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Blood Ties: Chimerism Can Mask Twin Discordance in High-Throughput Sequencing

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Twin studies have long provided a means to separate the contributions of genetic and environmental factors. A recent pioneering report by Baranzini et al. presented an analysis of the complete genomes and epigenomes of a monozygotic (MZ) twin pair discordant for multiple sclerosis. This failed to find any difference between the twins, raising doubts regarding the value of whole-genome twin studies for defining disease susceptibility alleles. However, the study was carried out with DNA extracted from blood. In many cases, the hematopoietic lineages of MZ twins are chimeric due to twin-to-twin exchange of hematopoietic stem cells during embryogenesis. We therefore wondered how chimerism might impact the ability to identify genetic differences. We inferred the blood chimerism rates and profiles of more than 30 discordant twin cases from a wide variety of medical conditions. We found that the genotype compositions of the twins were highly similar. We then benchmarked the performance of SNP callers to detect discordant variations using high-throughput sequencing data. Our analysis revealed that chimerism patterns, well within the range normally observed in MZ twins, greatly reduce the sensitivity of SNP calls. This raises questions regarding any conclusions of genomic homogeneity that might be drawn from studies of blood-derived twin DNA.

■ **Keywords:** chimerism, high throughput sequencing, shared circulation

Shared blood circulation during embryogenesis is found in most MZ twin pregnancies (Greaves et al., 2003; Gringras & Chen, 2001; Hall, 2003; Machin, 2009; Martin et al., 1997). About 70% of all MZ twin embryos are monochorionic (MC) indicating a split after four days post ovum fertilization. At this stage, placental progenitor cells have already detached from the inner cell mass (Gringras & Chen, 2001; Hall, 2003). This gives rise to a single placenta that feeds both twins and contains vascular anastomoses that enable transfusion of blood between the twins. Up to 1% of the total twin blood volume is exchanged daily and unbalanced flow between the twins can develop within weeks into twin-to-twin transfusion syndrome (TTTS; van Gemert & Sterenborg, 1998).

Shared blood circulation provides a documented anatomical route for trafficking of hematopoietic stem cells (HSC) between the twins (Greaves et al., 2003; Gringras & Chen, 2001; Hall, 2003; Machin, 2009; Martin et al., 1997). This creates chimeric hematopoietic systems, where stem cells from one twin are engrafted in the co-twin and vice versa. Therefore, post-twinning genetic variations that arise in one twin can also be detected in the blood system of the co-twin. This usually stands in contrast to other cells types, which show distinct genotypes.

Whole genome and epigenome sequencing of discordant MZ twins has the potential to isolate *de novo* modifiers with very high specificity. Remarkable advances in DNA sequencing have enabled us to observe such differences on a genome-wide scale (Metzker, 2010). A recent pioneering study analyzed the genomes and epigenomes of a MZ twin pair discordant for multiple sclerosis (MS) (Baranzini et al., 2010). This failed to find any difference between the twins despite excellent variation calling accuracy, casting doubts on the value of a whole twin genome sequencing approach. However, the study was executed using blood-derived DNA.

We explored the possibility that blood chimerism could impact the detection of genetic differences in blood-

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derived DNA by high-throughput sequencing and thus produce falsely null results. First, we determined the rate and profile of blood chimerism in twins. For this, we conducted a meta-analysis of blood chimerism in more than 30 cases of discordant twins from a wide variety of medical conditions. Second, we used real high throughput sequencing data to simulate the engraftment profile that was found in our meta-analysis and to evaluate its effect on finding discordant variations. Our study proposes that even a modest level of twin chimerism can significantly impact the ability to find discordant variations using high throughput data, thus suggesting caution regarding any conclusion about genomic homogeneity between twins that might be drawn from studies of blood-derived DNA.

Materials and Methods

Meta-Analysis of Chimerism Rates

A large number of studies have highlighted the potentially confounding effects of blood chimerism when studying genomic and epigenetic variations among discordant MZ twins (Bourthoumieu et al., 2006; Gringras & Chen, 2001; Kaminsky et al., 2009; Kaplan et al., 2010; Machin, 2009; Monteiro et al., 1998). However, to the best of our knowledge, the level of blood chimerism in MZ twins has never been systematically analyzed across conditions and cases. We therefore analyzed the engraftment levels between twins in sporadic medical cases that describe chimerism.

