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# Analphoid marker chromosome in a patient with hyper-IgE syndrome, autism, and mild mental retardation

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**Abstract:** Hyper-IgE syndrome with recurrent infections (HIES) is a primary immunodeficiency disease characterized by recurrent skin and lung abscesses and extreme elevations of serum IgE, but also involving dentition, bones, and connective tissue. Although the etiology of HIES is unknown, autosomal dominant inheritance has been observed in multiple kindreds. A 17 year old male with sporadic HIES, autism, and mild mental retardation was found to have a supernumerary marker chromosome in peripheral blood lymphocytes and skin fibroblasts. Microdissection and FISH analysis of the marker chromosome showed that it was derived from a small interstitial deletion of one homologue of chromosome 4q21. Lack of hybridization of probes specific for telomeres and alphoid centromeres, including a centromere 4 specific probe, established that the marker was an analphoid ring chromosome. Comparative genotyping of transformed B-cell subclones with (M+) and without (M−) the marker chromosome showed loss of the maternal alleles in M− cells between markers D4S1569 and D4S3010. FISH using YAC clones from 4q21 confirmed the size and location of the interstitial deletion. Thus our patient's phenotypes were associated with de novo formation of a marker chromosome containing 15–20 cM of DNA deleted from his maternally derived chromosome 4. Proximal chromosome 4q therefore is a candidate region for disease genes for both HIES and autism. Identification of genes disrupted or lost during the formation of the marker chromosome as well as linkage studies in kindreds with HIES or autism may help us to understand the etiology of these complex phenotypes. ***Genetics in Medicine*, 1999:1(5):214–219.**

**Key words:** *Analphoid, centromere, chromosome 4q21, immunodeficiency, immunoglobulin E, interstitial deletion, Job syndrome, marker ring chromosome*

Hyper-IgE syndrome with recurrent infections (HIES) (also called hyper-IgE recurrent infection syndrome and Job syndrome, OMIM #14,7060, #24,3700) is a multisystem disorder of unknown etiology affecting the immune system, bones, teeth, and connective tissue.[1] Although the specific gene defect is unknown, HIES can be inherited as an autosomal dominant trait with variable expressivity.[1] HIES is characterized by eczema from infancy, recurrent skin abscesses and pneumonia, and extremely elevated levels of serum IgE.[1–8] Characteristically, bacterial pneumonias in HIES fail to resolve normally and lead to persistent pneumatoceles. By middle adulthood, many patients succumb to complications of chronically superinfected pneumatoceles or progressive loss of lung function. In addition to immunologic defects, distinct nonimmunologic features are associated with HIES, including a characteristic facial appearance, increased nasal width, prominent forehead, high arched palate, retained primary dentition, recurrent bone fractures, scoliosis, craniosynostosis, and hyperextensibility.[1,9,10] Despite earlier observations of abnormal granulocyte chemotaxis in several HIES patients, no primary underlying immunologic defect has been defined.[4,5,11–13]

Since its first descriptions,[2–4] more than 200 cases of HIES have been published,[1,7,14] but no associated cytogenetic abnormalities have been found. We screened 52 unrelated HIES probands for cytogenetic anomalies and report here a unique patient with HIES and autism who had an analphoid marker ring chromosome derived from the long arm of chromosome 4.

## CASE REPORTS

The proband, a 17 year old African American male, was born to a 21 year old healthy mother, who has since given birth to two full siblings and two half siblings of the proband, all without medical or cognitive problems. He had neonatal eczema, and subsequently developed multiple facial and scalp abscesses from which *Staphylococcus aureus* was isolated. His

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*Received: April 3, 1999*

*Accepted: June 23, 1999*

total eosinophil count was 4,300 cells/μl (normal <640 cells/μl) and serum IgE level was 51,670 IU/ml (normal <130 IU/ml). At 1.5 years, he experienced his first pneumonia; his sixth and most recent pneumonia at age 16, caused by *S. pneumoniae*, developed into a pneumatocele. He also suffered from recurrent onychomycosis, chronic sinusitis, and otitis. At 5 years, he fractured the left distal femur while carrying a bag of groceries. The weight of the leg cast induced a second femoral fracture. At age 15, leg length discrepancy and 10° of scoliosis were noted. At 8, 9, and 12 years of age, nine primary teeth were extracted to make way for already erupting permanent teeth. At age 17 he still had nine retained primary teeth. Permanent tooth eruption has been delayed for age although tooth number, structure, and maturation were normal.[1]

