Reviewer's report

Title: Deletion Xq27.3q28 that includes IDS and FMR1 in female patient with global developmental delays and nonrandom X inactivation

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Reviewer: Pietro Chiurazzi

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MS. By Marshall et al. entitled “Deletion Xq27.3q28 that includes IDS and FMR1 in female patient with global developmental delays and nonrandom X inactivation”

This paper reports on a four year-old female patient with global developmental delay and a hemizygous deletion of Xq27.3q28. First of all I was surprised that the extent of the deletion (144,270,614-154,845,961) was only mentioned in the legend to Figure 3! This Figure should also show at least all those genes (of the >50 included) that are already known to cause human pathology.

The Authors emphasize the presence of both the FMR1 and IDS in the deletion interval and correctly recall that a few cases with deletions that included IDS and FMR1 have already been reported in females with developmental delay, features of Hunter syndrome, and nonrandom X inactivation of the NORMAL X chromosome. In these cases the variable phenotype is supposedly caused by the high percentage of cells lacking a functional copy of the FMR1 and IDS (and FMR2…) genes. The Authors then underline the difference of their patient who exhibits nonrandom (preferential) inactivation of the MUTANT X chromosome and (obviously) tested negative for Hunter syndrome. They also conclude that this finding “does not support a primary role of the Xq27-28 region in X inactivation”.

I have an easier explanation for their observation which they unfortunately disregard throughout the paper (only generically recalling that >50 genes are included in the deletion): the deletion of their patient extends far beyond IDS including practically all Xq28 and a number of other XLMR/ID genes such as MECP2 and IKBKG/NEMO. Deletions or loss-of-function mutations of at least the latter gene are known to cause extreme X-inactivation skewage because of the negative selection of cells expressing the MUTANT X-chromosome (see some of the PubMed abstracts below for a limited example). This most likely explains the mild phenotype of the described patient and all the manuscript should be restructured to take this into account (starting from the Title that surprisingly quotes only FMR1 and IDS). Please also do not use this case to disprove the alleged (but unlikely) role of the Xq27-28 region in X inactivation.


Novel splicing mutation in the NEMO (IKK-gamma) gene with severe immunodeficiency and heterogeneity of X-chromosome inactivation.
We report on a family with three stillborn males, three affected males who were small for gestational age and died within 8 months, and one male who died at age 5 years. This boy had cone-shaped teeth and oligoodontia. He had serious bacterial infections and inflammatory bowel disease. Mutations in the NF-kappaB essential modulator (NEMO) gene have recently been shown to be the cause of a group of ectodermal dysplasia and immunodeficiency disorders (EDA-ID). Analysis of the NEMO gene revealed a nucleotide change in the consensus sequence of the splicing donor site of exon 6 IVS6 + 5G --> A(1027 + 5G --> A), which has not previously been described in EDA-ID. RT-PCR analysis of fibroblast RNA from an aborted affected male fetus demonstrated a skipping of exons 4, 5, and 6 which resulted in a truncated protein of about 35 kDa revealed by NEMO antibody. The skipping of exons 4, 5, and 6 did not affect the ORF of the C-terminal of NEMO encoded by exons 7, 8, 9, and 10, which contains a coiled-coil motif (CC2), a leucin-zipper (LZ), and a zinc finger motif (ZF) sub-domains of NEMO. IkappaBalpha degradation was strongly impaired in the fetal fibroblasts, suggesting an impaired NF-kappaB signaling. One healthy carrier had a completely skewed X-inactivation pattern with the normal X active, whereas the two other carriers had a random X-inactivation pattern. This family may represent a new phenotype within the EDA-ID disorders. From the heterogeneity in X-inactivation phenotype, we conclude that this mutation is not deleterious enough to be lethal for peripheral blood cells. PMID: 16333836 [PubMed - indexed for MEDLINE]
inactivation in an incontinentia pigmenti female patient with immunodeficiency. Martinez-Pomar N, Munoz-Saa I, Heine-Suner D, Martin A, Smahi A, Matamoros N.

Incontinentia pigmenti is an X-linked genodermatosis, lethal in males. Affected females survive because of X-chromosome dizygosity and negative selection of cells carrying the mutant X-chromosome, and for this reason the skewed X inactivation pattern is often used to confirm the diagnosis. The most frequent mutation is a deletion of part of the NEMO gene (NEMODelta4-10), although other mutations have been reported. Mutations of NEMO which do not abolish NF-kappaB activity totally permit male survival, causing an allelic variant of IP called hypohidrotic ectodermal dysplasia and immunodeficiency (HED-ID). We present a non-classical IP female patient who also suffered transient immunodeficiency because of a late and progressive selection against peripheral blood cells carrying an active mutated X-chromosome. This finding suggests that in the absence of known mutation the X-inactivation studies used in genetic counselling can induce mistakes with some female patients. At the age of 3 years and 6 months, all immunodeficiency signs disappeared, and the X-chromosome inactivation pattern was completely skewed. The low T cell proliferation and CD40L expression corroborate the important role of NEMO/ NF-kappaB pathway in T cell homeostasis. The decreased NEMO protein amount and the impaired IkBalpha degradation suggest that this new mutation, NM_003639: c.1049dupA, causes RNA or protein instability. To our knowledge, this is the first time that selection against the mutated X-chromosome in X-linked disease has been documented in vivo.

