Vascular Ehlers Danlos syndrome: Pathways to Progress. Current state of knowledge, assessment, and treatment.

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Background

Arterial rupture, bowel rupture, rupture of the gravid uterus and striking tissue fragility are the classic hallmarks of the dominantly inherited vascular Ehlers Danlos syndrome, an uncommon genetic disorder that results from mutations in *COL3A1*, which encodes the chains of type III procollagen. Although some affected individuals may have other clinical manifestations that include unexplained extensive bruising, small and large joint hypermobility and subluxation, thin translucent skin, and spontaneous pneumothorax, the diagnosis frequently awaits a major and sometimes catastrophic event. Even the multi-generation family history of early death as a result of arterial rupture may not trigger the search for the diagnosis unless the individual or family is seen in an academic setting.

The rarity of this condition has meant that most individuals are seen and complications managed in isolation, centers for care are difficult to establish in the US because of distance, insurance, and medical specialty considerations. As a result, the development of a strategy for care, an approach to treatment, a true understanding of the natural history of the condition, the development of biologically based effective therapies, and early diagnosis have been restricted. To develop approaches to these concerns, an international group of some 30 clinicians, translational and basic scientists, along with representatives of some of the family peer groups met in Chicago to review current knowledge, consider some of the recent advances, and develop a strategic collaboration to understand molecular pathways to the generation of complications as a means to identify effective intervention. The meeting was sponsored by the Freudmann Fund for Research in Ehlers Danlos syndrome, EDS Cares, and the Ehlers Danlos National Foundation.

Summary of Presentations

Estimates of the incidence/prevalence of vascular EDS have ranged from about 1/50,000 to 1/million. Two strategies to measure the prevalence have been the use of diagnostic testing to provide a minimum prevalence estimate (Melanie Pepin, University of Washington; Danielle Macaya, GeneDx; Paul Coucke, University of Ghent; Bart Loeys, University of Antwerp), and the creation of clinical centers of excellence to which all individuals with the diagnosis are referred (Michael Frank, Xavier Jeunemaitre, Universite Paris Descartes; Julie DeBacker, University of Ghent; Diana Johnson, Glenda Sobey, University of Sheffield and the British Consortium for Rare Disorders). The diagnostic laboratories in the US have identified 2000-2500 individuals with vEDS based on counts in the groups at the meeting and surveys of others. This represents a minimum estimate of prevalence of about 1/125,000. Estimates from France, Britain, and Belgium based on clinical referrals are similar but are limited by lower ascertainment in families. Among those groups, generally mutations that result in substitution for glycine residues in the triple helical domain of type III procollagen (characterized by the repeating Gly-X-Y amino acid sequence) and splice site mutations that lead to exon skipping account for more than 90% of affected individuals. There is a significant under-representation of substitution of glycine by small residues (alanine, serine, cysteine) compared to the expected frequency and, in contrast to mutations seen in another member of the same gene clade, *COL1A1*, the frequency of heterozygous null mutations is lower than expected (Peter Byers, University of Washington).

There is nothing in the gene sequences that would predict these differences and it is likely that many of the individuals with these mutations do not come to clinical attention. In some families in which a proband was identified with a heterozygous null mutation other family members may have had no clinical complications even into their 8th decade. If these differences in behavior of mutations are considered, then the frequency of potentially deleterious variants must approach or exceed 1/50,000 in the populations. Survey of variants found in the ExAC data base of some 60,500 exome sequences from individuals not known to have single gene mendelian disorders identified 7 variants that would be classified as pathogenic or causative which far exceeds the estimate of prevalence obtained from clinical studies. This assessment is based on the assumption that the phenotypes of the missing mutations from clinical studies would have the same or similar clinical presentations. Instead, they could result in later onset of some of the same clinical features or, perhaps, in different phenotypes, governed by the nature and locations of the sequence alteration.

Parental mosaicism for the causative mutation was identified in more than 10% of families in which a child with an apparently *de novo* mutation was identified. The natural history of this group with respect to the development of isolated arterial events has not been assessed. There is stratification of the risk of a fatal arterial event based on the nature and type of mutation: exon skipping mutations have the largest effect, substitution of glycine by large hydrophobic residues and large charged residues are next, and those with least effect are quantitative mutations (Pepin, Frank, Leistritz, University of Washington). This effect is manifest by the age of the incident with a shift of about 15 years between the earliest and latest events.

These models of natural history derive from two different types of ascertainment—a cross-sectional view gained by diagnostic laboratories that are linked to single time assessments (Pepin, Macaya, Coucke, Loeys), and the longitudinal views afforded by centralized programs with regular assessments (Frank, Jeunemaitre—Paris; De Backer—Ghent; Diana Johnson, Glenda Sobey—Sheffield). Longitudinal studies (Frank) of arterial integrity, as part of a surveillance program in which the affected individuals are seen every 1-1.5 years found that arterial dissections can heal, that almost all adults have had dissections in the carotid artery usually in the extracranial portion and that most of those dissections were asymptomatic and stable. Almost all individuals in the study were treated with Celiprolol. Most people have slowly progressive changes that do not presage the time or the location of an arterial event. Indeed, it appeared that arterial events (dissection and rupture into a confined space) are generally handled medically. The general impression was that hospitalization due to catastrophic events was reduced after Celiprolol was introduced but the absence of a control group was a concern. Stenting has been used (Frank; James Black, Johns Hopkins) but concern about arterial integrity in the face of pressured stents has lead to decreased enthusiasm. When intervention has occurred, context seems to matter so that planned approaches rather than those done at the time of an event appear to have more success (Black; Sherene Shalhub, University of Washington) and that the nature of the underlying mutation matters—people with null mutations fare the best (Shalhub). The development of biological markers has lagged but the idea that new imaging technologies such as 4-D MRI might assist in the identification of sites of unusual hemodynamic properties, a possible indicator of rupture is appealing (Balu Niranjan, University of Washington).

The outcome of bowel rupture events may also differ, depending on the perspective. Cross sectional studies suggested that surgical intervention with creation of a blind loop and colostomy, followed a few months later by reconstitution of the bowel was an effective mode of treatment (Pepin). The longitudinal studies appeared to be less optimistic with a higher rate of failure of the reconstitution and an observable rate of re-rupture (Frank, Sobey). On the basis of those studies, there was a sense that partial or full colectomy at the time of the first rupture might be advantageous in the long run, but definitive data were lacking.

Recurrent hemoptysis emerged as a more frequent characteristic than previously recognized, often paired with spontaneous pneumothorax and the presence of unusual pulmonary nodules (Enid Neptune, Johns Hopkins). The source of the pathology, the frequency of this entity rather than uncomplicated pneumothorax, and the occurrence of hemopneumothorax appeared to be more frequent than previously appreciated. Histology of the lung was consistent with failure of adequate septation during development, leading to larger alveolar spaces that could alter pulmonary function. The question was raised whether pneumothorax could be a sentinel event and a forerunner of arterial compromise by dissection or rupture (Shalhub).

Pregnancy has, in some quarters, been considered contraindicated for women with vascular EDS. A fifty percent risk of transmission, and a perceived high death rate during pregnancy were the major issues and might lead to advice such as "do not get pregnant, if pregnant terminate the pregnancy, and if not, seek care elsewhere". In this context, the study of about 300 women with vascular EDS who had become pregnant and a similar number with vascular EDS who had not, failed to reveal a difference in apparent survival (Kaplan Meier curves) (Mitzi Murray, University of Washington). Uterine rupture was rare but could be lethal and except for tissue fragility in the birth canal, other complications were similar to those seen in women of the same age. Early C-section could not be assessed to benefit because of limited data but did reduce tissue damage. Few pregnancy complications were identified in women with null mutations.

Diagnostic studies in children proved to be controversial (Frank, Pepin, Johnson, Murray) and the argument for performing them under the age of 10 was often expressed by parents who indicated that it would remove about 50% of children from surveillance and provide them with control of the situation, even in the absence of definitive treatments. The other side of the question was the concern about stigmatization, the lack of definitive treatments, and implementations of restrictions in the absence of clear clinical data.

