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A Roadmap to Investigate the Genetic Basis of Bicuspid Aortic Valve and its Complications:

Insights From the International BAVCon (Bicuspid Aortic Valve Consortium)

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Abstract

Bicuspid aortic valve (BAV) is the most common adult congenital heart defect and is found in 0.5% to 2.0% of the general population. The term “BAV” refers to a heterogeneous group of disorders characterized by diverse aortic valve malformations with associated aortopathy, congenital heart defects, and genetic syndromes. Even after decades of investigation, the genetic determinants of BAV and its complications remain largely undefined. Just as BAV phenotypes are highly variable, the genetic etiologies of BAV are equally diverse and vary from complex

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inheritance in families to sporadic cases without any evidence of inheritance. In this paper, the authors discuss current concepts in BAV genetics and propose a roadmap for unraveling unanswered questions about BAV through the integrated analysis of genetic and clinical data.

Keywords

bicuspid; consortium; roadmap; valve

The most common adult congenital heart defect, a bicuspid aortic valve (BAV), is found in 0.5% to 2.0% of the general population (1,2), with a higher prevalence in men. BAVs result from abnormal aortic cusp formation during valvulogenesis, in which adjacent cusps fuse into a single cusp that is bigger than its counterpart but smaller than 2 normal cusps combined (2). Overall, “BAV” refers to a heterogeneous group of disorders distinguished by aortic valve malformations with associated aortopathy, congenital heart defects, and genetic syndromes. Despite years of investigation, researchers have yet to define genetic determinants of BAV and its complications, although most patients with BAV will develop complications requiring treatment. Just as BAV phenotypes are highly variable, the genetic etiologies of BAV are equally diverse and vary from complex inheritance in families to sporadic cases without evidence of inheritance (3).

CURRENT KNOWLEDGE OF THE GENETICS OF BICUSPID AORTIC VALVE GENETIC SYNDROMES

Although most patients with BAV have no extracardiac abnormalities, BAV also occurs as a component of known genetic syndromes (Table 1). The highest penetrance of BAV for a genetic syndrome occurs in women with Turner syndrome, which is caused by partial or complete absence of one X chromosome in women. BAV appears in >30% of women with Turner syndrome, and the prevalence of associated coarctation, aortic aneurysms, and acute aortic dissections exceeds that in sporadic BAV cases (4). In the general population, BAV is more prevalent in men (1% to 2%) than women (0.5%) (5), suggesting that the loss of genes on the X chromosome may predispose to BAV formation. The genes responsible for this effect have not been identified.

CONGENITAL HEART DEFECTS

Although the genetic links remain unknown, BAV is also seen with some congenital heart disorders involving the left ventricular outflow tract (LVOT), such as hypoplastic left heart syndrome, coarctation of the aorta, and some ventricular septal defects (6–8). Hypoplastic left heart syndrome and coarctation are significantly enriched among primary relatives of patients with BAV, and according to linkage analysis, mutations in the same genes probably caused some cases of hypoplastic left heart syndrome and BAV (9). One possible interpretation of these observations is that LVOT morphology and obstruction may also influence aortic valve development because of alterations in blood flow or shear stress (10).

FAMILIAL INHERITANCE

BAV occasionally demonstrates complex inheritance in large families without syndromic features. Autosomal-dominant transmission of BAV was observed in some 3-generation pedigrees, but no single-gene model clearly explains BAV inheritance (11–13). The prevalence of BAV stands nearly 10-fold higher in primary relatives of patients with BAV than in the general population (1). Inheritance is observed in >50% of families if associated nonvalvular complications are involved, such as aortic coarctation, thoracic aortic aneurysms, and mitral valve or ventricular septal defects, which are each found in 10% of family members (14). The heritability of BAV has been estimated to be as high as 0.89 in these studies, and multiple genetic alleles can interact to cause BAV or different congenital heart defects without BAV in the same family. Genetic, epigenetic, and environmental modifiers may be responsible for the variable penetrance and phenotypic expression.

Through linkage analysis of BAV pedigrees, investigators have uncovered multiple genetic loci for BAV. The most significantly associated genes were located on chromosomes 3p22 (*TGFBR2*), 5q15-21, 9q22.33 (*TGFBR1*), 9q34-35 (*NOTCH1*), 10q23.3 (*ACTA2*), 13q33-qter, 15q25-q26.1, 17q24 (*KCNJ2*), and 18q, with a maximal logarithm of odds score of 3.8 on chromosome 18q22.1 (13,15,16). Most families were identified because of thoracic aortic aneurysms and dissections (TAAD); BAV was found incidentally in some patients.

These studies, along with the diverse genetic syndromes associated with BAV, highlight BAV's substantial genetic heterogeneity and implicate many discrete genes in its formation. Importantly, BAV may not have been detected on the echocardiograms of index patients. Without definitive phenotypic classification of affected patients, linkage analysis offers substantially diminished power and has not generally succeeded in identifying genes that cause BAV without aortic or extravascular manifestations. *NOTCH1* remains the only gene for isolated BAV identified using linkage analysis and positional cloning strategies, but it is unlikely to cause more than a small proportion of familial cases (17,18).

In families with inherited BAV, the mutated gene often correlates with specific features and prognosis. *ACTA2* mutations cause a syndrome that may include BAV as well as thoracic aortic aneurysms, premature coronary artery disease, and cerebrovascular disease (19). *NOTCH1* mutations predispose to BAV with calcific aortic stenosis but are not associated with aortic disease or other extracardiac abnormalities. BAV occurs in 20% of cases of Loeys-Dietz syndrome, associated with craniofacial defects and connective tissue fragility with vulnerability to acute arterial dissections (20). Therefore, knowledge of the particular mutation in a family can direct clinicians to screen for additional clinical features in the proband and family members. As more BAV genes are discovered, genetic information will increasingly influence clinical decisions about diagnostic tests and therapies for patients with BAV.

Studies involving large cohorts of patients with BAV and thoracic aortic disease indicate that many types of genetic variation contribute to the risk for this complex disease, from common single-nucleotide polymorphisms to rare copy-number variants and chromosomal abnormalities (Fig. 1A). Mutations in the same gene appear to cause TAAD across an allelic spectrum ranging from recognizable genetic syndromes to sporadic disease. For example,

some mutations in *MYH11* cause autosomal-dominant inheritance of TAAD associated with patent ductus arteriosus, but rare duplications involving the same gene predispose to isolated, nonfamilial cases of aortic dissection (21–23). In the majority of these patients, the total burden of genetic variants and environmental factors, rather than a single variant or class of variants, contributes to TAAD risk.

Because BAV and TAAD frequently occur together, the genetic architecture of BAV may also consist of many different genetic variants that interact in an additive manner to increase risk for BAV and its complications (Fig. 1B). Therefore, successful gene discovery in BAV will require a combination of different methods, such as linkage analysis, positional cloning, copy-number analysis, genome-wide association (GWA) studies, and ultimately whole-genome sequencing as its cost continues to decrease.

FEATURES OF BICUSPID AORTIC VALVE THAT INFLUENCE GENE DISCOVERY

CLINICAL HETEROGENEITY

Reduced penetrance and variable expressivity present major impediments to identifying novel BAV genes in families (24). Thus, some subjects who transmit BAV to their offspring who are assumed to harbor causative genetic mutations may not have BAV themselves or may have other cardiovascular abnormalities. BAV disease also displays remarkable variation in the age of onset of complications, such as aortic valve disease or TAAD. Although BAV formation is genetically determined, the rate of progression to clinically evident disease or the development of aortopathy may be driven by interactions between genetic factors causing BAV, genetic factors associated with the development of aortic stenosis in tricuspid aortic valves, and well-established cardiovascular risk factors such as smoking, hypertension, and dyslipidemia (25,26). These factors heavily influence valve calcification leading to aortic stenosis or regurgitation (the most common indications for valve replacement surgery in patients with BAV) (27–30).

The interaction between genes and environment in influencing aortic valve complications is also apparent in mice, in which the development of calcific tricuspid aortic valve stenosis required both dietary modifications and specific genetic mutations (31). A recent GWA study demonstrated that an intronic genetic variant at the lipoprotein(a) locus promotes human aortic valve calcification (25). Distinct genetic variants and environmental hazards, independent of the factors that cause BAV formation, may influence the complications and outcomes of BAV disease and thus the recognition of BAV in an individual patient.

ANIMAL MODELS

The paucity of animal studies erects another obstacle to understanding BAV's etiology. It develops spontaneously in an inbred strain of Syrian hamsters but without valve or aortic complications typical of human BAV, and the underlying genetic defect is unknown (32,33). Targeted deletions of fibroblast growth factor, bone morphogenetic protein, and Notch pathway genes in mice produced BAV with incomplete penetrance, as well as various malformations of the LVOT and aorta (Table 2). This approach, if sufficiently scaled to

accommodate the large numbers of BAV candidate genes and pathways, can help prioritize candidate genes for further analysis.