PMID: 16228229 [PubMed - indexed for MEDLINE]

Molecular analysis of the genetic defect in a large cohort of IP patients and
identification of novel NEMO mutations interfering with NF-kappaB activation.

Ursini MV.

Incontinentia Pigmenti (IP) is an X-linked genodermatosis that is lethal for males and present in females with abnormal skin pigmentation and high variable clinical signs, including retinal detachment, anodontia, alopecia, nail dystrophy and nervous system defects. The NF-kappaB essential modulator (NEMO) gene, responsible for IP, encodes the regulatory subunit of the IkappaB kinase (IKK) complex required for nuclear factor kappaB (NF-kappaB) activation. We analyzed the NEMO gene in 122 IP patients and identified mutations in 83 (36 familiar and 47 sporadic cases). The recurrent NEMO exon 4-10 deletion that is the major cause of the disease was present in 73 females (59.8%). In addition 10 point alterations (8.2% of females) were identified: three frameshift, three nonsense, three missense and one in-frame deletion of a single amino acid. We measured the effects of these NEMO point-mutations on NF-kappaB signaling in nemo(-/-) deficient murine pre-B cells. A mutation in the N-terminal domain, required for IKK assembly, reduced but did not abolish NF-kappaB activation following lipopolysaccharide stimulation. Mutations that disrupt the C-terminal domain, required for the recruitment of upstream factors, showed lower or no NF-kappaB activation.

A phenotype score based on clinical features of our IP patients was applied for summarizing disease severity. The score did not correlate with mutation type or domain affected indicating that other factors influence the severity of IP. Such a factor is likely to be X-inactivation. Indeed, 64% of our patients have extremely skewed X-inactivation pattern (>80 : 20). Overall IP pathogenesis thus depends on a combination of X-inactivation and protein domain that recruit upstream factors and
activate NF-kappaB.


A recurrent deletion in the ubiquitously expressed NEMO (IKK-gamma) gene accounts for the vast majority of incontinentia pigmenti mutations.


Incontinentia pigmenti (IP) is an X-linked dominant disorder characterized by abnormal skin pigmentation, retinal detachment, anodontia, alopecia, nail dystrophy and central nervous system defects. This disorder segregates as a male lethal disorder and causes skewed X-inactivation in female patients. IP is caused by mutations in a gene called NEMO, which encodes a regulatory component of the IkappaB kinase complex required to activate the NF-kappaB pathway. Here we report the identification of 277 mutations in 357 unrelated IP patients. An identical genomic deletion within NEMO accounted for 90% of the identified mutations. The remaining mutations were small duplications, substitutions and deletions. Nearly all NEMO mutations caused frameshift and premature protein truncation, which are predicted to eliminate NEMO function and cause cell lethality. Examination of families transmitting the recurrent deletion revealed that the rearrangement occurred in the paternal germline in most cases, indicating that it arises predominantly by intrachromosomal misalignment during meiosis. Expression analysis of human and mouse NEMO/Nemo showed that the gene becomes active early during embryogenesis and is expressed ubiquitously. These data confirm the involvement of NEMO in IP and will help elucidate the mechanism underlying the manifestation of this disorder and the in vivo function of NEMO. Based on these and other recent findings, we propose a model to explain the pathogenesis of this complex disorder.


Survival of male patients with incontinentia pigmenti carrying a lethal mutation can be explained by somatic mosaicism or Klinefelter syndrome.

Incontinentia pigmenti (IP), or "Bloch-Sulzberger syndrome," is an X-linked dominant disorder characterized by abnormalities of skin, teeth, hair, and eyes; skewed X-inactivation; and recurrent miscarriages of male fetuses. IP results from mutations in the gene for NF-κB essential modulator (NEMO), with deletion of exons 4-10 of NEMO accounting for >80% of new mutations. Male fetuses inheriting this mutation and other "null" mutations of NEMO usually die in utero. Less deleterious mutations can result in survival of males subjects, but with ectodermal dysplasia and immunodeficiency. Male patients with skin, dental, and ocular abnormalities typical of those seen in female patients with IP (without immunodeficiency) are rare. We investigated four male patients with clinical hallmarks of IP. All four were found to carry the deletion normally associated with male lethality in utero. Survival in one patient is explained by a 47,XXY karyotype and skewed X inactivation. Three other patients possess a normal 46,XY karyotype. We demonstrate that these patients have both wild-type and deleted copies of the NEMO gene and are therefore mosaic for the common mutation. Therefore, the repeat-mediated rearrangement leading to the common deletion does not require meiotic division. Hypomorphic alleles, a 47,XXY karyotype, and somatic mosaicism therefore represent three mechanisms for survival of males carrying a NEMO mutation.

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Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests