568–4131259; hg19; NCBI build 37 (~150 kb; Figure 3a). This SRO is shared by 11 of 13 patients (excluding patient 6 partially and patient 10 totally, both of whom have normal head size) and by 13/14 patients in previously reported cases.^{18–22} This segment of ~150 kb includes one microRNA (*SNORD37*) and four RefSeq genes: *PIAS4*, *ZBTB7A*, *MAP2K2* and partially *EEF2*. Review of additional cases included in the literature and public databases such as DECIPHER and ISCA Consortium (with full clinical data) allowed us to narrow this SRO to a 113.5 kb segment (chr19:3979568–4093035; hg19; NCBI build 37) and excluded *EEF2* (Figure 2). Thus, 31/37 individuals shared this SRO and others overlapped it partially (case 4 in reference,²² patient 6 in our series and DECIPHER case 271675; Figure 2). On the other hand, three cases did not share the SRO (patient 10 in our series and DECIPHER cases 259222, 255689), although they shared some clinical findings, such as ID, wide nasal bridge, narrow forehead or vesicoureteral reflux, with the SRO's patients.

Characterization of mechanisms underlying interstitial 19p13.3 genomic rearrangements

We initially designed a custom aCGH with a significant coverage of 19p13.3 (chr19:1477536–6653608, hg19). Further, long-range PCR and automated sequencing were performed to precisely define the breakpoints in some cases (patients 1–7 and 12, Figure 3; patients 9 and 13, data not shown), followed by bioinformatic analysis in both breakpoints through several web tools showing that those lay next to highly homologous repetitive sequences of SINE or LINE elements, which could mediate those rearrangements directly. Results of repeat element analyses, breakpoints and sequences involved are summarized in Table 4, and showed in detail in supplementary data (Supplementary Figure S2). In addition, we also showed analysis of known repeat and genomic architectural elements, such as palindromic DNA or stem-loop structures within the deleted breakpoints that may also modulate in those non-recurrent genomic rearrangements (Supplementary Tables S2 and S3).

Table 1 Patients reported herein with 19p13.3 rearrangements

| Patients | Patient 1 | | Patient 2 | | Patient 3 | | Patient 4 | | Patient 5 | | Patient 6 | | Patient 7 | | Patient 8 | | Patient 9 | | Patient 10 | | Patient 11 | | Patient 12 | | Patient 13 | | Subtotal | Frequency, all cases ^a | Total (%) |
|---------------------------------|-----------|----------|-----------|----------|-----------|------------------------|------------------------|------------------------|-----------|----------|-----------|----------|-----------|----------|-----------|----------|-----------|----------|------------|----------|------------|----------|------------|----------|------------|----------|----------|-----------------------------------|------------|
| | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | 11del/2dup | 24del/3dup |
| Type of rearrangement | Y | Y | Y | Y | Y | ± | Y | Y | Y | Y | ± | Y | Y | Y | Y | Y | Y | Y | N | N | Y | Y | Y | Y | Y | Y | 11/13 | 24/27 | |
| Included in our SRO | Y | Y | Y | Y | Y | N | Y | Y | Y | Y | N | Y | Y | Y | Y | Y | Y | Y | N | N | Y | Y | Y | Y | Y | Y | 11/13 | 24/27 | |
| Includes P/AS4 | Y | Y | Y | Y | Y | N | Y | Y | Y | Y | N | Y | Y | Y | Y | Y | Y | Y | N | N | Y | Y | Y | Y | Y | Y | 11/13 | 24/27 | |
| Gender | F | F | F | F | F | M | F | F | M | M | M | M | M | F | F | F | F | F | F | F | F | F | F | F | F | F | 8F/5M | 15F/12M | |
| Age diagnosis | 7 yr | 9 yr | 10 yr | 5 yr | 3 yr | 5 yr | 5 yr | 5 yr | 3 yr | 3 yr | 5 yr | 5 yr | 5 yr | 6 yr | 10 yr | 10 yr | 10 yr | 10 yr | 6 yr | 6 yr | 6 yr | 6 yr | 6 yr | 6 yr | 6 yr | 6 yr | 3/13 | 4/14 | |
| Growth and develop | + | + | + | + | + | + | + | + | + | + | + | + | + | ++ | + | + | + | + | + | + | + | + | + | + | + | + | 13/13 | 27/27 | |
| Psychom develop delay | Mild | + | Mild | Mild | + | + | + | + | + | + | + | + | + | Mild | + | + | + | + | Mild | Mild | + | + | + | + | + | 13/13 | 26/26 | | |
| Intellectual disability | + | + | + | + | + | ++ | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 10/10 | 19/19 | |
| Speech delay | + p>97 | + p>97 | + p>97 | + p>97 | + p>97 | Normo | Normo | Normo | + | + | + | + | + | -4,4SD | + | + | + | + | + | + | + | + | + | + | + | 11/13 | 25/27 | | |
| Macro-microcephaly | + | + | + | + | + | relative | relative | relative | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 92/59 | | |
| Overgrowth synd testing | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 28/57 | | |
| Proportionate short stature | + | - | + | + | - | + | + | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | 8/11 | 72/72 | |
| Face | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 9/10 | 17/18 | |
| Hypertelorism | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3/9 | 12/20 | |
| Downslanting palpebral fissures | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2/20 | down | |
| Short palpebral fissures | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0/3 | 2/5 | |
| Ptosis | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1/9 | 4/12 | |
| Epicantal folds | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 3/9 | 5/11 | |
| Wide nasal bridge | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 10/10 | 19/19 | |
| Depressed nose and root | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 7/10 | 9/13 | |
| Philtrum anomalies | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | Short | 5/9 | 10/14 | |
| Thin upper lip | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 7/9 | 16/19 | |
| Ear anomalies | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 8/10 | 17/19 | |
| High or prom forehead | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 11/11 | 24/24 | |
| Neurology | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 12/12 | 21/25 | |
| Hypotonia | Timid | - | - | - | - | Aggressive/hyperactive | Aggressive/hyperactive | Aggressive/hyperactive | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 5/9 | 6/10 | |
| Behavior | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1/13 | 4/17 | |
| Hearing problems | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0/9 | 3/22 | |
| Others | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0/9 | 3/22 | |
| Cutis aplasia | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2/4 | 3/5 | |
| Urinary reflux | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4/4 | 13/14 | |
| Gastroesophageal reflux | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 6/6 | 13/15 | |
| Abnormal fingers/toes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 6/9 | 14/22 | |
| Feeding problems | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 6/9 | 7/10 | |
| Ophthalmologic abnormalities | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 6/9 | 7/10 | |
| Umbilical hernias | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 4/4 | 6/12 | |
| Sleeping disorders | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3/3 | 10/13 | |
| Heart disease | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 7/11 | 15/24 | |

