Super CHIP: Duplex Sequencing™ Reveals Clonal Dynamics in Blood that are Magnitudes more Complex than Previously Recognized

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Abstract

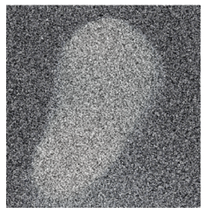
Hematopoietic cell transplantation (HCT) is used in the treatment of hematologic malignancies (HM). Clonal hematopoiesis of indeterminate potential (CHIP) is the age-associated process whereby healthy individuals accumulate low-frequency clones driven by mutations in genes recurrently mutated in HM. CHIP is associated with increased risk of cardiovascular disease and transformation into HM.

It has been hypothesized that CHIP clones present in HCT donors might disproportionately proliferate in the recipient due to bottlenecking and a relative growth advantage during immune reconstitution. Here we use ultra-sensitive Duplex Sequencing (DS) of genes associated with HM to investigate whether the clonal makeup of CHIP in prior donors themselves, differs from that of the engrafted donor-derived hematopoietic system multiple years after allogeneic transplant into a recipient.

We identify dozens of mutation-bearing clones in all donor samples sequenced, which are enriched for genes previously associated with CHIP. The extremely high sensitivity and specificity of DS reveals a far more complex landscape of competing ultra-low frequency driver mutations within individuals than observed in any prior studies.

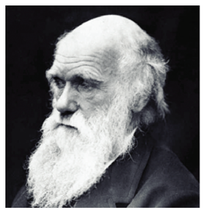
When comparing clonal dynamics between donors and recipients we observe no significant change in clonal makeup, frequency or mutation spectrum, regardless of age. This suggests that HCT does not accelerate CHIP evolution in transplant recipients, which is reassuring from the perspective of stem cell health, secondary cancer risk, and safety of using older donors.

Sequencing Errors Obscure Truth



Next-Generation Sequencing (NGS)

Single Strand Error-Corrected NGS

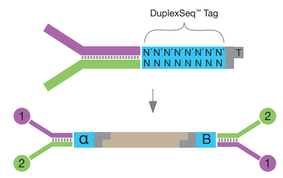


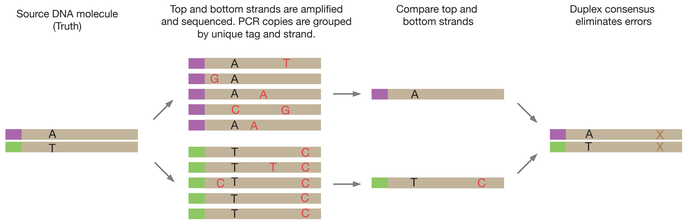
Duplex Sequencing

TwinStrand Duplex Sequencing™ Technology

A DuplexSeq™ Adapter has:

1. Identical (or relatable) degenerate tags in each strand.
2. An asymmetry allowing independent strand identification.

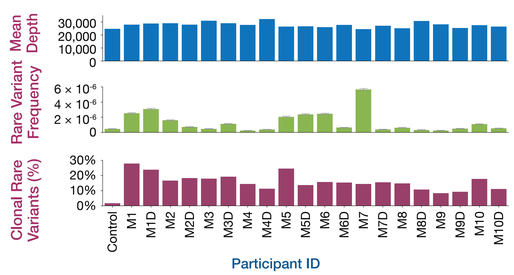




Donor-Recipient Characteristics

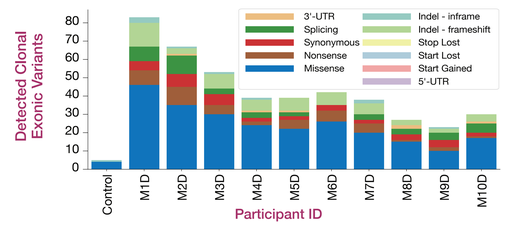
Unique Pair ID	Years from HCT	Disease	Stem cell source	HLA-match	Recipient age at HCT (years)	Donor ge at HCT (years)	Age difference between donor & recipient (years)
1	6.6	MDS	PBSC	Matched	48	84	36
2	45.7	PNH	BM	Matched	23	18	5
3	36.3	CML	BM	Matched	28	27	1
4	38.6	AML	BM	Matched	16	14	2
5	8	AML	PBSC	Matched	42	71	29
6	32.1	CML	BM	Matched	30	43	13
7	39	AA	BM	Matched	12	13	1
8	37.7	AA	BM	Matched	22	19	3
9	36.9	AA	BM	Matched	14	12	2
10	26.4	CML	BM	Mismatched	35	62	27

Duplex Sequencing: High Depth, Low Background



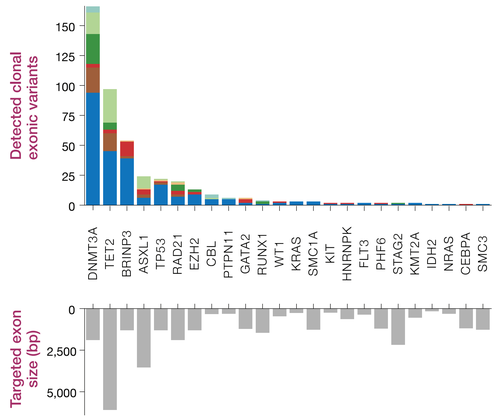
Barplots of mean Duplex Sequencing depth, frequency of rare variants, and the percent of rare variants that are clonal (multi-count) for all samples in project, including a healthy 18 year old control. Samples were sequenced to at least 24,000x mean Duplex depth. Paired donor and recipient samples generally had a similar proportion of clonal rare variants. Background mutation frequencies were below one-in-one million ($1x10^{-6}$) for many individuals, including the control. Up to 29% of rare variants were clonal in donors and recipients, with a much lower proportion in the much younger control.

