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REVIEW

The VACTERL association: mosaic mitotic aneuploidy as a cause and a model

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Abstract

While mitotic errors commonly cause aneuploid clones soon after conception, the embryos often normalize as clones are rapidly eliminated. Although generally considered benign, evidence suggests clone elimination as the primary cause of the vertebral, ano-rectal, cardiac, tracheo-esophageal, renal, and limb (VACTERL) association of anomalies, and possibly other adverse outcomes as well. Here, clone elimination-related development disruption at specific locations is used as the basis of a comprehensive theoretical VACTERL association model that also elucidates mitotic mosaic aneuploidy effects. For the association, the model explains random temporal and spatial origins during a limited time frame and overlapping clusters of component anomalies. It supports early developmental effects involving the stage of determination, where the position in a specific morphogen field controls what a cell will become and where it will be located. Developmental properties related to determination also create specific vulnerabilities to the midline and distal defects, the latter explaining exclusively radial and tibial defects with duplications and deficiencies. The model also supports isolated anomalies as part of the association and, for mosaic mitotic aneuploidy, indicates that clone elimination nears completion at the time of lower limb determination. Although mosaic clone elimination may cause other defects, occurrences in different developmental fields separate them from VACTERL anomalies. Clone elimination may also be related to risks for a single umbilical artery and for non-structural adverse pregnancy outcomes such as losses, prematurity, and growth delays, while a paucity of clone lethality in non-humans explains the rarity of the association and of single umbilical arteries in animals.

Keywords Cell fate · Determination · Mitotic aneuploidy · Mosaicism · VACTERL association

Introduction

Aneuploid clones commonly arise from mitotic errors soon after fertilization, with an estimated occurrence of 30% of all early embryos [1], but their reproductive implications are still uncertain. These clones are often rapidly eliminated, but this apparent normalization may not be as benign as generally assumed [2]. In particular, there are indications of a causal link between clonal elimination effects and the VACTERL association of **V**ertebral, **A**no-rectal, **C**ardiac, **T**racheo-**E**sophageal, **R**enal, and **L**imb anomalies (or, simply, VACTERL, or, the association, here), a common disorder with variable findings [3].

This connection is used to formulate a comprehensive model of VACTERL. Here, sequelae of aneuploid clone elimination at a specific location in the embryo physically interfere with the positional information that controls early development. This explains the random temporal and spatial origins of VACTERL defects during a limited window of time, overlapping clusters of anomalies, and developmental effects involving the stage of determination. A paucity of lethal clone effects in animals also accounts for their general lack of the VACTERL association.

The model suggests that mitotic mosaic clone elimination is nearing completion at the time of lower limb determination and may also account for some non-VACTERL malformations and for adverse pregnancy outcomes such as losses, growth delays, and prematurity. It indicates that single anomalies can be part of the VACTERL spectrum and explains why certain malformations are included, while others are not.

The present analysis begins with background information on the aneuploid clones and on VACTERL, which is then used to formulate a comprehensive theoretical model of the

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association, followed by some specific implications and confounding factors. Finally, other VACTERL models are discussed.

Mosaic mitotic aneuploidy (note: excludes confined placental mosaicism)

Mitosis in early human embryos is remarkably unstable, giving rise to frequent aneuploid clones. Methodological and other factors make occurrence rates difficult to assess, but such clones probably involve at least 30% of cleavage stage embryos and blastocysts [1]. Mechanisms causing the mosaicism include centrosome and mitotic spindle aberrations, chromosome cohesion defects, and relaxed cell cycle control with a weakening of several checkpoints [2].

These dysfunctions rapidly resolve and effectively halt the production of additional clones early on, e.g., with one mechanism, cell cycle control is probably re-established at the four to eight cell stage. Although mosaic clones can persist and affect the development and survival, they are typically eliminated, with the percentages of aberrant cells rapidly decreasing in several ways, including cellular fragmentation and/or lysis [4], apoptosis, and overgrowth.

It is generally thought that the process of elimination fully normalizes the embryo [2]. However, there are reasons to believe that this might not always be the case and that sequelae can occasionally include developmental anomalies such as VACTERL and other findings as well.

VACTERL properties

While different numbers of anomalies have been used to empirically diagnose VACTERL [3], the acronymic components are generally accepted as core findings with variable combinations. Statistical analysis confirms these defects as a distinct set of abnormalities instead of a non-specific grouping, while facial clefts [5] and neural tube defects [6] are negatively associated, and connections to other cephalic anomalies are reduced [7].