In all cases beside gene imprinting, the engraftment levels were calculated directly by comparing the marker level of the graft in the host twin. In cases of discordant twins for gene imprinting, the engraftment levels are given by: $EH = (1\text{-}x/0.5)$ and $EA = y/0.5$, where EH and EA are the engraftment levels in the healthy and the affected twin, and x and y are the methylation levels in the healthy and the affected twin, respectively.

Illumina Sequencing

We sequenced the exome of a mother and her child. One ug of blood derived DNA was prepared from whole exome sequencing using the Illumina platform as previously described (Edvardson et al., 2010). Each sample was sequenced on two lanes of Illumina GAIIx using 76bp paired-end protocol. The raw sequences were aligned using Bowtie (Langmead et al., 2009) to the NCBI36/HG18 human genome and converted to BAM-pileup files using the pileup command in the SAM-tools package (Li et al., 2009).

Chimerism Simulation

Chimerism was simulated by randomly interleaving the BAM-pileup files and generating two new BAM-pileup files reflecting the mixture of the sequencing results. All scripts and BAM-pileup files are available on request from the author. Consistent with the MS study, only positions with ≥ 11x coverage and Q ≥ 20 were considered. SNPs were called using either SNVmix (Goya et al., 2010) or VarScan (Koboldt et al., 2009), two well-tested analysis programs. For mirrored chimerism, the average results of the two programs with their default values are reported. For reciprocal chimerism, we tested each program under two conditions: 'off-the-shelf' (default) and tuned to the parameters in supplementary table 8 of Baranzini's study. The results of each condition are reported. In addition, we tested the stringent filtration method in the MS study, and called only SNPs when the read frequency of the non-reference allele differed by > 50% between the twins.

Evaluating the Predictive Positive Value of SNP Calling

We intersected the child's SNP calling results from the sequencing data to the child's results of an Affymetrix 250K genotyping array.

Results

We began the meta-analysis by reviewing 16 cases of MZ twins discordant for chromosomal abnormalities. These included trisomy 21 (Rogers et al., 1982), Turner Syndrome (Costa et al., 1998; Dallapiccola et al., 1985; Edwards et al., 1966; Fujimoto et al., 1991; Kaplowitz et al., 1991; Lespinasse et al., 1998; Pedersen et al., 1980; Potter & Taitz, 1972; Rohrer et al., 2004; Uchida et al., 1983; Weiss et al., 1982), Ring 18 syndrome (Hata et al., 1982), and other chromosomal rearrangements (Bourthoumieu et al., 2005; Marcus-Soekarman et al., 2004). The ratios of the two karyotypes in lymphocyte cell lines provided an estimate of the chimerism level.

We compared the level of the normal karyotype in the affected twin to the level of the pathogenic karyotype in the normal twin. We found a significant inverse correlation between the engraftment levels ($R^2 = 0.73$, F statistic $= 37.56$, $p < 10^{-4}$). To avoid potential confounding by somatic mosaicism, we repeated the statistical analysis only with the cases where skin fibroblasts showed distinct genotypes. This also revealed significant inverse correlation between the engraftment levels ($R^2 = 0.82$, F statistic $= 34.04$, $p < 10^{-3}$).

Our results indicate that the blood chimerism pattern is mirrored — the hematopoietic lineages of MZ twins have mixtures of genotypes. This is illustrated in the case of twins discordant for Down syndrome (Rogers et al., 1982). In this case, 16% of the lymphocytes of the healthy twin displayed the trisomy and 84% of the lymphocytes of the affected twin displayed the normal karyotype.

We also found that on average the engrafting levels of the normal karyotype in the affected individual are significantly higher than the engrafting of the pathologic karyotype in the healthy individual ($\mu_{\text{normal->pathogenic}} = 79\%$, $\mu_{\text{pathologic->normal}} = 19\%$, Wilcoxon-W statistic $= 4$, $p < 10^{-4}$), suggesting a proliferative advantage for the normal karyotype.