The proband's motor, language, social, and developmental milestones were delayed. Testing at ages 8 and 15 showed cognitive and adaptive functioning in the borderline to mild mental retardation range. He has required special education for disabilities in quantitative reasoning, arithmetic, reading, and spelling, but has demonstrated relative strengths in visual detail skills. He has a profound impairment in social interaction, minimal speech, or communication, and restricted patterns of interests consistent with autism.

## MATERIALS AND METHODS

### Blood samples and cell lines

Blood samples from the proband and his mother and a skin biopsy from the proband were obtained for genetic analysis with informed consent under an approved NIH protocol. The father could not be located. An Epstein-Barr virus (EBV) transformed B-cell line was established from the proband. After culture in limiting dilution, subclones of this line were further analyzed.

### Cytogenetic analysis

Karyotyping of high-resolution, G-banded metaphases was performed by standard methods.[15,16] Microdissection of 16 marker chromosomes was performed. The DNA was amplified with UN1 universal primers as described.[17] For FISH, amplified marker DNA as well as DNA from yeast artificial chromosome (YAC) clones was labeled with SpectrumOrange (Boehringer Mannheim, Mannheim, Germany) using a nick translation kit (Boehringer Mannheim). To each slide was applied 250 ng to 1 μg (for PCR product) or 5 μg (for YACs) of labeled probe, in which nonunique and nonspecific DNA had been blocked by preannealing with 50 μg human Cot-1 DNA (Life Technologies, Gaithersburg, MD). A telomere probe, a chromosome 4 alphoid centromere probe, and an All-Human-Centromeres probe were used according to manufacturer's instructions (Oncor, Gaithersburg, MD; now Vysis, Downers Grove, IL). YAC clones were selected according to their position on available physical maps [http://www.shgc.stanford.edu/Mapping/phys_map/Chr4YAC.html].[18]

### DNA analysis

Fluorescent primers for polymorphic markers from the long arm of chromosome 4 were sought from available genetic maps [http://www.marshmed.org/genetics][19] and purchased from Research Genetics (Huntsville, AL) and PE/Applied BioSystems (Foster City, CA). Genotyping was performed using conditions appropriate for each primer set. PCR reactions were analyzed in an ABI 377 sequencer (PE/Applied BioSystems). After data assembly with Collection and Analysis software, allele sizes were determined with help of the Genotyper program (all software from PE/Applied BioSystems).

## RESULTS

### Cytogenetic studies

Eight hundred G-banded metaphases from multiple blood samples, including 25 at 850-band resolution, and 26 metaphases from cultured skin fibroblasts of the proband were analyzed. In both tissues, 75% of the cells showed a supernumerary marker chromosome (Fig. 1A, *arrow*). Twenty-five percent of the cells appeared to have a normal karyotype (not shown). To address the structure of the marker chromosome, staining with a telomere probe was performed. In 20 metaphases, the marker was the only chromosome lacking telomeric repeat signals (Fig. 1 B), consistent with a ring chromosome derived from an interstitial deletion. In addition, the marker was directly observed to have a ring structure in a prometaphase spread (Fig. 1 C, *arrow*). To investigate the origin of the marker chromosome, it was microdissected, amplified by PCR, labeled, and used for FISH analysis. On control metaphases, the marker probe stained only a single region, chromosome 4q21, on both homologues (not shown). In the proband's cells that contained the marker chromosome, the probe stained only the marker plus one homologue of chromosome 4q21 (Fig. 1, C and D). The second homologue of chromosome 4 had no detectable staining. In the 25% of cells that were marker negative, again only one of the two chromosome 4 homologues stained (not shown). Thus, the marker represented material deleted from one copy of chromosome 4q21. All of the patient's somatic cells were therefore abnormal, 75% with a marker ring derived from 4q21, and 25% that had lost the ring and were thus deleted for one copy of 4q21 chromosomal material. In order to be distributed to daughter cells during mitosis, the marker chromosome must have contained a region with centromeric function. Centromeres are generally identified by the presence of repetitive DNA sequences known as alpha satellite, or alphoid, sequences. However, probes representing alphoid sequences from all of the human chromosomes, including a chromosome 4 specific alphoid centromere probe, failed to show detectable hybridization to the marker chromosome (Fig. 1 D and data not shown). An unidentified nonalphoid, or analphoid, repeat sequence or a low copy number of alphoid repeats functioning as a neo-centromere could have escaped detection.