However, with rare exceptions, mutations in genes that cause BAV when targeted in animals have not been found in humans with BAV. Therefore, typical animal models with single gene mutations may not be sufficient, and incorporation of multiple mutations or gene-environment interactions will likely be required to understand the complexity of human BAV disease.

CLINICAL IMPLICATIONS

Because BAV formation and disease involve many genes, developing clinical genetic tests to identify patients at risk for BAV-related complications, such as aortic stenosis or TAAD, will require considerable time and research. Until then, early identification and careful surveillance of at-risk family members will likely remain primary clinical strategies. The 2010 American College of Cardiology and American Heart Association guidelines for thoracic aortic disease provide a class I indication to image first-degree relatives of patients with BAV and TAAD to detect valvular and extravalvular complications (34,35). Affected relatives can then receive interventions to reduce traditional cardiovascular risk factors and, perhaps, emerging therapies targeted to prevent these complications. It is also recommended that every newly diagnosed BAV patient undergo a comprehensive clinical evaluation that includes complete imaging, at a minimum, of the heart and thoracic aorta to rule out other defects and additional imaging as warranted by examination or family history. Genetic tests are offered to patients with BAV when features of single-gene disorders or syndromes are present, and findings are confirmed in first-degree relatives whenever possible.

BICUSPID AORTIC VALVE GENETICS: ROADMAP TO DISCOVERY

With so many issues to be resolved, how can we unravel the genetic complexity of BAV? A proposed roadmap for translational applications of genetic discoveries is illustrated in the Central Illustration.

The International Bicuspid Aortic Valve Consortium (BAVCon) (Online Appendix) was recently founded to translate genetic discoveries into clinically useful diagnostic tests and therapies, as well as address broader challenges related to knowledge gaps in the pathophysiology and natural history of BAV disease. Designed as a registry for long-term surveillance, BAVCon is conceptually similar to the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC), which enrolled subjects with diverse forms of thoracic aortic disease, including BAV. The BAVCon Registry consists of independently arbitrated echocardiographic and computed tomographic images from more than 4,000 patients with BAV and tricuspid controls retrospectively enrolled at 16 centers in North America and Europe. Prospective collection of images and tissue samples from additional patients, and the eventual incorporation of GenTAC patients with BAV into BAVCon, will create a deeply phenotyped and collaborative resource for clinical, genetic, and molecular studies available to all BAVCon investigators.

The enormous diversity of BAV with respect to anatomic variation, coexisting abnormalities, and natural history poses a significant challenge to researchers whose goal is to identify genes that cause BAV or predict BAV-associated complications. To sort through this genetic complexity, large and ethnically diverse cohorts that are well characterized in terms of BAV anatomy, associated complications, and cardiovascular and noncardiovascular risk factors are needed. BAVCon will permit adequately powered GWA studies, which are particularly important for understanding and predicting endpoints of BAV disease, such as valve calcification and acute aortic dissection.

Similarly, well-characterized families predisposed to BAV and its complications inherited in a Mendelian manner are needed to identify genes with mutations that confer high disease risk. Investigators may need to follow this cohort for a decade or longer to capture outcomes, such as aortic valve stenosis, that are slow to develop. Designed with these challenges in mind, BAVCon represents the first worldwide registry of ethnically diverse patients with BAV characterized by experts in the field using stringent, uniform criteria and standardized measurements. Valve morphology and hemodynamic data will be precisely assessed using state-of-the-art imaging methods and will be correlated with these outcomes. Because different genetic variants may contribute to outcomes, we envision BAVCon as a rich and continuous source of future genetic studies.

Large families with inherited predisposition to BAV present the most promising opportunities for gene discovery, because family based studies optimize the statistical power to identify causative genetic variants. When BAV is inherited as an autosomal-dominant trait, the mutant gene can be traced from parents to offspring using segregation analysis, and the causative mutations can then be identified by sequencing the genes of distantly related subjects (Fig. 2A). The ability to sequence entire exomes or genomes of multiple family members has greatly reduced the time and labor required for gene discovery and has led to the discovery of several novel genes for TAAO (21,36–39).