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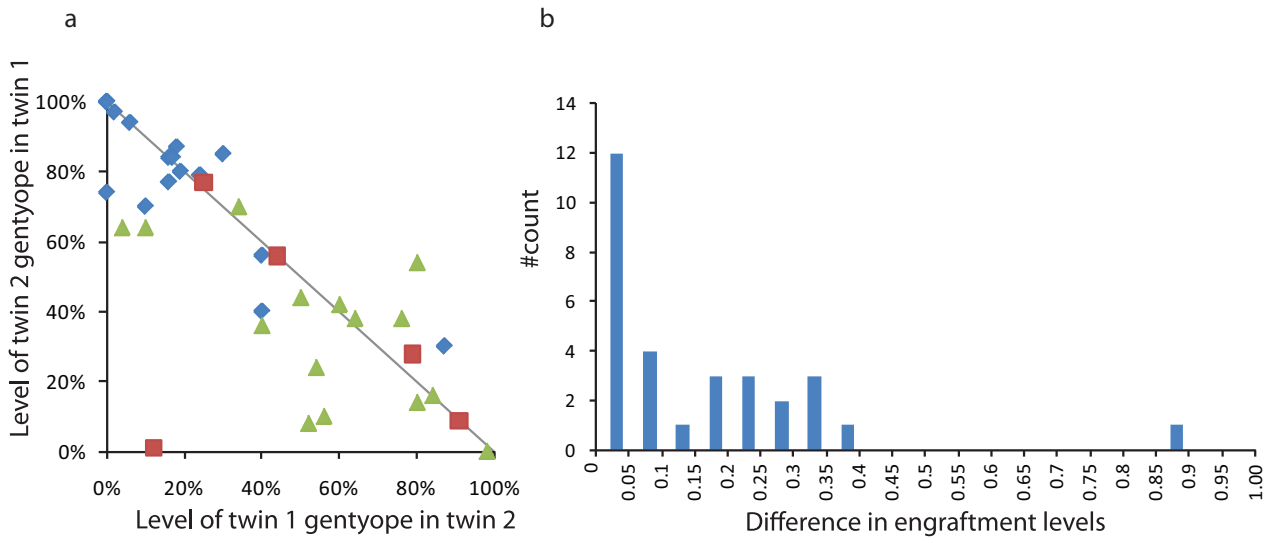

FIGURE 1

Summary of chimerism levels (a) Chimerism shows a mirrored profile. Each data point represents a twin case. Blue – cases of aneuploidy, Green – cases of discordant imprinting, Red – MC-DZ twins. Gray line indicates perfect mirrored chimerism. In disease cases, twin 1 is always the affected and twin 2 is the healthy twin (b) Distribution of the difference in genotype compositions in the twins' hematopoietic systems.

We analyzed 15 cases of discordant MZ twins for Beckwith–Wiedemann Syndrome (BWS) due to aberrant methylation in their KCNQ1OT1 gene (Bliek et al., 2009; Weksberg et al., 2002). We compared the similarity of the methylation levels in lymphoblast samples to fibroblast samples. Again, a significant level of correlation in the methylation levels was found within the discordant twins ($R^2 = 0.44$, F statistic $= 10.41$, $p < 10^{-2}$), indicating the same profile of mirrored chimerism. Unlike the cases with chromosomal abnormalities, the methylation profiles of the blood of the healthy twins were closer to methylation profile of the fibroblasts of their affected co-twins. This presumably reflects a proliferation advantage of the abnormal cells, which is concordant with the overgrowth phenotype of BWS.

We sought to evaluate the profile of blood chimerism in the absence of severe pathological alterations that may bias our analysis. Recently, Zech et al. reported a case of naturally conceived MZ twins discordant for sex chromosome due to reversion of an XXY zygote. Notably, their blood karyotype showed the same pattern of mirrored chimerism. The male twin displayed the XX karyotype in 28% of his nucleated blood cells and the female twin displayed the XY karyotype in 78% of her blood cells. The karyotypes in their umbilical cord fibroblasts were completely distinct and reflected their gender (Zech et al., 2008). We also analyzed 3 cases of heterosexual DZ-MC twins that were conceived by assisted reproductive technology (Ekelund et al., 2008; Souter et al., 2003; Williams et al., 2004). In these rare cases, the twin pairs also have a shared blood circulation through placental vascular anastomoses and develop blood chimerism. This setting provides an unambiguous data about blood chimerism, as hidden somatic mosaicism blood cannot affect the graft level. All three cases showed mirrored blood chimerism and distinct fibroblast genotypes. The average engrafting level of XY cells versus XX cells across the XXY and three DZ-MC cases was closer than in the pathologic cases ($\mu_{XX->male} = 60\%$, $\mu_{XY->female} = 42\%$), supporting our hypothesis that chimerism is shaped by clonal advantage. We also measured blood chimerism in a single case of male DZ-MC twins using blood groups (Aoki et al., 2006). Although the twins showed some blood chimerism, they did not display mirrored chimerism. The results of all the reviewed studies are summarized in Table 1 and Figure 1a.