Abbreviations: develop, development; F, female; M, male; Macro, macrocephaly; mo, months; N, no; NA, not applicable; NB, newborn; Normo, normocephaly; p, percentile; prom, prominent; psychom, psychomotor; synd, syndrome; Y, yes; yr, years. A frequency over 75% is marked in bold. +, feature present; -, feature absent; ++, feature severe.

^aIncludes 14 cases from the literature (13 deletions and 1 duplication, Table 2).

DISCUSSION

We delineate, review and refine recently described overlapping interstitial deletions/duplications within proximal 19p13.3 (genomic coordinates: 2329320–4996928; genome assembly hg19, NCBI build 37), showing a consistent genotype–phenotype correlation of 13 novel patients, using a ‘genotype first approach’. Thus, we propose a novel interstitial microdeletion/duplication syndrome at 19p13.3, centromeric to the 19p13.3 terminal microdeletions and microduplications.^{6,7}

Both deletions and duplications of this region result in some common, non-specific features present in many other microdeletion/duplication syndromes. Available clinical data presented here, together with cases in the literature^{8–22} and databases, demonstrate several consistent phenotypic findings for this interstitial 19p13.3 deletion: macrocephaly, typically combined with prominent forehead and bi-temporal narrowing; facial dysmorphic features such as hypertelorism, depressed nasal bridge and nasal root, short philtrum, thin upper lip and ear anomalies; and developmental and speech delay and ID. Similarly, all patients with duplications of the same region consistently showed microcephaly, dysmorphic facial features (wide nasal bridge, depressed nasal root and hypertelorism), feeding problems in infancy, DD and ID, although dysmorphic features and DD/ID were less severe than in the reciprocal deletions.

Genomic context, deletion size and genes implicated

The SRO for most cases (31 out of 37) presented here is a 113.5 kb region harboring three genes: *PIAS4*, *ZBTB7A* and *MAP2K2* (Figure 2). Although patient 6 (who partially shared the SRO), patient 10 and DECIPHER cases 255689 and 259222 did not share the SRO, they were included in this study because they share many clinical features with patients with deletion or duplication of the SRO. The three SRO genes are involved in diverse functions, including transcription, histone deacetylation and gene translation; *ZBTB7A*

(zinc finger and BTB domain containing 7A) is a transcriptional repressor belonging to the POK (POZ/BTB) family involved in adipogenesis. *ZBTB7A* may have an important role in neuronal development,²⁸ thus deletion of *ZBTB7A* might contribute to the DD in our patients. In addition, other similar members of this protein family, such as *ZBTB20*, *ZBTB38* and *ZBTB18*, are strong candidates for the DD in a new microdeletion syndrome at 3q13¹⁷ and have been associated with human height in GWAS studies²⁹ or with several features in 1q43-q44 microdeletion syndrome,³⁰ respectively. Dominant gain-of-function mutations of *MAP2K2* (mitogen-activated protein kinase kinase 2) cause cardiofaciocutaneous syndrome,³¹ and recently, haploinsufficiency of *MAP2K2* has been suggested as a new model of RASopathy in a series of seven patients with deletions.²² Finally, *PIAS4* (protein inhibitor of activated *STAT4*) encodes a RING finger (RNF) protein, which interacts with the androgen receptor (AR). *PIAS4* is also an E3 ligase involved in ubiquitin signaling pathways.³² Additional cases (showing almost the same phenotype) that either partially overlapped or did not include this 113.5 kb SRO region suggest that part of the phenotypic features could be explained by a ‘position effect’ or by additional genes close to this SRO (Figure 2). Therefore we hypothesize that the interval from *TLE2* to *CREB3L3* within 19p13.3, a highly conserved region among different species^{33,34} with a rich content in haploinsufficient genes (with 16/55 with a high likelihood of being haploinsufficient),³⁵ as a critical region responsible for most clinical features. Additional genes that may contribute to clinical features are (from centromere to telomere): *EEF2*, *DAPK3*, *NMRK2*, *ATCAY* and *NFIC*, among others (Figure 2), which are involved in cognitive impairment,³⁶ regulation of myogenic differentiation,³⁷ apoptosis and transcriptional regulation of canonical Wnt/ β -catenin signaling,³⁸ and autosomal recessive Cayman cerebellar ataxia,³⁹ respectively. *NFIC* is a member of the nuclear factor I (NFI) gene family necessary for optimal cellular gene expression,⁴⁰ similar to