Even if BAV inheritance is not observed, genetic tests of family members may still be informative. Genetic variants present in affected children but not their parents are referred to as *de novo* variants (Fig. 2B), which are generally prioritized because they tend to be more deleterious than inherited variations (40). Recent trio-based exome sequencing studies identified causal *de novo* mutations in several rare syndromes that are not inherited in families (41,42). Because dozens of *de novo* variants arise stochastically in each generation, candidate genes with *de novo* variants need to be validated by performing mutation analysis of independent groups of cases.

In contrast to families with inherited BAV disease, >80% of patients with BAV have no known affected relatives and are regarded as sporadic cases. Because linkage analysis is unfeasible to investigate single genetic variants in unrelated patients, case-control association methods such as GWA studies must be used (Fig. 2C). GWA studies typically require thousands of affected individuals and controls to generate meaningful results and are measured by indirect associations between the genuine risk variants and genotyped single-nucleotide polymorphisms, rather than by direct identification of the causal variants. Thus, GWA studies are most likely to identify genetic variants that increase susceptibility to BAV

or its complications. The size of the BAVCon cohort will provide sufficient power to discover these common but low-impact BAV-associated variants.

Among sporadic BAV cases, patients at the severe end of the BAV phenotypic spectrum are most likely to have highly penetrant mutations in single genes because they resemble patients with genetic syndromes or inherited BAV. This includes patients with unicuspid aortic valves, those with additional congenital malformations, and those who require early interventions for valve disease or TAAO manifesting in childhood or adolescence. Genomic comparisons between these subgroups and older patients with BAV without significant valve or aortic complications can uncover genetic variants that predispose to BAV-associated complications. In normal populations, these variants may only weakly influence the likelihood of developing disease and may be difficult to detect, but the effects of these variants are likely to be amplified in individuals with BAV, who tend to develop more pronounced aortic disease at younger ages. In other situations, this strategy of sampling at the phenotypic extremes led to the discovery of individually rare variants that would otherwise be missed by traditional GWA studies (43–46).

Genetic studies may lead to the discovery of novel genetic variants whose biological relevance to BAV disease is unknown. Leveraging systems biology to identify data patterns can help prioritize candidate genes. Systems analyses test associations of biological pathways, rather than single genes, with disease. For example, the discovery of *NOTCH1* mutations in BAV families led to the systematic investigation of Notch pathway genes in aortic valve development and calcification (47–49). De novo variants of genes that regulate histone modification were found to be enriched in patients with severe congenital heart disease (50). These pathways and others with relevance to BAV outcomes include genes involved in aortic valve calcification, connective tissue integrity, and aortic disease. Using systems-based approaches, BAV candidate genes can be ranked according to their biological functions; interacting genes in the same pathways can then be screened for mutations in BAV cases by sequencing or copy-number analysis.

Because BAV displays incomplete penetrance, a substantial proportion of BAV-associated genetic variants in sporadic cases may also be present in apparently unaffected subjects. Therefore, independent observations of genetic discoveries in separate cohorts of patients with BAV will be essential for the prioritization of candidate genes. With an enrollment of several thousand patients, BAVCon will provide an important platform for these types of validation studies.

Despite significant limitations in BAV animal models, targeted gene deletions in mice, zebrafish, and cultured aortic valve interstitial cells have provided useful insights into candidate gene involvement in LVOT or aortic valve development (3,51,52). Some of these methods can be developed into high-throughput assays to screen candidate genes for roles in BAV formation or disease progression. To investigate therapies for BAV-associated complications, improved genetic models are needed. “Knock-in” experiments, in which human mutations are introduced into homologous mouse or zebrafish genes, may result in more faithful models of human BAV phenotypes than current models with null mutations (53). Pathobiological studies may complement these strategies by validating candidate

genes' biological functions or identifying pathways that can be explored using systems methods. These approaches already have led to the discovery of new drug targets and genetic tests for inherited cardiomyopathies, and hold tremendous promise for BAV (54–56).

BACK TO THE BEDSIDE OF THE PATIENT WITH BICUSPID AORTIC VALVE

How then do we translate these discoveries into clinically meaningful tools for patients with BAV and their families? One approach is to develop genetic tests or biomarkers that identify patients with BAV who are at high risk for adverse outcomes such as aortic valve stenosis or TAAD. Selected patients with BAV can then be enrolled into clinical trials to assess novel preventative strategies or drug therapies on the basis of their individualized genetic risk profiles. Clinical trials will determine if selectively interfering with these pathways using this targeted approach can retard or prevent disease progression.