The emerging picture from these multiple lines of studies is that the level of blood chimerism in MC twins can substantially vary. However, the composition of the genotype mixture is highly correlated between twins. The difference in the genotype compositions betweem the twins was 10% on average, and in half of the cases the difference was less than 5% (Figure 1b).

Using high-coverage exome sequencing data of a parent-child pair, we simulated mirrored chimerism by mixing sequencing reads from each individual. To avoid biased results arising from confounding sequencing errors, we restricted our analysis to a subset of SNPs that were called with the highest confidence level in the absence of chimerism. The positive predictive value (PPV) of SNP calling in this subset was 99.6%, which is higher than the PPV of 98.6% in Baranzini's study. Thus, we structured our analysis to be highly conservative and to present every advantage for calling discordant SNPs in chimeric samples.

We tested increasing levels of dissimilarity between the genotype compositions with two initial conditions: (a) *no advantage* — a genotype mixture of 50%:50% (b) *patho–*

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TABLE 1

Graft Level Percentage Represents the Level of Markers Belonging to the Other Twin

Graft level	Condition	Comments	Reference
Discordant aneuploidy in MZ twins			
H:16% A:84%	Trisomy 21		(Rogers et al., 1982)
H:30% A:85%	Turner syndrome		(Rohrer et al., 2004)
H:18% A:87%	Turner syndrome		(Kaplowitz et al., 1991)
H:2% A:97%	Turner syndrome		(Uchida et al., 1983)
H:0% A:74%	Turner Syndrome		(Weiss et al., 1982)
H:10% A:70%	Turner Syndrome		(Pedersen et al., 1980)
H:87% A:30%	Turner Syndrome		(Potter & Taitz, 1972)
H:40% A:56%	46,XX,der(15)t(11;15)(p12;p11.2)	Affected twin showed a mild somatic mosaicism of 5%.	(Marcus-Soekarman et al., 2004)
H1:0% H2:0% A: 100%	Turner syndrome	MZ triplet with two males and one female.	(Dallapiccola et al., 1985)
H:17% A:84%	Dup(11)(p12p15)	Buccal smear and urine sediments of affected twin revealed somatic mosaicism of 68%.	(Bourthoumieu et al., 2005)
H:0% A:100%	Turner syndrome		(Edwards et al., 1966)
H:16% A:77%	Turner syndrome	Healthy twin showed mosaicism also on left arm fibroblasts.	(Costa et al., 1998)
H:24% A:79%	Turner syndrome		(Costa et al., 1998)
H:40% A:40%	Turner syndrome	Co-twin suffers from idic(Y)(p11). Skin fibroblasts and showed 78% of 45,X in fibroblasts.	(Fujimoto et al., 1991)
H:19% A:80%	Ring 18 syndrome	Skin fibroblasts revealed mosaicism in affected twin 51%.	(Hata et al., 1982)
H1:6% H2:6% A:94%	Turner syndrome	MZ triplet with two males and one female. H1 has mosaicism of 43%, H2 has mosaicism of 3% and A has 1% in skin fibroblasts.	(Lespinasse et al., 1998)
BWS in MZ twins			
H:84% A:16% H:60% A:42% H:80% A:16% H:76% A:38% H:50% A:44% H:64% A:38% H:34% A:70%	Discordant KCN1OT1 imprinting		(Bliek et al., 2009)
H:4% A:64% H:54% A:22% H:40% A:36% H:56% A:10% H:98% A:0% H:80% A:54% H:52% A:8% H:10% A:64%	Discordant KCNQ1OT1 imprinting		(Weksberg et al., 2002)
MZ twins discordant for gender (non-syndromic)			
F: 79% M:28%	MZ twins discordant to sex chromosomes		(Zech et al., 2008)
MC-DZ twins			
M1:12% M2:1%	IVF. MC-DZ twins	Chimerism based on detection of blood group markers.	(Aoki et al., 2006)
F:25% M:77%	IVF. MC-DZ twins		(Williams et al., 2004)
F:91% M:9%	IVF. MC-DZ twins		(Souter et al., 2003)
F:44% M:56%	IVF. MC-DZ twins		(Ekelund et al., 2008)

Note: H: Healthy; A: Affected; M: Male; F: Female.

logical setting — a genotype mixture of 80%:20% (see Methods). In both conditions, we found a dramatic drop in the sensitivity to detect discordant variations when the differences in the genotype composition were less than 10%, which we found in most chimerism cases (Figure 2). When we employed Baranzini's stringent filtration criteria, the sensitivity dropped to zero in all tested conditions (data not shown).

