Title

The Necessity For *in Vivo* Functional Analysis in Human Medical Genetics.

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Abstract

Approximately 50% of all congenital anomalies cannot be linked to any specific genetic etiology, but in recent years cost effective high throughput sequencing has emerged as an efficient strategy for identifying single nucleotide polymorphisms (SNPs) associated with disease. However, in many cases there is not enough evidence to determine if these SNPs underlie disease. To bridge this gap in our understanding advances in functional analyses are warranted. Several preclinical model systems are currently being utilized to provide such evidence, including the advantageous zebrafish embryo. While every system exhibits disadvantages and caveats, a new era of multidisciplinary research has evolved, which uses a broad spectrum of functional analysis tools. This approach will make it possible to identify potential therapeutic targets for both common and rare human disorders.
Keywords: Functional analysis, zebrafish, multiple congenital anomalies

1. Introduction

Complex monogenic disorders include those that are caused by mutations in a single gene, yet result in an array of phenotypes. Phenotypes associated with monogenic disorders are heterogeneous in nature, making diagnosis difficult such that patients are generally given the common diagnosis of multiple congenital anomalies (MCA). MCAs are defined by a single patient that presents with two or more structural malformations present at birth, further these abnormalities cannot be explained by an underlying syndrome (Ooki, 2012). MCAs can affect nearly every organ system and can include, but are not limited to brain malformations, intellectual disabilities, metabolic defects, loss of limbs, and structural heart malformations.

Nearly 200 single gene defects have been associated with rare Mendelian inherited diseases (Lee et al., 2014; Ng, Buckingham, et al., 2010; Rabbani, Mahdieh, Hosomichi, Nakaoka, & Inoue, 2012; Zhang, 2014). In most cases there are no formal functional analyses used to confirm the causality of such mutations. This lack of functional analyses represents a significant barrier towards the development of better treatment options. The question that remains is "What is the most informative, cost-effective approach to definitively label these mutations as causal?" While several approaches and model systems exist for such analyses, all of them exhibit multiple limitations when used individually. Therefore, an evolving interdisciplinary approach is needed to understand the pathways that regulate both normal development and disease.

2. How far can medical genetics take us?

2a: Improving medical treatment

Single gene defects that result in a myriad of manifestations represent a treatment conundrum. For example, "Are the phenotypes completely independent of one another?" or perhaps "Are several phenotypes the consequence of one primary phenotype?" How can we possibly differentiate these two alternatives solely based upon phenotype? The simple answer is that treatment options based upon phenotype data alone are insufficient to improve quality of life. Therefore, it is absolutely essential to combine genetic diagnoses with functional analyses in order to improve treatment of MCAs (Figure 1). This interdisciplinary approach is likely to yield significant advances because while phenotypes might overlap, the mechanistic basis of disease could be distinct, uncovering unique treatment options.

2b: Defects in cobalamin metabolism: an example for the need of functional analyses.

Cobalamin is the cofactor for the MTR enzyme, which converts homocysteine into methionine. Defects in one or more of the many enzymes involved in cobalamin processing, result in metabolic deficiencies. Individuals with cobalamin disorders present with a multiple congenital anomaly syndrome characterized by brain deficits, metabolic

defects, and craniofacial abnormalities (Carrillo-Carrasco, Adams, & Venditti, 1993). Prevailing hypotheses have suggested that the brain defects associated with cobalamin metabolism are a consequence of toxic metabolite accumulation, whereby defects in metabolism cause multiple unrelated phenotypes. However, new evidence suggests that this may not be the case (Huang et al., 2012) and provides strong evidence that many of the phenotypes associated with defects in cobalamin metabolism are in fact independent. For example, methylmalonic academia and homocysteinemia cblX type (*cblX*) is very similar clinically to methylmalonic acidemia and homocysteinemia cblC type (*cblC*) (Yu et al., 2013), however these two disorders are caused by mutations in the *HCFC1* gene or the *MMACHC* gene (Liu et al., 2010), respectively. Traditionally, both *cblC* and *cblX* have been treated in a similar manner. However, one might expect that the mechanistic basis of each disorder is completely unique because MMACHC is an enzyme in the cobalamin pathway (Hannibal et al., 2009) and HCFC1 is a transcriptional co-factor that regulates nearly 5000 different genes (Michaud et al., 2013, p. 1). In order to determine the distinct mechanisms regulating each phenotype, researchers have performed elegant functional analyses which have provided evidence that some phenotypes might be related, while others are likely completely unique (Gérard et al., 2015a, p. 1; Jolly et al., 2015, p. 1; Quintana et al., 2014, p. 1). These animal model studies substantiate clinical findings that demonstrate that a single treatment does not necessarily alleviate all phenotypes (Weisfeld-Adams et al., 2013). Together these data infer causality and uncover pathways that are essential for both development and disease. Importantly, they suggest that even the most similar of disorders should be considered independent when evaluating treatment options.

2c. Clinical Mendelian genetics: A pathway to drug discovery

Over the past two decades the total number of new pharmaceuticals reaching the market has steadily decreased (Frantz, 2004; Sams-Dodd, 2005). To alleviate this burden, a new train of thought is emerging, which indicates that the genetic basis of rare disorders is a novel approach towards identifying new drug targets for a vast array of disorders (Brinkman, Dubé, Rouleau, Orr, & Samuels, 2006). For example, manyof the genes associated with MCAs regulate basic cellular processes such as differentiation, proliferation, and apoptosis. These processes are central to ongoing homeostasis, and therefore, many of the genes identified using whole exome sequencing are also important in the formation and progression of more common disorders. For example, recently mutations in *NOTCH2* were associated with Hajdu- Cheney syndrome, a Mendelian inherited disease (Simpson et al., 2011). The Notch signaling pathway is one of the most commonly activated pathways in a wide variety of cancers and

the use of Notch pharmaceutical inhibitors alongside other cancer therapeutics can improve chemotherapy resistance (Armstrong & Zhang, 2015; Shimizu & Nakagawa, 2015; Yuan et al., 2015). Thus, identifying master regulators of developmental disease is likely to improve the outcome of more common disorders, such as cancer.

2d. Kabuki Syndrome: Mendelian genetics meets cancer

Kabuki Syndrome was first described in 1981 as an MCA characterized by a unique facial appearance, cardiac abnormalities, and mild to moderate intellectual disabilities (Niikawa, Matsuura, Fukushima, Ohsawa, & Kajii, 1981). Nearly 29 years later, whole exome sequencing identified mutations in *KMT2D* as the cause of Kabuki Syndrome (Ng, Bigham, et al., 2010). Since its original identification, whole exome sequencing/Sanger sequencing approaches have demonstrated that nearly 55-65% of all Kabuki cases are caused by mutations in *KMT2D* (Cheon et al., 2014; Lederer et al., 2012; Li et al., 2011; Miyake et al., 2013). However, very little is known about the underlying molecular mechanisms of the disorder, For example, "By what mechanisms does mutation of a single gene affect multiple different organ systems?"

KMT2D encodes a SET domain protein that regulates histone H3 lysine 4 tri- methylation (Smith, Lin, & Shilatifard, 2011), an epigenetic mark generally associated with transcriptionally active genes (Martin & Zhang, 2005). In cancer cells KMT2D interacts and binds to the regulatory DNA of nearly 2000 genes in the human genome (Guo et al., 2012). Furthermore these genes are among very diverse signaling cascades. These data provide evidence that mutations in *KMT2D* have the potential to impact a myriad of different pathways in a cell type dependent manner. Together, these data reveal a clear gap in our understanding of how mutations in *KMT2D* lead to such a heterogeneous disorder. To this end, several groups have begun to analyze the function of KMT2D and its highly related protein partner, KDM6A in an *in vivo* developmental context (Lan et al., 2007, p. 3; Lindgren et al., 2013; Van Laarhoven et al., 2015). Interestingly, animal model genetics has demonstrated that inhibition of histone deacetylase activity can attenuate some of the phenotypes associated with mutations in the murine *Kmt2d* gene (Bjornsson et al., 2014).