Similarly, genetic risk profiles may influence surgical decisions. For example, young patients with TAAD with normally functioning or mildly dysfunctional BAVs frequently receive valve-sparing aortic grafts, but we do not know which of them may require future valve replacements or more distal aortic repair (57,58). Genetic profiles may dictate when valve replacements are indicated during aortic repair and, most important, which aortas need to be repaired at the time of valve replacement, a subject of significant current controversy. These are only 2 of many potential examples that illustrate the impact of genetic discoveries on BAV diagnosis and therapies. BAVCon will provide us with unprecedented detail about subtypes of BAV disease through its large and well-phenotyped cohort of patients, making this type of comparative analysis possible for the first time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ABBREVIATIONS AND ACRONYMS

BAV	bicuspid aortic valve
BAVCon	International Bicuspid Aortic Valve Consortium
GWA	genome-wide association
LVOT	left ventricular outflow tract
TAAD	thoracic aortic aneurysms and dissections

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APPENDIX

For a list of the BAVCon sites and investigators, please see the online version of this article.

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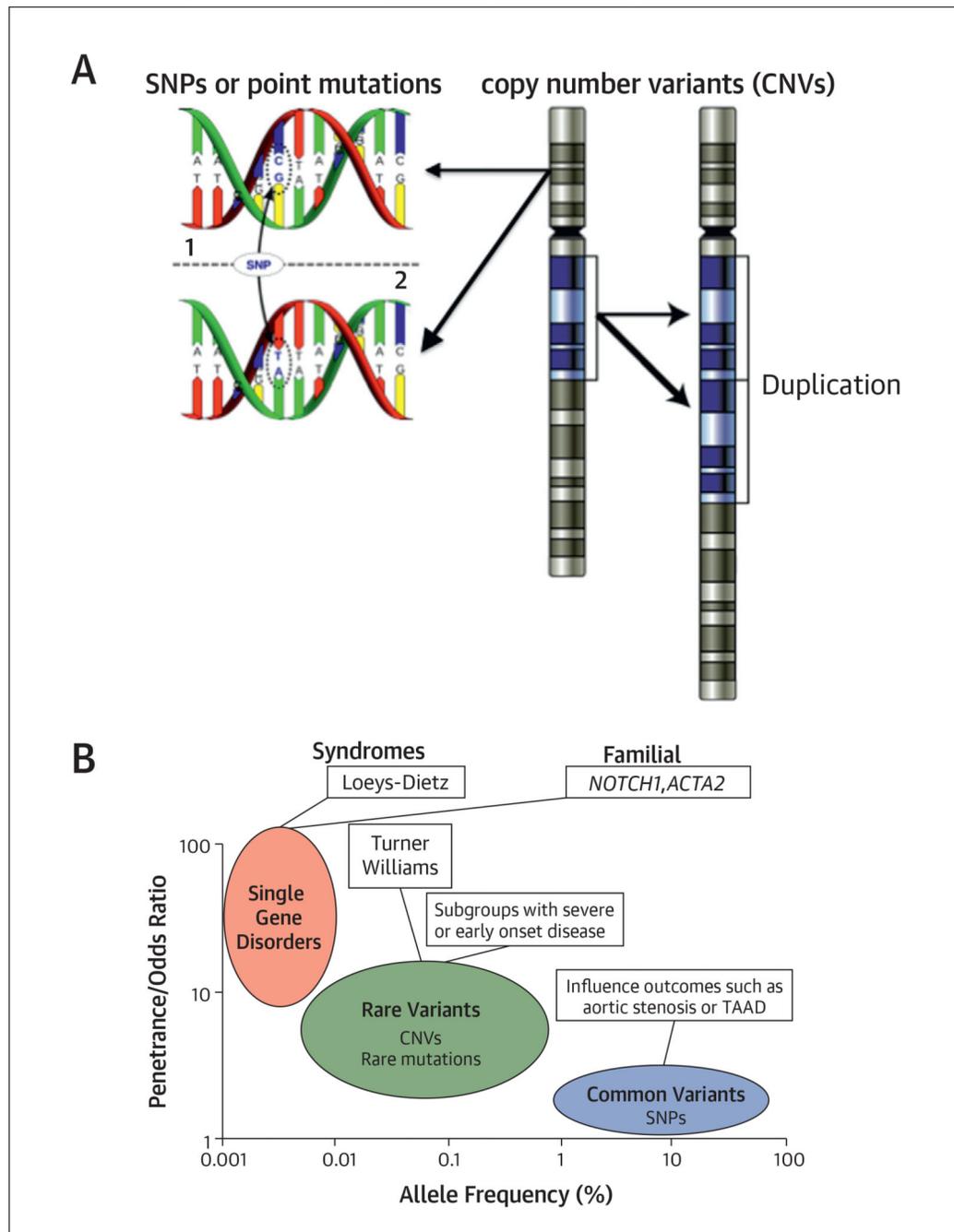