Other organ system involvement (ocular, skin, dentition for example) was not as extensively covered except to note that keratoconus may be more common, that carotid cavernous sinus fistula formation was unusually common in this group, and that alopecia and extensive (rather than “easy”) bruising were recognized features but their predictive value in terms of arterial compromise could not be assessed. Two birth defects, congenital hip dislocation and club foot are seen more often than expected.

Animal models of heritable connective tissue disorders (Marfan syndrome, osteogenesis imperfecta, dermatosparaxis and others) have played a key role in the characterization of the pathway from nucleotide change to phenotype and provided the experimental substrate to test candidate small molecule intervention. Animal model for vascular EDS had been limited to two lineages, both of which were heterozygous for mutations that led to haploinsufficiency. These could be valuable for the identification of modifying loci that increase the rate of aortic or small vessel dissection or rupture (Jefferson Doyle, Johns Hopkins, re: experience with Marfan syndrome) but do not represent the common mutations in this human disorder. Two long anticipated missense mutations, both in the first 50 residues of the triple helical domain, are now available (Jeunemaitre, Paris, Graham Rykiel—Johns Hopkins, Dietz laboratory). In the absence of provocative hypertension, neither developed lethal events within the first year of life. But hypertension provoked by angiotensin but not norepinephrine was rapidly lethal. Treatment with one model with beta blockade appeared to delay dissection/rupture and losartan also appeared to provide protection. Planned genetic and small molecule therapies in these animals are anticipated.

The two types of mouse models (haploinsufficiency and missense mutations in the triple helical domain) sample two classes of effects. Both haploinsufficiency models probably arise as a consequence of mRNA instability and the effect of activating the nonsense-mediated mRNA degradation pathway, in addition to production of half the normal amount of type III procollagen, can be assessed. The missense mutations are both close to the amino-terminal end of the triple helical domain and so the effect on triple helix folding is small, an effect on propeptide cleavage cannot be excluded, and determination of the effect of both reduction in secretion (one apparent effect) and intracellular accumulation is not clear. Other pathogenetic mechanisms that could reflect different classes of mutations include haploinsufficiency due to failure of protein folding with carboxy-terminal propeptide alterations and add an additional factor of activation of the unfolded protein responses. Exon-skipping mutations can lead to normal secretion of two classes of intact helical molecules, one of which (about 1/8th of the total) has three short chains, in addition to sometimes marked ER inclusion of poorly folded molecules. The effect of missense mutations closer to the carboxyl-terminal end of the triple helical domain can differ from those near the amino-terminal end as a consequence of sometimes dramatic ER inclusion.

Like all good conferences, this one succeeded both in the creation and validation of a research and clinical community and identified many unanswered questions that will direct research in the next few years. These include, but are not limited to the following:

* What is the frequency of deleterious mutations in *COL3A1* and do all mutations result in the a phenotype in the vascular EDS spectrum?
* Do the “missing” mutations appear in mildly involved individuals?
* Is surveillance beneficial to the affected individual?
* If so, what is the best modality to use?
* What are the criteria and tools for intervention?
* What is the natural history of vascular stents and coils?
* What procedures are most beneficial for GI ruptures?
* Does mutation type, location, nature help in the prediction of specific types of involvement?
* Are additional animal models (splice site mutations, C-terminal propeptide mutations, for example) justified to explore additional pathogenetic mechanisms and interventions?
* Will registries be beneficial to determine effects of drugs, surgery, and other treatments?
* Should additional trials for other agents be begun and, if so, what medications?
* Are they ways to rescue data about celiprolol treatment with outside controls?
* Is there a biological explanation for the downward spiral?
* Do different mutation classes (splice site, missense, protein nulls, RNA nulls) have different mechanisms and would treatment differ?
* What are the mechanistic pathways for each mutation type?
* Are there specific interventions derived from animal models?