Kabuki Syndrome affects a small fraction of individuals and the cost of drug discovery for such a small population of individuals may seem counterintuitive. However, somatic mutations in *KMT2D* have been found in several types of human cancer, including medulloblastoma, breast, colon, gastric, and non-small cell lung cancer (Ford & Dingwall, 2015; Je, Lee, Yoo, & Lee, 2013; Natarajan et al., 2010; Rabello, de Moura, de Andrade, Motoyama, & Silva, 2013; S. Yin et al., 2014). Therefore, it is likely that functional analyses of *KMT2D* will yield promising new drug targets for several different diseases (Figure 2).

3. The toolbox: past, present, and future

Human genetic approaches have vastly expanded in recent years, identifying nearly 1200 genes associated with human diseases and much of the success has come from studies using linkage analysis and positional cloning (Botstein & Risch, 2003). Linkage analysis has allowed for the identification of a number of genes including those that underlie cystic fibrosis (Kerem et al., 1989), ataxia talengiestasia (Savitsky et al., 1995), and even some hereditary forms of cancer (Fung et al., 1987; Miki et al., 1994). The basic principles of linkage analysis rely upon family heritability and concise phenotype data (Borecki & Province, 2008; E. W. Taylor, Xu, Jabs, & Meyers, 1997), however in many disorders the phenotypes are heterogeneous in nature, which can complicate linkage analysis studies. Furthermore, the resolution of linkage analysis is 1-10cM, which may include as many as a hundred candidate genes. However, these limitations of linkage analysis have been aided by the advent of cost effective Whole Exome Sequencing (WES) (Lee et al., 2014; Zhang, 2014). WES has proven highly effective at diagnosing monogenic disorders and is a preferred method for identifying single nucleotide polymorphisms (SNPs) associated with disease. Furthermore, WES can also be used to identify small insertions/deletions, and copy number variations, which adds to its utility (Hehir-Kwa, Pfundt, & Veltman, 2015).

A major limitation of WES is that one cannot detect changes in regulatory DNA or intergenic regions of DNA, but there is now a shift towards Whole Genome Sequencing (WGS) (Johansen Taber, Dickinson, & Wilson, 2014). The development of new bioinformatics technologies capable of analyzing WGS data will be needed and will likely lead to yet another explosion of genes and/or genetic elements linked to human disease, but translating these genetic findings into feasible treatment options will continue to remain a challenge.

4. Functional analysis: basic science meets target validation

4a. Why go beyond gene association?

SNPs occur nearly every 1000 base pairs (Aerts, Wetzels, Cohen, & Aerssens, 2002; Karki, Pandya, Elston, & Ferlini, 2015) and any two individuals can have 0.1% of their genome that differs. Most of these changes do not have on effect on disease outcome, therefore, in many cases we lack the confidence to label variants as mutations. How can we move past this dilemma? There are two primary means of labeling a SNP as pathogenic. The first, is finding the exact same SNP/mutation in multiple patients with overlapping phenotypes. For example, discovering that two siblings with the same disorder carry the same polymorphism provides strong evidence for causality (Gérard et al., 2015b, p. 1). Alternatively, it is possible to study large cohorts of unrelated individuals with the same disease and find that a large percentage of those individuals carry mutations in the same gene. However in some cases this approach is not feasible, because the disorders are so rare. For example, many new cases of children

with MCAs, are single cases (Coughlin et al., 2015; Yu, Geiger, Medne, Zackai, & Shaikh, 2014). Thus, in order to provide evidence for causality in these unique cases, functional analysis is of utmost importance.

4b. Can we answer causality using *in vitro* methodology?

Technology affords the ability to produce immortalized patient cell lines (Welte, Davies, Schäfer, & Regenbrecht, 2013), which provide an *in vitro* experimental model to examine gene function. For example, cell lines can be used to examine post translational modifications of histones, transcription factor binding, protein interactions, as well as the effects of a single mutation on global gene expression. By utilizing these *in vitro* technologies, pathways will be uncovered and the molecular effects of a single point mutation can be identified. Studies such as these will pave the way for testing potential therapeutics in immortalized cell lines. These data that will help identify new drug targets and hopefully, novel therapies for rare disorders. Furthermore, newer research producing patient derived induced pluripotent stem cells (Lin, Bolund, & Luo, 2015), cells that can be differentiated into multiple different lineages, will likely result in tremendous scientific advances. Experiments using cell lines will answer insightful and important questions regarding human disease, but most importantly these experiments will provide a foundation for the development of informed hypotheses, which can be tested in an *in vivo* context.

4c. Characteristics of an appropriate animal model?

MCAs are inherently developmental in nature and therefore, careful thought must be given when selecting a preclinical model system. Some recommendations to consider when choosing a preclinical model include:

1) Developmental processes are well characterized and conserved with human development.
2) Genetic manipulation of the species is easy and cost-effective.
3) Multiple biological processes can be studied with established techniques.
4) The number of animals needed to achieve an appropriate result can be readily obtained.

Furthermore, animals that offer a high fecundity, low cost of breeding and care, and have the potential for *in vivo* live imaging are of particular interest. Additionally, one should not rule out the use of invertebrate animals particularly because 75% of human genes have some degree of homology with the common fruitfly, *D. melangaster* ("Drosophila: , the golden bug, emerges as a tool for human genetics : Article : Nature Reviews Genetics," n.d.). Furthermore, invertebrates such as *D. melanogaster* and *C. elegans* can be adapted for large scale pharmaceutical screens (Ségalat, 2007).

5. Zebrafish: a missing link?

Zebrafish are a small vertebrate animal emerging as a cost effective

preclinical model. Traditionally mice are the preferred animal model because complete genome sequencing and comparison has demonstrated that the gene content in humans and mice is highly conserved ("Insights from human/mouse genome comparisons - art%3A10.1007%2Fs00335-002-4001-1.pdf," n.d.; Mouse Genome Sequencing Consortium et al., 2002). However, mice are cost and labor intensive to maintain and because exome sequencing has yielded a tremendous amount of data, they may not always represent an optimal model system. In comparison, zebrafish are a cost effective alternative and complete sequencing of the zebrafish genome has demonstrated that their DNA is 70% homologous to human DNA (Howe et al., 2013). Importantly, zebrafish have a similar number of chromosomes relative to humans and mice (Kalueff, Stewart, & Gerlai, 2014), adding to their exceptional utility. Additionally, using zebrafish for large high throughput screens costs 5-fold less than an equivalent screen using mice as a preclinical model (Kalueff et al., 2014).