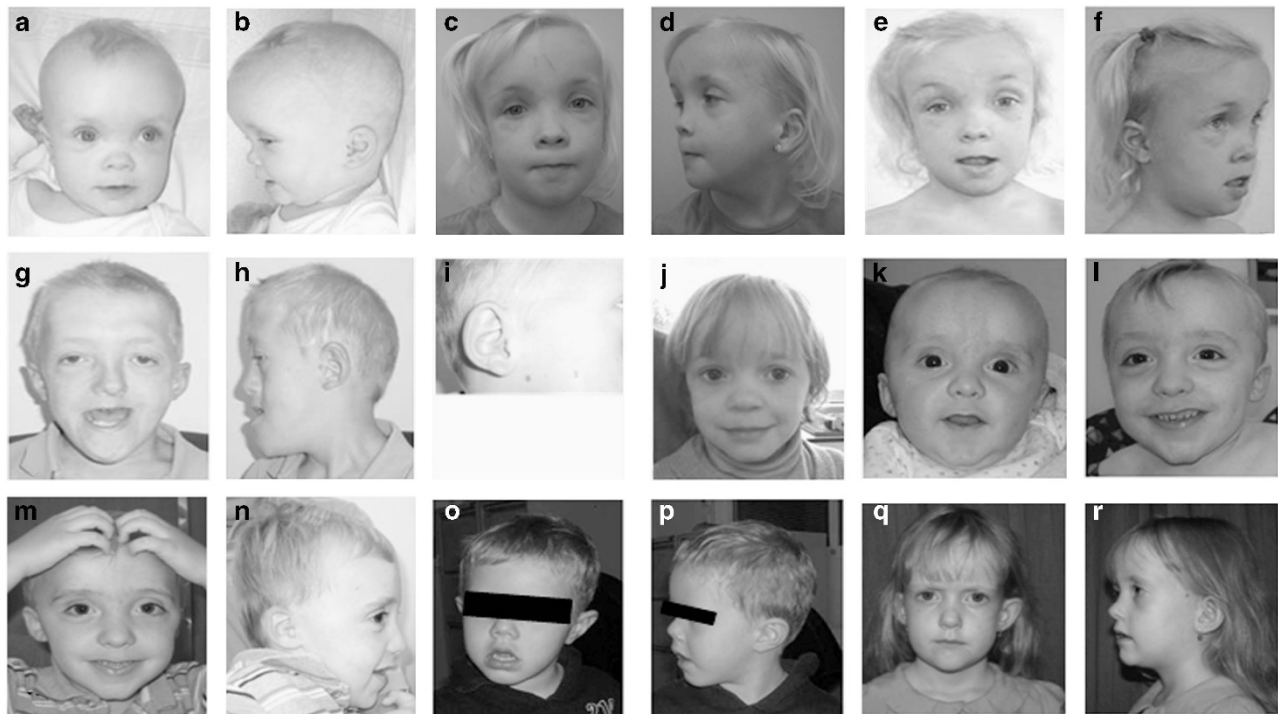


Figure 1 Facial photographs of individuals presented in this study. Patient 1 at age 8 months (a, b), 4 years (c, d) and 7 years (e, f); patient 3 at age of diagnosis, 10 yrs (g, h, i); patient 4 at age 5 years (j); patient 5 at age 7 months (k), 2 years (l) and 4 years (m, n); patient 6 at age 5 years (o, p); patient 13 at age 8 years (q, r).

Table 2 Patients reported in the literature with interstitial 19p13.3 rearrangements

| Patients | Al-Kateb <i>et al</i> , ¹⁸ | | De Smith <i>et al</i> , ¹⁹ | | Sigberg <i>et al</i> , ²¹ | | Rishg <i>et al</i> , ²⁰ | | Rishg <i>et al</i> , ²⁰ | | Nowaczyk <i>et al</i> , ²² | | Nowaczyk <i>et al</i> , ²² | | Nowaczyk <i>et al</i> , ²² | | Nowaczyk <i>et al</i> , ²² | | Frequency |
|--|---------------------------------------|--------------------------|---------------------------------------|-------------|--------------------------------------|----------------|------------------------------------|----------|------------------------------------|----------|---------------------------------------|----------|---------------------------------------|----------|---------------------------------------|----------|---------------------------------------|------------|----------------|
| | Deletion | Deletion | Deletion | Duplication | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | |
| Included in our SRO Includes PI/AS4 | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | ± | Y | Y | Y | Y | Y | 13del/1 dup |
| Gender | F | F | F | M | M | F | F | F | F | F | M | M | F | F | M | F | M | M | 7F/7M |
| Age at diagnosis | 4 yr | 17 yr | 2 yr | 9 yr | 9 yr | 23 yr | 2 yr | 2 yr | 6 yr | 6 yr | 10 yr | 5.9 yr | 3 yr | 4 yr | 6 yr | 2 mo | 5 yr | 2 mo-23 yr | |
| <i>Growth and develop</i> | | | | | | | | | | | | | | | | | | | |
| Psychom develop delay | + | Mild | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 14/14 |
| Intellectual disability | + | + | + | Mild | + | + | + | + | + | + | + | + | Mild | ++ | ++ | ++ | ++ | ++ | 13/13 |
| Speech delay | + | + | + | + | + | + | + | + | + | + | ++ | + | + | + | + | + | + | + | 9/9 |
| Macro-/microcephaly | +3SD | +4.5SD | +3SD | Micro | Micro | Macro relative | + | + | Macro | Macro | +2.5SD | -3.5SD | -2.5SD | +3.5SD | +5.5SD | +1SD* | +3.5SD | +3.5SD | 14/14 |
| Obesity | | + | | | | | | | | | | | | | | | | | 1/1 |
| Proportionate short stature | | - | | | | | | | | | | | | | | | | | 0/1 |
| Overgrowth synd testing | | + | | | | | | | | | | | | | | | | | 1/1 |
| <i>Face</i> | | | | | | | | | | | | | | | | | | | |
| Hypertelorism | + | | | | | | | | | | | | | | | | | | 8/8 |
| Downslanting palpebral fissures | + | Upslant | + | | | | | | | | | | | | | | | | 9/11down |
| Short palpebral fissures | | | | | | | | | | | | | | | | | | | 1/11 up |
| Ptoisis | | | | | | | | | | | | | | | | | | | 2/2 |
| Epicanthal folds | + | | | | | | | | | | | | | | | | | | 3/3 |
| Wide nasal bridge | + | + | | | | | | | | | | | | | | | | | 2/2 |
| Depressed nose and root | - | - | | | | | | | | | | | | | | | | | 9/9 |
| Philtrum anomalies | Prominent | Short | Short | Short | Short | Hypoplastic | Short | Short | Flat | Flat | | | | | | | | | 1/3 |
| Thin upper lip | Tented | + | + | + | + | | + | + | | | | | | | | | | | 5/5 |
| Ear anomalies | + | Dysplastic | + | + | + | + | + | + | + | + | + | + | ++ | ++ | + | + | + | + | 9/10 |
| High or prom forehead | + | + | + | + | + | + | + | + | + | + | ++ | ++ | + | + | + | + | + | + | 9/9 |
| <i>Neurology</i> | | | | | | | | | | | | | | | | | | | 13/13 |
| Hypotonia | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | 9/13 |
| Behavior | | Self injury/aggressivity | | | | | | | | | | | | | | | | | 1/1 |
| Hearing problems | Mild loss | - | Minor problems | | | | | | | | | | | | | | | | 3/4 |