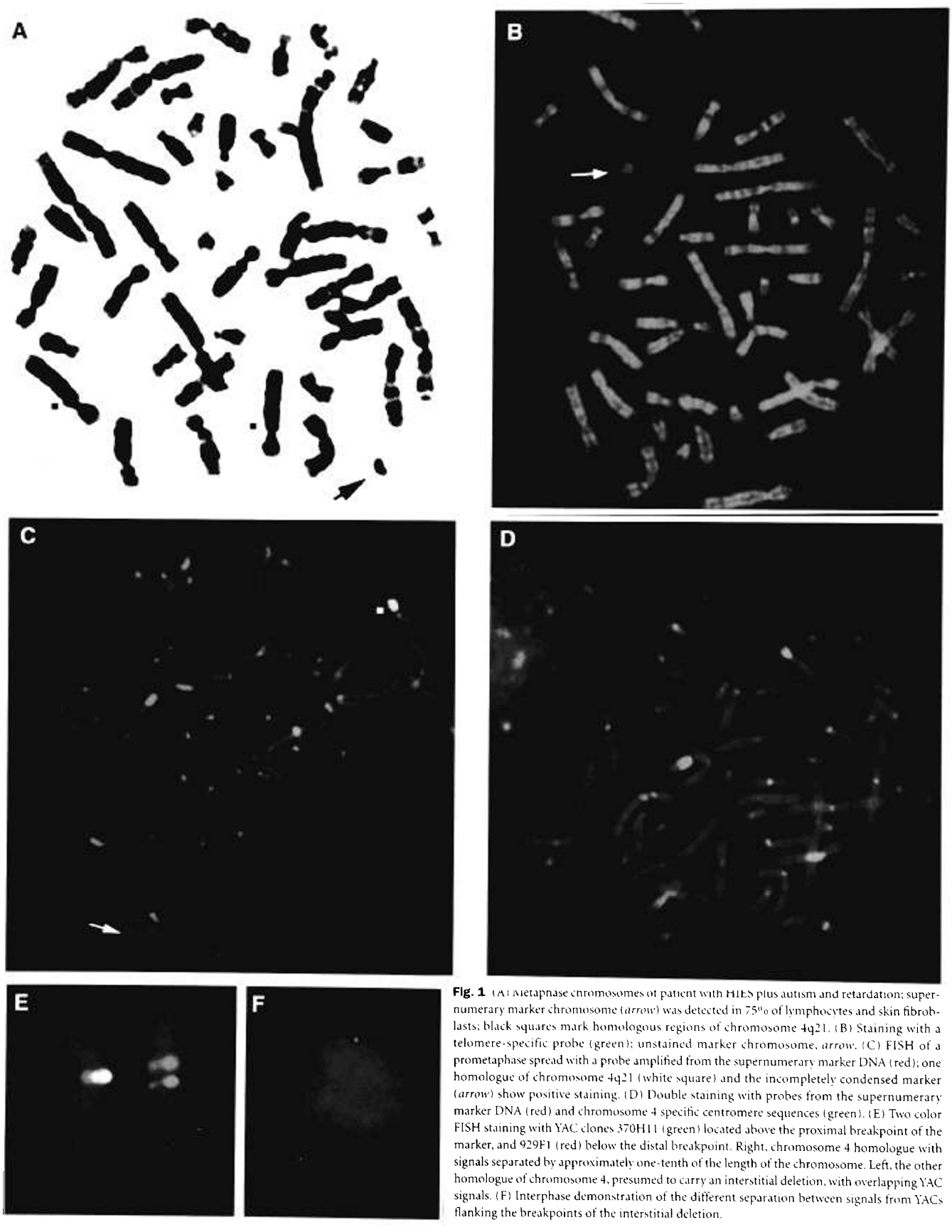


**Fig. 1** (A) Metaphase chromosomes of patient with HIES plus autism and retardation; supernumerary marker chromosome (*arrow*) was detected in 75% of lymphocytes and skin fibroblasts; black squares mark homologous regions of chromosome 4q21. (B) Staining with a telomere-specific probe (green); unstained marker chromosome, *arrow*. (C) FISH of a prometaphase spread with a probe amplified from the supernumerary marker DNA (red); one homologue of chromosome 4q21 (white square) and the incompletely condensed marker (*arrow*) show positive staining. (D) Double staining with probes from the supernumerary marker DNA (red) and chromosome 4 specific centromere sequences (green). (E) Two color FISH staining with YAC clones 370H11 (green) located above the proximal breakpoint of the marker, and 929F1 (red) below the distal breakpoint. Right, chromosome 4 homologue with signals separated by approximately one-tenth of the length of the chromosome. Left, the other homologue of chromosome 4, presumed to carry an interstitial deletion, with overlapping YAC signals. (F) Interphase demonstration of the different separation between signals from YACs flanking the breakpoints of the interstitial deletion.

### Molecular DNA studies

Subclones from limiting dilution of the EBV transformed cell line from the proband were analyzed by FISH to identify those that had retained (M+) or lost (M−) the marker chromosome. DNA samples prepared from the proband's M+ and M− subcloned cell lines were compared with genomic DNA from whole blood from his mother. Seventeen polymorphic markers on chromosome 4 between D4S2629 and D4S423 were heterozygous in proband M+ DNA. In contrast, M− DNA was hemizygous for the markers D4S398, D4S392, and D4S2963 on chromosome 4q21, but heterozygous for the more centromeric markers D4S2629 and D4S1596, as well as the more telomeric markers D4S3010 and D4S423 (Fig. 2). Thus the upper breakpoint of the marker region was between D4S1569 at 71.8 cM and D4S398 at 72.5 cM, and the lower breakpoint was between D4S2963 at 87 cM and D4S3010 at 90.3 cM from the top of chromosome 4.