The zebrafish genome has undergone duplication during evolution (J. S. Taylor, Van de Peer, Braasch, & Meyer, 2001). This genome duplication may appear on the surface as a potential drawback to their utility. However, not every gene has undergone duplication. Functional analysis of genes that have undergone duplication has demonstrated that in many cases the duplicated genes have split the function of a single human gene (Quintana et al., 2014; Van Laarhoven et al., 2015). The split function of genes lends itself well to the study of congenital disorders because in many cases specific strategies can be implemented to circumvent embryonic lethality

5a: Genetic manipulation Morpholinos

Morpholinos are short anti-sense oligonucleotides and are commonly utilized for transient loss of function assays (Eisen & Smith, 2008; Summerton, 2007). Recently, morpholinos were adapted to larger screens, demonstrating that they have significant utility in gene function assays (Melvin, Feng, Hernandez-Lagunas, Artinger, & Williams, 2013). However, in recent years their specificity has been questioned (Gerety & Wilkinson, 2011) and the development of new technologies has become a top priority in the field. Furthermore, because morpholinos are transient in nature they limit the types of experiments one can perform.

mRNA over-expression

Early embryo transient over-expression strategies can yield important information especially when combined with other methodologies including morpholino mediated knockdown. Over-expression of an RNA molecule in zebrafish can be performed quickly and easily by injecting *in vitro* synthesized mRNA into a single celled embryo. This methodology is most informative when mRNA is co-injected alongside morpholinos, whereby one can determine if the over expression of one gene effectively restores the phenotypes

associated with loss of a gene.

Take your pick: forward or reverse genetics TILLING

Targeting Induced Local Lesions IN Genomics (TILLING) is a very popular method of inducing mutations, it has been applied to many organisms including the zebrafish (Moens, Donn, Wolf-Saxon, & Ma, 2008; Winkler, Gscheidel, & Brand, 2011). The initial step of this technology is the generation of mutagenized individuals usually using N-ethyl-N-nitrosourea (ENU). Mutagenized individuals are then mated and the offspring screened for a desired phenotype, for example defects in neural crest cell migration. Follow up to initial mutation includes the identification of germline sequence specific mutations (Mathews et al., 2014; Snyder, Kearns, & Appel, 2012). The utility of TILLING has proven to be very valuable in the field of functional genetics and is applicable to several different species (Henikoff, Till, & Comai, 2004; Moens et al., 2008; Winkler et al., 2011).

Endogenous genome editing: A new frontier in zebrafish

Technology designed to induce targeted mutations in the zebrafish has been a major impediment to their overall utility, but the field is now on the cusp of effectively producing stable targeted germline mutations at a minimal cost. There are three very popular alternatives for endogenous genome editing in the zebrafish, all of which result in insertions and deletions (Figure 3).

One such strategy utilizes Zinc Finger Nucleases (ZFNs), proteins that have been reported to induce very high rates of targeted mutagenesis in fish and other species (Pelegri, 2011). A ZFN is produced by fusing the FokI endonuclease to a zinc finger protein. Zinc finger proteins, like transcription factors contain motifs that bind to DNA in a sequence specific manner. Therefore, upon expression, the zinc finger motif will bind to an endogenous target and the nuclease will induce double strand breaks in the DNA (Leong et al., 2011). The double strand brand breaks are generally repaired using non-homologous end joining, which is inherently error prone, thus leading to mutations. In zebrafish, *in vitro* synthesized mRNA encoding the ZFNs is injected at the single cell stage and is transmitted to individual cells, which if performed correctly includes germ cells. Induction of double strand breaks in the germline will ultimately produce targeted mutations. A very similar technology coined transcription activator like effector nucleases (TALENs) can also be utilized for highly efficient targeted genome editing (Sun & Zhao, 2013). TALEN technology also uses the FokI endonuclease fused to a DNA binding protein motif, the TAL effector DNA binding domain. TAL effectors are proteins that were originally identified in plant bacteria, they have a sequence specific amino acid code, which allows them to bind DNA in a sequence specific manner (Moscou & Bogdanove, 2009). The primary advantage of TALENs over ZFNs is the availability of cost effective high quality reagents (Clark, Voytas, & Ekker, 2011).

The CRISPR/Cas9 system is the newest and most cost effective genome editing technique applicable to zebrafish. This editing technology was developed from the normal adaptive immune defenses present in some bacteria. CRISPR/Cas9 refers to clustered regularly interspaced short palindromic repeats associated with the Cas9 endonuclease (Mali et al., 2013). It is based upon the naturally occurring short RNA molecules that mark foreign nucleic acids for degradation. Recent work has adapted this system to several preclinical models including human cell lines, *C.elegans*, and zebrafish (Blackburn, Campbell, Clark, & Ekker, 2013; Byrne, Mali, & Church, 2014; Hwang et al., 2013, 2015; Tzur et al., 2013; L. Yin, Jao, & Chen, 2015). Some of the advantages of CRISPR/Cas9 include the ease of creating target specific RNAs, the cost of producing target specific RNAs, and its ability to adapt to multiple biological systems.

5b: Understanding the dynamics of cells behavior: Genetics meets *in vivo* live imaging.

In the past, transgenesis in zebrafish was inefficient and relied primarily on the injection of plasmid DNA into a single celled embryo (Houdebine & Chourrout, 1991). However, over the past few decades the production of transgenic zebrafish has exploded primarily due to the advent of the Tol2kit, a multisite gateway recombinase construction kit that utilizes the Tol2 transposase (Kwan et al., 2007). This system produces a single "destination vector" using a simple recombination strategy. Destination vectors usually include sequences for tissue specific promoters, fluorescent reporters such as EGFP, and in some cases fusion proteins. These destination vectors are injected into single celled embryos alongside *in vitro* synthesized Tol2 transposase mRNA. The transposase effectively cleaves that endogenous DNA allowing integration of the plasmid DNA (Suster, Kikuta, Urasaki, Asakawa, & Kawakami, 2009). The Tol2 transposase system dramatically improves germline transmission efficiency when compared with the injection of linearized plasmid DNA (Kawakami, 2005).

In vivo live imaging has been used to study many biological processes including the nervous and hematopoietic system (Barbosa et al., 2015; Sanderson et al., 2015). For example, recent work utilized live imaging to investigate single cells in the developing spinal cord and/or their ability to produce myelin sheathes in response to neuronal activity (Hines, Ravanelli, Schwindt, Scott, & Appel, 2015; Kirby et al., 2006). The combination of *in vivo* live imaging and cell type specific transgenes has the potential to unravel complex biological questions in the coming years.

6. Conclusion

Tremendous advances in gene identification have yielded numerous genes associated with human disease. In many cases these novel candidate genes are associated with rare Mendelian inherited disorders and cannot be definitively labeled as causal. Functional

genetic approaches are needed in order to provide evidence that specific DNA variants are indeed mutations. Additionally, these preclinical models will enable geneticists to gain a more comprehensive understanding of the cellular and molecular mechanisms associated with disease. In many cases these cellular mechanisms are important for normal developmental processes and include pathways that are critical for the progression of more common disorders including cancer. Therefore, functional analysis of genes that cause rare disorders is likely to be applicable to the successful treatment of more common disorders, such as cancer.

Several model systems exist, both *in vivo* and *in vitro*, and each of them have individual disadvantages. Furthermore, there is evidence that the zebrafish preclinical model will play an integral role in our understanding of human disease. However, despite the advantages of zebrafish, it is clear that a comprehensive understanding of human disease can only be obtained from a collaborative interdisciplinary approach, which utilizes multiple methodologies to address hypotheses.