VACTERL anomalies arise early, during the stage of blastogenesis [8, 9]. Blastogenesis is dominated by the process of determination, where positional information controls cell fate and pattern. In other words, where a cell is located determines what it will become, in what context, and where it will finally be situated. This information is provided by gradients of signaling molecules or morphogens. Different morphogens form different developmental (also called determinative and morphogenetic) fields that organize development in a coordinated and interactive manner [10, 11].

Most components (VACTE) are midline. The exceptions, renal and limb, are often unilateral, with limb findings limited to radial and tibial duplications and deficiencies [12].

Locations and the number of findings vary [6, 7, 12–19], while timing differences for origins are apparent using cardiac anomalies as developmental stage markers [20].

For VACTERL overall, multiple developmental processes are affected during organogenesis [21], and physically adjacent components (e.g., tracheoesophageal and high vertebral anomalies [7]) may involve very different mechanisms.

Unlike genetic syndromes, where the presence or absence of one defect typically fails to affect the likelihood of another, the frequencies of the different associated VACTERL anomalies vary with the abnormality used for ascertainment [22], while taxonomic studies show overlapping clusters of anomalies [5–7, 13].

Given the complexities of VACTERL and its empiric origins, I have tried to formulate a comprehensive model that provides a theoretical justification for the association, as well as guidelines for future research.

A comprehensive model

Mosaic mitotic aneuploidy clone formation and elimination can be incorporated into a comprehensive model of VACTERL, and no clone properties are incompatible with a causal role.

Three aspects of VACTERL are central to the model:

1. Random temporal and spatial origins during a limited window of time [10–12];
2. Overlapping clusters of anomalies [9–12];
3. Early developmental effects involving the stage of determination [23].

With the suggested model, sequelae of aneuploid clone elimination disturb development at that location, leading to VACTERL anomalies in a minority of cases. The random origins of these clones [7, 8] explain the variable locations, extent, and developmental timing characteristic of VACTERL association anomalies.

At the same time, the specific physical locations of individual mitotic aneuploid clones impose spatial organization, with overlapping clusters of anomalies [9–11, 13], and biased findings related to ascertainment through different anomalies [22].

A limited early window of time for the origins and elimination of clones limits the primary effects to the stage of blastogenesis [8, 9], and to the process of determination, which can affect multiple later developmental processes occurring during organogenesis [11].

Sequelae of aneuploid clone elimination at a specific location can interrupt the positional information that controls development during determination. Sequelae could affect more than one potential anomaly at the same time, linking anomalies with otherwise independent origins.

Determination also involves two specific developmental vulnerabilities that are consistent with VACTERL findings [24]:

First is the midline [25], where most components (VACTE) arise. Besides being the earliest part of the body plan, the midline is developmentally unique. Instead of a self-contained developmental field, the midline is defined by its position between two other bilaterally symmetrical developmental fields. As a result, it has less positional information for the process of determination than other areas, creating a unique area of sensitivity to developmental disturbances [26].

Second, both theoretical models and experimental manipulations show that the most distal parts of developmental fields are preferentially affected by disturbances. For the limbs, the thumb and hallux (first toe) are the most distal areas during early development, explaining the characteristic radial and tibial findings [11], while with renal development, the metanephros, the earliest stage of the permanent kidney, is induced by the distal outgrowth of the ureteral bud [27].

Theoretical and experimental work also shows that both duplications and deficiencies can arise from similar disturbances of developmental fields [28], so that the thumb and the hallux can be underdeveloped or duplicated [29], while kidney defects include renal collecting system duplications and agenesis [14].

Two issues require further explanations:

First, VACTERL defects in a single patient can show significant physical separations despite localized causes. Although anomalies are most likely at or near the physical site of a disturbance, these separations are consistent with "ripple effects," reflecting the self-regulating nature of determinative fields, as a disturbance at one location destabilizes development over a broader area [11]. This phenomenon is especially prominent in two situations.

One involves the midline, which, as noted above, should be particularly vulnerable to disturbances of positional information [26].