FIGURE 1. Genetic Variants on the Spectrum

(A) The diversity of genetic variants in the human genome. (Left) A single-nucleotide variant at a specific location in the genome in 2 different individuals. Single-nucleotide variants are classified as single-nucleotide polymorphisms (SNPs), which are found in 1% or more of the general population, or rare variants, including disease-causing mutations. (Right) A chromosomal duplication that results in a copy gain of a chromosomal segment. Structural variants in the human genome include copy-number variants (CNVs) such as duplications and deletions, as well as copy-neutral events such as inversions and

translocations. **(B)** The genetic spectrum of bicuspid aortic valve (BAV) disease. The allele frequency equals the proportion of subjects harboring a genetic variant. Single-gene disorders with high penetrance tend to cause genetic syndromes with BAV or familial forms of BAV. Rare mutations such as CNVs are expected to cause early onset forms of BAV with severe manifestations but may not be recognizable as genetic syndromes. Common variation in genes that regulate aortic valve calcification or connective tissue integrity can modify the phenotype of BAV disease progression but are unlikely to cause disease on their own. Any combination of these variants may predispose to BAV in individual cases.

TAAD = thoracic aortic aneurysms and dissections. **Figure 1B** is adapted from McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet* 2008;9:356–69.

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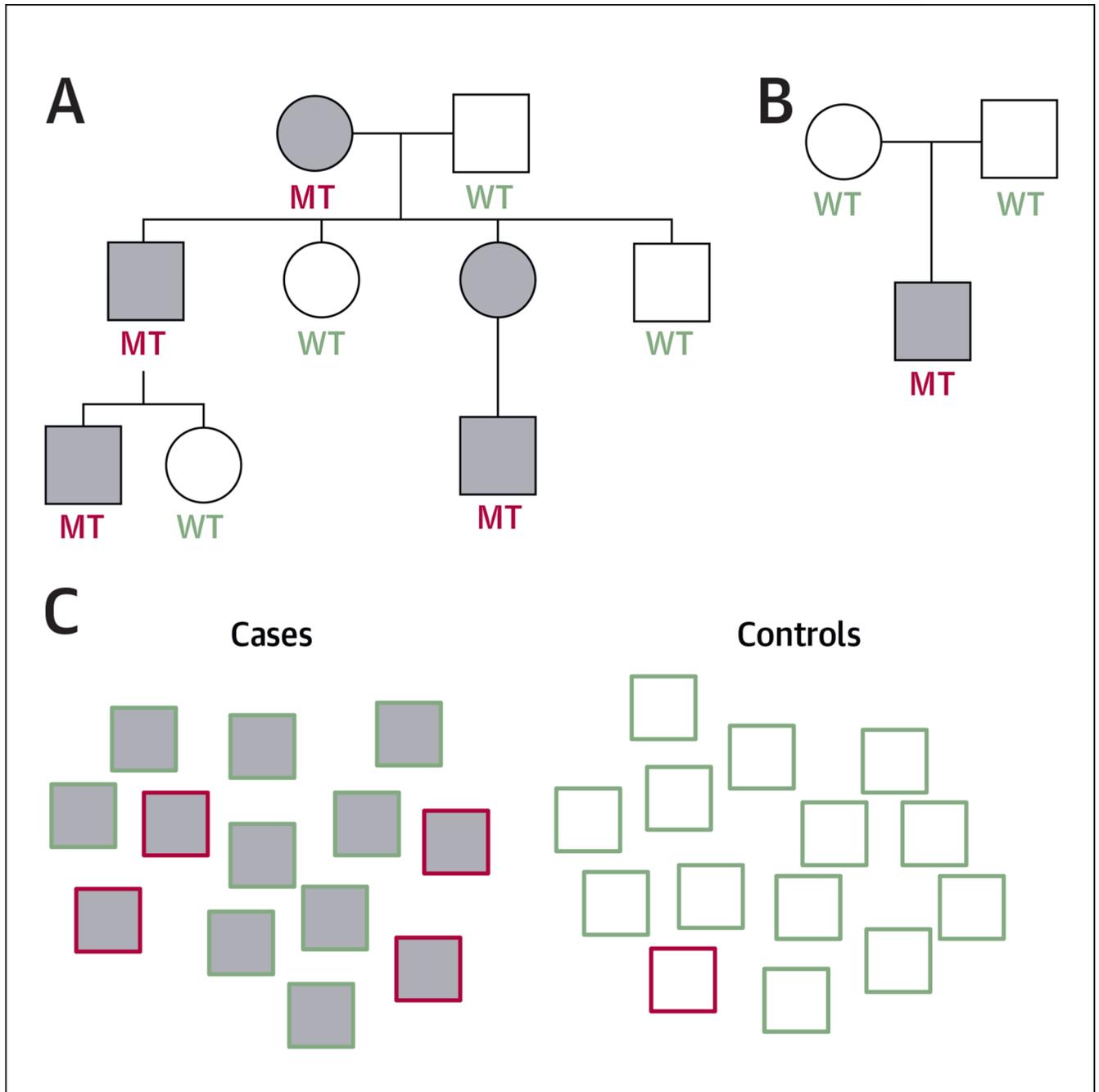