To ensure the robustness of our results, we also tested a model of reciprocal chimerism. In this model, the level of twin A genotype in twin B is identical to the level of twin B genotype in twin A. We found that even with a chimerism level of 10% most SNP differences between genomes were lost. With higher chimerism levels differences became almost completely obscured (Figure 3).

Discussion

This study presents a systematic analysis of twin blood chimerism across multiple conditions and tests its effect on finding discordant variations using high throughput

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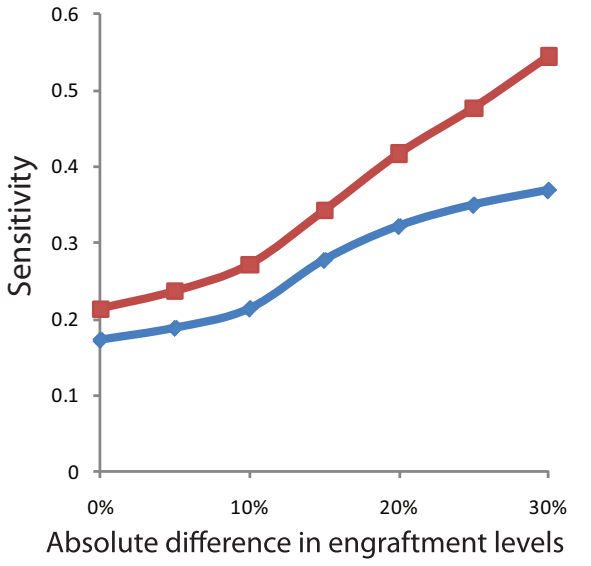

FIGURE 2

The effect of mirrored chimerism on calling discordant variations from high throughput sequencing data. The x axis represents the difference in the genotype compositions between the twins. Blue — pathological setting, Red — no clonal advantage setting.

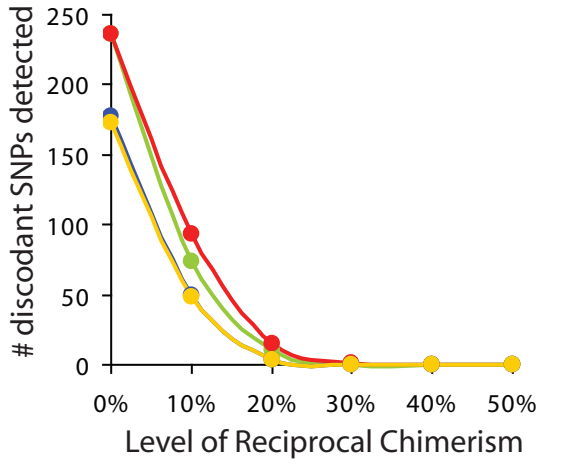

FIGURE 3

The effect of reciprocal chimerism on calling discordant variations from high throughput sequencing data. The x axis represents the absolute engraftment levels in each twin assuming that the levels are correlated. The graph shows the performance of two SNP callers, SNVmix (red, green) and VarScan (yellow, blue) using standard parameters (green, blue) and using the cutoffs in supplementary table 8 of Baranzini's study (red, yellow). The y axis shows the number of discordant SNPs detected.

sequencing data. In almost all cases, the chimerism patterns were mirrored and the compositions of the hematopoietic lineages of the twins were similar. This was found across multiple conditions and ages. Mirrored blood chimerism suggests that MC twins exchange their entire HSC repertoire during embryogenesis. The blood engraftment levels are shaped by clonal selection and after birth the grafts show long-term stability. Such long term stability of grafts is also found in other cases of blood transfusion-induced chimerism, for instance, in recipients of major blood volumes after trauma (Utter et al., 2004) and post-pregnant women that show fetus-derived DNA years after giving birth (Johnson & Bianchi, 2004).