The other concerns limb defects, which are physically separated from the other VACTERL anomalies. Here, a central disturbance can affect vulnerable distal positional information. Supporting central origins, 23 of 25 patients ascertained through arm anomalies also had costovertebral defects [16]. Similarly, there was "a strong association of bilateral limb defects with bilateral renal anomalies and unilateral with unilateral," with malformations elsewhere in almost all cases [17]. Since these two non-midline areas are far apart in development, correlations indicate a central effect on distal sensitivities.

The second general issue is the exclusion of other anomalies with very early developmental origins from VACTERL. This is understandable if defects arise in separate developmental fields. And here, while the primitive node alone induces the trunk, it interacts with the anterior visceral endoderm for head formation [30], creating a different positional information field. This alternative field controls the development of cephalic anomalies, so that even if clonal elimination also caused anomalies here, they would not associate with VACTERL findings. This explains the limited cephalad connections [7, 26], as with holoprosencephaly, which involves determinative patterning [31]. Timing may also minimize the overlaps here, since severe head anomalies should originate even earlier than VACTERL defects [32].

Other causes would also limit the connections to VACTERL, as with neural tube defects and facial clefts, where genetic and/or environmental factors are heavily involved [33, 34]. Similarly, limited links to truncal midline defects such as omphaloceles and diaphragmatic herniae [7], both alone and with neural tube defects and cleft lip/palate in the schisis-association of midline fusion anomalies [35], would reflect primary pathogenetic processes unrelated to determination [18].

Implications of the model

Besides organizing and explaining what is known about VACTERL and mosaic mitotic aneuploid clones, the model also has implications for understanding each of these:

1. Links between mosaic mitotic aneuploidy and VACTERL help define clonal elimination times: frequent arm anomalies in VACTERL predate rarer leg defects [29], suggesting that elimination is nearing completion at the time of lower limb determination.
2. For mitotic clonal mosaicism, defects such as VACTERL represent intermediary effects between major disruptive effects and normalization that may also involve further issues and findings, including isolated SUV (see item 7). Of greater concern, VACTERL anomalies, both isolated and combined, show increased prenatal lethality, growth delays, and prematurity [21, 36, 37], raising the possibility that clonal elimination is related to such adverse effects as well, even in the absence of VACTERL anomalies, although confounders make this difficult to evaluate, as described in the next section.

   In this respect, while significant losses have not been noted with genetic forms, idiopathic holoprosencephaly is almost always lethal early in gestation, with a total frequency of this finding of 1/16,000 live births and 1/250 conceptuses [38]. This may be similar to the phenomenon of VACTERL losses, even though the degree of lethality

is greater than with association anomalies [27, 36] for unknown reasons, although earlier timing for holoprosencephaly is one possibility.

3. Although idiopathic anomalies arising in different developmental fields are excluded from VACTERL, clone elimination effects should also apply to these areas, giving mitotic mosaic aneuploidy a more general role in the etiology of malformations. Holoprosencephaly is a likely example, with very early origins and a determination-based pathogenesis [38].
4. Although an association by definition includes at least two concordant findings, there is no pathogenetic reason to exclude isolated defects, and a similar epidemiology for both associated and isolated VACTERL anomalies supports a single disorder [36]. (Note: defects are etiologically heterogeneous, and VACTERL would include some, but not all, isolated occurrences.)
5. Given the rarity of VACTERL compared to the frequency of mosaic aneuploidy, mosaicism leads to malformations in only a small minority of embryos. With this limitation, clonal variables such as size and placement, and aneuploidy type, may be influential.
6. A role for clonal elimination explains a dearth of VACTERL in animals, with only one report—a cat with vertebral, anal, radial, cardiovascular, and renal defects [39]: clone-related lethality is part of a high rate of embryonic loss in humans compared to other mammals, losses with unique evolutionary justifications [40]. With this, infrequent mitotic aneuploidy effects in animals [2] should be reflected in lower incidences of VACTERL.
7. Similarly, although an atretic form is common in humans with and without VACTERL, idiopathic SUA [37] is exceptional in animals, with only a single English language report, a calf with other findings [41] indicating a developmental form instead. Human SUA correlates with decreased embryonic size [37], which is compatible with clone elimination volume loss, so that the same considerations noted above suggest that human idiopathic SUA mostly involves mitotic aneuploid clones.

Ancillary factors

Delineating VACTERL is complicated by confounders that are not causes per se but that non-specifically enhance the effects of the elimination of mitotic aneuploid clones. Two such factors are particularly common.