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References

Aerts, J., Wetzels, Y., Cohen, N., & Aerssens, J. (2002). Data mining of public SNPdatabases for the selection of intragenic SNPs. *Human Mutation*, *20*(3), 162– 173. http://doi.org/10.1002/humu.10107

Armstrong, A. J., & Zhang, T. (2015). Docetaxel Resistance in Prostate Cancer: Taking It Up a Notch. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. http://doi.org/10.1158/1078-0432.CCR-15-1613

Barbosa, J. S., Sanchez-Gonzalez, R., Di Giaimo, R., Baumgart, E. V., Theis, F. J., Götz, M., & Ninkovic, J. (2015). Neurodevelopment. Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain. *Science (New York, N.Y.)*, *348*(6236), 789793.http://doi.org/10.1126/science.aaa2729

Bjornsson, H. T., Benjamin, J. S., Zhang, L., Weissman, J., Gerber, E. E., Chen, Y.- C., … Dietz, H. C. (2014). Histone deacetylase inhibition rescues structural and functional brain deficits in a mouse model of Kabuki syndrome. *Science Translational Medicine*, *6*(256), 256ra135. http://doi.org/10.1126/scitranslmed.3009278

Blackburn, P. R., Campbell, J. M., Clark, K. J., & Ekker, S. C. (2013). The CRISPR System-Keeping Zebrafish Gene Targeting Fresh. *Zebrafish*, *10*(1), 116–118. http://doi.org/10.1089/zeb.2013.9999

Borecki, I. B., & Province, M. A. (2008). Linkage and association: basic concepts. *Advances in Genetics*, *60*, 51–74. http://doi.org/10.1016/S0065-2660(07)00403-8

Botstein, D., & Risch, N. (2003). Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nature Genetics*, *33*, 228–237. http://doi.org/10.1038/ng1090

Brinkman, R. R., Dubé, M.-P., Rouleau, G. A., Orr, A. C., & Samuels, M. E. (2006). Human monogenic disorders — a source of novel drug targets. *Nature Reviews Genetics*, *7*(4), 249–260. http://doi.org/10.1038/nrg1828

Byrne, S. M., Mali, P., & Church, G. M. (2014). Genome editing in human stem cells.*Methods in Enzymology*, *546*, 119–138. http://doi.org/10.1016/B978-0-12-801185-0.00006-4

Carrillo-Carrasco, N., Adams, D., & Venditti, C. P. (1993). Disorders of Intracellular Cobalamin Metabolism. In R. A. Pagon, M. P. Adam, T. D. Bird, C. R. Dolan, C.-T. Fong, R. J. Smith, & K. Stephens (Eds.), *GeneReviews™*. Seattle (WA):University of Washington, Seattle. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK1

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Cheon, C. K., Sohn, Y. B., Ko, J. M., Lee, Y. J., Song, J. S., Moon, J. W., … Jeong, S.-Y. (2014). Identification of KMT2D and KDM6A mutations by exome sequencing in Korean patients with Kabuki syndrome. *Journal of Human Genetics*, *59*(6), 321–325. http://doi.org/10.1038/jhg.2014.25

Clark, K. J., Voytas, D. F., & Ekker, S. C. (2011). A TALE of two nucleases: gene targeting for the masses? *Zebrafish*, *8*(3), 147–149. http://doi.org/10.1089/zeb.2011.9993

Coughlin, C. R., Scharer, G. H., Friederich, M. W., Yu, H.-C., Geiger, E. A., Creadon- Swindell, G., … Shaikh, T. H. (2015). Mutations in the mitochondrial cysteinyl- tRNA synthase gene, CARS2, lead to a severe epileptic encephalopathy and complex movement disorder. *Journal of Medical Genetics*, *52*(8), 532–540. http://doi.org/10.1136/jmedgenet-2015-103049

Drosophila: , the golden bug, emerges as a tool for human genetics : Article : Nature Reviews Genetics. (n.d.). Retrieved August 24, 2015, from http://www.nature.com/nrg/journal/v6/n1/full/nrg1503.html

Eisen, J. S., & Smith, J. C. (2008). Controlling morpholino experiments: don't stop making antisense. *Development (Cambridge, England)*, *135*(10), 1735–1743. http://doi.org/10.1242/dev.001115

Ford, D. J., & Dingwall, A. K. (2015). The cancer COMPASS: navigating the functions of MLL complexes in cancer. *Cancer Genetics*, *208*(5), 178–191. http://doi.org/10.1016/j.cancergen.2015.01.005

Frantz, S. (2004). FDA publishes analysis of the pipeline problem. *Nature Reviews Drug Discovery*, *3*(5), 379–379. http://doi.org/10.1038/nrd1404

Fung, Y. K., Murphree, A. L., T'Ang, A., Qian, J., Hinrichs, S. H., & Benedict, W. F. (1987). Structural evidence for the authenticity of the human retinoblastoma gene. *Science (New York, N.Y.)*, *236*(4809), 1657–1661.

Gérard, M., Morin, G., Bourillon, A., Colson, C., Mathieu, S., Rabier, D., … Benoist, J. F. (2015a). Multiple congenital anomalies in two boys with mutation in HCFC1 and cobalamin disorder. *European Journal of Medical Genetics*, *58*(3), 148– 153. http://doi.org/10.1016/j.ejmg.2014.12.015

Gérard, M., Morin, G., Bourillon, A., Colson, C., Mathieu, S., Rabier, D., … Benoist, J.F. (2015b). Multiple congenital anomalies in two boys with mutation in HCFC1 and cobalamin disorder. *European Journal of Medical Genetics*, *58*(3), 148– 153. http://doi.org/10.1016/j.ejmg.2014.12.015

Gerety, S. S., & Wilkinson, D. G. (2011). Morpholino artifacts provide pitfalls and reveal a novel role for pro-apoptotic genes in hindbrain boundary development. *Developmental Biology*, *350*(2), 279–289. http://doi.org/10.1016/j.ydbio.2010.11.030

Guo, C., Chang, C.-C., Wortham, M., Chen, L. H., Kernagis, D. N., Qin, X., … He, Y. (2012). Global identification of MLL2-targeted loci reveals MLL2's role in diverse signaling pathways. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(43), 17603–17608. http://doi.org/10.1073/pnas.1208807109

Hannibal, L., Kim, J., Brasch, N. E., Wang, S., Rosenblatt, D. S., Banerjee, R., & Jacobsen, D.W. (2009). Processing of alkylcobalamins in mammalian cells: A role for the MMACHC (cblC) gene product. *Molecular Genetics and Metabolism*, *97*(4), 260–266. http://doi.org/10.1016/j.ymgme.2009.04.005

Hehir-Kwa, J. Y., Pfundt, R., & Veltman, J. A. (2015). Exome sequencing and whole genome sequencing for the detection of copy number variation. *Expert Review of Molecular Diagnostics*, 1–10. http://doi.org/10.1586/14737159.2015.1053467

Henikoff, S., Till, B. J., & Comai, L. (2004). TILLING. Traditional mutagenesis meets functional genomics. *Plant Physiology*, *135*(2), 630–636. http://doi.org/10.1104/pp.104.041061

Hines, J. H., Ravanelli, A. M., Schwindt, R., Scott, E. K., & Appel, B. (2015). Neuronal activity biases axon selection for myelination in vivo. *Nature Neuroscience*, *18*(5), 683–689. http://doi.org/10.1038/nn.3992

Houdebine, L. M., & Chourrout, D. (1991). Transgenesis in fish. *Experientia*, *47*(9), 891–897.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), 498–503. http://doi.org/10.1038/nature12111

Huang, L., Jolly, L. A., Willis-Owen, S., Gardner, A., Kumar, R., Douglas, E., … Gecz, J. (2012). A Noncoding, Regulatory Mutation Implicates HCFC1 in Nonsyndromic Intellectual Disability. *American Journal of Human Genetics*, *91*(4), 694–702. http://doi.org/10.1016/j.ajhg.2012.08.011

Hwang, W. Y., Fu, Y., Reyon, D., Gonzales, A. P. W., Joung, J. K., & Yeh, J.-R. J. (2015). Targeted Mutagenesis in Zebrafish Using CRISPR RNA-Guided Nucleases. *Methods in Molecular Biology (Clifton, N.J.)*, *1311*, 317–334. http://doi.org/10.1007/978-1-4939-2687-9_21

Hwang, W. Y., Fu, Y., Reyon, D., Maeder, M. L., Tsai, S. Q., Sander, J. D., … Joung, J. K. (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology*, *31*(3), 227–229. http://doi.org/10.1038/nbt.2501

Insights from human/mouse genome comparisons -art%3A10.1007%2Fs00335-002- 4001-1.pdf. (n.d.). Retrieved August 24, 2015, from http://download.springer.com/static/pdf/244/art%253A10.1007%252Fs00335- 002-4001-1.pdf?originUrl=http%3A%2F%2Flink.springer.com%2Farticle%2F10.1007%2Fs00335-002-4001-1&token2=exp=1440449210~acl=%2Fstatic%2Fpdf%2F244%2Fart%25253A10.1007%25252Fs00335-002-4001-1.pdf%3ForiginUrl%3Dhttp%253A%252F%252Flink.springer.com%252Farticle%252F10.1007%252Fs00335-002-40011*~hmac=c9c2c701c8bfdf12588b6f783c8bc36e6a85fe7b8ff545e44c1d474c8b 308d14

Je, E. M., Lee, S. H., Yoo, N. J., & Lee, S. H. (2013). Mutational and expressional analysis of MLL genes in gastric and colorectal cancers with microsatellite instability. *Neoplasma*, *60*(2), 188–195.

Johansen Taber, K. A., Dickinson, B. D., & Wilson, M. (2014). The promise and challenges of next-generation genome sequencing for clinical care. *JAMA Internal Medicine*, *174*(2), 275–280. http://doi.org/10.1001/jamainternmed.2013.12048

Jolly, L. A., Nguyen, L. S., Domingo, D., Sun, Y., Barry, S., Hancarova, M., … Gecz, J. (2015). HCFC1 loss-of-function mutations disrupt neuronal and neural progenitor cells of the developing brain. *Human Molecular Genetics*. http://doi.org/10.1093/hmg/ddv083

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, *35*(2), 63–75. http://doi.org/10.1016/j.tips.2013.12.002

Karki, R., Pandya, D., Elston, R. C., & Ferlini, C. (2015). Defining "mutation" and "polymorphism" in the era of personal genomics. *BMC Medical Genomics*, *8*, 37. http://doi.org/10.1186/s12920-015-0115-z

Kawakami, K. (2005). Transposon tools and methods in zebrafish. *Developmental Dynamics: An Official Publication of the American Association of Anatomists*, *234*(2), 244–254. http://doi.org/10.1002/dvdy.20516

Kerem, B., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., … Tsui, L. C. (1989). Identification of the cystic fibrosis gene: genetic analysis. *Science (New York, N.Y.)*, *245*(4922), 1073–1080.

Kirby, B. B., Takada, N., Latimer, A. J., Shin, J., Carney, T. J., Kelsh, R. N., &

Appel, B. (2006). In vivo time-lapse imaging shows dynamic oligodendrocyte progenitor behavior during zebrafish development. *Nature Neuroscience*, *9*(12), 1506–1511. http://doi.org/10.1038/nn1803

Kwan, K. M., Fujimoto, E., Grabher, C., Mangum, B. D., Hardy, M. E., Campbell, D. S., …Chien, C.-B. (2007). The Tol2kit: a multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. *Developmental Dynamics: An Official Publication of the American Association of Anatomists*, *236*(11), 3088–3099. http://doi.org/10.1002/dvdy.21343

Lan, F., Bayliss, P. E., Rinn, J. L., Whetstine, J. R., Wang, J. K., Chen, S., … Shi, Y. (2007). A histone H3 lysine 27 demethylase regulates animal posterior development. *Nature*, *449*(7163), 689–694. http://doi.org/10.1038/nature06192

Lederer, D., Grisart, B., Digilio, M. C., Benoit, V., Crespin, M., Ghariani, S. C., … Verellen-Dumoulin, C. (2012). Deletion of KDM6A, a histone demethylase interacting with MLL2, in three patients with Kabuki syndrome. *American Journal of Human Genetics*, *90*(1), 119–124. http://doi.org/10.1016/j.ajhg.2011.11.021

Lee, H., Deignan, J. L., Dorrani, N., Strom, S. P., Kantarci, S., Quintero-Rivera, F., … Nelson, S. F. (2014). Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA*, *312*(18), 1880–1887. http://doi.org/10.1001/jama.2014.14604

Leong, I. U. S., Lai, D., Lan, C.-C., Johnson, R., Love, D. R., Johnson, R., & Love, D. R. (2011). Targeted mutagenesis of zebrafish: use of zinc finger nucleases. *Birth Defects Research. Part C, Embryo Today: Reviews*, *93*(3), 249–255. http://doi.org/10.1002/bdrc.20213

Lindgren, A. M., Hoyos, T., Talkowski, M. E., Hanscom, C., Blumenthal, I., Chiang, C., … Morton, C. C. (2013). Haploinsufficiency of KDM6A is associated with severe psychomotor retardation, global growth restriction, seizures and cleft palate. *Human Genetics*, *132*(5), 537–552. http://doi.org/10.1007/s00439-013-1263-x

Lin, L., Bolund, L., & Luo, Y. (2015). Towards personalized regenerative cell therapy: Mesenchymal stem cells derived from human induced pluripotent stem cells. *Current Stem Cell Research & Therapy*.

Liu, M.-Y., Yang, Y.-L., Chang, Y.-C., Chiang, S.-H., Lin, S.-P., Han, L.-S., … Liu, T.-T. (2010). Mutation spectrum of MMACHC in Chinese patients with combined methylmalonic aciduria and homocystinuria. *Journal of Human Genetics*, *55*(9), 621–626. http://doi.org/10.1038/jhg.2010.81

Li, Y., Bögershausen, N., Alanay, Y., Simsek Kiper, P. O., Plume, N., Keupp, K., … Wollnik, B. (2011). A mutation screen in patients with Kabuki

syndrome.*Human Genetics*, *130*(6), 715–724. http://doi.org/10.1007/s00439-011-1004-y Mali, P.,

Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., … Church, G.M. (2013). RNA-guided human genome engineering via Cas9. *Science (New York, N.Y.)*, *339*(6121), 823–826. http://doi.org/10.1126/science.1232033

Martin, C., & Zhang, Y. (2005). The diverse functions of histone lysine methylation. *Nature Reviews Molecular Cell Biology*, *6*(11), 838–849. http://doi.org/10.1038/nrm1761

Mathews, E. S., Mawdsley, D. J., Walker, M., Hines, J. H., Pozzoli, M., & Appel, B. (2014). Mutation of 3-hydroxy-3-methylglutaryl CoA synthase I reveals requirements for isoprenoid and cholesterol synthesis in oligodendrocyte migration arrest, axon wrapping, and myelin gene expression. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *34*(9), 3402–3412. http://doi.org/10.1523/JNEUROSCI.4587-13.2014

Melvin, V. S., Feng, W., Hernandez-Lagunas, L., Artinger, K. B., & Williams, T. (2013). A morpholino-based screen to identify novel genes involved in craniofacial morphogenesis. *Developmental Dynamics: An Official Publication of the American Association of Anatomists*, *242*(7), 817–831. http://doi.org/10.1002/dvdy.23969

Michaud, J., Praz, V., Faresse, N. J., Jnbaptiste, C. K., Tyagi, S., Schütz, F., & Herr, W. (2013). HCFC1 is a common component of active human CpG-island promoters and coincides with ZNF143, THAP11, YY1, and GABP transcription factor occupancy. *Genome Research*. http://doi.org/10.1101/gr.150078.112

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., … Ding, W. (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science (New York, N.Y.)*, *266*(5182), 66–71.

Miyake, N., Koshimizu, E., Okamoto, N., Mizuno, S., Ogata, T., Nagai, T., … Niikawa, N. (2013). MLL2 and KDM6A mutations in patients with Kabuki syndrome. *American Journal of Medical Genetics. Part A*, *161A*(9), 2234–2243. http://doi.org/10.1002/ajmg.a.36072

Moens, C. B., Donn, T. M., Wolf-Saxon, E. R., & Ma, T. P. (2008). Reverse genetics in zebrafish by TILLING. *Briefings in Functional Genomics & Proteomics*, *7*(6), 454–459. http://doi.org/10.1093/bfgp/eln046

Moscou, M. J., & Bogdanove, A. J. (2009). A simple cipher governs DNA recognition by TAL effectors. *Science (New York, N.Y.)*, *326*(5959), 1501. http://doi.org/10.1126/science.1178817

Mouse Genome Sequencing Consortium, Waterston, R. H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J. F., … Lander, E. S. (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature*, *420*(6915), 520–562. http://doi.org/10.1038/nature01262

Natarajan, T. G., Kallakury, B. V., Sheehan, C. E., Bartlett, M. B., Ganesan, N., Preet, A., … Fitzgerald, K. T. (2010). Epigenetic regulator MLL2 shows altered expression in cancer cell lines and tumors from human breast and colon.*Cancer Cell International*, *10*, 13. http://doi.org/10.1186/1475-2867-10-13

Ng, S. B., Bigham, A. W., Buckingham, K. J., Hannibal, M. C., McMillin, M. J.,Gildersleeve, H. I., … Shendure, J. (2010). Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nature Genetics*, *42*(9), 790–793. http://doi.org/10.1038/ng.646

Ng, S. B., Buckingham, K. J., Lee, C., Bigham, A. W., Tabor, H. K., Dent, K. M., … Bamshad, M. J. (2010). Exome sequencing identifies the cause of a mendelian disorder. *Nature Genetics*, *42*(1), 30–35. http://doi.org/10.1038/ng.499

Niikawa, N., Matsuura, N., Fukushima, Y., Ohsawa, T., & Kajii, T. (1981). Kabuki make-up syndrome: a syndrome of mental retardation, unusual facies, large and protruding ears, and postnatal growth deficiency. *The Journal of Pediatrics*, *99*(4), 565–569.

Ooki, S. (2012). Multiple Congenital Anomalies after Assisted Reproductive Technology in Japan (between 2004 and 2009). *International Scholarly Research Notices*, *2013*, e452085. http://doi.org/10.5402/2013/452085

Pelegri, F. J. (Ed.). (2011). Inducing High Rates of Targeted Mutagenesis in Zebrafish Using Zinc Finger Nucleases (ZFNs) - Springer. Humana Press. Retrieved from . http://link.springer.com/protocol/10.1007%2F978-1-61779-210- 6_20

Quintana, A. M., Geiger, E. A., Achilly, N., Rosenblatt, D. S., Maclean, K. N., Stabler,S. P., … Shaikh, T. H. (2014). Hcfc1b, a zebrafish ortholog of HCFC1, regulates craniofacial development by modulating mmachc expression. *Developmental Biology*, *396*(1), 94–106. http://doi.org/10.1016/j.ydbio.2014.09.026

Rabbani, B., Mahdieh, N., Hosomichi, K., Nakaoka, H., & Inoue, I. (2012). Next generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. *Journal of Human Genetics*, *57*(10), 621–632. http://doi.org/10.1038/jhg.2012.91

Rabello, D. do A., de Moura, C. A., de Andrade, R. V., Motoyama, A. B., & Silva, F. P. (2013). Altered expression of MLL methyltransferase family genes in breastcancer. *International Journal of Oncology*, *43*(2), 653–660. http://doi.org/10.3892/ijo.2013.1981

Sams-Dodd, F. (2005). Target-based drug discovery: is something wrong? *Drug Discovery Today*, *10*(2), 139–147. http://doi.org/10.1016/S1359-6446(04)03316-1

Sanderson, L. E., Chien, A.-T., Astin, J. W., Crosier, K. E., Crosier, P. S., & Hall, C. J. (2015). An inducible transgene reports activation of macrophages in live zebrafish larvae. *Developmental and Comparative Immunology*, *53*(1), 63–69. http://doi.org/10.1016/j.dci.2015.06.013

Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., … Shiloh, Y. (1995). A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science (New York, N.Y.)*, *268*(5218), 1749–1753.

Ségalat, L. (2007). Invertebrate animal models of diseases as screening tools in drug discovery. *ACS Chemical Biology*, *2*(4), 231–236. http://doi.org/10.1021/cb700009m

Shimizu, T., & Nakagawa, K. (2015). [Novel signal transduction pathways: the molecular basis for targeted cancer therapies in Hedgehog/Notch/Wnt pathway]. *Nihon Rinsho. Japanese Journal of Clinical Medicine*, *73*(8), 1342– 1348.

Simpson, M. A., Irving, M. D., Asilmaz, E., Gray, M. J., Dafou, D., Elmslie, F. V., … Trembath, R. C. (2011). Mutations in NOTCH2 cause Hajdu-Cheney syndrome, a disorder of severe and progressive bone loss. *Nature Genetics*, *43*(4), 303–305. http://doi.org/10.1038/ng.779

Smith, E., Lin, C., & Shilatifard, A. (2011). The super elongation complex (SEC) and MLL in development and disease. *Genes & Development*, *25*(7), 661–672. http://doi.org/10.1101/gad.2015411

Snyder, J. L., Kearns, C. A., & Appel, B. (2012). Fbxw7 regulates Notch to control specification of neural precursors for oligodendrocyte fate. *Neural Development*, *7*, 15. http://doi.org/10.1186/1749-8104-7-15

Summerton, J. E. (2007). Morpholino, siRNA, and S-DNA compared: impact of structure and mechanism of action on off-target effects and sequence specificity. *Current Topics in Medicinal Chemistry*, *7*(7), 651–660.

Sun, N., & Zhao, H. (2013). Transcription activator-like effector nucleases (TALENs): a highly efficient and versatile tool for genome editing. *Biotechnology and Bioengineering*, *110*(7), 1811–1821. http://doi.org/10.1002/bit.24890

Suster, M. L., Kikuta, H., Urasaki, A., Asakawa, K., & Kawakami, K. (2009). Transgenesis in zebrafish with the tol2 transposon system. *Methods in Molecular Biology (Clifton, N.J.)*, *561*, 41–63. http://doi.org/10.1007/978-1-60327-019-9_3

Taylor, E. W., Xu, J., Jabs, E. W., & Meyers, D. A. (1997). Linkage analysis of genetic disorders. *Methods in Molecular Biology (Clifton, N.J.)*, *68*, 11–25.

Taylor, J. S., Van de Peer, Y., Braasch, I., & Meyer, A. (2001). Comparative genomics provides evidence for an ancient genome duplication event in fish. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *356*(1414), 1661–1679. http://doi.org/10.1098/rstb.2001.0975

Tzur, Y. B., Friedland, A. E., Nadarajan, S., Church, G. M., Calarco, J. A., & Colaiácovo, M. P. (2013). Heritable custom genomic modifications in Caenorhabditis elegans via a CRISPR-Cas9 system. *Genetics*, *195*(3), 1181– 1185. http://doi.org/10.1534/genetics.113.156075

Van Laarhoven, P. M., Neitzel, L. R., Quintana, A. M., Geiger, E. A., Zackai, E. H., Clouthier, D. E., … Shaikh, T. H. (2015). Kabuki syndrome genes KMT2D and KDM6A: functional analyses demonstrate critical roles in craniofacial, heart and brain development. *Human Molecular Genetics*. http://doi.org/10.1093/hmg/ddv180

Weisfeld-Adams, J. D., Bender, H. A., Miley-Åkerstedt, A., Frempong, T., Schrager, N. L., Patel, K., … Diaz, G. A. (2013).Neurologic and NeurodeVeloPm-ental phenotypes in young children with early-treated combined methylmalonic acidemia and homocystinuria, cobalamin C type. *Molecular Genetics and Metabolism*, *110*(3), 241–247. http://doi.org/10.1016/j.ymgme.2013.07.018

Welte, Y., Davies, C., Schäfer, R., & Regenbrecht, C. R. A. (2013). Patient derived cell culture and isolation of $CD133^+$ putative cancer stem cells from melanoma. *Journal of Visualized Experiments: JoVE*, (73), e50200. http://doi.org/10.3791/50200

Winkler, S., Gscheidel, N., & Brand, M. (2011). Mutant generation in vertebrate model organisms by TILLING. *Methods in Molecular Biology (Clifton, N.J.)*, *770*, 475–504. http://doi.org/10.1007/978-1-61779-210-6_19

Yin, L., Jao, L.-E., & Chen, W. (2015). Generation of Targeted Mutations in Zebrafish Using the CRISPR/Cas System. *Methods in Molecular Biology (Clifton, N.J.)*, *1332*, 205–217. http://doi.org/10.1007/978-1-4939-2917-7_16

Yin, S., Yang, J., Lin, B., Deng, W., Zhang, Y., Yi, X., … Kong, X. (2014). Exome sequencing identifies frequent mutation of MLL2 in non-small cell lung carcinoma from Chinese patients. *Scientific Reports*, *4*, 6036. http://doi.org/10.1038/srep06036

Yuan, X., Wu, H., Xu, H., Xiong, H., Chu, Q., Yu, S., … Wu, K. (2015). Notch signaling: An emerging therapeutic target for cancer treatment. *Cancer Letters*. http://doi.org/10.1016/j.canlet.2015.07.04

8

Yu, H.-C., Geiger, E. A., Medne, L., Zackai, E. H., & Shaikh, T. H. (2014). An individual with blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) and additional features expands the phenotype associated with mutations in KAT6B. *American Journal of Medical Genetics. Part A*.
http://doi.org/10.1002/ajmg.a.36379

Yu, H.-C., Sloan, J. L., Scharer, G., Brebner, A., Quintana, A. M., Achilly, N. P., … Shaikh, T. H. (2013). An X-Linked Cobalamin Disorder Caused by Mutations in Transcriptional Coregulator HCFC1. *American Journal of Human Genetics*, *93*(3), 506–514.
http://doi.org/10.1016/j.ajhg.2013.07.022

Zhang, X. (2014). Exome sequencing greatly expedites the progressive research of Mendelian diseases. *Frontiers of Medicine*, *8*(1), 42–57.
http://doi.org/10.1007/s11684-014-0303-9

Figures and Figure Legends:
Figure 1:

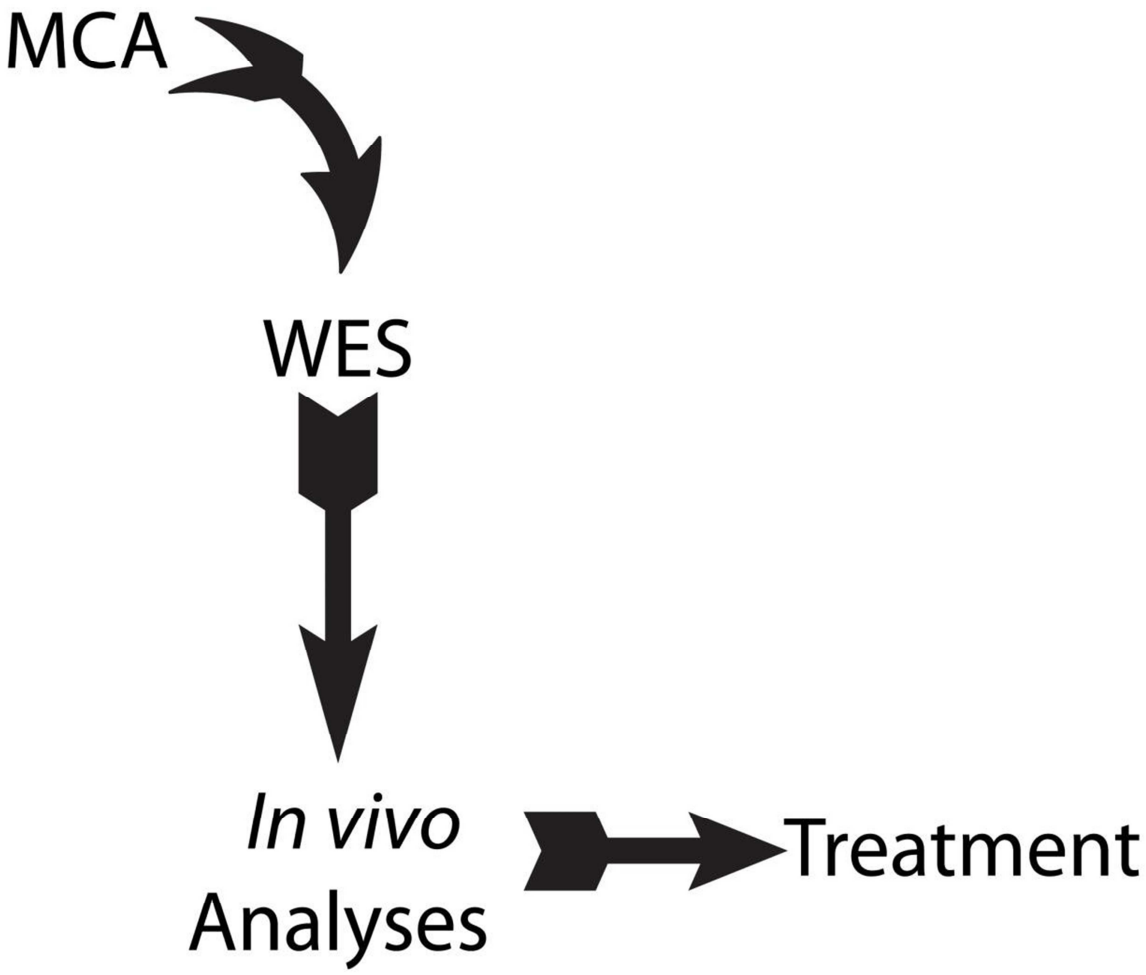

Figure 1: Functional Analyses: A pathway to better treatment options for complex disorders. Multiple Congenital Anomalies (MCA) are complex disorders that can be diagnosed using whole exome sequencing approaches (WES). Combining such genetic approaches with *in vivo* functional analyses represents a clear path towards our understanding of disease.

Figure 2:

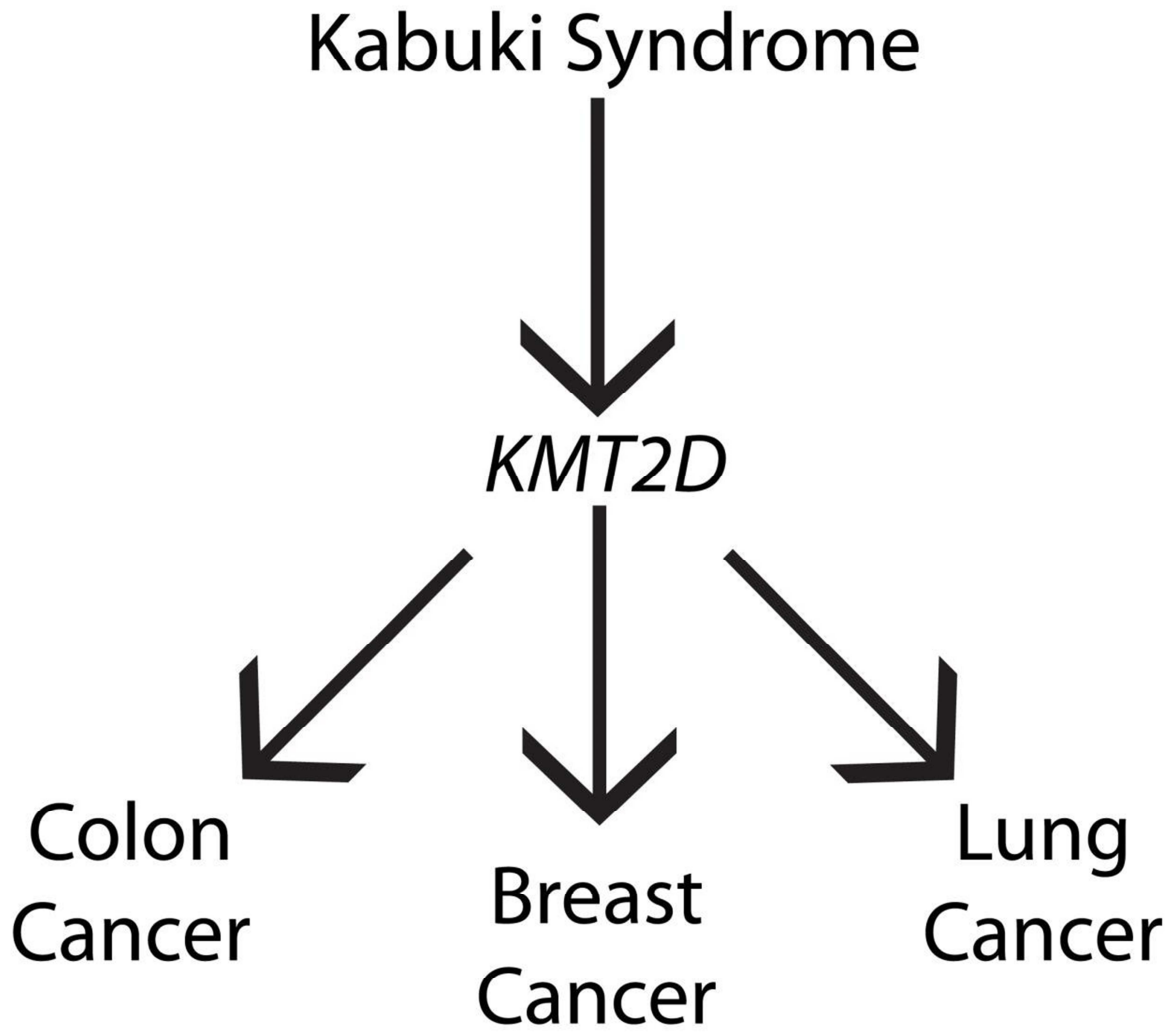

Figure 2: Germline mutations in *KMT2D* might reveal novel drug targets for several different cancers. Whole exome sequencing identified mutations in *KMT2D* as the cause of Kabuki Syndrome and functional analyses of *KMT2D* is likely to inform of the mechanisms regulating more common disorders, because somatic mutations of this gene have been reported in multiple different cancer types.

Figure 3:

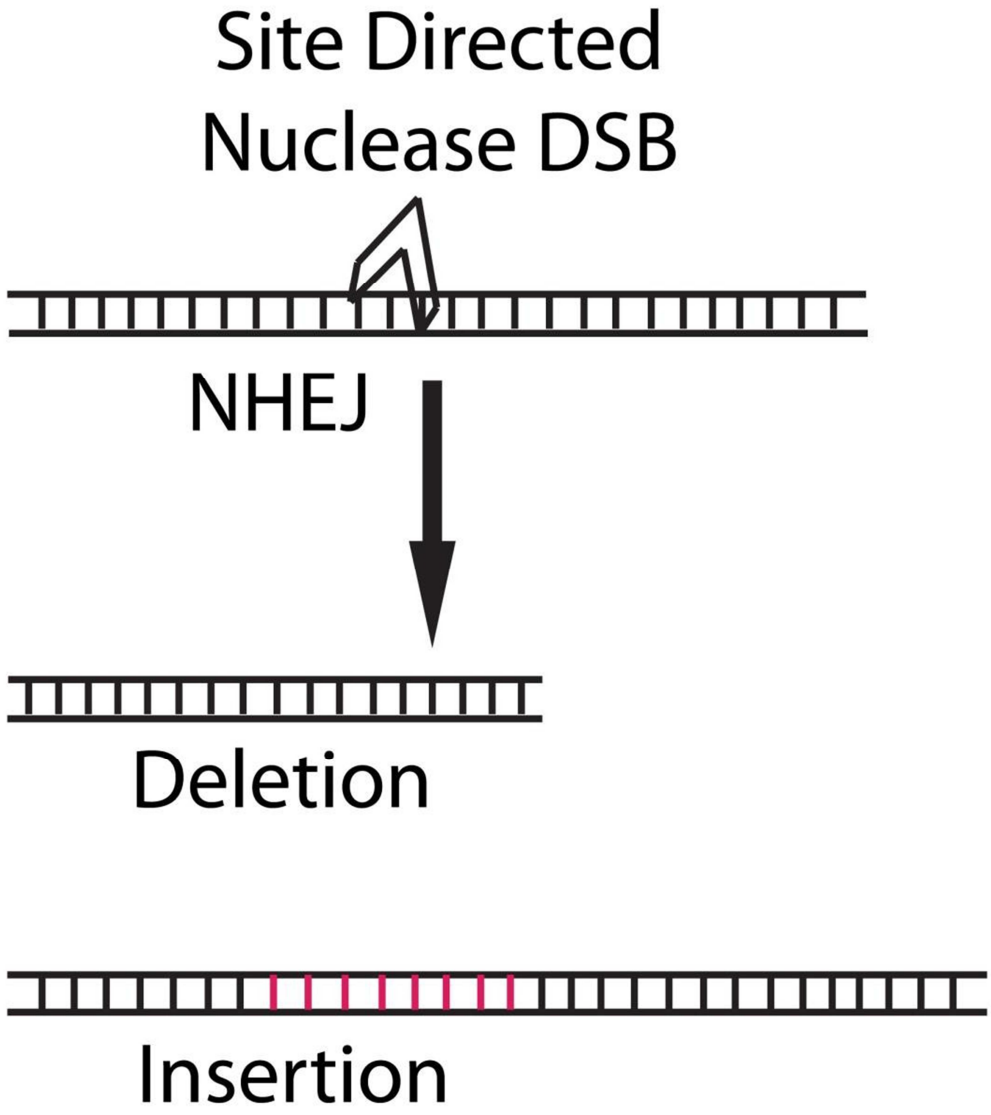

Figure 3: Manipulating non-homologous end joining (NHEJ) to induce site directed insertions and deletions. Several methodologies exist for modifying the zebrafish genome in a site specific manner. All of which target a specific nuclease to DNA and induce double strand breaks (DSB). These DSB initiates NHEJ, which does not effectively repair DNA, ultimately resulting in germline insertions or deletions.