The effect of mirrored chimerism on detection of discordant SNP variations is substantial. We found that the sensitivity dropped below 20% in the range of typical chimerism and zero sensitivity when more stringent calling was applied. With an expectancy of 70 *de novo* variations in a human genome (Roach et al., 2010), 20% sensitivity is prone to miss any post-twinning variations. Thus, our analysis proposes that blood-derived DNA is inadequate for whole genome sequencing of MZ twins.

The challenge of picking the right tissue for twin genomics is twofold. First, one should avoid tissues that contain high levels of hematopoietic cell lineages due to twin chimerism. Second, any post-twinning variation is likely to show some degree of somatic mosaicism and might be found only in certain cell lineages. Thus, it is highly important to sample a tissue that shows the discordant phenotype or developmentally close tissues if the affected organ is not accessible. One plausible solution is sampling buccal cells or skin cells. These types of tissue are high accessible and contain marginal amounts of transdifferentiated hematopoietic cells (Krause et al., 2001; Tran et al., 2003), which, according to our analysis, should not mask the post-twinning variations. In addition, buccal and skin are ectoderm-derived tissues and therefore may be a reliable proxy of post-twinning variations in neuronal tissues. Indeed, the genomics community might be reluctant to use these tissues due to the presence of exogenous DNA that can reduce the amount of informative sequence reads. However, increasing the sequencing power can compensate for that problem, whereas chimerism risks the entire study.

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References

Aoki, R., Honma, Y., Yada, Y., Momoi, M. Y., & Iwamoto, S. (2006). Blood chimerism in monochorionic twins conceived by induced ovulation: Case report. *Human Reproduction, 21*, 735–737.

Baranzini, S. E., Mudge, J., van Velkinburgh, J. C., Khankhanian, P., Khrebtukova, I., Miller, N. A., Zhang, L., Farmer, A. D., Bell, C. J., Kim, R. W., May, G. D., Woodward, J. E., Caillier, S. J., McElroy, J. P., Gomez, R., Pando, M. J., Clendenen, L. E., Ganusova, E. E., Schilkey, F. D., Ramaraj, T., Khan, O. A., Huntley, J. J., Luo, S., Kwok, P. Y., Wu, T. D., Schroth, G. P., Oksenberg, J. R., Hauser, S. L., & Kingsmore, S. F. (2010). Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. *Nature, 464*, 1351–1356.

https://doi.org/10.1375/twin.14.2.137 Published online by Cambridge University Press

Bliek, J., Alders, M., Maas, S. M., Oostra, R. J., Mackay, D. M., van der Lip, K., Callaway, J. L., Brooks, A., van 't Padje, S., Westerveld, A., Leschot, N. J., & Mannens, M. M. (2009). Lessons from BWS twins: Complex maternal and paternal hypomethylation and a common source of haematopoietic stem cells. *European Journal of Human Genetics, 17*, 1625–1634.

Bourthoumieu, S., Esclaire, F., & Yardin, C. (2006). Chimerism in twins: Caution is needed in interpretation of karyotypes. *American Journal of Medical Genetics A, 140*, 533–535.

Bourthoumieu, S., Yardin, C., Terro, F., Gilbert, B., Laroche, C., Saura, R., Vincent, M. C., & Esclaire, F. (2005). Monozygotic twins concordant for blood karyotype, but phenotypically discordant: A case of 'mosaic chimerism'. *American Journal of Medical Genetics A, 135*, 190–194.

Costa, T., Lambert, M., Teshima, I., Ray, P. N., Richer, C. L., & Dallaire, L. (1998). Monozygotic twins with 45,X/46,XY mosaicism discordant for phenotypic sex. *American Journal of Medical Genetics, 75*, 40–44.

Dallapiccola, B., Stomeo, C., Ferranti, G., Di Lecce, A., & Purpura, M. (1985). Discordant sex in one of three monozygotic triplets. *Journal of Medical Genetics, 22*, 6–11.

Edvardson, S., Shaag, A., Zenvirt, S., Erlich, Y., Hannon, G. J., Shanske, A. L., Gomori, J. M., Ekstein, J., & Elpeleg, O. (2010). Joubert syndrome 2 (JBTS2) in Ashkenazi Jews is associated with a TMEM216 mutation. *American Journal of Human Genetics, 86*, 93–97.

Edwards, J. H., Dent, T., & Kahn, J. (1966). Monozygotic twins of different sex. *Journal of Medical Genetics, 3*, 117–123.