Table 2 (Continued)

| Patients | Al-Kateb et al, ¹⁸ | De Smith et al, ¹⁹ | Siggeberg et al, ²¹ | Siggeberg et al, ²¹ | Risheg et al, ²⁰ | | Risheg et al, ²⁰ | | Risheg et al, ²⁰ | | Risheg et al, ²⁰ | | Risheg et al, ²⁰ | | Risheg et al, ²⁰ | | Frequency |
|------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|-----------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|---|-----------|
| | | | | | Pat1 | Pat2 | Pat3 | Pat1 | Pat2 | Pat3 | Pat4 | Pat5 | Pat6 | Pat7 | | | |
| Others | | | | | | | | | | | | | | | | | |
| Cutis aplasia | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | 1/1 |
| Urinary reflux | | | | | + | + | + | + | + | + | + | + | + | + | + | + | 3/13 |
| Gastroesophageal reflux | + | - | | | | | | | | | | | | | | | 1/1 |
| Feeding problems | + | - | + | | | | | | | | | | | | | | 9/10 |
| Abnormal fingers/toes | | | | | | | | | | | | | | | | | 8/10 |
| Ophthalmologic abnormalities | | | | | | | | | | | | | | | | | 1/1 |
| Umbilical hernia | - | | | | | | | | | | | | | | | | 2/8 |
| Sleeping disorders | - | | | | | | | | | | | | | | | | 7/10 |
| Heart disease | + | - | + | | | | | | | | | | | | | | 8/13 |

Abbreviations: develop, development; dysplast, dysplastic; F, female; M, male; Macro, macrocephaly; Micro, microcephaly; mo, months; N, no; prom, prominent; psychom, psychomotor; SD, standard deviation; synd, syndrome; Y, yes; yr, years; +, feature present; ++, severe feature; -, feature absent. (*) this proband has been born after 27 gestational weeks, showing low weight and height, but showed +1SD in OFC. Thus, is considered a relative macrocephaly.

NFIX, a strong candidate for many phenotypic findings similar to those reported here in the recently described 19p13.13 microdeletion/duplication syndrome (located ~9 Mb centromeric to our SRO).² *NFIX* has been also implicated in the autosomal dominant Marshall-Smith syndrome (MIM 602535) and in patients with Sotos-like syndrome (currently known as Sotos syndrome type 2; MIM 614753).¹⁰ Interestingly, deletion of *NFIX* is observed in 22/37 of patients discussed here (Figure 2). Altogether, these genes appear to be involved in important pathways that could contribute to developmental abnormalities. Additional cases will be needed to refine genes directly implicated in the complete phenotype.

The potential relevance of this ~1.1 Mb interval from *TLE2* to *CREB3L3* (that includes the SRO) is supported by its highly conserved nature in all vertebrates, from fish to mammals.^{33,34} Further support underlining the importance of this highly conserved region and its contribution to the clinical features in patients with 19p13.3 deletion/duplications is given by their haploinsufficiency (HI) score. HI score (defined by Huang and colleagues, 2010)³⁵ ranges from 0 to 100, where 0 means highly haploinsufficient and 100 not haploinsufficient. The region from *TLE2* to *CREB3L3* includes 16/55 potential HI genes with a score <50.³⁵

Chromosomal architecture context and mechanisms of rearrangement

The deletions and duplications at 19p13.3 in patients reported here were not flanked by segmental duplications and mostly unique to each patient, though some are in close proximity (see Figure 2, Table 3). This fact combined with the absence of low copy repeats within or flanking deleted/duplicated regions makes non-allelic homologous recombination (NAHR) an unlikely causative mechanism. Several mechanisms have been proposed for the formation of copy number alterations with non-recurrent breakpoints, and in most microhomology at breakpoint junctions have been invoked. They can be grouped as non-replicative (NAHR and others such as non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ)) or replicative-based repair mechanisms (fork stalling and template switching (FoSTeS), serial replication slippage (SRS), break-induced SRS and microhomology-mediated break-induced replication).⁴¹⁻⁴⁴

We hypothesize that most interstitial non-recurrent 19p13.3 rearrangements are a consequence of NHEJ and/or the alternative pathway MMEJ, although different patterns have been depicted. Indeed, although repetitive elements of the same family (SINE; Alu; patients 1, 4 and 5) or different family (DNA MER and LINE; patient 2) are present at both breakpoints, the level of sequence identity is probably too low for Alu/Alu-mediated NAHR. In addition, the 4bp, 3bp, 50 bp or 3bp of microhomology (patients 1, 2, 4 and 5, respectively) and/or some changes of nucleotides at the breakpoints (patients 2 and 5) may point to NHEJ or to MMEJ as mechanisms preferentially involved (Table 4, Supplementary Figure S2). In another group of patients, such as patients 6 and 7, only the distal breakpoint showed a repetitive element directly involved, although microhomology of 5- and 6 bp, respectively, and some nucleotide insertions observed at the junctions of these deletions, suggested also a MMEJ mechanism (Table 4, Supplementary Figure S2). Finally, a third group of patients (eg, patient 12) did not show any repetitive element directly involved, but close to them. In fact, breakpoints were flanked by AluJo and L1 (LIMB7) elements and the presence of 3 bp microhomology without any scar may point to NHEJ mechanism.

In silico genomic analysis of the breakpoints of the remaining patients (3, 9 and 13) revealed numerous highly repetitive sequences (SINES/LINES, see Table 4) that may be involved in the generation of such events. In fact, chromosome 19 contains 'Alus' in the highest

Table 3 Coordinates of imbalances at interstitial 19p13.3 region according to the HGVS and ISCN nomenclatures

| Patient | Genomic imbalance; inheritance | Size (Mb) | Type | CGH-array platforms and methods used |
|---------|---|---------------|----------------------|---|
| 01 | chr19:hg19:g(3,324,025_3,324,026)(4,870,882_4,870,883)del ISCN:19p13.3(3,324,026-4,870,882)x1 dn | 1.546 | Deletion | KaryoArray_v3.0 and custom 19p13.3 and Sanger sequencing |
| 02 | chr19:hg19:g(3,874,599_3,874,600)(4,183,343_4,183,344)del ISCN:arr[hg19]19p13.3(3,874,600-4,183,343)x1 dn | 0.309 | Deletion | KaryoArray_v3.0 and custom 19p13.3 and Sanger sequencing |
| 03 | chr19:hg19:g(3,227,720_3,234,036)(4,823,723_4,830,039)del ISCN:arr[hg19]19p13.3(3,234,036-4,823,723)x1 dn | 1.491 | Deletion | KaryoArray_v3.0 and custom 19p13.3 and Sanger sequencing |
| 04 | chr19:hg19:g(3,932,784_3,932,785)(4,523,183_4,523,184)del ISCN:arr[hg19]19p13.3(3,932,785-4,523,183)x1 dn | 0.590 | Deletion | KaryoArray_v3.0 and custom 19p13.3 and Sanger sequencing |
| 05 | chr19:hg19:g(3,279,941_3,279,942)(4,168,106_4,168,107)del ISCN:arr[hg19]19p13.3(3,279,942-4,168,106)x1 dn | 0.888 | Deletion | SignatureChipOS v2.0 12-plex, KaryoArray_v3.0, custom 19p13.3 and Sanger sequencing |
| 06 | chr19:hg19:g(3,789,486_3,789,487)(3,988,746_3,988,747)del ISCN:arr[hg19]19p13.3(3,789,487-3,988,746)x1 dn/chr19:hg19:g(46,121,111_4,612,112)(46,387,319-46,387,320)del ISCN:arr[hg19]19q13.32(46,121,112-46,387,319)x1 dn | 0.199/0.266 | Deletion/deletion | KaryoArray_v3.0 and custom 19p13.3 and Sanger sequencing |
| 07 | chr19:hg19:g(3,295,067_3,295,068)(4,996,928_4,996,929)del ISCN:arr[hg19]19p13.3(3,295,068-4,996,928)x1 dn | 1.700 | Deletion | SignatureChipWG v1.0.1; KaryoArray_v3.0 and custom 19p13.3 and Sanger sequencing |
| 08 | chr19:hg19:g(2,362,767_2,487,767)(4,882,351_5,007,351)dup ISCN:arr[hg19]19p13.3(2,487,767-4,882,351)x3 dn | 2.395 | Duplication | Qchip_v3.0 |
| 09 | chr19:hg19:g(3,184,457_3,190,773)(3,448,532_3,454,848)del ISCN:arr[hg19]19p13.3(3,190,773-3,448,532)x1 chr19:hg19:g(3,579,614_3,585,930)(4,621,011_4,627,327)del ISCN:arr[hg19]19p13.3(3,585,930-4,621,011)x1 dn | 0.257/1.035 | Deletion/deletion | SignatureChipWG v1.0.1, and custom 19p13.3 |
| 10 | chr19:hg19:g(2,294,320_2,329,320)(3,808,325_3,843,325)dup ISCN:arr[hg19]19p13.3(2,329,320-3,808,325)x3 dn | 1.479 | duplication | SignatureChipOS v2.0 12-plex |
| 11 | chr19:hg19:g(3,944,568_3,979,568)(4,131,259_4,166,259)del ISCN:arr[hg19]19p13.3(3,979,568-4,131,259)x1 | 0.151 | Deletion | SignatureChipOS v2.0 12-plex |
| 12 | chr19:hg19:g(3,451,210_3,451,211)(4,600,362_4,600,363)del ISCN:arr[hg19]19p13.3(3,451,211-4,600,362)x1 dn | 1.149 | Deletion | SignatureChipOS Version 4.0 and custom 19p13.3 and Sanger sequencing |
| 13 | chr19:hg19:g(3,397,174_3,403,490)(3,405,207_3,411,524)dup ISCN:arr[hg19]19p13.3(3,403,490-3,405,207)x3 chr19:hg19:g(3,406,937_3,413,253)(4,195,610_4,201,926)del ISCN:arr[hg19]19p13.3(3,413,253-4,195,610)x1 | 0.00177/0.781 | Duplication/deletion | SignatureChipOS Version 4.0 and custom 19p13.3 |

Abbreviations: dn, *de novo*; HGVS, Human Genome Variation Society; ISCN, International System for Human Cytogenetic Nomenclature. Data are from UCSC Genome Browser (February 2009 assembly, hg19, NCBI build 37).