[19] These data were consistent with the hypothesis that the marker was a chromosomal segment derived from a 15–20 cM region of 4q21. Maternal genomic DNA was analyzed at the same polymorphic loci for comparison with the proband's genotype. As shown in Figure 2, at D4S398, D4S392, and D4S2963, which gave hemizygous signals in the proband's M− DNA, the alleles that were retained were not carried by the mother, indicating that the maternally inherited chromosome 4 was the one that had undergone deletion and marker formation in the proband.

### FISH studies with YAC clones

DNA from yeast clones containing YACs from proximal chromosome 4q[18] were labeled and used for FISH on metaphases from the proband. YAC clones 822D10, 256A10, 826F5, and 370H11, located proximal to D4S1569 stained both homologues of chromosome 4. YAC clones 898H4 and 915B1, located distal to D4S398, stained the marker chromosome and only one homologue of chromosome 4 (not shown). Therefore, the latter two YACs contain DNA involved in the interstitial deletion that gave rise to the marker chromosome. YAC clone 929F1, located distal to D4S3010, again stained both copies of chromosome 4 but did not stain the marker chromosome. To estimate the physical size of the deleted DNA fragment, two color FISH was performed using YAC clone 370H11 proximal to the interstitial deletion and YAC clone 929F1 distal to the interstitial deletion. In one homologue, the two signals were separated by approximately 10% of the length of the chromosome, whereas the other homologue demonstrated overlapping signals (Fig. 1E). Similarly, interphase signals consistently showed different distances separating the signal pairs (Fig. 1F). In metaphases from healthy controls, these YAC markers were equally separated on both chromatids, supporting the hypothesis of interstitial deletion coincident with the formation of the marker chromosome in the proband.