Ekelund, C. K., Skibsted, L., Sogaard, K., Main, K. M., Dziegiel, M. H., Schwartz, M., Moeller, N., Roos, L., & Tabor, A. (2008). Dizygotic monochorionic twin pregnancy conceived following intracytoplasmic sperm injection treatment and complicated by twin-twin transfusion syndrome and blood chimerism. *Ultrasound in Obstetrics and Gynecology, 32*, 832–834.

Fujimoto, A., Boelter, W. D., Sparkes, R. S., Lin, M. S., & Battersby, K. (1991). Monozygotic twins of discordant sex both with 45,X/46,X,idic(Y) mosaicism. *American Journal of Medical Genetics, 41*, 239–245.

Goya, R., Sun, M. G., Morin, R. D., Leung, G., Ha, G., Wiegand, K. C., Senz, J., Crisan, A., Marra, M. A., Hirst, M., Huntsman, D., Murphy, K. P., Aparicio, S., & Shah, S. P. (2010). SNVMix: Predicting single nucleotide variants from next-generation sequencing of tumors. *Bioinformatics, 26*, 730–736.

Greaves, M. F., Maia, A. T., Wiemels, J. L., & Ford, A. M. (2003). Leukemia in twins: Lessons in natural history. *Blood, 102*, 2321–2333.

Gringras, P., & Chen, W. (2001). Mechanisms for differences in monozygous twins. *Early Human Development, 64*, 105–117.

Hall, J. G. (2003). Twinning. *Lancet, 362*, 735–743.

Hata, A., Suzuki, Y., Kuroki, Y., & Matsui, I. (1982). Ring-18 Mosaicism in Identical-Twins. *Japanese Journal of Human Genetics, 27*, 166–167.

Johnson, K. L., & Bianchi, D. W. (2004). Fetal cells in maternal tissue following pregnancy: what are the consequences? *Human Reproduction Update, 10*, 497–502.

Kaminsky, Z. A., Tang, T., Wang, S. C., Ptak, C., Oh, G. H., Wong, A. H., Feldcamp, L. A., Virtanen, C., Halfvarson, J., Tysk, C., McRae, A. F., Visscher, P. M., Montgomery, G. W., Gottesman, II, Martin, N. G., & Petronis, A. (2009). DNA methylation profiles in monozygotic and dizygotic twins. *Nature Genetics, 41*, 240–245.

Kaplan, L., Foster, R., Shen, Y. P., Parry, D. M., McMaster, M. L., O'Leary, M. C., & Gusella, J. F. (2010). Monozygotic twins discordant for neurofibromatosis 1. *American Journal of Medical Genetics Part A, 152A*, 601–606.

Kaplowitz, P. B., Bodurtha, J., Brown, J., & Spence, J. E. (1991). Monozygotic twins discordant for Ullrich-Turner syndrome. *American Journal of Medical Genetics, 41*, 78–82.

Koboldt, D. C., Chen, K., Wylie, T., Larson, D. E., McLellan, M. D., Mardis, E. R., Weinstock, G. M., Wilson, R. K., & Ding, L. (2009). VarScan: Variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics, 25*, 2283–2285.

Krause, D. S., Theise, N. D., Collector, M. I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S., & Sharkis, S. J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell, 105*, 369–377.

Langmead, B., Trapnell, C., Pop, M., & Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology, 10*, R25.

Lespinasse, J., Gicquel, C., Robert, M., & Le Bouc, Y. (1998). Phenotypic and genotypic variability in monozygotic triplets with Turner syndrome. *Clinical Genetics, 54*, 56–59.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics, 25*, 2078–2079.

Machin, G. (2009). Non-identical monozygotic twins, intermediate twin types, zygosity testing, and the non-random nature of monozygotic twinning: A review. *American Journal of Medical Genetics C Seminars in Medical Genetics, 151C*, 110–127.

Marcus-Soekarman, D., Hamers, G., Velzeboer, S., Nijhuis, J., Loneus, W. H., Herbergs, J., de Die-Smulders, C., Schrander-Stumpel, C., & Engelen, J. (2004). Mosaic trisomy 11p in monozygotic twins with discordant clinical phenotypes. *American Journal of Medical Genetics A, 124A*, 288–291.

Martin, N., Boomsma, D., & Machin, G. (1997). A twin-pronged attack on complex traits. *Nature Genetics, 17*, 387–392.