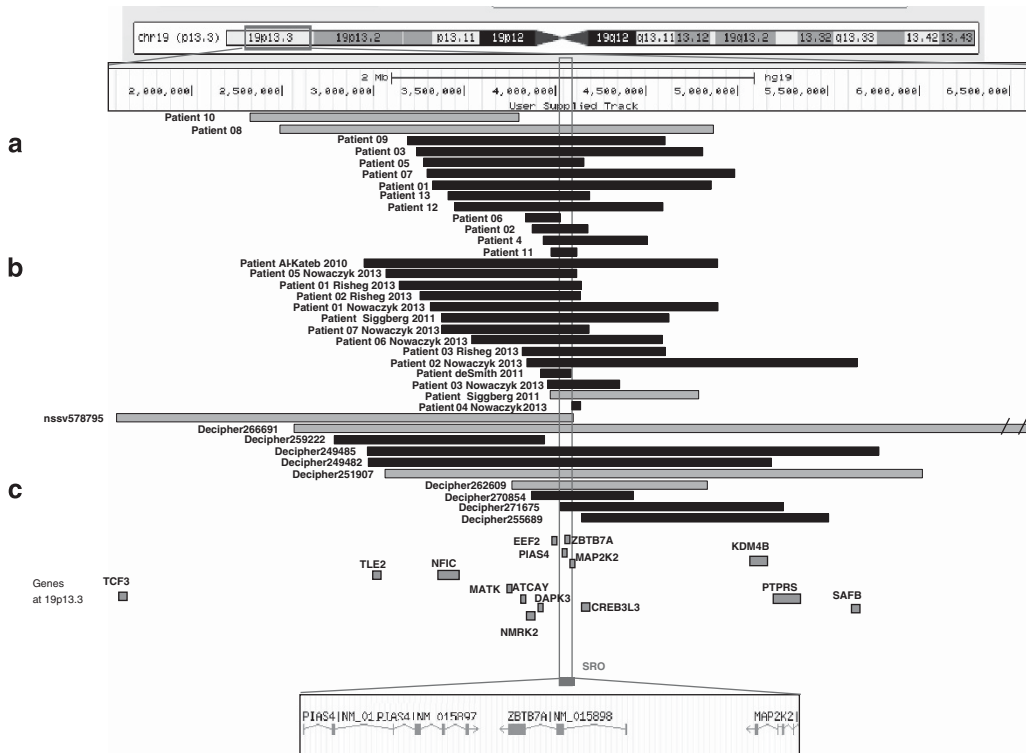


Figure 2 Graphical representation of interstitial 19p13.3 deletions and duplications; genes within the minimal region of overlap. SRO (chr19: 3979568–4093035; hg19; NCBI build 37). Patients are from our series (a); previous reports (b) and public databases, such as DECIPHER and ISCA consortium (c). Gray; duplications, Black; deletions.

density, comprising 27.2% of chromosome.⁴⁵ In patients 9 and 13 a replicative mechanism may be suggested by the complex genomic rearrangement observed (deletion-normality-deletion and duplication-normality-deletion, respectively; see Supplementary Figure S3). Additional cases must be analyzed for a more complete understanding of the mechanisms of deletion and whether genomic architecture in this region predisposes to genomic rearrangements, as previously suggested in other chromosomal regions.⁴⁶ In fact, additional analysis of sequence elements at both breakpoints revealed repetitive elements in all cases, sequences forming nonB-DNA conformations and sequence motifs that may lead to genomic instability and subsequently genomic rearrangements by promoting double strand breaks (DSBs) or by stalling the replication fork as we discussed for the *FOXL2* locus⁴⁷ (also see Supplementary Tables S2).

Clinical findings and genotype correlation with head circumference

Macrocephaly can be caused by disruption of a broad spectrum of genes and biological functions. There are a few microdeletion/duplication syndromes with macrocephaly listed in the DECIPHER database: the 1q21.1 microduplication, 8p23.1 duplication, 19p13.13 deletion and Sotos syndrome. In contrast, microcephaly is listed in 16 syndromes in the DECIPHER database. We add the interstitial 19p13.3 microdeletion/duplication syndrome to the differential diagnosis of macrocephaly or microcephaly, respectively. A similar phenomenon of ‘mirror’ head size phenotypes has been previously reported with genomic rearrangements such as the 19p13.13 microdeletion/duplication syndrome,² the 1q21.1 microdeletion/duplication syndrome,⁴⁸ and the Sotos syndrome/5q35 duplication syndrome.⁴⁹

A comparative analysis of almost all deleted and duplicated patients pointed to *PIAS4* as a strong candidate for abnormal head size (see Figure 3, dashed line), as all patients but one with abnormal head size had deletion or duplication of this gene. In agreement with this, injection of high doses of *PIAS* into early frog embryos led to reduced head structures with a general and reduced embryonic body length.³⁴ In addition, most patients without rearrangements of *PIAS4* are normocephalic (Figure 3 panel b). Further support for *PIAS* genes' implication in head size in humans was obtained reviewing our own database for macrocephalic patients. Indeed, we found a patient with a 3.68 Mb 7p13-p12.3 deletion (43 511 010-47 209 011, genome assembly hg19, NCBI build 37), which included *ZMIZ2*, a *PIAS*-like family gene⁵⁰ (see Supplementary Figure S4), as well as that other patients with rearrangement at 7p13-p12.3; cases nssv578186 and nssv584525 and DECIPHER 1224, 1223, 1222, 1221, 1220 (deletions) and nssv579045 (duplication) also showed macrocephaly and microcephaly, respectively.