FIGURE 2. Experimental Designs to Identify Disease-Causing and Susceptibility Genetic Variants

(A) Example of a 3-generation pedigree with autosomal-dominant inheritance. Affected patients are **gray**. Mutation carriers (MT) and subjects without mutations (WT) are highlighted in **red and green**, respectively. In this instance, the penetrance of the mutation is complete, and the mutant gene can be mapped to a segment of the genome using linkage analysis. (B) Example of a trio. De novo mutations in the affected offspring that are not found in the parents may be prioritized for analysis in independent groups of cases. In this

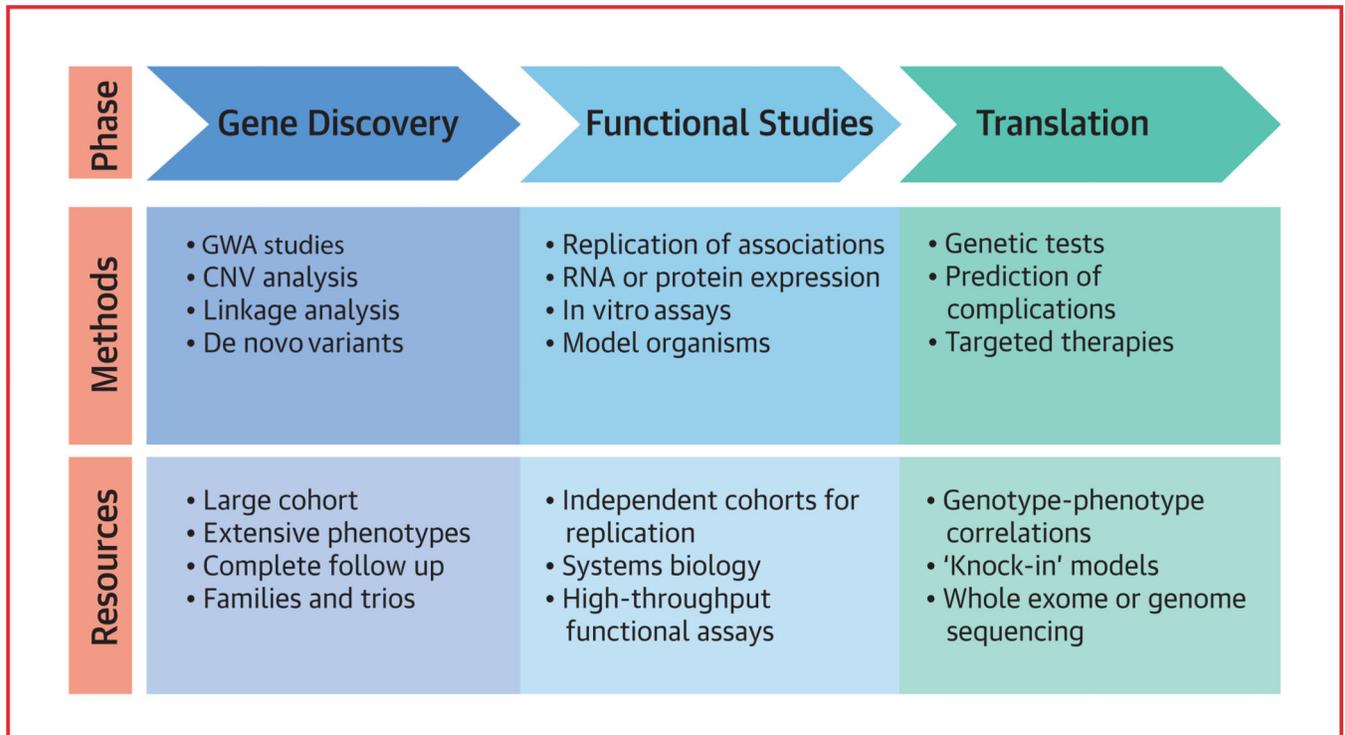
situation, the penetrance of the phenotype in the parents is uncertain and may confound the results. (C) Example of a case-control association study. The genotypes of large groups of cases (**gray**) and controls (**white**) are compared at sites across the genome. The **red border** identifies subjects who carry the risk allele that is being tested for association. Variants that are enriched in cases compared with controls are considered to be associated with the disease. In this case, there is a 4-fold enrichment of the variant among the cases compared with controls.