## DISCUSSION

Patients with any syndrome plus additional findings, particularly cognitive impairment, may be suspected to have cytogenetic abnormalities. Our proband was unique in that he had sporadic, typical HIES, but also had intellectual impairment with autistic features, not part of the HIES phenotype either in our 44 patient NIH cohort[1] or in >200 cases reviewed in the literature.[6–8,14] However, in view of the cytogenetic abnormality in our patient, it may be possible to link the immunologic and cognitive phenotypes to a genomic location. During the marker chromosome formation, loci might have been lost or disrupted, leading to the HIES and autism phenotypes.

The underlying causes of HIES and of autism could be single gene defects or contiguous gene deletion or dysregulation syndromes. Therefore, 4q should be considered a candidate region for linkage studies of HIES and autism. Our preliminary linkage analysis in familial HIES has demonstrated a multipoint LOD score of 3.69 for linkage of HIES to markers on proximal 4q.[20] In addition, a recent genome wide linkage scan for familial autism revealed multiple markers with positive

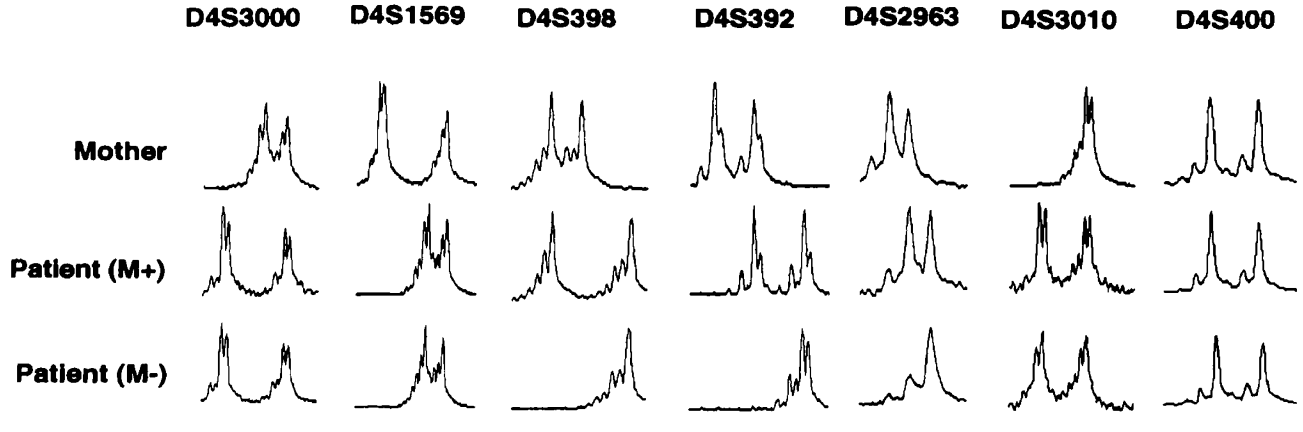


**Fig. 2** Comparative genotyping of DNA from mother and from patient lymphocyte clones with marker (M+) and without marker (M−). Polymorphic markers D4S398, D4S392, and D4S2963 were hemizygous in M− subclone; retained allele was not the maternally inherited allele. Marker order from centromeric (left) to telomeric (right).