Metzker, M. L. (2010). Sequencing technologies: The next generation. *Nature Reviews Genetics, 11*, 31–46.

Monteiro, J., Derom, C., Vlietinck, R., Kohn, N., Lesser, M., & Gregersen, P. K. (1998). Commitment to X inactivation precedes the twinning event in monochorionic MZ twins. *American Journal of Human Genetics, 63*, 339–346.

Pedersen, I. K., Philip, J., Sele, V., & Starup, J. (1980). Monozygotic twins with dissimilar phenotypes and chro-

mosome complements. *Acta Obstetricia et Gynecologica Scandinavica, 59*, 459–462.

Potter, A. M., & Taitz, L. S. (1972). Turner's syndrome in one of monozygotic twins with mosaicism. *Acta Paediatrica Scandinavica, 61*, 473–476.

Roach, J. C., Glusman, G., Smit, A. F., Huff, C. D., Hubley, R., Shannon, P. T., Rowen, L., Pant, K. P., Goodman, N., Bamshad, M., Shendure, J., Drmanac, R., Jorde, L. B., Hood, L., & Galas, D. J. (2010). Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science, 328*, 636–639.

Rogers, J. G., Voullaire, L., & Gold, H. (1982). Monozygotic twins discordant for trisomy 21. *American Journal of Medical Genetics, 11*, 143–146.

Rohrer, T. R., Gassmann, K. F., Rauch, A., Pfeiffer, R. A., & Doerr, H. G. (2004). Growth of heterokaryotic monozygotic twins discordant for Ullrich-Turner syndrome during the first years of life. *American Journal of Medical Genetics Part A, 126A*, 78–83.

Souter, V. L., Kapur, R. P., Nyholt, D. R., Skogerboe, K., Myerson, D., Ton, C. C., Opheim, K. E., Easterling, T. R., Shields, L. E., Montgomery, G. W., & Glass, I. A. (2003). A report of dizygous monochorionic twins. *New England Journal of Medicine, 349*, 154–158.

Tran, S. D., Pillemer, S. R., Dutra, A., Barrett, A. J., Brownstein, M. J., Key, S., Pak, E., Leakan, R. A., Kingman, A., Yamada, K. M., Baum, B. J., & Mezey, E. (2003). Differentiation of human bone marrow-derived cells into buccal epithelial cells in vivo: a molecular analytical study. *Lancet, 361*, 1084–1088.

Uchida, I. A., deSa, D. J., & Whelan, D. T. (1983). 45,X/46,XX mosaicism in discordant monozygotic twins. *Pediatrics, 71*, 413–417.

Utter, G. H., Owings, J. T., Lee, T. H., Paglieroni, T. G., Reed, W. F., Gosselin, R. C., Holland, P. V., & Busch, M. P. (2004). Blood transfusion is associated with donor leukocyte microchimerism in trauma patients. *Journal of Trauma, 57*, 702–707.

van Gemert, M. J., & Sterenborg, H. J. (1998). Haemodynamic model of twin-twin transfusion syndrome in monochorionic twin pregnancies. *Placenta, 19*, 195–208.

Weiss, E., Loevy, H., Saunders, A., Pruzansky, S., & Rosenthal, I. M. (1982). Monozygotic twins discordant for Ullrich-Turner syndrome. *American Journal of Medical Genetics, 13*, 389–399.

Weksberg, R., Shuman, C., Caluseriu, O., Smith, A. C., Fei, Y. L., Nishikawa, J., Stockley, T. L., Best, L., Chitayat, D., Olney, A., Ives, E., Schneider, A., Bestor, T. H., Li, M., Sadowski, P., & Squire, J. (2002). Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Human Molecular Genetics, 11*, 1317–1325.

Williams, C. A., Wallace, M. R., Drury, K. C., Kipersztok, S., Edwards, R. K., Williams, R. S., Haller, M. J., Schatz, D. A., Silverstein, J. H., Gray, B. A., & Zori, R. T. (2004). Blood lymphocyte chimerism associated with IVF and monochorionic dizygous twinning: Case report. *Human Reproduction, 19*, 2816–2821.

Zech, N. H., Wisser, J., Natalucci, G., Riegel, M., Baumer, A., & Schinzel, A. (2008). Monochorionic-diamniotic twins discordant in gender from a naturally conceived pregnancy through postzygotic sex chromosome loss in a 47,XXY zygote. *Prenatal Diagnosis, 28*, 759–763.

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