In contrast, other facts do not support *PIAS4*'s role in head size in humans. Heterozygous and homozygous knockout mice for *PIAS4* did not show phenotypic changes.⁵¹ However, no brain weight measures were taken in the study (Dr Grosschedl's personal communication). In addition, normocephalic patients have *PIAS4* deletions or duplications; 11 of 48 patients in this review had a head size that did not correlate with *PIAS4* copy number changes (see Figure 3, mostly in normocephalic individuals in panel b; with asterisks). However, some were evaluated using low-resolution arrays (case 4 in reference²² and DECIPHER 255689) or have additional (case 3 in reference²²) or very large chromosomal rearrangements that extend toward the centromere (Figure 3 panel b). We speculate that very large deletions and

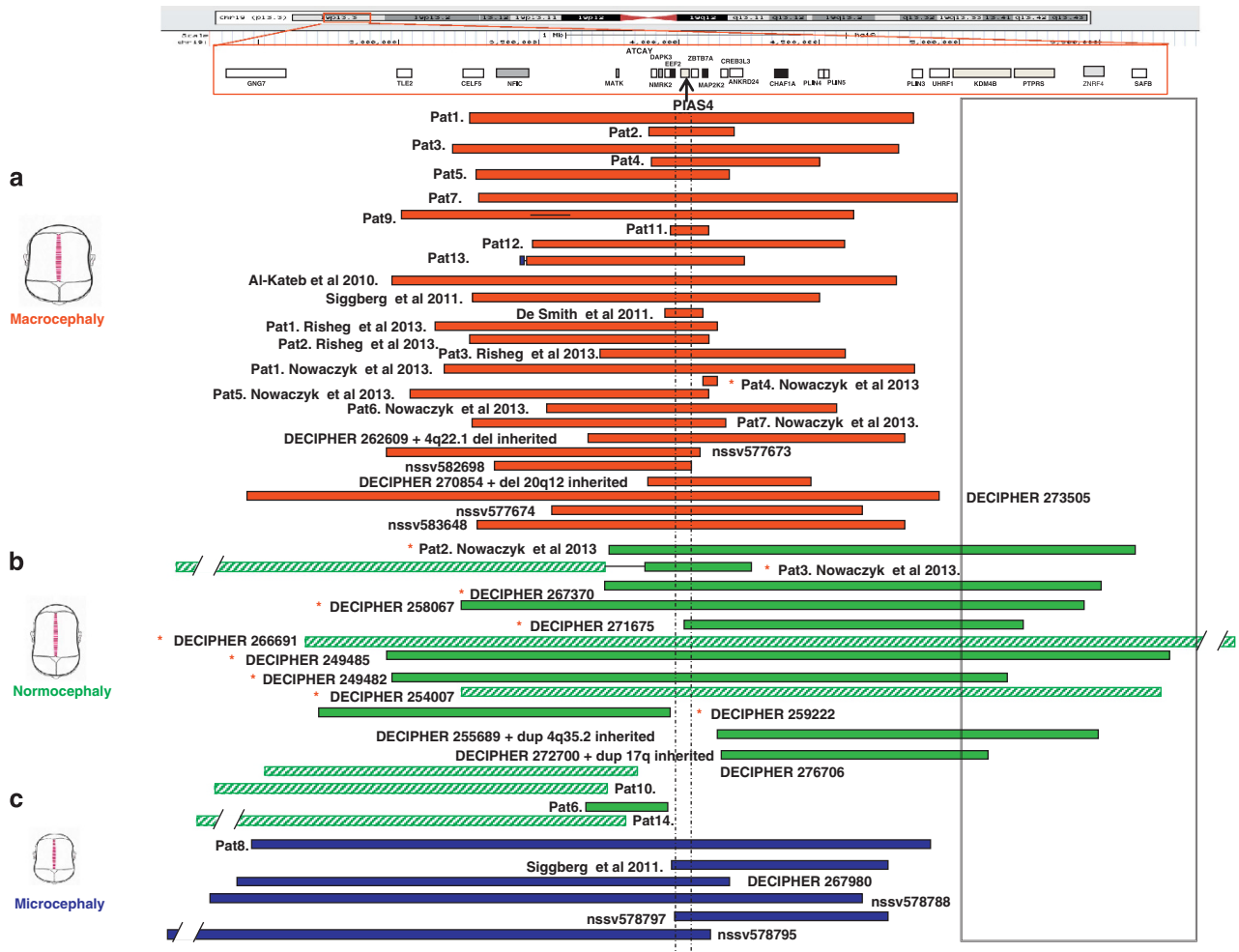


Figure 3 Graphic representation of the minimal region of overlap (SRO, dashed line) responsible for macro/microcephaly in interstitial 19p13.3 microdeletion/duplication syndrome (by means of aCGH). The SRO included only one gene, *PIAS4*. (a) Almost all patients with macrocephaly (including relative macrocephaly, red bars) have deletions affecting *PIAS4*. In contrast, all patients with microcephaly (blue bars) have duplications of *PIAS4*. Patients in panel b, with either deletions (full green bars) or duplications (striped green bars), are normocephalic. The majority of these CNAs either do not include *PIAS4* or extend further towards the centromere than the individuals with head size abnormalities. Cases where *PIAS4* deletion/duplication status does not correlate with head size (mainly in the normocephalic group) are illustrated by an asterisk (*). The solid black line outlines a putative region which may negate effects of *PIAS4* dosage changes. Pat, Patient; nssv, ISCA consortium patients.

duplications extending toward the centromere past genomic coordinate 5 000 000 (at 19p13.3 band; hg19, NCBI 37) may modify the head size phenotype of *PIAS4* dosage change owing to the existence of putative dominant-negative regulator genes within this area. *KDM4B*, *PTPRS* and *SAFB* are predicted haploinsufficient genes³⁵ that might be strong candidates for this effect. Mouse knockout studies reveal that these genes are involved in abnormal head morphology (*Kdm4b*), decreased brain size by weight (*Ptprs*), or in smaller fetal size (including head; *Safb*) compared with littermates (Mouse Genomic Informatic database, MGI; and Mouse Genome Database, MGD; web pages).