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CENTRAL ILLUSTRATION. Roadmap to Translate Genetic Discoveries Into Clinical Applications

“Knock-out” models include tissue-specific and whole-organism deletion of genes. “Knock-in” models are generated by introducing a specific human sequence variant into an orthologous gene. In real time, these processes would most likely occur simultaneously. CNV = central nervous system; GWA = genome-wide analysis.

TABLE 1**Human Genetic Syndromes That Include Bicuspid Aortic Valve**

Syndrome	Genetic Defect	Clinical Features	OMIM No.
Turner	Monosomy X	Short stature, infertility, coarctation	—
Loeys-Dietz	<i>TGFBR1</i> , <i>TGFBR2</i> mutations	TAAD, craniosynostosis, bifid uvula, skeletal defects	609192, 608967 (type 1) 610168, 610380 (type 2)
DiGeorge	22q11.2 deletion	Truncus arteriosus, tetralogy of Fallot, craniofacial defects	188400 (DiGeorge) 192430 (VCF)
Familial TAAD	<i>ACTA2</i> mutations (10%–15%)	TAAD, Premature coronary artery disease, cerebral aneurysms	607086 (AAT1) 607087 (AAT2) 132900 (AAT4) 608967 (AAT5) 611788 (AAT6) 613780 (AAT7) 614816 (LDS type 4)
Andersen-Tawil	<i>KCNJ2</i> mutations (60%)	Dysmorphic features, cardiac arrhythmias, periodic paralysis	170390 (LQTS 7)
Larsen	<i>FLNB</i> mutations	Craniofacial and skeletal defects	150250
Kabuki	<i>KMT2D</i> , <i>KDM6A</i> mutations (70%)	Mental retardation, hearing loss, coarctation	147920 (type 1) 300867 (type 2)

AAT = aortic aneurysm, familial thoracic; LDS = Loeys-Dietz syndrome; LQTS = long-QT syndrome; OMIM = Online Mendelian Inheritance in Man; TAAD = thoracic aortic aneurysms and dissections; VCF = velocardiofacial syndrome.

TABLE 2

Transgenic Mouse Models That Include Bicuspid Aortic Valve

Allele	Penetrance	Other Phenotypes	Ref. #
Gata5 ^{tm1.1Nemr}	7/28	LVH	(47)
Tg(GATA5-cre) ^{1Krc} /Acvr1 ^{tm1Vk}	3/22	Perimembranous VSDs	(59)
Nos3 ^{tm1Unc}	5/12	None	(60)
Hoxa1 ^{tm3Mrc}	4/17	IAA, VSD	(61)
Tbx1 ^{tm1Pa}	5/34	IAA, TOF, coronary anomalies	(62)
Hoxa3 ^{tm1(cre)Moon} /Fgf8 ^{tm2Moon}	7/33	IAA, coronary anomalies	(63)
Nkx2-5 ^{tm1(cre)Rjs} /Frs2 ^{tm1Fwan}	20%	OA, DORV	(64)
Nkx2-5 ^{tm1Wehi} * Nkx2-5 ^{tm4Rph} *	11/100 2/98	Conduction defects, PFO	(65)

Allele information is taken from Mouse Genome Informatics (<http://www.informatics.jax.org>).

* Heterozygotes.

DORV = double-outlet right ventricle; IAA = interrupted aortic arch; LVH = left ventricular hypertrophy; OA = overriding aorta; PFO = patent foramen ovale; TOF = tetralogy of Fallot; VSD = ventricular septal defect.