CHIP Variants Identified in Every Sample



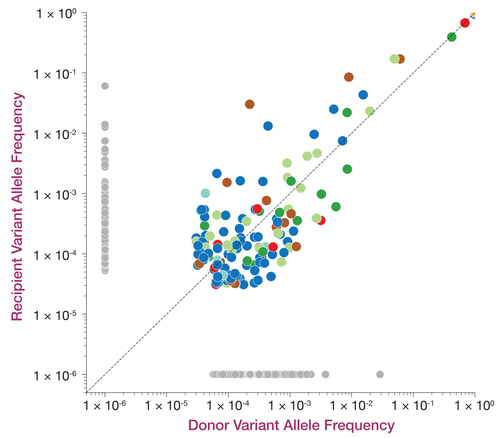
Dozens of multi-count (clonal) variants from known CHIP genes were identified in each donor, and only 6 in the control. Variants were colored by predicted effect on the canonical transcript. The majority were missense or nonsense, indicating *in vivo* selection for those that alter function of the resulting protein.

Abundant Mutations in Canonical CHIP Genes



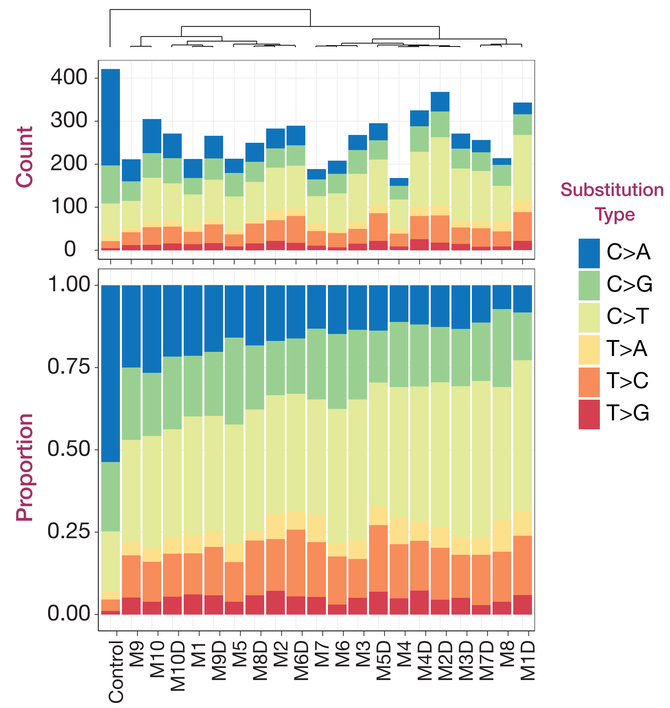
Total number of multi-count (clonal) variants from donors in known CHIP genes. Variants are colored by predicted effect on the canonical transcript. There was little correlation between gene size and the number of variants detected, arguing against sise-mediated bias. Compared to other approaches used in previous studies, Duplex Sequencing observed many more clonal variants in all patients and genes.

Complex Donor/Recipient Clonal Dynamics



Measure of change in allele frequency in variants shared between donors and recipients. As in previous figures, variants are colored by predicted effect on the canonical transcript. Many variants increased or decreased in frequency, with the majority present at VAF < $1x10^{-3}$. Some are unique to donor or recipient (along axes), but there was not a consistent pattern of increased frequency in the recipient. This suggests that HTC does not bias age-associated expansion of clonal variants.

No Unique Base Substitution Signature in HCT Recipients



Unsupervised heirarchical clustering of the DNA base substitution spectrum in all donors, recipients, and the control. There was no strong base substitution signature unique to HCT recipients, but all donor and recipient samples show an aging signature relative to the 18-year old control. Donors and recipients ranged in age from 49-91 years old at the time of biopsy.

Conclusions

- Duplex Sequencing detects multiple ultra-low frequency clonal CHIP mutations in all individuals, including the healthy 18-year old control.
- Duplex Sequencing detects drastically more CHIP mutations per individual than in previous studies, and at much lower frequencies.
- Background mutation frequency in the control is $4.7x10^{-7}$.
- Tracking of ultra-low frequency clonal dynamics between donor and recipient samples reveals that there is no strong bias for clonal expansion of CHIP variants after HCT.
- Duplex Sequencing identifies enough rare frequency variants to calculate base substitution spectra for all samples. However, there is little difference between donor and recipient spectra. The predominant base substitution signature in all samples is driven by aging.
- The sensitivity of Duplex Sequencing may help determine whether specific CHIP mutations are associated with stronger risk of HM or cardiovascular disease.

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