One is embryonic hypocellularity, with decreased cell numbers for a particular developmental stage. This phenomenon is consistent with higher VACTERL rates with monozygotic twinning, maternal diabetes, and certain types of (non-mosaic) aneuploidy with such reductions [37], since a smaller embryo would increase the relative impact of a clone of a specific size. Elimination of a clone should, to some extent, also decrease the mass of the embryo, contributing to hypocellularity, so that a larger clone would theoretically be more likely to lead to VACTERL, and to related findings (below), than a smaller one.

Although a normal size can be restored, early reductions may persist long enough to affect vascular flow dynamics, resulting in a single umbilical artery, a frequent concomitant of VACTERL, as a secondary atresia instead of a primary malformation [37]. These types of circulatory effects may contribute to VACTERL cardiovascular anomalies, which are the most common associated defects [3].

The other confounder involves epigenetic disturbances that increase embryonic and fetal vulnerabilities to developmental and other perturbations by modifying the actions of multiple genes, usually by affecting DNA methylation. This increases the risks for VACTERL and other malformations and for adverse pregnancy outcomes such as losses, prematurity, growth delays, and perinatal difficulties [36, 42]. These epigenetic factors would obscure possible clone related predispositions to the same adversities.

One additional possible confounder involves genes and other factors, such as copy number variations [43], that may increase vulnerabilities to specific VACTERL components.

Other explanations

The suggested model involves exogenous disturbances of developmental programming. The main alternatives are intrinsic genetic factors, which are wanting in several respects.

First, while the Fanconi anemia complementation group is an apparent genetic form, added findings, such as hematologic disorders, indicate intrinsic issues with the process of determination instead of exogenous teratogenic "hits." In these circumstances, Fanconi anemia also shows biases, such as isolated radial abnormalities as the most common finding, which differ from those of VACTERL [44].

Second, in terms of general relationships, some common VACTERL combinations, such as tracheoesophageal and high vertebral findings, are rare with Mendelian disorders [23], and individual anomalies found together often involve separate developmental issues difficult to explain through single genes. Genetic causes also fail to account for the frequent adverse pregnancy outcomes [36], ascertainment biases, and clustering seen with VACTERL [9–12, 22].

Finally, putative examples of familial or genetic VACTERL are uncommon, and either lack certain VACTERL anomalies or show them rarely. These cases typically lack the distinctive VACTERL limb findings [42], and are more compatible with midline effects [25, 26], while key

spatial and temporal relationships between the association anomalies [15, 20] have not been demonstrated.

Still, causal heterogeneity is possible, so that rare mutations [45] can mimic VACTERL. However, the issues cited above indicate that this accounts for only a small minority of cases at best and fails to explain the broad range of findings.

Conclusions

A theoretical model of the VACTERL association based upon the effects of mitotic aneuploid clone elimination during the stage of determination accounts for all major empiric properties, to wit:

1. A specific set of correlated anomalies, with others largely excluded
2. A preponderance of midline anomalies
3. Connections between the non-midline arm and renal defects and midline disorders
4. Exclusively radial and tibial limb findings, with both duplications and deficiencies
5. Statistical correlations defining the overlapping clusters of defects
6. Variable numbers and combinations of findings
7. Involvement of multiple unrelated developmental processes
8. Variable developmental timing within a limited temporal window

The model also has several implications, including:

1. The inclusion of isolated defects as part of the spectrum of VACTERL findings.
2. The formation of malformations unassociated with VACTERL through the same clone related mechanism, but in different developmental fields.
3. A relationship between mosaic aneuploid clone sequelae and non-structural adverse pregnancy outcomes, such as losses, prematurity, and growth restrictions.
4. Completion of early mitotic aneuploid clone elimination shortly after the time of lower limb determination.
5. That a rarity of VACTERL in animals is consistent with lower rates of mitotic mosaic aneuploidy effects.
6. A similar rarity of idiopathic SUA in animals suggests that most such human SUA involves mitotic mosaic aneuploidy.

Overall, this model provides a theoretical justification for VACTERL as a distinct, albeit variable disorder, with a specific cause and a defined pathogenesis, instead of an empiric or statistical set of findings. It also indicates a greater role for mitotic mosaic aneuploidy in the genesis of adverse pregnancy outcomes than previously suggested.

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Compliance with ethical standards

Conflict of interest The author declares that there are no conflicts of interest.

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