LOD scores on 4q including markers located on our proband's marker chromosome region; however, significant linkage with a $p$ value of 0.0199 was achieved only with marker D4S1535 on 4q35.[21]

Our proband's marker chromosome was derived from an interstitial deletion of chromosome 4q21. Genotyping of polymorphic markers in M+ and M− B-cell subclones revealed that the marker formation was a de novo event involving breakpoints between D4S1569 and D4S398 proximally and D4S2963 and D4S3010 distally on the maternally derived chromosome 4. According to available maps,[19] D4S398 and D4S2963 are 14.5 cM, and D4S1569 and D4S3010 are 18.5 cM apart. Two color FISH staining with YACs derived from above and below the breakpoints[18] demonstrated an interstitial deletion of approximately 10% of the length of the chromosome. Chromosome 4 is estimated to have a genetic distance of 211 cM.[19] Thus deletion of the size observed would be equivalent to approximately 20 cM. This value is in close agreement with the mapped genetic distances of the markers involved in the loss of heterozygosity.

Marker chromosomes have been found in approximately 1 in 2,500 amniocenteses and 1 in 10,000 living individuals, sometimes inherited from healthy parents.[22] Most contain duplicated chromosomal material including alphoid repeats associated with centromeres, and even the unusual analphoid markers are often composed of inverted duplications.[23] Mental retardation, the most common phenotype associated with de novo marker chromosomes, is thus generally attributed to partial trisomy. There have been five reported cases of marker chromosomes derived from proximal chromosome 4q,[24-27] all of them larger than our proband's, more proximally located, and containing alphoid repeat sequences specific for the chromosome 4 centromere. Clinically, these ring formations were associated with severe embryopathy with alobar holoprosencephaly in one case;[26] mental retardation with moderate dysmorphology in two cases;[24,27] and a normal phenotype in one case.[25]

In contrast to the above cases, we could not detect alphoid sequences by FISH in the marker in our proband, and we do not know if it contains duplicated DNA. However, 25% of our proband's lymphocytes and fibroblasts lacked the marker, probably due to somatic mitotic nondysjunction, and were thus monosomic for 4q21. In this regard, reported interstitial deletions might shed light on the expected phenotype of the partial 4q monosomy of our proband. There are more than 20 published cases with interstitial deletions involving 4q11 to 4q21.1.[28–48] Taken together, these reports suggest that deletions involving only 4q11-22 were compatible with life, whereas larger deletions involving 4q13.2 to 4q23 produced severe abnormalities with fetal or infantile demise. The smaller deletions, reported in patients between 7 months and 17 years of age, were all associated with facial anomalies and, in all but one patient,[46] severe mental retardation was observed. The autosomal dominant Piebald trait, due to mutations in the c-kit proto-oncogene in 4q12,[49-51] occurred in the cases in which this locus was deleted. Other than retardation, the phenotypes of previously reported patients with small 4q deletions did not resemble that of our patient; the reported facial anomalies were not characteristic of HIES, and neither autism nor immunologic abnormalities suggestive of HIES were noted.

Given that HIES can be inherited as an autosomal dominant trait, pathologic mechanisms for HIES in our patient include: (a) haploinsufficiency due to disruption of gene(s) at the upper or lower boundaries of the deletion; (b) alteration of a gene by truncation or fusion with another gene, resulting in production of a toxic protein with a dominant negative effect; (c) haploinsufficiency due to loss of genetic material, so far undetected, at the marker breakpoints or elsewhere if the marker formation was a complex event involving multiple DNA breaks and rejoinings; (d) altered expression of gene(s) on the marker or nearby on 4q21 due to disruption of a locus control region or imprinted region. The lack of a HIES phenotype in other published cases with 4q deletions argues against the hypothesis of haploinsufficiency causing HIES. However, the partial monosomy of cells that have lost the marker ring may contribute to the cognitive aspects of our patient's phenotype.

This case illustrates the benefit of cytogenetic analysis in a unique patient with a defined syndrome plus an additional phenotype. The pursuit of transcripts within the region of the proband's marker chromosome, and particularly at the marker breakpoints, may help to identify a disease gene for HIES.

**Acknowledgments**

The authors thank Michael Bittner and Yuan Jiang for assistance with microdissection, Evgenia Pak for assistance with FISH, Anne O'Connell for dental evaluation, Maximilian Muenke for helpful discussions, and the patient and his mother for their participation. B.G. was supported by Deutsche Forschungsgemeinschaft, Grant No. GR1617/1-1 and the Immune Deficiency Foundation.

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