The specific mechanism of how *PIAS4* or other RNF proteins may be involved in macro/microcephaly is unknown, but *PIAS4* is highly conserved from zebrafish to mammals.^{33,34} One hypothesized mechanism is disruption of AR-mediated transcription, which is a common feature of PIAS-like proteins.⁵² A second hypothesis is disruption of bone morphogenetic protein-signaling pathways, as the RING domain of *PIAS4* is involved in its suppression.⁵³ Third, *PIAS4* may be involved in the WNT signaling pathway through its interaction with several transcription factors,^{32,54} and *WNT* is important for

several aspects of facial morphogenesis. Fourth, *PIAS4* is an E3 ubiquitin ligase, and haploinsufficiency of E3 ubiquitin ligases in plants and *Drosophila melanogaster*^{55–57} leads to organ overgrowth. Interestingly, individuals with haploinsufficiency of *RNF135* or *RNF125*, which encode other RNF proteins, have been associated recently (via alteration of ubiquitin signaling pathways) by us and others with macrocephaly and overgrowth in patients with dysmorphic features and ID.^{58,59} Thus, additional efforts will be necessary to definitively establish how *PIAS4*, *PIAS*-like or other RNF proteins, may be involved in regulation of human head size.

In summary, after identifying 13 unrelated patients and reviewing others previously reported, we further delineate a novel interstitial microdeletion/duplication syndrome at 19p13.3 (different from subtelomeric microdeletions) whose clinical features overlap many other known microdeletion/duplication syndromes, such as anomalies of head size (macrocephaly in deletions and microcephaly in duplications), pointing to *PIAS4* as a putative candidate. This study also provides detailed clinical information for genitivics to assist in the evaluation, diagnosis and management of individuals with similar genomic rearrangements.

Table 4 Detection of breakpoints and junction fragment analysis in some interstitial 19p13.3 deletion patients

| Patient | Genomic rearrangement | Deletion size | | Micro-homology at the breakpoint | Change of the sequence at the breakpoint | Repetitive elements at the breakpoints distal/proximal | Most probable Mechanism |
|-----------------|---|---------------|--|----------------------------------|---|--|--|
| | | (Mb) | | | | | |
| 01 | chr19 hg19:g.(3,324,025_3,324,026)_ (4,870,882_4,870,883)del | 1.546 | TTTC | | Not detected | AluJr/AluSp | NEHJ or MMEJ |
| 02 | chr19 hg19:g.(3,874,599_3,874,600)_ (4,183,343_4,183,344)del | 0.309 | TGG | | chr19 hg19:g.3874596delT | DNAMer82/L2b (LINE) | NEHJ or MMEJ |
| 03 ^a | chr19 hg19:g.(3,227,720_3,234,036)_ (4,823,723_4,830,039)del | 1.491 | | | | SINE/SINE | NEHJ or MMEJ |
| 04 | chr19 hg19:g.(3,932,784_3,932,785)_ (4,523,183_4,523,184)del | 0.590 | CCGCCTCGGCCTCCAA; AGTGCTGGGATTACAGG CGTGAG | | Not detected | AluX3/AluX | MMEJ |
| 05 | chr19 hg19:g.(3,279,941_3,279,942)_ (4,168,106_4,168,107)del | 0.888 | CCACTGTGCC ATG | | chr19 hg19:g.(3279937dupT; 3279938A>T) | AluX/AluSq | NEHJ or MMEJ |
| 06 | chr19 hg19:g.(3,789,486_3,789,487)_ (3,988,746_3,988,747)del | 0.199 | CCCAG | | chr19 hg19:g.(3789465GGGAAC- CAAGGTCTTGAG> TCCTAGCACCGGGCCCTGTA; 3789485dupAT) | AluSc8/near to DNAMer113 | MMEJ |
| 07 | chr19 hg19:g.(3,295,067_3,295,068)_ (4,996,928_4,996,929)del | 1.700 | GCCCTG | | chr19 hg19:g.4996938du pXCCTCXTT | DNAMer20/- | MMEJ |
| 09 ^a | chr19 hg19:g.(3,184,457_3,190,773)_ (3,448,532_3,454,848)del chr19 hg19:g. (3,579,614_3,585,930)_ (4,621,011_4,627,327)del | 0.257/1.035 | | | | Near toGC-rich/ between AluSX1 and MIR3/Near toC-rich/ AluSq2 | Replicative –based repair mechanism |
| 12 | chr19 hg19:g.(3,451,210_3,451,1211)_ (4,600,362_4,600,363)del | 1.149 | GCA | | Not detected | near L1mb7(LINE)/near to AluIu | NEHJ |
| 13 ^a | chr19 hg19:g.(3,397,174_3,403,490)_ (3,405,207_3,411,524)dup chr19 hg19:g. (3,406,937_3,413,253)_ (4,195,610_4,201,926)del | 0.00177/0.781 | | | | MIR/near toMIRC near to AluX/near to AluX/AluSg | Replicative –based repair mechanism |

^acases under *in silico* analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank Drs J Andrieux, N Van der Aa, L Bird, T Cole, K Neas, F Faletta, T de Ravel, N Morrison, four useful and additional information required for several patients in free Databases. This work was supported by a grant from REDES/FIBHULP08 of the Fundación para la Investigación Biomédica Hospital Universitario la Paz, the ENDOSCREEN project from Comunidad Autónoma de Madrid, FIS 011/2491 from ISCIII and the EUCID-COST Action BM-1208.

AUTHOR CONTRIBUTIONS

MAM, MPB, EV and JN performed the microarray analysis. JAT did the punctual mutational analysis of *PIAS4* gene. MLT, AD and BF did the cytogenetic and FISH studies. IRA and MVFM did Sanger sequencing analysis. IRA and RM did the detection of breakpoints and the junction fragment analysis. RMA and MCC performed the aCGH studies. JN, FSS, SGM, PDL, AD, MC, AP, LD, MO, MCSH, ECF, ASJ, GG, LA, CHE, SS, ED, XL, HD, DBB, SV, MBD, JWE, SR, CAVV, FFR did the clinical characterization of the patients described herein. JAR, KWG, PL and JN wrote the paper in consultation with all the other authors.

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